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EFFECT OF CEPHALOSPORINS ON (BIOFILM PRODUCTION AND PROTEASE) ACTIVITIES BY SOME BACTERIA ISOLATED FROM OTITIS MEDIA

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Abstract:

30 samples (swab) were collected from patients suffering from Otitis media. Swabs were implanted on the culture media blood agar and MacConkey agar to isolate the bacteria and to diagnose them using microscopic, culture and biochemical tests and confirmed by the Vitck-2 system. Of the total, 18 isolates were selected which belong to 8 (26.6%) *Staphylococcus aureus*, 5 (16.6%) *Klebsiella pneumonia*, and 4 (13.3%) *Escherichia* coli. All isolates were investigated for sensitivity to (18) antibiotics, six of them from the cephalosporins group The results showed that all isolates were 100% resistance to Cefotaxime, Ceftazidime, Cefixime, Ceftriaxone, Cefepime, Cefoxitin, Aztronam, Ampenicillin, while the isolates showed the lowest percentage of resistance to Imipenem (4.54) %, while all differences showed a clear difference in some of their resistance. Of the antagonists (Vancomycin, Erythromycin, Rifampin) with a percentage of (95.45, 27.27, 18.18)%, respectively. but for concentrations (4–16)µg/ml were ineffective for some of them. The (Minimal inhibitory concentrations) MIC test indicated that it ranged between (4–32)µg/ml for Ceftriaxone and (16–32)µg/ml for Ceftazidime. All isolates were shown ability have (100%) activity to produce (Biofilm) was tested on the Congo red agar (CRA) medium, and ability to produce a protease enzyme by (72.22%) on Skim milk agar medium. The results showed a effect of inhibitory concentrations of Ceftriaxone on the activity of biofilm production and the protease enzyme, further the results of this study showed that the following concentrations (1024, 512, 256 and 128)µg/ml were lethal to isolates, while (32–64)µg/ml were inhibitory, Also, the molecular diagnosis shown results of Agarose - gel electrophoresis of both (normal case) *S. aureus*, E. coli and Kl. pneumonia and heal isolates observed the presence of chromosomal and plasmid DNA bands in the normal status but alone chromosomal DNA bands occur with the isolates deal in Ceftriaxone at levels of (32–128)µg/ml. In the study of the effect of some Gram-negative bacteria by using IL-2 human. It can be used in the diagnosis of inflammation of the Otitis Media.

Key words: Cephalosporins, Otitis media, Biofilm, protease, *E. coli, Staphylococcus aureus, Klebsiella pneumonia*.

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Introduction:

Otitis Media

Otitis Media (OM) is defined as an infection of the mucous membranes lining the middle ear cavity by pathogens. The bacterium is at the forefront of the important causes of its occurrence (1).Otitis Media is the second most common childhood disease after upper respiratory tract infection. The middle ear is part of the upper respiratory tract (URTI). Therefore, an injury to the respiratory tract will have a role in Otitis media (2). As otitis is the most common bacterial infection in children under the age of five years, the disease may develop with complications, including hearing loss, at a critical stage in the child's learning of speech and language.

The researchers explained the mechanism of Otitis media taking place by entering the bacteria colonizing the nasopharynx into the middle ear through the Eustachian tube after avoid normal immunity. It blocks bacteria from entering the middle ear by the epithelium that lines the Eustachian tube. However, the viral respiratory infection destroy the ciliated mucous system and devastate the defense fibers that prevent bacterial penetration.

In addition to the loss of the Eustachian tube function that leads to the reduction of middle ear pressure that pushes mucus, nasopharyngeal secretions and bacteria to the middle ear and this engenders an perfect environment for secondary bacterial infection (4)

The invasion of the nasopharynx of the middle ear and its multiplication is characterized by the bacterial pheno type in response to the differences of the nasopharynx from the mask of the middle ear, according to the response of the host present to the replicating bacteria through the release of many particles outside and inside cells and this chain of events leads to the disease and in the end the occurrence Otitis media . The existing host of the multiplying bacteria by liberating many particles outside and inside cells, and this chain of events leads to disease and ultimately the occurrence of otitis media (5).

The importance of losing the function of the Eustachian tube can be indicated as a result of the occurrence of otitis media. As the natural function of the canal is to regulate the pressure and make it equal to the external pressure, as well as to protect the ear from secretions from the nasopharynx. It is an indirect course of middle ear secretions into the nasopharynx (6).

Studies have shown how viral infections lead to increased loss of eustachian tube function, and with it, increased bacterial colonization in the nasopharynx. The second route of infection is the tympanic mebranc. If this membrane represents a protective barrier to the middle ear from the external environment. When a hole or defect occurs in it, it provides a pathway for the bacteria present in the external auditory canal (extenal audiory meatus) to facilitate the middle ear. Otitis media can be classified into several types and depending on the disease, disease severity and symptoms, it is classified into: -

First: - Acute Otitis Media: symbolized by (AOM), which is a severe inflammation of the middle ear, often caused by viral infections, and is characterized by redness and swelling of the tympanic membrane. The bacterial infection has been established in 70 of the injuries (9). And that the most common bacterial pathogens in otitis reduction were Notypable Haem. Streptococcus pneumonia, lnfluenza moroxella cataharalis

Second: - Gonorrhea Otitis Media:

It is symbolized by OME, and it is also called sectory otitis media, and sought to have auriculitis. otorrhea while there are no signs and symptoms of infection, such as fever. Ear pain. And irritability (trritability). Acute injury results as an inflammatory response or independently of loss of Eustachian tube function and with it, hearing loss (11).

Third: - Chronic Suppurative Otitis Media:

It is symbolized by (CSOM), and it is a chronic inflammation of the middle ear and the visceral cavity. It is characterized by frequent ears clotting through the perforated tympanic membrane.

Several bacterial strains were isolated from Otitis media, the most important of which was Staphylococcus aureus, Psendomonas aeruginosa Diphtheroides, Klebsiella pneumonia, Proteus ssp.

Anaerobic bacteria such as Propiobacterium peptostreptococcus were isolated (7).

If these types do not have a natural shape in the outer ear. But it may reproduce with wounds and infections. When there is high humidity in the ear (12).

Although the mortality rate is low in people with Otitis media, its prevalence in infants and children of older age makes it a major public health problem(3).

Predisposing factors

• Age

Studies indicate the prevalence of Otitis media in both sexes and different ages, especially among children 6-18 months old (13).

In a study conducted by (14). It was mentioned that 22% of acute ear infections occur during the first year of life, and its cause (2.10.15)% during the second, third and eighth years of the child's life, respectively. Also found (15). Children between the ages of 6-11 months are more prone to complications from acute Otitis media and after infection of the upper respiratory tract.

• Sex

Recent studies have indicated a convergence of the rate of infection with ear infection between males and females (16). It is evident that some previous studies show that the incidence of infection is higher among males (17).

• Host factors

Many factors related to the host's immune system are involved in the occurrence and development of infection, including: -

Immune systems are considered mature for infants or patients with insufficient immune systems associated with immune defects, as is the case with AIDS or diabetes (DIABETES) (18). Also, children who suffer from vitamin A deficiency. In addition to the respiratory tract infection, they are more susceptible to acute ear infection (14).

• Genetic factors

Studies have indicated the absence of specialized genes related to otitis media. However, these studies suggested the existence of a genetic relationship to aroma factors (Rist Factor) that appears when a family member suffers from acute otitis media. As the risk of infection increases for the rest of the family (19).

• Faulty Eustachian channel functionality

Influenza infections of the upper respiratory tract lead to respiratory epithelial cell inflammation. It causes loss of eustachian tube function, which makes the host possible increase in acute bacterial otitis media development (6).

• **Envioronmental factors include:**

Infant feeding method, Possive Smoke Exposure, Low Socioeconomic Status and
Sensitivity this allergy in children is described as one of the risk factors for Otitis media because it causes swelling of the mucous membrane of the upper respiratory system, and may cause a defect or loss of the function of the eustachian tube, similar to what is observed in the case of viral infections of the upper respiratory tract, as the body's response to allergies may cause a decrease in the effectiveness Mucinous cilia that allow increased bacterial colonization of the upper respiratory tract (22).

• **Other factors**

It is noted that the incidence of middle ear infection increased in winter (13). The researcher, when studying people with Otitis media in Nasiriyah, found that the number of infected people was large during the winter months, the percentage of injured persons was 40% compared to the summer months, which reached 15.5%.
The researcher (23) found that water entering the ear during swimming in baths and beaches is one of the main causes of most cases of infection with Psendomonas aeruginosa.

Microorganisms causing Otitis Media

Chronic suppurative Otitis is caused by several microorganisms, including the aerial bacteria Escherichia, Staphylococcusaureus, and pseudomnas aeruginosa, Klebsiella spp. Protens mirabilis.
Microorganisms causing Otitis Media in this study:

• **Staphylococcus aureus**

Staphylococcus aureus is the second most common pathogen isolated from chronic diseases of the middle ear, its incidence of injuries has been estimated at 15-30% (39).
S. aureus belongs to the Microcoaceae family. They are motile, not forming whiteboards, facultative anaerobes, and their cells are arranged in cells, single, pairs, or tetrapods, chains, and form of clusters. (15).
S. aureus generates infections through its ability to spread in tissues thanks to its production of many extracellular materials, As well as its production of important enzymes, including Haemolysin, Lipase, and protease as well as Coagulase, Nuclease, Hy lourindase, and other virulence factors it contains some of its strains on the capsule resisting the process of phagocytosis by cells (PMN) Poly Morpho Nuclear (NUTROPHIL). As well as its a pathogen in hospital infections, as it can colonize medical devices and equipment by acquiring more resistance to antimicrobials through the formation of the biofilm.

• **Klebsiella spp.**

Klebsiella spp. belong to the Enterobacteriaceae family, and this genus includes gram-negative bacilli, not motile usually cyclic, can be distinguished by their large colonies that are gray and sometimes white, mucous (muicod) on laboratory media, especially if it contains a percentage of sugar, especially since it has the ability to ferment sugars, including lactose.
K.pnenmonia has many harmful factors that contribute to disease, including the Capsular Antigen, especially since the capsule is very necessary for its pathology, as it protects it from the process of phagocytosis by PMNS and on the one hand it prevents the killing of bacteria mediated by serum factors.

The Klebsiella fimbria (Pilli) cells that contribute to the process of bacterial attachment to the surface of the host tissues, the capsule and the fimbria are structural components that are fixed on the surfaces of K.pneumonia cells and have an important role in conditioning and pathogenesis (40).
Klebsiella can be isolated from clinical and non-clinical models, and many recent studies have praised that this bacterium is largely responsible for hospital-borne infections such as septicemia (Bacteriema) and abscesses (Sepsis), especially in immunosuppressed persons (41). (C), in its study of some pathogens of chronic suppurative ear infection in Baghdad, found that the percentage of K. pnenomonia isolates was 6.02%.

• Escherichia coli

E. coli belong to the Enterobacteriaceae family, facultative anaerobic, gram negative, causing mixed-acid fermentation in anaerobic conditions, the optimum temperature for their growth is 37˚C, while several laboratory strains can grow at different heat up to 49˚C, the flagellation arrangement In E. coli, oceanic arrangement petrichons (41).
Coli strains cause many infections such as gastroenteritis, urinary tract infections, and cerebral meningitis in infants, and cause septicemia and pneumonia caused by Gram negative pnenmonia (42).
(A), in its study of some causes of ear infection, stated that E. coli isolates represented (4.29)% of the total bacterial isolates.

Bifilm formation

It is considered as one of the virulence factors and is formed by many types of microorganisms. It is a collection of microbial cells, that form on the surfaces, and are associated with them in a way that is difficult to remove, and they are surrounded by a filling of polysaccharide (43).
The process of biofilm formation and adhesion on living and non-living surfaces is related to a group of factors, namely the affinity of the surfaces of bacterial cells to water, the presence of adhesion factors, as well as the different properties of the surfaces.
The formation of the biofilm increases the resistance of bacteria to antimicrobials. The ability of bacteria to form a biofilm has been observed on various surfaces, starting with the intravascular catheter and even the pacemaker leads (44).

Protease production

The production of proteolytic enzyme by bacteria is one of the important virulence factors possessed by bacterial species, which helps them in causing infection. As well as causing many changes in the tissues that are infected with these bacterial species, and most of these enzymes are of the type secreted outside the cell so that they can be isolated from the culture medium (45).
Protease enzymes have different characteristics depending on their effectiveness and composition, and they can be classified according to the molecular weight, the charge they carry, the base material they work on, or the active site (active site). Inhibitors, while there are compounds that have the ability to increase the speed of the enzymatic reaction, they are called activators (46).

The Cephalosporins

The third-generation cephalosporins are concerning broad-spectrum antibiotics that possess activity against both gram-negative and gram-positive bacteria. However, these drugs are more

effective against gram-negative organisms and bacteria that are resistant to the first and second-generation cephalosporins (47). The past decade, resistance dominance in hospital-acquired infections has risen considerably; Infections caused by resistant organisms are thought to result in prolonged hospitalization, and higher morbidity and mortality. Third-generation cephalosporins were started with cefotaxime 30 years ago, resistance in bacterial species emerged a few years later due to selective pressure exerted by these new cephalosporins (48). At present, Antibiotic resistance considered a global health emergency and extended-spectrum beta-lactamase producing bacteria that can resist third-generation cephalosporins are on the rise and increasing with time (47). Cephalosporins are bactericidal; acts by disrupting the synthesis of the cell wall layers, as well as they have the same mode of action as other β-lactam antibiotics like penicillin but are less susceptible to β-lactamases. During the past fifteen years, dissemination and developing of β-lactam resistance in nosocomial gram-negative bacteria and Pseudomonas aeruginosa became a serious problem globally, especially the increasing resistance to third and fourth generation cephalosporins (48). As with most beta-lactam antibiotics, third-generation cephalosporins are generally well tolerated and characteristically have a low toxicity profile.

Some examples of infections that Cephalosporins can treat include:

Skin or soft tissue infections, urinary tract infections (UTIs), strep throat, ear infections as (Otitis Media), pneumonia, sinus infections, meningitis, and gonorrhea.
They can be taken orally or injected into a vein (intravenous injection), depending on the infection. Healthcare providers use cephalosporins to treat a variety of bacterial infections, especially for people who are allergic to penicillin, another common antibiotic. [49].

- Oral Cephalosporins are generally used for simple infections that are easy to treat. For example, a routine case of strep throat might be treated with a course of oral cephalosporins.
- Intravenous (IV) cephalosporins are used for more severe infections. This is because IV antibiotics reach your tissues faster, which can make a big difference if you have a serious infection, such as meningitis.

Material and Methods

- **Swabs Collection:**

30 swabs were collected from patients attending the outpatient clinic of the Ear, Nose and Throat Division who suffer from ear infections and under the supervision of specialized doctors at Hospital Baghdad, using sterile cotton swabs.

- **Swabs Culture:**

Cotton swabs were cultured immediately after collection on the culture media that included the medium of blood agar and the medium of Maconkey agar, then the dishes were incubated at (37°C) for 24 hours and the bacte rial isolates were initially diagnosed according to the phenotypic characteristics of the colonies' shape, strength and color, and other diagnostic tests were completed for them.

Diagnostic Testes:

• Microscopic test

A part of the growing bacterial culture was transferred on the nutrient agar medium and placed on a clean glass slide and fixed and stained with gram stain, to observe the shape, size and ability of bacteria to be stained with gram stain (50).

• Biochemical tests: were identified to the level of subspecies using the conventional biochemical and morphological test and then confirmed by the Vitck2 system.

D- Biofilm production test:

Detection of the ability of bacteria to produce biofilm by Congo red agar (CRA) method.

Congo red dye was prepared by dissolving it in water in an autoclave at a temperature of 121˚C for 15 minutes, then adding agar after cooling it at 55˚C. dark ends of growing determined the biofilm production (Todar,2007).

E- Protease test

Skim milk agar, this medium was used to investigate the ability of the bacterial isolates under study to produce the protease enzyme. Investigation of bacterial isolates producing proteinase

The diameter of the inhibition zone was measured, then the isolates that gave the widest diameter of the decomposition zone were selected.

F- Growth on Mannitol - Salt agar test:

which is a selective medium for the isolation of Staphylococcus Spp. (Because it contains a high salt concentration and also to observe the ability of bacteria to ferment mannitol sugar (50).

G- Growth on Eosin-methylene Blue (EMB):

Intestinal family isolates were cultured on EMB medium, as E.coli isolates give a green metallic sheen, which distinguishes them from other genera of the intestinal family. As for Klebsiella Spp. so its colonies are mucoid, and they do not show metallic luster (50).

Antimicrobial Susceptibility test:

The disk diffusion test method was used according to method (54) to test the sensitivity of the isolates under study to antimicrobials using Muller-Hinton Agar medium, as follows:-

1- Number 3-5 of colonies with the same phenotypic traits grown on the nutrient agar medium were transferred by the standard culture vector into a tube containing 5 ml of nutrient broth.

2- The tubes containing the liquid bacterial culture were incubated at 35°C for 24 hours.

3- Transferring part of the liquid culture to tubes containing the physiological saline solution, and the turbidity of the growth was compared with the turbidity of the standard turbidity constant solution (Mcfarland standard No. 0.5 which gives 1.5 x 108 CFU/ml).

4- Transfer 100 µl of the bacterial suspension with a microtiter pipette, then spread it with a glass spreader on the surface of the Muller-Hinton agar medium homogeneously, then leave the plates to dry at room temperature for 10-15 minutes.

5- After that, the antibacterial tablets were transferred by sterile forceps to the dishes with 6-5 tablets per dish, then the dishes were incubated at a temperature of 35°C for 24 hours.

6- The results are read by measuring the diameter of the damping areas in mm around the antibacterial tablets and compared with (NCCLS, 2002) (51).

Determination of minimal inhibitory concentration of antimicrobials

The agar dilution method was followed for a number of cephalosporins under study, as stated in () and as follows:

• Multiple serial concentrations of antibacterial agents were prepared, the value of which ranged between 1024-2 µg/ml.

• The medium of Muller-Hinton aquarium was prepared in clean glass bottles of 20 ml per bottle, then sterilized with sterilizer and the media were cooled to 45°C.

• Antibiotics were added, the media was shaken well, poured into sterilized dishes, and kept at 4°C until use.

• Bacterial cultures were prepared at the age of (24-18) hours and compared with a standard turbidity constant solution.

• Withdrawal of 5 microliters of the above bacterial cultures, and of all isolates, by means of a micropipette, and inoculated with it in Müller-Hinton agar media containing cephalosporins at different concentrations.

• The media was left for a while at room temperature until the surface of the dishes dried before inverting, and then incubated at 37°C for 24 hours.

• According to MIC, it is the lowest concentration of anti-bacterial that prevents the appearance of a clear growth of bacteria.

Detection of Enzymatic activity after the addition of antimicrobial agents

The aim of this test is to investigate the ability of local isolates to produce biofilm and protease enzyme after adding antibacterials. In this assay, cephatrixone and ceftazidime compounds were used, as they were used in concentrations lower than the minimum inhibitory concentration for each isolate, which is the concentration that allows bacterial growth.

A fixed volume of bacteria 0.1 ml was used, compared with the standard turbidity constant and added to a fixed volume of Sub-MIC antibacterial (1 ml). The mixture was incubated for 24 hours at 37°C. Withdraw a volume of 0.1 ml of the mixture by means of a microtiter pipette and streak the Kongo Red agar medium and the agar milk screening medium. The results are read the next day, which indicates the ability of the isolates under study to produce biofilm and protease enzyme after exposure to the antigen.

Detection IL-2 in serum

The study involved drawing blood samples from all patients with Otitis media, drawing 3 ml of blood, isolating blood and keeping serum in cryopreservation for IL-2 measurement by ELISA assay.

3- Results and discussion

Isolation and diagnosis

The bacterial isolates were initially identified on the basis of their phenotypic characteristics in the culture media. S. aureur colonies were characterized by being smooth, elevated, forming golden pigments, and were surrounded by a region of complete hemolysis on the center of the blood agar base, as in figure (1), and they did not grow on the center of the MacConkey because it contains bile salts that inhibit the growth The bacteria positive for the cram stain, either on the

medium of mannitol salt agar, which is a selective medium for it was fermented mannitol sugar, transforming the color of the medium to yellow as in Figure (2).

As for E. coli colonies, they were characterized by being fermented for lactose sugar on MacConkey Agar, flat, dry, pink and surrounded by a dark pink area as a result of the precipitation of bile salts, and the use of EMB blue medium was diagnosed when it showed a green metallic luster as in Figure (3).

K. pneumonia showed mucous colonies on the Maconkey medium and did not appear the green metallic luster on the E.M.B. medium, and these results were compatible with what was mentioned in (6).

Figure (1) S.aureus colonies produce the hemolytic enzyme (hemolysin) on blood agar

Figure (2) S.aureus colonies fermenting mannitol sugar on Mannitol Salt Agar

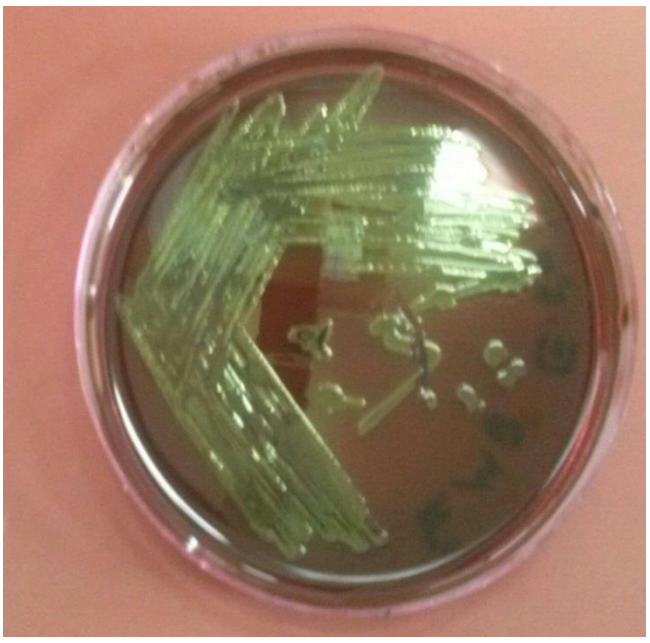

Figure (3) E. coli colonies on E.M.B. medium showing green metallic sheen

Figure (4) K. pneumoniae mucoid colonies fermented lactose on MacConkey medium.

After the isolates were subjected to microscopic and other diagnostic tests, biochemical tests were tested and as observed in Table (1-3), the isolates belonging to the genus S. aureus For blood, positive for catalase and negative for oxidase.
Also, the diagnostic tests for the intestinal family showed that the isolates of type K. pneumoniae and negative for Cram stain, positive for catalase, Voges - Proscure and citrate utilization, negative for oxidase, Indole production and methyl red.
Isolates of E. coli showed positive for catalase, indole and methylase production, but negative for Voges - Proscure tests, and consumption of citrate and oxidase.

Table (3-1): Microscopic and biochemical tests performed on bacterial isolates isolated from middle ear infections

Types of isolates / The exams	S.aureus	K.pneumonia	E.coli
Microscopic test	Cocci cluster G+ve	Coccobacilli G-ve	Coccobacilli G-ve
Catalase test	+	+	+
Oxidase test	_	_	_
Indole production	#	_	+
Methyle red test	#	_	+
Vogas- Proskaur test	#	+	_
Citrate utilization test	#	+	-
Growth on mannitol salt medium	+ Fermented mannitol sugar	#	#

+: The result is positive -: The result is negative #: Not tested

Testing the sensitivity of bacteria isolated from middle ear infections to antibiotics

• Bacterial sensitivity test using the disc diffusion method.
This assay was conducted to investigate the sensitivity of the isolates to (18) antibiotics, six of which are from the group of cephalosporins: Cefotaxime, Ceftazidime, Cefixime, Ceftriaxone, Cefepime, Cefoxitin and other antibiotics belonging to the beta-lactam groups and quinolines, and others are: Imiflopenamin, Azizronamin, and Azizil Ampenicillin, Levofloxacin, Vancomycin, Erythromycin, Rifampin, the results were recorded by measuring the diameters of inhibition formed around the discs and comparing them with international tables (NCCLS, 2002).
All isolates showed 100% resistance to Cefotaxime, Ceftazidime, Cefixime, Ceftriaxone, Cefepime, Cefoxitin, Ampenicillin, while the isolates showed the least resistance to Imipenem (4.54)%, and for Ciprofloxacin (36.36)%, Levo18. These results were in agreement with the findings of d)), as the percentage of resistance of isolates to anti-cephalosporins was 100% and to anti-ciprofloxacin was 33%, and the isolates were resistant with close proportions ranging between (43-18)% to the antigens (Nitrofurantoin, doxycyclin, Gentamycin, Aztronam), while Some isolates showed a clear difference in their resistance to each of the antigens (Vancomycin, Erythromycin, Rifampin) with a percentage of (95.45, 27.27, 18.18)%, respectively.

Determination of (MICs) for Cephalosporins

MIC is defined as the minimum inhibitory concentration of the antibacterial agent that inhibits the growth of bacteria outside of vivo (in vitro). The MIC values of the bacterial isolates were

determined by serial multiplicative concentrations on Muller-Hunton agar medium, as the components of this nutrient influence the MIC results. This medium is suitable for conducting antimicrobial tests because it contains small amounts of NaCl with appropriate amounts of Mg, Ca, which have an effect on the effectiveness of the antimicrobial. The size of the bacterial inoculum affects the MIC values, as the MIC value increases with the increase in the size of the bacterial inoculum ( ).

The effects of inhibitory concentrations of Ceftriaxone on the activity of biofilm production and the protease enzyme were determined. Inhibition of biofilm and protease production was observed. The results of this study showed that the following concentrations (1024, 512, 256, 128) µg/ml were lethal to the isolates, while (32 - 128) µg/ml were inhibitory, and as for the concentrations (4 - 32) µg/ml were not effective for some of them. The MIC test showed that it ranged between (4- 23) µg/ml for (Ceftriaxone) and (16-23) µg/ml for (Ceftazidime).

The effect of (MICs) of Cephalosporins on biofilm formation activity.

Antimicrobials have multiple effects on bacterial cells even when used at low concentrations, and these effects were studied in detail at the cellular metabolic level, which showed that all these compounds - regardless of their receptors on the surface of the bacterial cell or their mechanism of action, have the ability to provoke or induce Gene cloning of some virulence factors when antibiotics are used at low concentrations.

Bacterial virulence factors such as biofilm formation and protease production reflect the organism's ability to cause pathogenic effects in the host.

Our study included exposing the bacterial isolates to the MIC of two antibiotics from the cephalosporins group, Ceftazidime (Ceftriaxone), and observing the activity of these isolates after exposure to the antibiotic as in figures (5, 6) for K. pneumonia ,(7, 8) for E. coli and (9, 10) for S. aureur.

Figure (5) Biofilm formation by K. pneumonia on Congo red agar medium

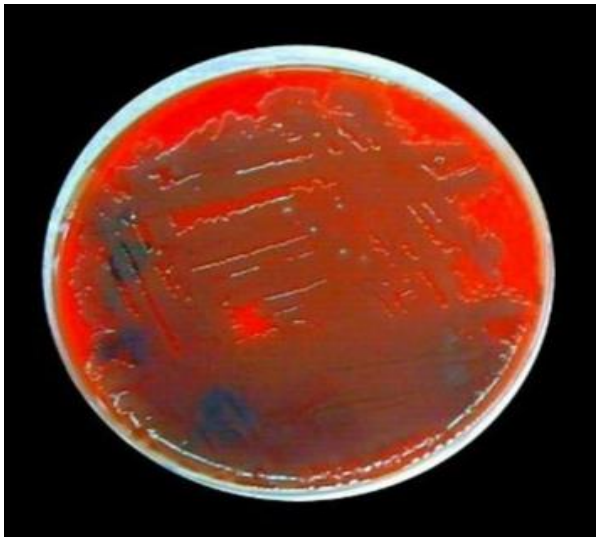

Figure (6) Inhibition of biofilm formation by K. pneumonia on Congo red agar medium after treatment with 32µg/ml Ceftriaxone

Figure (7) Biofilm formation by E. coli on Congo red agar medium

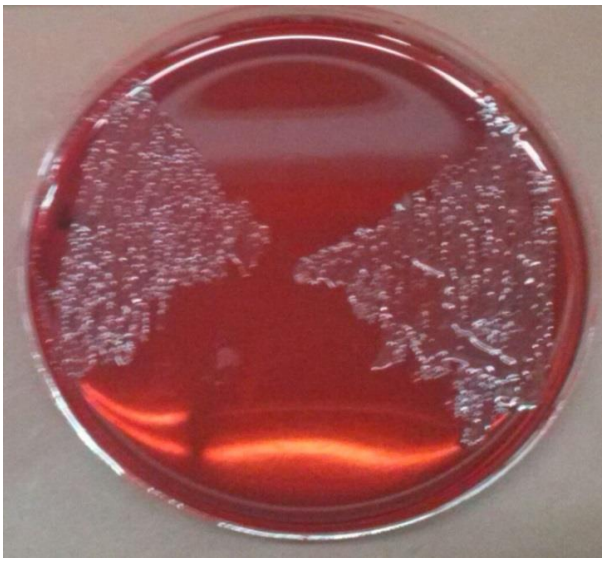

Figure (8) Inhibition of biofilm formation by E. coli on Congo red agar medium after treatment with 32µg/ml Ceftriaxone

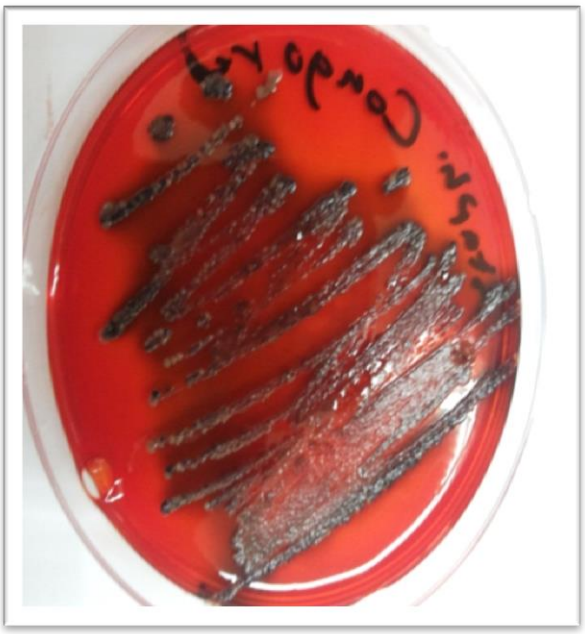

Figure (9) S. aureus bacteria forming the biofilm on the Congo red agar medium

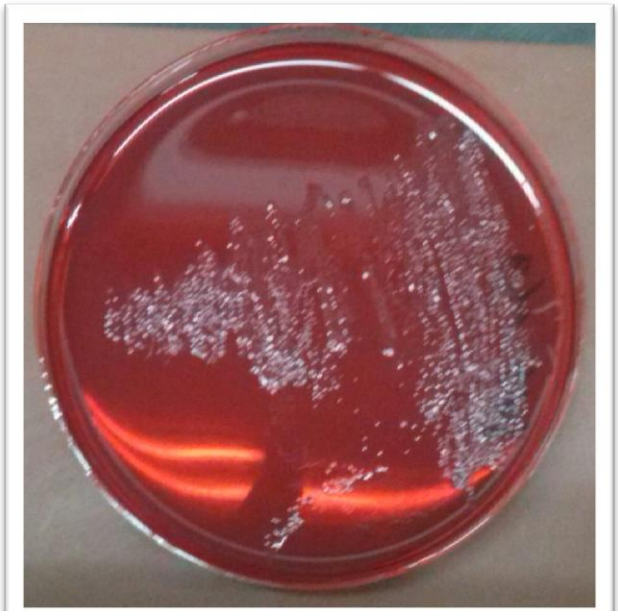

Figure (10) Inhibition of biofilm formation by S. aureus on Congo red agar medium after treatment with 32µg/ml Ceftriaxone

Effect of minimum inhibitory concentration MIC of cephalosporins on protease enzyme activity.

Our study included exposing the bacterial isolates to the minimum inhibitory concentration (MIC) of the aforementioned antagonists, and observing the effect of these concentrations on the activity of the protease enzyme. for all selective isolates.

Our study included exposing MIC bacterial isolates to the aforementioned antigens and observing the effect of these concentrations on protease enzyme activity, and their effect appeared to be inhibited by the appearance of microbial growth around the pits in a medium

DNA analysis

The results of DNA –analysis of both normal and cured isolates showed the prescence of chromosomal and plasmids bands in (normal case) while only chromosomal bands observed in E. coli isolates treated with Ceftriaxon at concentrations (32-128 µg/ml) as in figure (11) ,the absence of plasmids was correlated with the absence of protease production and biofilm formation by E. coli isolates as mentioned below which explain the fact of their genetics ,they may be ,in most probable plasmids determined neither than chromosome .
lastly ,the results observed Ceftriaxon their effect on the virulence factors particularly protease and biofilm formation because the mutagenic effect on the specific genes of it production in E. coli isolates in our study.

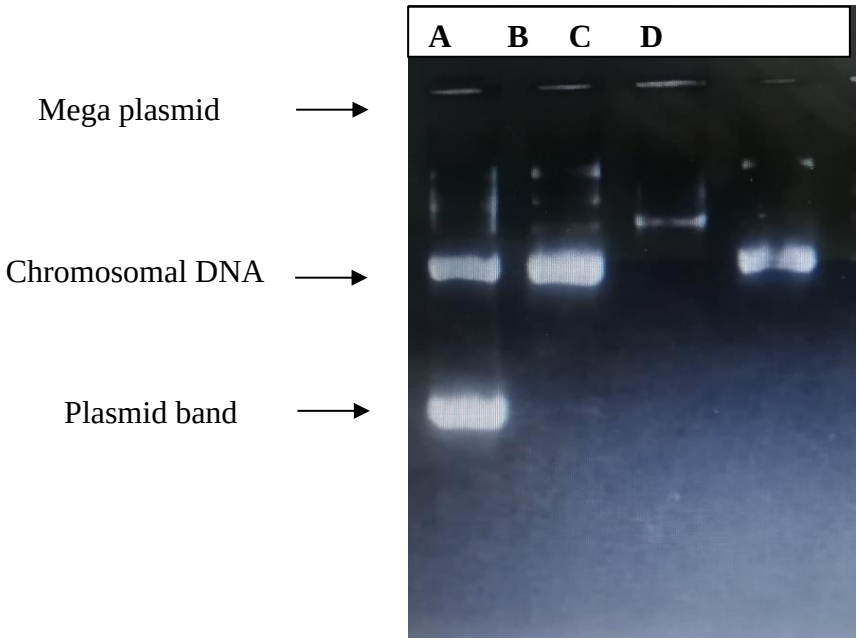

Figure (11) : Agaros gel electrophoresis of chromosomal and plasmid DNA isolated from *E. coli* for (line A) normal and curing *E. coli* isolates treated with with Ceftriaxon at concentrations (32-128µg/ml) panel (B,C,D)

Interleukin-2 concentrations were calculated in the serum of patients and healthy controls by the effect of Gram-negative bacteria, as in figure (12) It found that there were no significant differences in concentrations between patients and control t =8.183 (P = 0 .0038), where the results for patients were 0. 172 ± 0.0181 (mean ± SD), while the concentration of interleukin-2 in healthy subjects was 0.075 ± 0.0129 (mean ± SD).

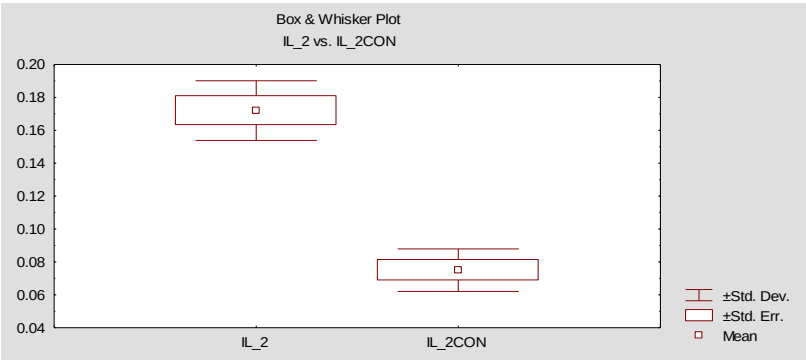

Figure (12) Interleukin-2 concentration in patients with Otitis media and control

Conclusion

1- The bacteria S. aureus is the most common species in causing middle ear infections(Otitis media), followed by K. pneumonia and E. coli in the isolates under study.
2- Cephalosporin antibiotics are more effective in the growth of bacterial species isolated from Gram-positive and Gram-negative middle ear in high concentrations.
3- Continuously different minimum inhibitory concentrations as a result of bacterial resistance mutations.
4- Biofilm formation and production of protease enzyme increases bacterial resistance when random use of antibiotics.

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COMPARISON BETWEEN CARDIAC ENZYMES IN PATIENTS WITH HYPOTHYROIDISM AND HYPERTHYROIDISM

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Abstract:

Thyroid hormones modify each cardiovascular system component, it's essential for the function and development off the cardiac system. Thyroid hormones and the cardiac enzyme were measured in (120) Iraqi women aged (20-65) years in three groups: patients with hypothyroidism, hyperthyroidism, and control. Thyroid hormones (TSH, T3, and T4) were measure by ELISA by using a procedure of TOSOH,CHINA, also, cardiac enzymes were determined by biochemical assay of Biosystem company, Barcelona. The results showed the level of CK enzyme increasing non significantly (53.61) between groups in hyperthyroidism (G1), hypothyroidism(G2) and was (151.40 ±8.86uk) and (127.80 ±21.82uk) respectively compared with control was (G3) (100.60 ±18.80 uk),also the level of Troponin- I enzyme increasing non significantly (213.42) between groups was in(G1) (430.20 ±53.38) (Pg/UL), (G2) was (369.20 ±75.75) (Pg/UL) and (G3) (275.60 ±76.18).In comparison the study showed decreasing non significantly in cardiac enzymes as AST and ALT. It concluded that non-significant effect of thyroid hormones on the level of cardiac enzyme in both patients with hypothyroidism and hyperthyroidism.

Key words: Hyperthyroidism, Cardiac Enzyme, Hypothyroidism, Hormones, Troponin I.

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Introduction:

Thyroid hormone (TH) controlson metabolism necessary for good growing, normal development also in the adult arrangement of metabolism (1,2). Thyroid hormone associates body weight with spending of energy (3). Hyperthyroidism state that increasing in thyroid hormone level supports a hypermetabolicrang by amplified resting energy expending, weight or mass loss, decreasing in levels of cholesterol, augmented lipolysis also gluconeogenesis (4).In contrast, hypothyroidism state describes levels of thyroid hormone decreasing, which is related to hypometabolism level by condensed resting energy expending, the addition of weight, augmented levels of cholesterol, condensed of lipolysis and gluconeogenesis (5). TH excites lipogenesis and lipolysis, though when levels of TH are elevated, leads to the loss of fat (6). TH affects the switch of metabolic processes and pathways by the regulation of energy levels (7). Its controls metabolism mainly during actions in organs as the brain, white and brown fat, liver, heart, and skeletal muscle (8).Hypothyroidism is a common clinical disease with inconstant incidence. It has an influence on the functions of the heart through its effect on cardiac contractility, also vascular resistance, heart rhythm, and blood pressure (9).Hypothyroidism is related to cardiac output decreasing because of weakness relaxation of smooth muscle and reduction of endothelial nitric oxide availability (10).Patients with hyperthyroidism have heart rate increasing; also, pulse amplitude increasing, and augmented cardiac output which is similar to form of enlargedin adrenergic activity (11), in spite of the normal or tiny level of catecholamines concentration. Hormonal reasons are affected such as the elevated rang of atrial natriuretic peptide, vasodilating polypeptide adrenomedullinand endothelin-1(12). The aim of study that investigates of comparison between cardiac enzymes in patients with hypothyroidism and hyperthyroidism.

Material and methods

Procedures and Experimental design: The study was achieved in the biology department /Collage of Science in University of Baghdad. The study trials involved 120 Iraqi women aged (20-65) years, which divided into three groups: 40 women with hyperthyroidism (G1), 40 women with hypothyroidism (G2), and 40 healthy women or control (G3), all blood samples were collected from hospitals in Baghdad.

Collection of Blood Trials: Blood was taken from women of three groups by vein puncture via syringe, venous blood into tube minus anticoagulant, blood samples had to be clot for 15-25 min at room temperature. Then separated the serum by centrifuge at 3500 (rpm)for10-15 minutes to measure thehormonal and biochemical parameters.

Biochemical and Hormonal assays:

Hormonal assays included thyroidstimulating hormone (TSH), alsoT3 and T4by technique ELISA, the kit specific for human according to the manufacturer's instructions of TOSOH, China.At the same time, Creatine Kinase (CK), Troponin I, Aspartate transaminase (AST)in aadition toalanine aminotransferase (ALT)were determined by biochemical assay of Biosystem company, Barcelona.

Statistical Examination

Program statistical analysis system [SAS], It used to distinguish the influence of different parameters in the study, also using LSD (ANOVA) to study associate in gamong parameters significantly.

Results

The results in study discovered no significant effect P-value(0.749) between age in Hyperthyroidism (G1)(45.00 ±4.94), Hypothyroidism (G2)(42.00 ±4.04) and normal groups (G3) (39.80 ±5.32)Table 1.

Table 1: Comparison between different groups in Age

Groups	Mean ± SE of Age (year)
Hyperthyroidism (G1)	45.00 ±4.94
Hypothyroidism (G2)	42.00 ±4.04
Normal (G3)	39.80 ±5.32
LSD value	14.803 NS
P-value	0.749
NS: Non-Significant.	

When the comparison of hormones levels in different groups in this study , TSH showed high differences significantly** (P≤0.01) (45.846) **in(G1)was (0.047 ±0.02)(mIu/ml).While TSH level(G2)was (87.00 ±25.76) (mIu/ml) and in (G3)(3.99 ±0.66) (mIu/ml), the level of T3 appeared non significantly (4.345) between groups (G1), (G2) and (G3) were(1.892 ±0.34), (4.81 ±2.34)and(4.81 ±2.34) respectively, also the results revealed that the level of T4 was high differences significantly** (P≤0.01)(49.358)between groups in (G1)was (73.50 ±27.64), while in(G2) was (5.08 ±1.70) and in(G3)was(7.00 ±1.58)Table 2.

Table 2: Levels of TSH,T3, T4 hormones between groups

Groups	Mean ± SE		
	TSH (mIu/ml)	T3(ng/ml)	T4(ug/dl)
Hyperthyroidism(G1)	0.047 ±0.02 b	1.892 ±0.34	73.50 ±27.64 a
Hypothyroidism(G2)	87.00 ±25.76 a	4.81 ±2.34	5.08 ±1.70 b
Control (G3)	3.99 ±0.66 b	4.81 ±2.34	7.00 ±1.58 b
LSD value	45.846 **	4.345 NS	49.358 **
P-value	0.0020	0.328	0.0163
significant difference. ** (P≤0.01).			

The levels of cardiac enzymes CK and Troponin- I differences in this study showed the level of CK enzyme increasing non significantly(53.61) between groups in(G1), (G2) and were (151.40 ±8.86)(uk) and (127.80 ±21.82)(uk) respectively compared with (G3) as a control was 100.60 ±18.80(uk). Also the level of Troponin- I enzyme increasing non significantly (213.42) between groups were (430.20 ±53.38)(Pg/UL),(369.20 ±75.75)(Pg/UL)and (275.60 ±76.18) in(G1), (G2) and (G3) respectivelyTable3

Table 3: levels of cardiac enzymes CK and Troponin- I between groups

Group	Mean ± SE	
	CK (uk)	Troponin I (Pg/UL)
Hyperthyroidism(G1)	151.40 ±8.86	430.20 ±53.38
Hypothyroidism(G2)	127.80 ±21.82	369.20 ±75.75
Control (G3)	100.60 ±18.80	275.60 ±76.18
LSD value	53.61 NS	213.42 NS
P-value	0.161	0.317
NS: Non-Significantly.		

The study showed decreasing non significantly (20.586) of AST enzyme level (27.40 ±7.92)(U/L), (28.20 ±7.69) (U/L)and (44.00 ±3.43)(U/L) in Hyperthyroidism(G1) and Hypothyroidism(G2) compared with control (G3)respectively, also level of AST was decreasing significantly (13.496)which were(24.20 ±42)(U/L),(30.40 ±5.98)(U/L) and (39.28 ±1.46)(U/L)in Hyperthyroidism (G1) and Hypothyroidism (G2) compared with control (G3), respectively Table 4.

Table 4: levels of cardiac enzymes AST and ALT between groups

Group	Mean ± SE	
	AST(U/L)	ALT (U/L)
Hyperthyroidism(G1)	27.40 ±7.92	24.20 ±42 b
Hypothyroidism(G2)	28.20 ±7.69	30.40 ±5.98ab
Control (G3)	44.00 ±3.43	39.28 ±1.46 a
LSD value	20.586 NS	13.496 *
P-value	0.182	0.048
significant difference.* (P≤0.05).		

Discussion

The results in the study discovered no significant effect P-value (0.749) between age in (G1), (G2) and normal groups (G3),Table 1,while however, other studies disagree with this study which showed that elderly patients might perhaps with higher of TSH levels in the deficiency of thyroid disease (13,14).

TSH showed high differences significantly** (P≤0.01) (45.846) ** in (G1) was (0.047 ±0.02) (mIu/ml). while TSH level in (G2) was (87.00 ±25.76) (mIu/ml) and in (G3) was (3.99 ±0.66) (mIu/ml),the level of T3 appeared non significantly (4.345) between groups (G1), (G2) and (G3) were (1.892 ±0.34), (4.81 ±2.34) and (4.81 ±2.34) respectively, also the results revealed that the level of T4 was high differences significantly** (P≤0.01) (49.358) between groups in Hyperthyroidism (G1) was (73.50 ±27.64), while in Hypothyroidism (G2) was (5.08 ±1.70) and in Control (G3) was (7.00 ±1.58).

Hyperthyroidism is described through a low level of TSH besides the elevated level of thyroid hormones from thyroid gland: tri-iodothyronine (T3) also thyroxine (T4) (15).The greatest cause of Graves' disease tailed after toxic nodular goiter. In addition to significant such as thyroiditis, drug, iodine-induced and artificial consumption of excess thyroid hormones (16). The hypothyroidism is well-defined that TSH levels is above normal range and concentrations of

thyroxineless than the normal values (17).Hypothyroidism is mostly found in humans with autoimmune diseases, like diabetes type (1), coeliac disease, and happen in several autoimmune endocrinopathies. In addition to, Downs' syndrome persons may be had an augmented risk factor of hypothyroidism. While smoking and modest spirits consumption are related to a condensed risk factor of hypothyroidism(18).Biochemically, Frequently, TSH level is slightly elevated, maybe because bioactivity decreasing (19.20).In iodine-sufficient zones, the furthermost common reason hypothyroidism is Hashimoto's disease.(21).

The levels of cardiac enzymes CK and Troponin- I differences in this study showed the level of CK enzyme increasing non significantly (53.61) between groups in (G1), (G2) and were (151.40 ±8.86) (uk) and (127.80 ±21.82) (uk) respectively compared with (G3) as control was 100.60 ±18.80 (uk), also the level of Troponin- I enzyme increasing non significantly (213.42) between groups were (430.20 ±53.38) (Pg/UL), (369.20 ±75.75) (Pg/UL) and (275.60 ±76.18) in (G1), (G2) and control (G3) respectively

Hypothyroidism reasons of secondary dyslipidemia, estimation of thyroid function is required when starting hypolipidemic treatment. Failure in thyroid function is related with augmented concentration of CK (22). The effects of preliminary stat in therapy with undiagnosed hypothyroidismmay considerably increase CK level (23).

The changing happen of sarcolemmal membranes paid to decrease in adenosine triphosphate compound (ATP) levels outside a serious edge has advanced as probable mechanisms of muscle participation. ATP decreasing is lead to the hypometaboliccase in hypothyroidism due to changed cellular permeability then outflowincell enzymes (24). Increasing in muscle mass also decreasing in clearance enzymes clearance cause hypothyroidism lead to increasing of CK enzyme level in skeletal muscle [25].In another study that detected creatine kinase activity significantly decreasing in hyperthyroidism in contrast to control[26]. In hyperthyroidism,it may perhapsbecause enzyme degradation increasing, muscle worsening or muscle bulk decreasingduringhypermetabolic [27].

In hypothyroidism have CK levels increasing, because increasing in CK production, or increasing inpermeability of cellular membrane and decreasing in enzyme clearance. In recent times, troponins TandI have briefly studied as nature of cardiac specificity. Types of cardiac troponins in cardiac injury are markers described sensitive and specific [28].Hyperthyroidism has largeinfluences on circulatory system. Cardiac markers as NT-proBNP also troponin I have confirmed useful to determining myocardial disease but not lengthily examined in hyperthyroidism [29].

The study showed other type of cardiac enzymes as AST and ALT which was decreasing non significantly (20.586) of AST enzyme level (27.40 ±7.92) (U/L), (28.20 ±7.69) (U/L)and (44.00 ±3.43) (U/L) in (G1) and (G2) compared with (G3) respectively, also level of AST was decreasing significantly (13.496) which were (24.20 ±42) (U/L), (30.40 ±5.98) (U/L) and (39.28 ±1.46) (U/L) in (G1) and (G2) compared with (G3) respectively, The relationship between thyroid hormones, and liver and heart enzyme levels as CK, ALT, AST and troponins has been well documented through its importance as biomarkers of liver and heart injury.

Thyroid hormone effects on heart structure and its specialized conducting coordination mechanism. In addition to thyroid hormones direct influences on cardiovascular function lead to differences in level of cardiac markers, also secondary influences on autonomic nervous coordination also the renin-angiotensin-aldosterone (RAAS)pattern, addition to vascular compliance, renal function and vasore activity(30).

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SYNTHESIS AND CHARACTERIZATION OF SOME NEW 1,3-OXAZEPINE COMPOUNDS

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Abstract:

In this study, bis imines were synthesized by reacting aldehyde with diamine in the presence of chloroformat (55-60˚C). In moderate yield (78-82) %, and then used in the preparation of 1, 3-Oxazepine compounds by reacting with phathalic anhydridein Benzene at (80-85 ˚C). In moderate yield (83-88) %.These vehicles have been verified using(FT-IR).

Key words: Characterization, Compound, Oxazepine.

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Introduction:

Oxazepine is a heterocyclic ring consisting of 7 atoms.The heptagonal Oxazepine ring consist of two oxygen and nitrogen partical at positions 1 and 3, respectively, plus the five carbon atoms. 1,3-Oxazepine is part of several heterocyclic Oxazepines[1]-[6]. The main structure of the heptagonal ring is 1,3-Oxazepine-4, 7-diones along with two carbonyl groups. The preparation of Oxazepine compounds was confirmed and documented in several ways, as it was synthesized by pericycliccyclo addition of Imines or hydrazine with phthalic,succinicandmaleic anhydride[7][8][9][10][11],as well as by method of green chemistry [12][13]. Conventional methods of synthesis of the Oxazepine ring are limited[14].Recently, a cycloaddition reaction was used, a kind of cyclic reacting that was use in the prepare of 1, 3-Oxazepine ring[15][16],where this reaction is one of the unlimited reactions and gives multiple and different derivatives of 1, 3-Oxazepine. The preparation of these compounds is a class of cyclic reactions that are classified as (5+27), implying a five-atom component plus a diatomic component resulting in a seven-atom ring [17][18]. Oxazepine derivatives have shown broad biological activity against severalkinds of bacteria, in addition to the use of these derivatives asinhibitor of some enzymeactivity [19][20]. Oxazepines and their derivatives contain some the importancebiological and pharmacology activity[21]like enzyme inhibitor [22], painkilling[23], alleviates depression [24], and psychoactive drugs[25]. Amoxapine could be a sort of medicine called a tri-ring antidepressant that is use to treatment symptoms of anxiety, agitation, and depression [26].

Imines or Schiff bases contain an active group (C=N-). These compounds are synthesized by an acid catalyst condensation react between primary amineand aromatic ketone or aldehyde[27][28][29][30]. The mechanism of this interaction is well known [31]. Due to their high elasticity and diversified formational sides, extensive totals of imines were prepare and Study its complex behavior [32][33]. Moreover imines show a diversity of important biologic activity, such as, anti a bacterial infection[34], antifungals [35], anti of virus viral inflammation of the liver (MHV)[36], inhibitor of herpes simple infection kind 1 (HSV-1) and glandularvirus kind 5 (Ad 5) [37], anti-cancer[38] , anti-mosquito larva [39]and activities of pesticides herbs [40]. Also,imines are very important intermediates compound in the prepare of some biological active compounds such as beta-lactams [41].

2. Experiential:

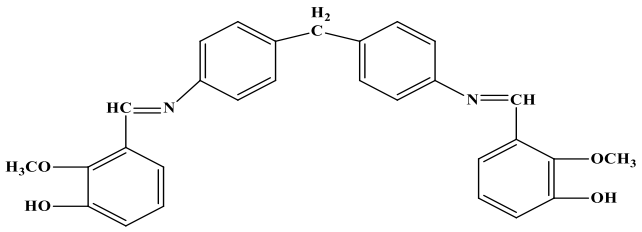
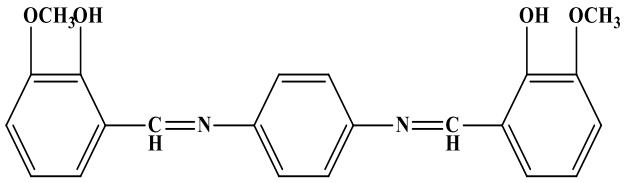
All chemical was in detector degree except if otherwise specified stated and obtained from Sigma-Aldrich. Silica gel (Merck 7736), and silica gel plates for column and thin layer TLC chromatography was Aldrich products, iodine vapor was used to detect the separate components. Use for drying organic solutions anhydrous sodium sulfate. Also, the IR spectrophotometer was used Perkin-Elmer (FT-IR) spectrophotometer, in the (4000-400) cm-1 rang using (KBr, disk), in college of Science, the department of chemistry, Thi-Qar University, Iraq. And melting points was measured used a melting point device SMP 31, in College of pharmacy, the Department of Chemistry, University of Thi-Qar, Iraq.

2.1.The general method for the synthesis of imines [42][43]

In general, Bis imines was synthesized by reaction between amine and aldehyde, this mixture was heated in (20 ml) of chloroform with the addition of 4-6 drops of glacial acetic acid in a water bath at a temperature of (55-60°C).The react mix was reflux for (20 min) with stirrer.

And the Advance of the interaction was checked by TLC. After finishing, the dissolvent has been evaporate and then recrystallized in a suitable solvent.

Table (2-1): The chemical Structures of the prepared bis-imines compounds 2(a,b).

No.	Name of bis-imines compound	Structure
2a	3,3'-((1E,1'E)-((methylenebis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))bis (2-methoxyphenol)	
2b	6,6'-((1,4-phenylenebis(azanylylidene))bis(methanylylidene))bis(2-methoxyphenol)	

The following methods of imines preparations are:

2.1.1Synthesisof 3, 3'-((1E, 1'E)-((methylenebis(4,1-phenylene))bis,(azanylylidene))bis,(methanylylidene)),bis(2-methoxyphenol) (2a)

4-Hydroxy-3-Methoxy benzaldehyde (0.304 g, 2 mmol) contain three drops of glacial acetic acid was dissolve in ethanol (15mL), then 4,4diamnodiphenyl methane (0.198g, 1mmol) was dissolve in ethanol (15mL) and then added drop wisdom. The react mix was reflux with stirrer in a water bath at a temperature of (75°C) for (12 min),then the reaction mix was leave to cool at room temperature and the dissolvent evaporate.The precipitate was filtering and wash well with cold ethanol; Yield = 78.5%, m.p. = 283-285 0C. IR (ῡ,,cm-1,,KBr disk):1618 (,C=N,).

2.1.2 Synthesis of 6, 6'-((1,,4-phenylene bis(azanylylidene)),bis,(methanylylidene))bis,(2-methoxyphenol) (2b)

4-Hydroxy-3-Methoxy benzaldehyde (0.304 g, 2mmol) contain three drops of glacial acetic acid were dissolve in ethanol (15mL), then phenylen1-4di amine(0.108g, 1mmol) was dissolve in ethanol (15mL)and then added drop wisdom. The react mix was refluxwith stirrer in a water bath at a temperature of (75°C) for (15 min), thenthe reaction mixwas leave to cool at room temperature and the dissolvent evaporate. The precipitate was filtering and wash well with cold ethanol; Yield = 82%, m.p.= 208-210 0C . IR (ῡ, cm-1, KBr disk):1607(C=N).

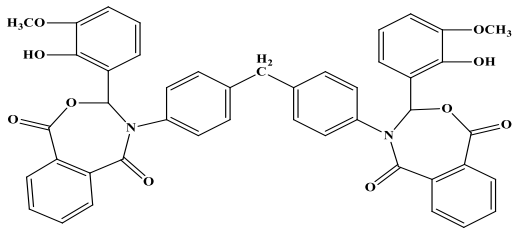
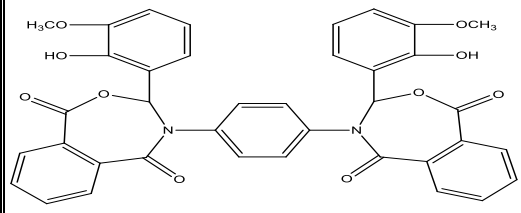
Table (2-2): Show physical properties for imines 2(a,b):-

Imine (a-b)	m.p °C	Yield %	Color	Solvent of recrystallization
2a	283-285	78.5%	Yellow	Ethanol
2b	208-210	82%	Orange	Ethanol

2.2 The general method for the synthesis of heterocyclic compounds (1, 3-oxazepine) [44]

In general, 1, 3-Oxazepine were prepared by reaction the mixture of Bisimines that prepared with phathalic anhydride in (20 mL) of dry Benzene.The react mix was reflux for (6 hr)with stirring. The mix was leave to cool down at room temperature and dissolvent evaporate and then recrystallized from a suitable solvent.

Table (2-3): The chemical Structures of the prepared 1, 3-Oxazepine compounds 3(a,b).

No.	Name of 1,3 oxazepine compound	Structure
3a	4,4'-(methylenebis(4,1-phenylene)),bis,(3-(2-hydroxy-3-methoxyphenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione)	
3b	4,,4'-(1,4-phenylene),bis,(3-(2-hydroxy-3-methoxyphenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione)	

The following methods of 1,3oxazepine preparations are:

2.2.1Synthesis of 4,,4'-(methylenebis(4,1-phenylene)),bis,(3-(2-hydroxy-3-methoxyphenyl)-3,4-dihydrobenzo[e][1,3]Oxazepine-1,5-dione)(3a)

Schiff base derivative (0.466 g, 1mmol) and phathalic anhydride (0.296 g, 2mmol)is dissolved in (20mL) of dry benzene. The reaction mix is refluxed with stirring at (85ºC)for(6hr). The reaction mix is leave to cool down at room temperature, a colored precipitate is formation, and it is filtered and recrystallized using dioxin.

2.2.2 Synthesisof 4,,4'-(1,4-phenylene),bis,(3-(2-hydroxy-3-methoxyphenyl)-3,4-dihydrobenzo[e][1,3]Oxazepine-1,5-dione)(3b)

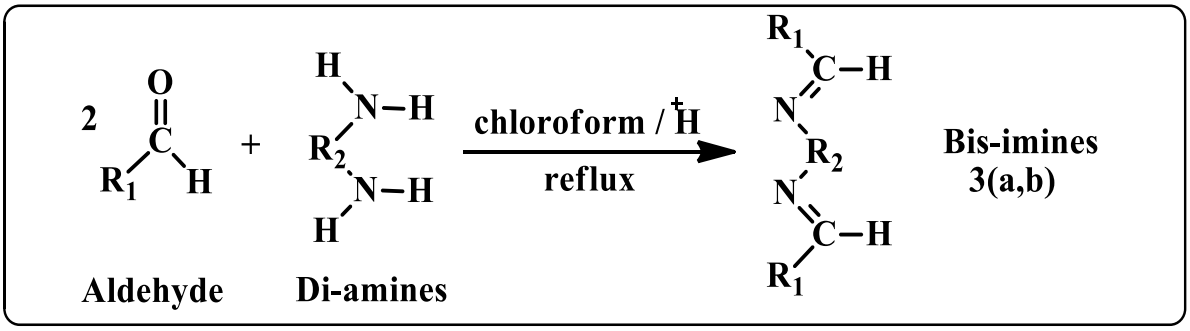
Schiff base derivative 2a (0.374g,1 mmol) and phathalic anhydride (0.296g, 2mmol)isdissolvedin(20mL) of dry benzene. The reaction mix is refluxed with stirring at 85ºC for (6hr).The reaction mix is leave to cool at room temperature, a colored precipitate is formation, and it is filtered and recrystallized using dioxin.

Table (2-4):shows physical properties for1,3-Oxazepines:-

Comp.	m.p °C	Yield	Color
3a	276-279	83%	Ruby red
3b	205-207	88%	Faind Orange

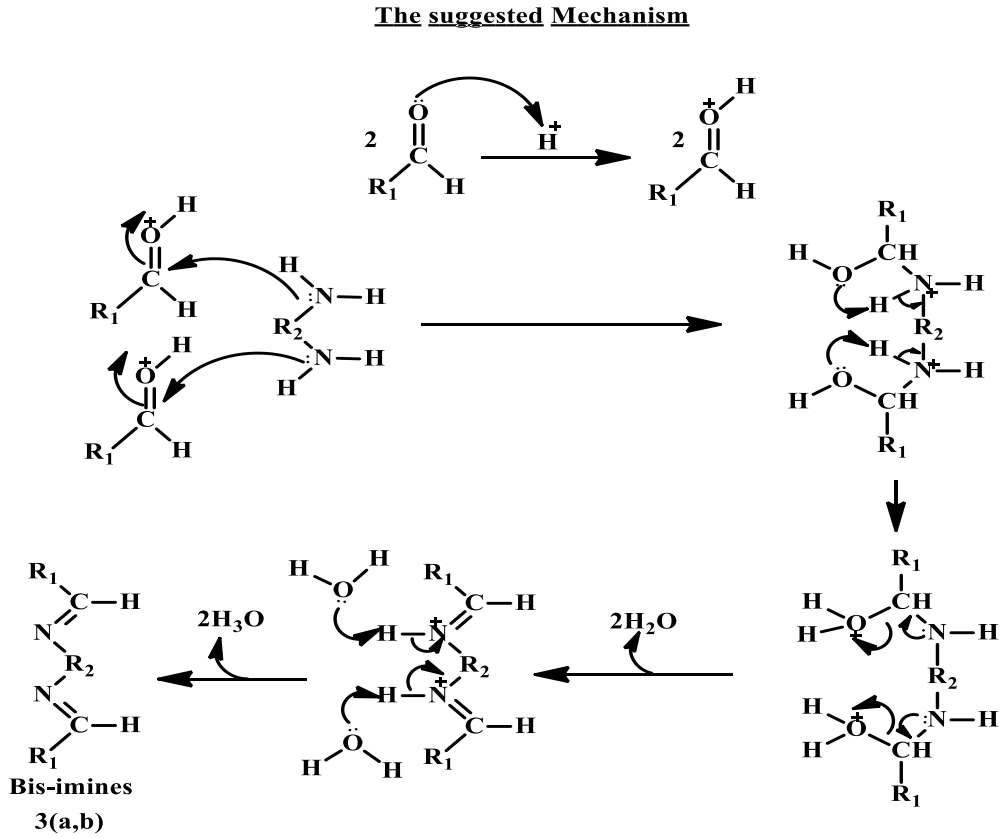
3. Results and Discussion:

In this study the 1,3-Oxazepine weresynthesized in a few steps.The target molecules were divided into two main parts. The first part includes.The required Various Schiff bases2(a,b),it was synthesized by reacting equal amounts of aromatic amines and appropriate aromatic aldehydes in refluxing chloroform, according to the scheme(3-1).



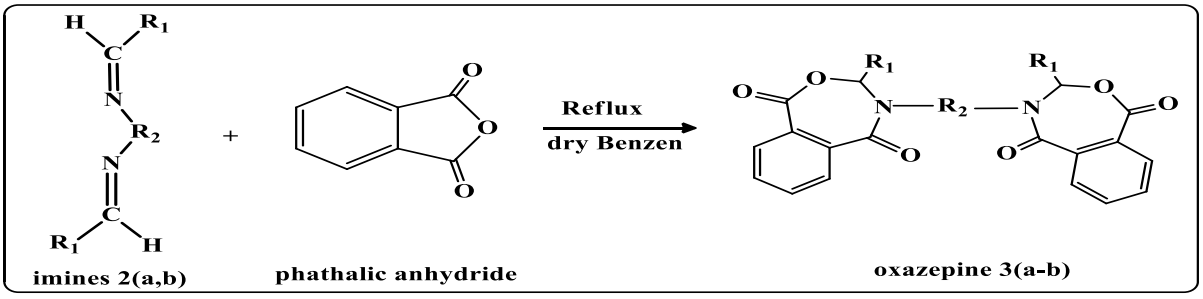
Scheme (3-1)

The proposed mechanics of the preparation are as in the scheme (3-2):



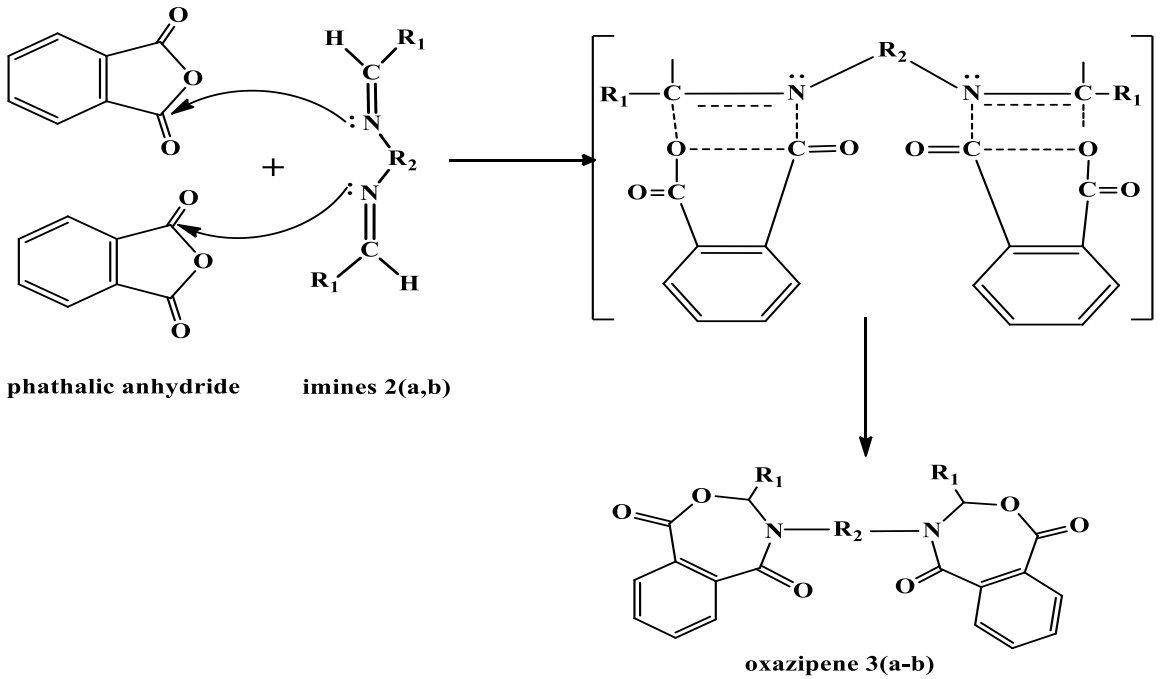
Scheme (3-2)

The second part is the portion which contains the 1,3-Oxazepine3(a,b). This part was synthesized through reacting phathalic anhydride with bis-imines2(a,b) in Benzene at (80-85° C), according to thescheme (3-3).



Scheme (3-3)

The mechanism of the cyclic interaction between a phthalytic anhydride and an imine group to synthesize the 1, 3-Oxazepine rings systematically investigated as (5+2) cycloaddition. The break and formed of bonds happen at the same time and thus the reaction returns cross one cyclic transition state and there is no chance for formation an intermediate as it was shown in scheme(3-4)[45].



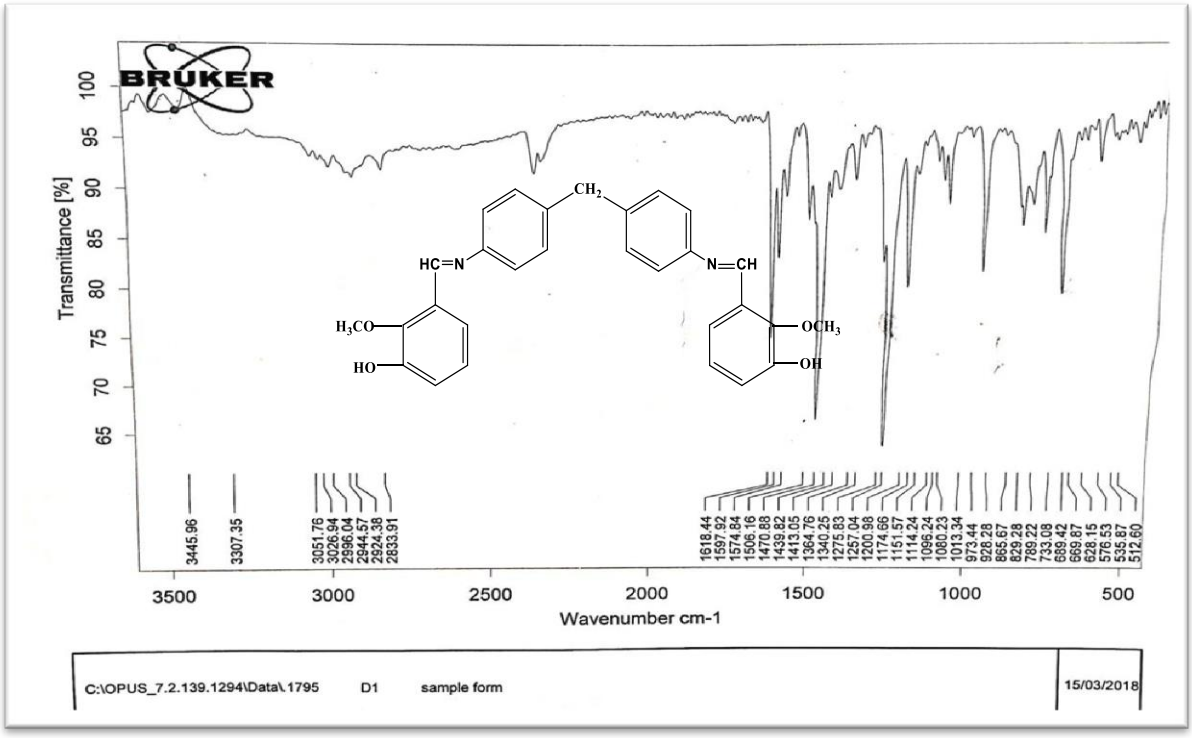
Scheme (3-4)

3.1 Infrared spectra (FT-IR)

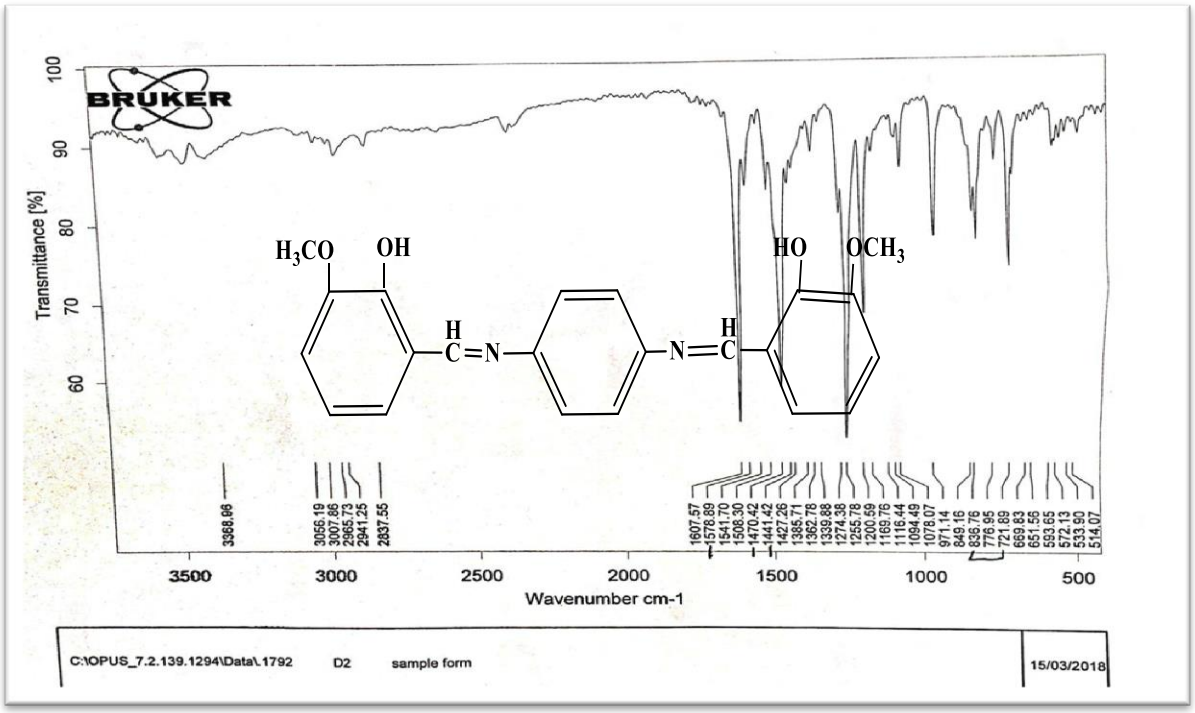
The IR spectra of the bis imines2(a,b), as KBr disc are shown in figures (3-1),(3-2) , the IR spectra of bis imines show absorption range at (1618-1607) cm-1 correspond to the Schiff base (C=N) group [46][47][48] . While this bands disappear and two bands appeared at (1695-1692) cm-1 back to (C=O lactone) group[49][1], and (,1657-1677,)cm-1 back to (C=O lactam) group[47][48][49] of 1,3-Oxazepinecompounds 3(a,b). This information above evidence to formation of compounds 2(a-b), 3(a-b) other information of functional groups appeared in Table (3-1)

Table (3-1) the major FT-IR absorption (cm-1) of compounds

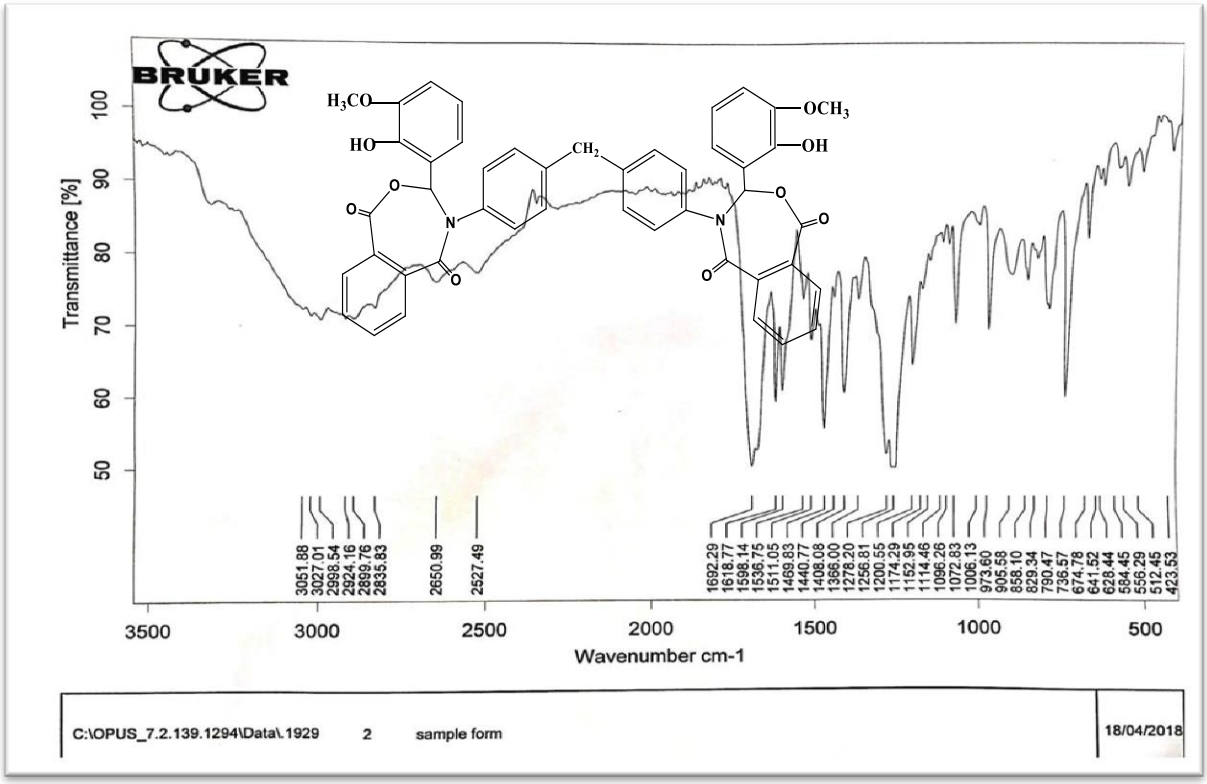
NO	Aromatic C-H	Aliphatic C-H	Lactone C=O	Lactam C=O	C=N	Aromatic C=C	Aromatic C-H
2a	3026	2996	------	------	1618	1597 1470 1413	829 789 733
2b	3007	2965	-------	------	1607	1578 1470 1441	836 776 721
3a	3007	2968	1695	1657	-------	1595 1582 1547	835 797 738
3b	3027	2998	1692	1677	------	1598 1536 1511	858 829 790



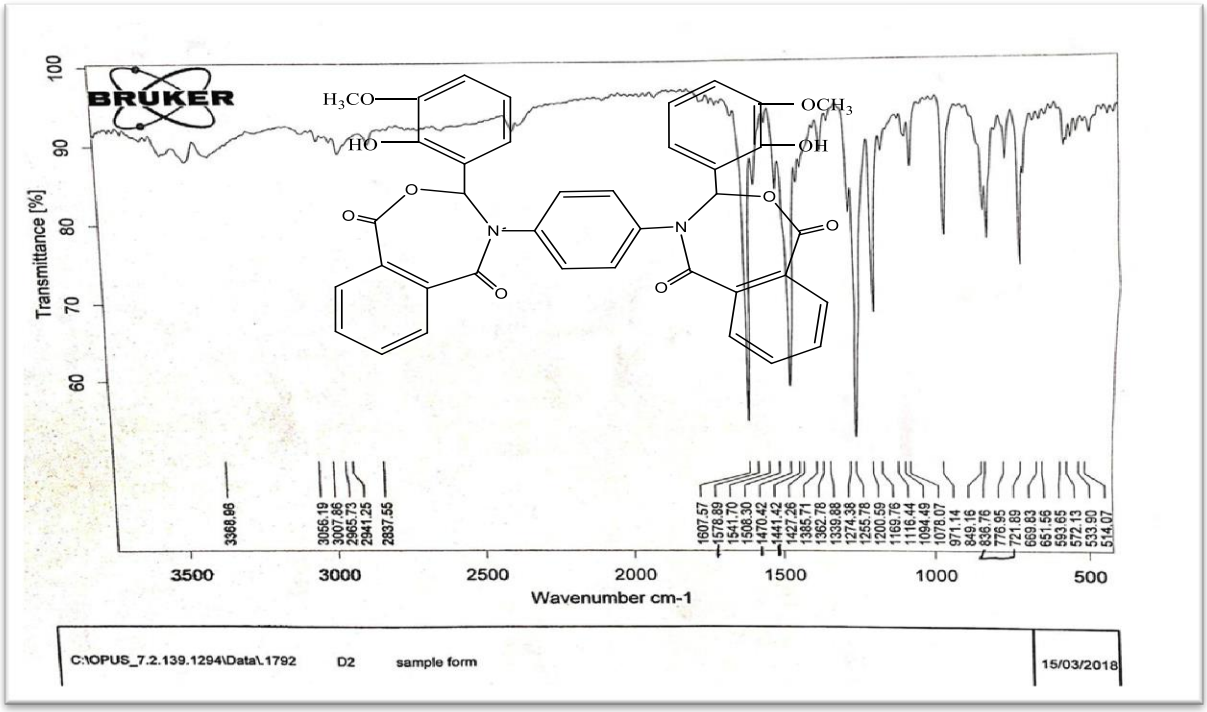
Schem (1): FT- IR spectrum of compound (2a)



Schem (2) : FT- IR spectrum of compound (2b)



Schem (3): FT- IR spectrum of compound (3a)



Schem (4): FT- IR spectrum of compound (3b)

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EFFECT OF THE DEPOSITION TEMPERATURE ON THE OPTICAL PROPERTIES FOR ZNO:BTHIN FILMS OBTAINED BY (LPCVD)FOR SOLAR CELL

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Abstract:

We are studying the effect of the deposition temperature on the optical properties for ZnO:B thin films on glass substrates obtained bylow pressure chemical viper deposition (LPCVD), to develop layers with characteristics that best meet the requirements for use in solar cell.According to the depositiontemperaturein the range (185-195 ℃), we obtainedthat ,when (T=195℃),the optimum value of the Haze factor about 25.7% ,ZnO:B thin film thickness increased, which leads to decrease the value of sheet resistance,and the transmittance decreases ,so the optimum temperature in the range (90-95℃).

Key words: Zinc Oxide, LPCVD ,Solar Cell, Haze Factor.

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Introduction:

One of the many applications of zinc oxide (ZnO) thin films is their use as transparent electrical contacts and buffer layers in thin-film solar cells (TFSC) [1]. This material is subject to requirements for ensuring conductivity, transparency, low reflection, and technological compatibility with the technology of forming active semiconductor layers [2]. Another requirement is the prevalence of all the components that make up the material. In this regard, the use of a layer of indium tin oxide (ITO) containing indium, whose reserves in the world are very limited, is not desirable. Therefore, layers of zinc oxide ZnO doped with boron were used as a conducting transparent electrode[3]. This material, compared to tin oxide SnO2, can significantly reduce optical losses in the region of 550-700 nm.
ZnOhas a wide band gap (about3.3eV) at 300 K [4], high optical transparency in the visible range (400nm – 800nm) more than (85%) [5]. Currently, ZnO is used to create various sensors and as a transparent conducting contact in solar panels [6].
The aim of this research is to study effect of deposition temperature on the optical properties for ZnO:B thin films obtained by (LPCVD)and choose the optimum temperature to improve I-V characteristics silicon solar cell.

1. Experimental details

The most widely used in the technology of thin film solar cells is (LPCVD) ,which allow as
to obtain ZnO films doped with boron with a developed pyramidal texture, uniform thickness and with high scattering [7,8].

Table 1. The conditions for the deposition of ZnO:B films.

Thin film	Zn(C2H5)2st .cm3/min.	H2, st.cm3/min.	H2O, st.cm3/min.	B2H6, st.cm3/min.	Temperature , ˚C	Time,s
ZnO	650	100	800	60	185,90,95	530

Vapors of diethyl-zinc[Zn(C2H5)2]and (H2O) are used as precursors,B2H6 is used to obtain layers with high conductivity, (H2) to maintain uniform temperature distribution and nitrogen (N2) to maintain a constant pressure to improve uniform thin film thickness. The optical properties were measured with the help of using the SENSOL - H setup, which allows us to obtain the transmission, reflection, and scattering spectra of films. The study of the structural features of the samples of ZnO:B was carried out at room temperature on the LabRam HR800 of the (Horiba JobinYvons company with the LabSpec software.

2. Results and discussion

Figure (1, 2) and according to the data from Table 2, it is observed that ,with an increase in temperature the total transmission and the diffuse transmission increases.

Table 2. The results of optical and structural parameters for ZnO:B with difference temperature

parameter	T1=85℃	T2=90℃	T3=95℃
Time deposition, s	530	530	530
Thickness ,nm	1288	1394	1752

Transmittance (400-900)nm ,%	85.2	83.5	77.8
Diffuse transmission	5.8	11	23.6
Haze (600nm), %	7	13.5	25.7

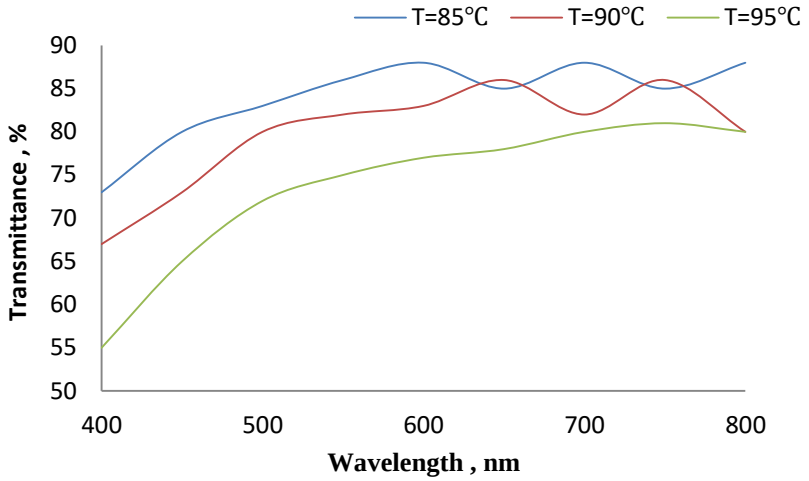

Figure1. The total transmission spectra of ZnO:Bthin film with a difference temperature

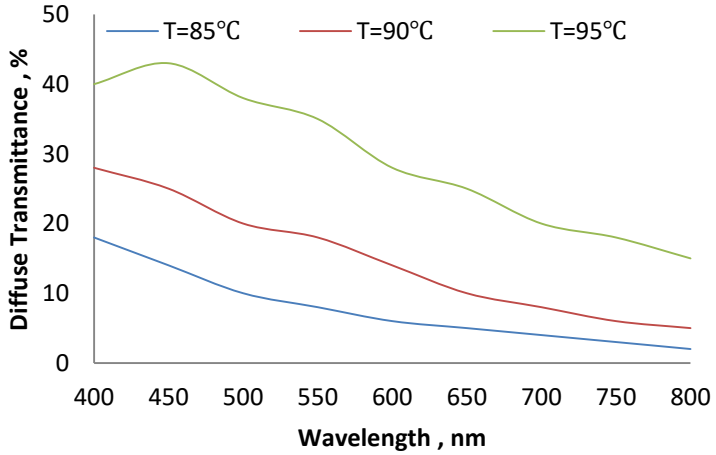

Figure2. The diffuse transmission spectra of ZnO:B thin film in a difference temperature

According to the data presented in Table 1 for the ZnO:B samples, we observed that with an increase in the temperature of the deposition process, the thickness of the deposited films increases at the same deposition time. With increasing film thickness, optical parameters such as Tfull and Tdiff decrease due to an increase in absorption and the size of the polycrystalline pyramidal structure on the film surface. An increase in the root-mean-square roughness leads to an increase in the degree of dispersion, which is a positive property in the manufacture of solar cell. An important optical parameter for ZnO:B, used as a transparent conductive contact for the manufacture of solar cell, is the degree of scattering or Haze factor you can find it by (Haze $\frac{T_{full}}{T_{diff}}\ x100\%$) , according to the obtained spectra of the degree of scattering (Figure 3) and their calculated average spectral values (Table 2), we observed that with an increase in the temperature of deposition of ZnO:B films, the degree of scattering increases.

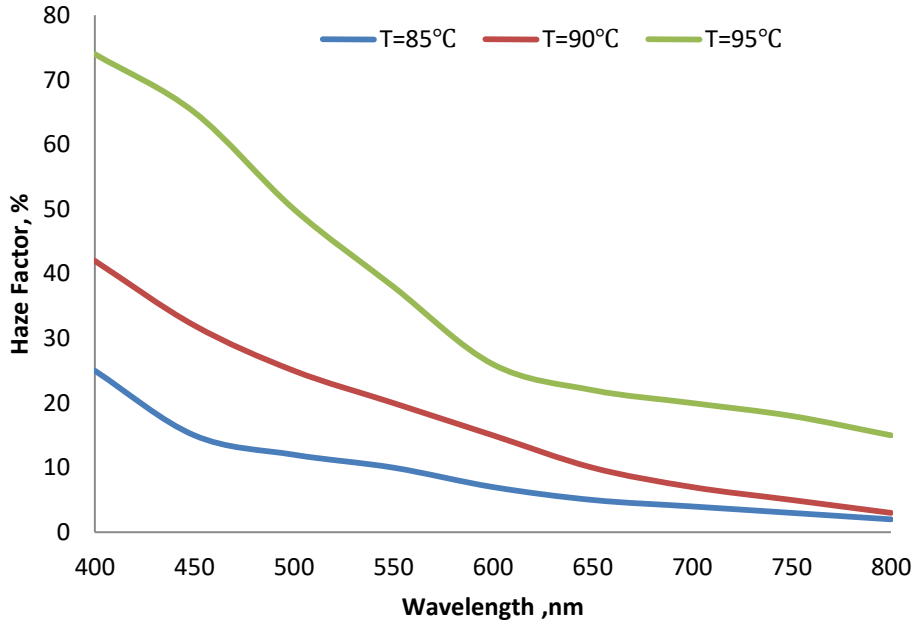

Figure3. Haze factor ofZnO:B with a difference temperature as a function of wavelength
An increase in the degree of scattering characterizes an increase in the size of the polycrystalline pyramidal structure on the surface of the ZnO:B layer obtained by LPCVD.

According to the Raman spectrum of the samples ZnO:B (Figure 4), pronounced peaks are observed at 457, 549, 794 and 1097 cm-1. The maxima at 549, 794 and 1097 reverse centimeters occur due to the substrate (glass) and the atmosphere in which the measurements took place, that is, air, mainly oxygen manifests itself at 1097 cm-1. With an increase in temperature the deposition , the qualitative change in the ZnO:B is not observed, but only the intensity has increased. This shows the advantage of the LPCVD method, its uniformity of deposition. At the same time, no serious structural changes are observed for each series of samples, since the growth of the structure occurred with an increase in zinc oxide crystallites.

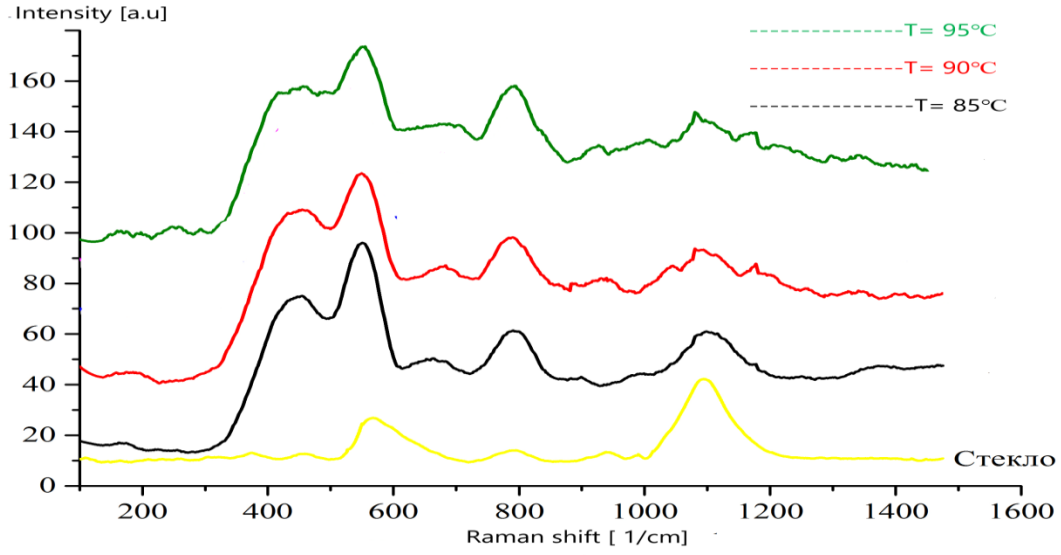

Figure 4. Raman spectrum with difference temperature for ZnO:B thin films

According to the Raman scattering spectra (Figures 4), with an increase in the temperature, the thickness of the films increased, the structure became the most defective, which characterizes an increase in the density of localized states in the band gap that arise due to defects and impurities. In addition, the number of defects associated with free oxygen atoms increase , but due to the low density of their location. The defects associated with free excitons did not change for samples (T1 ,T2), and their influence significantly decreased for sample T3.

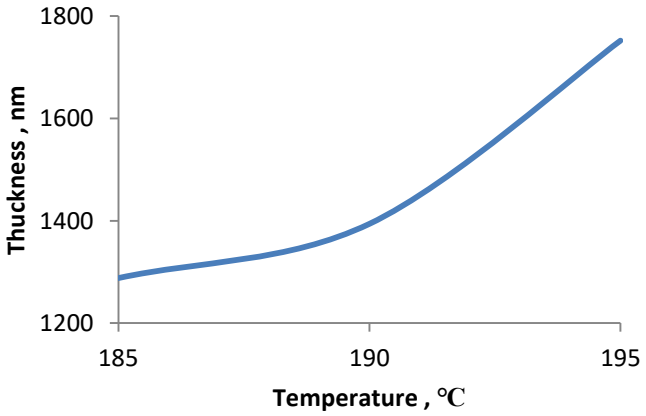

Figure 5.Thickness of ZnO:B as function of temperature

Since the thickness of the films increased with an increase in the temperature (Figure 5), and consequently the number of defects in the structure became greater, their influence is not so significant compared to the entire structure as a whole.

Conclusion

In general, a change in some parameters leads to a decrease in other parameters of the ZnO:B, in this regard, it is necessary to find the optimal deposition parameters, which was done in this work on a small number of samples for testing the methods of studying ZnO:B. When analyzing the results obtained, the optimal parameters for the deposition of thin films of transparent conductive zinc oxide doped with boron are the deposition time of 530 seconds at a temperature of 195 °C.

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V. P. Afanasiev, I. N. Kashkul, S. E. Kumekov, N. V. Mukhin and E.I.Terukov, 2017 Glass Phys. Chem. 3 272–5

EFFECT OF THE ENERGY DRINK (TIGER) ON THE PARAMETERS OF LIPID PROFILEIN THE FEMALE ALBINO MICE

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Abstract:

The aim of research is to clarify effect of an energy drink (Tiger) on Lipid profile physiological parameters in female mice for a period of four weeks. Adults female mice were used in research and divide to two groups. The first group is the control group that give distilled water (D.w.) for four weeks and the second group was treated group with tiger concentration dose 1.5 ml/mg for period of four weeks. After the end of the dosing period, sacrifices animals and the blood samples are collected without anticoagulant, and blood serum is obtained and kept at− 20 °C for biochemical tests. The research was seen that there was the significant increase ($p<0.05$) in a cholesterol and the high-density lipoproteins, with a significant decrease ($p< 0.05$) in value of triglycerides and a very low-density lipoprotein(VLDL), a Low density lipoprotein (LDL) was showed non –significant ($P\geq0.05$) in the dosed group as compare as the control group.

Key words: Energy Drink (Tiger), Cholesterol(CHO), Triglycerides (TG), High-Density Lipoprotein (HDL), Very Low-Density Lipoprotein (VLDL), The (LDL) Low-Density Lipoprotein.

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Introduction:

Energy drinks are the new consumer product that similar to soft drinks, they have materials and contain high doses of caffeine(1). Most energy drinks are sweet drinks have 80-320mg of caffeine per serving, there are many compounds found in energy drink including taurine, vitamins, guarana, ginseng, glucuroncolactone(2).
Energy drinks consumed by adults, teens and children(3), that consumption were related to several diseases including brain growing and cardiovascular functioning.
The severe effects to consume caffeine and take energy products that cause to increase heart rate, blood pressure (4). Blood pressure elevated moderately harmless in healthy adults,but the effect caffeine on children growth because they have a small body and unable to tolerate the drug response, for that some countries decided not to sell the energy drink for minors and the others countries have done so too (5). The study of Thomson and Scheiss explained the used of an energy drink drive teenagers and children, the adverse effect, the consumption of 3mg/kg/day.When combined with substances found in diet (6).The cardiac effects are increased when caffeine and taurine are digested together (7). The caffeine consumed alone that caused increase heart rate and blood pressure (8). Through laboratory studies explained when the caffeine or energy drink is digested with alcohol that induce clearly to drink more alcohol(9). Caffeine is a chemical composition made up of 1,3,7-trimethylxanthineMethylxanthine which has the same composition of purines, adenosine, xanthine, and uric acid. In humans, the caffeine is rapidly absorbed through gastrointestinal tract. The caffeine founds in coffee was absorbed rapidly than the caffeine in cold drinks. Due to the several reasons, the lower temperature of the drinks can reduce blood flow rate in the intestines, phosphoric acid in cold drinks can reduce gastric emptying; absorption rate may increase with caffeine dose, the sugar in cold drinks can discourage gastric emptying of caffeine and delay absorption (10). Caffeine dispersed and penetrated the vital membranes of the body, like the blood brain barrier and the placenta barrier, so it doesn't aggregate in the organs or tissues (11).
Sweden study was appeared a strong association was found between energy drinks and dental erosion, the study of Marshall et al showed the same observations in American children(12).Energy drink consumption related to 2.4-fold an increase in dental erosion. Because it has a low pH and the high sugar content (13). Pinto et al found the energy drink can lead to cervical hypersensitivity via removing the surface layer (14).

Material and Methods

Energy drink was found in the market,she was prepared 1.5 ml/mg (15).the experiment was happened in the animal house in the department of biology.college of science for women /university of Baghdad .Ten female mice weighting 25- 30 mg used for study.the animals were placed in cages for the purpose of adaptation in a room with appropriate temperature. They were kept in a good health condition.Animals have been preserved on natural 12h.Light and 12h.dark,they received a balanced diet ,water and libtium during the trial period. the mice were divided in two groups (n=5) and treated four weeks as follows : control group (c) received distal water orally daily for four weeks and treated group (T) received energy drink (tiger) intraperitoneal injection at dose.1.5 ml (15). after the trial period end that it led to the collection of blood samples using a heart puncture without anticoagulant for biochemical tests.The serum separates from coagulant blood by centrifugation at 5000 rpm for 10 minutes and stored at -20 c in order to study the following: Triglyceride by using enzymatic assay kit (16) a cholesterol by

using enzymatic assay kit (17), the high density lipo protein (HDL) by using enzymatic assay kit (18) the Low density lipoproteins (LDL) by (19), and a very low density lipoproteins (VLDL) by (19).

The Statistical Analysis

statistical analysis system-sas(2012)program is used to determine the effects of an energy drink (tiger) on the parameters used for the study. T- test used in significantly comparing between means in this study (20).

Results

The results of the current study showed the physiological parameters increased significantly ($P<0.05$) and it appeared in the cholesterol concentration in treated group (106.02 +_ 0.82).mg/ dl as compare with control group (154.70+_0.82)mg/dL.the triglyceride concentration was significant decrease ($p<0.05$) in dosed group (115.96+-0.67)mg / dL as compared with control group (127.12 +_0.69)mg/ dL.after 30 days there was the significant increase in HDL(high density lipoproteins) value ($p<0.05$) in the dosed group (50.30+-0.94) mg/ dL as compared to the control group (41.50+_ 0.61) mg/dL,there is no – significant change ($p>0.05$) in the low density lipoproteins (LDL) in the treated group (86.70+-0.75) mg/dL as compared to the control group (85.26+-0.76) mg/ dL, while the very low density lipoproteins (VLDL) concentrations appeared significant decrease ($p<0.05$) in the dosed group (21.80 +- 0.62) mg/dL as compared to the control group(26.30+_0.98)mg/dL. The results was shown in table -1-.

Table-1- effect of the tiger (energy drink) on the profile lipid parameters in female mice in 1.5ml/kg B.W.in cholesterol, triglyceride,,a (LDL)low density lipoproteins , (VLDL)a very low density lipoproteins and(HDL) a high density lipoproteins parameters in control and the treated group.

Group	Mean ± SE (mg/dl)				
	Cholesterol	Triglyceride	HDL	LDL	VLDL
Control	154.70 ±0.82	127.12 ±0.69	41.50 ±0.61	85.26 ±0.76	26.30 ±0.98
Treated	160.02 ±1.52	115.96 ±0.67	50.30 ±0.94	86.70 ±0.75	21.80 ±0.62
T-test	3.997 *	2.220 **	2.590 **	2.461 NS	2.686 **
P-value	0.0201	0.0001	0.0001	0214	0.0042
* (P≤0.05),** (P≤0.01).					

Discussion

Both cholesterol and triglycerides are fatty substances known as lipids. But, triglycerides are in the form of fats while cholesterol is in the form of awaxy substance fats. That similar fat and found in all cell of the body. The liver can make cholesterol which is an important part of the walls of cells and nerves.

Cholesterol plays an important role in the function of the body such as in the digestion and in the hormones production . it produces by the body,when we eat the animal food cholesterol is obtained through it,Pure cholesterol cannot be mixed or dissolved in the blood. Therefore,in the liver the cholesterol bundle with triglycerides and proteins in the carriers are called

lipoproteins. The lipoproteins move from a mixture of fats to areas throughout the body. An increase in the level of triglyceride leads to heart disease.

The decrease of triglycerides rate the reason may be due to decrease the fatty diet by the animal, unsaturated fats can decrease of triglycerides rate.(21).

When we were taken energy drink, the caffeine was begun enter in the blood stream that led to increase blood pressure and heart rate and the effect in the eyes that led to liver releases the large amount of sugars in the blood stream and led to the receptor in the brain prevent drowsiness so the liver responds to this by turn sugar to lipids or fats,the body was released all water in the energy drink by the urination.

The caffeine consumption that led to decrease of bile acids and neutral sterols that may be led to increase the cholesterol rate.

Consuming saturated fats may cause an increase of total cholesterol and an increase of HDL thereby increasing the ratio of total cholesterol and HDL, so the risk of atherosclerosis is .Energy drink is kind of beverage that contains stimulants such as caffeine, which is marketed as providing physical stimulation and mental as energy ,the large amounts of caffeine consumed through of an energy drinks are associated with a series of side effects such as seizures, diabetes mellitus, cardiac abnormalities or mood and behavioral disorders, particularly in children, adolescents and young adults as well as those taking medications. observed by the same treatment. The lipid profile is essential for the dyslipidemia and associated with atherosclerosis, diabetes, obesity and other degenerative disorders.

The level of a high density lipoprotein was an associated with an inverse relationship with a risk of cardiovascular disease It is known that these changes in blood lipids related to doubling of the chance of stroke,hardeningand narrowing of the arteries, as well as a heart or stroke energy drink.(22).

A very low density lipoprotein produced through liver and releases in the blood stream. The vldl particle can carry triglycerides.

VLDL and LDL were called bad cholesterol in sometimes, that led to the build –up the plaque in arteries called atherosclerosis. the plaque isa sticky substance that is present in the blood and made of several materials such as fat, cholesterol, calcium, and other materials . the plaque can cause hardening and narrowing of the arteries that limiting the flow of oxygen-rich blood to the body that cause coronary artery disease and other heart diseases.

The VLDL and the triglycerides are linked together when the level of triglycerides in the blood decrease that leads to a decrease of vldl in the blood stream(23), this is an indication of vldl – cholesterol level known the low level of cholesterol in the blood plasma was mainly to reducing or eliminating in the major inherited risk factor related to premature heart disease and stroke (24).

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EFFECT OF DIFFERENT FREEZING PERIODS OF SERUM SAMPLES ONCOMMON BIOCHEMICAL TESTS

http://dx.doi.org/10.47832/MinarCongress3-6

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Abstract:

Blood samples are often collected, stored for certain periods and analyzed in the laboratory, and this affects the results of the analysis. The aim of this study was to determine the analytical persistence of serum samples expose to storage temperature range and times prior to analysis.

Materials and methods: Serum samples were obtained from 36 persons. After measuring the fresh sample, the rest of the serum sample was divided into 5 groups and stored at -20 ˚C. A group of sera was kept frozen for up to 42 days then analyzed for stability. A total of 9 chemistry analytics were assayed(glucose, urea, uric acid, total protein, albumin, calcium, lactate dehydrogenase (LDH), and alkaline phosphatase) at each time point. The results were compared with those obtained from the initial analysis of fresh samples.

Results: Among the analytics studied, cholesterol was stable in all conditions. Glucose, urea, uric acid, total protein, albumin, calcium, lactate dehydrogenase (LDH), and alkaline phosphatase were changed significantly ($P < 0.005$).

Conclusions: These results can be used to determine which chemical analysis gives incorrect results when exposed to different storage times but some of the chemical analysis was not affected by the delay and storage conditions for up to 6 weeks prior to analysis.

Key words: Serum, Stability, Storage Time, Storage Temperature Range.

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Introduction:

Stability is the ability to keep the concentrations of the affected analytic to a minimum within the limits of acceptable variation during the study period (Association of Normalization, 2009).A common problem in clinical laboratories is maintaining the stability of serum analytes during sample storage. Samples are usually stored in the door (4–8°C) of a refrigerator for short durations or in a freezer (−20°C) for longer time periods. Thus, the temperature at which the samples are stored constitutes an important preanalytical variable that may affect analysis results in the clinical biochemistry laboratory setting (Kashawa et al, 2017). Some studies have shown that 75% of sample handling errors occur in the pre-analysis phase (Cuhadar et al, 2013). In practice, it is possible to re-analyze the stored biological samples to confirm the results previously obtained or for further investigation. However, the stability of the analyzes must be ensured before results are disclosed or further analyzes are performed (CuhadarS,et al.2013). This study was designed to determine the effect of freezing and storage time on biochemical analysis in human serum. Blood sugar, urea, uric acid, total cholesterol (TC), total protein, calcium, albumin, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were tested.

Aim of the study

To determine the impact of freezing of serum at different storage time on stability of a number of biochemistry analytics.

Material and method

Subjects

36 persons were unrolled in this study. Sampling was performed at the National Center of Hematology. Samples were collected from each person for routine examination ordered by the physician.

Method

After the serum samples separated and biochemical analysis were conducted, the remaining serum for each sample was divided into five parts, which were placed in an Eppendorf tube and freezen for different storage times. Then destroying the sample that was analyzed, and after one day of storing the samples, the same process is carried out on the remaining parts of each sample. Concentration changes of biochemistry parameters (sugar, blood urea, uric acid, cholesterol, protein, albumin, calcium, lactate dehydrogenase, and alkaline phosphatase) were evaluated by a spectrophotometer and changes were recorded.

Statistical analysis

Serum concentration of analysis are shown as the mean and standard deviation.The variation in the analytics due to freezing are expressed as mean percentage change with a " + " for an increase and a " - " for decrease compared with two freeze. Analysis of data was carried out using available packing of SPSS-24(Statistical Packages for Social sciences-version 24) Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range(minimum-maximum values).

Results

Concentration differences in serum samples were measured for the nine biochemical parameters from Day zero and (day 1, day 2, week 2 and 6). A comparison of concentration for biochemical investigation was shown (Table 1). Blood sugar levels, when measured after 6 weeks there was significant difference from previous measurements(figure 1 and table 2). In addition, blood urea levels after 6 weeks showed significant difference from previous measurements as shown in(figure 2 and table 3). The results of uric acid and albumin showed significant difference and altered throughout the period of freezing and storage time shown in (figures 3,4 and tables 4, 5). However there was no significant difference in shown cholesterol and other analytics over the period of freezing and storage time shown in(figure 5 and table 6). On the other hand, concentrations of protein were significantly different from previous measured and altered throughout the period of freezing and storage time as shown in(figure 6 and table 7). Calcium showed a significant difference in concentrations for weeks 2, 4,and 6 in comparison with day zero. Calcium stability on day two did not changes as shown in (figure 7 and table 8). there were significant differences in LDH on days two and weeks 2,4 and 6 from day zero(figure 8 and table 9). Regarding the alkaline phosphatase, there was a significant difference in weeks 2 and 4 from day zero. In addition and there was a significant difference in week four from the previous measurements as shown in (figure 9 and table 10).

Table 1:Comparison of biochemical analytics at different storage times

Mean±SD (Range)	Day 0	Day 1	Day 2	Week 2	Week 4	Week 6
Sugar (mmol/L)	5.90±2.36 (3.2-16.4)	6.01±1.67 (4.0-11.3)	5.98±2.07 (4-15)	5.87±1.67 (4.0-11.9)	5.71±1.52 (3.9-11.4)	6.26±2.61 (3.9-19.6)
Urea (mg/dL)	5.84±2.72 (2.8-20.0)	5.71±1.62 (2.1-10.2)	5.92±1.90 (2.6-11.4)	6.22±1.92 (2.7-12.2)	5.60±1.79 (2.1-11.1)	6.07±1.90 (2.8-11.6)
Uric Acid (µmol/L)	316.44±76.05 (173-461)	364.39±108.85 (228-777)	360.94±81.11 (240-564)	367.81±103.94 (221-708)	357.36±86.05 (213-553)	377.11±82.45 (220-552)
Cholesterol (mmol/L)	4.48±1.18 (1.9-6.8)	4.57±1.11 (2.0-6.7)	4.59±1.05 (2.5-7.1)	4.51±1.35 (1.2-6.7)	4.64±1.17 (2.3-7.2)	4.67±1.29 (2.3-7.3)
Protein (mg/dL)	68.08±8.70 (49-86)	69.36±9.91 (46-87)	68.81±9.06 (46-83)	70.89±10.70 (45-92)	73.19±8.90 (51-89)	77.00±9.32 (57-102)
Albumin (mg/dL)	39.25±6.29 (24-53)	43.53±11.58 (25-83)	44.17±9.11 (30-86)	44.42±8.57 (25-65)	45.58±6.72 (29-61)	46.08±7.22 (31-63)
Ca (mmol/L)	2.02±0.22 (1.3-2.5)	2.29±0.45 (1.5-3.4)	2.12±0.30 (1.5-2.7)	2.28±0.41 (1.4-3.1)	2.34±0.31 (1.6-3.1)	2.36±0.32 (1.8-3.2)
LDH (U/L)	198.72±44.42 (100-265)	205.75±46.60 (125-323)	227.39±44.73 (113-340)	222.42±42.28 (160-305)	226.00±38.60 (129-289)	234.33±37.02 (177-304)
Alkaline Phosphatase (U/L)	156.69±75.97 (66-469)	157.47±84.10 (66-510)	164.03±82.60 (74-522)	171.03±84.18 (80-544)	123.81±61.74 (58-320)	113.33±46.35 (72-243)

The mean blood sugar was significant (p-value 0.05) after 6 weeks of storage as shown in (table 2 and figure 1)

Table 2: Concentrations of sugar significant of serum samplesat different storage times.

Period	Blood SugarRange Mean±SD (mmol/L)
Day 0	5.90±2.36 (3.2-16.4)
Day 1	6.01±1.67(4.0-11.3)
Day 2	5.98±2.07 (4.0-15.0)
Week 2	5.87±1.67(4.0-11.9)
Week 4	5.71±1.52(3.9-11.4)
Week 6	6.26±2.61(3.9-19.6)#
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

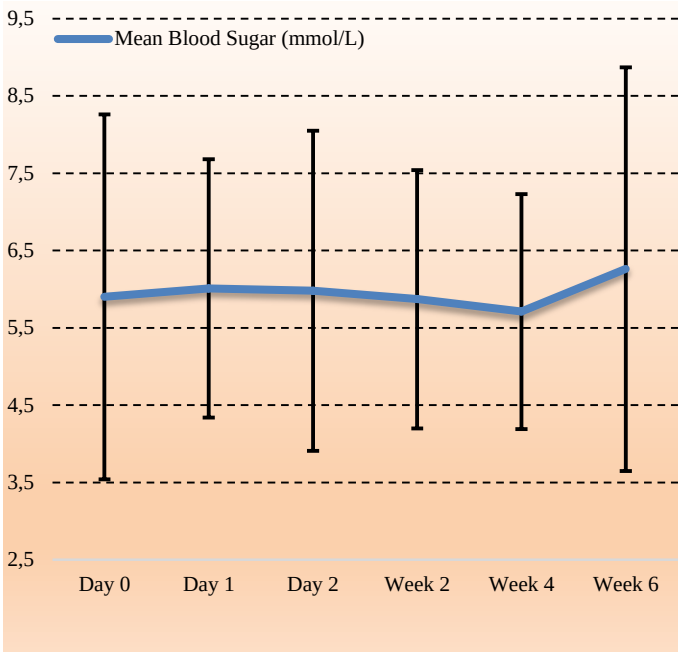

Figure 1: Blood sugar change over different storage time.

The mean blood urea was significant (p-value 0.05) after 6 weeks of storageas shown (in table 3 and figure 2)

Table 3: Concentration of urea significant of serum samples at different storage time.

Period	Blood Urea Range Mean±SD(mg/dL)
Day 0	5.84±2.72(2.8-20.0)
Day 1	5.71±1.62 (2.1-10.2)
Day 2	5.92±1.90 (2.6-11.4)
Week 2	6.22±1.92 (2.7-12.2)
Week 4	5.60±1.79 (2.1-11.1)
Week 6	6.07±1.90 (2.8-11.6)#
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

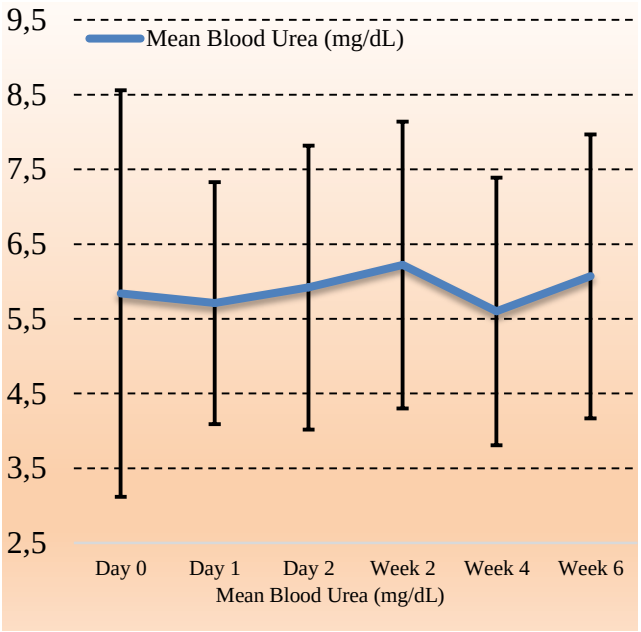

Figure2: Blood urea change over different storage time

The mean uric acid was significant(p-value 0.05) after 1,2 days and 2,4,6 weeks of storage shown in (table 2 and figure 3)

Table 4: Concentration of serum uric acid significant of serum samples at different storage time.

Period	Uric acid Mean±SDμmol/l Range
Day 0	316.44±76.05 (173-461)
Day 1	364.39±108.85 (228-777)*
Day 2	360.94±81.11 (240-564)*
Week 2	367.81±103.94 (221-708)*
Week 4	357.36±86.05 (213-553)*
Week 6	377.11±82.45 (220-552)*
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

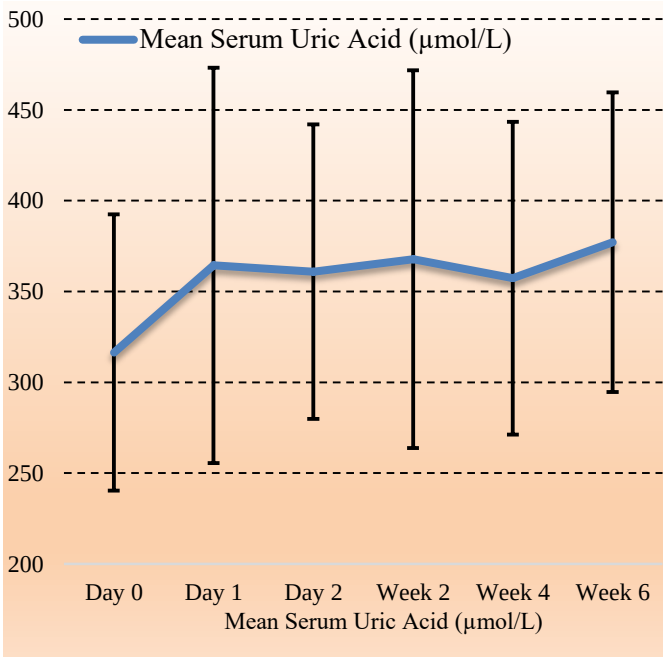

Figure3: Serum of uric acid change over different storage time

The mean serum cholesterol was non- significant (P-value>0.05) after 6 weeks of storage as shown in (table 5 and figure 4).

Table 5: Concentration of cholesterol significant of serum samplesat different storage time

Period	Serum cholesterol Range Mean±SD(mmol/L)
Day 0	4.48±1.18 (1.9-6.8)
Day 1	4.57±1.11 (2.0-6.7)
Day 2	4.59±1.05 (2.5-7.1)
Week 2	4.51±1.35 (1.2-6.7)
Week 4	4.64±1.17 (2.3-7.2)
Week 6	4.67±1.29(2.3-7.3)
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

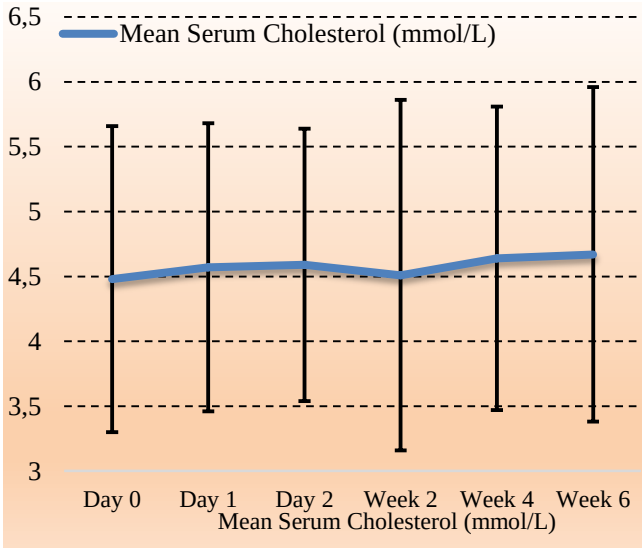

Figure4: Serum cholesterol change over different storage time

The mean serum protein was significant (p-value 0.05) after 2, 4, 6 weeks of storage as show in (table 6 and figure 5)

Table 6: Concentration of serum protein Significant of serum samples at different storage time

Period	Serum protein Mean±SD(mg/dL)	Range
Day 0	68.08±8.70	(49-86)
Day 1	69.36±9.91	(46-87)
Day 2	68.81±9.06	(46-83)
Week 2	70.89±10.70	(45-92)*
Week 4	73.19±8.90	(51-89)*
Week 6	77.00±9.32	(57-102)*#

-Data Mean±SD were presented as (Range)

*Significant difference from Day 0 using Paired-t-test at 0.05 level

#Significant difference from previous measurement using Paired-t-test at 0.05 level

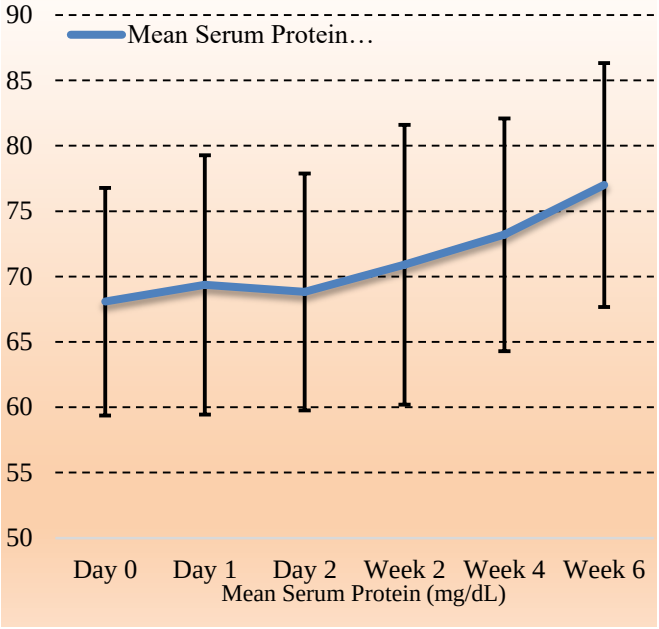

Figure5: Serum cholesterol change inover different storage time.

The mean serum albumin was significant(p-value 0.05) after 1,2 days and after 2, 4, 6 weeks of storage as show in (table 7 and figure 6)

Table 7 : Concentration of serum albumin Significant of serum sample at different storage time

Period	Serum albumin Range Mean±SD(mg/dL)
Day 0	39.25±6.29 (24-53)
Day 1	43.53±11.58(25-83)*
Day 2	44.17±9.11 (30-86)*
Week 2	44.42±8.57 (25-65)*
Week 4	45.58±6.72 (29-61)*
Week 6	46.08±7.22 (31-63)*

-Data Mean±SD were presented as (Range)

*Significant difference from Day 0 using Paired-t-test at 0.05 level

#Significant difference from previous measurement using Paired-t-test at 0.05 level

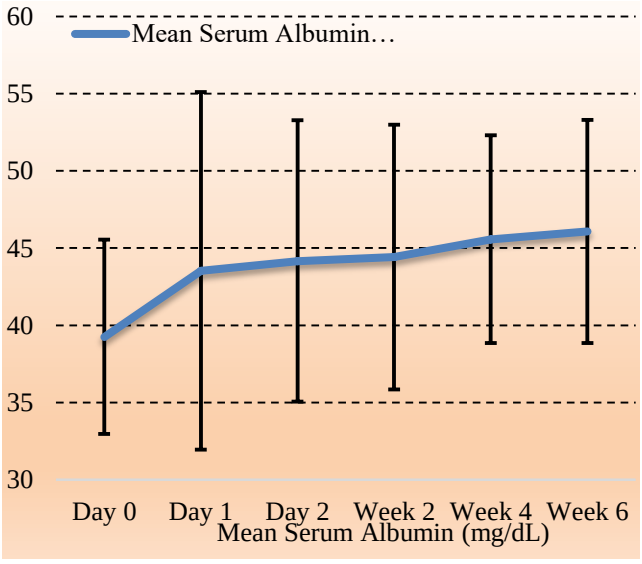

Figure 6: Serum albumin change over different storage time

The mean serum calcium was significant(p-value 0.05) after 1 days and after 2 , 4, 6 weeks of storage as shown in (table 8 and figure 7)

Table 8: Concentration of serum calcium significant of serum sample at different storage time

Period	Serum Ca Range Mean±SD(mmol/L)
Day 0	2.02±0.22 (1.3-2.5)
Day 1	2.29±0.45 (1.5-3.4)*
Day 2	2.12±0.30 (1.5-2.7)
Week 2	2.28±0.41 (1.4-3.1)*
Week 4	2.34±0.31 (1.6-3.1)*
Week 6	2.36±0.32 (1.8-3.2)*
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

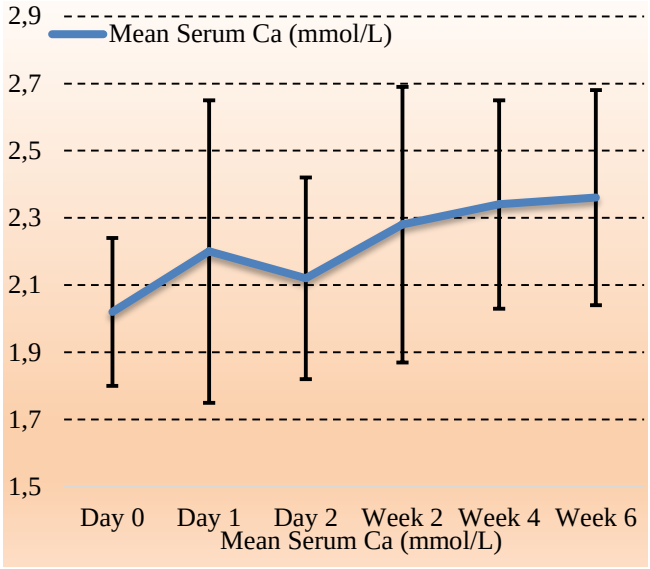

Figure 7: Serum calcium change over different storage time

The mean serum LDH was significant(p-value 0.05) after 2 days and after 2,4, 6 weeks of storage as shown in (table 9 and figure 8)

Table 9: Activity of serum LDH significant of serum sample at different storage time

Period	LDH (U/L) Range
Day 0	198.72±44.42 (100-265)
Day 1	205.75±46.60 (125-323)
Day 2	227.39±44.73 (113-340)*
Week 2	222.42±42.28 (160-305)*
Week 4	226.00±38.60 (129-289)*
Week 6	234.33±37.02 (177-304)*
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

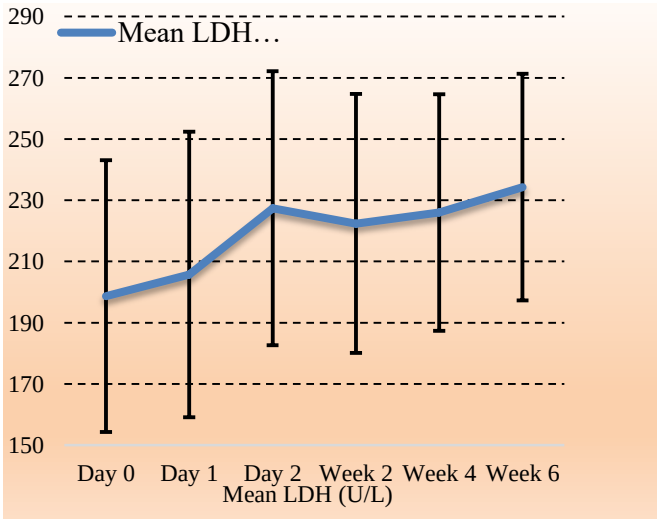

Figure 8 :Serum LDH change in different storage time

The mean serum alkaline phosphatase was significant(p-value 0.05) after 2, 4, weeks of storage as shown in(table 10 and figure 9)

Table 10: Activity of serum alkaline Phosphatase significant of serum sample at different storage time

Period	Alkaline PhosphataseRange (U/L)
Day 0	156.69±75.97 (66-469)
Day 1	157.47±84.10 (66-510)
Day 2	164.03±82.60 (74-522)
Week 2	171.03±84.18 (80-544)*
Week 4	123.81±61.74 (58-320)*#
Week 6	113.33±46.35 (72-243)
-Data Mean±SD were presented as (Range)	
*Significant difference from Day 0 using Paired-t-test at 0.05 level	
#Significant difference from previous measurement using Paired-t-test at 0.05 level	

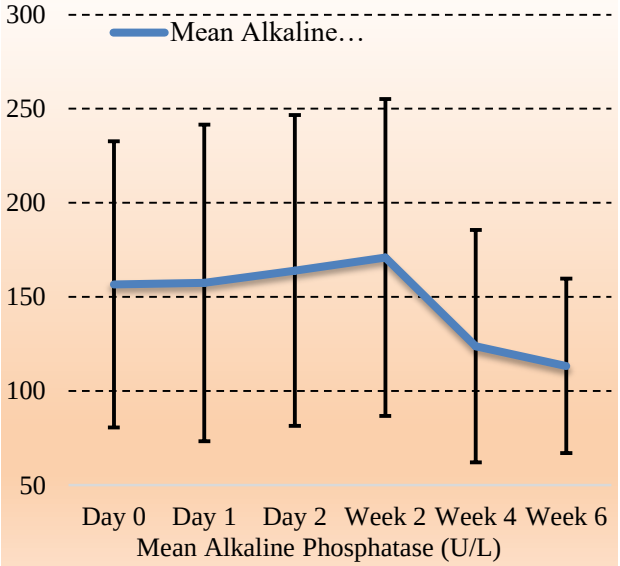

Figure 9:Serum alkaline phosphatase change in activity over different storage time

Discussion

This study was conducted to investigate the effects of storage time on biochemical analytics before analysis the findings of this study showed that some of the analytics were stable in samples stored at -20 ° C during the study period as blood glucose and urea,the results of the analysis did not change after 4 weeks of storage at -20°C. This is consistent with the results of (Kachhawa, et al 2017).

Previous studies (Cuhadar S, et al 2012 and 2013) showed that serum concentrations of uric acid, albumin and calcium were unstable after 48 hours of storage at −20 °C this is in agreement with our results in this study, which showed a change in uric acid, albumin and calcium concentration after days 1,2 and weeks 2,4; however, these changes were significant in comparison with other day zero. The temperature at which samples are stored constitutes an important pre-analytical variable that may affect analysis in clinical biochemistry laboratory setting and there was a clear effecton the analytical persistence a serum specimen if exposed to variable temperatures and a long storage period(Cuhadar et al 2013. and Paltiel et al 2008).

In the current study, there were no significant differences in serum total protein on (day 1 and day 2) compared to fresh samples (day zero) but there was a significant difference between the serum protein level in the (day zero) and serum protein stored for weeks 2,4 and 6 weeksthis is consist with other study when serum was storage at 4 °C, non-significant different occurred in protein level. This suggests the stability of proteins in serum samples is temperature-dependent.Steps should be taken to prevent delays in sample processing to minimize the degradation of proteins(Rai AJ et al 2005 and Kachhawa, et al 2017).

Multiple time points in which the levels of (LDH) were assessed. It was observed that there were statistically significant differences between the serum LDH levels in fresh samples and samples stored for 2 days and 2, 4, and 6 weeks. This is due to the enzymatic cleavage of precursor

molecules. Exchange of substances between serum and erythrocytes occurs due to prolonged contact between them, which causes dilution or may even lead to an increase in the concentration of an analytic in the serum(Heins et al 1995).

Results showed a statistical difference between levels of serum ALP in (day zero) and the same serum stored for ALP in (2,4 weeks). Among the factors that affect the concentration of drug components and analytical values in addition to the stability of enzymes in serum samples are the temperature and the period during which the samples are stored. This differences in the activities of ALP may be due to the presence of different enzymes that differ in their densities at different temperatures (Divya PD et al,2014).

In this study total cholesterol (TC) was measured, results for serum cholesterol showed non-significant differences levels in (1,2 days and 2,4,6 weeks) compared to day zero, this is consistence with the results of (Paltiel et al 2008,and Cuhadar et al 2013).

The previous study investigated the effects of time, temperature, type of tube, and the delay before centrifugation on the concentration of 81 analytics, and found that most of the analytics they tested remained stable up to 24 h before centrifugation at all storage time. A significant difference were found in some analytics due to:

- Prolonged contact of serum and plasma with cells and leakage of intracellular constituents
- Degradation of peptides and proteins by blood enzymes
- Glycolysis in cells
- Some changes were temperature-dependent(Oddoze et al2012)

Conclusion

There was statistically significant differences in the analytical persistence of biochemical analytics in serum when stored at -20 c for different storage periods. In this study uric acid, calcium, albumin, and enzymes(LDH and ALP) are the least stable.Therefore, it is best to perform the analysis of the serum samples immediately after the separation process to ensure that the calculated values are accurate and obtain convincing results.

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SOFTSWISH NEURAL NETWORK APPROXIMATION WITH ZUI-CUI MODULUS

http://dx.doi.org/10.47832/MinarCongress3-7

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Zainab Abdulmunim SHARBA[2]

Abstract:

Until today, many formulas of neural networks are defined to be used for function approximation, they vary with respect to the weights, activation functions and other standards. Moreover, researchers have studied the approximation of different spaces of functions. In this paper, we approximate functions from multivariate $L_p$spaces with a neural network with a new defined form of Swish function, named SoftSwish. Also, multivariate Zou-Cui modulus is introduced to express the degree of approximation by our Swish neural network that we call "SoftSwish Neural Network"..

Key words: Approximation, Neural Network, Swish, Modulus of Smoothness.

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Introduction and Preliminaries:

Artificial Neural Network (ANN) is an artificial intelligent method, that is created to understand the abilities of learning realizing, which is one of the characteristics of human brain to discover new information automatically and generate new information. In recent decades, neural networks have enjoyed a big evolution in the field of functions approximation.

In neural networks, the activation function plays a crucial role. Sigmoid has long been one of the most popular activation functions in neural network behaviour, but its small derivative causes disappearing gradients, making it less ideal for learning, see (X. He & Xu, 2007; Klambauer et al., 2017).

In(Glorot, 2011), the authors introduced another activation function called ReLU. In the positive region, it has an identity derivative and therefore it gives less susceptible to vanishing gradients.Therefore, the function has become the most common recently.

Due to the success of ReLU, numerous adaptations have been proposed in(Bhaya & Sharba, 2020; Dittmer et al., 2019; K. and Z. X. and R. S. and S. J. He, 2015), but did not reach the same popularity of Sigmoids, this is due to the simplicityof the mathematical formulas of each. Moreover, none hasgotten the same tractionbecause gains appear inconsistent or negligible across the data set and models.

There are certain properties that are characterized by the activation functions which are considered important for successful learning, such as derivative and monotonicity, and whether their range finite or not.

Ramachandran et al(Ramachandran, n.d.) introduced the Swish activation function, which is related to Sigmoidal function simply by the formula

$$\sigma_j(x) = x_j.sigmoid(x_j)$$

In their paper, the researchers showed the powerful characters that explains why their Swish should be preferred among other activations including ReLU and the general Sigmoid.

Many moduli of convexity developed and characterized to match the Banach spaces (Zuo & Cui, 2009) and quasi-Banach spaces(Kwun et al., 2018). In the multivariate spaces, the activation function should be defined accurately to generate a multivariate neural network so it could be the approximation of a multivariate function. In (Anastassiou, 2011) defined the the sigmoid activation function in the space of $\prod_{i=1}^{n}[a_i, b_i]$ and approximate the functions from $C(\prod_{i=1}^{n}[a_i, b_i])$ by his SoftMax neural network. The degree of Anastassiou's approximation is in terms of the first modulus of smoothness $\omega_1\left(f, \frac{1}{n}\right)$. Later, the authors in (Almurieb & Bhaya, 2020), defined Soft Max neural network, then they estimate the degree of approximation with the kth order modulus of smoothness $\omega_k\left(f, \frac{1}{n}\right)$.

2. Construction of SoftSwish Neural Networks

The general form of any neural network is given by

$$N_d(x) = \sum_{j=0}^{d} c_j \sigma_j(w_j \cdot x + \mathrm{b}_j) \qquad 2.1$$

b_j, c_j are constants in $\mathbb{R}, w_j$are the weights, x_j are the inputs, , σ_j is the activation function.

We begin by defining the new activation function in $\mathbb{R}$
Let

$$\emptyset_j(x_j) = x_j\sigma_j(x_j) + \sigma_j(x_j)\left(1 - x_j\sigma_j(x_j)\right) \qquad 2.2$$

For purposes of function approximation, it is better to use (2.2) than the Sigmoid and Swish functions for several reasons appeared in the properties below,

For any $j = 1, \dots, d, \emptyset_j$ satisfies

(i) $$\sum_{j=1}^{d} \emptyset_j(x_j) \leq 1 \qquad 2.3$$

Proof

$$\sum_{j=1}^{d} \emptyset_j(x_j) = \sum_{j=1}^{d} x_j\sigma_j(x_j) + \sigma_j(x_j)\left(1 - x_j\sigma_j(x_j)\right)$$

$$\leq d\sum_{j=1}^{d} \sigma_j(x_j) + \sum_{j=1}^{d} \sigma_j(x_j)\left(1 - d\sum_{j=1}^{d} \sigma_j(x_j)\right)$$

$$= 1\,,$$

since that

$$\sum_{j=1}^{d} \sigma_j(x_j) = 1$$

Also, it is easy to prove that

(ii) $$\int_{-1}^{1} \emptyset_j(x_j)\,d\,x_j = 1 \qquad 2.4$$

The multivariate neural network with inputs $\boldsymbol{x} = (x_1, \cdots, x_d)$ from $[-1,1]^d = [-1,1] \times \cdots \times [-1,1]d$-times. The multivariate Swish activation function is given by

$$\emptyset(\boldsymbol{x}) = \emptyset(x_1, \cdots, x_d) = \prod_{j=1}^{d} \emptyset_j(x_j) \qquad 2.5$$

We call $\emptyset(\boldsymbol{x})$ in 2.5, SoftSwish like SoftMax in the sigmoid case.

More properties that are similar to those of univariate case of $\emptyset_j$ are hold for multivariate case of $\emptyset$ as follow

$$\text{(i)} \sum_{|\boldsymbol{x}-\boldsymbol{k}|\leq 1} \emptyset(\boldsymbol{x} - \boldsymbol{k}) = \sum_{|x_j - k_j|\leq 1} \prod_{j=1}^{d} \emptyset_j(x_j)$$

$$\leq \prod_{j=1}^{d} \sum_{|x_j-k_j|\leq 1} \emptyset_j(x_j-k_j) \leq 1$$

$$\text{(ii)} \int_{-1}^{1} \emptyset(\boldsymbol{x}) d\,\boldsymbol{x} = \int_{-1}^{1} \cdots \int_{-1}^{1} \prod_{j=1}^{d} \emptyset_j(x_j)\, d\, x_j$$

$$\leq \prod_{j=1}^{d} \int_{-1}^{1} \cdots \int_{-1}^{1} \emptyset_j(x_j) d\, x_j = 1$$

We reach to define the multivariate neural network with respect to the function $\boldsymbol{f} \in L_p[-1,1]^d$, where $\boldsymbol{f} = (f_1, \cdots, f_d)$

$$N_d(\boldsymbol{x}, \boldsymbol{f}) = \sum_{\boldsymbol{k}} c\boldsymbol{f}(\boldsymbol{k})\, \emptyset(\boldsymbol{x} - \boldsymbol{k}) \qquad 2.6$$

$$= \sum_{k_1} \cdots \sum_{k_d} c \prod_{j=1}^{d} f_j(k_j) \emptyset_j(x_j - k_j)$$

where $c = \frac{\|\boldsymbol{f}(x)+tf(y)\|_p^p + \|\boldsymbol{f}(x)-tf(y)\|_p^p}{2nC^p}$

3. Modulus of Smoothness

Moduli of smoothness of functions measures how smooth the function is. Many mathematicians defined several types of moduli. The most important is that Ditzian and Totik. In 1980 Ivanov defined moduli which he characterized as the best algebraic approximation in *Lp* space see (Klambauer et al., 2017).

We choose Zuo-Cui modulus in the quasi-Banach space from(Zuo & Cui, 2009). Actually, we need to define the multivariate Zuo-Cui modulus for functions f from L_p space as follow

$$\zeta^{(k)}(\boldsymbol{f}, t) = \sup_{x,y\in[-1,1]^d} \left\{ \frac{\|\boldsymbol{f}(\boldsymbol{x}+t\boldsymbol{y})\|_p^p + \|\boldsymbol{f}(\boldsymbol{x}-t\boldsymbol{y})\|_p^p}{2C^p} \right\} \qquad 3.1$$

where $0 < t < 1.$

4. Application to Function Approximation by SoftSwish Neural Network

Neural networks have been widely used in the field of approximation of functions. The following theorem is the existence theorem of such a neural network that approximate $L_p$functions. The degree of neural approximation is estimated here in terms of modulus of smoothness.

Theorem

For anyconvex function $\boldsymbol{f} \in L_p[-1,1]^d$, there is a neural network of the form (2.1) s.t. $\|\boldsymbol{f} - \boldsymbol{N}\|_p^p \leq C\zeta^{(p)}(t)$

Proof

Since f is convex, then

$f(x+ty) \leq f(x) + tf(y)$

So by 2.1-2.5 and 3.1, we have

$$\|f - N\|_p^p = \left\| \sum_k cf(k)\, \emptyset(x-k) - f(x) \right\|_p^p$$

$$\leq \sum_k \int_{-1}^{1} |cf(k)\, \emptyset(x-k) - f(x)|^p dx$$

$$\leq \sum_k \frac{\|f(x) + tf(y)\|_p^p + \|f(x) - tf(y)\|_p^p}{2nC^p} \int_{-1}^{1} |f(k) - f(x)|^p |\emptyset(x-k)|^p dx$$

$$\leq \sum_{k_1} \cdots \sum_{k_d} \frac{\|f(x) + tf(y)\|_p^p + \|f(x) - tf(y)\|_p^p}{2nC^p} \prod_{j=1}^{d} \int_{-1}^{1} |f_j(k_j) - f_j(x_j)|^p |\emptyset(x_j - k_j)|^p \, dx_j$$

$$\leq C(p,|k|)\zeta^{(p)}(f,t) \blacksquare$$

Conclusions

We realize that we can use neural networks with an appropriate activation function for approximating continuous functions. In our work, we introduce function approximation on Lp space by using a new multivariate formula of Swish activation function. New version of direct inequality using neural networks with generalized Swish activation function in terms of Zuo-Cui modulus of smoothness can be estimated. It open doors wide for more theorems and applications.

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EFFECT OF IRAQI DESERT TRUFFLE AND SOME TYPE OF HONEY AGAINST SOME BACTERIA

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Abstract:

The present study is interested in studying the effect of some natural nutrients on some types of bacteria , especially truffle and honey as food and nutritional value in our ancient parents. A water extract was prepared from the fruits of the black truffle and then studied the effect of this extract against *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*. The results showed that the water extract had a clear inhibitory effect on all the bacteria except *P. aeruginosa*, which showed resistance to the extract. The study also examined the effect of five types of honey in the crude (non-diluted) and diluted form 1: 1 (v: v) obtained from home honey bee, mountain honey, pine honey, grape leaves honey and German honey against the isolates under study. In the our study were showed that the types of honey have a broad and diversity of inhibition against microorganisms under study ,The raw Germany honey (non-diluted) showed high inhibition of *P. aeruginosa*as 25 mm inhibition zone .In comparison, the same type of honey showed 17 mm inhibition zone against *S. aureus* when diluted it ,while *P.aeruginosa* were showed resistance .The results showed that pine tree honey was the best honey used in the study. It showed a clear inhibitory effect on all the isolates used in the test in the crude and dilution form (1:1) without any resistance from any of the isolates.

Key words: Truffle, Water extract, Antibacterial, Pathogenic Bacterial.

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Introduction:

The wrong use of antibiotics led to the appearance of resistance by microorganisms that cause problems in clinical treatment. Therefore, it requires developing antibacterial agents to treat and prevent infection, such as the use of truffles , honey and other natural substances(Al-Jabri,2005;Nggaz and Fortas,2013).

The truffle desert is a fungus used in medicines and as food by ancient people in Iraq, Saudi Arabia and other countries .Depending on the local researcher, this truffle rarely grows in a unique environments in raining season between February to April (Malik *et al.*,2018).Honey is a sticky natural sweet material have a high osmotic effect ,lower pH value and H_2O_2.These properties, as well as its plant nature ,make it have an antiflammatory effect.(Szweda,2017).

Recently, due to the increase in the occurrence of cases of resistance against antibiotics by bacteria in our country, which led to increased severity of disease and the difficulty of treatment , and as far as the subject is related to our competence as microbiologist , the study focused on the get an active extract by easy preparation method and detection of the activity of this extract as well as investigate the effectiveness of different types of honey in raw and diluted form 1:1(v:v)concentration against following bacteria : *Escherichia coli , Klebsiella pneumonia ,Pseudomonas aeruginosa* and *Staphylococcus aureus* by using effective method .

Materials and Methods

Materials

Samples:

1- Desert truffle

Fresh fruiting bodies of truffle were purchased from the Mosul truffle marker located in the " Al-Yabisat" during march in (2019), then they were washed carefully to remove soil. We choose the fruiting bodies have dark brown color with middle size harvested from" Al-Hather"figure (1) .

Figure (1): Fresh fruiting bodies of truffle

2- Honey

Five samples of honey were collected from different origin as following:

1-House honey:-we harvested personally from honey bees house located in the Al-Rifaaei.

2-Mountainous honey: the honey were bought from Sulaimaniyah market.
3-Grape leaves trees honey: the honey were obtained from Al-Rashidiya farm.
4-Pine tree honey: the honey were obtained from Hawei Al Kaneisa.
5-Germany honey:-the Germany honey were obtained personally from German farm and transferred into the Iraq by use sterile bottle specialized for honey.

Pathogenic bacteria

Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, staphylococcus aureus were obtained from some of microbiological masters of department of science/Mosul University.

Preparation of bacterial species suspension

The overnight broth bacterial cultures made by inoculated loopfull of tested bacteria into nutrient broth and then incubated 24 hour at 37°C ,then the broth cultures were diluted by distill water and Suspension of different strains of the tested bacteria were made by diluted broth culture with distill water and compared with 0.5 McFarland tube that equal $1.5x10^5$ CFU/ml cells. (Collee *et al.*, 1996).

Preparation of truffle aqueous extract

For preparation of aqueous extract, fruiting bodies were weighed, washed and homogenized in distilled water (1:3 w/v) by using a household blender. the homogenate is put in bottle in the refrigerator for a 12 h. ,filtered through medical gauze and then centrifuged at 2500 rpm. for 30 min to separates insoluble materials. The supernatant was collected and dried by using watch class exposed to fan. Then collected the extract and put in sterile container until used, Figure (2)

The truffle extract 200 mg/ ml concentration.

Prepared it by dissolved 2 gm of water extract in 10 ml of sterile distill water Figure (3).

Figure (2): Truffle aqueous extract

Figure (3): the truffle extract 200mg/ml concentration

The agar well diffusion assay for test truffle aqueous extract

The suspension of the different bacterial species equal 1.5×10^5CFU/ml cells in comparison McFarland tube were cultured on Mueller Hinton agar medium through 0.1 ml of it into plate and left under dry .A well of 5 mm was made in the agar plate using sterile yellow tips. The truffle extract 200 mg/ ml concentration was added into well of agar medium. Control plate was made without truffle aqueous extract.
All plates (test plate and control) were incubated for 24h at 37°C.after incubation, the antibacterial activity of aqueous extract was determined by measuring the diameters of inhibition zones (Saddiq *et al.*, 2016). The results were photographed.

The agar well diffusion assay for test honey sample extract

The suspension of the bacterial species 1.5×10^5 CFU/ml cells were inoculated on Mueller Hinton agar by spread 0.1 ml of suspension on agar plate. Honey samples was added into well (5 mm) on agar plate. The volume of honey samples equal the size of well because the honey have high viscosity and very difficult to measure the volume by micropipette. Control plate was made without honey
All plates (test plate and control) were incubated for 24h at 37°C.after incubation, the antibacterial activity of honey was determined by measuring the diameters of inhibition zones. the results were photographed.

Result

Antimicrobial activity of truffle aqueous extract by Agar well diffusion assay

The result of antibacterial effect of truffle aqueous extract against bacterial isolates under test shows a significant inhibitory activity zones against four isolate from five isolates were used in test as shows in the table (1)

Table (1) the inhibitory activity zones of aqueous extract against some isolates

Isolates	Inhibition zones(mm)
Enterococcus faecalis	14
E. coli	10
K. pneumoniae	14
P. aeruginosa	0
S. aureus	16

We found that the aqueous extract very active against *Staphylococcus aureus* in inhibitory diameter zone about 16 mm, following by 14 mm diameter inhibitory zone against *Enterococcus faecalis* and *Klebsiella pneumoniae*, while 10 mm diameter inhibitory zone against *E.coli*, but didn't appear any inhibition zone with *Pseudomonas aeruginosa* as shows in (figure 4). Our results agreed with the results of the many researchers that the truffles extract has a wide effectiveness towards many pathogens (Malik *et al.*, 2018; Neggaz and Fortas ,2013) and did not agree with the results of the researcher(Saddiq *et al.*,2016) when using the aqueous extract towards the bacteria *Pseudomonas aeruginosa*, he explained that the extract was actually gave a zone of inhibition as 19,21 and 28 towards three different strain of *Pseudomonas aeruginosa*,these differences may be due to the type of truffle and geographic Location of harvest
.

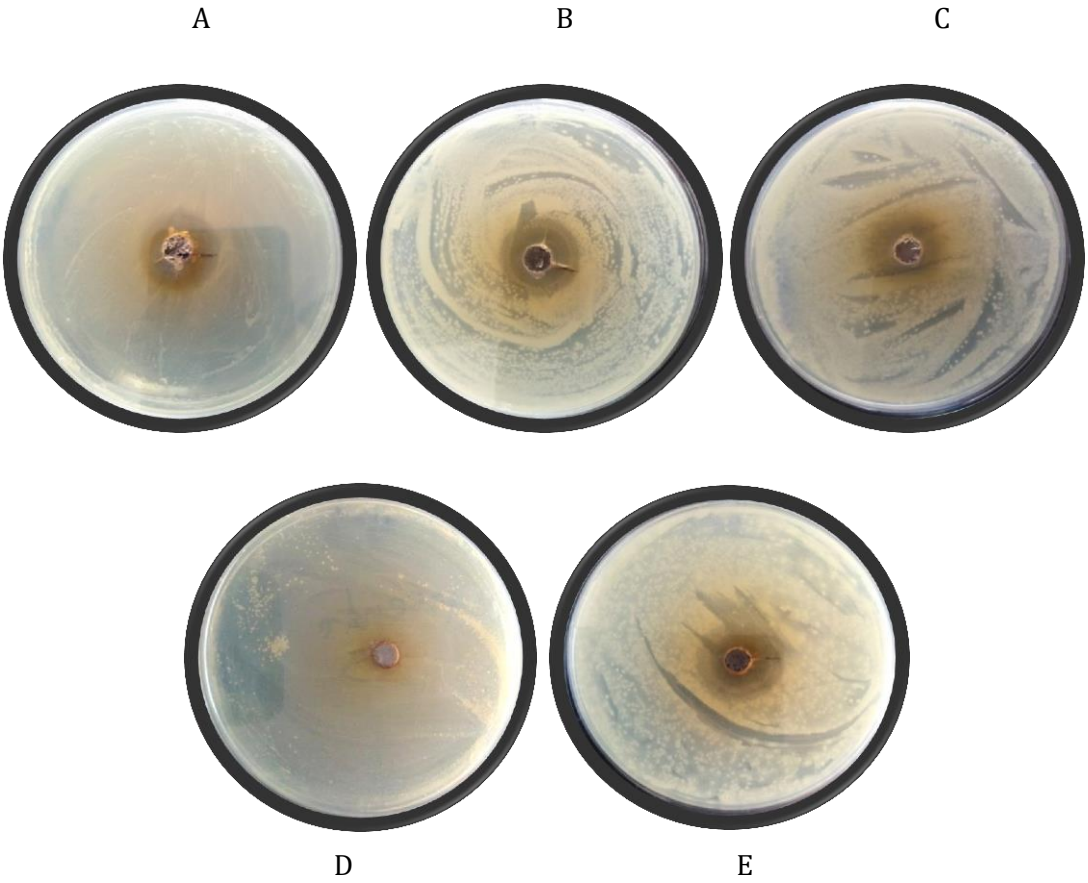

Figure (4) the inhibitory activity zones of truffle aqueous extract against following isolates:-A-Enterococcus faecalis , B- *E. coli* , C- *K. pneumoniae* , D- *P. aeruginosa* , E- *S. aureus*

Antimicrobial activity of honey by Agar well diffusion assay

All crude honey samples used in the experiment exhibited remarkable inhibitory zone against isolates were used as shows in the table (2).Our result reveled that *P.aeruginosa* have the highest inhibitory diameter zone in 3 types of honey in crude form as 25 mm with Germany honey,22 mm with pin trees honey, and 17 mm with home honey while *Enterococcus feacalis* showed resistant against mountainous honey ,grape leave trees honey and Germany honey (Figure 5).

The result of 1/1 (v/v) diluted honey types also showed remarkable inhibition zone with some diversity as mentioned in table (3).we observed that *S.aureus* exhibited the highest inhibitory diameter zone in Germany honey as 17mm while *P.aeruginosa* and *Enterococcus feacalis* exhibited resistance to home, mountain and Germany honey (Figure 6).Among the collection of crude honey used in the test, Germany honey exhibited the highest inhibitory diameters zone against *P. aeruoginosa* with 25mm while the lower inhibitory zone showed in mountainous honey against *S. aureus* as 8 mm . Germany honey in 1:1 (v:v) diluted form exhibited the highest inhibitory diameters zone against *Staphylococcus aureus* with 17mm while the lower inhibitory zone as 5 mm in mountainous honey against Klebsiella pneumoniae and *E. coli*.

Table(2) the inhibition zones of honey types in concentrated form against some type of isolates

Honey types	isolates	Inhibition zone mm
Home honey	*Enterococcus feacalis*	9
	E. coli	14
	Klebsiella pneumoniae	16
	P. aeruginosa	17
	S. aureus	13
Mountain honey	*Enterococcus feacalis*	0
	E. coli	18
	Klebsiella pneumoniae	13
	P. aeruginosa	12
	S. aureus	8
Pine tree honey	*Enterococcus feacalis*	13
	E. coli	12
	Klebsiella pneumoniae	13
	P. aeruginosa	22
	S. aureus	13
Grape leave trees honey	*Enterococcus feacalis*	0
	E. coli	15
	Klebsiella pneumoniae	16
	P. aeruginosa	13
	S. aureus	14
Germany honey	*Enterococcus feacalis*	0
	E. coli	17
	Klebsiella pneumoniae	16
	P. aeruginosa	25
	S. aureus	10

Table(3): the inhibition zones of 1:1(v:v) dilution of honey types against some type of isolates

Honey types	isolates	Inhibition zone mm
Home honey	*Enterococcus feacalis*	0
	E. coli	10
	Klebsiella pneumoniae	10
	P. aeruginosa	0
	S. aureus	16
Mountain honey	*Enterococcus feacalis*	0
	E. coli	5
	Klebsiella pneumoniae	5
	P. aeruginosa	0
	S. aureus	7

Pine tree honey	*Enterococcus feacalis*	6
	E. coli	7
	Klebsiella pneumoniae	12
	P. aeruginosa	7
	S. aureus	14
Grape leave trees honey	*Enterococcus feacalis*	7
	E. coli	9
	Klebsiella pneumoniae	7
	P. aeruginosa	10
	S. aureus	10
Germany honey	*Enterococcus feacalis*	0
	E. coli	15
	Klebsiella pneumoniae	14
	P. aeruginosa	0
	S. aureus	17

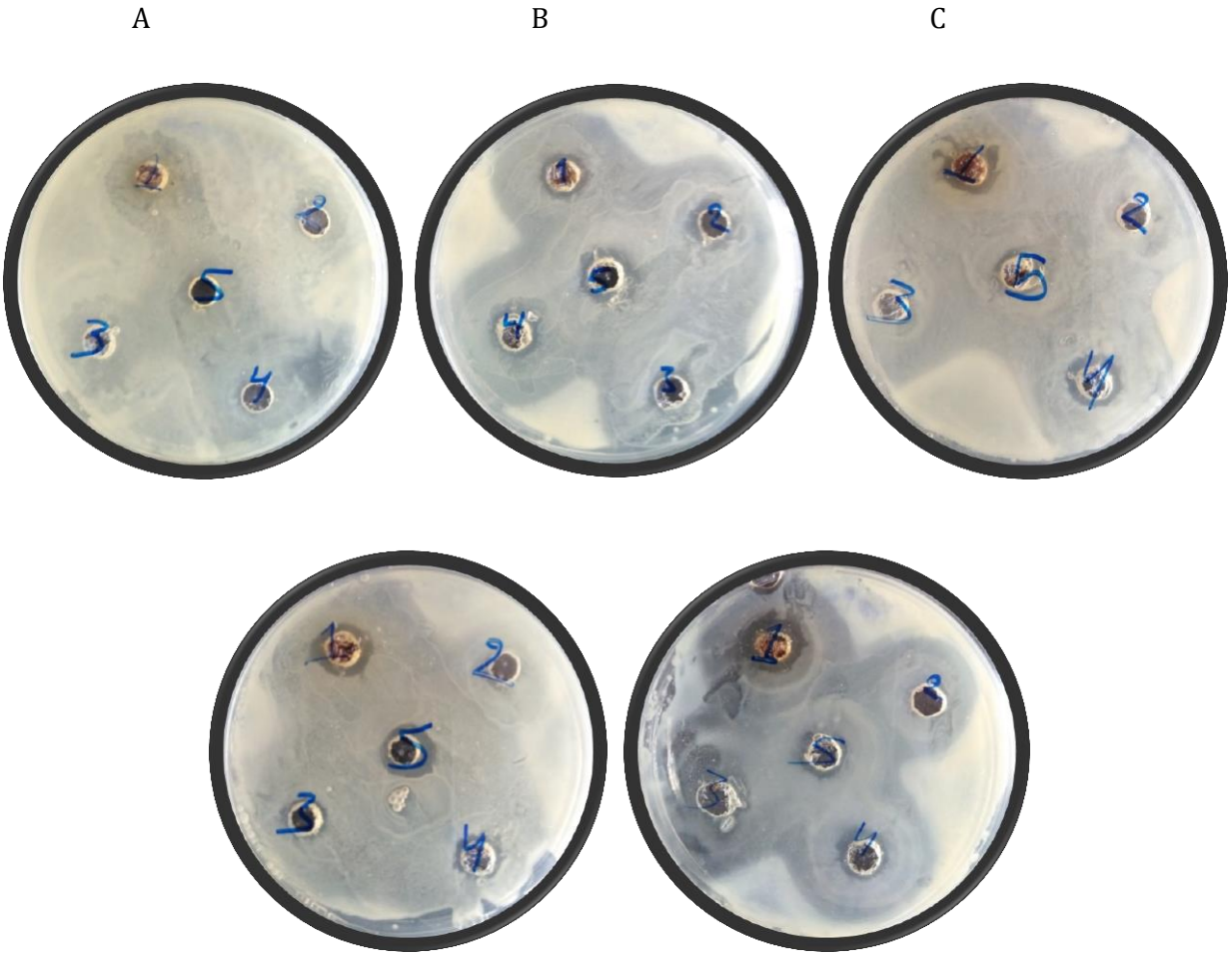

Figure (5): Inhibitory zones of crude honey types of against following bacteria
A- *Enterococcus faecalis* , B- *E. coli* , C- *K. Pneumoniae* , D- *P. aeruginosa*, E- *S. aureus*.

Honey types:

1. Home honey
2. Mountain honey
3. Pine tree honey
4. Grape leave trees honey
5. Germany honey

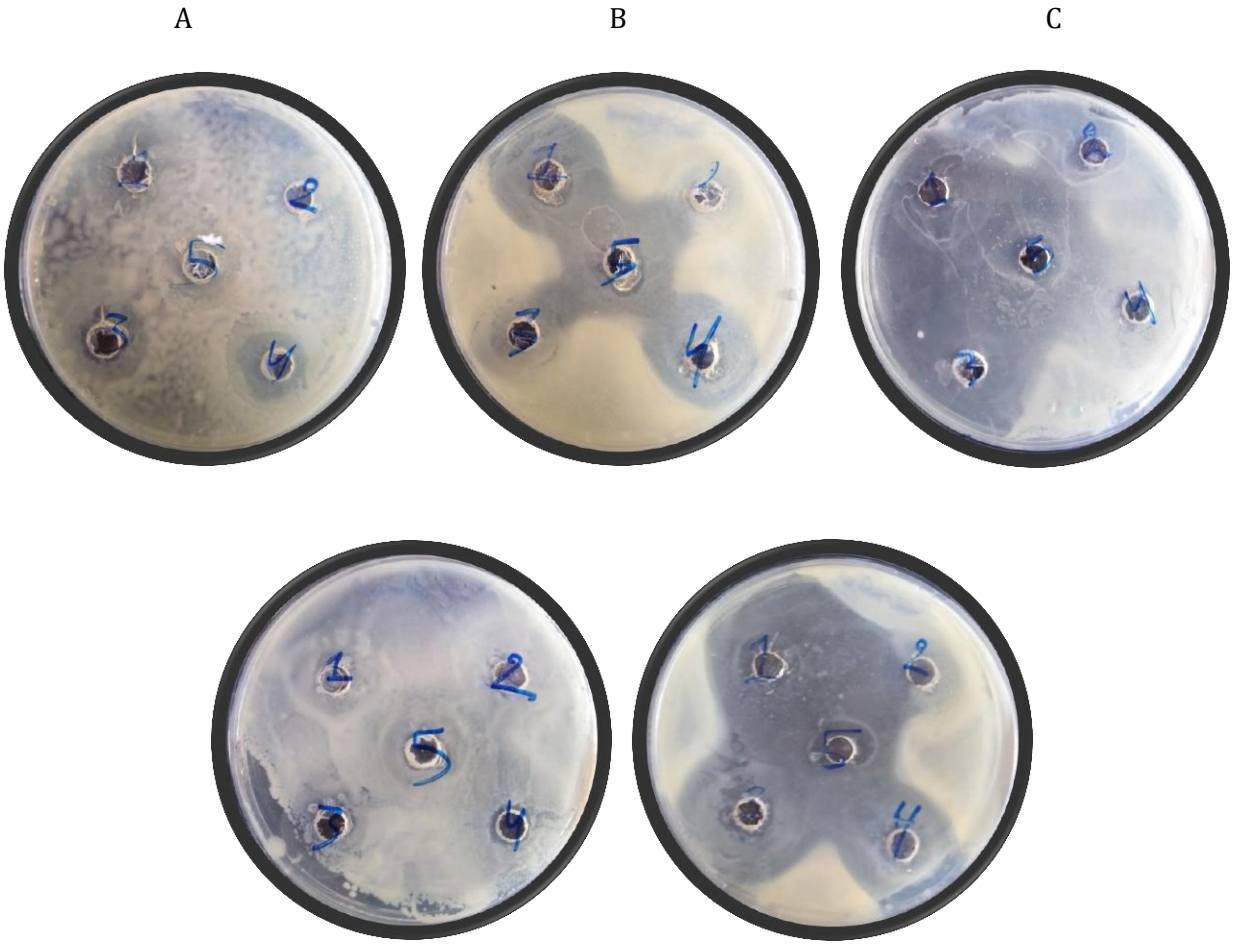

Figure 6(1.5×10^5 CFU/ml cells): Inhibitory zones of 1:1 dilation honey types of against following bacteria

A- *Enterococcus faecalis* , B- *E. coli* , C- *K. pneumoniae* , D- *P. aeruginosa* , E- *S. aureus*

Honey types

1. Home honey ,2. Mountain honey ,3. Pine tree honey ,4. Grape leave trees honey
5. Germany honey

Discussion

In the our study ,the antimicrobial effect of aqueous extract of truffle fruiting bodies against various pathogenic bacteria allow us to conclude that the extract exhibited antibacterial activity that support traditional use of the truffle water in the treatment of some disease.It is noteworthy that our study is one of the few studies in this field in Iraq due to the short harvest season , decomposition of truffle and economic cost of it.

Our study revealed that P.aeruginosa were more sensitive at concentrated honey in contrasts with the 1:1 (v:v)dilution honey ,these finding render may be to the osmotic effect of the honey in the concentrated form than diluted form or may be to the other antibacterial factors present in the concentrated honey such as pH. Many researchers (Zainol *et al*., 2013; Molan,1992) pointed out that very high osmotic pressures coupled with high acidity are the two main factors contributing to the antibacterial properties of honey at concentrated form but when honey is diluted to certain extents, glucose oxides will be activated and start to utilize glucose to produce H_2O_2. At this point, the antibacterial activity of honey will gradually shift from osmotic and pH-dependent to peroxide-dependent.

Our finding reflect that the mountainous honey in dilution form didn't have antibacterial activity against *Enterococcus faecalis* and *P. aeruginosa* and exhibit a small inhibition zone against *E. coli*, *K. pneumoniae*, and *S.aureus*, this may be render to the industrial cheating of mountainous honey.

While the other honey types shows varying degree of inhibition activity in concentrated and dilution form against *Enterococcus faecalis* , *E. coli* ,*S. aureus*, *K. Pneumoniae* , and *P. aeruginosa* ,Some of types were very active in concentrated form while other very active in diluted form with some diversity. These finding might be due to differences between bacterial species under test towards the osmotic effect, pH and H_2O_2 which are unsuitable for bacterial growth . Also, our results show that the honey of pine leaves is more effective against the all bacterial isolates under test in its concentrated and non-concentrated form than the other types of honey (Figure 5 ,6), since no resistance isolates it .This may be due to the extent of variation in antibacterial component to this type of honey.

Two important enzymes known to contribute to the major biological activities of honey are bee-origin glucose oxidase and floral-origin catalase. These enzymes are crucial in determining the level of peroxide activity in honey which underlies numerous biological functions, including antibacterial potency.

Conclusion

We found Effectiveness of the water extract of black truffle in inhibiting bacterial isolates *Enterococcus faecalis, Escherichia coli , Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureu*. Conclusion From the results of the investigation of the inhibitory action of five types of honey towards the bacteria under study, the different types of honey have different inhibitory effect against different bacteria, and when made compare of the results of the inhibition of honey types against isolates under study showed that honey pine trees is the best quality in its inhibitory effectiveness compared to other types of honey, whether it is raw or diluted.

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ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND THE ANTAGONISTIC EFFECT OF LACTOBACILLUS IN FIGHTING *SHIGELLA* SPP. ISOLATED FROM DIARRHEIC CHILDREN

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Abstract:

Background: *Shigella* is an major source of bacterial gastroenteritis in lack of health awareness in society. The handling of shigellos is mostly requires antibiotics. The using of probiotic of Lactic acid bacteria possess the counter effect against many dangerous bacterial pathogens which associated with gastroenteritis like *Shigella* spp.

Aim: the purpose of this study to estimate the influence of lactic bacteria of the genus Lactobacillus on the population *Shigella* as a main pathogen involved in gastroenteritis in children.

Patients and methods: A total of 50 stool specimens were collected during the period September2019 to January 2020 from diarrheic children patients age range(1-3)years. Standard bacteriological methods were used to isolate, identify, and determine the antimicrobial susceptibility pattern of *Shigellaisolates*, and we used fresh culture of Lactobacillus 24 h (previously isolated as member of fecal microbiota from healthy person and identified by molecular assay). Then we done centrifugation to obtain supernatant which have test bioactive materials like bacteriocin. These bacteriocin materials subjected to own antibacterial activity against other bacterial pathogen like *Shigella* spp. By using agar diffusion method .

Results: All the 14 *Shigella* spp. isolates show 100% resistance to nalidixic acid,. cotrimoxazole , and High resistance to ciprofloxacin (85%), and moderate resistant of ampicillin (64%). In agar- well diffusion method indicated the high antagonistic activity of the strains of Lactobacillus 2, 3, and 4 isolated from health GI tract against all *Shigella* spp., as a result of their activity the total elimination of *Shigella* within 24 h was observed.

conclusion: the *Shigella* spp. Strains exhibited antibiotic resistant against more one type of antiobiotic The lactobacilii strains tested during this study showed strong antimicrobial activity against *Shigella* spp.

Key words: Shigellos is, Lactobacillus, 16S Rrna.

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Introduction:

Shigellos is is a worldwide general community health problematic. "shigellos is" is involved for around 165 million cases yearly, of which 98.8% are in evolving states as documented by epidemiological reports. *Shigella* : S. dysenteriae, S. flexneri, S. boydiiand S. sonnei are four species able to cause the infection, are divided into different serotypes dependingon the structure of O-specific polysaccharide of the LPS. The direct contact with an sick person or by eating contaminated food and water with *Shigella* represent the basicway of spread of this microbe [1]. Children <5 years of age , it is the target group and the highest risk of shigellos is, nearly 69% of the cases.[2] In evolving countries, majority of *Shigella* infections is due to endemic shigellos is. The reliable method for diagnosis of shigellos is is culture for isolation of microorganism from feces. The gastroenteritis with *Shigella* can be managed with rehydration; however, antibiotics have verified to decrease severity, period, and prevent dangerous health complications. Attempting treating by administering antibiotics requires full knowledge of the type of local strains involved in many cases of gastroenteritis . Especially important is the awareness of the global emergence of multidrug-resistant (MDR) *Shigella*[3].
The use of probiotics to cure a wide variety of disorder in the past decade. As part of efforts to find an alternative to antibiotic treatments as well as the lack of impact treatments for GI illnesses. While there are increasing reports of the efficacy of probiotics in the treatment of diseases such as "pouchitis [4, 5], diarrhea [6], and irritable bowel syndrome" [7]
Probiotics are living microorganisms that have the ability to alter gene expression under different environmental conditions.Probiotics are specific strains that possess a number of disease-fighting mechanisms. For example, modify in output TNF by strains of Lactobacillus reuteri identified strains that were modulator in immune system [8].

Material and Methods

The study period extended from September2019 to January 2020during this period 50 fecal swabs were collected from diarrheic children attended to Maternity and pediatric Hospital, Hilla city , Iraq

1-Culture media preparation

1-1Muller Hinton agar

This medium was prepared according to the manufactured company (Hi Media , India)
1-2 Man-Rogosa –Sharp (MRS)
This medium is specialized for lactic acid bacteria growing , it was prepared according to the manufactured company (Hi Media , India)

2-Isolation and growing of Lactobacillus spp.

Lactobacilli were isolated from stool of health individuals(10 volunteers) , the stool specimens were culture on the MRS agar under anaerobic condition at 37C0 for 24 hrs. The isolates were diagnosed by the molecular detection of 16S/23S rRNA gene.

3- molecular assay of Lactobacillusspp. on the genus level

Bacterial Genomic DNA of lactobacillus spp. were separated according to the procedure supplied by the of the manufactured institutes (Promega, USA).Primers dissolved and prepared according

to manufactured company (Alpha-USA). which was designed by Dubernet et al.(2002)[9] to amplify the spacer region(250bp) between 16S and 23Swithin the bacterial genomic sequences. The forward primer sequence, LbLMA1(5-CTCAAAACTAAACAAAGTTTC-3) and the revers primer sequence, R16 (5-CTTGTACACACCGCCCGTCA-3).
The total volume of PCR mixture was 25 µl, composed forward primer 1µl and the reverse primer 1µl, 5µ1 of template DNA specimen, 12.5µl of master mix (Promega-USA) and 5.5µ1 of Nuclease free-water. The PCR amplification was achieved with the thermo cycler PCR machine according to the following conditions: the denaturation at 95ºC for 5 min,l cycle followed by 35 cycles of 95°C for 30sec, 55°C for 30 sec, and 72°C for 30 sec; a final extension step for 7 min at 72°C.Positive PCR replicon size was well-known by 1.5% agarose gel electrophoresis at 80 Volts for 50 min staining with ethidium bromide under UV light, with DNA marker 100-2000t5rt46bp[9].

4- Isolation and growing of *Shigella* spp.

For the purpose isolating *Shigella* spp. the feces swabs were collected from 50 diarrheic children, cultured on the primary culture media (salmonella –*Shigella* agar, macConkey agar) and aerobically incubated overnight at 37C patients. *Shigella* spp. diagnosed by the classical biochemical tests and confirmed by the Api20 system (bioMerieux, France). From a purified single colony, bacterial inoculum was made at 1.5* 108 for antibiotic susceptibility test

5-Preparation of bioactive materials (cell free culture of Lactobacillus culture)

One ml of an overnight Lactobacillus growth was used to seed 100 ml of MRS broth, and the culture was conducted at 25 C0 for 24 hours. After centrifuging the culture, the supernatant was filtered through a 0.2 l pore size filter to obtain a cell-free solution. The pH of the supernatant was adjusted to 6.5. [10]

6- Antimicrobial assay

The statement has been approvedthe Clinical and Laboratory Standards Institute (CLSI) guidelines,antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar The antibiotics used were ampicillin AMP(10mcg), cotrimoxazole COT(25mcg), ciprofloxacin CIP(5mcg), nalidixic acid NA(30mcg) purchased from India HiMedia company. The indicator strain (*Shigella* spp.) overnight culture was used to impregnation Muller Hinton agar at 37C for the agar-well diffusion experiment. Agar plates were cut into 5mm diameter wells, and 50 µl of filtered culture supernatant was poured to each well and allowed to diffuse into the agar for 3 hours at 4C. The plates were then incubated at 37°C for 24 hours to allow the bacteria to grow.

Statistical analysis

The statistical analysis in the current study was based on percentages to calculate the frequency of the bacteria concerned in the study

Results

The stool samples which were collected from healthy people given six isolates of Lactobaci1lusspp (60%) These isolates diagnosed by the amplification of intergenic spacer 16S rRNA/23S rRNA in genomic DNA (Figure-1)

Out of 50 stool sample only 14 specimens give positive results for *Shigella* spp. (28%). All the 14 (100%) *Shigella* spp. isolates show resistance to nalidixic acid,. cotrimoxazole , and12 (85%) isolates revealed High resistance to ciprofloxacin , out of 14 isolates 9 isolates (64%) are resistant to ampicillin. Other part of this study is to evaluate the antagononicity of Lactobacillus probiotic strains against *Shigella* spp. All the Lactobacillus spp. strains were shown to create a bacteriocin-like substance, exhibited a inhibitory activity against test bacteria . The *Shigella* spp exhibited more sensitivity toward lactobacillus bacteriocin , the inhibition zones was 20-26 mm.

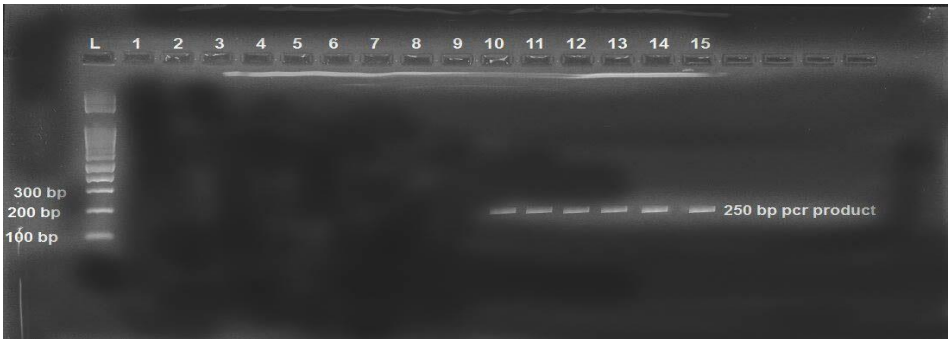

Figure -1: 1.5% Agarose Gel e1ectrophoresis of PCR product of l6s/23s ribosomal RNA intergenic spacer region (250 bp) for Lactobacillus , M: refer to the DNA size marker 100 – 2000bp; 1ane: 10-15 refer to the samples. PCR products visualized under U. V 1ight, after staining with ethidium bromide

Discussion

The *Shigella* bacteria infection is one of the most important infections that affect children in unurbanized areas. Although the infection may recover on its own, it remains necessary to carry out an exit culture for the purpose of ascertaining the type of bacteria and for the purpose of limiting the spread of the microbe[3].

the results of the current study regarding resistance to antibiotics by *Shigellaiolates* are similar with studies conducted by [11] they found that, 42% of isolates of S. flexneriwere sensitive to ciprofloxacin and cotrimoxazole, but all isolates of S. sonnei were resistant to ciprofloxacin and cotrimoxazole

The use of probiotics in the prevention and treatment of gastrointestinal illnesses has gotten a lot of attention in the last two decades. Several methods for lacotobacilli's antibacterial activity against Gram-negative bacteria have been proposed, including "synthesis of organic acids, undissociated organic acid molecules, and bacteriocin, competition for adhesion sites, and co-culture". [12]

Entero-invasive S. sonnei induces inflammatory damage of the intestinal epithelium, resulting in acute recto-colitis and potentially fatal consequences. However, nothing is known about *Shigella* sonnei's antimicrobial action in vitro. *Shigella* (mainly S. sonnei).These bacteria remain the primary cause of gastrointestinal sickness in the United States, and are classified among the three main causes of this.[13]

The lactobacilii strains tested during this study showed strong antimicrobial activity against *Shigella* spp.

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EFFECT OF LASER SHOTS ON THE OPTICAL PROPERTIES OF FE2O3: CUO THIN FILMS PREPARED BY PULSE LASER DEPOSITION TECHNIQUE

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Abstract:

CuO-doped Fe2O3 thin films were deposited onto glass substrates using the Pulsed Laser Deposition (PLD) process at room temperature and a vacuum of 10-2 mbar, utilizing a Nd:YAG laser with a wavelength of 1064 nm, an average frequency of 6 Hz, and a pulse duration of 10 at various laser pulses (300,400 and 500 and).The effect of number of pulsed laser shots on the optical properties of the films was invesigated. UV-VIS spectrophotometer mentioned that the transmittance increases to 90 % when decresing the number of the laser shots. Furthermore, The optical measurements indicate that the Fe2O3:CuO films have a direct Egopt that diminishes as the number of laser pulses increases. The band gap energy of the Fe2O3:CuO found was 3.01 eV. This value was reduced significantly to 3.0 by increasing the number of laser blasts. However, optical constants such as the refractive index (n), the extinction coefficient (k), and the dielectric constant (r, I rise in a predictable manner as the number of laser flashes increases.

Key words: CuO –doped Fe2O3; PLD technique ; Thin Film; Optical Properties; Band Gap.

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Introduction:

Metal oxides nanoparticles have been intensively studied in the last decade,because of their sizes, morphology and structure.[1]Due to its high sensitivity to combustible gases, rapid reaction time, and long-term stability, iron oxide thin film (Fe2O3) may be utilized in a variety of applications such as gas sensor [2]. Due to its large optical band gap (Eg = 1.9 eV), strong optical absorption coefficient (a = 105cm-1) for wavelengths > 600 nm, and ability to exhibit both conductivity types by employing a suitable doping element, the photo electrochemical solar cell is well suited for solar energy conversion [3]. Ferromagnetic films are used in a wide variety of applications, including microwave devices and high-density recording medium [4].

As well as, the CuO is a p-type semiconductor with a bandgap value confined between 1.2-2.0 eV [5].It can be used in solar cells due to their high absorption in the visible region .Moreover, it can be used as an active substance in the gas sensor due to its high stability underexposure for many gasses, low-cost material and low base resistivity changes[6]. Also, Gas sensor [7] .To synthesize the Fe2O3:CuO compound, many different chemical and physical methods have been reported, such as Sol gel, chemical vapor deposition, electro-deposition, thermal oxidation, sputtering process and spray pyrolysis.The PLD approach has been extensively employed in recent years for the production of many types of thin films, particularly oxides of several metals and semiconductors. The current work focuses on the doping of iron Oxide thin films with CuO using the PLD technique. The effect of various pulsed lasers on diverse samples of Fe2O3 thin films has been examined and described in terms of morphology and optical characteristics. Section 2 details the experimental procedures. Section 3 contains the findings and conclusions.

2.Experimental Part

Pellets of (Fe2O3)0.85(CuO)0.15 nanoparticles were prepared by 2g maxing the powder togther and then the pressing process was carried out using an electro-hydraulic press with a pressure of (5 tons) and for a period of (10 minutes), with pellets diameter of (1.5 cm) and a thickness of (3 mm). then The thin films of (Fe2O3)0.85 :(CuO)0.15were prepared using PLD technique (DIAMOND-288) of 1064 nm wavelength, 10 ns pulse duration and 500 mJ pulse at different Laser pulses (300,400 and 500). The surface morphology properties of thin films was examined using Atomic Force Microscope (AFM), type (AA3000 Scanning Probe Angstrom Advance Inc.) . The thickness of thin films about 250 nm was measured using the reflectance probe (SR300 Angstrom Sun Technologies). The optical properties such as transmission absorpttion cofficient and optical constants have been investigated

3.Results and Discussion

Optical Properties

UV-V absorption measurements were used to determine the electrical structure and size effect of as-prepared nanoparticles.

Transmittance:

Figs.(1)shows the optical transmittance spectrum as a function of wavelength of Fe2O3)0.85(CuO)0.15thin films at different laser shots (300,400 and 500) in the wavelength range of (365-1100) nm.The results indicate that the films are transparent to visible light and infrared regions of the electromagnetic spectrum, with a sharp cut-off wavelength of about 500

nm. The transmittance value falls as the number of pulsed lasers rises.This implies that the film has a high level of UV and near-visible light absorption. This increase in optical transmittance caused by the thickness effect results in a reduction in structural homogeneity and crystallinity[8,9].

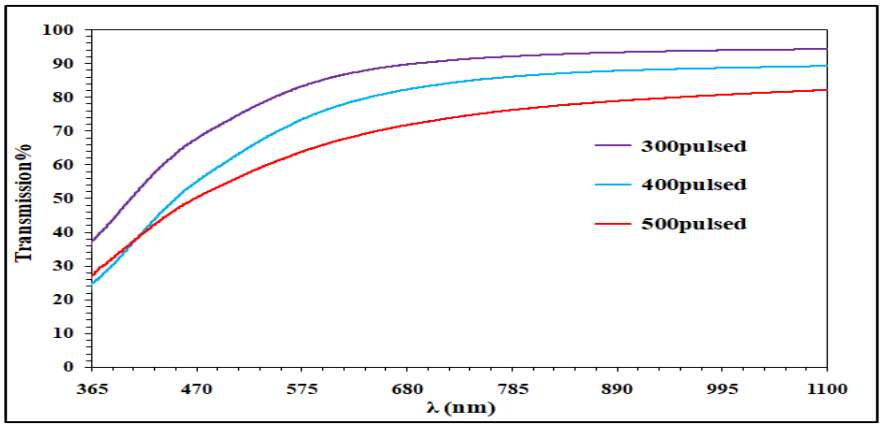

Fig.(1) The Transmittance spectrum as a function of wavelength of (Fe2O3)0.85 : (CuO)0.15 thin films at different number of laser shots

Absorption Coefficient

The absorption coefficient(α) is calculated byusing equation[10]

$$\alpha = \frac{2.303A}{t} \qquad (1)$$

where A: is the Absorbance and t: is the thicknessof the thin films

The figure represents the fluctuation in the absorption coefficient (α) as a function of wavelength for (Fe2O3)0.85:(CuO)0.15thin films exposed to a variety of laser pulses (2). The absorption coefficient (α) is higher than (104cm-1) in this figure, indicating that the electronic transitions were direct. In general, the absorption coefficient reduces as the wavelength increases, as seen in the figure. Additionally, as a result of the thickness effect, the absorption coefficient (α) increases as the number of laser shots increases. This may be ascribed to an increase in layer particle size and density, as well as to the light scattering effect, which accounts for the high surface roughness [11].

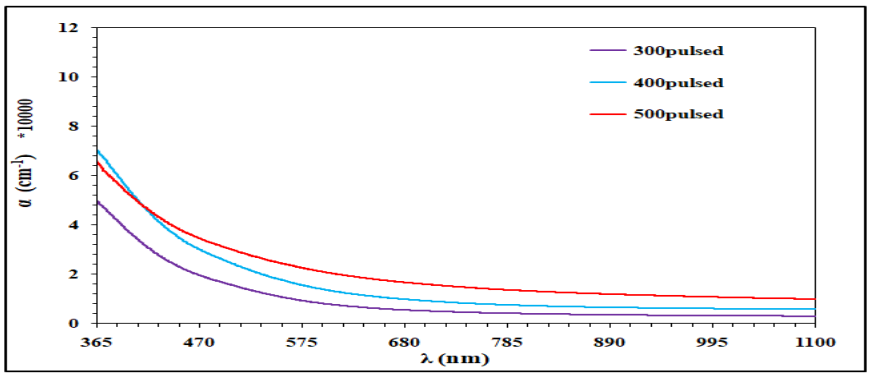

Fig.(2). The absorption coefficient spectrum as a function of wavelength for(Fe2O3)0.85 :(CuO)0.15thin films at different number of laser shots

The Optical Band Gap

The energy gap values are known to be dependent on the crystal structure of the film in general. Crystal regularity also influences the arrangement and distribution of atoms inside the crystal lattice. The optical band gap of a films is calculated using the Tauc model in the area of high absorption using the relationship[12].

$$\alpha h\nu = B(h\nu - Eg)\ r \quad (2)$$

Where hν denotes the photon energy, Eg denotes the optical energy gap, B denotes a constant, and r denotes the kind of electronic transition, with r = 1/2 for direct permitted transitions and r= 2/3 for prohibited transitions.The change of (αhν)2as a function of photon energy for (Fe2O3)0.85 :(CuO)0.15thin films is shown in Fig (3).

The optical energy gap is calculated by extrapolating the portionat (α=0)using the Tauc relation. It is discovered that the relation for r=1/2 produces linear dependency, which accurately represents the permitted direct transition. As seen in the figure, the optical energy gap diminishes as the number of laser pulses increases. This is because the concentrated density of states near the band boundaries increases with thickness, lowering the value of Eg. Additionally, the direct band gap decreases as the number of laser pulses increases.

The results of energy gap value shown in the table(1)

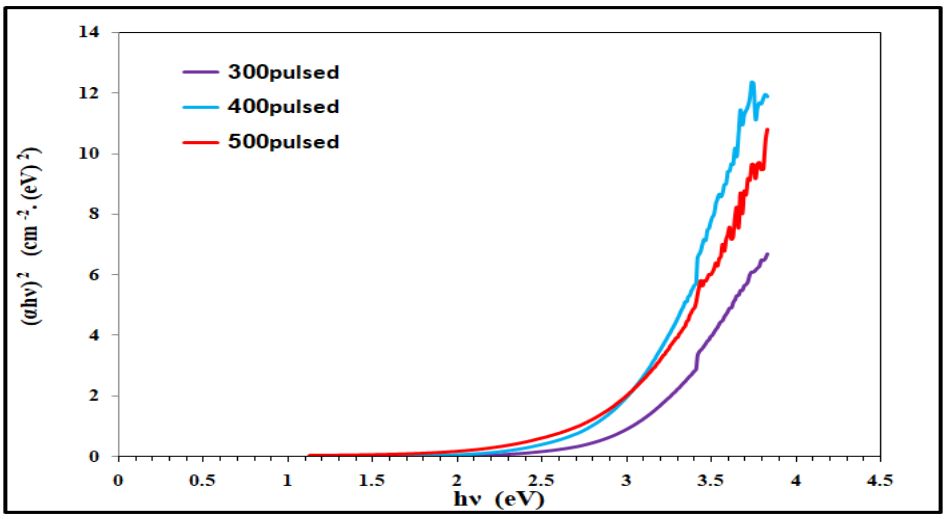

Fig.(3) (α hν)2 as a function of (hν) for (Fe2O3)0.85 :(CuO)0.15 thin films at different number of laser shots

Extinction Coefficient

The extinction coefficient (K) indicates the amount of energy absorbed by a thin film material, which is equivalent to the attenuation of an electromagnetic wave passing through a material. It was determined using the relation [13].

$$K = \frac{\alpha\lambda}{4\pi} \quad (3)$$

Fig.(4) showsthe variation of extinction coefficient as function of wavelength for Fe2O3)0.85 :(CuO)0.15thin films preparedat different number of laser shots (300,400 and 500) .It is seen that the extinction coefficient behaves just like the absorption coefficient (α) because they are

joined by previous relation. It is obvious that the extinction coefficient rises with the number of laser blasts, which is related to the increase in thickness [14]. The table below contains the results of the K value calculation (1).

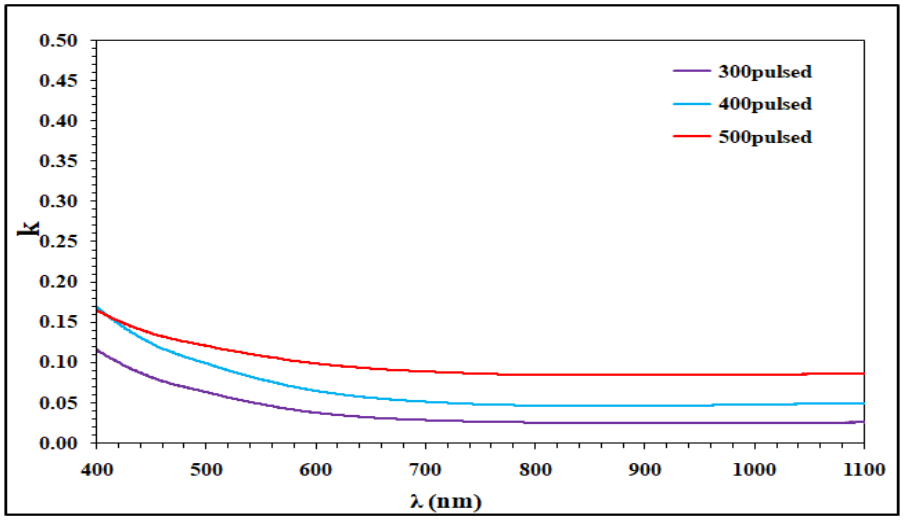

Fig. (4) Extiction coefficient variation as a function of wavelength for (Fe2O3)0.85 :(CuO)0.15thin films at various laser shot counts

The Refractive Index

The thin films' refractive index (n) was calculated using equation [15].

$$n=\frac{\sqrt{1+R}}{\sqrt{1-R}} \qquad (4)$$

As seen in Fig. (5), the number of pulsed lasers has an effect on the refractive indices of the films. As the number of laser shots rises, the refractive index increases, and as the wavelength increases, the refractive index falls, as indicated in the Table (1).This behavior is expected and accompanies the increment of number of laser shots (with narrow energy gap) in (Fe2O3)0.85 :(CuO)0.15thin films according to inverse relation between the refractive index and energy gap.

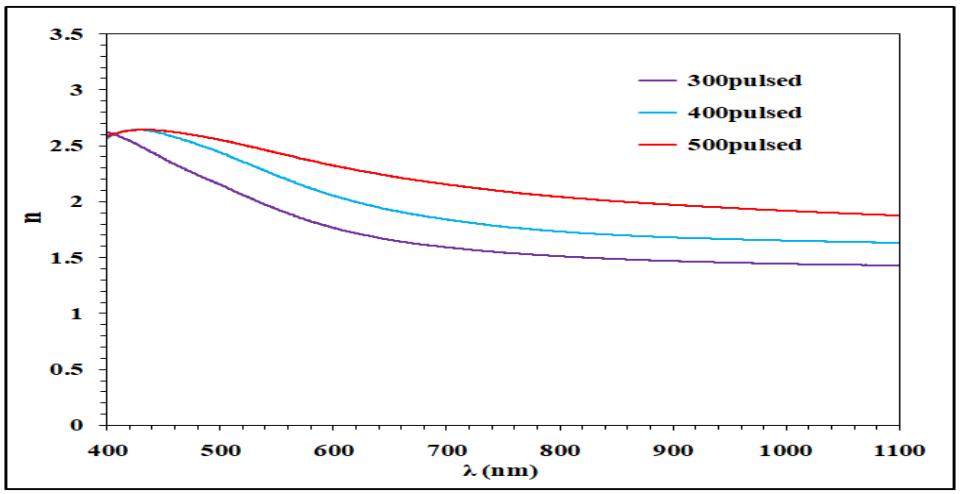

Fig. (5) The refractive index fluctuation as a function of wavelength for thin films of (Fe2O3)0.85 :(CuO)0.15 at various laser shot counts

Dielectric Constants

Calculate the real portion of the dielectric constant using the following equations [16]:

$$\varepsilon_r = n^2 - k^2 \qquad (5)$$

Fig.(6) illustrates the variation of (εr) as a function of wavelength.Because k2 is lower than n2, the behaviour of εr is comparable to that of the refractive index, according to equation (5). The figure clearly shows that (εr) grows as the number of laser shots increases.

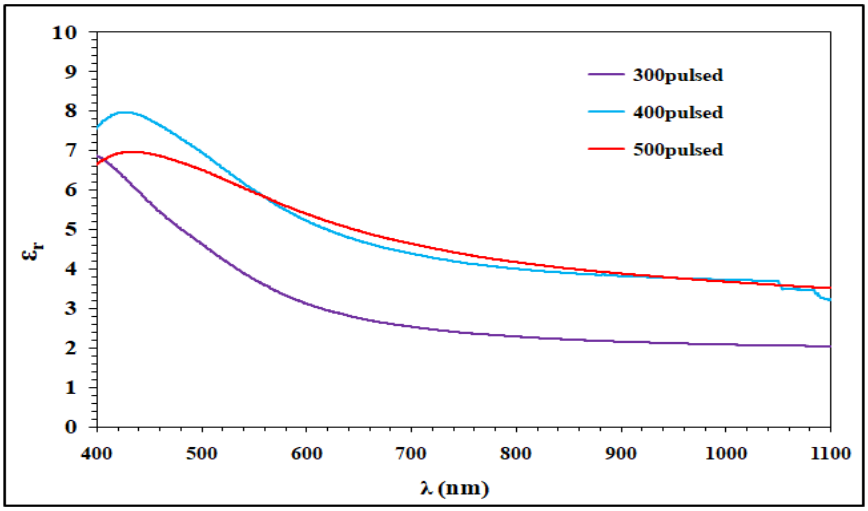

Fig (6).Variation of (εr) as a function of wavelength for thin films of (Fe2O3)0.85 :(CuO)0.15at various laser shots

The imaginary part of dielectric constant can be calculated by using [16]

$$\varepsilon_i = 2nk \qquad (6)$$

The change of (εi) as a function of wavelength is seen in Figure (7). In general, the figure depicts a reduction in (εi) as the wavelength increases.Also, the imaginary part of dielectric constant εi reveals the same behavior of Kwith the variation of number of laser shots as clear from Table (1).It is increses with incresing of number of the laser shots. (εi) can be explained in the same way as n and k.

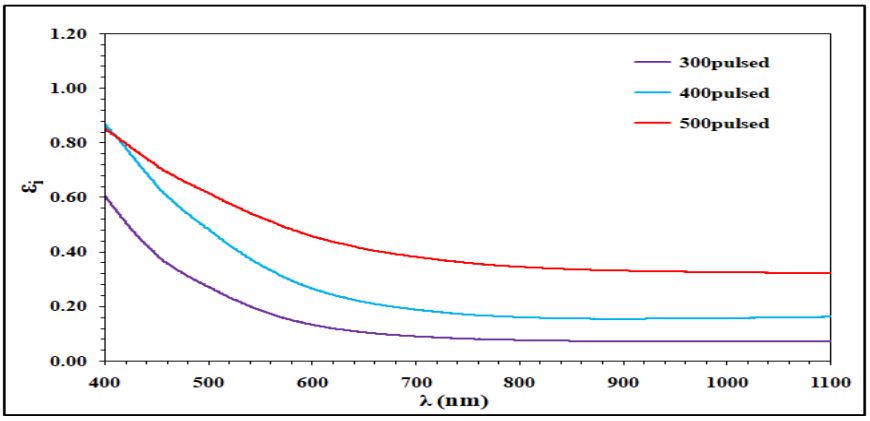

Fig. 10. Variation of (εi) as a function of the wavelength for (Fe2O3)0.85 :(CuO)0.15thin films at various laser shot counts

Finally we can conclude the results the optical parameter and theoptical constants at λ=500 nm for (Fe2O3)0.85 :(CuO)0.15thin films at different number of laser shots İn the Table (1).

Table (1):- Optical properties and Direct allowed band gap (Fe2O3)0.85 :(CuO)0.15thin films at different laser shots and wavelength 500 nm

No. of pulsed	T%	α (cm-1)	K	n	εr	εi	Eg (eV)
300	72.91	15796	0.063	2.153	4.631	0.271	3.10
400	60.97	24737	0.098	2.442	6.951	0.481	3.05
500	54.63	30232	0.120	2.554	6.508	0.615	3.00

Conclusion

On a glass substrate, thin films of (Fe2O3)0.85:(CuO)0.15 were produced using the PLD approach to examine the impact of the number of laser pulses on the optical characteristics of the films.The absorption coefficient spectrum was determined using a UV-Visible spectrometer.The optical studies of (Fe2O3)0.85 :(CuO)0.15 thin films show that the transmission spectrum is in the visible region and its values are higher than 90% with a sharp cut-off wavelength values approximately 500 nm. The absorption coefficient (α) of the thin films is more than 104 cm-1, indicating a high chance of a direct electronic transition.
The forbidden energy gap for permitted direct transition reduced as the laser shot intensity increased. The optical band gap gained from Tauc plot reduces from 3.10 eV for Fe2O3 :CuO prepared at laser shot 300 to 3.0 for films prepared at 500 laser shot . Additionally, optical constants such as the real and imaginary dielectric constants were measured, which revealed that they dropped as the wavelength increased while increasing as the number of laser flashes increased. In the future, (Fe2O3)0.85:(CuO)0.15 may be utilised to significantly increase the efficiency of solar cells or the responsiveness of gas sensor devices.

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EFFICIENT IMAGE ENCRYPTION SCHEMES BASED ON DEVELOPING AES ALGORITHM WITH FUZZY FUNCTION

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Abstract:

This paper suggests three image encryption schemes based on developing ofAdvance Encryption Standard (AES) algorithm with the employment of fuzzy function for diagnoses medical images. In the three proposed image encryption methods, the image is firstly scrambled using the: a) First Algorithm: start with applied AES algorithm then the result modulated with Fuzzy function; .b) Second Algorithm: start with modulated Fuzzy function then the result applied AES algorithm. c) Third Algorithm: star with combine between modulated Fuzzy function with each operations of AES algorithm. The usage of a fuzzy map as a pre-processing scrambling stage along with the AES algorithm gives the advantages of both the noise immunity from the fuzzy map and the security encryption from the AES algorithm. Furthermore, the Triangular Membership Function (TMF) parameters are utilized as additional extra keys that improve the security of the image encryption methods. The analysis results of the security as well as evaluate the efficiency of developed algorithms detect that the cipher text image acquired is the similar as the plaintext image and fuzzy set theory was suitable for apply as round function in the design of other block image ciphers. Moreover, the security properties, demonstrated that our designs were highly secure and robust against possible image cryptographic attacks. Finally, the NIST statistical test for randomness as well as performance comparison between proposed schemes with identical methods ciphers image revealed that the proposed algorithms were quite secure, efficient, and faster than the conventional block ciphers.

Key words: Image encryption, Advance Encryption Standard (AES), Fuzzy set theory, Triangular Membership Function (TMF), Peak Signal-to-Noise Ratio (PSNR), National Institute of Standards and Technology (NIST).

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Introduction:

Normally, 0 and 1 numeric is a degree of membership for an object ,this mean that, 0 the object is not belong to the set,1 means the object is belong to the set and in between means the object is partially in the set. The description of this fact in mathematic denoted as, If U is a universal set of objects, then a fuzzy set A in U is $A = \{(x, \mu_A(x)) | x \in U\}$, where : universe of discourse, and $\mu_A: U \rightarrow [0,1]$. $\mu_A(x)$ is said to be membership where

$$\mu_A(x) = \begin{cases} 1 \; if \; x \in A \\ 0 \; if \; x \notin A \end{cases}$$

The membership functions that characterize the blurry groups and the assembly's used are the foundation of fuzzy sets and fuzzy logical systems [1].
Triangular Membership Function (TMF) as shown in Figure 1, canmake of lines, and realized by the series of linear equations as:

$$M_A(x) = \begin{cases} \frac{x - x_1}{x_2 - x_1} \\ \frac{x_3 - x}{x_3 - x_2} \end{cases} \qquad (1)$$

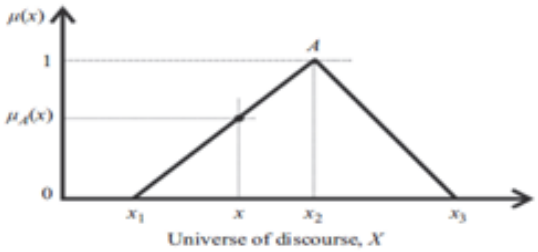


Figure 1. Shows a Triangular fuzzy membership function [2]

Where the parameters x_1, x_2 and $x_3$represent the position of fuzzy membership map A in the X universe. In fact, these parameters represent the function of membership A and show us its location in the opposite universe. It is enough to change parameter values to determine a new membership map of a similar format or to change the location of the speech. This is why the parameter formulas are important for representing membership maps. Equation can be used as a parameterized membership function that represents ambiguous subsets of the triangular type. Equation (1) shows that x_2 is a convergence point and equation (2) can be satisfied as long as x1 ≤ x2 and x ≤ x2.

$$M_A(x) = \left(\frac{x - x_1}{x_2 - x_1}\right) < \left(\frac{x_3 - x}{x_3 - x_2}\right) \qquad (2)$$

$$M_A(x) = \left(\frac{x3 - x}{x3 - x2}\right) < \left(\frac{x - x1}{x2 - x1}\right) \qquad (3)$$

Similarly, equation (3) is satisfied as long as x ≥x2 and x2 ≤ x3. i.e, the output is equal to the smaller part of (2) or (3). Nevertheless, these equations give a negative output when x< x1 or x > x3. Since the membership scores are set at a time interval [0,1], negative outputs must be changed to 0. Consequently, the maximum value must be set between 0 and output from (2) or (3). Accordingly, (1) can be converted to the figure in (4):

$$M_A(x) = \text{Max}\left(\min\left(\frac{x-x1}{x2-x1}\frac{x3-x}{x3-x2}\right)\right) \quad (4)$$

TMF is simple to model and very easy to simulate. The sharp peak cans them to effective to any changes even if they are very small. Thus, sharp peak produces triangle membership functions critical to the changes in the fragile variable x [2].

The National Institute of Standards and Technology (NIST) began the search for an alternative to the Data Encryption Standard (DES) in 1997. The Advanced Encryption Standard (AES) is the new standard, as shown in Figure 2, developed by Joan Damen and Vincent Ragman. Encoding/decrypting data in 128-bit by using clusters 128-bit (10-bit), 192-bit (for 12 rounds) and 256-bit (14-round) key sizes; therefore each round includes stages in to different processing consists by (substitution, conversion, conversion, mixing of ordinary text of income, and conversion) for final output of encoded text. this process can be more secure than DES and 3DES, moreover, (general design, flexibility and availability) worldwide for free, Figure 2. Shows AES algorithm[3].

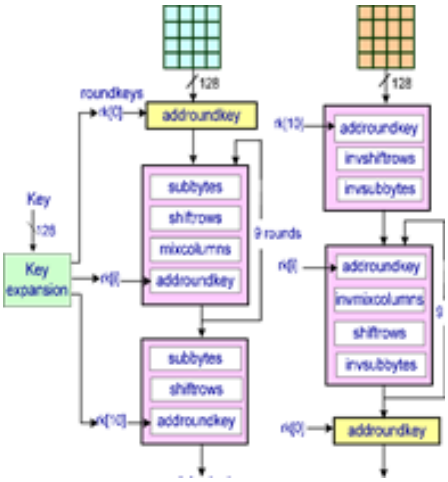


Figure 2. Shows AES algorithm [6]

Image encryption with the continuous development of information technology, ensuring information security has become an important issue. Image is widely used as a multimedia tool; therefore this article proposes a chaotic image encryption algorithm based on developing of AES algorithm with the employment of fuzzy function for diagnoses medical images. The rest of this paper as the following, section 2 summarized some of related work, section 3 provided schemes and models methods with the mathematical basis of proposed algorithms, while section 4 contented some results and discussions for the proposed algorithm, finally, some conclusion was describe in section 5.

2. Related work

Encryption of the image has become an essential way to secure image information with the high frequency of multimedia information exchange on the Internet. Moreover, image encryption based on fuzzy logic sets theory has become a rich research area in the field of computer security and cryptography. In the following, some of the published works in this area are review.

Abdurrahim Toktas, Uğur Erkan & Deniz Ustun, 2021 [4], proposed image encryption in scheme based on Optimal Chaotic Map (OCM) has been derived by multi-objective optimization strategy

through artificial bee colony (ABC) algorithm. First of all, the empirical model for the OCM with four unknown variables, and then, these variables are optimally found out using ABC for minimizing the multi-objective function composed of the information entropy and Lyapunov Exponent (LE) of the OCM.
are optimally found out using ABC for minimizing the multi-objective function composed of the information entropy and Lyapunov Exponent (LE) of the OCM. The OCM can show better chaotic attributes in the evaluation analyses using metrics, such as "bifurcation, 3D phase space, LE, Permutation Entropy (PE) and Sample Entropy (SE)". The encrypting performance of the OCM demonstrated on straightforward IES and verified by various cryptanalyses in many research studies, as well.
Verified by various cryptanalyses that compared with many reported studies, as well.
Noura Khalil, Amany Sarhan Mahmoud A. M. Alshewimy, 2021 [5], suggested an effective chaotic color/grayscale image encryption algorithm. The propose algorithm used hybrid 2D composite chaotic map in order to combined with (sine/cosine) cross-chaotic map. The process of transformation required to scramble the image as a confusion phase. The diffusion phase in 1D has been combined with (Logistic-Tent) chaotic map in order to used to generate a chaotic self-diffusion matrix that is bitwise XORed with the scrambled image to produce final encrypt of image.
a chaotic self-diffusion matrix that is bitwise XORed with the scrambled image to produce the final cipher image. The proposed algorithm combines the merits of both 1D and 2D chaotic maps; it has a simple structure, easy implementation, and excellent chaotic features making its chaotic orbits more unpredictable for introducing more security. The encryption algorithm has a simple structure, easy implementation, and more secure .The proposed algorithm has a high degree of security level, and it can compete with other encryption algorithms.
Xingyuan Wangab, Nana Guana, Jingjing Yanga, 2021,[6] recommend a chaotic image encryption algorithm based on scrambling and diffusion operations. They propose a new one-dimensional chaotic system with better chaotic performance. A novel random block strategy designed based on the new chaotic map. Bit-level confusion method is adopted using random block strategy.
C. Madan Kumar, R. Vidhya & M. Brindha, 2021, [7], They proposed a unique chaotic image encryption on the basis of Enhanced Thorp Shuffle and Zig-zag Scan based Convolution (ETS-ZSC). A one-dimensional chaotic map utilized for both shuffling the plain image and producing the critical grid for the convolution activity. The substitution operation performed in two ways: forward substitution and reverse substitution with zigzag scan. The original seed of the logistic map created from the hyper chaotic system by matching with the plain image to overcome the differential attacks.
Jilei Sun, 2021,[8], proposed an associated color chaotic image encryption algorithm according to a two-dimensional chaotic system and random XOR diffusion. Firstly, the initial value of the 2D-Logistic-Sine-Coupling map (2D-LSCM) generated by the SHA-256 and the keystream generated by the 2D-LSCM. The three channels (red, green, blue) of the color image are processed into a matrix. The keystream used in the color image scrambling phase according to the cyclic shift. Third, do random XOR diffusion according to the mathematical expression of the 2D-LSCM. In this step, nonlinear diffusion is used, and the scrambling is included in the diffusion. Each pixel value of the ciphertext is XORed by three values, which are the key stream, a pixel value of the scrambled image and the position of the pixel value is different from the position of the ciphertext pixel value, and a non-adjacent ciphertext pixel value.

3. Schemes and Models Methods

The mathematical basis of proposed algorithms wasprovide in this section, some of the planners used to structure modern restore block ciphers and modes of procedure. Further, it represents the design of a new efficient and secure block cipher called Fuzzy-AES algorithm.[9] Two major parts are producing by the proposed algorithms: Fuzz set theory and AES algorithm, which is used to implement the encipherment and decipherment image processes, while the keysteam generated depend on hard mathematical operations. The methodology for Fuzzy-AES demonstrated in Figure 3.

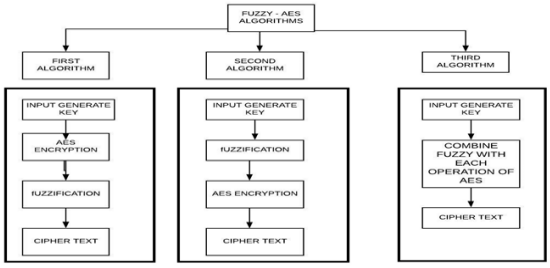
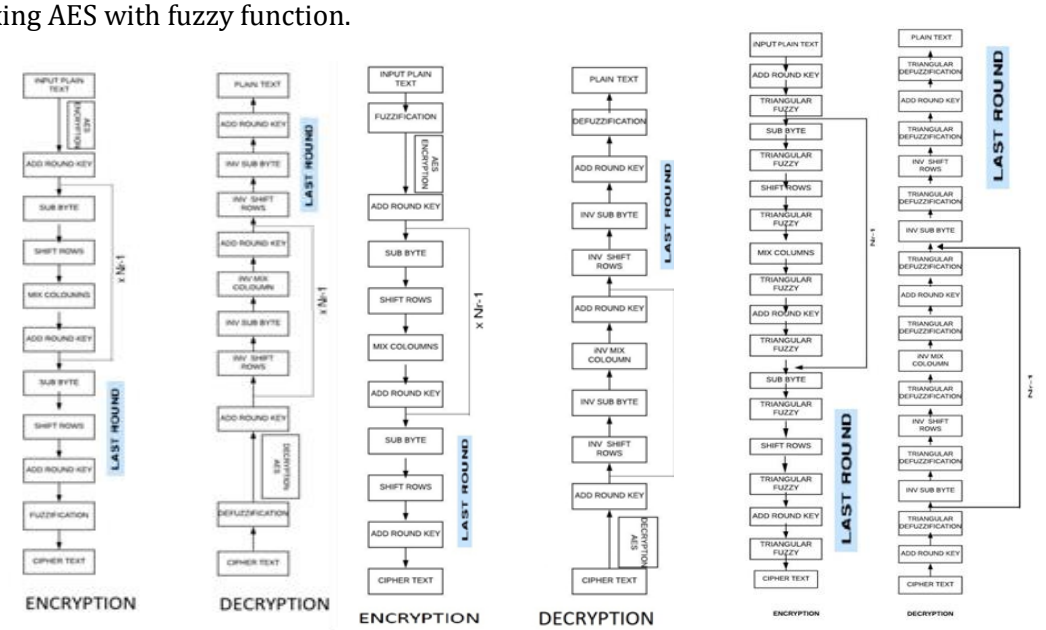


Figure 3. Block diagram of Fuzzy-AES algorithms[9]

In this paper, we applied three encryption algorithmsapproaches mixing AES with fuzzy function, for encryption images.

3.1 The Construction of Proposed Image Encryption Systems

The structure design of the three propose image encryption algorithms, as illustrated in Figure 4 below, show that, it was modern technique based on mixing AES with fuzzy function.



a. 1st algorithm b. 2nd algorithm c. 3rd algorithm

Figure 4. Shows the structure design of three image algorithms.

As demonstrated in Figure1, these algorithms depend on several mathematical steps:

Step 1: Use the Triangular fuzzy membership function
Step 2: Enter the initial parameters values.
Step 3: Apply the Triangular fuzzy membership map with AES algorithm to generate a sequence of new values of x, which are real numbers between 0 and 1.
Step 4: Find the Exponential to generate the extended values exp(x).
Step 5: Find the Floating-Point Representation group of random numbers.
Step 6: Find the Random Keystream based on the random Matrix Table.
Step 7: Utilize the key for the encryption and decryption processes.

3.2 The Test Vectors for Executing Propose System

The design and implementation of the recommended schemes of digital image encryption system was dedicated in this section. In generally, the suggested system encrypted a colored squared digital image utilities the advantage of fuzzy function and AES algorithm properties to make the encipherment more protected and strong against security attacks. In the following subsections, test vectors show the results of implementing propose algorithm for the key generation steps and encryption/decryption processes.

3.3 Key Generation Steps

As shown in Figure 1, the key generation is presenting by the following steps:
1. Enter the parameters of Triangular Fuzzy Membership (TFM) Map to get a good randomness, as shown in Figure 5.

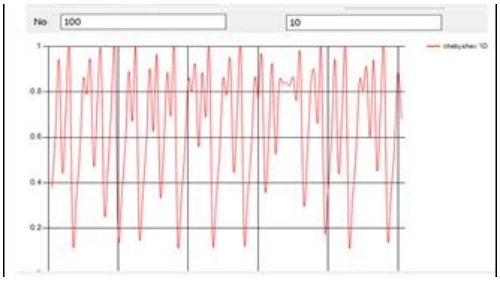


Figure 5. Show the parameter of Triangular Fuzzy Membership (TFM) Map

2. The output of TFM map is passing through the exponential function [exp(x)] to get wide range of input as shown in Figure 6.
3. Then the result of step 2 is mapping into words to fit the required domain of the used multiplexed.

No	X	Exp(x)	Machine Words
0	0.378286738313747	1.4597814581445	401485D0635B18
1	0.604907566120297	1.83108294696228	40152E58A1887490
2	0.942699401149351	2.56690117050383	4018CE3CC928003C
3	0.446447899221967	1.56275126487814	4014CCBA4BB22C18
4	0.71332552526167	2.04076660751219	401BB52BEA7B73
5	0.999042637777578	2.71568069343945	4019045CF566EB24
6	0.143701165915936	1.15453904135163	4015C1C48EEC7F
7	0.332850639597567	1.39493893426892	40148FAD8DF43B30
8	0.5372317106248	1.71126302780316	401502C1880BAE70
9	0.855583954498669	2.35274787721323	4018805434C441AC

Figure 6.Shown the exp(x) and machine words steps.

4. The next stage was passing the dictionary words into the multiplexer in order to produce the secret keystream. multiplexer stage works to find the random keystream based on the random Matrix Table , as shown in Figure 7 , generation such keystream represented as a main goal of the propose algorithm such that the input machine words to get the random keystream.

	A	B	C	D	E	F
0	1093	1073	1047	1126	1027	844
1	1096	1021	1138	1130	1115	1069
2	1144	827	1108	887	1132	1064
3	1006	993	1140	1111	1031	667
4	1089	1019	1135	1131	1113	1067
5	1145	879	1074	1128	1143	1070
6	1081	962	1041	944	1112	1141
7	1084	942	1050	1133	905	1142
8	1085	826	1063	1129	1028	866
9	1094	1020	1139	1134	1117	1068

Figure 7 Shown the Random Matrix Table.

5. The cipher image after applied encryption process shown in Figure 8

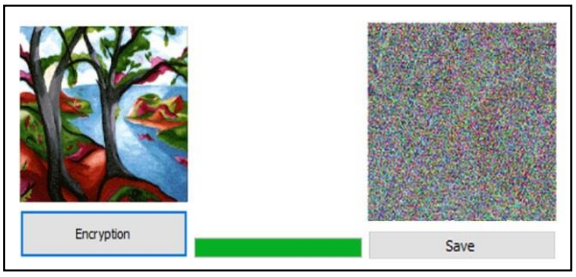


Figure 8. Shown the cipher image after encryption.

3.4 Decryption Process

For decryption process, as shown in Figure 9, the receiver employs the output of stream generator is xor'ed sequentially item to item with cipher image to produce the plain image.

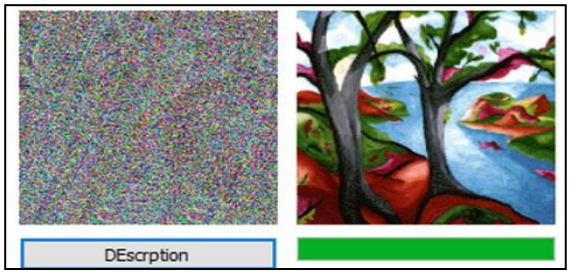


Figure 9. Shown the plain image after decryption

4. Results and Discussions

Some results as well as security analysis of the proposed image encryption algorithms was discussion in this section. In general, for test a good image encryption schemes is that the cipher image should be completely different from its plain form There are many measurements for encrypted images such as PSNR, MSE,error calculation, NIST and Histogram Analysis to measure the efficient of suggested schemes. The following subsections summarized these measurements.

4.1 Peak Signal-to-Noise Ratio (PSNR)Analysis

The mathematical definition of PSNR is: [10]

$$PSNR = 2log_{10}(255/\sqrt{MSE}) \quad (5)$$

$$MSE = \frac{1}{M \times N}\sum_{i=1}^{m}\sum_{j=1}^{n}(P_0(i,j) - (P_1(i,j))^2 \quad (6)$$

Where M and N represented the width and the height of the test image, respectively.
$P_0(i,j)$ and $P_1(i,j)$ represented the pixel values of the plain image and cipherimage, respectively.
MSE represented the mean squared error between the plain image and the cipher image.
For better encryption security, the value of MSE should be large and PSNRshould be small value.

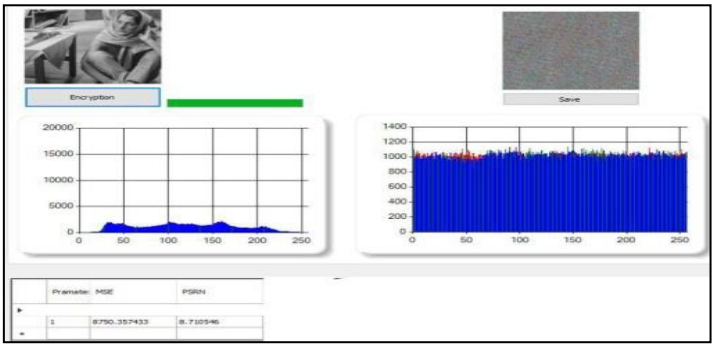


Figure 10 The Histogram , MSE and PSNR for the Plain Image and Cipher Image.

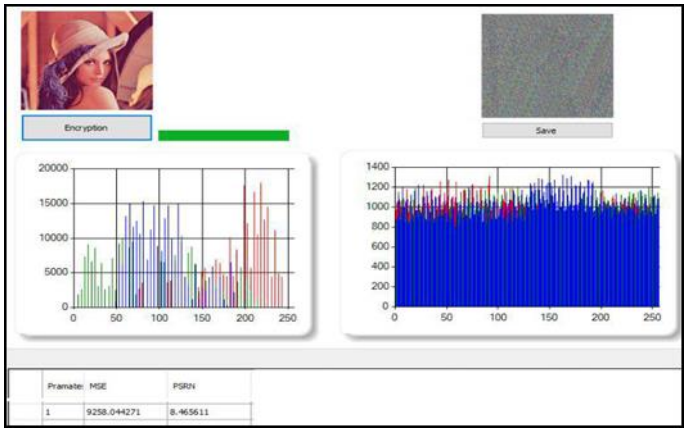


Figure 11. The Histogram , MSE and PSNR for the Plain image

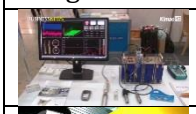
The follwoing table illiestled the MSE and PSNR value for different plain images

Table 1. Shows MES and PSNR value

Image (256x256)	MSE	PSNR
Cameramen	1.1876x10^4	7.4729
House	8.3572x10^3	8.8275
Lena	8.9389x10^3	8.6487

The follwoing table illiestled the error calculation

Table 2. shows error calculation

Original image	Reconstructed image	Error value	Match value
		0.124102	99.863542
		0.131604	99.857081
		0.141047	99.840957

To validate the performance of the proposed schemes. The comparison simulation results obtained with other recent approaches was give, in terms of correlation coefficients, NPCR, UACI and entropy as shown in table 3.

Table 3. Performance comparisonbetween proposed schemes with identical methods

image Lena256×256	Parameter	Ref [13]	Ref [14]	Ref [15]	Our Method
	Correlation vertical	0.0019	0.0019	0.0011	0.00083
	Correlation horizontal	-0.0230	0.0230	0.0018	-0.0135
	Correlation diagonal	-0.0034	0.0011	-0.0012	0.0078
	Entropy	7.9974	7.9975	7.9994	7.9990
	UACI	33.5100	33.5851	33.4365	33.5510
	NPCR	99.6200	99.5193	99.6166	99.9100

4.2 NIST Statistical Suite Tests

Furthermore, the keystream ,which has been used in all image encryption algorithms pass all statistical tests in NIST package [11],[12]for randomness as shown in Table 4.

Table 4. NIST Statistical Suite Tests for the Keystream.

Statistical Tests	P- Values	Result
Frequency	0.501233	success
Block Frequency (128)	0.516010	success
Runs	0.534119	success
Long Runs of Ones (10000)	1.000000	success
Rank	0.000000	success
Spectral DFT	0.371050	success
Non-Overlapping Templates	1.000000	success
Overlapping Templates (9)	1.000000	success
Universal (7)	0.610548	success

Linear Complexity (500)	1.000000	success
Serial 1 (16)	0.488669	success
Serial 2 (16)	0.487541	success
Approximate Entropy (10)	1.000000	success
Cumulative – sums Fwd	0.538015	success
Cumulative – sums Rev	0.527721	success
Random Excursions	0.931073	success
Random Excursions Variant	0.960143	success

4.3 Histogram Analysis

The histogram analysis test is the excellent tools for evaluating good cipher image. In this test, the encipher image should be hide the redundancy of the original image and seem as uniformed distribution. Figure12, summarized the difference between the plain images and their encrypted images when applied the suggested schemes.

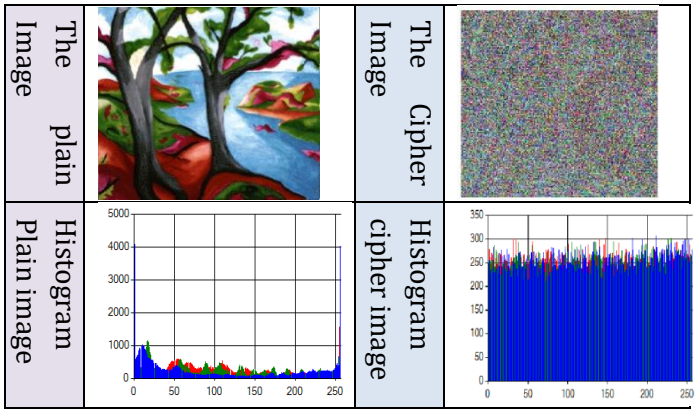


Figure 12. The Histogram for the Plain Image and Cipher Image

Conclusion

The suggestion of an efficient image encryption schemes based on developing AES algorithm with fuzzy set function was propose in this paper. We initiate that, fuzzy logic map is an emerging rule of secure sharing as well asthe usage of a fuzzy map as a pre-processing scrambling stage along with the AES algorithm gives the advantages of both the noise immunity from the fuzzy map and the security encryption from the AES algorithm. Thesuggested schemes completely focused on secure participation applying fuzzy logic that planned based on share generation and reconstruction. These suggested schemes increase the level of security when fuzzy logic utilized.Moreover, the Triangular Membership Function (TMF) parameters are employee as additional extra keys that improve the security of the image encryption methods. Because fuzzy logic has not exact value (approximately between zero and one) therefore, confidential data can be transfer in secure way and make the interrupted of an unauthorized person is very difficult. These methods improves the performance, gets reconstructed image with very less noise ,highly secure and robust against possible image cryptographic attacks .Thus, Fuzzy logic mixed with secret sharing concept is a good way to enhance of image encryption for secret data transfer.

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SUSTAINABLE ADAPTATION FOR CONTEMPORARY ARCHITECTURE BUILDINGS

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Abstract:

The concept of sustainable adaptation is used as a means of dealing with aging buildings and the possibility of exploiting existing vacant buildings and reusing them through possible strategies to deal with aging buildings. Hence the general problem of research in (the complete lack of knowledge of the impact of sustainable adaptation in contemporary architecture buildings), and a research problem represented by (the lack of knowledge about a method that demonstrates the impact of sustainable adaptation mechanisms on the building to reach sustainable adaptive buildings in contemporary architecture), in order to reach the aim of the research in (building a theoretical framework on the concepts of sustainable adaptation in the obsolete building in contemporary architecture and the mechanisms for achieving it to reach a sustainable architecture using the perspective of energy efficiency and natural resources consumption), where the research assumed that (the possibility of achieving sustainable adaptation by achieving sustainability and continuity in the life of the building, and the sustainability is enhanced through the application of sustainable adaptation mechanisms for obsolete buildings in contemporary architecture), that the research design plan is using a selected sample of examples and the use of comparative analysis between them in the application Mechanisms of sustainable adaptation to reach a measurement ruler to test the research hypothesis and reach the final results and conclusions.

Key words: Sustainable Adaptation, Obsolescence, Existing Building, Adaptation Mechanisms, Contemporary Architecture.

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التكيف المستدام لمباني العمارة المعاصرة

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الملخص:

يتم استخدام مفهوم التكيف المستدام كوسيلة للتعامل مع تقادم المباني وإمكانية استغلال المباني القائمة الشاغرة وإعادة استخدامها من خلال الاستراتيجيات الممكنة للتعامل مع المباني المتقادمة. من هنا ظهرت المشكلة العامة للبحث في (النقص المعرفي الكامل لتأثير التكيف المستدام في مباني العمارة المعاصرة)، وبمشكلة بحثية متمثلة بـ (النقص المعرفي حول منهج يوضح تأثير آليات التكيف المستدام على المبنى للوصول إلى مبانٍ متكيفة مستدامة في العمارة المعاصرة)، من أجل الوصول إلى هدف البحث في (بناء إطار نظري حول مفاهيم التكيف المستدام في المبنى المتقادم في العمارة المعاصرة وآليات تحقيقه للوصول إلى عمارة مستدامة باستخدام منظور كفاءة استهلاك الطاقة والموارد الطبيعية)، حيث افترض البحث بأن (إمكانية تحقيق التكيف المستدام من خلال تحقيق الديمومة والاستمرارية في حياة المبنى ويتم تعزيز الديمومة من خلال تطبيق آليات التكيف المستدام للمباني المتقادمة في العمارة المعاصرة)، وذلك بأن تكون خطة تصميم البحث باستخدام عينة منتخبة من الأمثلة واستخدام التحليل المقارن بينها في تطبيق آليات التكيف المستدام للوصول إلى مسطرة قياس لاختبار فرضية البحث والوصول إلى النتائج والاستنتاجات النهائية

الكلمات المفتاحية: التكيف المستدام، التقادم، المبنى القائم، آليات التكيف، العمارة المعاصرة.

مقدمة:

منذ اوائل العقد الأول من القرن الحادي والعشرين كانت هناك استجابة واضحة لأهمية الاستدامة داخل البيئة المبنية واهمية الطاقة الكامنة داخل المباني القائمة، وبالتالي يؤثر عمر وجودة المخزون من المباني في منطقة ما على مقدار التكيف الذي يتم اجراؤه، وعليه سيتم توضيح مفهوم التكيف في المباني القائمة المكونة من منظومات متعددة.

مفهوم التكيف في المباني القائمة:

يرتبط مفهوم التكيف في المبنى القائم بـ (تغيير الاستخدام الوظيفي مع ثبات منظومة الهيكل الانشائي ونسيج المبنى بشكل أساسي وتغير المنظومات الأخرى بدرجات ونسب متفاوتة.
ويأتي مفهوم التكيف أحيانا ضمن مفاهيم (التجديد وإعادة الاستخدام التكيفي وإعادة التأهيل والتحويل والتعديل التحديثي والترميم)، فالتكيف في المبنى القائم (هو أي عمل لمبنى فوق مستوى الصيانة لتغيير قدرته أو وظيفته أو أداءه) بمعنى (أي تدخل لضبط أو تطوير مبنى ليتناسب مع متطلبات المالك المتغيرة) (Wilkinson , Remoy & Langston , 2014 , p.4).

التكيف المستدام sustainable adaptation:

هو إمكانية المبنى على تقليل التأثير البيئي من خلال تكيف المباني بدلا من الهدم والبناء الجديد، حيث تلعب صناعة المباني دورا رئيسيا للحد من الآثار السلبية على البيئة.
فالاستدامة sustainability: هي تلبية احتياجات الحاضر دون المساس بقدرة الأجيال القادمة على تلبية احتياجاتهم الخاصة (WCED , 1987).
والمبنى المستدام sustainable building: هو خلق بيئة مبنية صحية باستخدام مبادئ فعالة من حيث الموارد وقائمة على البيئة (Kibert , 2005 , p. 21)، حيث اعتمد (Hill & Bowen , 1997, p. 237-239) اربعة مبادئ لمفهوم المبنى المستدام وهي: الاستدامة الاجتماعية، الاستدامة الاقتصادية، الاستدامة التقنية والاستدامة الفيزيائية الحيوية، حيث يوفر مفهوم المبنى المستدام الإطار العملي لتوجيه تنفيذ المباني.

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أما العمارة المعاصرة تعرف (بأنها التطور التكنلوجي السريع للعمارة في الحالة الراهنة واستخدام أشكال معقدة ومواد بنائية جديدة وتأثيرات نحتية عالية ومفهوم عدم التوازن واللاثبات وكل ذلك ادى إلى توضيح ملامح العمارة المعاصرة) (شيرزاد، 2002، ص 174- 175).

والعمارة المعاصرة كما وضحها (د. فاروق حيدر) في موسوعته (موسوعة العمارة الحديثة والمعاصرة وروادها (حيدر، 2014، ص 1493- 1494) هي: حركة ما بعد الحداثة التي بدأت تنميتها خلال الخمسينات بالاتجاهات التجريبية من خلال عمارة الحداثة المتأخرة واستمرت كمصدر الهام الهندسة المعمارية المعاصرة اليوم بأفكار جديدة وتحت مسميات كثيرة منها: (العمارة التفكيكية، العمارة البيولوجية، العمارة الجينية، عمارة النانو) وحركات هذه الأنواع من العمارة ماهي إلا احد حركات عمارة الأشكال غير المعتادة المؤلفة من نظام البناء الايكولوجي للشكل الحيوي من أجل النمو العضوي للمباني والمدن.

لذا فالعمارة المعاصرة هي امتزاج المفاهيم المعاصرة بالمنتج المعماري ومن هذه المفاهيم:

مفاهيم العولمة وأثرها في العمارة من خلال المشاركة في التصميم و الخلفية الثقافية للمصمم

مفاهيم التنمية المستدامة

مفاهيم التقنية العالية حيث اصبح التوجه الأكثر نحوها من خلال استخدام منظومة الإدارة في المباني (.B.M.S)، كذلك تعد العمارة جزء من منظومة متكاملة للتفاعل مع الثقافات والحضارات ولا تنفصل عنها فبالإضافة إلى وظيفتها الأساسية فأنها تعكس الهوية والدين.

ونظراً لأن العمارة هي أم الفنون فهي تتأثر بنقل الخيال من الفن والفكر كما تتأثر بمهارة الانسان وابداعه في ظل التكنولوجيا الحديثة والتطور الاجتماعي والاقتصادي والثقافي والسياسي على مر السنين، حيث يرى البعض ان العمارة الحديثة هي ناطحات السحاب المنشأة بالحديد والزجاج أو المفاهيم الجديدة للتجمعات الفراغية، واعتبرت العمارة الحديثة هي العمارة الناتجة من افكار بنظريات جديدة وبتكنلوجيا متطورة ومواد تخدم الانسان، كما تشمل الشكل الذي يتبع الوظيفة التي لها اصول عصرية مع اهتمامها ببساطة الشكل والتجريد الهندسي المرتبط بالعمارة المتحررة من الزخرفة مع اعتبار المنازل آلات للمعيشة.

اما عمارة ما بعد الحداثة التي توصف أحيانا بالعمارة المعاصرة فقد ارتبط تفاعلها بالمجتمع لربط علاقة الانسان بمحيطه من التغيرات البيئية التي بنيت بصفة أساسية على استراتيجيات التنمية المستدامة والعمارة الخضراء، ولذلك وجد عصر.الاليكترونات جيلا معماري يدين بالولاء لبرامج الحاسب الآلي الحديثة منذ بداية القرن الحادي والعشرين والتي اصبحت أساس اسلوب ثورة المعلومات للمباني والإنتاج المعماري كأداة تصميمية ناتجة من خلط ودمج المعلومات الإلكترونية لمعرفة أحاسيس الجهاز العصبي للانسان، ومع ذلك فالعديد من المعماريين يعتقدون ان التقدم الرهيب في التكنولوجيا قد يعد سلاحاً لإنتاج قيم أفضل في الحياة لكل البشر لذلك تطورت نظريات ما بعد الحداثة بأشكالها العديدة لتناسب هذا العصر (حيدر، 2014، ص 1492-1494).

لذا فإن مباني العمارة المعاصرة تحتاج في تصميمها وتنفيذها ان تصمم وتنفذ بشكل تكنولوجي مستدام من خلال تصنيف المبنى إلى منظومات وهذه المنظومات يمكن من خلالها تحقيق التكيف المستدام للمبنى كاملا، وهذه المنظومات هي: (منظومة الغلاف الخارجي، منظومة الهيكل الانشائي، منظومة الخدمات)، وإذا حصل تقادما في اي منظومة من هذه المنظومات يمكن بسهولة ومرونة أحداث التغيير أو التعديل فيها دون ان يؤثر ذلك في منظومات المبنى الأخرى اذا كانت كفؤة في أداء عملها، مما يحقق التكيف المستدام في المباني القائمة دون الحاجة إلى الهدم الكلي وكذلك تحقيق أقل استهلاك للطاقة وتقليل نسب انبعاثات الكربون من المباني القائمة.

يعد التكيف مستداما بطبيعته لأنه ينطوي على استخدام أقل للمواد واستهلاك أقل للطاقة وتلوث أقل أثناء البناء , Johnstone) (Bullen , 2007, p.30)(1995,p.22، حيث تعتبر الطاقة المخزونة كبيرة في المباني، فالتكيف يوفر ما يقارب 95% من الطاقة المخزونة (Binder , 2003)، حتى لو كانت تكاليف التكيف الاقتصادية عالية، كون ان عملية الهدم تعد مهدرة للمواد مالم يتم إعادة استخدامها أو إعادة تدويرها (Ball , 2012 , p. 111).

ويعد اهم جانب لتحسين استدامة البيئة المبنية هو تكيف المباني القائمة من خلال إعادة تدويرها .p , 2011 , Cooper) (161، حيث ان مستخدمي المباني يبدؤون بتكيف مبانيهم منذ بداية بناءها وبمرور الوقت تبدأ فائدة أي مبنى تقل لوظيفته الأصلية، حيث تعرف هذه العملية بالتقادم وتمثل نقصا في المنفعة، وسيتم لاحقا توضيح معنى التقادم في المبنى.

دوافع تكيف المباني القائمة:

كان الهدم هو مصير المباني الفائضة عن الحاجة أو المتهرئة في المناطق الحضرية حتى أواخر السبعينات، وبعد الحرب العالمية الثانية كان هناك نموا حضريا سريعا، وبالإضافة لذلك ادى امتلاك السيارة إلى الحاجة إلى إعادة تخطيط المخططات العامة للمدن

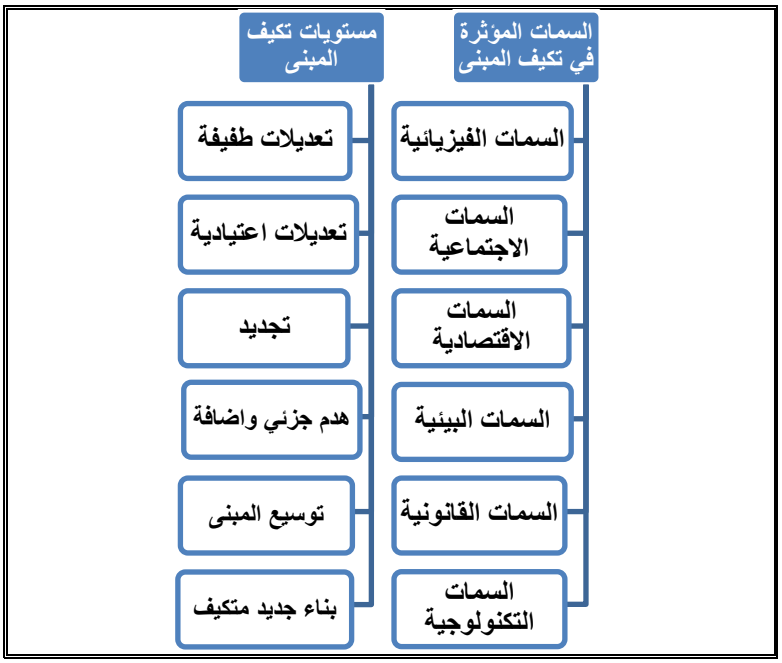
الحالية لاستيعاب نظام الطرق المتوسع، حيث ان الاسباب الآتية مجتمعة تجعل عملية تكيف المبنى وإعادة التأهيل ضمن إطار التطوير المستدام، والتي تتوضح بالآتي (Brooker & Stone, 2005 , p.73-74):

الزمن: عادةً ما يكون إعادة تأهيل العمل القائم أسرع من البناء الجديد.
الأداء: إن الحاجة إلى تحسين الأداء الصوتي أو الحراري أو الهيكلي للمبنى القائم غالباً ما يكون سبباً أو رد فعل للقيام بأعمال التكيف، فالاستهلاك المفرط للطاقة في المبنى قد يدفع في الكثير من الأحيان إلى تغييرات مثل تجديد نظام التدفئة وتحسين الكفاءة الحرارية للمحيط.
تغيير الاستخدام: عندما يكون المبنى فارغاً لفترة طويلة فقد لا يكون استخدامه السابق مطلوباً، لذا قد تكون هناك حاجة لإعادة الاستخدام التكيفية لضمان استمرار وجود فائدة لتشغيل المبنى القائم.
الحفاظ: يمكن للأسباب الثقافية وكذلك التقنية ان تؤثر وبشكل متكرر على قرار التكيف لأي مبنى قائم بدلاً من البناء الجديد وقد تكون الأهمية التاريخية أو المعمارية للمبنى سبباً كافياً للحفاظ عليه وإعادة تكيفه.
الاستدامة: إعادة الاستخدام أو إعادة النهوض بالمباني القديمة هي أكثر ملائمة للبيئة من البناء الجديد، حيث ان البناء الجديد ينطوي على إضافة مباني بفعآليات جديدة ويتوسع إلى استهلاك بالطاقة والانبعاثات الكربونية أكثر من التكيف. (Graves, Phillipson ,2000, p. 46)

مستويات التكيف في المبنى القائم:

يتخذ تكيف المبنى القائم ثلاثة أشكال رئيسة هي: - التغييرات في الوظيفة - التغييرات في الحجم، - التغييرات في الأداء، ويعد كل مستوى من مستويات التغيير سمة أساسية للقدرة على التكيف، حيث انها (القدرة على التغيير الطفيف أو الكبير في المبنى)، وهذه المستويات هي (Kincaid , 2012 , p. 25) ، (Doghlas , 2006 , p.11):

المستوى الأول: هو تغيير الاستخدام مع الحد الادنى من التدخل بسبب المرونة الموجودة في المبنى.
المستوى الثاني: المبنى مخصص للتكيف مع تغيير بسيط، أي قابلية المبنى على التحويل (تقنيا – وظيفيا).
المستوى الثالث: المبنى يحتاج درجة أعلى من التدخل ويشار اليه عادة بإسم الترميم (التجديد) أو التعديل التحديثي.
المستوى الرابع: يتضمن المبنى عمليات هدم محددة، أي قابلية المبنى على التفكيك بأمان وكفاءة لكامله أو لجزء منه.
المستوى الخامس: يتضمن امتداد (توسيع) للمبنى، أي قابلية المبنى على زيادة الحجم أو السعة أو إمكانية تزحيف صغير في مخطط الفضاء لإعادة تكوين المخطط العام وجعله أكثر فعالية.
المستوى السادس: هو الهدم وإعادة التطوير ويتم اختياره عندما تكون الظروف الاجتماعية والاقتصادية والبيئية والتنظيمية والمادية للمبنى في نهاية دورة حياته، وبمنفعة قليلة أو يفتقر للمنفعة، وذلك بتفصيل المواد والمكونات من أي مبنى مهدم والتي تكون قابلة لإعادة المعالجة والتدوير. (Bottom ,1999, p.348)
ويوضح دافي (Duffy, 1993, p.28) ان الاهم هو الاخذ بالاعتبار المباني التي تكون في (مستوى التغيير) بمرور الزمن، وهذا يتطلب نظرة أكثر شمولية للمباني لوضعها في سياقاتها التاريخية والمادية والزمنية، فالقدرة على التكيف هي العملية التي يتم بها صناعة المبنى للاستجابة لتلك التغييرات.
يمثل الشكل (1) تمثيلا تخطيطيا ثابتا يحلل البيانات ويبين مدى تأثير كل سمة من السمات المؤثرة على تكيف المبنى بمستوياته المختلفة، على سبيل المثال، يمكن ان تشمل (المستوى الاول – التعديلات الطفيفة) تجهيز الطوابق في مبنى مكتبي مثلا، و(المستوى الثاني – التعديلات الاعتيادية) يمكن ان تشمل الامتداد العمودي لمبنى مكتبي حالي من خلال طابق اضافي، تم تحليل التعديلات السابقة لتحديد اهم السمات.
الشكل (1) نموذج تخطيطي يوضح اهم السمات المؤثرة على تكيف المبنى ومستويات التكيف المعتمدة، المصدر: الباحثة عن (Reomy,2016,p.46)



وتعتمد دورة التكيف والصيانة للمبنى على عدة عوامل:

- الغرض أو وظيفة المبنى
- جودة المبنى (حالته واهميته المعمارية)
- جودة استخدام المبنى أو اساءة استخدامه
- المتطلبات القانونية خصوصا متطلبات الصحة والسلامة
- متطلبات المستخدمين / المالكين

وحسب ما اشار (Duffy , 1993 , p.35) إلى ان دورة الصيانة والتكيف في المبنى تختلف حسب وظيفة المبنى، لذا لا يمكن إطالة عمر المبنى إلا من خلال التكيف وهذا ما يجعل البيئة المبنية أكثر استدامة.

مميزات الحاجة إلى التكيف في المبنى القائم:

هناك عدة مميزات للحاجة إلى التكيف: (Douglas ,2006 , p. 155)

مميزات تقنية: يمكن استخدام الهيكل القائم والنسيج المحيط بالمبنى القائم لتوفير إحاطة للمكان وتقديم الحماية للعمل وتخزين المواد وبذا يحتاج المبنى فقط إلى تعديله لتلبية أعمال التكيف المقترحة دون الحاجة إلى شراء وتثبيت مكونات جديدة.

مميزات مكانية: يمكن احتساب خصائص الفضاءات مكانياً بشكل أفضل من خلال تكيفها، حيث انه يمكن لمالك المبنى الاستفادة الكاملة من الموقع القائم على خلاف المبنى الجديد كون انه قد يتعين إعادة تعيين المبنى إلى خط بناء آخر لتلبية مقترحات وقوانين الطرق والبناء الخاصة بالسلطات المحلية. (Scottish civic Trust , 1981)

مميزات بيئية: إن المظهر الجيد والمحسّن يمكن ان يتحقق للمبنى المتكيف اذا تم تصميم المبنى وتنفيذه بعناية ودقة حيث يبدو المبنى أفضل من ذي قبل، ولهذا سيكون تأثيره تأثيراً ايجابياً على الخصائص المحيطة، علاوة على ذلك فأن المبنى المتكيف يجب أن يكون أكثر كفاءة من ذي قبل وخاصة عندما تكون الاستدامة معياراً رئيسياً.

ونظراً للقدرة الحرارية العالية للمباني التقليدية وكذلك استجابتها الحرارية البطيئة فأنها تتمتع بفعالية جيدة في الحفاظ على الطاقة حيث تميل المباني التقليدية إلى ان تكون بنوافذ صغيرة واضاءة وتهوية طبيعيتين مما يؤدي إلى الاقتصاد في استهلاك الطاقة.(UNEP,2001 , Climate Change 2001)

لذا فالتكيف يعد معياراً مهماً للاستدامة وذلك لأنه يقلل من استهلاك الطاقة ويقلل من توليد النفايات ومن الحاجة إلى استخدام مواد جديدة ومن الطاقة اللازمة لإنتاجها ونقلها.

مميزات اجتماعية: من الأفضل الحفاظ على طابع المناطق الاجتماعي والطرق وخصوصيتها وذلك من خلال تكيف مبانيها حيث توفر المباني القديمة راحة نفسية بسبب خصائصها المميزة. (McGregor & Then , 1999)، وهناك العديد من المزايا المعمارية والثقافية والتأريخية لتكيف المباني. لذا فأن الحفاظ على المبنى يزيد من أهميته في العالم المتقدم.

كيفية اتخاذ القرار لتكيف المبنى القائم:(Douglas ,2006 , p.39) .

هناك عدة اعتبارات يجب اخذها بعين الاعتبار عند اتخاذ القرار من أجل التكيف وهي:

المتطلبات العامة: يمكن للاستشاريين المهنيين كالمعماريين والمساحين ان يقدموا مساهمة كبيرة في عملية صنع القرار للتكيف، فمن المهم ان يدركوا احتياجات المستخدم في جميع الأوقات. ولأغلب المستخدمين ومالكي المباني فأن المتطلبات الرئيسية الآتية تكون ضرورية لتكيف المبنى وتحقيق الاستدامة وهي:

- يجب ان يكون عمر المبنى طويلاً (ان يكون متيناً) مقاوما للتآكل.
- أن يكون للمبنى قابلية ملائمة وتوسع (ان يكون قابلا للتكيف) لاستيعاب التغييرات المستقبلية.
- يجب ان يكون باستهلاك منخفض للطاقة (ان يكون كفوءا حرارياً) بتكاليف تشغيل قليلة.
- ان يكون مقاوما للرياح وللماء (ان يكون مانعا لتسرب الماء)
- يجب ان يوفر بيئة داخلية آمنة وصحية (ان يكون مريحا)

المتانة: بالنسبة للمباني السكنية فأن العمر الافتراضي لا يقل عن 30 سنة ويتوقع ان يتبعها عملية تكيف، اما بالنسبة للمباني غير السكنية حيث تكون الاستجابة لتأثيرات السوق كبيرة فأن معدل التكيف أو القابلية للتغيير تكون عادةً أعلى بكثير.

القدرة على التكيف: من الناحية المثالية، يجب ان يكون المبنى قادراً على استيعاب التغيير في المستقبل للسماح بالتعديل اوالتغيير للاستخدام المختلف.

نظرا لتغيير الطلب على مساحة المبنى يجب ان تكون الخصائص قادرة على الاستجابة لهذه التغييرات لتفادي التكرار.

كفاءة الطاقة: منذ ازمة النفط في اوائل سبعينات القرن العشرين التفت العالم إلى ضرورة الحفاظ على الوقود غير المتجدد، وان احدى الطرق الرئيسة للقيام بذلك هي تحسين نظام العزل الحراري والتدفئة ضمن المبنى، هذا إلى جانب تدابير الاستدامة الأخرى. (Graves, Phillipson ,2000, p. 76)

مقاومة الظروف المناخية: ان احد عوامل النجاح لأي مبنى تم تشييده حديثاً هو صموده أمام متغيرات المناخ وخاصة مياه الامطار وما يترتب عليها من تسرب للمياه إلى داخل المبنى أو تعاني من انابيب خدمتها، حيث ان هذا التسرب للماء لا يتسبب في تدمير المبنى والانهاءات والخدمات فحسب بل قد يعطل استخدام المبنى وعادةً ما يعزى التسريب في النسيج إلى الحشوات الرديئة التركيب أو إلى المواد المقاومة للرطوبة. ((Endean ,1995 ,p. 33 ، كذلك الدعامات والفتحات في اجزاء المبنى تكون أكثر عرضة لتسلل المياه، كل هذه المواقع قد تزيد من خطر تغلغل الرطوبة من خلال مياه الأمطار.

ويمكن ان يحدث ضغطاً أو انفجاراً في أنابيب المياه نتيجة لمفأصل التثبيت الرديئة والى الصمامات التالفة التي تصيب الأنابيب، مما يؤدي إلى زيادة حجم المياه أو كميتها ويتم تصريفها بالضغط العالي على الأنابيب ويؤدي إلى تلفها اذا لم يتم اكتشافها قبل فترة من الوقت.

الراحة: وتمثل الهدف الرئيس، وخاصة عندما تزداد المشاكل التي يعاني منها المبنى القائم وتصل إلى حد يعد كمتلازمة المبنى المريض (SBS) عملية مهمة وبشكل متزايد في بعض المباني وخاصة التجارية، وهناك العديد من المشاكل الأخرى المرتبطة بالوهج ومستويات الاضاءة وانبعاثات الغازات وجودة الهواء الداخلي (Anon , 2000b)، عندها لابد من التحرك نحو تكيف المبنى واعتماد سياسة مستدامة للمنشأ في ملخص تصميم التكيف للحد من هذه المشاكل والوصول إلى راحة الشاغلين.

التقادم Obsolescence:

هو فقدان المنفعة بسبب تطوير خدمات محسنة ومتوفرة، لكنه ليس خسارة المنفعة بسبب التدهور الطبيعي أو الاضمحلال ، ويكون بعدة أنواع (Khalid , 2004, p. 39-40):

- التقادم المادي: هو تآكل المباني أو الاجزاء المكونة لها.
- التقادم الوظيفي: هو ان تنتفي الحاجة إلى الوظيفة الأصلية للمبنى.
- التقادم الاقتصادي: يكون عند ازالة الأساس المنطقي الاقتصادي للمبنى
- التقادم الموقعي: هو عندما لا يكون موقع المبنى مناسبا بمرور الوقت.

حيث يمكن ان يؤثر أي نوع من التقادم على المبنى وفي أي وقت خلال دورة حياته مما يتطلب إجراءات نحو التكيف بدلا من الهدم، ويكون التكيف اما بتغيير الاستخدام أو ضمن الاستخدام الحالي. (Kincaid , 2012)، وهذا يعتمد على الاستخدام الأفضل من خلال تقييم استخدام الارض وتنسيقها مع الاراضي المجاورة أو تكون مكملة لها.

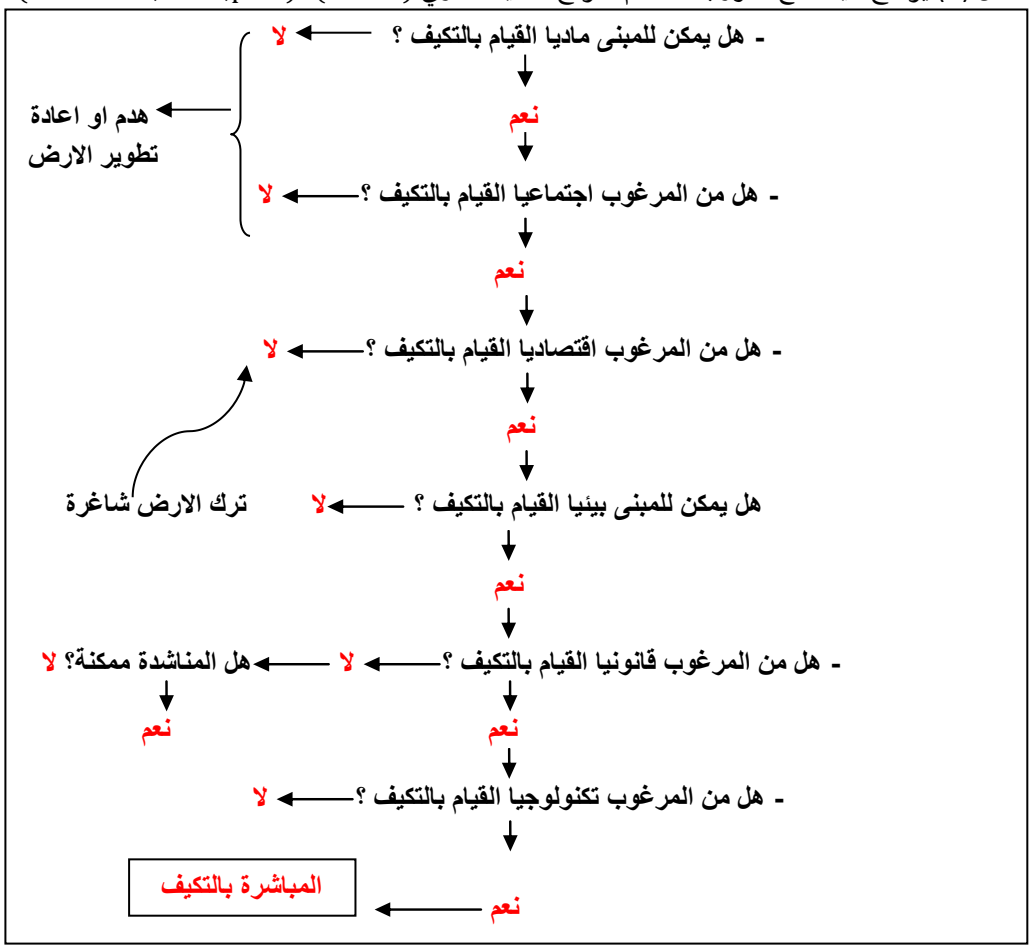
نموذج ARP لتقييم المباني من أجل التكيف:

(ARP) (Adaptive Reuse Potential) إمكانية إعادة الاستخدام التكيفية: وهي إمكانية ميل الأصل إلى إعادة التدوير لأداء وظيفة مختلفة بشكل كبير مع الحفاظ على الخصائص الأساسية للمبنى الأصل في مكانها الصحيح.
تتميز إمكانية إعادة الاستخدام التكيفية عن إعادة التدوير في أنها يمكنها التعديل لتلائم غرضا جديدا، إلا أن إعادة معالجتها لا تؤدي إلى فقدان الشكل الأصلي.
يمكن لـ (ARP) تحقيق نتائج التكيف من خلال الحفاظ على المواد لأنه يتضمن إعادة استخدام الكل بدلا من بعض الاجزاء التابعة له، وبذا تكون إعادة الاستخدام التكيفية مهمة عندما تصبح الوظيفة الأصلية للمبنى الأصل متقادمة، وهناك حاجة إلى وظيفة أخرى، حيث تكون الحالة المادية للمبنى الأصلي جيدة، أو ان الارض التي يقع عليها المشروع في موقع جيد ومهم وفيها إمكانية وصول مناسبة من وسائل النقل، أو ان قيمة المبنى مهمة من أجل الحفاظ على نسبة كبيرة من الهيكل الانشائي وغلاف المبنى (Langston et al. , 2014 , p.187)
تم استخدام نموذج (ARP) لتقييم المباني من أجل التكيف في أكثر من دولة، وهو تقدير العمر المادي المتوقع للمبنى والعمر الحالي له (Langston et al. , 2008 , p.1-4).

تقييم التكيف بالنسبة للمبنى القائم:

يمثل النموذج المحدد لتقييم التكيف في المبنى القائم، الشكل (2) وهو (Prelimary Adaptation Assessment Modeler) نموذج تقييم التكيف الاولي PAAM، إطار ا معرفيا يتضمن الصفات المتعددة والمستويات المختلفة للتكيف الممكن تحقيقها والتي تبين انها الأكثر اهمية من خلال تحليل محاولات التكيف السابقة للمبنى القائم، ويعتمد هذا النموذج إلى حد كبير على نموذج (Chudley 1981) الذي تم تعديله ليلائم الاستدامة ، ويعتمد المفهوم الأساسي للإطار المعرفي على تسلل القرارات التي يجب اتخاذها لإجراء تقييم اولي لمدى ملائمة المبنى للتكيف. (Remoy & Van der Voordt ,2007 , p.72), (Ball 2002, p.13).
يقوم PAAM بتقييم ما اذا كان التكيف سيوفر مبنى يلبي التوقعات التكنولوجية على انها مهمة، فاذا كانت جميع المراحل تفي بالشروط فيمكن إجراء تكيف للمبنى بدرجة معقولة وتحقق النجاح ، ان الهدف الرئيسي من PAAM هو انه يمكن استخدامه من قبل غير الخبراء لإجراء تقييم اولي لمدى ملائمة المبنى لتعديلات التكيف.

الشكل (2) يوضح الية صنع القرار باستخدام نموذج التكيف الأولي (PAAM)، (Wilknson ,2014,p.43)



التكيف في المباني الجديدة:

أبرز المؤشرات المستخلصة:

بعد استعراض أبرز المفردات البحثية الخاصة بالتكيف في المبنى القائم المعاصر وأبرز آليات التكيف وطرق تقييم المباني من أجل التكيف، لابد من بيان المحاور الرئيسية التي تم تطبيقها على الأمثلة المنتخبة بغية الوصول إلى أبرز عناصر التكيف المستدام لمباني العمارة المعاصرة.

ويوضح الشكل (3) أبرز حالات المبنى التي يمكن أحداث التغيير فيها لتحقيق التكيف وأبرز الآليات الممكن اتباعها لتحقيقه، وامكانيات الحلول المستقبلية للمبنى.

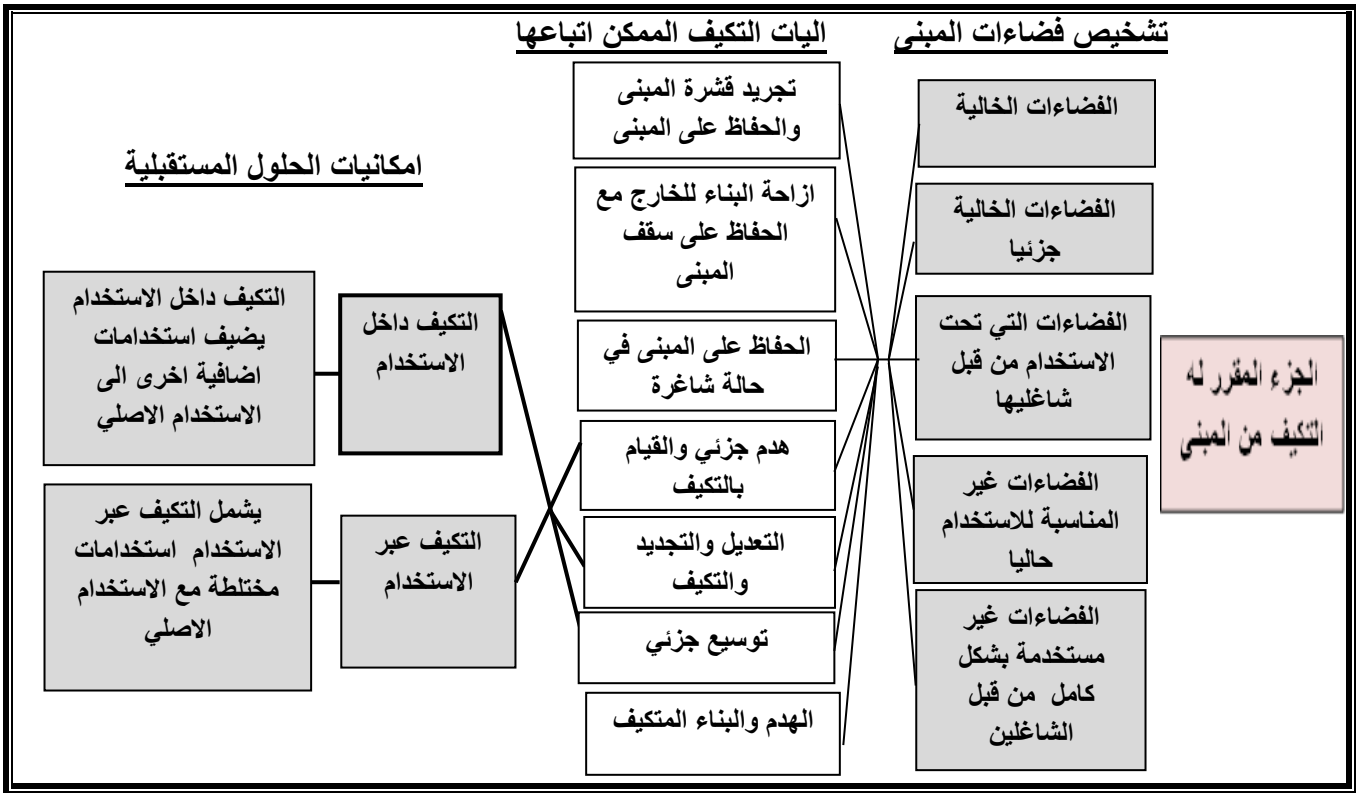
الشكل (3) يبين أبرز حالات المبنى والآليات المتبعة لتحقيق التكيف

الحالات الدراسية المنتخبة للتكيف المستدام في مبان قائمة:

تم انتخاب حالات دراسية تبين العديد من عناصر التكيف المستدام ضمن مستويات الاستدامة البيئية والاجتماعية والاقتصادية، وتوضح وصفا عاما للمشاريع المنتخبة وما هي عناصر التكيف المستدام التي تمت في كل مبنى، وهي:

مبنى 406 شارع كولينز (406 Collins Street):

هو مبنى نموذجي لناطحة سحاب في الستينيات من القرن الماضي الشكل (4) ، وقد تم بناؤه من هيكل فولاذي وخرساني، بواجهة بسيطة (غير مزخرفة) مع شريط من النوافذ في كل طابق وعلى شارع كولينز، وفي عام 1961 تم توسيع المبنى بإضافة اربعة طوابق. الميزة الوحيدة للمبنى الأصلي لعام 1897 هو " تمثال اطلس" وهو عبارة عن لوح زخرفي في الجزء العلوي للمبنى والذي يقع الآن



على مستوى الشارع عند المدخل. كانت منظومة (HAVC) نموذجية في الستينات، وفي عام 2006 تم اتخاذ القرار بضرورة اخضاع المبنى لعملية تكيف منظومة الخدمات وخاصة منظومة (HAVC) لانها وصلت إلى نهاية عمرها التشغيلي ومن أجل تحسين كفاءة استخدام الطاقة في المبنى وتحقيق الحد الادنى من استهلاك الطاقة وتقليل البصمة الكربونية واستخدام مصادر الطاقة الخضراء.

واجهت عملية التكيف للمبنى جملة من التحديات، منها الحاجة إلى الحفاظ على الخدمات الموجودة فيه، إضافة إلى الكلف المطلوبة والتي تم الحصول على جزء منها من صندوق المباني الخضراء إلا انها لم تغط النفقات، إضافة إلى تقليل تأثير أعمال التكيف على مستخدمي المبنى، حيث:

يجب خفض استهلاك الطاقة إلى النصف أو ربما أقل بنسبة 25% قبل التكيف

تحقيق استخدام منخفض للمياه في المبنى.

هناك اختلافات في نطاق درجة الحرارة الداخلية، إلا ان ثقافة المستخدمين ستسمح بتقبل درجات الحرارة المحيطة الأكثر دفئا في الصيف والبرودة في الشتاء بتوفير كبير في الطاقة.

مع تحسينات HAVC التي تم اجراؤها، فأن الصيانة ستكون أسرع واقل تكلفة.

الشكل (4) الواجهة الأمامية لمبنى 406 شارع كولينز
https://www.google.com/url?sa=t&source=web&rct=j&url=https://www.commercialrealestate.com

وكانت عناصر التكيف المستدام للمبنى هي:

منظومة تكييف الهواء VRV

- طوابق ذات وظائف تخصصية
- التنظيف الليلي الآلي
- تظليل داخلي وخارجي للباحة
- متحسسات ضوء للحركة في السلالم والمصاعد
- اضاءة عالية الكفاءة في المناطق العامة
- منظومة تحكم في إدارة المباني على الانترنت (BMCS)

مبنى 500 شارع كولينز (500 Collins Street):

هو تكيف واسع النطاق لتحقيق كفاءة الطاقة والمياه على مستوى اقتصادي عالي أثناء أعمال التكيف الشكل (5). اكتمل في السبعينيات من القرن الماضي، اشتهر مبنى 500 شارع كولينز بجودة خدماته ومعايير البناء الحديثة، وبحلول عام 2002 انخفض المبنى إلى مستوى منخفض من خلال التقادم والشيخوخة، وعلى الرغم من هذا الانخفاض بقي المستخدمون بسبب حجم المبنى والتكوين والموقع الممتازين والإدارة السليمة للمبنى، قبل التكيف كان المبنى من 23500 م 2 ، يتألف من الفضاءات المكتبية و140 موقف للسيارات وخمسة فضاءات تجارية.

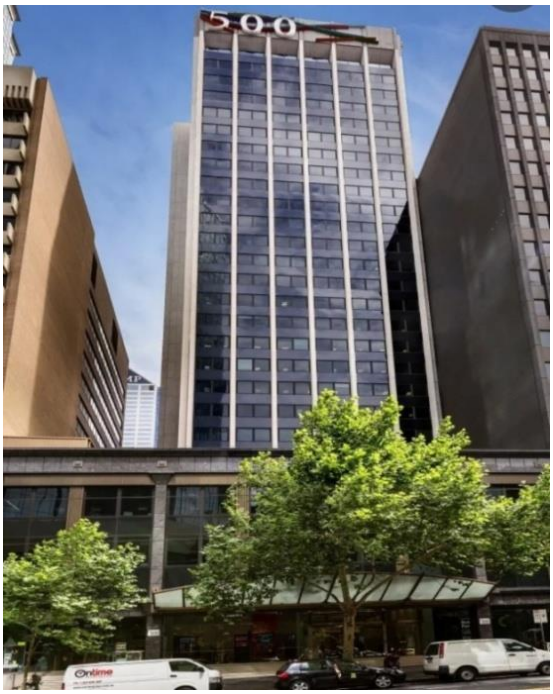
بدأ المشروع في منتصف عام 2003 واكتمل في اوائل 2011 حيث تم تسليمه على ثلاث مراحل للسماح للمبنى المشغول بالكامل تقريبا بالعمل خلال فترة التشغيل، ومراحل التكيف هي:

المرحلة الاولى: كانت استبدال المصنع وتحديثه وتجديد الواجهة.

المرحلة الثانية: زيادة مساحة الفضاءات التجارية إلى اقصى حد وإعادة تهيئة موقف السيارات.

المرحلة الثالثة: تطوير طابق المكاتب بشكل تدريجي، واكمال أعمال الانهاء لكل طابق.

الشكل (5) الواجهة الأمامية لمبنى 500 شارع كولينز
www.commercialrealestate.com.au/property/500-collins-street-melbourne-vic-



وتم تطوير الواجهة من خلال استبدال الألواح المزججة بألواح الجدران المصنوعة من الالمنيوم وأصلاح وتجديد الاعمدة الرأسية وإعادة الطلاء، وتم تطوير مداخل الباحة والاماكن العامة وتطوير منظومة HAVC بكفاءة اكبر ويتضمن مراوح لنشر الهواء البارد حول محيط المبنى حيث تكون الاحمال الشمسية عالية، إضافة إلى تركيبات مياه المطر وألواح ومعدات الاضاءة. وتمت معالجة النفايات من خلال إعادة التدوير في الموقع مع حوالي 80% من نفايات البناء التي تم إعادة تدويرها.

تضمنت تحسينات الاستدامة العامة للمبنى المتكيف:

- تقليل الطاقة الكامنة في المبنى باستخدام مواد خالية من PVC قدر الامكان
- استخدام مواد منخفضة المركبات العضوية القابلة لإعادة التدوير.
- اختيار مواد متينة ومن المصادر المستدامة.
- تشجيع استخدام الدراجات من خلال توفير مساحة توقف للدراجات الهوائية وتكون آمنة وتتسع لـ 82 دراجة.
- تحسين جودة البيئة الداخلية عن طريق زيادة الهواء النقي بنسبة 50% والتبريد بالاشعاع (انابيب التبريد)، واستخدام مواد منخفضة المركبات العضوية المتطايرة ضمن الفضاءات الداخلية وتقليل مستويات الضوضاء الداخلية المحيطة
- تم تجديد منظومة التحكم في المبنى.
- تم استبدال لوحة المفاتيح الكهربائية، وتوفير القياس الفرعي لكل جزء من المبنى لتمكين مراقبة الطاقة بشكل فعال
- كانت الاهداف هي تحقيق معيار بناء من الدرجة الاولى، لتحقيق درجة عالية من الكفاءة البيئية سواءا أثناء أعمال التكيف أو ما بعد التكيف.

كانت عناصر التكيف المستدام للمبنى:

- استخدام مبردات VSD [5]الموفرة للطاقة

[5] VSD: Variable Speed Drive (محرك متغير السرعة)

- غلايات تعمل بالغاز
- انابيب تبريد (فعالة وذاتية)
- ألواح شمسية تخدم 25% من متطلبات المياه الساخنة
- تجهيزات اضاءة منخفضة الطاقة
- خزانات مياه لتجميع مياه الامطار لري المناطق الطبيعية
- أجهزة تقييد تدفق المياه على جميع التركيبات
- التحدي الرئيسي للتكيف هو المستخدمين في الموقع وذلك باستمرار تشغيل الخدمات القائمة أثناء تركيب منشآت جديدة.

وكانت نتائج هذا التكيف للمبنى كما يلي:

- تم تصميم نموذج للطاقة لتحقيق انخفاض بنسبة 30%في تكييف الهواء وانخفاض بنسبة 50% في الاضاءة وانخفاض بنسبة 15% في المياه الساخنة
- تم تصميم انابيب المياه لتحقيق وفورات بنسبة 40-50%
- تم تحقيق انخفاض في الاجازات المرضية بنسبة 44% بسبب تحسين راحة المبنى وجودة الهواء
- يرجع انخفاض تكاليف الصيانة إلى التخفيض في التصنيع وزيادة كفاءة معدات المبنى وتحسين المراقبة من خلال منظومة إدارة المباني
- تم الحفاظ على دعم المستخدمين للمبنى خلال فترة التكيف بمعدل اشغال لا يقل عن 70%
- حصل المبنى على تصنيف (5 نجوم خضراء)(5-star Green Star Office Design v1)

الاستنتاجات

بالنسبة للمباني القائمة: يكون من الأفضل للمباني القائمة القديمة، والتي تعد "خام حضري"، ان يتم تخصيصها إلى تطبيقات جديدة بدلا من هدمها أو تفكيكها للحصول على المواد الخام، ويكون الأفضل ترك الهيكل الانشائي الأساسي والمظهر السليم للمبنى وتغيير استخدامه بشكل تكيفي ، حيث توفر إعادة استخدام المباني القديمة دون هدم فرصة كبيرة للحفاظ على الموارد وما يرتبط بها من تصنيع وتجميع للطاقة، وبالتالي يمكن ان يتم إجراء التعديلات الطفيفة أو الكبيرة للمبنى من خلال فحص إمكانية المبنى من أجل التكيف.

يتطلب التكيف في المبنى ادخال التحسينات ليس فقط على الركائز البيئية والفيزيائية والاقتصادية بل ايضا على الجانب الاجتماعي.

وجود توافق في الآراء من الادبيات والمراجع حول معنى القدرة على التكيف، حيث اعتمدت على مصطلحات منها فكرة التكيف مع التغيير بمرور الوقت، المرونة، طول عمر المبنى.

تعتمد أساس فكرة التكيف على تطبيق المفهوم في مراحل مبكرة من تصميم المباني لتحقيق أعلى مستوى من التكيف ضمن المبنى.

يعتمد مستوى التكيف المطلوب للمبنى على مزيج من الاحتياجات المحددة لكل مبنى منها الداخلية (للمستخدمين)، والخارجية (احتياجات السياق) أو المتطلبات العامة للمدينة من المباني، لذا فأن تحديد هذه الاحتياجات لاستيعاب التغيير المستقبلي يعد امرا ضروريا في توفير مستوى مناسب من التكيف في المباني، لأن كل مشروع يتطلب حلولا تصميمية تختلف من مبنى إلى آخر.

يشهد القرن الحادي والعشرين تغييرات (اجتماعية وبيئية واقتصادية) (محصلة ثلاثية) والتي ستغير مفهوم المباني بشكل كبير، حيث (تصميم وإنتاج أسرع، اعتماد منهج التصنيع والتنفيذ خالي من الهدر، ازدياد الطلب على البنية التحتية القابلة لإعادة التشكيل، تزايد الاهتمام بأداء الطاقة والتركيز على المباني خالية الكربون، إضافة إلى التشجيع على العمارة المفتوحة كونها أساس عملية التحول والتطور.

يعتمد نجاح المبنى في تحقيق الأداء العالي في التكيف من خلال جعل غلافه مستقلا عن هيكله الانشائي وبتوفير إمكانية وصول إلى منظومة الغلاف من داخل المبنى ومن خارجه لتسهيل الصيانة والتعديل، وباستخدام مواد متينة وذات مظهر جيد لاستيعاب التقادم في العمر بأمان.

يرتبط نجاح تكامل عمل منظومات المبنى لتحقيق التكيف بطبيعة وسلوك مستخدمي المبنى وان معرفة احتياجاتهم وتوقعاتهم تعد من الامور المهمة في تطوير المباني من أجل التكيف.

من أجل تحقيق التكيف في المباني من الأفضل اعتماد المصممين والمعماريين إلى تصنيف المبنى إلى منظومات بشكل مستويات تعتمد على خصائص المبنى للاستجابة للتغييرات.

من أجل التصميم لتحقيق التكيف لابد من التركيز عل ثلاثة متغيرات رئيسية في التصميم تتغير وتتطور بمرور الوقت وهي: (عمر المبنى أو المكون، الوظيفة، الأداء الاجتماعي (المستخدمون)).
لتحقيق التكيف على المصمم ان يراعي سلسلة من الأحداث وكيث سيتحول المبنى مع المستخدمين ضمن الفضاء أو الأداء أو التوسع أو الاستخدام أو التوقيع بدلا من التركيز والاهتمام على شكل المبنى أو كيف سيعمل في مكان واحد في هذه اللحظة.

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