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STUDY THE PROTECTION EFFECT OF ALCOHOLIC EXTRACT OF IRAQI PROPOLIS ON SOME OF LIVER AND KIDNEY FUNCTIONS AT MALE ALBINO RATS ADMINISTERED WITH A COMBINATION OF AMOXICILLIN/CLAVULANIC ACID (AUGMENTIN) ANTIBIOTIC

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ABSTRACT

The current study was conducted at the Animal House return to Life Sciences department / Faculty of Education for Girls from the period 25/1/2015 to 1/1/2016 and the study included the use of 40 male laboratory rats of the Sprague-Dawley strain divided into four major groups: the first group was the negative control group administered with normal saline solution(0.9%) , the second group was the positive control group treated orally with only a combination of Amoxicillin/clavulanic acid (Augmentin drug 2.232 mg/kg) , the third group was received the drug Augmentin (2.232 mg/kg) plus propolis ethanolic extract concentration of(100 mg/kg), and finally the fourth group which administered with extract of alcohol propolis only concentration of (100 mg/kg). The treatments of all study groups were continued for a period of seven days and three times/day orally.

During the present study, the total weights of animals as well as liver and kidney weights were measured, as estimation of the activity of some hepatic enzymes (ALT , AST and ALP) and also the concentrations of some biochemical criteria, which including: total bilirubin, total protein, albumin , globulin ,creatinine, urea, glutathione(GSH) and malondialdehyde(MDA) ,as well as the study included the histological examination of livers and kidneys of animals under study.

The results of the current study were showed :the treatment with drug was caused a significant ($P< 0.05$) rise in body weight, the liver and kidneys weights , the activity of liver enzymes(ALT , AST and ALP) as well as the concentrations of total bilirubin , creatinine , urea and (MDA) at the Augmentin treated group(positive control group) when

compared with the other groups, while the concentrations of total protein, albumin , globulin and (GSH) showed a significant decrease ($P< 0.05$)at the same group as compared with the rest of the groups.

The histological examination of livers of Augmentin treated animals pointed to obtain many of pathological changes included, necrosis of some liver cells and severe degeneration of the others, congestion and hemorrhage within the central vein as well as expansion or widening, severe aggregation or gathering of inflammatory cells within the tissues of liver.

As for the slides of animals kidneys that administrated with Augmentin has included, ruptured or destruction the wall of the renal glomerulus, severe degeneration of the renal cells , congestion as well as hemorrhage within the renal glomerulus and connective tissue finally, widening of the collecting tubules.

It was concluded from the present study that the treatment with propolis has shown an ameliorative impact on body weight, as well as the weights of livers, kidneys and some of biochemical standards of the blood, in addition to the preventive effects of propolis in protecting the histological structure of both liver and kidney from the toxic and detrimental influences of Augmentin, that was proved by maintaining biochemical blood standards levels touched upon the current study, so we recommend with propolis administration synchronous when Augmentin drug treatment.

INTRODUCTION

Augmentin which is composed of Amoxicillin added clavulanic acid ,is a synthetic penicillin recently used for treatment of respiratory tract , urinary tract , skin and soft tissue inflammations, dental infections as well as sinusitis and other injuries (Gorden 2010) , also Augmentin was broadly used by Dentists in Spain since 2005 (Roda *et al*., 2007 , Berlado *et al* ., 2013).It possesses a potent efficiency as a bactericidal against all of gram negative as well as most of the gram positive bacteria(Finlay *et al*.,2003).Also Augmentin reveals an antimicrobial efficacy in the treatment of some disorders such as chancroid , gonorrhea, pneumonia(acquired from community),chronic bronchitis state(the case of acute exacerbations),the acute inflammation of otitis media and others(De Koning *et al*.,1981;Fast *et al*.,1982; Olayinka and Olukowade,2010). Furthermore, this drug uses in the veterinary medicine for the treatment of numerous conditions. A recent study has been demonstrated that Augmentin revealed high activity against *Enterococcus faecalis* (Carlapati *et al*., 2016).

Generally , Augmentin is an antibiotic that is orally and intravenously taken (Davis *et al.* , 1988), it's a mixture of tri hydrate amoxicillin (a β-lactam antibiotic), as well as the potassium clavulanate (a β-lactamase inhibitor) .Augmentin derived from 6-aminopenicillanic acid(the basic nucleus of penicillin) , it's a broad spectrum antibiotic that is commonly used worldwide has been showed a high efficiency against bacterial species that are amoxicillin resistant β-lactamase producers (Olayinka and Olukowade,2010;Gillies *et al.*, 2014) , besides drug efficiency ; Augmentin possesses many of side effects such as vomiting , diarrhea , loss of appetite , trash , also it can induce hyper sensitivity reactions and pseudo membranous colitis (Andrade *et al*., 2005 ; Gordon *et al*., 2010; Chalasani *et al*., 2008) ,in addition to numerous adverse effects on some of body systems and organs such as skin , digestive system , liver and blood (Stephens *et al*., 2013 ;Berlado *et al*., 2013) , the chemical structure of Augmentin shown in figure(1) :

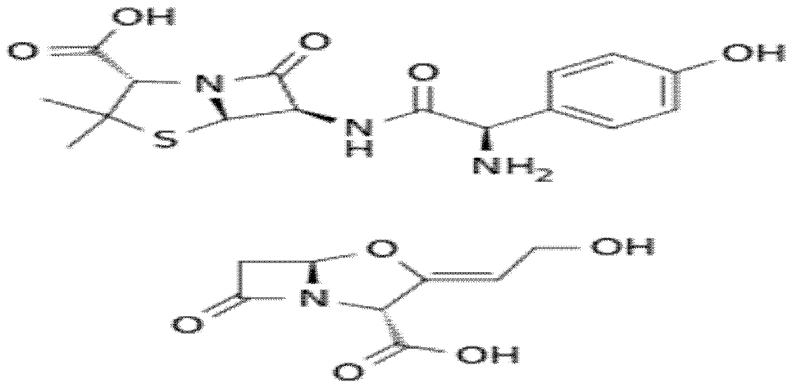


Figure (1): the chemical structure of Augmentin drug (Gillies *et al*., 2014)

Some studies found that Augmentin causes liver tissue infection in different patterns, with unknown mechanisms, may the metabolic interference play an important role in the infection, or the immune factors are the most important in cholestatic pathogenesis (Gresser, 2001). The liver fibrosis conjugated with Augmentin uptake mostly result in cholestatic disorder, the total bilirubin levels ranging between 0.6 – 36.9 mg/ dl (Lucena *et al* ., 2006) . Other studies showed that Augmentin treatment causes high recession in bile secretion while bilirubin levels rise to 50.75 mg/dl (Jose *et al* ., 2010) , the latent period between drug uptake and appearance of symptoms occurred in 3-4 weeks as an average (Andrade *et al*., 2005) . In case of stopping Augmentin uptake the liver is no longer effected (Lucena *et al*. ,2006), Other studies suggest that treatment of liver damage due to Augmentin use is performed

by treatment with glucocorticoid (Tajiri and Shimizu ,2008).

Propolis (Bee glue) is a natural product of honey bee , propolis extract is such a natural complicated formula containing amino acids , phenols , phenolic acid aceters , terpins ,flavonoids , caffeic and cinnamic acid, these materials well approved to show medical and therapeutic characteristics , the propolis undergo transformation into a viscous liquid material if subjected to high temperatures (more than 20ºC) and it can solidify in lower grade (Simon-Fenstron and Spivak, 2010; Durgun and Durmus,2004 ; Khayyal *et al*.,1993) .

Propolis is used by bees to keep their cells intact (Greenaway *et al*., 1990) as the bees collecting propolis from tips of trees , plant buddings , and other plants such as Poplar trees and chestnuts so it's used to seal unwanted pores in bees cells especially pores less than 6 mm , another important advantage for propolis in preventing entrance of parasite and other pathogens because it can inhibit bacterial and fungal growth , as well as enclosing dead insects to prevent their decay inside the bees cells (Simon- Fenstron and Spivak, 2010).

Types of propolis differ in one another in their smell , cell composition , and also it vary between seasons , generally it's dark brown in color , but it can be present in green , red , black or pale yellow depending on the bees' nutrition source (Mesquita and Francison, 1995). The propolis chemical structure is very complex and differed according to geographical areas, and it's composed of 50% resin and green balsam, 30% wax, 10% essential and aromatic oils, 5% pollens, 5% other ingredients including organic waste products (Viuda –Martos *et al*., 2008). Propolis used for various medical purposes due to its natural active materials which approved to possess antimicrobial characteristics (Koo *et al*. ,2000;Cardoso *et al*.,2016) and in treatment of Glutathione consumption inside the hepatocytes as well as free radical scavenging (Nirala and Bhadauria, 2008).

The past studies found that propolis play an important role in balancing body antioxidant systems ; as it possesses super antioxidant effect for lipid peroxidation in the cell membranes (Bhadauria *et al*. , 2008) it was used since thousands of years in treatment of oral pains , ulcers ,and genital disorders for it's pharmaceutical , immune activator , anti-inflammatory, anti-cancer , and anti-bacterial effects (Bankova *et al*., 2000 ; Fearnely ,2001).

Aims of study

1- Diagnosis or detection of the clinical symptoms due to Augmentin usage through the

performance of histological and biochemical study which including the estimation of liver functions by the measuring of some liver enzymes activity such as (AST, ALT, and ALP), as well as total bilirubin, total protein, albumin, and globulin concentrations in the serum.

2- Evaluation the kidney functions through the assessment of urea and creatinine levels in the serum, in addition to the examination of renal tissues.
3- The present study also aimed to determine the levels of oxidative stress standards after the administration of Augmentin drug.
4- Studying the possible protective effects of natural product of honey bee (Iraqi prpolis) on the hepatic and renal tissues against the probable deleterious impacts of Augmentin antibiotic uptake.

MARTIALS AND METHODS

1-Preparation of laboratory animals:

40 albino male rats whom belong to Sprague- Dawley strain used in the current study , their weights ranging between (200 – 250)g , less than 12 weeks in age , the laboratory animals brought from National Center For Control and Pharmaceutical Research in Baghdad into the Animal House in Educational Faculty for Girls/Kufa university , under laboratory conditions care like fixed lighting (13 hours lighting / 11 hours darkness) , adequate ventilation , conditional room , with temperature grade between (23-28) ºC . The cages floor was covered with sawdust , the sawdust floor displaced twice to three times a week to keep the cages clean , the rats fed an water and specialized feed enriched with protein until the animals get a three months(sexually matured) ,then it has been subjected to the present research.

2-Propolis: brought by Montaser AL-Husseinawi beehives in AL-Najaf AL-Ashraf/ Kufa, to ensure it is purity (free of other materials).

3-Preparation of ethanolic propolis extract : Iraqi propolis was collected from Montaser AL-Husseinawi in AL-Najaf AL-Ashraf / Kufa , the propolis was cut into small pieces by electric mixer , then 30g of propolis was solubilized in adequate amount of 70% ethanol, the size was completed to 100ml of ethanol to create propolis with concentration of 30% , the extract was maintained in dark containers in room temperature with intermittently shaking (daily shaking for several minutes) to ensure exposure of whole propolis powder to ethanol , the mixture was completely solubilized and filtered by using Watman No.42 filter papers according to

Boyanova *et al*.(2005).The filtered product was lyophilized with freeze dryer in central biochemistry laboratory –Pharmacy Faculty –Kufa university at -20 ºC. The dried extract (the lyophilized product) kept in freezer at 4 ºC until usage (Oršolić *et al*., 2013).

4-Augmentin Drug : the Augmentin drug (a combination of Amoxicillin/clavulanic acid) was brought with a dosage of (625mg/ kg) from Baghdad Pharmacy located in Al-Najaf Al-Ashraf / AL-Askan street.

5- Preparation of the therapeutic dosage of Augmentin drug: Augmentin with dosage of 625 mg/ kg was grinded into a powder , the required dose in the current study was determined depending on human therapeutic dosage after calibration it with the weight of rat according to the following equation : -

$$\textbf{Therapeutic dosage for rat}=\frac{625\ mg/kg\times 250gm}{70000gm}=2.232\ mg/kg$$

625mg/ kg= Therapeutic dosage for human
250gm=Average weight of rat
70000gm= Average weight of human

6- Experimental groups:

First group: this group composed of ten rats administered with normal saline (0.9%) every eight hours (three times daily) for seven days, regarded as a negative control group.

Second group : all of the ten rats were subjected to Augmentin uptake (2.232mg/kg) every eight hours daily(three times) for seven days , this is represented a positive control group.

Third group: this group was treated with ethanolic extract of propolis with a concentration of 100 mg/kg in addition to Augmentin drug (2.232mg/kg) every eight hours (three times daily) for seven days.

Fourth group: the group was submitted to the ethanolic propolis extract uptake (100 mg/kg) which was previously prepared every eight hours (three times daily) for seven days. The administration process was performed orally for all groups of treatments.

7-Animals sacrificing and blood samples collection:

At the end of administration process and already after 24 hours after the last dosage for every group , the weights of all rats was measured and recorded, by the using of diethyl ether as a narcotic agent , every rat was fixed with pins on cork plate ,then the blood was taken directly from the hearts by the heart puncture to get an adequate amount of blood (5 ml) , the taken blood was directly transferred into gel tubes which were anticoagulant free , the rats were anatomized by cutting the abdominal cavity ,both liver and kidney were cut and extracted, after the adipose tissue was removed from the required organs , organ's weight were recorded by using a sensitive balance , and it was kept in plastic

containers which containing formalin (_10% concentration) to be used for the histological study .

9- Preparation of Serum Samples:

The blood collected in gel tubes were centrifuged at 3000 rpm for 10 minutes, then the serum was transferred into serum tubes to be kept in -4ºC for the blood biochemical assessment.

10-Biochemical study:

A-Determination of serum alanine aminotransferase (ALT) and aspartate amino-transferase (AST) enzymatic activity:

The activity of enzymes: ALT and AST was determined through the using of kits were equipped by biomerieux, France Company . The method of Reitman and Frankel (1957) was adopted, which based on the following reactions:

GPT: Alanine + alpha - ketoglutarate ⟷ pyruvate + glutamate

GOT: Aspartate + alpha - ketoglutarate ⟷ Oxaloacetate + glutamate

The levels of both ALT and AST were evaluated at the absorbency of (505) nm.

B-Determination of serum alkaline phosphate activity (ALP):

According to the procedure of Kind and King (1954) the enzymatic activity of ALP was estimated, as well as the kit of Biomerieux, France Company was used . The method was based on the following reaction:

$$\text{Phenylphosphate} \xrightarrow[\text{pH = 10}]{\text{Alkaline phosphate}} \text{phenol + phosphate}$$

The color developed was read by the using of spectrophotometer at the absorbency of (510) nm.

C-Determination of total serum bilirubin concentration:

The concentration of total bilirubin was estimated by the using of Biolabo , France Company kit according to Walters and Gerarde (1970) method ,and the absorbency was measured at (550) nm .

D-Determination of total protein concentration in the serum:

To determine the total protein level in the serum, the Biuret reagent and kit of Biolabo,France Company was used. The total protein was evaluated according to West *et al.* (1966) method. The absorbency was read by the using of spectrophotometer at the absorbency of (550) nm.

E-Determination of albumin concentration in the serum:

According to Silverman *et al.* (1970) method the albumin level was evaluated by the forming of the chemical compound called Bromocresol green at PH=4.2 and absorbency of (630) nm.

F-Determination of creatinine concentration in the serum:

The creatinine level estimated according to Jaffe (1972) method (the method of colorimetric alkaline picrate), which based on the formation of creatinine-picrate complex, as well as the kit was used of Biolabo, France Company. The absorbency was (490) nm.

G-Determination of urea concentration in the serum:

The kit of Biomerieux, France Company and the method of Patton and Crouch (1977) were used to assess the urea level in serum at the absorbency of (580) nm.

H-Determination of reduced glutathione (GSH) concentration in the serum:

To determine the (GSH) level in serum, the Baker *et al*. (1990) method used and the kit of Biomerieux, France Company was obtained. The absorbency was measured between (405 – 414) nm by the using of spectrophotometer.

I-Determination of malondialdehyde (MDA) concentration in the serum:

The MDA level has been estimated according to the method of Armstrong and Browne (1994), the kit purchased from Biomerieux, France Company, at the absorbency ranged between (530 – 540) nm.

11-The histological study:

The histological sections of both liver and kidney were prepared according to Bancroft and Stevens (1982) method.

12-The statistical analysis:

The result of present study were statistically analyzed by the using of mega-stat program and (ANOVA) method, then the least significant difference (L.S.D) was used at the level of ($P<0.05$) (Morgan *et al*.,2010).

RESULTS

1-Effect of Augmentin and propolis on the body weight:

The result in figure (2) was revealed a significant increase ($P< 0.05$) in body weight at the positive control group when compared with other treated groups , while the results were showed no significant differences ($P> 0.05$) in body weight at the negative control group in comparison with the two groups ,that were treated with Augmentin + propolis and propolis only. As well as there is no significant difference ($P> 0.05$) in this parameter when the group was treated with Augmentin + propolis compared with the group of propolis only.

2- Effect of Augmentin and propolis on liver weight:

Concerning to the figure (3) , there was a significant difference ($P< 0.05$) in the liver weight at the positive control group comparable with other groups , while there is no significant difference ($P> 0.05$) in the

liver weight at the negative control group when compared with the Augmentin + propolis group and the group of propolis only . Also there is no significant change (P> 0.05) when the Augmentin + propolis treated group compared with group of propolis.

3- Effect of Augmentin and propolis on kidney weight:

The results were revealed a significant increment (P< 0.05) in the kidney weight at positive control group in comparison with the other treated groups, but there was no significant difference (P> 0.05) in this parameter when the negative control was compared with the Augmentin + propolis group and the group of propolis only. As well as there is no significant difference (P> 0.05) when the group treated with Augmentin + propolis compared with the group of propolis as shown in figure (4).

4-The biochemical parameters of blood:

A- The effect of Augmentin and propolis on ALT activity :

In relation to ALT, the results were showed a significant increment (P< 0.05) in the ALT activity at the positive control group comparable with the all groups of treatment , while there was a significant decrement (P< 0.05) in the activity of ALT at the group of propolis only when compared with the other two groups : negative control and Augmentin + propolis.

As well as there was no significant change (P> 0.05)in the ALT activity when the Augmentin + propolis group was compared with negative control group as shown in figure (5).

B – The effect of Augmentin and propolis on AST activity:

The findings have shown in figure (6) revealed a significant increment (P< 0.05) in the AST activity at positive control group in comparison with the other groups , in contrast there was a significant decrement (P< 0.05) in the activity of AST at the propolis treated group as compared with the both : negative control group and Augmentin + propolis group.

In addition, there was no significant change (P> 0.05) in the activity of ALT when the Augmentin + propolis group was compared with the negative control group.

C – The effect of Augmentin and propolis on ALP activity:

The data of present study were noticed a significant increase (P< 0.05) in the ALP activity at the positive control group comparable with the all other groups , while there was a significant decrease (P< 0.05) in this parameter at the propolis treated group when compared with the two groups : negative control and Augmentin + propolis.

In contrast, there was no significant difference (P> 0.05) in the ALP activity when the Augmentin + propolis group

compared with the negative control group, figure (7).

D - The effect of Augmentin and propolis on total bilirubin concentration:

As shown in figure (8) there was a significant increment (P< 0.05) in the total bilirubin concentration at the positive control group as compared with the other groups of treatment.

On the other hand there was a significant decrement (P< 0.05) was revealed in this parameter when the propolis treated group compared with the negative control group and the group of Augmentin + propolis, while there was no significant change (P> 0.05) in the total bilirubin concentration when the Augmentin + propolis group compared with the negative control group .**E – The effect of Augmentin and propolis on total protein concentration:**

The current study was appeared a significant decrease (P< 0.05) in the total protein concentration at the positive control group compared with the other treated groups.

As well as there was a significant increase (P< 0.05) in this parameter at propolis group in comparison with the all groups of treatment, while the results showed no significant changes (P> 0.05) in this standard when the negative control group was compared with the group of Augmentin + propolis as shown in figure (9) .

F - The effect of Augmentin and propolis on the albumin concentration:

The statistical analysis of the results revealed a significant decrement (P< 0.05) in the albumin concentration at the positive control group in comparison with the other groups , but there was a significant increment (P< 0.05) in this index when the propolis treated group compared with the other groups of treatments .

There was no significant change (P> 0.05) in the albumin concentration when the negative control group compared with the Augmentin + propolis treated group, figure (10).

G - The effect of Augmentin and propolis on globulin concentration :

Regarding the globulin concentration, the results shown in figure (11) revealed a significant decrease (P< 0.05) in the globulin concentration when the positive control group was compared with other groups , as well as there was a significant increase (P< 0.05) in this property at the propolis treated group comparable with the other groups of treatment .

Furthermore, there was no remarkable change (P> 0.05) in globulin concentration when the negative control group compared with the Augmentin + propolis treated group.

H - The effect of Augmentin and propolis on creatinine concentration:

The data of present study was noticed a significant rise (P< 0.05) in the creatinine concentration at the positive control group as compared with the other groups , in contrast there was a significant decrease (P< 0.05) in this concentration at the propolis treated group comparable with the negative control group and the group of Augmentin + propolis.

On the other hand, the results showed a significant (P< 0.05) change when the Augmentin + propolis treated group was compared with negative control group as shown in figure (12).

I - The effect of Augmentin and propolis on urea concentration:

When the positive control group compared with the other groups of treatment, there was a significant rise (P< 0.05) revealed in the urea concentration, while there was a significant decrement (P< 0.05) in this concentration was showed at propolis treated group as compared with the other two groups: negative control and Augmentin + propolis treated group.

Moreover, there was no significant change (P> 0.05) in this parameter when the Augmentin + propolis group compared with the negative control group as shown in figure (13) .

J - The effect of Augmentin and propolis on GSH concentration:

The result of the current study referred to a significant decrement (P< 0.05) in the glutathione concentration at the positive control group in compared with the other groups of treatment , conversely there was a significant increment (P< 0.05) in this concentration at the propolis treated group as compared with the other groups of treatment .

In addition, there was a significant difference (P< 0.05) in this marker when the negative control group compared with Augmentin + propolis treated group, figure (14).

K - The effect of Augmentin and propolis on MDA concentration:

The results in figure (15) indicated that there was a significant rise (P< 0.05) in the MDA at the positive control group as compared with other groups , while there was a significant decrease (P< 0.05) in this parameter when the propolis treated group compared with the other both groups : negative control and Augmentin + propolis.

As regard to MDA level, there was no significant change (P> 0.05) when the Augmentin + propolis treated group compared with the negative control group.

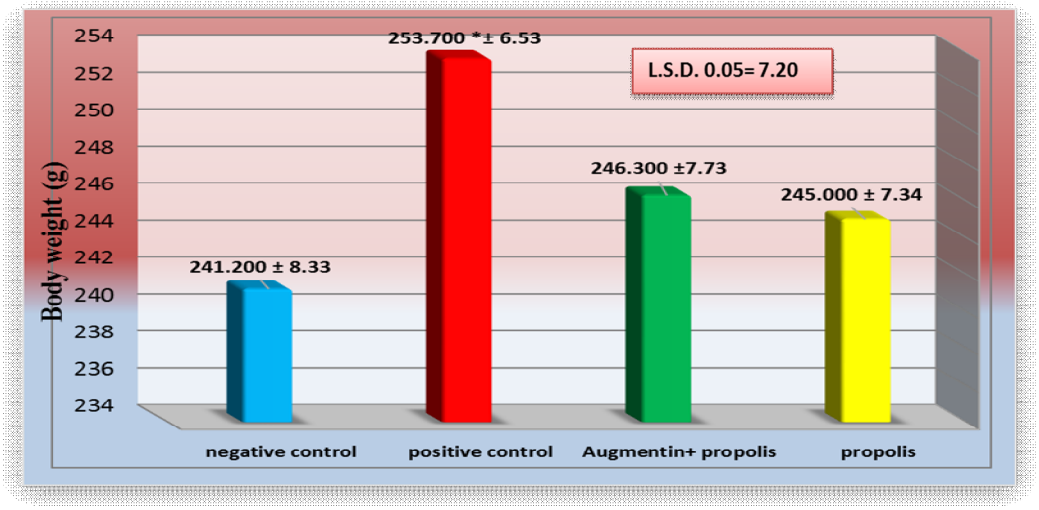


Figure (2): comparison between different groups according to the body weight
Values: represented as (mean + standard error), *: means a significant difference between groups at (P< 0.05)

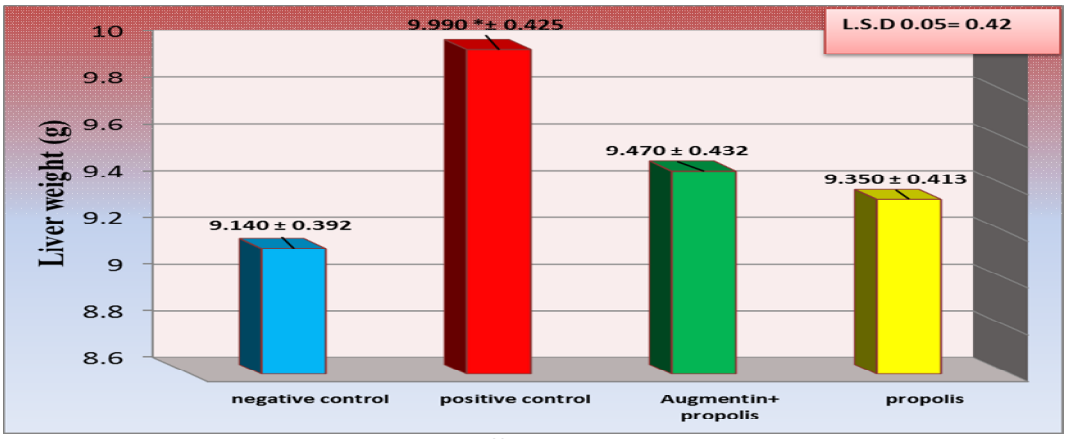
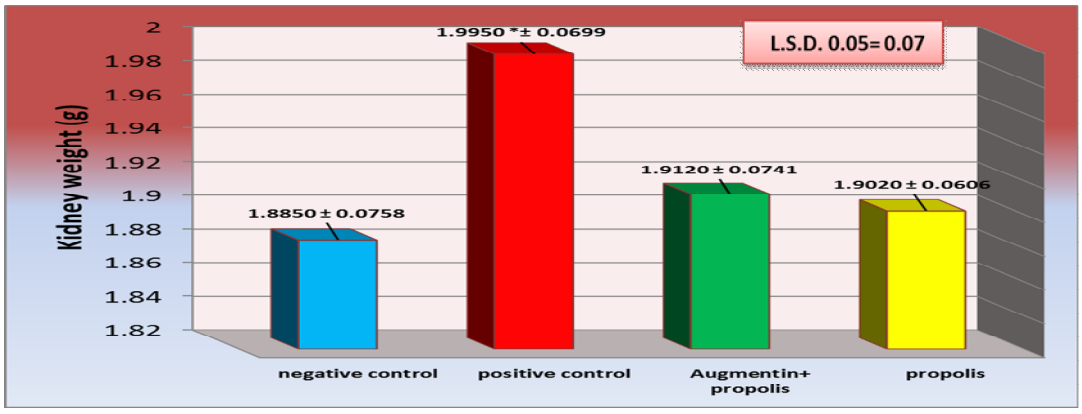
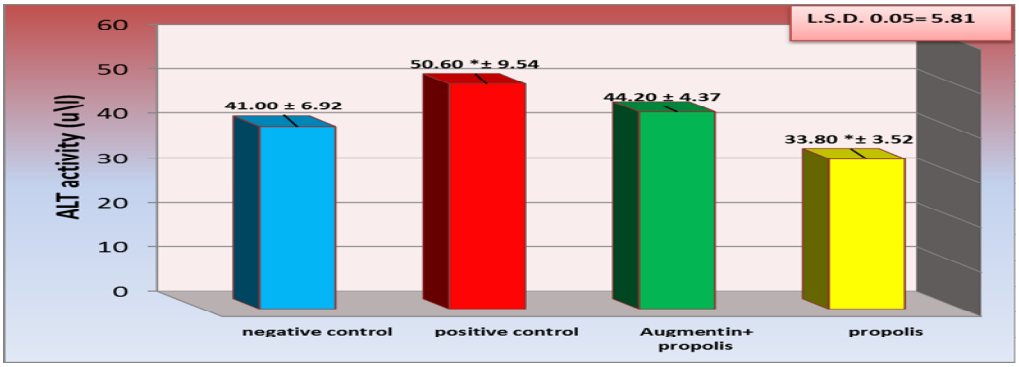


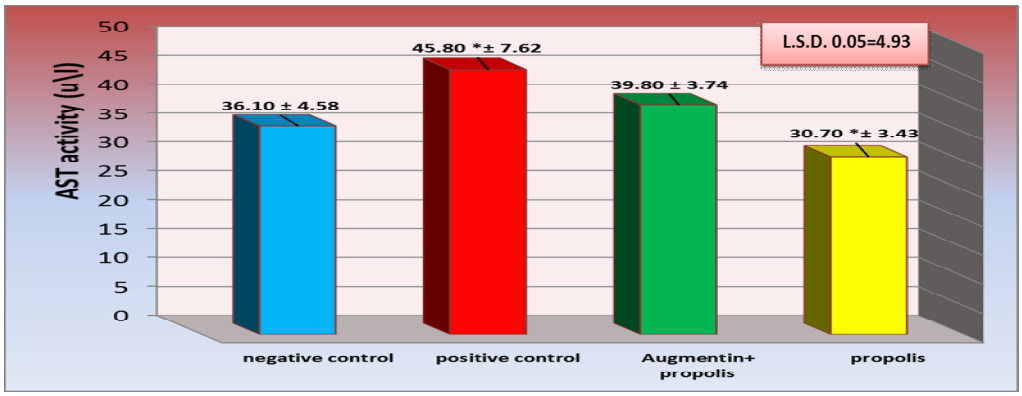
Figure (3): comparison between different groups according to the liver weight
Values: represented as (average + standard error), *: means a significant difference between groups at (P<0.05)



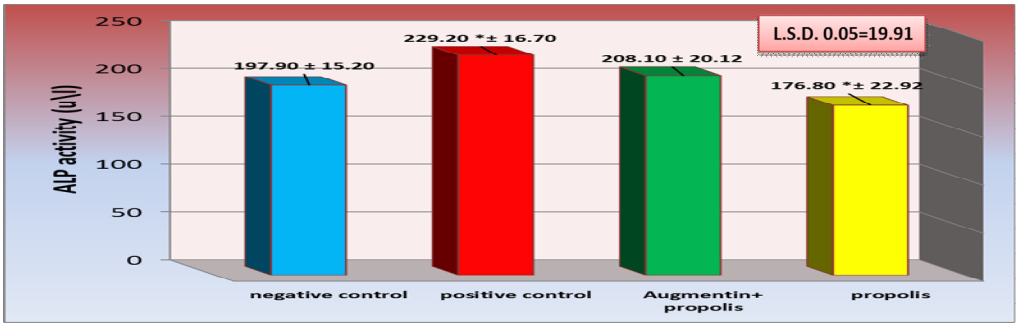
Figure(4):comparison between different groups according to the kidney weight
Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05)



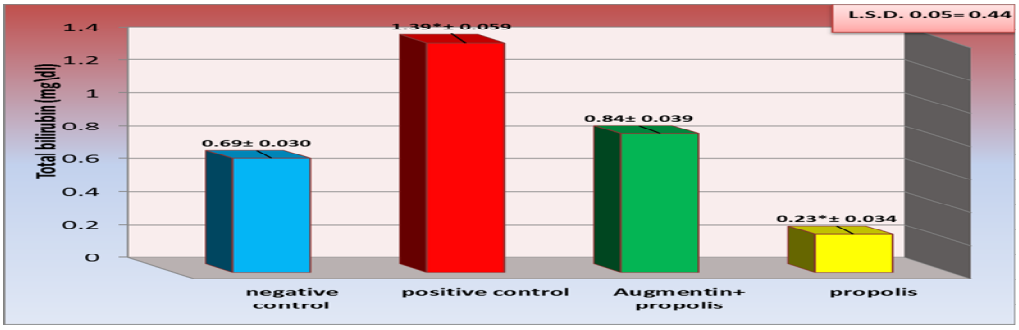
Figure(5):comparison between different groups according to the ALT activity
Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



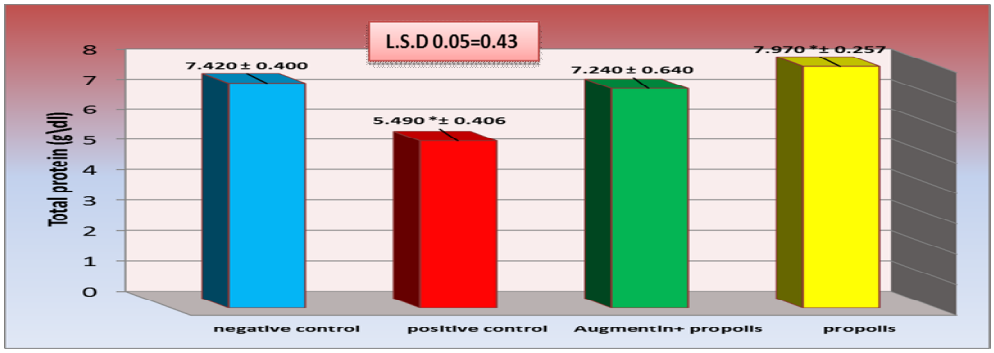
Figure(6):comparison between different groups according to the AST activity
Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



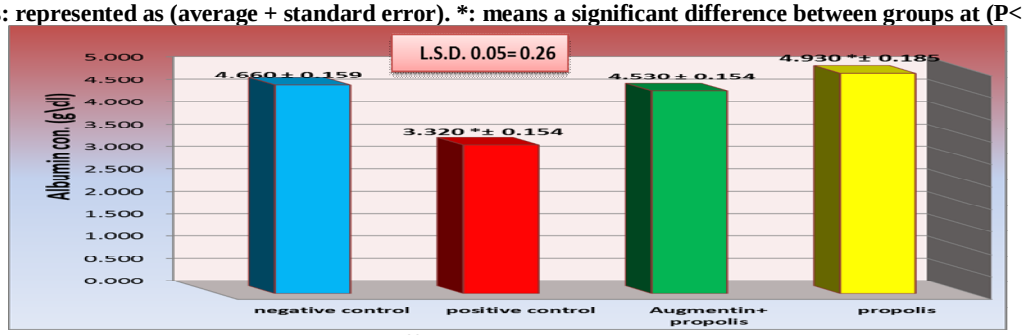
Figure(7):comparison between different groups according to the ALP activity
Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



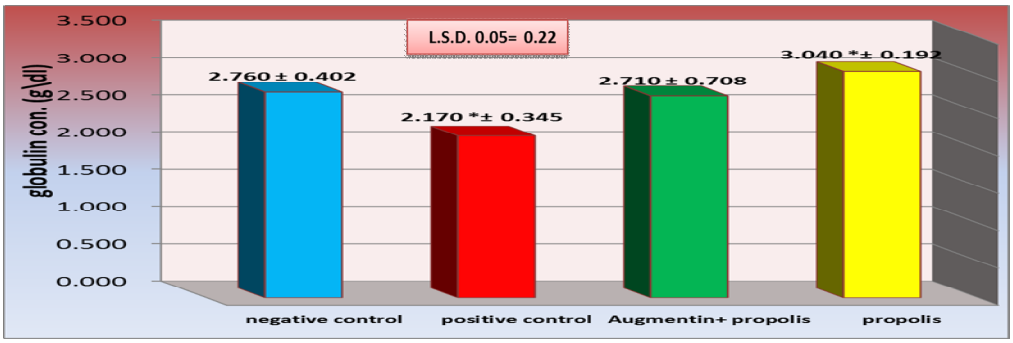
Figure(8):comparison between different groups according to the total bilirubin concentration
Values: represented as (average + standard error), *: means a significant difference between groups at (P< 0.05).



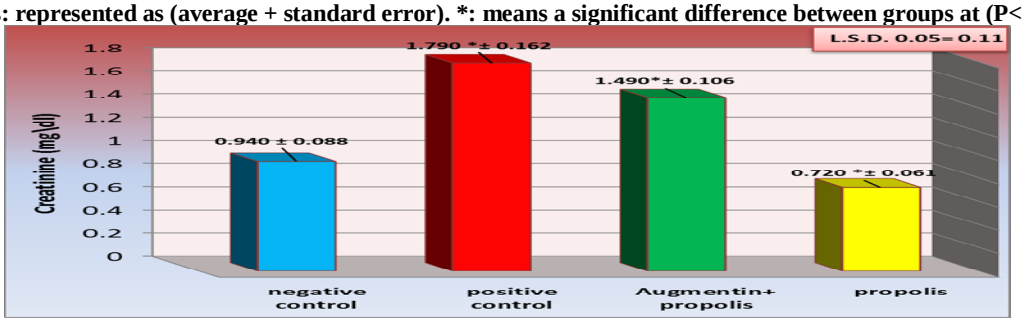
Figure(9):comparison between different groups according to the total protein concentration. Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



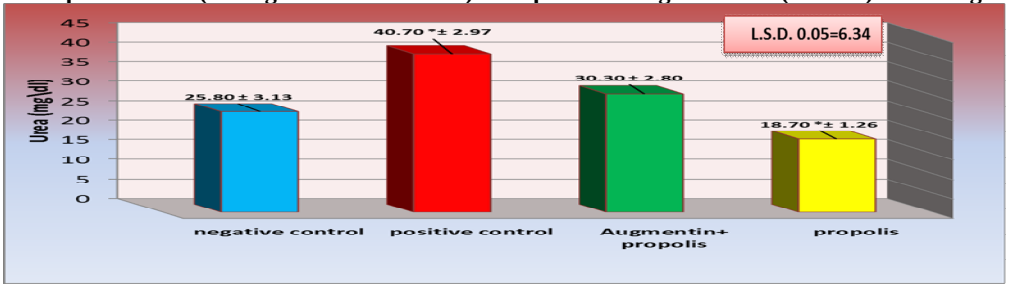
Figure(10):comparison between different groups according to the albumin concentration. Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



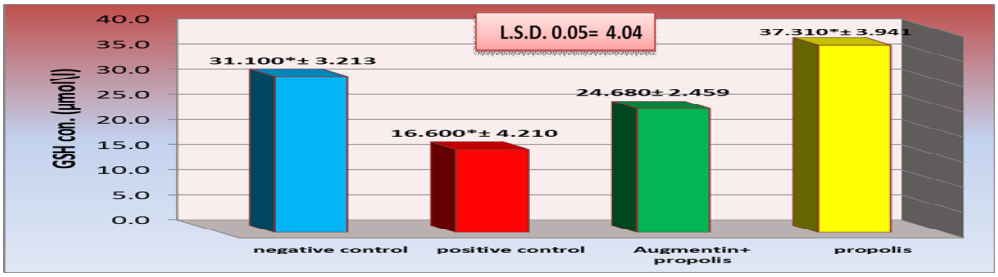
Figure(11):comparison between different groups according to the globulin concentration. Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



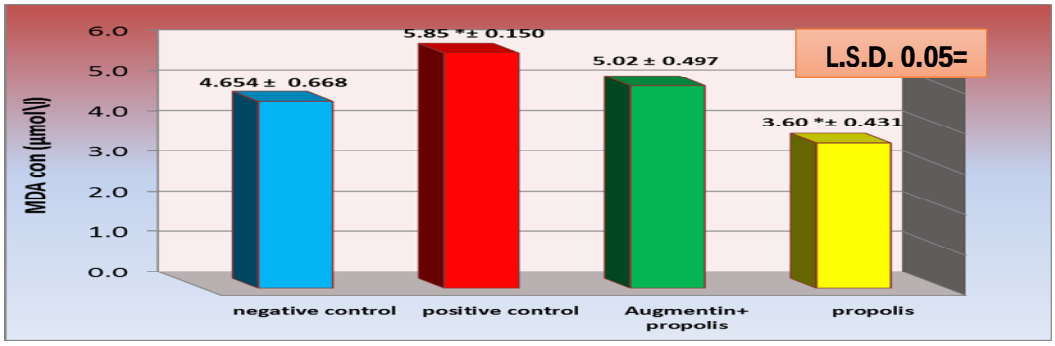
Figure(12):comparison between different groups according to the creatinine concentration. Values: represented as (average + standard error). *: represents a significant at (P< 0.05) between groups.



Figure(13):comparison between different groups according to the urea Concentration Values: represented as (average + standard error), *: means a significant difference between groups at (P< 0.05).



Figure(14):comparison between different groups according to the GSH concentration. Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).



Figure(15) :comparison between different groups according to the MDA concentration . Values: represented as (average + standard error). *: means a significant difference between groups at (P< 0.05).

5-The histological examination of liver and kidney sections at male albino rats:

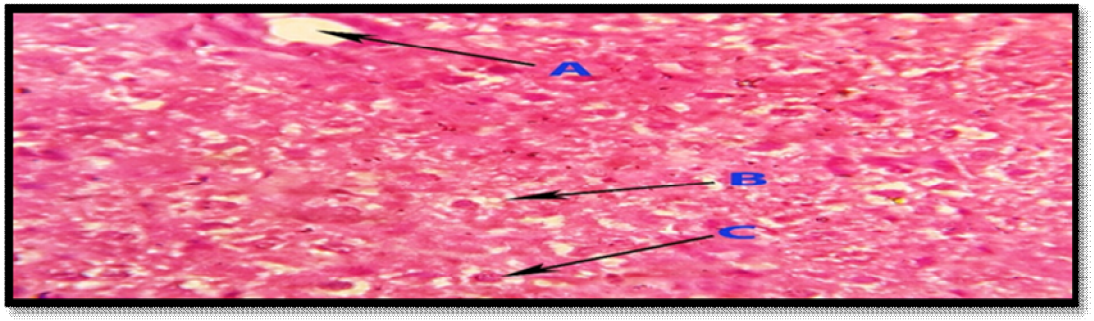


Figure (16): section in the liver at negative control group: central vein (A) , normal hepatocytes (B) , normal nucleus of hepatocytes(C) .Staining: hematoxylin – eosin (100X)

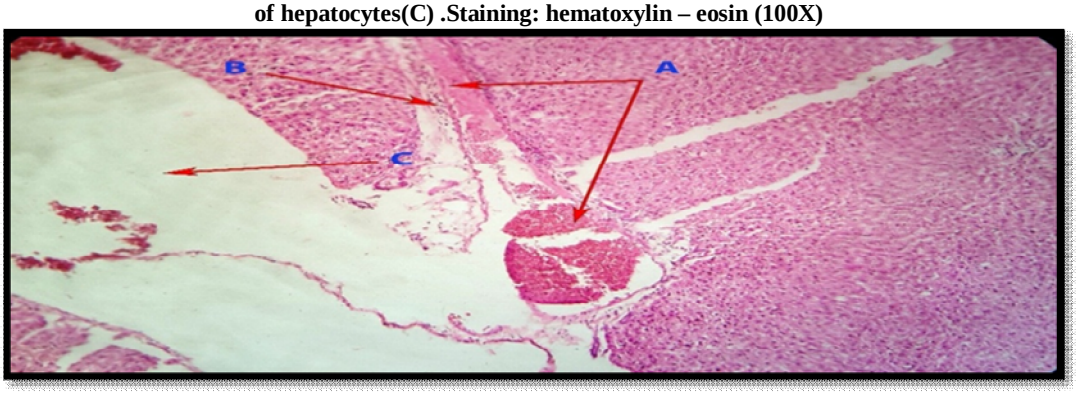


Figure (17): section in the liver at positive control group (Augmentin group): congestion and hemorrhage in the central vein (A) , aggregation of inflammation cells (B), severe degeneration of hepatocytes(C) .
Staining: hematoxylin – eosin (100X)

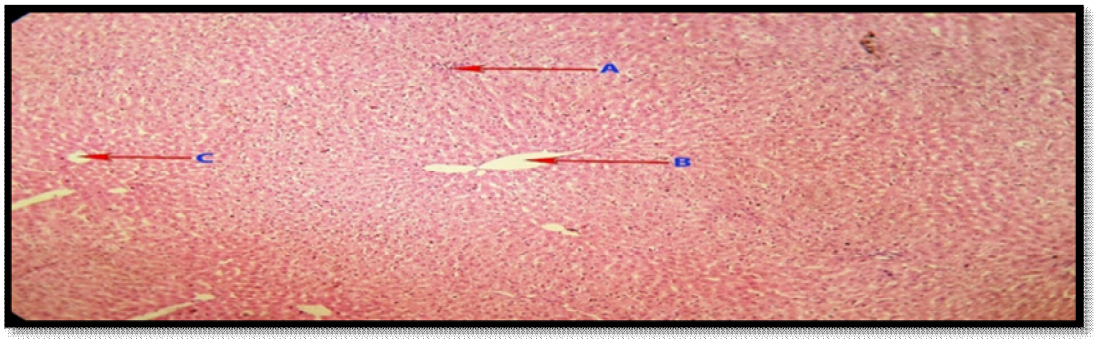


Figure (18): section in the liver at Augmentin + propolis group: mild aggregation of inflammation cells (A), mild widening of the central vein (B), normal central vein (C).
Staining: hematoxylin – eosin (100X)

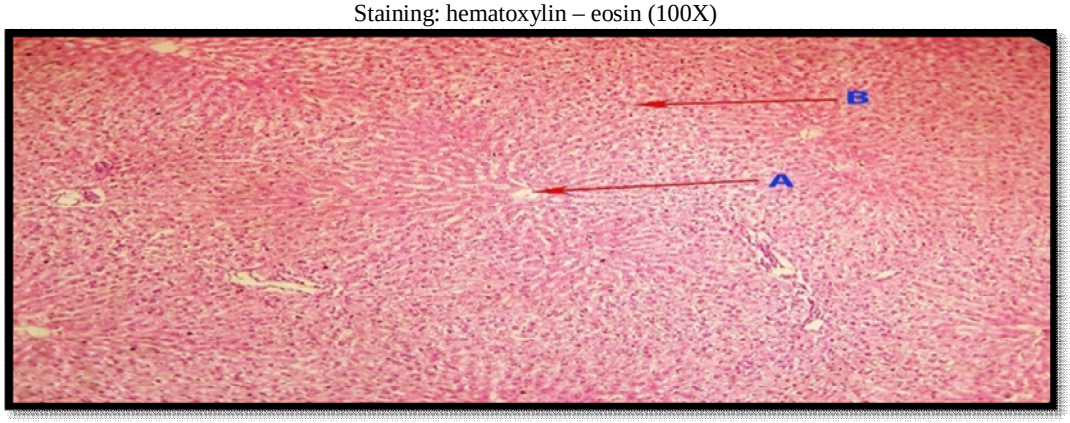


Figure (19): section in the liver at propolis treated group: normal central vein (A) , normal hepatocytes (B).Staining : hematoxylin – eosin (100X)

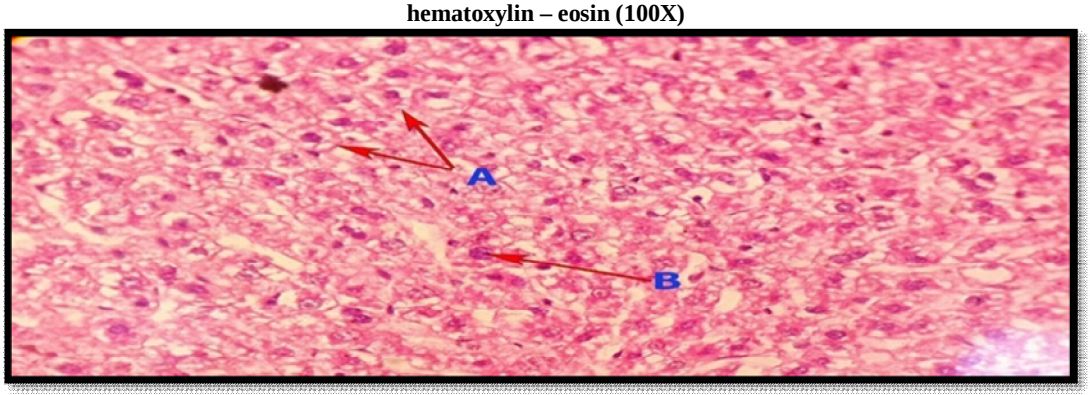


Figure (20): section in the liver at negative control group: normal hepatocytes (A) , normal nucleus of hepatocytes (B). Staining: hematoxylin – eosin (400X)

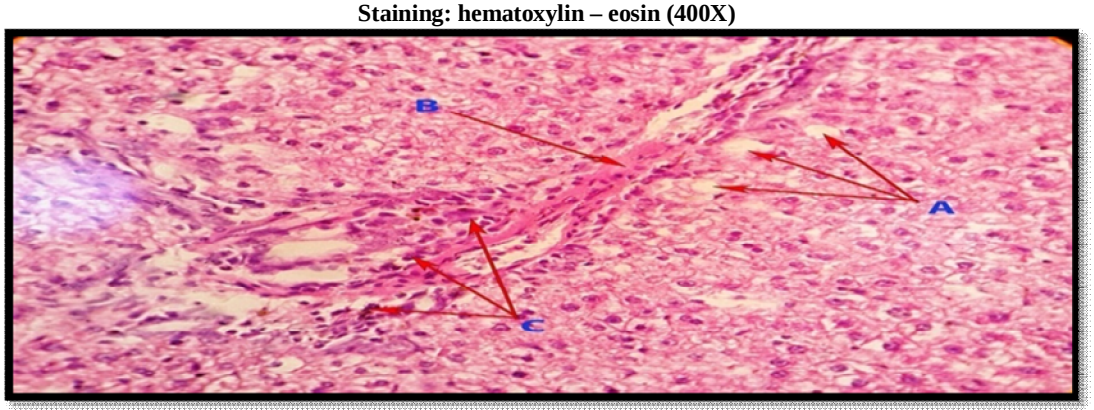


Figure (21): section in the liver at positive control group (Augmentin group) : necrosis of the hepatocytes and nuclei egression(A), hemorrhage in the central vein (B) , severe invasion of inflammation cells (C).
Staining: hematoxylin – eosin (400X)

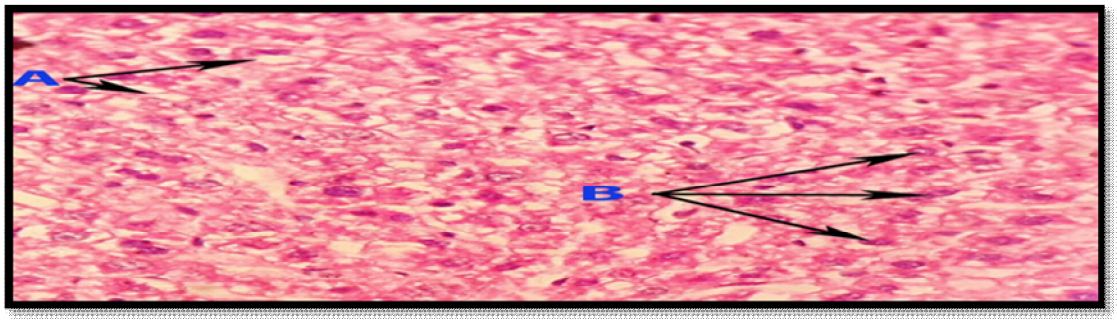


Figure (22): section in the liver at Augmentin + propolis group: mild necrosis of the hepatocytes (A), normal nuclei of hepatocytes (B).
Staining: hematoxylin – eosin (400X)

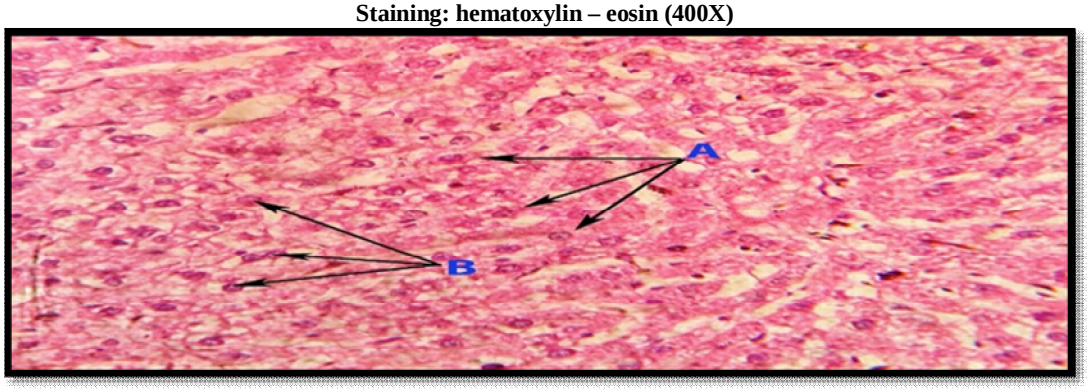


Figure (23): section in the liver at propolis group: normal hepatocytes (A) , normal nuclei of hepatocytes (B) .
Staining: hematoxylin – eosin (400X)

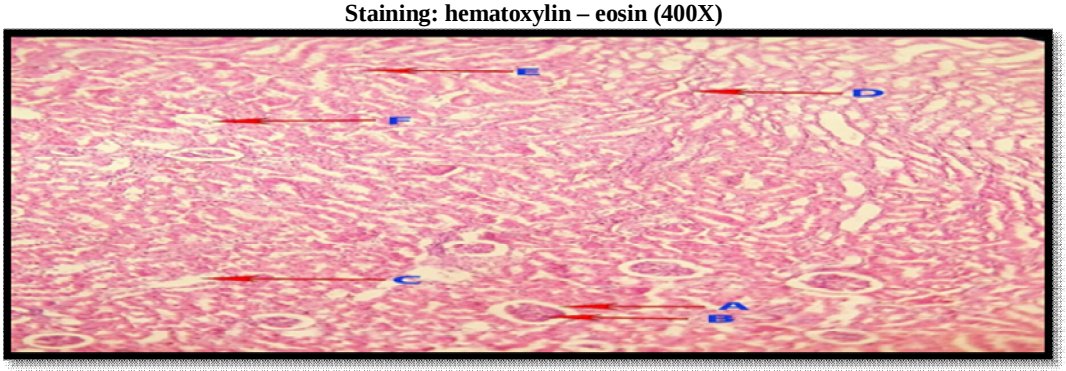


Figure (24): section in the kidney at negative control group: normal capsule of glomerulus (A) , normal renal glomerulus (B), collecting tubules (C) , arterioles of renal glomerulus (D), proximal convoluted tubules with brusher border (E) , distal convoluted tubules (F).
Staining: hematoxylin – eosin (100X)

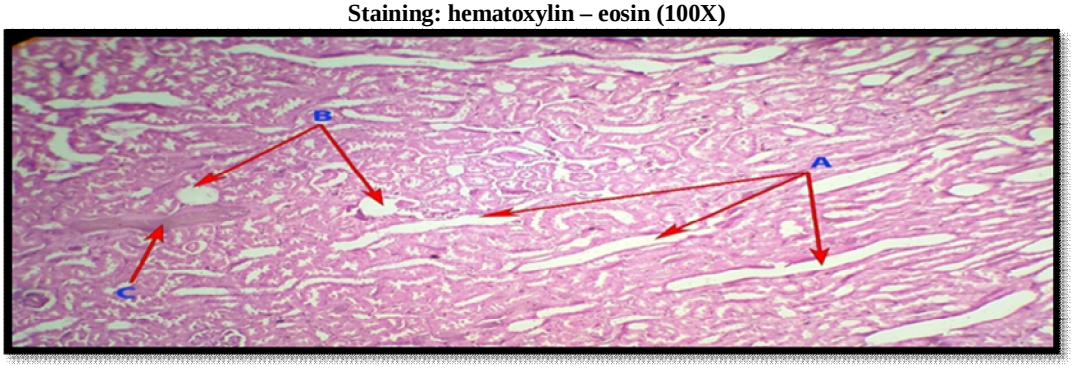


Figure (25): section in the kidney at positive control group (Augmentin group) : widening of the collecting tubules (A), severe degeneration of renal glomerulus (B), hemorrhage in the connective tissue (C).
Staining: hematoxylin – eosin (100X)

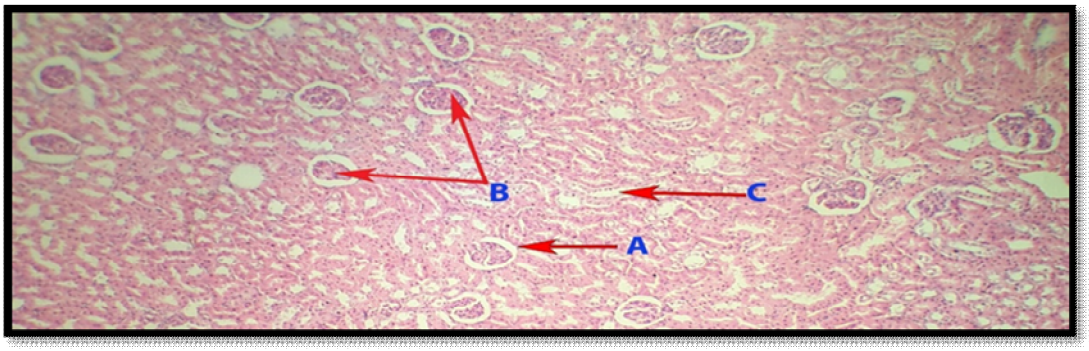


Figure (26): section in the kidney at Augmentin + propolis group: normal capsule of renal glomerulus (A), normal renal glomerulus (B), normal collecting tubules (C).
Staining: hematoxylin – eosin (100X)

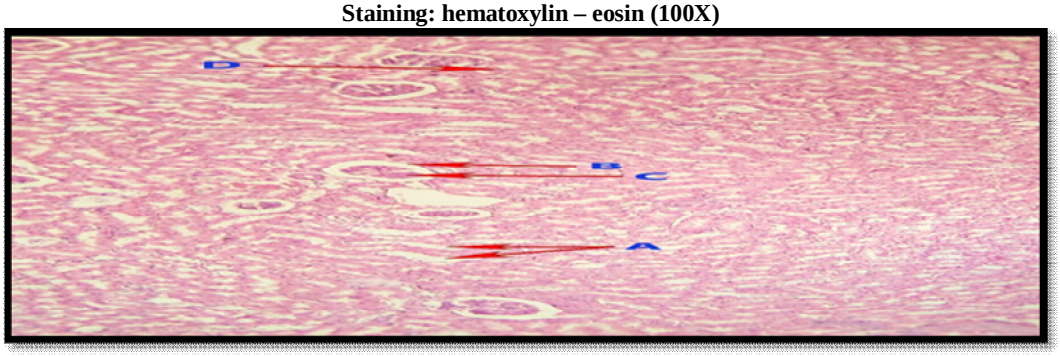


Figure (27): section in the kidney at propolis group: collecting tubules (A), capsule of renal glomerulus (B) , normal renal glomerulus (C), proximal convoluted tubules with brush border (D).
Staining: hematoxylin – eosin (100X)

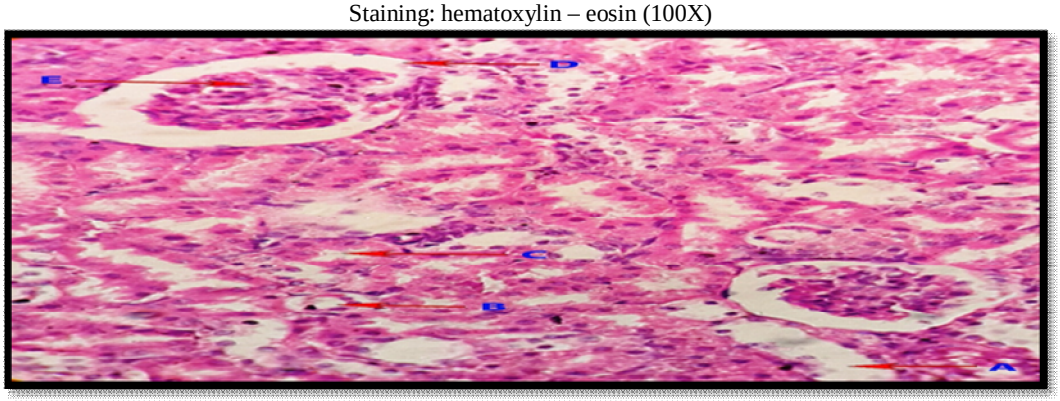


Figure (28): section in the kidney at negative control group: collecting tubules (A),normal distal convoluted tubules (B),normal proximal convoluted tubules (C),normal capsule of renal glomerulus (D), normal renal glomerulus (E).
Staining: hematoxylin – eosin (400X)

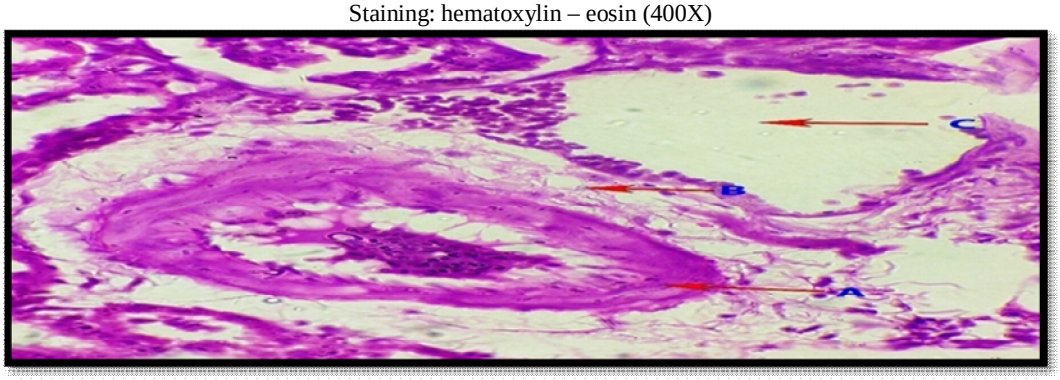


Figure (29): section in the kidney at positive control group (Augmentin group) :widening and congestion of the renal glomerulus (A) , destruction of renal glomerulus capsule (B), severe degeneration of renal glomerulus cells and nuclei egression (C).
Staining: hematoxylin – eosin (400X)

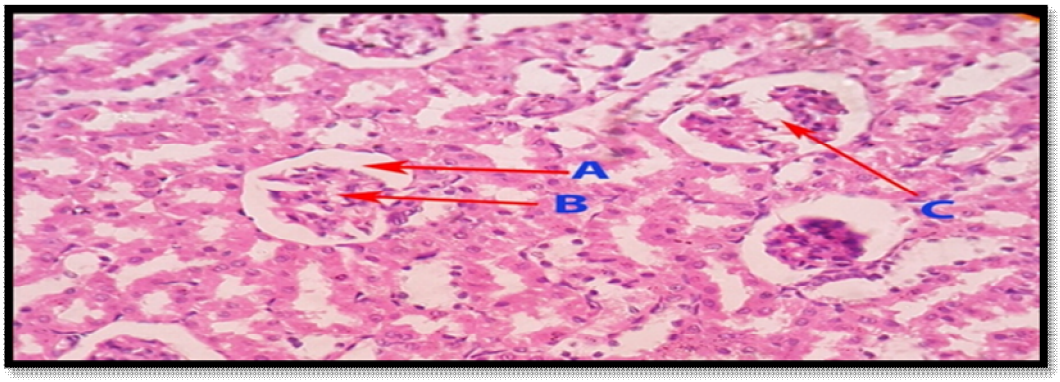


Figure (30): section in the kidney at Augmentin + propolis group: normal renal glomerulus capsule (A), normal renal glomerulus (B), mild necrosis of the renal glomerulus cells (C). Staining: hematoxylin – eosin (400X)

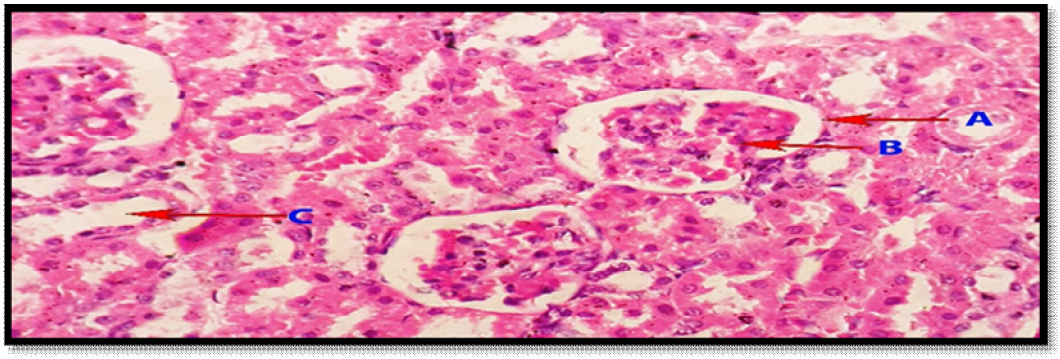


Figure (31): section in the kidney at propolis group: normal renal glomerulus capsule (A), normal renal glomerulus (B), normal collecting tubules (C).
Staining: hematoxylin – eosin (400X)

DISCUSSION:

1- The effect of Augmentin and propolis on body weight :

Treatment with Augmentin causes a significant rise in the body weight of rats in contrast with other experimental groups , this belong possibly to the Augmentin drug uptake which may stimulate the biological synthesis of: total cholesterol , triglycerides as well as low density lipoproteins and may be caused a significant increment in the body weight that was confirmed by numerous studies (Olayinka and Olukowade,2010; Olayinka *et al.,2012*),or could be occurred due to the significant rise in the weights of livers and kidneys of Augmentin treated animals that was recorded in the current study.

On the other hand, the propolis group showed a significant decrease in body weight when compared with the positive control group , this may attribute to the active chemical compounds of propolis represented by 50% resin , 30 % wax , 10 % essential and aromatic oils, 5% pollens and 5% other components including: proteins , polysaccharides and minerals (Pietta *et al.*, 2002), as well as propolis contains many phenolic constituents such as flavones , phenolic acids and phenolic acid

aceters (Falcao *et al*., 2010) which may work all together to decrease the concentrations of total cholesterol, triglycerides , low density lipoproteins significantly . Furthermore, the propolis can stimulate the synthesis of high density lipoproteins which may result in fatty acids removal from the cellular tissues and excluding it outside the body, so this appear as decrease in body weight significantly.

2- The effect of Augmentin and propolis on the liver weight

The results of current study referred to the presence of significant increase in the liver weight at positive control group as compared with the other groups , possibly this may belong to the Augmentin side influences on the histological structure of liver , Augmentin treatment causes partial or complete necrosis of some hepatocytes and destruction of the other accompanied by exodus of the nuclear contents due to lipid peroxidation that would be one of the Augmentin adverse effects , besides an accumulation of metabolic end products of this process inside the hepatic tissues , as the histological examination of rat's slides treated with Augmentin indicated to a congestion and hemorrhage in the hepatic vein, beside it's enlargement or widening . All these cellular pathogenic effects may play an important role in the remarkable increase of the liver weight.

In contrast , the both groups (Whom treated with Augmentin and propolis as well as Augmentin alone) showed a significant decrease in the liver weight as compared with the positive control group , this is probably due to its various chemical composition including vitamins: A , B1 , B2 ,B12 , and C ,also other active enzymes as Glucose 6-phosphate dehydrogenase ,moreover, the propolis contains amino acids , compound esters which composed of mono alcohols and rare minerals as K , Fe, Na, Zn ,Cu , Ca and I (Dobrowalski *et al*.,1991) , that play a major role in protecting all of the body tissues from Augmentin deleterious effects especially the accumulation of fatty acids inside the cells , so the liver weight was significantly decreased.

3- The effect of Augmentin and propolis on the kidney weight

Regarding the kidney weight ,the current study was indicated a significant rise in kidney weight at the positive control group in contrast with the negative control and other groups , this may be result of the antibiotic that used in the this study ,the Augmentin treatment causes rupture of glomerulus capsule, glomerular cells necrosis and destruction ,also the histological sections of Augmentin treated kidney tissues showed congestion inside the renal glomerulus in addition to hemorrhage in the connective tissue ,so these

pathological changes may result in the significant increase in kidney weight .

On the other side, kidney weight decrease was observed in Augmentin – propolis treated group and prpolis group in compared with the positive control group , it may attribute to the propolis contents of flavones , esters, caffeic acid , ferolic acid and other chemical components (Velikova *et al*., 2000) ,that may provide a sufficient protection for renal tissues from Augmentin lipid peroxidation which result in accumulation of damaging free radicals inside the cells ,thus the glomerular capsule remain intact as well as the glomerular cells could not enlarged and no remarkable rise in the kidney weight occurred.

4- The effect of Augmentin and propolis on AST, ALT and ALP activity in serum :

The findings of current study demonstrated a significant rise in the enzymatic activity of ALT and AST at the positive control group , other past studies have been indicated the same result (Olayinka and Olukowade, 2010 ; Olayinka *et al*., 2012),also the present study noted a significant increment in ALP activity, this may due to high Augmentin toxicity against the hepatocytes, which lead to rupture and damage of hepatocytes membranes as well as necrosis and destruction of hepatocytes that result in leakage of these enzymes into the cytoplasm then to the blood stream ,so their activities significantly rise. These enzymes are the sensitive indicators in the diagnosis of various liver diseases ,when the hepatocytes membranes destroyed these enzymes liberated into the blood stream ,so it can be used as markers to detect the kind of liver infection (Kuriakose and Kurup,2010).Other studies referred that Augmentin drug causes significant increase in the liver enzymes (ALT and AST)due to hepatocytes destruction ,in addition to other cytological effects such as liver necrosis (Macfarlane *et al*., 2000) .Another study has been suggested that, ALP enzyme is one of the highly sensitive enzymes in liver infections , liberates into the blood stream with tremendous amounts when the liver subjected to the oxidation stress (Uskokovic-Marcovic *et al* ., 2007) .

It is possible that the rise in hepatic enzymes activity caused by increasing in the concentration of malondialdehyde (MDA) at the positive control group which was recorded in the current study ,the latter is a major product of lipid peroxidation process that lead to increase free radical levels inside the hepatocytes , which in turn acting on attacking the hepatocytes to cause more damage and liver injury , especially that glutathione concentration greatly decreased in the same group , which represents the first line defense in scavenging the free radicals and excluding

it outside the living body (Gumieniczek ,2005) so the hepatocytes subjected to the detrimental effects of Augmentin.

On the other side , there was no significant difference when the Augmentin + propolis treated group compared with the negative control group , which lead to suggestion that propolis shows high efficacy in hepatocyte protection from the damage caused by lipid oxidation this may belong to the antioxidant activity of propolis extract against the different types of free radicals (Kolankaya *et al*., 2002;Selamoglu *et al*.,2015Graikou *et al*.,2016).Or may be due to its content of active chemical components as: carbohydrates, proteins, amino acids ,vitamins, flavonoids compounds ,minerals, volatiles and others (Astudillo *et al.*, 2000; Marcucci *et al*.,2001;Falcao *et al*.,2016).

5- The effect of Augmentin and propolis on total bilirubin concentration :

The results showed an increase in the level of total bilirubin in serum at the positive control group compared to other groups, it was agreed with many studies (Olayinka and Olukowade, 2010 ; Olayinka *et al*., 2012), which may be due to the toxic effects of the drug and the consequent breaking of hepatic cell membranes by the process of peroxidation of lipid and therefore increased production of different types of reactive oxygen species such as: hydrogen peroxide interactive , hydroxyl radical , radical anion of superoxide, peroxynitrite and other harmful free radicals that break down the large biological molecules like fats, proteins, carbohydrates as well as DNA bases and thus destroy the whole of liver cellular contents resulting in a significantly increase in total bilirubin level at the blood stream and this may be has actually happened in the current study.

The total bilirubin level in the serum is a sensitive diagnostic tool to detect the hepatic jaundice (Walker and Edwards, 1999). It also has many antioxidant properties, so it represents a defensive line to protect the tissues with non-processing of antioxidant systems (Temme *et al*., 2001).

Also the increment in total bilirubin concentration can attribute to increase the rate of decomposition of red blood corpuscles by Augmentin treatment for the possibility of cellular membranes necrosis of red blood corpuscles, also stopping the glycoproteins biosynthesis and therefore cell death as well as the resulting high level of total bilirubin in the blood. Or it could be interpreted as internal or external diverticulitis may occur at different places of hepatic bile duct which reflects the high level of total bilirubin.

The data of the current study also demonstrated a reduction in the level of total bilirubin at the group that treated with Augmentin + propolis and with propolis

only as compared with the positive control group and this decrease may be due to the ameliorative or the curative impacts of propolis extract content of antioxidants that have revealed high effectiveness in inhibiting the lipid peroxidation of Augmentin uptake and subsequently decrease the concentration of total bilirubin ,Dash *et al*.(2007) have been reported that the good control of total bilirubin level also the activity of alkaline phosphatase indicated to secretory mechanism improvement of the hepatocytes. Or may be explained to the efficiency of the propolis chemical components -which have been mentioned already- protect the hepatocytes also ensure continued their functional performance effectively and thus lower the level of total bilirubin in the serum significantly.

As for the decline in the level of bilirubin only at propolis treated group compared to other groups, it is possibly due to the protective and therapeutic effects of propolis, it contains ten phenolic compounds (Santos *et al*., 2003) and several studies have been postulated that flavonoids of the most effective chemical compounds in propolis that stimulate the biological effectiveness of vitamin C (Koo *et al*. , 2000), so it probably already has shown a positive action in protecting the hepatocytes and continuity of their normal functions.

6- The effect of Augmentin and propolis on total protein , albumin and globulin concentrations :

The total protein concentration was recorded a significant decline in serum at the positive control group compared to other groups, perhaps due to damaged liver tissues partly or wholly that induced by the drug treatment, and since the liver is the main manufacturer of all types of liver proteins so that damage of tissues negatively reflected on the lower level of total protein in serum. Or possibly attribute to the low serum albumin concentration that being represents the largest of total protein concentration (Al-muhammadi, 1998), so the low significant level was adversely reflected on the total protein concentration in serum.

With respect to the observed fall in serum albumin concentration may also explain to the histopathological changes noted in the liver tissues which represented ,the cellular membranes rupture, necrosis of hepatocytes ,nuclei egression , widening of central hepatic vein , congestion and hemorrhage were revealed many detrimental influences on the manufacturing process for this protein, so its concentration in the serum significantly decreased, Valero *et al.* (2016) have been demonstrated that the concentrations of plasma proteins showed a remarkable rise or reduction as a result of inflammations as well as injuries.

Or likely to be caused by the impact of the drug on renal tubules , thus may be changed the cell membranes permeability and by this way the molecules of albumin could leave the blood stream and the concentration significantly decreased in the serum.

On the other hand, the concentration of globulin showed a significant decline was registered in the group administered with the drug, and that may be suggested to many reasons including: the direct effect of the drug uptake by hepatic and renal destruction tissues which may cause an increment in deamination process of amino acids or a notable reduction in the biological synthesis of total protein molecules. While the indirect effect of the drug, perhaps by inhibiting the effectiveness of the immune system, which may be exposed to the bad influences of the Augmentin also lipid peroxidation process and subsequent major release of free radicals which may have caused damage or impairment in immune tissues as part of globulins (immunoglobulin) result from the immune system so it will probably be significantly decreased levels as a result.

Concerning to the propolis group and group of propolis + Augmentin were revealed significant increment in the concentrations of total protein, albumin and globulin, this may be because the biological efficiency of propolis, as it works to speed up the construction or manufacturing of proteins inside the cells (Gabrys *et al*., 1986), or perhaps because it contains one of the most vital and active compounds are flavones, which are soothing compounds and stimulate the growth process of new tissues as well as wound healing acceleration (Nevas and Mari , 2006), so it is likely that important properties may cause this rise has been observed in the present study.

7- The effect of Augmentin and propolis on creatinine and urea concentrations:

The findings of the current study showed a significant rise in the levels of creatinine and urea at the positive control group in compared with the rest of the other treated groups, the result agreed with most updated many studies (Olayinka and Olukowade, 2010; Olayinka *et al*., 2012),this probably explain to the renal glomerulus capsule damage , the total and partial destruction of functional units (renal glomeruli), as well as the hemorrhage inside and outside the renal glomeruli (in the connective tissue) affecting high levels of creatinine and urea in the serum , some of scientific sources indicated that the biological activity of nephron greatly reduced in the case of renal failure which could lead to significant increase in the creatinine and urea concentrations at serum as a result of low removal rate from the blood due to glomerular filtration rate reduction, so that

those standards are directories and clinically important indicators in the assessment of renal failure degree (Guyton and Hall, 2006). As regard to the significant rise in serum creatinine level, may be because erosions or, ulcers in the internal tissues of the kidney or, lower level of blood perfusion for kidney or, blockage in the lower urinary tract this is due to the influence of medicinal drugs (Cameron and Greger, 1988) and these pathological changes might be actually occurred in the current study because the Augmentin antibiotic treatment.

Or possibly attribute to a contraction or shrinkage in the mesangial cells of the glomerulus that interferes with many factors such as creation or liberation of the factor that acts as stimulator for platelets (PAF) and (Thromboxane A2) (Dos-Santos *et al*., 1991; Martinnez-Salgado *et al*., 2002), in addition to the release of different types of free radicals (Pedraza-Chaverri *et al*., 2003) and coincide with the phospholipase enzyme activation which used as biological indicator in the diagnosis of glomerulus mesangial cells shrinking and the resulting fall in activity of both glomerular filtration rate and ultrafiltration (Dos-Santos *et al*., 1991; Martinnez-Salgado *et al*., 2002), thus leading to low level filtering Kidney notably excretion and absorption processes in the renal tubules because the poisoning and death of cells (Dos-Santos *et al*., 1991).

And just the opposite of what was seen in the groups: treated with Augmentin + propolis and propolis only, as results indicated a significant decline in serum creatinine as well as urea concentrations, and probably explain it to the chemical content of alcoholic extract of propolis which is like a natural antibiotic shows no harmful effects on the body tissues for being non-toxic material (Hartwich *et al*., 2000;Morawiec *et al*.,2015), it works on repair and restoration of various body tissues, including the liver and kidney (Nevas and Mari , 2006), or because the preventive and the curative potentialities of flavonoids as has been confirmed by the medical research of Koo *et al*. (2000) in addition to the other ingredients of propolis and perhaps reflected on the Kidney's filtration performance improvement and therefore lower levels of creatinine and urea in serum.

8- The effect of Augmentin and propolis on GSH and MDA concentrations:

During this study , the glutathione level at the positive control group was significantly decreased ,that is apposite of increasing in malondialdehyde at the same group , the result of the present study showed in agreement with many studies(Olayinka and Olukowade, 2010; Olayinka *et al*., 2012) .

This may belong to the evidential scientific truth that glutathione is a preventive compound ,it is responsible for the detoxification process of various detrimental materials such as xenobiotic, free radical species and others, thus it provides the body tissues with the sufficient protection against Augmentin metabolism by acting as a reduced agent, interacts with free radicals to form complexes which lead to glutathione molecules consumption with significant decrease (Li *et al*., 2003). Glutathione represents the first line defense against different types of free radicals , acting by attacking and removal of free radical species from the living cells , low glutathione levels refer to more consumption by reactive oxygen species such as :hydrogen peroxide , radicals of alkoxy and hydroxyl which may attack the biological molecules especially the bases of DNA and also the thiol group containing compounds, Gumieniczek (2005) has been observed that glutathione level is decreased in the kidney due to peroxide free radicals removal .

Also Augmentin side effects might possibly progress to the second line defense represented by glutathione – dependent enzymes that participate in detoxification of secondary metabolites in lipid peroxidation process which in turn increase the production of free radicals and consumption of glutathione molecules subsequently ,an opposite for that exposure of rat liver to Augmentin in the current study causes significant rise in MDA levels as lipid peroxidation side product , this may be occurred as a result of membranes destruction that lining the hepatic and renal cells , besides the partial necrosis and total damage of the other cells , as well as exit of nuclei and cytoplasmic contents to the extracellular spaces which act as obvious markers to the failure of anti-oxidant defense mechanisms including the first and the second line resulting in a remarkable increase in MDA level at serum ,Jovanovic and Jovanovic (2013) reported that MDA is a good marker of local destruction of tissues as well as the liver injuries.

In relation to the Augmentin + propolis treated group ,it was revealed a notable rise in GSH level and decrease in MDA level in contrast with the positive control group, this result may refer to obvious improvement in oxidative stress standards due to antioxidants contained in propolis composition , represented by alkaloids and flavonoid compounds , various vitamins and other substances (Kolankaya *et al*., 2002) , which play a vital role in free radicals scavenging and removal outside the cells , so it showed improved and curative effects on the hepatocytes and renal tissues as well as the other tissues of the body, beside it is active contribution in tissue

protection against Augmentin toxic effect resulting in glutathione level rise as a protective system against various toxicants , this is possibly occurred in our study .

9- The effect of Augmentin and propolis on liver and kidney tissues :

Histological examination for hepatic sections at the positive control results showed occurrence of many pathological effects in the liver tissues , which seems obvious in the hepatocytes necrosis , and destruction of the other cells with egression of the nuclear material , central veins enlargement with congestion and subsequent hemorrhage , beside accumulation of many inflammatory cells due to Augmentin treatment deleterious effects in destroying the lipid molecules of hepatocyte lining membranes as a result of lipid peroxidation process , followed by continuous rise in various free radicals production , which in turn causes additional damaging influences(intracellular and extracellular) through destruction of hepatic tissue structures, attacking of macromolecules and degradation of nuclear and cytoplasmic contents , moreover, unfortunate glutathione low levels that indicate the absence of first line defense against various reactive oxygen species so their effects were duplicated , especially where GSH level decrease was accompanied with rise MDA concentrations which adversely reflected on the hepatocytes .

In contrast, the histological examination of Augmentin + propolis treated group as well as the group of propolis only were revealed the absence of negative cytological effects on the hepatic tissues ,this is possibly caused by improvement impacts of propolis , which limiting the Augmentin bad influences through maintaining of intracellular liver protein levels which possess the capability in rendering toxic compounds into intoxicants, inhibition the activity of many drugs and steroid hormones by converting the later into water – soluble compounds to make it easier in excluding from the cell of organism (Dufour, 2001) in this way , the cellular membranes remain intact with suitable permeability without subjected to the damages corresponding to Augmentin uptake ,Ashry and his colleagues(2012) have been confirmed that the propolis showed hepatoprotective effects against monosodium glutamate induced detrimental impacts at the rats. Another study also reported that the propolis revealed preventive influences against the hepatic damaging effects of N-Nitro –L-arginine methyl ester at rats (Talas *et al*.,2013).

On the other hand, the renal tissues of Augmentin treated group revealed many side effects due to Augmentin antibiotic administration including: rupture of

glomerulus capsule, partial necrosis of some glomeruli and complete destruction of the other, beside nuclear contents egression as well as hemorrhage inside the glomerulus and connective tissue also collecting tubules widening ,which are probably accompanied with Augmentin uptake as the hepatic tissue results were revealed .Regarding the Augmentin + propolis group and propolis treated group ,there is no obvious effect on the renal tissues at these groups, this indicates that Augmentin treatment coincident with propolis might provide the sufficient protection for renal tissues and increase drug renal removal as well as the filtration outside the body through improving the glomerular filtration rate due to the presence of active ingredients such as, various polyphenols , phenolic aldehydes , coumariens , amino acids , sterols and inorganic materials , some researches referred that Iraqi propolis contains: chrysin ,quercetin , galangin , apigenin as well as kaempferol ,flavonoid compounds , some acids as coumaric acid , ferulic and caffeic acids (Ali *et al*., 2012), which may possess important role in reducing Augmentin detrimental effects on renal tissues to minimal value at the Augmentin treated rats.

CONCLUSIONS:

1- The current study revealed that treatment with Augmentin (in 2.232 mg/ kg dosage) was accompanied with observation of many deleterious effects which reflected on the body weight, and the weights of some organs as liver and kidney by increasing in the weights of the mentioned organs.

2- Augmentin treatment causes negative impacts on the biochemical parameters through increasing the liver enzymatic activity (ALT, AST, and ALP), total bilirubin, malondialdehyde , urea , creatinine concentrations and opposite decrease in the total protein , albumin , globulins and glutathione concentrations.

3- Continuous treatment with Augmentin for seven days causes appearance of numerous pathological changes in the renal and hepatic tissues at male rates, despite the use of the suitable therapeutic dosage.

4- Synchronous Augmentin and propolis oral uptake showed obvious improvement in the blood biochemical standards through maintenance of hepatic enzymes (ALT, AST, and ALP), total bilirubin, urea, and creatinine concentrations in their normal values, as well as through GSH levels increasing and decrease in MDA levels.

5- Ethanolic – propolis extract revealed a protective effect for liver and kidney tissues against the Augmentin toxic effects which result in a functional ameliorative in their performance.

RECOMMENDATIONS

1.Based on this study results we recommend in limit the use of Augmentin

even though the therapeutic dose, or as recommended by the physician due to its harmful and toxic effects .

2. The continuous use of Augmentin administration must be accompanied with propolis uptake to provide an adequate protective coverage against drug side impacts for various body organs especially liver and kidney where the drug metabolized and filtered.

3. Further extensive studies for Augmentin influences on blood physiological properties including: RBCs count, hemoglobin concentration, packed cell volume, total and differential WBCs count et cetera.

4. More focusing on characterization for Augmentin treatment effects on the immune system through serum interleukins measurement

5. Studying the Augmentin influences on other body organs such as the heart, lungs, pancreas, spleen and others.

6. Detection or investigation about the Augmentin effects on blood biochemical indices, hepatic and renal tissues at female rats and comparing it with our study to diagnose drug influences on both genders.

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