DOI: 10.21608/pssrj.2023.195832.1228

Effect of Propolis and Albumin Mixture on Hepato-Nephrotoxicity Induced by Lead in Rats

Ryeaan A.M .S Abdel Rahem[1], AmgadI. M. Khedr[2], Shaimaa Hassan Negm[1], Donia Abdel Azim Mostafa[1]

[1]Home Economics Dept., Faculty of Specific Education, Port-Said University.

[2]Pharmacognosy Dept., Faculty of Pharmacy, Port-Said University.

rayyaan8@gmail.com, amged.ibrahim@pharm.psu.edu.ed, shaimaa_a_negm@yahoo.com, dmostafaa825@gmail.com.

https://pssrj.journals.ekb.eg
ISSN: 2682-325X
ISBN: 2536-9253
ORCID: 0009-0007-7388-9575
DOI 10.21608/pssrj.2023.195832.1228
Vol: 21 – Issue: 21

Effect of Propolis and Albumin Mixture on Hepato-Nephrotoxicity Induced by Lead in Rats

Ryeaan A.M .S Abdel Rahem[1], AmgadI. M. Khedr[2], Shaimaa Hassan Negm[1], Donia Abdel Azim Mostafa[1]

[1]Home Economics Dept., Faculty of Specific Education, Port-Said University.

[2]Pharmacognosy Dept., Faculty of Pharmacy, Port-Said University.

rayyaan8@gmail.com, amged.ibrahim@pharm.psu.edu.ed, shaimaa_a_negm@yahoo.com, dmostafaa825@gmail.com.

Abstract

Lead is a natural occurring element that have a high atomic weight and is wide distributed in the environment, with a high degree of toxicity that affect health. This study investigated the effect of mixtures of propolis and albumin in renal hepatotoxicity by lead (Pb) in rats. Lead toxicity was evaluated in rats, after one dose of 0.5 ml (Pb) last 12 days. 25 female albino rats were used, average weight 180±10 g, divided to 5 groups each of 5 mature rats, the first group control(g1) lead toxicated group with 0.5 ml lead(g2), group (3) fed a stable food +0.5 ml (Pb) +150mg/kg propolis+5mg albumin, group (4) fed stable food +0.5ml (Pb)+200mg/kg propolis+ 5mg albumin, group (5) fed stable food +0.5ml (Pb) +300mg/kg propolis+5mg albumin mixture treatment for 5 weeks. Blood picture, renal and liver function, antioxidant, glutathione peroxidase, albumin and FSH were investigated. The obtained results confirm the values of the propolis and albumin mixtures as hematinic potentials, improve hepato-renal functions, elevate antioxidant and while mixtures reduced FSH. It is concluded that propolis and albumin may improve health problems, confirm hematinic potential, hepato-renal function and immunity.

Key words:

Lead Intoxication, Propolis, Albumin, Health, Rats, Immunity.

تأثير خليط البروبوليس والألبومين على السمية الكبدية الكلوية المُحدثة بالرصاص في الجرذان

ريعان ع.م.س.عبد الرحيم، أمجد إ. م. خضر، شيماء حسن نجم، دنيا عبد العظيم مصطفى
قسم الاقتصاد المنزلي، كلية التربية النوعية، جامعة بورسعيد
قسم العقاقير، كلية الصيدلة، جامعة بورسعيد
rayyaan8@gmail.com, amged.ibrahim@pharm.psu.edu.ed,
shaimaa_a_negm@yahoo.com, dmostafaa825@gmail.com.

مستخلص البحث:

الرصاص عنصر طبيعي ذو وزن ذري مرتفع وانتشار واسع في البيئة، وله درجة عالية من السمية تؤثر على الصحة. بحثت هذه الدراسة في تأثير خليط من البروبوليس والألبومين في التسمم الكلوي الكبدي بالرصاص (Pb) لدى الجرذان. قُيِّمت سمية الرصاص لدى الجرذان بعد جرعة واحدة مقدارها 0.5 مل (Pb) لمدة 12 يومًا. استُخدمت 25 أنثى من الجرذان البيضاء، بمتوسط وزن 180 ± 10 جم، قُسِّمت إلى 5 مجموعات، كل مجموعة مكونة من 5 جرذان ناضجة. المجموعة الأولى (g1) مجموعة ضابطة (g2) مُسمَّمة بالرصاص بجرعة 0.5 مل (رصاص)، المجموعة (3) غُذيت بغذاء ثابت +0.5 مل (رصاص) + 150 ملجم/كجم من البروبوليس + 5 ملجم من الألبومين، المجموعة (4) غُذيت بغذاء ثابت +0.5 مل (رصاص) + 200 ملجم/كجم من البروبوليس + 5 ملجم من الألبومين، المجموعة (5) غُذيت بغذاء ثابت +0.5 مل (رصاص) + 300 ملجم/كجم من البروبوليس + 5 ملجم من الألبومين، بمزيج علاجي لمدة 5 أسابيع. تم فحص صورة الدم، ووظائف الكلى والكبد، ومضادات الأكسدة، وبيروكسيديز الجلوتاثيون، والألبومين، وهرمون .FSH أكدت النتائج المتحصل عليها قيم مزيج البروبوليس والألبومين كعاملين في تحسين وظائف الكبد والكلى، ورفع مستوى مضادات الأكسدة، بينما خفض المزيجان مستوى .FSH وخلصت النتائج إلى أن البروبوليس والألبومين قد يُحسّنان المشاكل الصحية، ويؤكدان إمكانية تحسين وظائف الكبد والكلى، وتحسين المناعة.

الكلمات المفتاحية:

التسمم بالرصاص، البروبوليس، الألبومين، الصحة، الجرذان، المناعة

Introduction

Propolis is a resinous mixture that is produced by honey bees by mixing saliva and beeswax with the combined secretions of tree buds or other plant sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is used in small openings, while large areas are filled with beeswax. The color of propolis varies according to its botanical source. dark brown is the most common color. Propolis is viscous from 20 °C, while at lower temperatures it becomes brittle and hard **(Micheal *et al.*, 2017).** The word propolis is taken from the Greek, where (propolis) denotes "at the entrance" and "polis" of the community "or" city", which means that it is a natural product used to defend the hive. Another name for it is bee glue. This name is due to its waxy nature and properties. Mechanical, bees use propolis to build and repair their hives also to close openings and cracks, to smooth the inner walls of the hive and as a protective barrier against external invaders, such as snakes, lizards, etc., or against wind and rain, and propolis also has a beneficial effect on human health. Propolis has been widely used in ancient times, especially in folk medicine for the treatment of many diseases.

The Egyptians used bee glue to embalm their dead bodies because they knew very well its properties that used as an antipyretic agent. Greek and Roman physicians used it as an antiseptic for the mouth and for healing in the treatment of wounds, prescribed for the topical treatment of skin and mucous wounds **(Vijay, 2013)**. Due to its popularity in folk medicine, propolis has become the subject of extensive pharmaceutical and chemical studies over the past 30 years. Numerous studies have demonstrated its diver's pharmaceutical activities: antibacterial, anti-fungal, anti-viral, anti-inflammatory, liver, anti-oxidant, anti-tumor, etc., this raw has been invested. For a long time by different countries in the treatment of many diseases due to its antioxidant content, in addition, propolis is used as stimulant agent for the immune system, tissue regeneration and capillary strengthening **(Mansour *et al.*, 2017)**. Most research on bee propolis has focused its efforts on identifying the chemical constituents and understanding the biological activity of whole extracts and active compounds. A lot of this has to do with identifying new compounds that have potential applications for human health. Over the years many reviews have examined this topic; She added a continuous update of the numbers of new chemical compounds that were discovered in the propolis samples they collected around the world and often included measures of biological activity. More than 300 compounds with variable biological activities were identified in the propolis samples **(Micheal *et al.*, 2017).**

Propolis is the third most important component of bee products. It consists mainly of resin 50% **(Decastro, 2001).** Phenolic compounds, esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes and alcohols are the important organic compounds found in propolis. There are also twelve different flavonoids: pinocymbrine, acacitin, chrysin, rutin, luteolin, kaempferol, apigenin, myricetin,

catechin, naringenin, galangin, and quercetin; Two phenolic acids, caffeic acid and cinnamic acid. One silybin derivative called resveratrol has been detected in propolis extracts by capillary electrophoresis. **(Huang *et al.*, 2014).** Propolis contains some minerals like Mg, Ca, I, K, Cu, Zn, Mn, and Fe in addition. It contains some enzymes such as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid propolis phosphatase contains copper 26.5 mg/kg, manganese 40 mg/kg and ash residues contain aluminum, vanadium, strontium and silicon **(Lotfy, 2006).**

Albumin is a component of blood serum as it contains large amounts of protein. Albumin is the main protein found in the blood, with another large group of proteins being globulins. Albumin is produced primarily in the liver, at a rate of about 12 g per day, and makes up 25% of the total protein production in the liver. Also, the breakdown of a large amount of albumin takes place in the liver as well, after an average lifespan of 17-20 days. Most albumin (about 60% of it) is found in body fluids outside the blood vessels, while the remaining 40% is found in blood serum. The normal serum albumin concentration (level) is 3.5-5.5 g/dL. The total level of protein in the serum is 5.5-9.0 g/dL **(Science, 2019)** and albumin has two basic functions in the human body albumin acts nonspecifically as a transport protein for numerous substances including free fatty acids, certain ions (e.g., Ca++, Zn++), bilirubin. It contributes to the oncotic pressure of plasma and maintaining the distribution of extracellular fluid between the vascular and extravascular compartments.

Albumin level in the body can be affected by a number of health conditions such as having a history of liver disease, since albumin is made in the liver. It can also be low in persons with a history of certain kidney diseases that cause protein to be lost in urine **(UNC, 2017).**

Heavy metals are naturally occurring elements that have a high atomic weight and a density five times higher than that of water. Various medical, industrial, domestic, agricultural and technological uses have lead to its wide spread in the environment; There has been growing concern about their potential side effects on human health and the environment. Its toxicity depends on various methods including the dose, method of exposure, and chemical species, as well as the age, gender, inherited characteristics, and nutritional status of those at risk. With high poisoning levels of arsenic, cadmium, chromium, lead, and mercury, they are among the oldest metals of public health importance. Accordingly, these metals have been documented as systemic toxicants known to induce many organ infections, even at lower exposures. It is also classified as a carcinogen (known or probable) to humans by the United States Environmental Protection Agency and the International Agency for Research on Cancer **(Sutton *et al.*, 2014).** Therefore, the purpose of this study was to evaluate the effect of propolis and albumin mixture on renal hepatotoxicity induced by lead in rats.

the main research **Citrasari *et al.*, 2017)** was to investigate the protective effect of the propolis extract of Apis mellifera, on the histological changes in the kidneys induced by lead acetate in mice. The results of the study showed that lead administration caused lipid accumulation, which reduced the activity of antioxidant enzymes, which can led to lipid peroxidation and cause necrosis. The proximal tubular cells suffer more from the injury as they perform the function of reabsorption, active transport, and secretory lipid oxidation resulting in the substitution of divalent cations such as Ca+2 and monovalent cations such as Na+. the influx of calcium (Ca+2) into the cytoplasm will increase cell permeability to maintain the normal organization of intracellular water. Thus, the intracellular sodium (Na+) will be increased and the water will be move through the cell membranes, causing the cells to swell. Tubal swelling can be reversible if the cause of the injury is not exaggerated. But if the cells have already crossed the point of no return, irreversible change occurs and the cell death becomes the antioxidants present in propolis, such as: CAPE, flavonoids, isoflavone and other phenolic compounds capable of modifying SOD. The SOD enzyme will remove the superoxide (O2) as free radical by covering it to H2O2. Then it will be converted into H2O and O2 by the enzyme catalase (CAT). While the enzyme GPx that causes glutathione (GR) donates its electron and combines with another molecule of glutathione to form glutathione disulfide (GSSG), the oxidized form of glutathione flavonoids also provides protection from oxidative stress by terminating the free radical chain reaction. The activities related to flavonoids and their phenolic compounds are caused by their ability to chelate metal ions and to detect free radical types, such as single oxygen, single oxide anions, hydroxyl radicals, etc. Pyrethroids are used to control a wide range of agricultural insects, vector insects, and eliminate veterinary pets by topical application. Cypermethrin (CYP) is widely used in agricultural and other applications. The aim of this study was to study the protective effect of propolis and curcumin (CUR) on amelioration of histological, structural, biochemical, and oxidative changes in their liver and kidneys induced by (CYP) in female albino rats. Biochemical changes were assessed by measuring liver function (ALT, AST, and ALP), renal function index (urea and creatinine), hepatotoxicity, histotoxicity, chemical toxicity and post administration (CYP), the results of the study showed that the use of (PRO and CUR) is beneficial for improving biochemical, histological, and infrastructure changes in the liver and kidney of rat-induced (CYP) due to its antioxidant effects. The current study demonstrated that the PRO is most effective in improving histological, histochemical, and (CYP) infrastructure changes in the liver and kidney (**Saied, 2017**). Cirrhosis is a major health problem estimated to affect more than 100 million people. This study was conducted in order to evaluate the effect of cirrhosis caused by ($CC1_4$) on immune organs and protective role of propolis in treating these negative effects. The results of that study showed that tetrachloride carbon ($CC1_4$) results in hepatotoxicity as a result of free radicals and the liver is not the only target organ of (CC14) but it also affects many parts of the body. The results of this study

showed that propolis works as a liver protection factor because it contains phenolic elements and antioxidants liver **(Ahmed, 2019).**

Antioxidants have received great attention in recent times because they have proven highly effective in preventing and treating many diseases such as cancer, vascular and cardiovascular diseases. Therefore, this study was conducted to find out the effect of the aqueous extract of propolis and pollen on the toxic effect of platinum. This is because they contain phenolic compounds that have a high efficiency in the Photoht against many causes of cancer in addition to being an anti-oxidant and targeted knowledge of the genetic toxicity of bone marrow cells and liver and kidney tissues of mice that were injected with the substance platinum in the peritoneum. The results of that study showed changes in the liver and kidneys Genetic and cellular toxicity was also evaluated by examining chromosomal aberrations in the bone marrow as a result of sepsin platinum poisoning it also showed that treatment with propolis extract led to an improvement in various histological changes in all organs. It also showed that bee gum extract has a high efficiency in resisting these genetic toxic effects in mouse marrow cells by reducing chromosomal changes and high rate of cell division coefficient **(Ahmed, 2019).** Aluminum is one of the toxic heavy metals and it is the most abundant mineral in the earth's crust. Therefore, current research has been conducted to evaluate the toxicity of aluminum chloride on female white mice and to evaluate the potential use of bee glue (propolis) in improving and preventing aluminum toxicity. Data obtained from the current study revealed that aluminum chloride causes histological and biochemical changes in the liver of mice. These histological and biochemical changes significantly increase the plasma amines (ALT and AST), alkaline phosphatase (ALP), acid phosphatase (AP), glucose, albumin accompanied by a significant decrease in the liver and tissues. These changes are attributed to tissue damage and impaired liver functions caused by Aluminum chloride has histological and biochemical changes in the kidneys in mice. These tissue changes are accompanied by a significant increase in the level of plasma creatine, urea, uric acid, cholesterol and triglycerides caused by aluminum chloride and tissue and cellular changes in bone marrow in mice. These changes include a significant increase in the number and size of fat cells, the results showed that treatment of the aluminum chloride group with propolis shows an improvement in the bone marrow in relation to fatty and cellular cells, and appears to recover the lobes of the nucleus and their size, as well as an improvement in the liver and kidney tissues **(Badr, 2004).**

Aim of the study:

Investigate the effect of mixtures of propolis and albumin on rats inductedwith Renal hepatotoxicity by (Pb) Lead.

Material and methods

Experimental animals:

The experimental female rats 25 of the albino strain were places in well-ventilated wire cages, and all animals were in a healthy condition. All rats were fed on basal diet for a week before the start of the experiment to adapt .The basal diet(gm/kg diet) consisted of 140 gm casein (>80%protein),100 gm sucrose ,50gm corn oil, 50gm cellulose, 35 gm mineral mixture, 10 gm vitamin mixture,1.8 gm L-cystine,2.5 gm choline bitartrate and the remainder is corn starch.Diet will be formulated according to **(Reeves et al.,1993)** After a period of adaptation, 25 rats were divided into 5 groups (5 rats of each) as follows:

Group (1) is fed with basal diet for 5 weeks.

Group (2) as the same of group 1 with 0.5ml of lead acetate (one dose).

Group (3) as the same of group 2 with 150mg/kg propolis from body weight+5mg of albumin (mixture 1).

Group (4) as the same of group 2 with 200mg/kg propolis from body weight+5mg of albumin (mixture 2).

Group (5) as the same of group 2 with 300mg/kg propolis from body weight+5mg of albumin (mixture 3).

At the end of the experiment rats were fasted overnight and sacrificed in order to determine blood picture, albumin also to determine liver enzymes (AST, ALT), kidney function (urea, creatinine), glutathione peroxidase, Testosterone and follicle-stimulating hormone (FSH) using commercial kits. Blood samples were put in tubes containing heparin, then centrifuged at 4000 RPM for 15 minutes, then plasma was stored at (-20°C) until analysis.

Chemical analysis:

Complete blood picture using coulter counter.

Albumin test was determined according to (**Chernecky and Berger, 2013)**

Liver function tests (AST, ALT) were determined according to **(SCE, 1974).**

Urea and creatinine were analyzed using a methods described by **(Patton and Crouch, 1977 and Henry, 1974),** respectively**.**

Testosterone and FSH were determined according to (**Wheeler، 1995).**

Glutathione peroxidase(GR) activity was determined using the method of **(Paglia and Valentinme, 1967).**

All results were expressed as means ± SD, and difference were considered statistically significant at $P \leq 0.05$.

Experimental design:

Twenty-five female albino rats were used in this experiment, weighing average 180±10g and were 3 months of age. They were bred in 5 cages (5 rats of each) in a room with a controlled temperature of (21°c) and 55% humidity and rats were kept on a 12 hr light cycle. Lead acetate was dissolved in distilled water to be given in (0.5ml dose) to rats of groups from 2 to 5. While control rats received only distilled water without lead (pb), during five weeks of the experiment research.

Results

Concerning the females, the infection decreased blood hemoglobin, hematocrit % and RBCs count, this decrease was significant in case of blood hemoglobin; and non-significant in case of hematocrit and RBCs count compared with negative control group. Similarly, both mixtures 1 and 2 significantly decreased blood hemoglobin and hematocrit % in comparison with negative control group. Again, only mixture 3 elevated blood hemoglobin, hematocrit % and RBCs count to the extent that made it insignificantly altered when compared with negative control values. **(Tables 1-3)**.

Table (1): Effect of propolis and albumin on hemoglobin of albino female rats treated with lead acetate

Groups	Hemoglobin (g/dl plama) (Mean ±SD)	MCV (fl) (Mean ±SD)	MCH(pg) (Mean ±SD)	MCHC g/dl (Mean ±SD)	RDW-cv (Mean ±SD)	RDW-SD (Mean ±SI
ve Control	16.60± 1.09^{a}	61.30±5.56^{a}	19.78±1.81^{a}	32.28±3.32^{a}	18.98±1.63^{a}	33.94±3.51
ead toxicated group	15.02 ± 1.07^{bc}	56.62±6.29^{a}	18.30±1.49^{ab}	32.32±3.50^{a}	14.98±1.09^{c}	23.96±4.04
roup 3: Mix 1	14.00 ± 1.05^{cd}	55.56±6.96^{a}	17.88±1.00^{b}	32.30±3.19^{a}	17.04±1.12^{b}	29.96±5.12
roup 4: Mix 2	13.02 ± 0.88^{d}	57.62±7.00^{a}	18.64±1.30^{ab}	32.30±2.90^{a}	17.06±1.20^{b}	27.02±4.55
roup 5: Mix 3	15.60 ± 0.99^{ab}	60.58±6.67^{a}	19.52±1.37^{ab}	32.22±3.29^{a}	17.04±1.12^{b}	28.98±4.68

Means in the same column with different superscript letters were significant at P< 0.05.

Table (2): Effect of propolis and albumin on Haematocrit activity of albino Female rats treated with lead acetate

Groups	Hematocrit % (Mean ± SD)
-ve Control	51.50 ± 6.12^{a}
Lead toxicated group	46.46 ±7.69^{ab}
Group 3: Mix 1	43.38 ±8.87^{ab}
Group 3: Mix 2	40.30±8.23^{b}
Group 3: Mix 3	48.30±9.91^{ab}

Means in the same column with different superscript letters were significant at P< 0.05.

Table (3): Effect of propolis and albumin on RBCs count activity of albino Female rats treated with lead acetate

Groups	RBCs count (Mean ± SD)
Control negative	8.38 ± 1.71[a]
Lead toxicated group (5.ml/kg Lead acetate)	8.20 ± 1.68[a]
Mix 1 (5.ml/kg Lead acetate+ 150mg/kg of Propolis of body weight + 5mg of albumin daily)	7.78 ± 1. 13[a]
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	6.98 ± 1.16[a]
Mix 3 (5.ml/kg Lead acetate+ 300mg/kg of Propolis of body weight + 5mg of albumin daily)	8.02 ± 1.60[a]

Means in the same column with different superscript letters were significant at $P< 0.05$.

Regarding to females, the situation was different, the +ve control group and the mixtures 1 and 2 significantly decrease the blood platelets count compared with negative control group. Only mixture 3 which significantly increased the blood platelets count compared with the other four groups **(Table 4)**.

Table (4): Effect of propolis and albumin on Platelets count activity of albino Female rats treated with lead acetate

Groups	Platelets count (Mean ± SD)
Control negative	**736.40 ± 15.56 [b]**
Lead toxicated group(5.ml/kg Lead acetate)	**585.20 ± 14.99 [c]**

Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	**426.20 ± 11.22 d**
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	**578.00 ± 15.02 c**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	**789.20 ± 22.48 a**

Means in the same column with different superscript lettersxfd were significant at P< 0.0

Concerning the female WBCs results, the infection and the mixture 2 showed non-significant decrease in WBCs count compared to negative control group. While groups 1 and 3 showed significant increase and significant decrease in WBCs count compared to negative control values, respectively. **(Table 5)**.

Table (5): Effect of propolis and albumin on WBCs count of albino Female rats treated with lead acetate

Groups	WBCs count (Mean ± SD)
Control negative	5.10 ± 0.92^{b}
Lead toxicated group(5.ml/kg Lead acetate)	3.92 ± 0.75^{bc}
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	8.30 ± 1.32^{a}
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	4.60 ± 0.70^{b}
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	3.40 ± 0.64^{c}

Means in the same column with different superscript letters were significant at P< 0.05.

In female rats, Neutrophil count in inducted and mixture 3 groups which recorded a significant increase in blood neutrophil count compared to negative

control group. Regarding to segmented neutrophils counts in female rats, different outcomes were recorded; the infection significantly reduced segmented neutrophils count compared with negative control group. Also, mixtures 1 and 3 significantly elevated while mixtures 2 significantly decreased segmented neutrophils count compared with lead toxicated group values **(Table 6)**.

Table (6): Effect of propolis and albumin on Neutrophil count of albino Female rats treated with lead acetate

Groups	Neutrophil count (Mean±SD)
Control negative	0.00 ± 0.00^{b}
Lead toxicated group (5.ml/kg Lead acetate)	0.98 ± 0.22^{a}
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	0.00 ± 0.00^{b}
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	0.00 ± 0.00^{b}
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	1.02 ± 0.19^{a}

Means in the same column with different superscript letters were significant at $P< 0.05$.

The female response was different, the infection and mixtures 1 & 2 induced significant increases while mixture 3 induced significant decreases in blood lymphocyte count compared with lead toxicated group values **(Table 7)** in female rats, such alterations were not observed; only the mixture 2 significantly decreased the monocyte count when compared with negative control count **(Table 7)**. Once more, the female response was dissimilar. The infection caused a significant increase in female blood eosinophils compared with negative control value. Whereas, all the 3 mixtures induced uneven significant decreases in eosinophil count compared with lead toxicated group.

Table (7): Effect of propolis and albumin on Lymphocytes count of albino Female rats treated with lead acetate

Groups	Lymphocytes count (Mean±SD)
	Female

Control negative	44.96 ± 4.77 c
Lead toxicated group(5.ml/kg Lead acetate)	59.98 ± 6.58 b
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	55.00 ± 6.54 b
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	76.98 ± 6.98 a
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	44.00 ± 4.76 c

Means in the same column with different superscript letters were significant at P< 0.05.

Concerning the female GR, the infection induced a significant decrease in GR activity when compared with that of negative control. While, all mixtures supplementations significantly increased the GR capacities compared with the GR activity in intoxicated group**(Table 8)**.

Table (8): Effect of propolis and albumin on antioxidant activity (GR) of albino Female rats treated with lead acetate

Groups	**GR (U/L serum) (M±SD)**
	Female
Control negative	610.00 ±69.15 **a**
Lead toxicated group (5.ml/kg Lead acetate)	210.02 ± 19.83 **d**
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	480.02 ± 48.73 **b**
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	300.04 ± 29.77 **c**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	510.02 ± 56.01 **b**

Means in the same column with different superscript letters were significant at P< 0.05.

As for female rats, both creatinine and urea were decreased by lead intoxication, while creatinine was significantly decreased; the urea was insignificantly decreased by lead intoxication. All used mixtures didn't induce a significant alteration in serum

creatinine and urea concentrations when compared with intoxicated group. Collectively, the least blood creatinine and urea were recorded in groups treated with mixtures 1 and 3**(Tables 9,10)**.

Table (9): Effect of propolis and albumin on creatinine of albino female rats treated with lead acetate

Groups	**Creatinine (mg/dl plama)(Mean±SD)**
	Female
Control negative	2.20 ± 0.46 **a**
Lead toxicated group(5.ml/kg Lead acetate)	1.60 ± 0.43 **b**
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	1.52 ± 0.44 **b**
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	1.90 ± 0.34 **ab**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	1.72 ± 0.42 **ab**

Means in the same column with different superscript letters were significant at P< 0.05.

Table (10): Effect of propolis and albumin on urea of albino rats treated with lead acetate

Groups	**Urea (mg/dl plama) (M ± SD)**
Control negative	71.20 ± 14.86 **a**
Lead toxicated group (5.ml/kg Lead acetate)	60.00 ±12.02 **ab**
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	54.00 ± 10.00 **b**
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	66.20 ± 11.61 **ab**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	51.80 ± 9.47 **b**

Means in the same column with different superscript letters were significant at P< 0.05.

The lead intoxication induced a significant elevation in the serum ALT levels in female rat when compared with that in the control negative group. All used mixtures (1, 2 and 3) significantly reduced the serum ALT**(Table 11)**.

Table (11): Effect of propolis and albumin on alanine transaminase (ALT) of albino Female rats treated with lead acetate

Groups	ALT(U/L plama)(M±SD)
Control negative	134.80 ± 29.06 **bc**
Lead toxicated group(5.ml/kg Lead acetate)	377.40 ± 49.53 **a**
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	108.60 ± 20.14 **c**
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	140.00 ± 19.35 **bc**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	160.40 ± 25.92 **b**

Means in the same column with different superscript letters were significant at $P< 0.05$.

The lead group recorded significant elevation in the serum AST levels in female rat when compared with that in the control negative group. All used mixtures (1, 2 and 3) significantly reduced the serum AST concentrations when compared with those in lead toxicated group **(Table 12).**

Table (12): Effect of propolis and albumin on aspartate transaminase (AST) of albino Female rats treated with lead acetate

Groups	AST(U/L plama) (M±SD)
	Female
Control negative	151.20 ± 16.81 **b**
Lead toxicated group(5.ml/kg Lead acetate)	227.60 ± 20.11 **a**
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	91.60 ± 20.82 **c**

Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	150.60 ± 18.68 **b**
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	143.40 ± 16.77 **b**

Means in the same column with different superscript letters were significant at P< 0.05.

Regarding to the blood albumin concentration in female rats, there were no significant changes between control, intoxicated and mixture 3 supplemented groups. Meanwhile, mixtures 1 and 2 showed a significant elevation in blood albumin compared with lead toxicated group**(Table 13)**.

Table (13): Effect of propolis and albumin on albumin count of albino Female rats treated with lead acetate

Groups	**Albumin(g/dl plama) (M±SD)**
Control negative	2.38 ± 0.42 b
Lead toxicated group(5.ml/kg Lead acetate)	2.52 ± 0.45 b
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	3.72 ± 0.61 a
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	3.54 ± 0.54 a
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	2.60 ± 0.23 b

Means in the same column with different superscript letters were significant at P< 0.05.

As for FSH concentration of female rats: Lead intoxication caused a significant increase in FSH compared to control group, while mixtures 1,2,3 reduced FSH due

to different on centration and mixture 3 Recorded the highest concentration **Table (14).**

Table (14): Effect of propolis and albumin on follicle-stimulating hormone (FSH) of albino rats treated with lead acetate

Groups	FSH (U/L) (M±SD)
Control negative	5.00±0.73 [b]
Lead toxicated group(5.ml/kg Lead acetate)	14.98±1.54 [a]
Mix 1 (5.ml/kg Lead acetate+ 150 mg/kg of Propolis of body weight + 5mg of albumin daily)	2.00±0.45[c]
Mix 2 (5.ml/kg Lead acetate+ 200 mg/kg of Propolis of body weight + 5mg of albumin daily)	1.98±0.44[c]
Mix 3 (5.ml/kg Lead acetate+ 300 mg/kg of Propolis of body weight + 5mg of albumin daily)	3.08±0.48[c]

Means in the same column with different superscript letters were significant at P< 0.05.

Histopathological study of liver tissue sections:

Staging and grading of liver lesions were categorized depending on the following tables **(Tables 15, 16)**.

Table (15): Modified staging: architectural changes, fibrosis and cirrhosis

Lobular architecture	Sc
Normal (absence of fibrosis)	0
Fibrous expansion of some portal areas	1
Fibrous expansion of most portal areas, with portal-portal septa	2
Fibrous extension of portal spaces with portal-portal and portal-central septa, with possible nodule formation	3
Cirrhosis with predominant nodular areas in relation to the remaining lobules	4

Table (16): Modified histological activity index (HAI) grading: necro-inflammatory scores.

A. Periportal or periseptal interface hepatitis (piecemeal necrosis)	Degree
• Absent	0
• Mild (focal, few portal areas)	1
• Mild/moderate (focal, most portal areas)	2
• Moderate (continuous around 60% of tracts or septa)	3
• Severe (continuous around >50% of tracts or septa)	4
B. Confluent necrosis	
• Absent	0
• Focal confluent necrosis	1
• Zone 3 necrosis in some areas	2
• Zone 3 necrosis in most areas	3
• Zone 3 necrosis+ occasional portal-central (P-C) bridging	4
• Zone 3 necrosis+ multiple P-C bridging	5
• Panacinar or multiacinar necrosis	6
C. Focal (spotty) lytic necrosis, apoptosis and lobular inflammation	
• Absent	0
• One focus or less per 10X objective	1
• Two to four foci per 10X objective	2
• Five to ten foci per 10X objective	3
• More than ten foci per 10X objective	4
D. Portal inflammation	
• Absent of portal lymphocytes	0
• Mild number of portal lymphocytes	1
	2

• Moderate number of portal lymphocytes Marked number of portal lymphocytes • Strongly marked number of portal lymphocytes	3 4

0 = no lesion,
1 + = less than 25% of tubules show evidence of ATI,
2+ = 25%-50% of tubules show evidence of ATI ,
3+ = 50%-75% of tubules show evidence of ATI
4+ = Complete atrophy of the tubules.

The scoring system for evaluation of glomerular changes was as follow: **

Class I: mild or nonspecific changes on light microscopy

Class II: diffuse mesangial expansion; IIa: mild mesangial expansion in > 25% of the observed glomeruli; IIb: severe mesangial expansion in > 25% of the observed glomeruli

Class III: nodular sclerosis

Class IV: advanced glomerulosclerosis, > 50% globally sclerosed glomeruli.

The interstitium was observed for presence or absence of chronic inflammation.

Histopathological study of renal tissue sections:

Renal acute tubular injury (ATI) was assessed for the following pathological changes: flattening of tubular epithelial cells, necrosis of cells, loss of brush border and apical vacuolization. Pathological changes were scored by a semi-quantitative method, with score (0 to 4+) according to the degree of renal tubular damage as following: **(Table 17)**.

Table (17): The interstitium was observed for presence or absence of chronic inflammation.

Group	Liver		Kidney		
	Grading	**Staging**	**Glomeruli**	**Tubules**	**Interstitial inflammation**
1 CF	-Hydropic degeneration and steatosis	0	Class I	0	No

	-Lobular inflammation (1 focus)….Grade 1				
2 CM F	-Hydropic degeneration. No steatosis -Lobular inflammation (2 foci) …Grade 2	0	Class I	2+	No
3 Ttt-1F	- Mild hydropic degeneration. No steatosis - Lytic necrosis (1 focus) …Grade 1	0	Class IIa	2+	Focal
4 Ttt-2F	-Hydropic degeneration. Mild steatosis - No lobular inflammation …Grade 0	0	Class IIa	1+	Focal Area of hemorrhage
5 Ttt-3F	-Hydropic degeneration and steatosis -Lobular inflammation (1 focus)….Grade 1	0	Class I	2+	No

1 CF

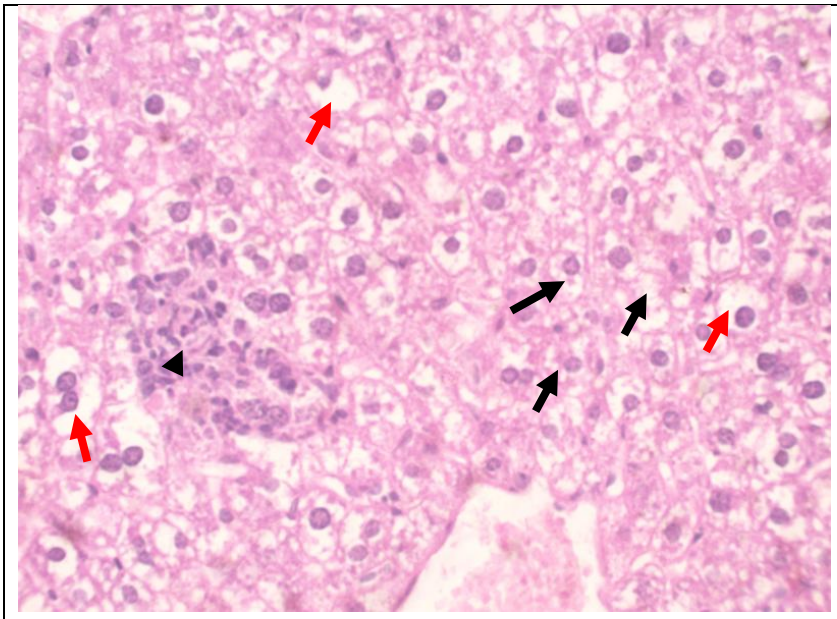


Photo (15): There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes. A focus of lobular inflammation is seen (Arrowheads) (H&E, 40x)

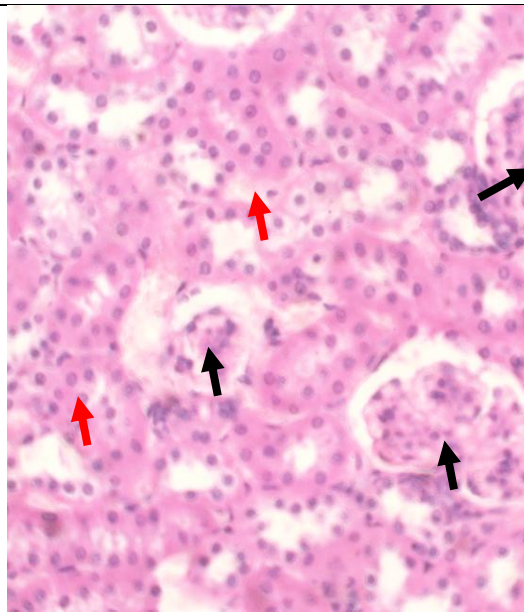
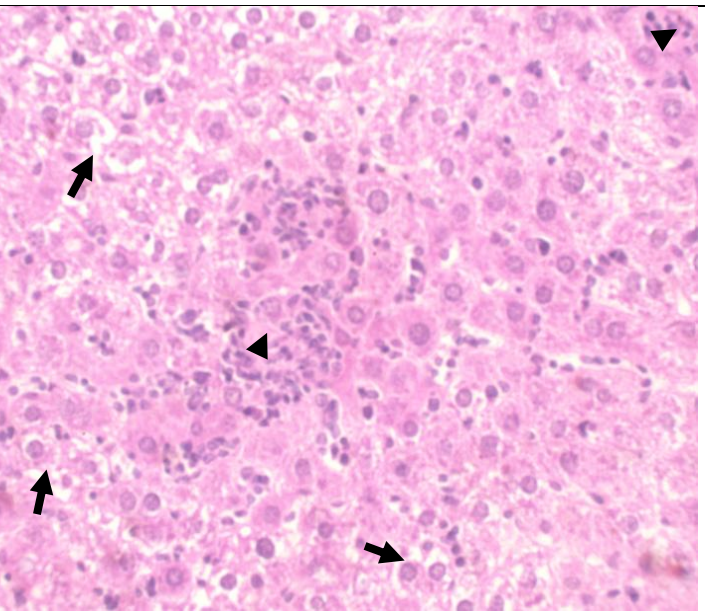


Photo (16): Uniform renal tissue with no glomerular (Black arrows) or tubular (Re (H&E, 40x)

2 CM F



19): There is hydropic degeneration (Black arrows). ci of lobular inflammation are seen (Arrowheads) 40x)

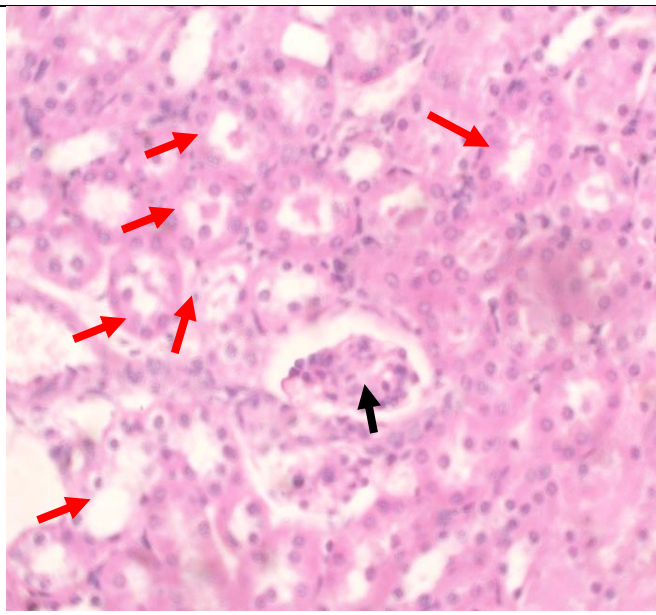
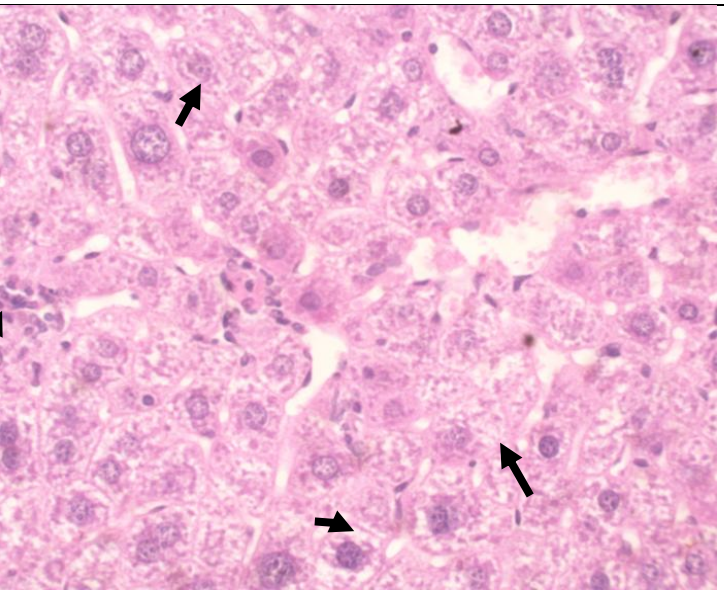
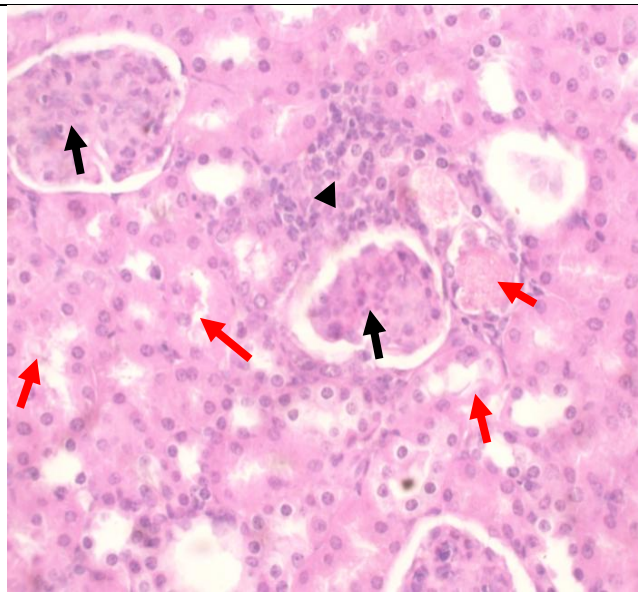


Photo (20): Glomeruli (Black arrows) are uniform no evidence of injury. Many tubules show evidenc acute injury (Red arrows) (H&E, 40x)

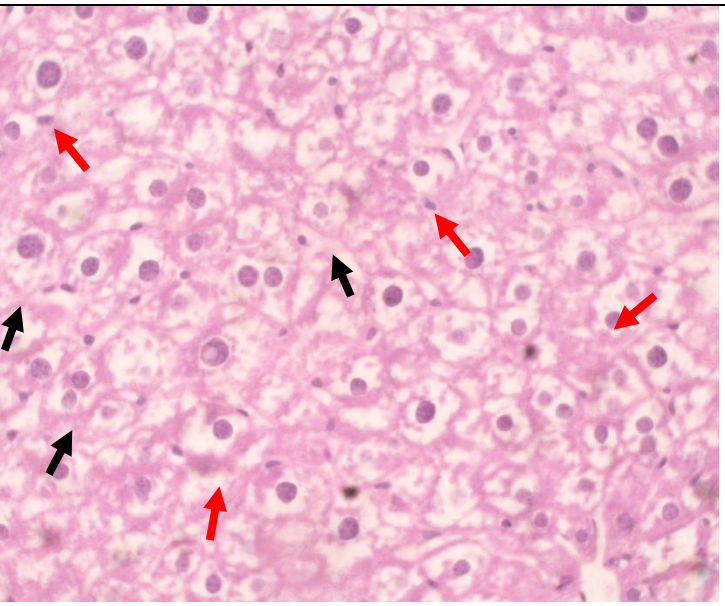
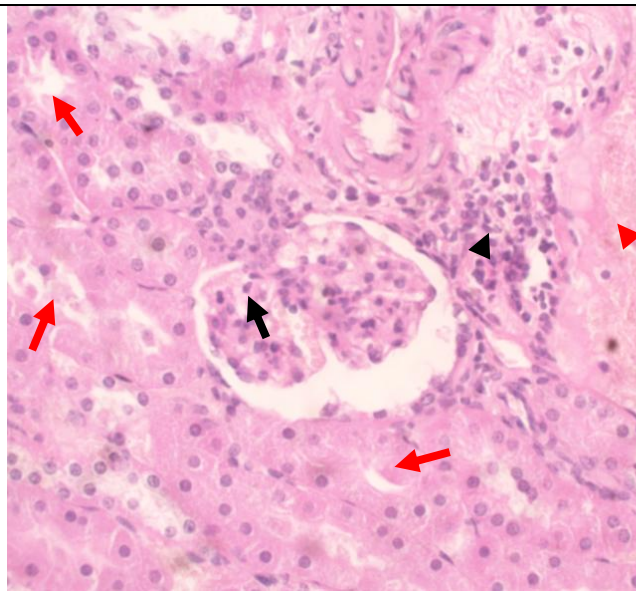
Treatment 1F



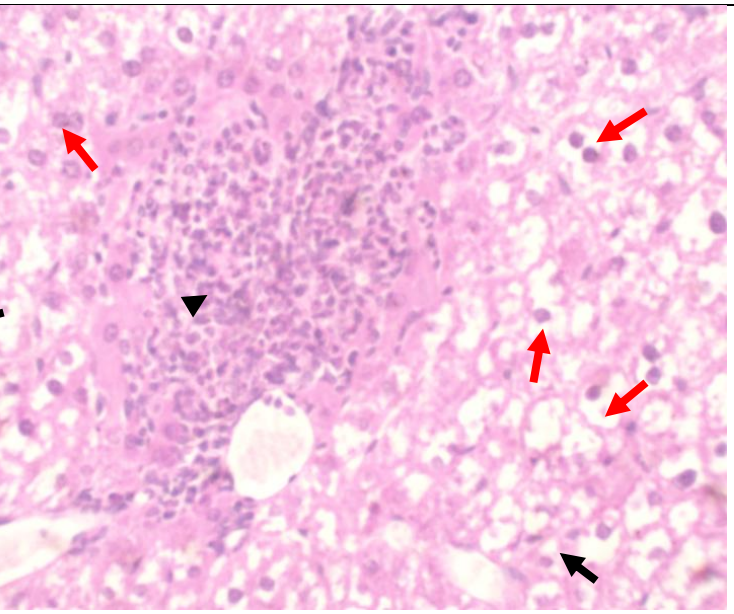


23): There is hydropic degeneration (Black arrows) and s (Red arrows) in hepatocytes. A focus of lytic necrosis (Arrowheads) (H&E, 40x)	**Photo (24):** Few glomeruli show mild mesangial expansion (Black arrows). Many tubules show ev of acute injury (Red arrows). There is a focus of interstitial inflammation (Arrowhead) (H&E, 40x

Treatment 2F

	
(29): There is hydropic degeneration (Black arrows) atosis (Red arrows) in hepatocytes (H&E, 40x)	**Photo (30):** Glomerulus shows mild mesangial expansion (Black arrow). Few tubules show evid acute injury (Red arrows). There is a focus of int inflammation (Black arrowhead) and hemorrhage arrowhead) (H&E, 40x)

treatment 3F



(31): There is hydropic degeneration (Black arrows) eatosis (Red arrows) in hepatocytes (H&E, 40x)

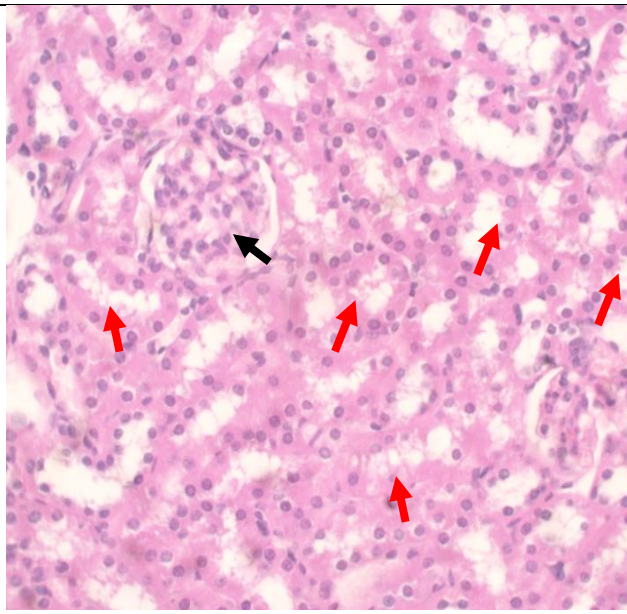


Photo (32): Glomeruli (Black arrows) are unifor with no evidence of injury. Many tubules show evidence of mild acute injury (Red arrows) (H& 40x)

Discussion

Concerning the females, the induction decreased blood hemoglobin, hematocrit % and RBCs count, this decrease was significant in case of blood hemoglobin; and non-significant in case of hematocrit and RBCs count compared with negative control group. Similarly, both mixtures 1 and 2 significantly decreased blood hemoglobin and hematocrit % in comparison with negative control group. Again, only mixture 3 elevated blood hemoglobin, hematocrit % and RBCs count to the extent that made it insignificantly altered when compared with negative control values.

Citrasari *et al.*, (2017) reported that lead may induce breakage of red blood cells of the non-significant changes of the Hb, hematocrit and RBCs may be induced due to the effect of the propolis plus albumin, which improved the blood cells number and elements leading to the return of the tested variables to normal values. Propolis has protective effect of these variables **(Mahmoud, 2006).** Some investigators reported that propolis may be used in the construction and repair of their hives, for

sealing openings and cracks and act as a protective barrier against external invaders and that propolis is an excellent healing product and provide beneficial effect on human health **(Amir, 2004; Citrasari *et al.*, 2017 and Jon, 2021)**

Table (1) showed that in case of the inducted lab animals with lead (pb) there occurred significant changes in RDW-CV and RDW-SD that indicated a great damage due to the infection with lead (pb), whereas, in case of the three mixtures used of propolis and albumin, the parameters return to the control state, indicating an improvement of the supplementation. In case of RBCs (MCV, MCH, MCHC), Table (1) revealed that in MCV, decreased significantly in inducted animals, and the three mixtures used to mean that the MCV did not return to the control volume which is a negative result. As for MCH, results indicated a lowering of hemoglobin per corpuscles after infection and supplementation which indicated a negative result, and that lead induce a high damage action. In case of the results of differential WBCs, Table (7) showed that lymphocytes increased significantly in case of the lead toxicated group, and that the mixtures of group one, two and three, induced a higher evaluation of lymphocyte that override lead toxicated group and the control group, which is an improvement of immunity, as lymphocyte produced antibodies that help destroying microorganisms due to the action of propolis and albumin **(Barrett *et al.*, 2010).**

Table (7) in female rats, Neutrophil count in inducted and mixture 3 groups which recorded a significant increase in blood neutrophil count compared to negative control group. Concerning the female WBCs results, the infection and the mixture 2 showed non-significant decrease in WBCs count compared to negative control group. While groups 1 and 3 showed significant increase and significant decrease in WBCs count compared to negative control values, respectively. These results are in accordance with **(Ahmed, 2019). Ganong, (2000)** stated that WBCs passed the ability of secreting proteolytic enzymes that induced lysis of microorganisms and elevate immunity of the body and health by combating diseases.

Regarding to females, the situation was different, the infection and the mixtures 1 and 2 significantly decrease the blood platelets count compared with negative control group. Only mixture 3 which significantly increased the blood platelets count compared with the other four groups. This result indicated that propolis and albumin supplementation may stimulate increasing platelets number that help in blood clotting and may secrete serotonin and other vasoconstrictors to constrict blood vessels **(Barret *et al.*, 2010).**

Concerning the female GR, the infection induced a significant decrease in GR activity when compared with that of negative control. While, all mixtures supplementations significantly increased the GR capacities compared with the GR activity in intoxicated group. (**Bratter *et al.*, 1999)** demonstrated that propolis may possess diverse pharmaceutical activities: included antioxidant and anti-

inflammatory effect, and **(Mansour *et al.*, 2017)** added that propolis is used as stimulant agent for the immune system, which might induce resistance to infection. **(Deniz *et al.*, 1997)** reported the mechanism of action of glutathione peroxidase in protecting RBCs against hemolysis, as oxidized glutathione is reduced to reduced glutathione (2G-SH) catalyzed by glutathione reductase, in turn reduced glutathione removes H_2O_2 from the RBCs in a reaction catalyzed by glutathione peroxidase, the enzyme acting as natural antioxidant, that attack peroxides and H_2O_2, also glutathione peroxidase affect incidence of cancers due to its content of selenium.

Tables (9,10) As for females, both creatinine and urea were decreased by lead intoxication, while creatinine was significantly decreased, the urea was insignificantly decreased by lead intoxication. All used mixtures didn't induce a significant alteration in serum creatinine and urea concentrations when compared with intoxicated group. Collectively, the least blood creatinine and urea were recorded in groups treated with mixtures 1 and 3.

The results are in accordance with **(Jarrar, 2003 and Flora *et al.*, 2006),** while in case of the mixture used number one and two a decreased variables were recorded a higher creatinine and urea concentration compared to the control group **(Murray *et al.*, 2006)** stated that the increased creatinine is recorded in acuter chronic renal insufficiency and impairment of renal function induced by some drugs. They added that the increase urea above normal in renal nephritis acute and chronic renal failure and urinary tract obstruction. They also added that decrease creatinine and urea below normal values might be due to hepatic failure or nephrosis.

The lead intoxication induced a significant elevation in the serum ALT and AST levels in female rat when compared with that in the control negative group. All used mixtures (1, 2 and 3) significantly reduced the serum ALT and AST concentrations when compared with those in intoxicated group.

Albumin is the major protein in the plasma and may comprise 60% of the total plasma protein. Liver produce about 12g of albumin/day which act as important binder to various ligands such as free fatty acids and certain steroid hormones, also it is the major determinant of plasma osmotic pressure **(Guyton and Hall, 2006).** The data presented in Table (13) indicated that albumin did not change in concentration in case of the lead toxicated group compared to the control group, while in case if the three mixtures used, there was a significant increased albumin, which might be due to the present of albumin in the different mixtures used with propolis to the administered lead (pb) in rats.

Also elevated albumin may be due to dehydration, shock, hemoconcentration or in case of intravenous administration of albumin and albumin may be increased in case of acute and chronic glomerulus-nephritis, nephrosis or chronic hepatic insufficiency or due to heavy metal administration such as lead or mercury which

may induce organ damage or can be carcinogens **(Haouas *et al.*, 2014 and Sutton *et al.*, 2014).**

As for FSH concentration of female rats: Lead intoxication induced a significant increase FSH compared to control group, while mixtures 1,2,3 reduced FSH due to different concentration and mixture 3 Recorded the highest concentration. FSH is responsible for the early growth of ovarian follicles in female, and lead acetate significantly increase FSH while mixtures reduce the hormone according the concentration.

In case of T. Testosterone concentrations result showed that no result was recorded. Which is also reported by **(ATSDR, 2021)** other natural product may decrease testosterone such as Hyphaene The baica extract. These results are also reported by **(Murray *et al.*, 2006)** and (Hatterjea, 2006), (Eman, 2019) added that propolis work as liver protector. This is also reported by **(Taib *et al.*, 2004; Sidhu and Nehru 2004).** Similar findings were attained affecting renal hepatotoxicity by heavy metals including lead, aluminum, arsenic and mercury, as these metallic elements are considered systematic toxicants that induce multiple organ damage, these histopathological changes seemed to be reflected on the serum analysis of rats and lab animals secreted by the inducted organs such as AST, ALT and urea, creatinine **(Sobhy *et al.*, 2003; Haouas *et al.*, 2014; Sultan *et al.*, 2014 and Saied, 2017)** added that lead is one of the important heavy metal poisoning, which is used in many purposes such as in building, and also used in paints and batteries. When inducted, lead affect the liver and the kidneys and is secreted in urine, they also added that organs, especially liver and kidney are the most organs affected by lead intoxication and produce many damages in these organs detected by high secretions of enzymes and production of (ROS).

Liver is one of the main important organs which may be affected by lead poisoning in treated rats, due to its storage in the liver cells, also the liver is very important organ for detoxication of toxic substances, this reflects the possible lead effects on liver hepatocytes, induce by the storage action of lead in liver **(Patrick, 2006).**

The microscopic examination of the kidneys of control group, revealed uniform renal tissues with no evidence of glomerular or tubular injury. The renal tissues were lined with simple epithelium with rounded nuclei the glomeruli were formed from glomerular tufts and bowman's capsule with clear space Photo (1), histopathology in Photo (2) show uniform glomeruli with no evidence of injury. while many tubules show evidence of acute injury. treated rat with mixture (1) show few glomeruli with mild mesangial expansion, many tubules show evidence of acute injury, and there is a focus of interstitial inflammation treated rats with mixture (2) show the glomerulus with mild mesangial expansion few tubules with evidence of

acute injury, and there is a focus of interstitial inflammation and hemorrhage .in case of mixture (3) treatment Photo. show uniform glomeruli, no evidence of injury with many tubules with mild acute injury.

liver of rats in Photo (1) shows hydropic degeneration and steatosis in hepatocytes, and a focus of lobular inflammation.

Photo (2) show there is hydropic degeneration two foci of lobular inflammation are seen Treatment rat's Photo (4) show hydropic degeneration post mixture (1) treatment and steatosis in hepatocytes, and a focus of lytic necrosis seen. Treatment with mixture (2) show a hydropic degeneration and steatosis in hepatocytes Treatment with mixture (3) show a hepatocytes degeneration and steatosis in hepatocytes kidney and liver of rats treated with lead revealed degenerative change in hepatocytes and in many tubules due to lead injury.

Together with interstitial inflammation and lobular inflammation manifested due to lead toxic effect these histopathological changes seemed to be reflected on the activities of AST, ALT and levels of urea and creatinine, and that propolis and albumin limited the toxic effect of lead in rats.

Conclusion:

The obtained results confirm the value of propolis as a natural supplement in conjunction with albumin as hematinic potentials, improve the hepato-renal functions, and structure of liver and kidney, immunity. The mixture may be of value in treatment of some health problem such as reproductive problems, and hepato-renal functions (AST, ALT, urea and creatinine) and albumin in regulation of body osmosis, with hematinic potential and phagocytic activity. While lead may be considered a strong hepatotoxic agent. However, more studies are needed to explore mechanism of lead actions.

References

1-Ahmed, E., 2019. Studying the molecular mechanisms underlying immune productive effects of propolis in albino mice Thesis, Ass. Univ. Fac. of Science, Zoology Dep.

2-Amir, H., 2004. Physiologic and histologic studies on the effect of propolis on some Organs of albino rats treated with aluminum Thesis, Sohag Univ. Fac. Of Science, Zoology Dep.

3-ATSDR, 2021. Environmental health and medicine education, physiological effects. Atsdr.cdc.gov.

4-Barrett, R., Barman, S. and Boitona, S., 2010. Ganong's review of medical physiology Mc Graw Hill, Lange, USA

5-Bratter, C., Tregel, M. and Liebenthal, C., 1999. Prophylactic effectiveness of propolis for immune-stimulation Forsch komplementarmed 6,256.

6- Chatterjea, M., Shinde, R., 2006. Text book: medical Biochemistry. JAYPEE medical publishers, New Delhi.

7- Chernecky, C.C., and B.J. Berger, 2013. Albumin - serum, urine, and 24-hour urine. In: Chernecky CC, Berger BJ, eds. Laboratory Tests and Diagnostic Procedures. 6th ed. St Louis, MO: Elsevier Saunders, 110-112.

8-Citrasari, H., Wiwik, M. and Hami, P., 2017. Protective effect of propolis in kidney mice induced by lead acetate. J of Int. dental and Med. Research 10, 1060.

9-Decastro, H.2001. Propolis: biological and pharmacological activities. Annual review of Bio medical sciences, 3, 49.

10-Deniz, S., Arzu, S. and Fegen, I., 1997. Lipid peroxidation and antioxidant status in experimental animal, Effects of aging and hypercholesterolemic diet. Clin. Chim Acta 265,77.

11-Flora, S., Flora, G., Saxena, G., 2006. Environmental occurrence, health effects and management of lead poisoning. Amsterdam, Netherland, El sevier science.

12-Ganong, W., 2000. Medical physiology. A lange medical book, USA.

13-Guyton, A. and Hall, J., 2006. Textbook of medical physiology. El sevier Saunders, USA.

14-Haouas, Z., Saliem, A. and Zidi, I., 2014. Hepatotoxic effects of lead acetate studies. J of cytology and histology 5:5

15- Henri, R., 1974. Determination of serum creatinine. Saunders, CO, London.

16-Huang, S., Zhang, C. and Wang, K., 2014. Recent advances in the chemical composition of propolis Molecules, 19,19610.

17-Jarrar, B., 2003; Histological alterations induced by lead. Ann Saudi Med. 23,10

18-Jon, J., 2021. What are the benefits of propolis? Medical news today . https://www.medicalnewstoday.com/articles/propolis

19-Mahmoud, L., 2006. Biological activity of propolis in health and disease. As. Pac. J of cancer prevention, 7,23.

20-Mansour, A., Yazum, B., Khalid, A., 2017. Ameliorative effect of propolis against hepato-renal Alteration induced by methotrexate. Int. J. Morphol 35, 764.

21-Micheal, S., Finstrom, R., Bora, M., 2017. Propolis counteracts some threats to honey bee health. Pub med. 80 20046.

22-Murray, R., Bender, D. and Weil, A., 2006. Harper's illustrated biochemistry. Lange, USA.

23-Paglia, D. and Valentinme, W., 1967. Studies on the quantitative and qualitative characterization of glutathione peroxidase. K lab clin med 70, 158.

24-Patrick, I., 2006. Lead toxicity, a review of literature Altern med review 11,2

25-Patton, C., and Crouch, S., 1997. Determination of serum urea. Anal chem. 49:464.

26-Reeve,S.P.G.,Rossow K.L.and Lindlauf J.(1993):Development and testing of the AIN-93purified diets for rodents: results on growth,kidney calcification and bone mineralization in rats and mice.J.Nutr.Vol.123,pp.1923-1931.

27-Saied, L., 2017. Histopathological, ultrastructural, biochemical studies of liver and kidney of albino rats exposed to cypermethrin and protective effect of propolis and curcumin. Thesis, Beni Suef Univ. Fac. of Science.

28- Salib, Y., Michael, H., Eskander, E., 2013. Antidiabetic properties of flavonoid isolated from Hyphaene Thebaic epicarp on alloxan induced diabetic rats. Pharmacognosy Res. 5:22.
29-SCE, 1974. Recommended method for determination of four enzymes in blood Scand J Clin Invest 33,29.
30- Science. Uo Babylon. Edu, ig. 2019, Dep. Of Chemistry.
31-Sidhu, B. and Nehru, B., 2004. Lead intoxication: histological damage in rat cerebrum, cerebellum J. trace element Exp. Med. 17, 45
32-Sobhy, H., Mohamed, E. and Mansour, M., 2003. Influence of Jojoba supplement on body gain, functions of organs Biochemical parameters and pathological alterations in rats. Kafr El Sheikh, Vet Med. J., 961, 982.
33- Sultan, J., Paul, B., Clement, G., 2014. Heavy metal toxicity and the environment. (NCBI.gov) doi: 101007/978-3.
34-Taib, N., Jarra, B. and Mubarek, M., 2004. Ultrastructural alterations in hepatic tissues of white rats induced by lead Saudi J. Biol. Sci 11,11.
35-Unc. Kidney.center. Org/files/2017/10/albumin
36-Vijay, D., 2013. Propolis a wonder bee product and its pharmacological potentials Advances in pharmacological. Sc. 2013, 308249.
37-Wheeler, M., 1995. The determination of bioavailable testosterone and FSH . Ann clin biochem 32:345.