Middle East Journal of Applied Sciences
Volume: 12 | Issue: 04| Oct. – Dec. | 2022

EISSN: 2706 -7947 ISSN: 2077- 4613
DOI: 10.36632/mejas/2022.12.4.43
Journal homepage: www.curresweb.com
Pages: 614-627

Effect of Mixture of Propolis and Albumin on Renal Hepatotoxicity by Lead (Pb) In Male Rats

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Received: 05 July 2022 **Accepted:** 22 August 2022 **Published:** 20 Dec. 2022

ABSTRACT

Lead is a natural occurring element that has a high atomic weight and is wide distributed in the environment, with a high degree of toxicity that affects 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 male albino rats were used, average weight 180±10 grm, divided to 5 groups each of 5 mature rats, the first group control(g1) infected 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 Mixure treatment for 5 weeks. Blood picture, renal and liver function, antioxidant, glutathione peroxidase, albumin and T-Testosterone were investigated. The obtained results confirm the values of the propolis and albumin mixtures as hematinic potentials, improve hepato-renal functions, elevate antioxidant and male hormone testosterone. It is concluded that propolis and albumin may improve health problems, confirm hematinic potential, hepato-renal function, immunity and male hormone production.

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

1. Introduction

Propolis or bee gum is a resinous mixture produced by honey bees by mixing saliva and beeswax with combined secretions from tree buds. Sap flows. Or other plant sources. It is used as a sealant for unwanted open spaces in the cell. Propolis is used in small gaps, while larger areas are filled with beeswax. Its color varies depending on its botanical source. With dark brown being the most common. Propolis is viscous at and above (20°C-68°F), while at lower temperatures, it becomes hard and brittle (Micheal, *et al.,* 2017). The word propolis is derived from the Greek, where (propolis) denotes "at the entrance to "and "polis for" society "or "Medina", which means that this natural product is used in hive defense. Another name of propolis is bee glue. Because of its waxy nature and mechanical properties, bees use propolis in the construction and repair of beehives – to close openings and cracks, soften interior walls and as a protective barrier against outside invaders. Such as snakes, lizards, etc., or against wind and rain, and provide a beneficial effect on human health. Since ancient times propolis has been widely used, specially in folk medicine to treat many diseases. Egyptians used bee glue to embalm their carcasses because they know very well its putrid properties that propolis is used as an antipyretic agent. Greek and Roman physicians used it as an oral antiseptic and as an antiseptic and healing product in treating wounds, prescribed for the topical treatment of cutaneous and mucosal wounds (Vijay, 2013).

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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 divers 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). The majority of research on bee propolis has focused on identifying chemical components, and understanding the bioactivity of whole extracts and active compounds. A lot of that has to do with identifying new compounds that could be used as human health applications. Numerous reviews have examined this topic over the years; constantly updating numbers of new chemical compounds that have been discovered in propolis samples collected around the world and often include measures of biological activity over 300 compounds with variable biological activities were identified in propolis samples (Micheal *et al.,* 2017).

Propolis is the third most important ingredient in bee products. It is mainly composed of resin (50%), (Decastro, 2001). Phenolic compounds, esters, flavonoids, terpenes, beta steroids, aromatic aldehydes and alcohol are the important organic compounds found in propolis. Twelve different flavonoids, they are: Pinocembrin, Acacetin, Chrysin, Rutin, Lotolin, Kempferol, Apigenin, Myricetin, Catechin, Naringenin, Galangin, and Quercetin; two phenolic acids, caffeic acid and cinnamic acid. One derivative of silipine called resveratrol was discovered in propolis extracts by capillary electrophoresis (Huang *et al.,* 2014). Propolis contains some minerals like Mg, Ca, I, K, Cu, Zn, Mn, as well as Fe. It includes several enzymes like 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 (Mahmoud, 2006).

Albumin is a component of blood serum, which contains large amounts of protein. Albumin is the main protein found in blood, and another large group of proteins are globulins. Albumin is produced in the liver mainly, at a rate of approximately 12 g per day, and it constitutes 25% of the total protein production in the liver. Also, the breakdown of a large part of the albumin takes place in the liver, too, after an average life of 17-20 days. Most of albumin (about 60% of it) is found in bodily fluids outside the blood vessels, while the remaining 40% is found in blood serum. The concentration (level) of intact serum albumin is 3.5-5.5 g/dl. The total level of protein in the serum is 5.5-9.0 g/dl, (Uobabylon, 2019) and albumin has two basic functions in the human body albumin acts nonspecifically as a transport protein for numerous substance 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 levels in the body can be influenced via many health conditions like having a chronicle of liver complaints, due to albumin being generated in the liver. It can also be minimal in people with a chronicle of kidney complaints that leads to a shortage of protein in the urine (UNC, 2017).

Heavy metals are naturally existing elements that have a high atomic weight and a density five times higher than water. The various industrial, domestic, agricultural, medicinal and technological uses have led to its wide spread in the environment; increasing worry about their prospect impacts on human health and the environment. Their toxicity relies on various ways including the dosage, way of exposure, and chemical species, in addition to the age, sex, inherited characteristics, and nutritional situation of risky persons. Due to their high levels of poisoning, arsenic, cadmium, chromium, lead, and mercury rank among the seniority metals that are of public health significance. These mineral metals are documented as systemic toxicants that are known to stimulate several organ injuries, even at a lower degree of exposure. They are also labeled as human carcinogens (known or potential) depending on the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer (Tchounwou *et al*., 2014).

Investigate the effect of mixtures of propolis and albumin on rats infected with renal hepatotoxicity by (Pb). Lead. Associated studies: the main research (Citrasari *et al.,* 2017) was to study the preservative impact of the propolis extract of *Apis mellifera*, on the histological variations in the kidneys stimulated 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 leads to necrosis. The proximal tubular cells suffer more from the damage as they perform the role 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 raise cell permeability to preserve the common organization of intracellular water. Thus the intracellular sodium (Na+) will be raised and the water will be move through the cell membranes, causing the cells to swell. Tubal swelling can be reversible if the reason of the damage is not exaggerated. But if the cells have formerly crossed the point of no return, constant variation occurs and the cell death becomes the antioxidants present in propolis, like: CAPE, flavonoids, isoflavone and another phenolic compound able to modifying SOD. The SOD enzyme will eliminate 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 (GSH) donates its electron and combines with other molecule of glutathione to form glutathione disulfide (GSSG), the oxidized form of glutathione flavonoids also provides protection from oxidative stress via terminating the free radical chain reaction. The activities related to flavonoids and their phenolic compounds are caused by their capability to chelate metal ions and to detect free radical types, like 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.

Saied (2017) demonstrated that the PRO is most effective in improving histological, histochemical, and (CYP) infrastructure changes in the liver and kidney. 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 (CC14) on immune organs and protective role of propolis in treating these negative effects. The results of that study showed that tetrachloride carbon (CC14) 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 fight 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, 2009). 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 male 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 (Amir, 2004).

The propose of this study was to study the protective impact of propolis and curcumin (CUR) on amelioration of histological, structural, biochemical, and oxidative changes in their liver and kidneys induced by (CYP) in male 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**.**

2. Material and methods

2.1. Experimental animals

The experimental 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 a standard food for a week before the start of the experiment to adapt. After a period of adaptation, 25 rats were divided into 5 groups (each group of 5 rats) as follows:

Group (1) is fed with staple food.
Group (2) is fed staple food + 0.5ml (pb) one dose.
Group (3) is fed staple food + 0.5ml (pb)+150mg/kg propolis from body weight+5mg of albumin.
Group (4) is fed staple food + 0.5ml (pb)+200mg/kg propolis from body weight+5mg of albumin.
Group (5) is fed staple food + 0.5ml (pb)+300mg/kg propolis from body weight+5mg of albumin.

Mixture treatment for 5weeks

- Blood collection: rats were sacrificed in order to determine blood pictures, albumin alalso to determine liver enzymes (AST, ALT), kidney function of urea, creatinine, glutathione peroxidase and T. Testosterone 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.

2.2. Chemical analysis

- Complete blood picture using coulter counter.
- Albumin test according to Chernecky and berger (2013)
- Liver function tests (AST, ALT) using SCE (1974).
- Kidney function (urea) using colorimetric method of Patton and Crouch (1977), creatinine using colorimetric method (Henry, 1974).
- Testosterone using kits according to Wheeler (1995) glutathione peroxidase activity using the method of Paglia and Valentine (1967).

2.3. Experimental design

25 male albino rats were used in this experiment, weighing average 180±10grm and were 3 months of age. They were bred in 5 cages of 5 rats 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 ml dose) to the infected and mixtures used rats, while control rats received only distilled water without lead (pb), during five weeks of the experiment research.

2.4. Statistical analysis

Statistical package for social science, the results were expressed as means ± SD, and difference were considered statistically significant at $P \leq 0.05$.

3. Results

Table (1) showed that lead acetate induced a significant decrease in complete blood picture, propolis and albumin mixtures showed a decreased values followed by elevated values due to increased concentration.

Table (2) showed that lead intoxication induced a significant decreased glutathione peroxidase and albumin values, while mixture of propolis and albumin led to increases glutathione and albumin in different rats.

Table (3) showed that lead intoxication led to significant elevation of liver and renal function tests, while propolis and albumin mixtures induced different degrees of decreased concentrations of variables.

Table (4) showed that testosterone concentrations decreased in the infected rats with lead, and propolis and albumin induced a gradual increased testosterone concentration due to different doses.

Table 1: Complete blood picture of rat groups

Variables	Control G1	Infected G2	Mixture 1 (G3)	Mixture 2 (G4)	Mixture 3(G5)
Hb g/dl	15.48 ± 0.47	15.02 ± 0.47	12.92 ± 0.41	15.02 ± 0.48	16.40 ± 0.51
PCV%	48.10 ± 3.14	46.60 ± 3.35	35.98 ± 2.35	46.50 ± 3.62	50.78 ± 5.24
Rbcs M/cml	8.48 ± 0.78	8.02 ± 0.72	6.56 ± 0.43	8.90 ± 0.56	9.42 ± 0.58
MCV (fl)	56.58 ± 2.68	58.12 ± 2.85	55.10± 2.94	52.14± 2.12	54.10± 2.64
MCH (pg)	18.22 ± 0.72	18.80 ± 0.65	17.72 ± 0.59	16.90 ± 0.52	17.44 ± 0.60
MCHC g/dl	32.18 ± 1.41	32.34 ± 1.52	32.22 ± 1.39	32.30 ± 1.50	32.24 ± 1.33
RDW-cv	14.68 ± 0.63	15.00 ± 0.47	16.06 ± 0.60	17.08 ± 0.55	17.02 ± 0.50
RDW-SD	24.04 ± 1.83	23.98 ± 1.41	30.00 ± 1.99	29.98 ± 1.82	31.00 ± 1.95
Wbc. t/cml	7.20 ± 0.55	3.92 ± 0.31	2.80 ± 0.20	4.30 ± 0.29	6.82 ± 0.55
Segmental%	33.00±1.55	39.00±1.90	27.00±1.45	31.02±1.26	13.98±0.69
Lymphocytes%	55.02 ±2.63	49.98 ± 2.44	60.00 ±3.21	59.98 ±2.45	78.98 ± 3.86
Monocytes%	8.02±0.30	9.98±0.37	8.98±0.35	4.00±0.18	4.02±0.17
Eosinophil%	3.96±0.17	1.00±0.09	3.00±0.13	4.00±0.09	2.00±0.09
Platelets (cml)	482.00 ± 11.92	585.40 ± 15.18	544.40 ± 14.11	650.40 ± 17.15	746.00 ± 19.01

Table 2: Presented glutathione peroxidase and albumin of different groups of rats

Variables	Control G1	Infected G2	Mixture 1 (G3)	Mixture 2 (G4)	Mixture 3(G5)
Glutathione Peroxidase mu/ml	599.98 ±30.47	420.02 ± 17.73	866.04 ± 39.33	409.96 ± 18.24	499.98 ± 24.55
Albumin g/dl	2.94 ± 0.24	2.70 ± 0.21	3.32 ± 0.25	3.46 ± 0.25	4.02 ± 0.16

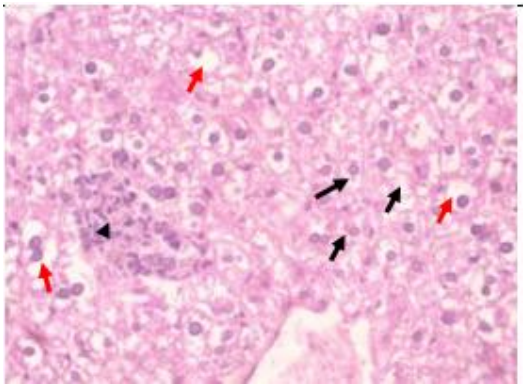
Table 3: Liver function and kidney function of rats groups

Variables	Control G1	Infected G2	Mixture 1 (G3)	Mixture 2 (G4)	Mixture 3(G5)
Creatinine	1.20 ± 0.07	2.80 ± 0.26	1.70 ± 0.16	1.48 ± 0.13	2.38 ± 0.27
Urea mg/dl	51.20 ± 2.40	66.20 ± 6.12	54.00 ± 3.96	51.40 ± 3.56	73.40 ± 6.70
GPT(ALT) IU/l	126.60 ± 12.14	133.00 ± 7.76	119.00 ± 9.98	115.40 ± 7.20	151.60 ± 10.94
GOT(AST) IU/l	146.80 ± 7.50	117.80 ± 4.73	93.80 ± 5.92	96.80 ± 5.41	146.80 ± 6.70

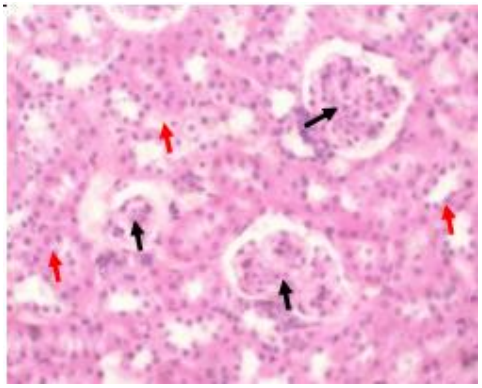
Table 4: Testosterone concentrations of the different groups of rats

Variables	Control G1	Infected G2	Mixture 1 (G3)	Mixture 2 (G4)	Mixture 3(G5)
Testosterone ng/ml	2.18±0.19	3.12±0.1	5.04±0.31	3.98±0.25	4.28±0.35

1 CF

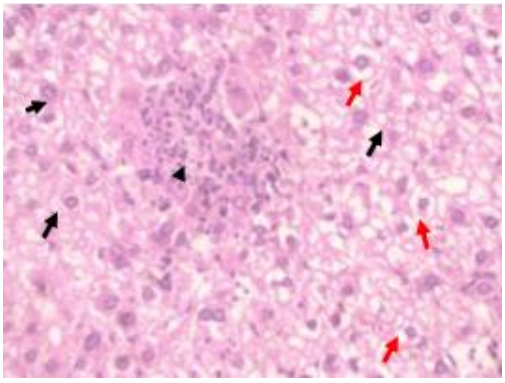


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes. A focus of lobular inflammation is seen (Arrowheads) (H&E, 40x),

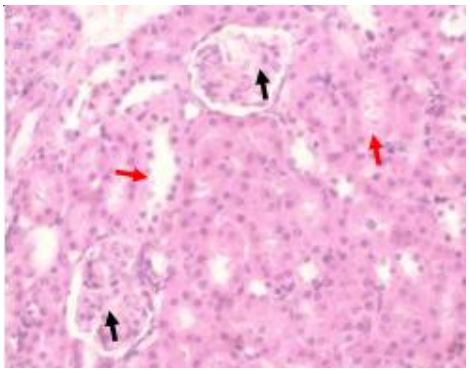


Uniform renal tissue with no evidence of glomerular (Black arrows) or tubular (Red arrows) injury (H&E, 40x)

1- 1 CL

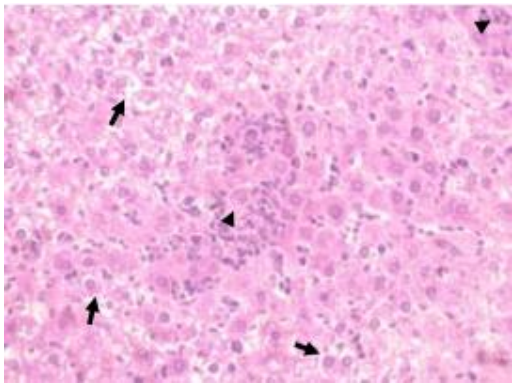


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes. A focus of lobular inflammation is seen (Arrowheads) (H&E, 40x).

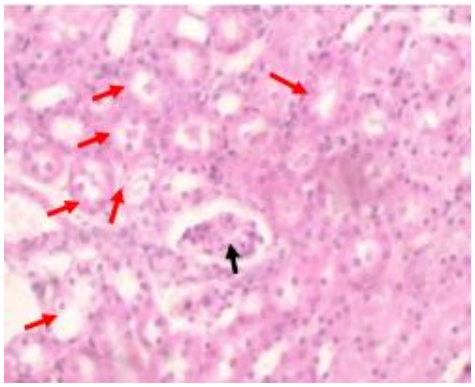


Glomeruli (Black arrows) are uniform with no evidence of injury. Few tubules show evidence of mild acute injury (Red arrows) (H&E, 40x)

2- 2 CM F

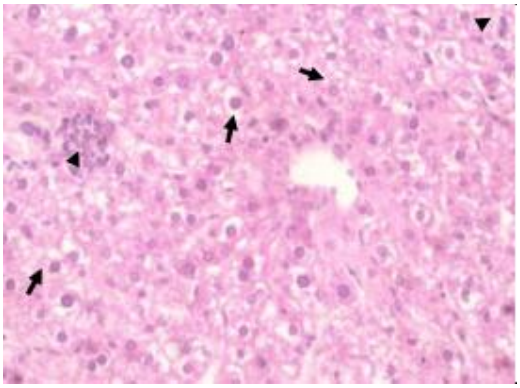


There is hydropic degeneration (Black arrows). Two foci of lobular inflammation are seen (Arrowheads) (H&E, 40x)

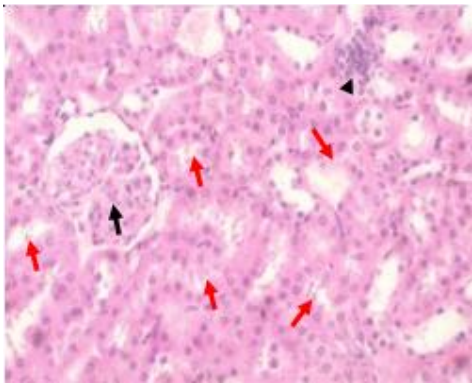


Glomeruli (Black arrows) are uniform with no evidence of injury. Many tubules show evidence of acute injury (Red arrows) (H&E, 40x).

3- 2 CM L

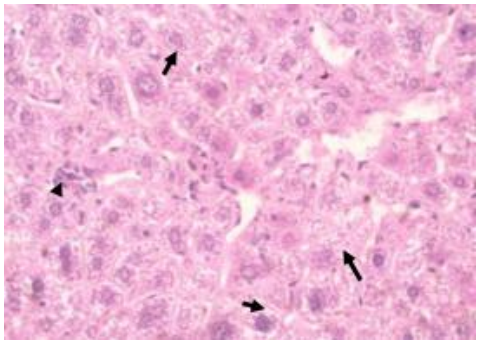


There is hydropic degeneration (Black arrows). One focus of lobular inflammation is seen (Arrowheads) (H&E, 40x).

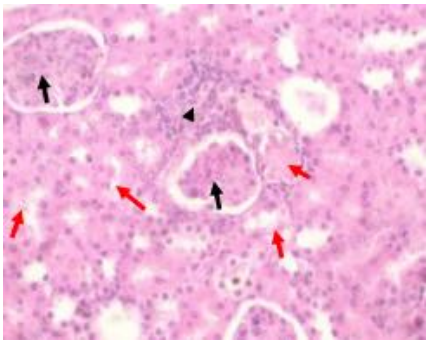


Few glomeruli show mild mesangial expansion (Black arrows). Many tubules show evidence of acute injury (Red arrows). There is a focus of interstitial inflammation (Arrowhead) (H&E, 40x).

4- 3 Treatment 1F

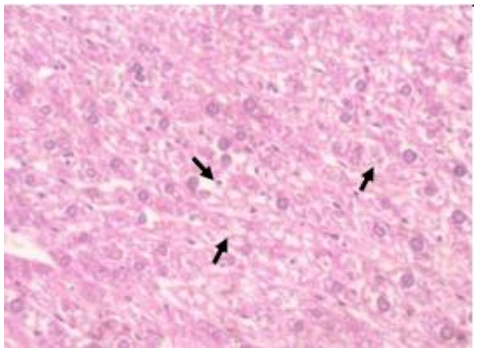


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes. A focus of lytic necrosis is seen (Arrowheads) (H&E, 40x).

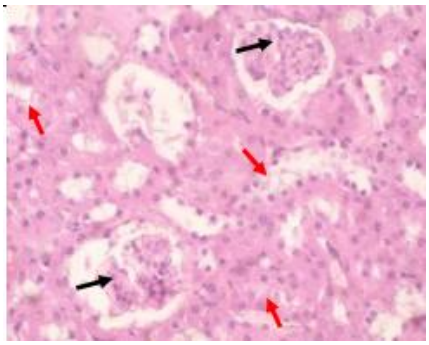


Few glomeruli show mild mesangial expansion (Black arrows). Many tubules show evidence of acute injury (Red arrows). There is a focus of interstitial inflammation (Arrowhead) (H&E, 40x).

5 3Treatment 1L

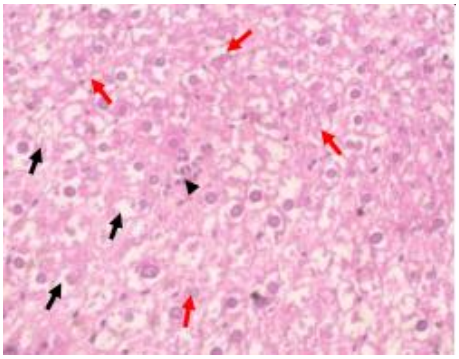


There is hydropic degeneration (Black arrows). No evidence of steatosis or lobular inflammation (H&E, 40x).

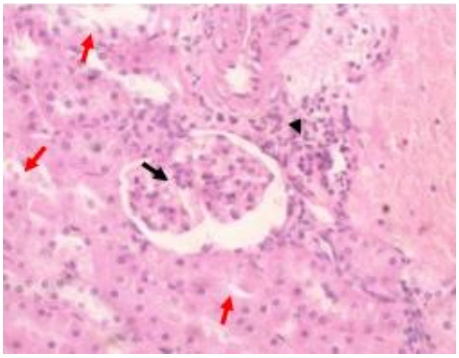


Glomeruli (Black arrows) are uniform with no evidence of injury. Few tubules show evidence of mild acute injury (Red arrows) (H&E, 40x).

6- 4 Treatment 2L

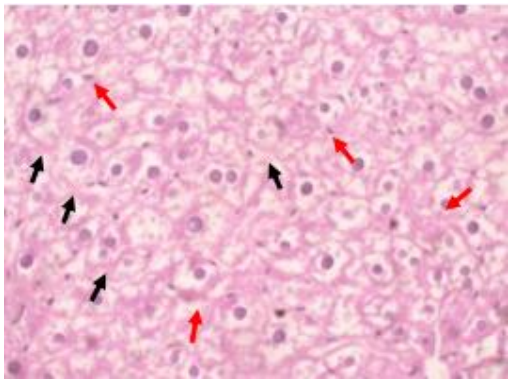


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes. One focus of lytic necrosis is seen (Arrowhead) (H&E, 40x)

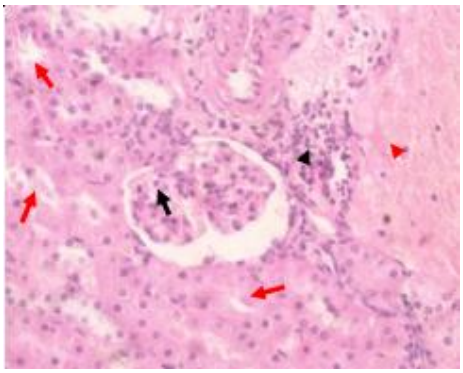


Glomerulus shows mild mesangial expansion (Black arrow). Few tubules show evidence of acute injury (Red arrows). There is a focus of interstitial inflammation (Arrowhead) (H&E, 40x).

7- 4 Treatment 2F

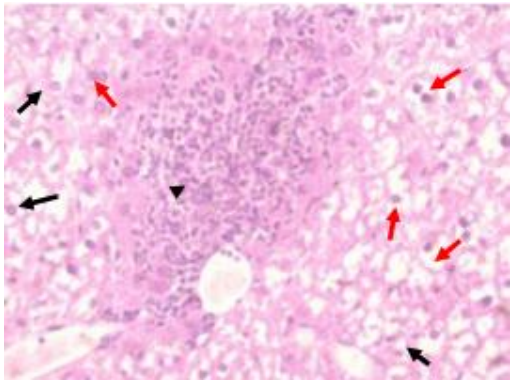


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes (H&E, 40x).

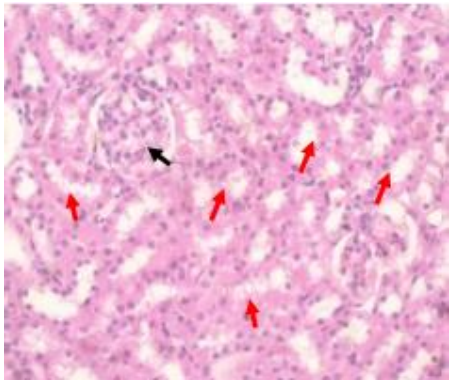


Glomerulus shows mild mesangial expansion (Black arrow). Few tubules show evidence of acute injury (Red arrows). There is a focus of interstitial inflammation (Black arrowhead) and hemorrhage (Red arrowhead) (H&E, 40x).

8- 5 treatment 3F

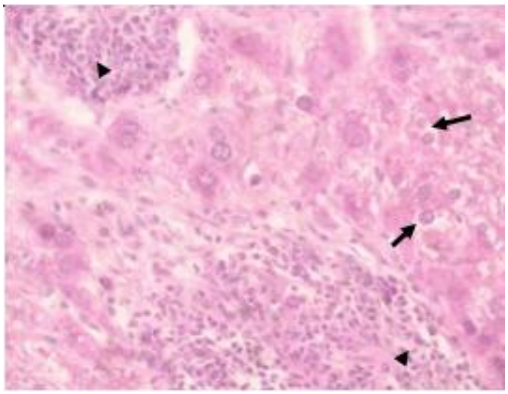


There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes (H&E, 40x).

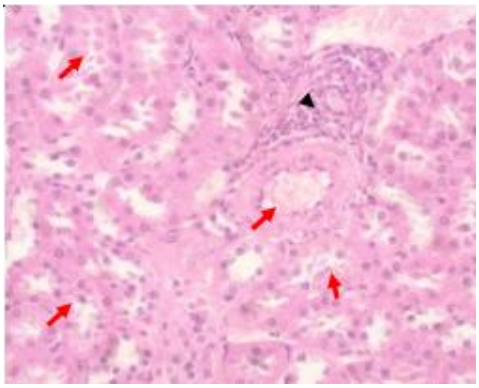


Glomeruli (Black arrows) are uniform with no evidence of injury. Many tubules show evidence of mild acute injury (Red arrows) (H&E, 40x).

9- 5 treatment 3L

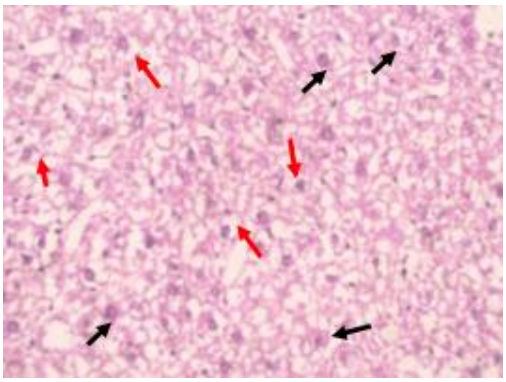


There is hydropic degeneration (Black arrows). Two foci of lobular inflammation are seen (Arrowheads) (H&E, 40x).

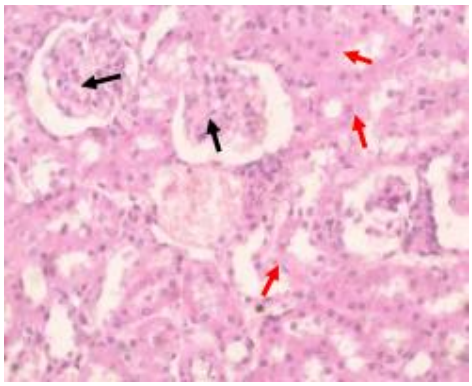


Glomeruli show no signs of injury. Few tubules show evidence of acute injury (Red arrows). There is a focus of interstitial inflammation (Black arrowhead) (H&E, 40x).

10- FCM3



There is hydropic degeneration (Black arrows) and steatosis (Red arrows) in hepatocytes (H&E, 40x).



Glomeruli (Black arrows) are uniform with no evidence of injury. Some tubules show evidence of mild acute injury (Red arrows) (H&E, 40x)

3.1. Histopathological examinations

For the sample fixation a 10% formalin solution was used for 24 hrs and then the sample was embedded in paraffin wax. Transverse sections of 3µm were prepared from all embedded paraffin wax blocks, fixed on clean glass slide, stained by hematoxylin and eosin (H&E) and investigated by pathologist.

3.2. Histopathological study of liver tissue sections:

Staging and grading of liver lesions were categorized depending on the following Tables (5, 6)

Table 5: Modified staging: architectural changes, fibrosis and cirrhosis.

Lobular architecture	Score
• 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 6: 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
• Moderate number of portal lymphocytes	2
Marked number of portal lymphocytes	3
• Strongly marked number of portal lymphocytes	4

3.3. Histopathological study of renal tissue sections

Renal acute tubular injury (ATI) was assessed for the following pathological changes (Table 8): 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: *

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 extension; IIa: mild mesangial extension in > 25% of the noticed glomeruli; IIb: severe mesangial extension in > 25% of the observed glomeruli
Class III: nodular sclerosis
Class IV: advanced glomerulosclerosis, > 50% globally sclerosed glomeruli.

Table 8: The interstetium was observed for presence or absence of chronic inflammation.

Group	**Liver**		**Kidney**		
	Grading	**Staging**	**Glomeruli**	**Tubules**	**Interstitial inflammation**
1 CF	-Hydropic degeneration and steatosis -Lobular inflammation (1 focus)….Grade 1	0	Class I	0	No
1 CL	-Hydropic degeneration and steatosis -Lobular inflammation (2 foci) …Grade 2	0	Class I	1+	No
2 CM F	-Hydropic degeneration. No steatosis -Lobular inflammation (2 foci) …Grade 2	0	Class I	2+	No
2 CM L	-Hydropic degeneration. No steatosis	0	Class IIa	2+	Focal

	-Lobular inflammation (1 focus) …Grade 1				
3 Ttt-1F	- Mild hydropic degeneration. No steatosis - Lytic necrosis (1 focus) …Grade 1	0	Class IIa	2+	Focal
3 Ttt-1L	-Hydropic degeneration. No steatosis - No lobular inflammation …Grade 0	0	Class I	1+	No
4 Ttt-2L	-Hydropic degeneration. Mild steatosis - Lytic necrosis (1 focus) …Grade 1	0	Class IIa	1+	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
5 Ttt-3L	-Hydropic degeneration and steatosis -Lobular inflammation (2 foci) …Grade 2		Class I	2+	Focal
FCM3	Hydropic degeneration and steatosis - No lobular inflammation …Grade 0		Class I	1+	No

4. Discussion

Table (1) indicated that hemoglobin (PCV) hematocrit value and Rbcs count were in the normal value in case of the control group, as for the infected group with lead (pb), it decreased significantly, and for the first mixture another lowering levels of the tested variables. In case of the second and third mixtures, there were a significant increased Hb, hematocrit and Rbcs count. 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.

And induced propolis 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 (Jon, 2021; Amir, 2004; Citrasari *et al*., 2017).

Table (1) showed that in case of the infected 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, increased significantly in infected animals, and the Mixture one and two significant decreased, MCV, MCH and MCHC, while Mixture three increased significantly. In case of the results of differential Wbcs, Table (1) showed that lymphocytes decreased significantly in infected group with lead, and that the mixtures of group one, two and three, showed significant increase lymphocyte, 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 (1) showed that infected group significantly increased segmented neutrophil compared to control group, and another decreased level of segmented neutrophil was noted in cases of the mixtured group one, two and three, indicating a lesser effect against inflammation due to propolis and albumin administration.

Table (1) showed a significant decreased white blood corpuscles in case of the infected group, while in mixture groups one, and two decease was reported. Mixture three showed a significant increase Wbcs numbers, indicating that the propolis and albumin administrations improved the numbers of Wbcs, and hence an improvement in immune response that approaches the data of the control group, which indicated an improvement in health. These results are in accordance of that of (Ahmed *et al.,* 2019). Ganong (2000) stated that Wbcs possed the ability of secreting proteolytic enzymes that induced lysis of microorganisms and elevate immunity of the body and health by combating diseases.

Platelets as reported in Table (1) showed tendency to a significant increased number in case of the infected group. Also another increased level in case of the first, second and third mixtures. This result indicated that propolis and albumin supplementation may stimulate increasing platelets number that help in blood clotthing and may secrete serotonin and other vasoconstrictors to constrict blood vessels (Barret *et al.,* 2010).

Glutathione peroxidase (Table 2) showed a significant decreased concentration in the infected group, followed by a significant elevated concentration in case of the 1st and 3rd mixtures groups'. While the second mixture groups showed a significant decreased glutathione peroxidase compared to the control group. The different in glutathione peroxidase results may be due to the difference in concentrations of propolis and albumin.

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 (G S S G) 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. Albumin is the major protein in the plasma and may comprise 60% of the total plasma protein. The 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 (2) indicated that albumin decreased in concentration in case of the infected 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 glomeruls-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; Tchounwou *et al.,* 2014).

The effect of propolis and albumin on renal toxicity, indicated by renal function of creatinine and urea, Table (3) showed that in case of the infected rat with lead, there was a significant increase in creatinine and urea concentration compared to the control group. The results are in accordance with (Flora *et al.,* 2006; Jarrar, 2003) 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 Mixture three showed increased concentration of urea and creatinine. Murray *et al.,* (2009) 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.

In case of the results of liver function test (Table 3) GPT increased significantly in case of infected group and GOT decreased with lead, which indicated an elevation of the enzymes in the blood and necrosis or disease of the liver and the elevate GPT more than GOT recorded may be caused due to acute infectious hepatitis. The three mixtures show the same results as kidney.

These results are also reported by Murray *et al.,* (2009); Ahmed, 2019) added that propolis work as liver protector.

This is also reported by Taib *et al.* (2004) and Sidhu *et al,* (2004). Similar findings were attained affecting renal hepatotoxicity by heavy metals including lead, aluminum, arsenic and mercury, as these mineral elements are documented as systematic toxicants that stimulate several organ injuries, these histopathological changes seemed to be reflected on the serum analysis of rats and lab animals secreted by the infected organs such as AST, ALT and urea, creatinine (Sobhy *et al.,* 2003; Saied, 2017;Tchounwou *et al.,* 2014; Haouas *et al.,* 2014) 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 infected, lead affect the liver and the kidneys and is secreted in urine, they also added that organs, specially liver and kidney are the most organs affected by lead intoxication and produce many damage in these organs detected by high secretions of enzymes and production of (ROS).

The 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 reflect the possible lead effects on liver hepatocytes, induce by the storage action of lead in liver (Patrick, 2006).

Table (4) showed that T. Testosterone concentrations significantly increased in case of the infected and mixtures one, two and three compared to control rats. While a gradual increased levels of T. Testosterone concentrations in case of mixture 1,2 and three, which indicated that propolis added to albumin may led to increase male sex hormone levels in relation to male rats. This result was also reported by researchers that propolis plus albumin stimulate the release of male sex hormone (ATSDR, 2021) other natural product may increase testosterone such as Hyphaene Thebaica extracts (Salib *et al.,* 2013).

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, immunity and productive hormone testosterone.

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. Histology study revealed that there are inflammation of lobule of liver and glomerula of kidney post lead administration, while reduce post mixed treatment according to doses.

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