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## Study the Effect of Propolis and Beeswax on Liver Disorder in Carbon Tetrachloride-Induced Hepatic Rats

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**Abstract:**

The present study was designed to study the effect of propolis and bees powder on hepatic rats. Forty-eight adult male albino rats were used in study, their weighting were (150±10g) divided into eight groups, sex rats e One of them they were kept as a positive control –ve group, while the o seven groups were injected by Carbon Tetrachloride (CCl<sub>4</sub>) to induce disorder. One of them was kept as a control (+) group and the other six gr treated with propolis, beeswax and mixture of them at concentrations 2.5% 5% from the main diet. Serum lipid profiles [total cholesterol (TC), triglycer (TG), low-density lipoprotein (LDL-c), very low-density lipoprotein (VLD high-density lipoprotein (HDL-c)], glucose levels, catalase (CAT) enzym superoxide dismutase (SOD) enzyme, liver enzyme activities (ALT, AST, ALP), and kidney functions (creatinine, uric acid, and urea levels) v measured at the end of experiment. From the obtained results it coul concluded that feeding rats on propolis and bees wax at 5% level car significant (P≤0.05) increase in HDL-c, catalase (CAT) and superoxide dismu (SOD) enzymes, but with significant (P≤0.05) decreases in liver functions, kid functions, lipid profile, serum glucose and malonaldehyde enzyme as comp with control (+ve) group, which reflects the powerful nutraceutical therap effect for feeding on propolis and bees wax for improving the biochem parameters which detected in liver illnesses.

**Keywords:** Liver disorder, Honey products, Rats, Biochemical analysis.

**Introduction**

The liver is the body's largest organ and is involved in drug metabolism. Hepatocytes are responsible for the detoxification of a wide range of medications, vitamins, hormones, and environmental toxins, as well as the metabolism of amino acids and ammonia, as well as biochemical oxidation processes. The liver is the first line of defense and appears to be the most frequently harmed organ by industrial toxins (1). Occupational and environmental liver illnesses can cause a wide range of symptoms, from asymptomatic elevated liver enzymes to acute liver failure, cirrhosis, and cancer. Most industrial chemicals, including solvents, cause

hepatocellular necrosis and have dose-dependent hepatocyte cytotoxicity (2). The liver, being a vital organ, is frequently subjected to a variety of dangers. The liver's functioning can deteriorate because of injury, which can lead to organ failure (3). Pathogenic bacteria and viruses, hepatotoxins, drug overdose and duration, obesity and malnutrition, alcohol, autoimmune disorders, type 2 diabetes, and genetic factors have all been suggested as possible risk factors for the development of liver illnesses (4). In humans and animal models, carbon tetrachloride (CCl<sub>4</sub>) is a known hepatotoxicant. It has been effectively utilized as a model and to assess hepatoprotective drugs in hepatotoxicity studies (5). After 24 hours, the CCl<sub>4</sub> caused liver cell damage, which was followed by a considerable increase in serum alanine amino transferase ALT activity and hepatic lipid peroxidation. Increased lipid peroxidation via a process that has been proposed to explain the progression of liver damage, fibrosis, and eventually cirrhosis in experimental animals and alcoholic liver disease (6).

The word propolis comes from the Greek morpheme 'Pro', which means "in front of" or "at the entrance of," and the morpheme 'polis,' which means "community" or "city," and refers to a hive defense material (7). Worker bees collect propolis from a variety of plant resinous secretions such as mucilage, gums, resins, and lattices, as well as leaf buds of various plant species such as palm, pine, alder, poplar, beech, conifer, and birch, and combine it with salivary and enzymatic secretions (8). It's also known as "Bee-glue," a natural resin (wax-like) substance found in bee hives that honey bees use as a cementing agent to bind open areas and cracks in their hives (9). Honey bees utilize propolis as an antiseptic to protect their larvae, honey stores, and comb from microbial illnesses, as well as to safeguard their hives by closing fractures, sealing voids, and smoothing the internal walls of their hives (10). Polyphenol (flavonoids, phenolic acids, and esters), phenolic aldehydes and ketones, and other compounds are found in propolis. The following is a breakdown of the percentages of various substances: Resins and vegetable balsam account for 50%, Bee wax for 30%, pollen for 5%, essential and aromatic oils for 10%, and other organic components account for the remaining 10% (11). According to Bankova, (12), isolated phenolic components from Brazilian propolis have hepatoprotective effects. Wagh, (13), also found that propolis extract had a protective effect against CCl<sub>4</sub>-induced hepatorenal oxidative stress and damage. Propolis has a hepatoprotective effect in rats suffering from liver damage caused by allyl alcohol CCl<sub>4</sub> and paracetamol. Propolis also exhibited favorable benefits on histological and biochemical indicators of non-alcoholic fatty liver disease (NAFLD), according to Kismet et al., (14), these effects were linked to propolis' antioxidant and anti-inflammatory properties. This work was conducted to study the effect of varying concentrations of propolis and beeswax on biochemical changes of hepatic rats.

## **Material and Methods**

### **Materials**

Propolis and bees wax were obtained from local market in Menoufia Governorate in 2021.

A total of 48 adult normal male albino rats Sprague Dawley strain their weighting were  $150\pm 10$ g and were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Casein, cellulose, choline chloride and DL methionine powder were obtained from Morgan Co. Cairo, Egypt.

El-Gomhoria company for Trading Drugs, Chemical and Medical Instruments, Cairo, Egypt, provided the carbon tetra chloride (CCl<sub>4</sub>) and other chemical kits which used in this study. According to Passmore and Eastwood (15). CCl<sub>4</sub> was used as a hazardous agent for liver poisoning.

## **Methods**

### **Preparation of propolis and beeswax powder**

The dried propolis and beeswax were ground in a grinder (Braun Biotech International GMBH. D.34212 Melsungen, Germany) to pass through a 1.6 mm sieves and stored at - 12°C until used.

### **The induction of experimental liver damage:**

Liver damage was induced in normal healthy male albino rats by addition of 0.2 ml/ 100g of rat's weight CCl<sub>4</sub> dissolved in paraffin oil Diao et al., (16), Carbon tetrachloride was injected two times per week for 2 consecutive weeks. Liver fibrosis was detected by determined liver functions in rat's serum.

### **Experimental design:**

Animal House, Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Egypt, carried out and approved the research.

Forty-eight adult male white albino rats, "Sprague Dawley" strain, 10 weeks old, weighing ( $150\pm 10$ g), were used in this experiment. For adaptation, all rats were fed a 7-day basal diet as described by Reeves (17). The rats were divided into two groups at random. The first group, the negative control group (n = 6), was given only the standard diet. Hepatic rats (n=42) were the second main group. In this group, rats were given 0.2 ml of CCl<sub>4</sub> per 100g of body weight. The following were used to divide hepatic rats into seven sub-groups, each with six rats:

Hepatic rats fed only the standard diet were employed as a positive control group. Hepatic rats in sub-group 2 were fed a standard diet supplemented with 2.5 percent propolis powder. Hepatic rats fed a standard diet supplemented with 5% propolis powder (subgroup 3). Hepatic rats fed a standard diet supplemented with 2.5 percent beeswax powder (sub-group 4). Hepatic rats fed a standard diet supplemented with 5% bees wax powder (sub-group 5). Hepatic rats were fed on a standard diet supplemented with a 2.5 percent mixture of propolis and beeswax powder (sub-group 6). Hepatic rats were fed a standard diet supplemented with a 5% mixture of propolis and beeswax powder.

### **Blood sampling**

Animals were starved for 12 hours and then scarified at the end of the four-week experiment. Blood samples were taken from the portal vein and placed in dry, clean centrifuge tubes for serum separation. Blood samples were centrifuged for 10 minutes at

4000 rpm to separate the serum. Serum samples were kept frozen at -20 °C until chemical analysis according to Schermer, (18).

### **Biochemical analysis**

Serum glucose was measured using the modified kinetic method according to Kaplan, (19) by using kit supplied by spin react. Spain.

The serum alanine aminotransferase (ALT), serum aspartates aminotransferase (AST), and serum alkaline phosphatase (ALP) were measured using the methods described by Hafkenscheid (20); Clinica Chimica Acta, (21) and Moss (22).

Serum total cholesterol was determined according to the colorimetric method described by Thomas (23). Serum triglycerides was determined by enzymatic method using kits according to the Fossati and Pricipe, (24). HDL-c was determined according to the method described by Grodon and Amer, (25). VLDL-c was calculated in mg/dl according to Lee and Nieman, (26) using the following formula:  $VLDL-c \text{ (mg/dl)} = \text{Triglycerides} / 5$ . LDL-c was calculated in mg/dl according to Lee and Nieman, (26) as follows:

$$LDL-c \text{ (mg/dl)} = \text{Total cholesterol} - HDL-c - VLDL-c.$$

According to the method, serum urea and serum creatinine were determined using an enzymatic technique Patton & crouch, (27) and Henry, (28). While serum uric acid was measured using a calorimeter using the method of Barham and Trinder, (29).

Catalase (CAT), superoxide dismutase (SOD) and malonaldehyde (MDA) were determined in stomach tissue according to the methods described by Hu (30), Jentzsch et al., (31) and Makarova et al., (32), respectively.

### **Statistical analysis**

The data were analyzed using a completely randomized factorial design SAS, (33) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ( $P \leq 0.05$ ) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

### **Results and Discussion**

The effect of propolis, beeswax, and their mixture as powder on hepatic rats' liver functions (AST, ALT, and ALP) is shown in Table (1). It's evident that the positive control group had the highest AST levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 181.13 and 134.66 U/L, respectively. On the other hand, the treated groups' maximum AST levels were found in 2.5 percent beeswax, while the lowest value was found in a 5 percent mixture, with significant differences ( $P \leq 0.05$ ). The mean values were 180.13 and 135.90 U/L, respectively.

There were no statistically significant differences ( $P \leq 0.05$ ) between the negative control, 5% propolis, and 5% combination. Additionally, no significant differences ( $P \leq 0.05$ ) were found between the positive control and 2.5 percent beeswax groups. In terms of ALT, the positive control group had the highest levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 45.00 and 23.76 U/L, respectively.

On the other hand, the treated groups' maximum ALT levels were found in 2.5 percent beeswax, while the lowest value was found in a 5 percent mixture, with significant differences ( $P \leq 0.05$ ). The mean values were 44.33 and 21.66 U/L, respectively. Positive control and 2.5 percent beeswax groups showed no significant differences ( $P \leq 0.05$ ). Also, no significant differences were found between the 5 percent propolis and 2.5 percent combination groups.

In case of ALP the highest levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were  $317.33a \pm 1.52$  U/L and  $216.80e \pm 3.60$  U/L, respectively.

**Table (1): Effect of different levels of propolis, beeswax and their mixture as powder on liver functions of hepatic rats**

Parameters	AST	ALT	ALP
Groups	(U/L)	(U/L)	(U/L)
Control group (-)	134.66 <sup>e</sup> ±1.25	23.76 <sup>e</sup> ±2.95	216.8 <sup>e</sup> ±3.60
Control group (+)	181.33 <sup>a</sup> ±1.51	45.00 <sup>a</sup> ±1.73	317.33 <sup>a</sup> ±1.52
G <sub>3</sub> (2.5% Propolis powder)	155.83 <sup>c</sup> ±1.55	36.43 <sup>c</sup> ±3.12	277.83 <sup>c</sup> ±2.66
G <sub>4</sub> (5% Propolis powder)	139.33 <sup>e</sup> ±4.50	29.66 <sup>d</sup> ±1.52	219.93 <sup>e</sup> ±0.90
G <sub>5</sub> (2.5% Beeswax)	180.13 <sup>a</sup> ±2.10	44.33 <sup>a</sup> ±2.51	314.00 <sup>a</sup> ±2.64
G <sub>6</sub> (5% Beeswax)	170.76 <sup>b</sup> ±2.15	40.43 <sup>b</sup> ±1.46	283.96 <sup>b</sup> ±4.05
G <sub>7</sub> (2.5% Mixture)	145.66 <sup>d</sup> ±4.93	31.83 <sup>d</sup> ±1.61	226.00 <sup>d</sup> ±2.64
G <sub>8</sub> (5% Mixture)	135.9 <sup>e</sup> ±1.41	21.66 <sup>e</sup> ±1.52	212.13 <sup>f</sup> ±0.90
LSD ( $P \leq 0.05$ )	3.731	4.850	4.510

Each value represents the mean  $\pm$  SD of three replicates.

Mean under the same column superscribed with different letters are different significantly ( $P \leq 0.05$ ).

The maximum levels of treated groups were found in 2.5 percent beeswax, while the lowest value was found in a 5 percent mixture, indicating significant differences ( $P \leq 0.05$ ). The average values were 314.00 and 212.13 U/L, respectively. The positive control and 2.5 percent bee wax groups showed non-significant changes. Furthermore, there is no significant differences ( $P \leq 0.05$ ) between the negative control and the propolis 5 percent groups. This result agreed with Nassar et al., (34), found that honey and bee products, such as propolis and beeswax, have antioxidant properties and protect the injured liver, resulting in a significant reduction in serum ALP, indicating hepatoprotective efficacy. Also, in agreement with El Menyiy et al., (35), who found that using propolis extract reduces the increased liver enzymes in diabetic rats significantly.

The effect of propolis, beeswax, and their mixture on hepatic rats' serum glucose levels is shown in Table (2). It's obvious to notice that the positive control group had the highest glucose levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ), which were 123.33 and 88.00 mg/dl, respectively. On the other hand, the 2.5 percent beeswax had the highest glucose levels, while the 5 percent mixture had the lowest, with significant differences ( $P \leq 0.05$ ), which were 119.46 and 85.00 mg/dl, respectively, there

is no statistically significant changes between the negative control, 5% propolis, and 5% mixture. Also, no significant differences ( $P \leq 0.05$ ) were found between the positive control and 2.5 percent beeswax groups. In addition, no significant differences are found between the 2.5 percent propolis and 5 percent beeswax groups. These findings confirmed those of Rivera-Yaez et al., (36), who found that an ethanolic extract of propolis considerably reduced blood glucose levels and body weight loss in diabetic mice. This is due to propolis' chemical structure and high concentration of phenols and flavonoids. In addition, Hassan et al., (37) observed that blood glucose levels dropped in experimental groups (hepatitis male rats) that were infected and treated with propolis when compared to the positive control group.

**Table (2): Effect of different levels of propolis, beeswax and their mixture as powder on glucose level hepatic rats**

Parameters	Glucose level (mg/dl)
Control group (-)	98.00h $\pm$ 2.64
Control group (+)	153.33a $\pm$ 1.52
G3 (2.5% Propolis powder)	132.66c $\pm$ 2.08
G4 (5%Propolis powder)	119.66e $\pm$ 0.51,
G5 (2.5% Beeswax)	139.46b $\pm$ 1.51
G6 (5% Beeswax)	124.43d $\pm$ 2.61
G7 (2.5% Mixture)	114.16f $\pm$ 1.04
G8 (5% Mixture)	105.00g $\pm$ 1.23
LSD ( $P \leq 0.05$ )	4.216

*Each value represents the mean  $\pm$  SD of three replicates.*

*Mean under the same column superscribed with different letters are different significantly ( $P \leq 0.05$ ).*

Data tabulated in Table (3) show the effect of propolis, beeswax and their mixture as powder on serum lipid profile (T.C, TG, HDL-c, LDL-c and VLDL-c) of hepatic rats. It's clear to notice that the positive control group had the highest serum total cholesterol levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 152.93 and 83.63 mg/dl, respectively. On the other hand, the 2.5 percent beeswax had the highest serum total cholesterol levels, while the 5 percent mixture had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 141.20 and 84.43 mg/dl, respectively.

The data also revealed that the positive control group had the highest serum triglyceride levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 155.66 and 63.33 mg/dl, respectively. On the other hand, the treatment groups' serum triglyceride levels were highest for 2.5 percent beeswax and lowest for a 5 percent mixture, with significant differences ( $P \leq 0.05$ ). The mean values were 109.30 and 64.00mg/dl, respectively.

Our findings revealed that the negative control group had the greatest serum HDL-c, whereas the positive control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean

values were 56.00 and 24.43 mg/dl, respectively. On the other hand, the 5 percent mixture recorded highest serum HDL-c levels, and 2.5 percent beeswax provided the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 55.36 and 31.00 mg/dl, respectively. The data also revealed that the positive control group had the highest serum LDL-c, whereas the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The average readings were 97.40 and 14.96 mg/dl, respectively. Furthermore, the 2.5 percent beeswax treatment group had the highest serum LDL-c values, while the 5 percent mixture had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 88.26 and 16.26 mg/dl, respectively.

The highest value for VLDL-c was recorded by the positive control group, whereas the lowest value was recorded by the negative control group, with significant differences ( $P \leq 0.05$ ). The mean values were 31.13 and 12.66 mg/dl, respectively. Other than that, the 2.5 percent beeswax had the highest serum VLDL-c levels, while the 5 percent mixture had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 21.93 and 12.80 mg/dl, respectively. These findings matched those of El Menyiy et al., (35), who discovered that using propolis extract lowers TC and TG levels in diabetic rats. Also, using propolis extract results in a considerable reduction in LDL cholesterol and a significant increase in serum HDLc.

Our findings are also consistent with those of Samadi et al., (38), who found that propolis supplementation reduces total cholesterol and LDL-c concentrations after 3 months of intervention at a daily dose of 900 mg. Propolis reduces lipid peroxidation, according to Ahmed et al., (39). As a result, propolis has the potential to minimize coronary artery disease by reducing lipid oxidation and normalizing lipids.

In hypercholesterolemic animal models, propolis lowered total cholesterol and LDL-c while increasing HDL-c concentrations, according to Kismet et al., (14). Oladayo, (40) found that propolis (200 and 300 mg/kg.BW) reduced very low-density lipoproteins (VLDL) while increasing blood levels of high-density 451 lipoprotein (HDL) in diabetic rats induced by alloxan.

**Table (3): Effect of different levels of propolis, beeswax and their mixture on serum lipid profile of hepatic rats**

Groups	Parameter (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control group (-)		83.6g±2.47	63.3e±7.63	56.0a±3.6	14.96g±4.63	12.66e±1.52
Control group (+)		152.9a±3.33	155.6a±3.7	24.4e±3.3	97.40a±2.74	31.13a±0.75
G3 (2.5% Propolis powder)		108.9d±1.45	88.6cd±1.5	38.7c±2.19	52.46d±2.40	17.73cd±30
G4 (5% Propolis powder)		90.5f±2.55	81.6d±4.16	52.33a±05	21.83f±1.53	16.33d±0.6
G5 (2.5% Beeswax)		141.2b±1.90	109.3b±2.5	31.0d±3.00	88.26b±4.04	21.93b±0.7
G6 (5% Beeswax)		123.2c±2.42	92.4c±2.51	36.5c± 2.91	68.23c±2.67	18.46c±0.51
G7 (2.5% Mixture)		100.2c±2.42	82.0d±4.35	47.1b±0.85	37.43e±1.20	16.4d±0.87
G8 (5% Mixture)		84.4g±1.62	64.0e±4.40	55.3a±2.09	16.2g±3.27	12.8e±0.88

LSD ( $P \leq 0.05$ )	3.950	7.318	1.521	1.663	1.135
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Each value represents the mean  $\pm$  SD of three replicates.

Mean under the same column superscribed with different letters are different significantly ( $P \leq 0.05$ ).

The effect of propolis, beeswax, and their mixture on kidney function levels (serum urea, uric acid, and creatinine) in hepatic rats is shown in Table (4). It's evident that the positive control group had the highest serum urea levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 46.93 and 21.60 mg/dl, respectively. The highest serum urea levels of the treatment groups were found in 2.5 percent beeswax, while the lowest value was found in a 5 percent mixture, with significant differences ( $P \leq 0.05$ ). The mean values were 43.16 and 21.63 mg/dl, respectively.

The data also revealed that the positive control group had the highest serum uric acid levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 3.10 and 1.66 mg/dl, respectively. On the other hand, the treatment groups' serum uric acid levels were highest for 2.5 percent bee wax and lowest for a 5 percent mixture, with significant differences ( $P \leq 0.05$ ). The mean values were 2.93 and 1.70 mg/dl, respectively. The data also revealed that the positive control group had the highest serum creatinine levels, while the negative control group had the lowest, with significant differences ( $P \leq 0.05$ ). The mean values were 0.80 and 0.16 mg/dl, respectively.

On the other hand, with significant differences ( $P \leq 0.05$ ), the highest serum creatinine levels of treated groups were reported for 2.5 percent beeswax, while the lowest value was observed for a 5 percent mixture. The mean values were 0.70 mg/dl and 0.23 mg/dl, respectively. These findings corroborated those of El Menyiy et al., (35), who found that using propolis extract reduced diabetic rats' serum creatinine and serum urea levels significantly. In addition, Omnia et al., (41) found that giving propolis to rats injected with TAA resulted in a substantial decrease in serum urea, uric acid, and creatinine concentrations as compared to the TAA group. In addition, Zhu et al., (42) investigated the effects of propolis on rats with experimentally produced type 1 diabetes mellitus and found that levels of blood urea nitrogen and urine micro albuminuria-excretion rate were reduced. Furthermore, propolis reduced oxidative stress in the kidney to varying degrees.

**Table (4): Effect different levels of propolis and beeswax and their mixture powder on kidney functions of diabetic rats**

Parameters	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Control group (-)	21.60d $\pm$ 1.53	1.66c $\pm$ 0.15	0.16e $\pm$ 0.05
Control group (+)	46.93a $\pm$ 2.60	3.10a $\pm$ 0.10	0.80a $\pm$ 0.10
G3 (2.5% Propolis powder)	30.53bc $\pm$ 6.38	2.46b $\pm$ 0.20	0.50bcd $\pm$ 0.20
G4 (5%Propolis powder)	24.70cd $\pm$ 1.57	1.80c $\pm$ 0.17	0.33de $\pm$ 0.05
G5 (2.5% Beeswax)	43.16a $\pm$ 2.15	2.93a $\pm$ 0.11	0.70ab $\pm$ 0.10
G6 (5% Beeswax)	35.23b $\pm$ 0.40	2.76a $\pm$ 0.20	0.63abc $\pm$ 0.15
G7 (2.5% Mixture)	28.06cd $\pm$ 2.76	2.30c $\pm$ 0.05	0.40cde $\pm$ 0.10



Parameters	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
	Mean±SD	Mean±SD	Mean±SD
G8 (5% Mixture)	21.63d±0.64	1.70c±0.26	0.23de+0.11
LSD (P≤0.05)	5.140	0.290	0.206

Each value represents the mean ± SD of three replicates.

Mean under the same column superscribed with different letters are different significantly (P≤0.05).

The effect of propolis, beeswax, and their mixture as powder on oxidative enzymes in hepatic rats is shown in Table (5). It's obvious to note that the negative control group had the highest serum SOD, while the positive control group had the lowest, with significant differences (P≤0.05). The mean values were 11.73 and 2.46 u/mg, respectively. On the other hand, the treatment groups' serum SOD levels were highest for the 5 percent mixture and lowest for 2.5 percent beeswax, with significant differences (P≤0.05). The mean values were 32.41 and 20.28 u/mg, respectively.

The data also revealed that the negative control group had the highest serum CAT, while the positive control group had the lowest, with significant differences (P≤0.05). The mean values were 24.36 and 3.53 u/mg, respectively. Furthermore, the highest serum CAT levels of treated groups recorded for 5% mixture, while the lowest value recorded for 2.5% beeswax with significant differences (P≤0.05). The mean values were 23.70 and 9.40 u/mg, respectively.

In case of MDA the highest value recorded for positive control group, while negative control group recorded the lowest value with significant differences (P≤0.05). The mean values were 92.36 and 55.03 nmol/mg, respectively.

With significant differences (P≤0.05), the highest blood MDA levels of treated groups were observed for 2.5 percent beeswax, while the lowest value was reported for a 5 percent mixture. The mean values were 78.66 and 54.33 nmol/mg, respectively. This conclusion matched with Hassan et al., (37), who evaluated the effect of propolis on hepatitis male rats and found that propolis administration reduced free radical activity and enhanced the activity of enzymes superoxide dismutase, glutathione-S-transferase, and catalase in body tissues.

In compared to TAA-treated rats, Omnia et al., (41) found that treating hyperammonemic rats with propolis resulted in a considerable increase in liver and brain SOD and CAT activity. In comparison to the TAA-treated group, there was a significant drop in L-MDA concentration in the liver, kidneys, and brain. These findings are also in line with those of Anilakumar and Khanum (43), who found that pre-treatment polyphenols extracted from bee wax significantly reduced HCH-induced changes in hepatic MDA, CAT, and SOD and maintained an antioxidant status comparable to control conditions, possibly due to its anti-oxidative properties. Cuesta et al., (44), for example, found that preventive usage of beeswax at 200 mg/kg resulted in a significant rise in catalase and SOD levels, as well as a decrease in malondialdehyde levels in CCl4-induced hepatic rats.

**Table (5): Effect of propolis and beeswax and their mixture as powder on oxidative enzymes of hepatic rats**

Parameters	SOD (u/mg)	CAT (u/mg)	MDA (nmol/g)
Groups	Mean±SD	Mean±SD	Mean±SD
Control group (-)	11.73a ±0.76	24.36a±0.56	55.03f±2.30
Control group (+)	2.46d±0.47	3.53 e±0.41	92.36a±2.30
G3 (2.5%Propolis powder)	8.93b±1.83	14.13c±2.80	69.23d±1.00
G4 (5% Propolis powder)	10.96ab±0.11	22.16a±1.80	56.96f ±2.93
G5 (2.5% Bees wax)	3.66d±0.85	9.40d±1.34	78.66b±2.70
G6 (5% Bees wax)	6.06c±1.23	11.76cd±1.68	73.13c±2.74
G7 (2.5% Mixture)	9.83ab±1.02	18.90b±1.01	61.10e±1.85
G8 (5% Mixture)	11.16ab±0.98	23.70a±1.40	54.33f±0.58
LSD (P≤0.05)	1.739	2.706	3.810

Each value represents the mean ± SD of three replicates.

Mean under the same column superscribed with different letters are different significantly (P≤0.05).

### Conclusion

Our results showed that feeding experimental animals with propolis, beeswax and their mixture as powder caused a significant (P≤0.05) increase in HDL-c, superoxide dismutase and catalase, but with a significant (P≤0.05) improvement in liver and kidney functions by decreasing ALT, AST, ALP, creatinine, uric acid, urea. Also, a significant (P≤0.05) decrease in blood glucose and serum lipid profile compared to the group control (+ve), reflecting the strong therapeutic effect of feeding on propolis, beeswax and mixture as powder for the treatment of hepatic rats.

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## دراسة تأثير البروبوليس و شمع العسل علي الخلل الكبدي الحاد في فئران التجارب

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الملخص العربي:

صممت الدراسة الحالية لدراسة تأثير مسحوق البروبوليس وشمع العسل ومخلوطهم على الفئران المصابة بتلف الكبد. تم استخدام ثمانية و أربعين فأر من ذكور الفئران الألبينو البيضاء في هذه الدراسة يتراوح أوزانها (10±150 جم) وتم تقسيمها الى 8 مجموعات 6 فئران في كل مجموعة، تركت إحداها كمجموعة ضابطة سالبة، أما المجموعات السبعة الأخرى تم حقنها بواسطة رابع كلوريد الكربون وذلك لإحداث الإصابة باضطراب الكبد وتركت إحداهم كمجموعة ضابطة موجبة، بالنسبة للست مجاميع الأخرى تم إضافة مسحوق البروبوليس وشمع العسل وخليط منهم بنسبة 2,5% ، 5% من الوجبة. تم قياس دهون الدم [الكوليسترول الكلي (TC) ، الدهون الثلاثية (TG) ، البروتين الدهني منخفض الكثافة (LDL-C) ، البروتين الدهني منخفض الكثافة جدا (VLDL-C) ، البروتين الدهني عالي الكثافة (HDL-C)] ، مستويات الجلوكوز ، والانزيمات المؤكسدة (كتاليز، السوبر أوكسيد ديسميوتيز، المالنالدهيد) ونشاط إنزيمات الكبد (ALT ، AST ، ALP) ووظائف الكلى (اليوريا، حمض اليوريك ، الكرياتينين) في نهاية التجربة. من النتائج التي تم الحصول عليها يمكن استنتاج أن التغذية على مسحوق البروبوليس وشمع العسل ومخلوطهم أدت عند مستوى 5% زيادة في البروتين الدهني عالي الكثافة و إنزيمي الكتاليز والسوبر أوكسيد ديسميوتيز وانخفاض في باقي التحاليل وذلك مقارنة بالمجموعة الضابطة الموجبة، حيث أدي العلاج بواسطة مسحوق البروبوليس وشمع العسل إلي تعزيز وظائف الكلى والكبد عن طريق خفض إنزيمات ألانين أمينو ترانسفيريز ، أسبرتات أمينوترانسفيريز، ألكالين فوسفاتيز، أيضا خفض مستوي اليوريا والكرياتينين وحمض اليوريك وخفض مستوى سكر الدم وخفض إنزيم المانولدهيد. هذه النتيجة تعكس التأثير التغذوي العلاجي لمسحوق البروبوليس وشمع العسل لتحسين المقاييس البيوكيميائية المصاحبة للأمراض الكبدية.

الكلمات المفتاحية: تلف الكبد – منتجات العسل - الفئران - التحاليل الكيميائية والحيوية.