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Original article

A STUDY ON THE EFFECT OF ETHANOLIC PROPOLIS EXTRACT ON LIVER FUNCTION IN RATS EXPOSED TO CARBON TETRACHLORIDE

By
Salem S. Elwahaishi[1], Mohammed E.abdalmalik[2]
1, Department of Therapeutic Nutrition, Faculty of Health Sciences, Misurata University, Libya
2-Libyan Authority For Scientific Research.

A B S T R A C T

This study aimed to evaluate the hepatoprotective effect of the ethanolic extract of propolis against liver injury induced by carbon tetrachloride (CCl_4) in adult male albino rats (Rattus norvegicus strain). To determine the median lethal dose (LD_{50}) of CCl_4, an initial experiment was conducted using four groups (n = 5 per group), which received increasing oral doses of CCl_4. The animals were monitored for behavioural changes and mortality. The LD_{50} was estimated at 4 ml/kg, while 1.25 ml/kg was identified as the critical toxic dose based on both experimental observations and previous studies. Following this, 25 rats aged 12–14 weeks and weighing 182 ± 22.5 g were randomly divided into five groups (n = 5 per group): a negative control group, a CCl_4-intoxicated group, and three treatment groups receiving different concentrations (1%, 2%, and 3%) of ethanolic propolis extract. Hepatotoxicity was induced by oral administration of CCl_4 at the toxic dose (1.25 ml/kg) twice weekly for four weeks.

Biochemical analyses were performed to evaluate liver enzyme levels (serum alanine aminotransferase, serum aspartates aminotransferase, serum alkaline phosphatase, lipid profile (triglycerides, total cholesterol, Very Low-Density Lipoprotein), and other vital indicators including urea, creatinine, and uric acid. The CCl_4 group exhibited significant elevations ($P < 0.05$) in liver enzyme levels, urea, creat, and uric acid, indicating hepatic injury. Treatment with the ethanolic extract of propolis, especially at the 1% concentration, resulted in notable improvements in liver function markers, bringing values closer to those of the control group ($P > 0.05$).In conclusion, the ethanolic extract of propolis demonstrated a significant hepatoprotective effect against CCl_4-induced toxicity, likely due to its antioxidant constituents, including phenolic compounds and flavonoids. These findings highlight its potential as a natural therapeutic agent for liver damage caused by chemical toxins.

KEY WORDS: Effect, Alcoholic, Extract, Propolis, Vital, Indicators, Rats, Carbon Tetrachloride

INTRODUCTION

The liver weighs around 1500 grams, making it the largest organ by mass in the human body. As a critical organ responsible for processing and metabolizing all types of chemicals and drugs that enter the body, liver metabolic activity can lead to acute liver injury, necrosis, and changes in important normal liver functions. Hepatic damage is a serious condition and can be fatal. The liver has a number

of functions, including the metabolism of carbohydrates, fats, and proteins, detoxifying, glycogen storage, bile production, and regulation of hormones and plasma levels [1].

Liver diseases can be hereditary or caused by a variety of causes that harm the liver. There are many kinds of liver diseases which can arise from viruses, damage from medications and toxic exogenous agents, obesity, diabetes or autoimmune attacks where the immune system attacks the liver itself. [2]. If not properly managed, these conditions could be dangerous and could even lead to irreversible damage to the liver or biliary tree [2]. There are a variety of reactive species that are formed and participate in many biochemical processes in the body, including respiration. Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH), as well as reactive nitrogen species (RNS), such as the nitroxyl radical [3,4]. These reactive species can generally be divided into two groups based on the arrangement of their outer-shell electrons. The first group is called free radicals, most of which are derived from oxygen. It is estimated that about 1–3% of the oxygen used in cellular respiration within the mitochondria can be converted into free radicals [5]. A free radical is defined as any atom, molecule, or ion that contains one or more unpaired electrons. Free radicals are unstable and highly reactive, with a strong tendency to interact with biological molecules in our bodies, reacting vigorously with surrounding molecules in an attempt to reach a stable state [6]. The second category includes non-radical compounds that have an unsaturated electron shell, similar to reactive free radicals, but the electrons in these compounds are arranged differently from those in free radicals (in pairs rather than singly). Therefore, non-radicals are much less reactive than free radicals. A good example of a non-radical is hydrogen peroxide [7,8]. The accumulation of these reactive oxidant species free radicals in concentrations exceeding the antioxidant defense system's ability to neutralize them leads to reactions with many biological molecules and damage to the organism, resulting in oxidative stress, which causes damage to cellular systems and cellular injury [9]. Carbon tetrachloride (CCl_4) is one compound whose toxicity mainly works through the production of free radicals, as it produces the trichloromethyl radical (CCl_3), which, in the presence of oxygen, produces the more toxic trichloromethyl peroxyl radical (CCl_3O_2) [10] Significant liver damage appeared within 24 hours of carbon tetrachloride (CCl_4) administration, as indicated by a marked increase in serum alanine aminotransferase (ALT) activity and increased lipid peroxidation in liver tissue. This oxidative stress is likely to play an important role in the sequence of liver injury events that lead to fibrosis and, ultimately, cirrhosis in experimental animal models of alcoholic liver disease [11].

For thousands of years, humans have used natural substances, including herbs, for healing. Until the Industrial Revolution, this practice was very common, and at that time included some chemically synthesized active compounds that served as substitutes for materials found in medicinal plants. The word (Propolis) comes from the Greek word (Pro) meaning at the entrance and (Polis) meaning city or community. Propolis is a natural protective substance used by bees to safeguard their colonies [12].

Corresponding author: Salem Elwahaishi Email:salem.elwahaishi@misuratau.edu.ly
ORCD: "https://orcid.org0000.0002.1593.4215
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Although bees collect propolis from a variety of resinous secretions derived from plant-based gums, mucilage, resins, and latex, they also gather resinous secretions from the buds of many plants such as palm and pine, as well as alder, beech, coniferous trees, and birch. Bees then mix these resinous substances with their saliva and enzymes to produce propolis [13].

Propolis, commonly known as "bee glue," is a resinous, wax-like material found in honey bee hives. It serves as an adhesive that benefits the colony, used as a sealant to cover openings and cracks, helping to maintain hive structure and protect the colony from enemies [14].

MATERIALS AND METHODS

All experimental procedures were carried out in the laboratories of the Faculty of Pharmacy, Misurata University, Libya, in accordance with the regulations and ethical guidelines of the institution.

This study aimed to investigate the hepatoprotective effects of ethanolic propolis extract in male albino rats, using commercial Chinese propolis powder and treating them with carbon tetrachloride (CCl_4).

The 70% ethanolic extract was prepared by soaking 30 grams of propolis in 100 ml of ethanol for 14 days, then filtering and concentrating it using a rotary evaporator [15]. The ethanolic extract was stored in a cold room at 4°C until use adult male Sprague Dawley rats (182 ± 23 g) were randomly selected for this study and housed under standard laboratory conditions in the Animal House, Faculty of Pharmacy, Misrata university, from 26/4/2025 to 26/6/2025. Hepatotoxicity was induced using CCl_4 administered orally at a dose of 0.125 ml/100 g body weight, twice a week for four weeks, followed by a treatment period of four weeks.

The hepatotoxicity group was separated into five groups:

Group 1: Negative control; received only water and standard feed.

Group 2: Positive control; received carbon tetrachloride (CCl_4) 1.25 ml/kg mixed with olive oil (1:1).

Group 3: CCl_4 + 1% ethanolic propolis extract.

Group 4: CCl_4 + 2% ethanolic propolis extract.

Group 5: CCl_4 + 3% ethanolic propolis extract.

After the treatment period of 28 days, during which 4 ml/kg was administered once daily, blood samples were taken from the portal vein, then the serum was separated and stored at −20°C. The serum alanine aminotransferase (ALT), serum aspartates aminotransferase (AST), and serum alkaline phosphatase (ALP) lipid profile; Total cholesterol (TC), Triglyceride (TG), Very-low-density lipoprotein, VLDL-C), and kidney function markers (urea, serum uric acid and creatinine) were measured using the Mindray BS-240 analyzer. VLDL-c was calculated in mg/dl according to Lee and Niemanusing the following formula: VLDL-c (mg/dl) = Triglycerides / 5,[16].

Data were analyzed statistically using CRD in the SAS software and then differences among mean were identified using the LSD test at a significance level of $P \leq 0.05$. One-way ANOVA and Kruskal-Wallis Test-Mann-Whitney Test was also performed using SPSS software.

RESULTS AND DISCUSSIONS

Table 1 shows the effects of ethanolic propolis extract at different concentrations on liver enzyme activities (AST, ALT, and ALP) in rats.

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ORCD: "https://orcid.org0000.0002.1593.4215

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The positive control group (treated with CCl_4 only) exhibited a marked elevation in enzyme levels, with mean values of 2076.42 ± 1218 U/L for AST, 1873 ± 1037 U/L for ALT, and 710.44 ± 307.17 U/L for ALP, indicating significant liver injury compared to the negative control group. Administration of ethanolic propolis extract at concentrations of 1%, 2%, and 3% led to a noticeable reduction in enzyme levels relative to the positive control. The 1% treatment group showed mean values of 190.3 ± 190 U/L for AST, 66 ± 33.89 U/L for ALT, and 283.5 ± 85.5 U/L for ALP. Similarly, the 2% group recorded 351.96 ± 86 U/L (AST), 276 ± 76.16 U/L (ALT), and 470 ± 93 U/L (ALP), while the 3% group showed values of 276.93 ± 37.46 U/L (AST), 235 ± 80.85 U/L (ALT), and 288 ± 68.8 U/L (ALP). Statistical analysis showed that the positive control group had significantly higher liver enzyme levels compared to all other groups ($P \leq 0.05$), whereas no significant differences ($P \geq 0.05$) were observed between the 1% propolis-treated groupand the negative control. This result agreement with many studies. Propolis possesses antioxidant, anti-inflammatory, antibacterial, and antifungal properties due to its flavonoids and phenolic acids, protecting cells from free radical damage [17]. Animal experiments demonstrated that propolis reduces liver damage caused by CCl_4, improves biochemical markers, liver tissue integrity, and mitigates oxidative stress [18, 19.] Propolis also improves liver function, reduces fibrosis and histopathological changes induced by CCl_4, and supports anti-inflammatory effects [20, 21] Different doses of propolis have proven effective in protecting against CCl_4-induced hepatotoxicity, including improving liver enzyme levels and reducing tissue damage [22, 23] Overall, propolis is a promising natural agent for preventing and mitigating the harmful effects of CCl_4 on the liver.

Table (1): Effect of different levels of propolis alcoholic extract on liver functions of hepatic rats

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1: Control group (-)	$117.35^{a} \pm 19.01$	$47.05^{a} \pm 7.29$	$306.98^{a} \pm 69.43$
G_2:Control group (+)	$2076.42^{b} \pm 1218$	$1873^{b} \pm 1037$	$710.44^{a} \pm 307.17$
G_3 :(1% Pro ethanolic ex)	$190.3^{ac} \pm 190$	$66^{a} \pm 33.89$	$283.5^{a} \pm 85.5$
G_4 :(2% Pro ethanolic ex)	$351.96^{c} \pm 86$	$276^{c} \pm 76.16$	$470^{a} \pm 93$
G_5 :(3% Pro ethanolic ex)	$276.93^{c} \pm 37.46$	$235^{c} \pm 80.85$	$288.0^{a} \pm 68.80$

As shown in table (2), administration of ethanolic propolis extract at concentrations of 1%, 2%, and 3% influenced serum lipid levels in rats treated with carbon tetrachloride (CCl_4). The positive control group (CCl_4 only) exhibited slight changes in lipid parameters compared to the negative control group. Total cholesterol (TC) levels were quite similar between the two groups, with the positive control group recording 65.24 ± 21.60 mg/dl and the negative control group showing 64.42 ± 7.22 mg/dl. However, triglyceride (TG) levels were lower in the positive control group (61.64 ± 12.79 mg/dl) compared to the negative control group (71.57 ± 22.94 mg/dl). As for the concentration of very low-density lipoprotein

Corresponding author: Salem Elwahaishi Email:salem.elwahaishi@misuratau.edu.ly
ORCD: "https://orcid.org0000.0002.1593.4215

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cholesterol (VLDL-c), it was slightly lower in the CCl_4 group (12.32 ± 2.56 mg/dl) compared to the negative control (14.31 ± 4.59 mg/dl). For both triglycerides and total cholesterol, no statistically significant differences were observed between the two control groups ($p > 0.05$).

Upon treatment with propolis extract at concentrations of 1%, 2%, and 3%, noticeable increases were observed in all measured lipid parameters. These increases were statistically significant at all concentrations except for the 1% extract group in the case of total cholesterol, where no significant difference was found compared to the control groups. Specifically, in the 1% group, total cholesterol, triglycerides, and VLDL-c levels were 88.54 ± 26.68, 124.74 ± 23.1, and 24.94 ± 4.66 mg/dl, respectively. In the 2% group, the values rose to 99 ± 7.07, 132.86 ± 24.16, and 26.57 ± 4.83 mg/dl, respectively. The 3% group recorded similar levels of 92.08 ± 15.44 for total cholesterol, 118.43 ± 31.50 for triglycerides, and 23.69 ± 6.31 mg/dl for VLDL-c.

Despite these numerical increases, statistical analyses revealed significant differences in TC, TG, or VLDL-c levels between the treated groups and the negative control group ($p \leq 0.05$), indicating a degree of consistency. Additionally, no significant difference in total cholesterol was found compared to the 1% propolis group, reflecting a relatively consistent effect across the different concentrations. The results showed no significant differences between the negative control group and the positive control group, which does not agree with some previous studies that reported significant differences in the lipid profile between the two control groups. Furthermore, the findings revealed that treatment with propolis led to a significant increase in certain components of the lipid profile, which is inconsistent with most previous studies that have reported a role for propolis in reducing or improving lipid parameters. Propolis is known for its antioxidant, anti-inflammatory, antibacterial, and antifungal properties, attributed to its content of flavonoids and phenolic acids that protect cells from free radical damage [17]. Animal studies have confirmed that propolis reduces liver damage induced by carbon tetrachloride (CCl_4), improves biochemical markers, preserves liver tissue integrity, and alleviates oxidative stress [18, 19]. It has also been shown to improve liver function, reduce fibrosis, and limit histopathological changes caused by CCl_4, in addition to supporting anti-inflammatory effects [20, 21]. Various doses of propolis have demonstrated effectiveness in protecting against CCl_4-induced hepatotoxicity by improving liver enzyme levels and minimizing tissue damage [22, 23]. Overall, propolis appears to be a promising natural agent for the prevention and mitigation of CCl_4-induced liver damage.

Table (2): Effect of different levels of propolis, on serum lipid profile of hepatic rats.

Parameter Groups	TC (mg/dl)	TG (mg/dl)	VLDL-c (mg/dl)
G1:Control group (-)	64.42a±7.22	71.57a±22.94	14.31a±4.59
G2:Control group (+)	65.24a±21.60	61.64a±12.79	12.32a±2.56
G3 (1% Pro ethanolic ex)	88.54a±26.68	124.7b±23.1	24.94b±4.66
G4 (2% Pro ethanolic ex)	99b±7.07	132.86b±24.16	26.57b±4.83
G5 (3% Pro ethanolic ex)	92.08b±15.44	118.43b±31.5	23.69b±6.31

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As shown in table 3, analysis of kidney function parameters revealed that the positive control group (G2) exhibited significant increases ($P < 0.05$) in urea (44.81 ± 4.54 mg/dl), uric acid (2.70 ± 0.37 mg/dl), and creatinine (0.75 ± 0.05 mg/dl) compared to the negative control group (G1), which recorded the lowest values for these parameters (25.48 ± 2.05, 1.83 ± 0.59, and 0.37 ± 0.15 mg/dl, respectively), indicating clear renal toxicity induced by CCl_4.

Treatment of rats with ethanolic propolis extract at concentrations of 1%, 2%, and 3% resulted in variable improvements in kidney function parameters. For urea, the negative control group (G1) differed significantly from all other groups. However, for creatinine, G1 was statistically similar to the 1% propolis group (G3), which recorded a creatinine level of 0.29 ± 0.12 mg/dl, indicating partial renal protection at this concentration. The 2% and 3% groups showed slightly higher creatinine levels (0.66 ± 0.01 and 0.68 ± 0.06 mg/dl, respectively), with relatively low uric acid levels (2.04 ± 0.14 and 2.09 ± 0.74 mg/dl) and urea levels of 42.68 ± 5.64 and 40.05 ± 5.51 mg/dl, respectively.

These results indicate that the ethanolic propolis extract exhibited partial nephroprotective effects against CCl_4-induced toxicity, with the 1% concentration showing the best statistical agreement for creatinine with the negative control group, while all treated groups differed significantly from G1 in terms of urea levels This finding is consistent with some previous studies that reported significant differences in kidney-related biomarkers between the control groups. The results also showed that treatment with propolis led to a significant recovery, which aligns with most previous studies that highlighted the role of propolis in reducing or improving vital indicators. Propolis is known for its antioxidant, anti-inflammatory, antibacterial, and antifungal properties, which are attributed to its content of flavonoids and phenolic acids that help protect cells from free radical damage [17]. Animal studies have confirmed that propolis reduces liver damage caused by carbon tetrachloride (CCl_4), improves biochemical parameters, and alleviates oxidative stress [18, 19].

Table 3: Effect different levels of propolis on kidney functions of rats

Parameters Groups	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
	Mean ± SD	Mean ± SD	Mean ± SD
G1:Control group (-)	25.48a ±2.05	1.83a±0.598	0.37a±0.15
G2:Control group (+)	44.81b ±4.54	2.70b ±0.37	0.75b±0.05
G3: (1% Pro ethanolic ex)	42.43b±13.31	0	0.2880a±0.1196
G4: (2% Pro ethanolic ex)	42.68b ±5.64	2.04a±0.14	0.66d±0.01
G5 :(3% Pro ethanolic ex)	40.05b±5.51	2.09a ±0.74	0.68bd±0.06

CONCLUSION

The results indicate that the alcoholic extract of propolis has a partial protective and therapeutic effect against carbon tetrachloride (CCl_4)-induced hepatic and renal toxicity in rats. Treatment with propolis at a concentration of 1% led to a significant reduction in elevated liver enzyme levels (AST, ALT, and ALP) observed in the positive control group, indicating a clear hepatoprotective activity. Notably, the 1% concentration was the most effective in restoring liver enzyme

Corresponding author: Salem Elwahaishi Email:salem.elwahaishi@misuratau.edu.ly
ORCD: "https://orcid.org0000.0002.1593.4215
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levels closer to those of the negative control group, which may be attributed to the presence of phenols and flavonoids in propolis. Regarding kidney function, administration of propolis extract at concentrations of 2% and 3% resulted in decreased blood levels of uric acid, while the 1% concentration reduced creatinine levels compared to the CCl_4-only group. Although these levels did not fully return to normal values, the reduction, particularly in uric acid, suggests a mitigating effect of propolis on renal toxicity, which may be attributed to the antioxidants present in the alcoholic extract that help improve liver function. However, the alcoholic propolis extract induced noticeable changes in blood lipid parameters. Levels of total cholesterol and triglycerides (VLDL-c) increased significantly in the 2% and 3% groups compared to the control groups, while some improvement was observed in the 1% treated group. These findings suggest that the effect of propolis on lipid metabolism is complex and may be dose-dependent or related to metabolic changes during the recovery phase.

Overall, the study supports the therapeutic potential of the alcoholic propolis extract, particularly at the 1% concentration, in alleviating CCl_4-induced liver and kidney damage. Nevertheless, its effect on lipid metabolism requires further investigation to determine whether these changes are beneficial, neutral, or potentially harmful in the long term.

These results are consistent with many previous studies, as propolis possesses antioxidant, anti-inflammatory, antibacterial, and antifungal properties due to its content of flavonoids and phenolic acids, which protect cells from free radical damage [17]. Animal studies have demonstrated that propolis reduces CCl_4-induced liver damage, improves biochemical markers, preserves liver tissue integrity, and alleviates oxidative stress [18,19]. Furthermore, propolis improves liver function, reduces fibrosis, and limits histopathological changes induced by CCl_4, in addition to exerting anti-inflammatory effects [20,21]. Various concentrations of propolis have been shown to be effective in protecting against CCl_4-induced hepatotoxicity, including improving liver enzyme levels and reducing tissue damage [22,23].

Overall, propolis is considered a promising natural agent for preventing and mitigating the harmful effects of carbon tetrachloride on the liver.

RECOMMENDATIONS:

1.Therapeutic Potential: Ethanolic propolis extract, especially at a concentration of 1%, shows promise as a natural agent for mitigating liver and kidney damage caused by chemical toxins. It could be considered for further development in hepatoprotective and nephroprotective therapies.

2.Dose Optimization: While 1% propolis was effective, its influence on lipid profile parameters indicates the need for dose optimization. Future studies should explore lower or intermediate concentrations, as well as alternative dosing regimens, to minimize potential side effects.

3.Mechanistic Studies: Further research is recommended to investigate the underlying mechanisms by which propolis modulates lipid metabolism and influences enzymatic activity in hepatic and renal tissues.

4.Extended Evaluation: Long-term studies assessing histopathological changes, oxidative stress markers, and inflammatory responses would help validate the protective effects observed and clarify any delayed effects or adaptations over time.

Corresponding author: Salem Elwahaishi Email:salem.elwahaishi@misuratau.edu.ly
ORCD: "https://orcid.org0000.0002.1593.4215

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5.Comparative Studies: It would be beneficial to compare propolis extract with standard pharmaceutical hepatoprotective and nephroprotective agents to evaluate its efficacy and safety profile in a broader therapeutic context.
6.Clinical Translation: Before recommending propolis for human use, clinical trials are necessary to confirm its safety, efficacy, and appropriate dosing in human subjects, considering variability in propolis composition depending on geographical and botanical sources.

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ORCD: "https://orcid.org0000.0002.1593.4215
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