



# مجلة التربوي

## مجلة علمية محكمة تصدر عن كلية التربية جامعة المرقب

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## Hepatoprotective Potential of Propolis on Carbontetrachloride-Induced Hepatic Damages in Rats

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**ABSTRACT:** The human body is exposed nowadays to increasing attacks by toxic compounds in polluted air, industrially processed foods, alcohol and drug consumption that increase liver toxicity, leading to more and more severe cases of hepatic disorders. The paper aims to examine the effects of bees propolis against liver damage in wistar rats with carbon tetrachloride induced hepatotoxicity, by analyzing the biochemical determination (total protein, albumin, total bilirubin levels) and antioxidant enzymatic (nitric oxidase (NO), Glutathione-S-transferase (GST)). The experiment was carried out on four groups of female wistar rats. Hepatic lesions were induced by intraperitoneal injection carbon tetrachloride (dissolved in an equal volume of olive oil) 0.5ml/kg were administered three times per week for two weeks. Biochemical results reveal that therapy with propolis has a positive effect on improving the parameters such as; total protein, albumin, total bilirubin levels and antioxidants such as; nitric oxidase, Glutathione-S-transferase.

**Keywords:** Carbon tetrachloride, Liver injury, Propolis, Free radicals.

### INTRODUCTION

Various substances are known to cause liver damage. One of these chemicals is carbon tetrachloride (CCl<sub>4</sub>) which is a xenobiotic that induces hepatotoxicity in humans as well as in animals [1]. Liver is one of the most important internal organs in human body with multiple functions such as detoxification, protein synthesis, and production of biochemicals necessary for digestion etc. [2]. In addition, liver is also the most vulnerable organ attacked by chemical toxic agents [3]. The liver plays an important role in many metabolic processes such as glycemic control, detoxification of xenobiotic, synthesis of lipoproteins, hormones and enzymes [4]. Carbon tetrachloride (CCl<sub>4</sub>) is one of the most extensively studied hepatotoxicants and the mechanism by which CCl<sub>4</sub> causes hepatotoxicity is well documented [5]. CCl<sub>4</sub> has been commonly used as a hepatotoxin in experimental hepatopathy [6] because it induced a cirrhotic response in animals which is similar to human cirrhosis of the liver [7]. Free radicals formed during biotransformation process of CCl<sub>4</sub> are more reactive and toxic than parent compound. Biotransformation of CCl<sub>4</sub> occurs in endoplasmic reticulum and isoenzyme implicated in this process is cytochrome P4502E1 (CYP 2E1) [8].

Propolis is a complex resinous substance manufactured by honeybees (*Apis mellifera*, L.) from the collection of leaf buds and cracks in the bark of various plants that are transformed in the presence of bee enzymes [9]. This product is widely used in traditional medicine and has been the subject of intense pharmacological and chemical studies for the last 30 years [9,10]. Amongst its biological and pharmacological properties, the antibacterial, antifungal, antiviral, anti-inflammatory,

hepatoprotective, antioxidant and antitumor power activities stands out [11]. Biological activities of propolis mainly depend upon the presence of more than 300 compounds including flavonoids, phenolics and their esters in particular [12].

## METHODS AND CHEMICALS

Wister albino rats (180 - 200 g) were obtained from the Animal House, Faculty of pharmacy, University of Elmergib, Libya. Animals were maintained on standard diet and housed, in polystyrene cages in room free from any source of chemical contamination, artificially illuminated (12 h dark/light cycle) and thermally controlled ( $25 \pm 2^\circ\text{C}$ ). All animals received human care in compliance with the guidelines of the Ethics committee.

### Experimental design:

The present study was designated to determine the toxic effects of  $\text{CCl}_4$  on rats and the possible protective role of bee's propolis. Therefore, rats were randomly divided into 4 groups each group containing 6 female rats:

**Group (1):** Control group: Untreated control, fed in normal diet.

**Group (2):** Olive oil group: Rats were injected intraperitoneal (ip), 3 times per week with olive oil (0.5 ml/kg body Weight ( b.wt)) for 2 weeks.

**Group (3):**  $\text{CCl}_4$  group: Rats were injected (i.p) 3 times per week with (0.5 mL/kg b.wt)  $\text{CCl}_4$  dissolved in an equal volume of olive oil, (1:1 v/v) 3 times per week for 2 weeks.

**Group (4):** Propolis group: Rats were injected (i.p) with (0.5 mL/kg b.wt)  $\text{CCl}_4$  dissolved in an equal volume of olive oil, (1:1 v/v) plus bees propolis (200 mg/kg b.wt) daily by orally for 2 weeks [13].

### Materials

All chemicals were of the highest commercially available purity. The kits for all biochemical estimation (total protein, total bilirubin, albumin, NO, and GST) were purchased from Biodiagnostics Co. (Cairo, Egypt). Activities of enzymes were determined using commercial kit.

Carbon tetrachloride ( $\text{CCl}_4$ ) solution used through this work obtained from Research labs and olive oil was obtained from herbal markets, Libya

Propolis was obtained from pharmacy at Tripoli, Libya. So that propolis dose was given daily by orally at (200 mg/kg b.wt), and Propolis extract was prepared by the method of [14]. Under sterile conditions 16.8 mg of the brown powder of propolis was dissolved in 10 mL distilled water and mixed vigorously for 10 min. Finally, this suspension was centrifuged at 1000 rpm for 10 min in room temperature. The supernatant was collected and stored under freezing condition at  $-20^\circ\text{C}$  until used.

**Statistics:** Data are expressed as mean  $\pm$  SD of six animals used in each group. Statistical analysis was carried out by one way analysis of variance (ANOVA) considered significant at  $P \leq 0.05$  followed by Student's t-test [15].

## RESULTS

Animals treated with  $\text{CCl}_4$  alone (**G3**) showed significant elevation ( $P < 0.05$ ) in total bilirubin level accompanied with a significant decrease in albumin, total protein levels compared with those of the control group (**G1**). On the other hand, animals received

CCl<sub>4</sub> plus propolis extract(**G4**) showed significant improvements in all of these liver function tests compared with those of animals received CCl<sub>4</sub> alone (**G3**)(table 1).

Group	Total bilirubin (mg/dl)	Albumin (g/dl)	Total protein (mg/dl)
<b>G1</b>	0.40 ± .089	3.483 ± 0.299	5.116 ± 0.325
<b>G2</b>	0.357 ± .067	3.250 ± 0.251	5.375 ± 0.350
<b>G3</b>	0.652 ± .092*	2.216 ± 0.353*	3.481 ± 0.967*
<b>G4</b>	0.470 ± .035**	3.028 ± 0.215*	4.832 ± 0.0231**

**Table 1. Effects of propolis extract on total protein, total bilirubin, and albumin on rats liver treated with CCl<sub>4</sub>:**

Data are expressed as mean±S.D. (n = 8 in each group), \*: P< 0.05 versus control group, \*\*: P< 0.05 versus CCl<sub>4</sub> group.

As shown in table 2, animals treated CCl<sub>4</sub> alone (**G3**) showed a significant (P<0.05) increase in NO level and a significant (P<0.05) decrease in GST concentration and activities compared with those of control group(**G1**). On contrast, the administration of propolis extract resulted in significant improvement (P<0.05) in all of these parameters compared with those of rats administered CCl<sub>4</sub> (**G3**).

Group	NO (IU/L)	GST (IU/L)
<b>G1</b>	15.47 ± 4.30	652.78 ± 149.62
<b>G2</b>	13.48 ± 3.16	646.04 ± 105.54
<b>G3</b>	33.48 ± 9.77*	358.13 ± 64.62*
<b>G4</b>	17.82 ± 3.09**	499.22 ± 49.08**

**Table 2. Effects of propolis extract on nitric oxide (NO), and glutathione-s-transferase (GST) on rats liver treated with CCl<sub>4</sub>:**

Data are expressed as mean±S.D. (n = 8 in each group), \*: P< 0.05 versus control group, \*\*: P< 0.05 versus CCl<sub>4</sub> group.

**Table 3.** Asignificant negative correlation was found between albumin, NO and T.bilirubin, GST and T.protein, NO (r = - 0.636, p =0.000& r = - 0.382, p = 0.045& r = - 0.637, p = 0.000) respectively. In addition, asignificant positive correlation was between T.bilirubin, NO and albumin, GST (r = 0.679, p = 0.000& r = 0.502, p = 0.007). However, a non- significant correlation was recoded between T.protein, GST.



Markers		NO (IU/L)	GST (IU/L)
<i>T. Bilirubin</i> (mg/dl)	r	.679**	-.382*
	P value	.000	.045
	N	33	28
<i>Albumin</i> (g/dl)	r	-.636**	.502**
	P value	.000	.007
	N	33	28
<i>T. Protein</i> (mg/dl)	r	-.637**	.348
	P value	.000	.069
	N	33	28

**Table 3. Correlations between antioxidants and liver functions:**

Correlation coefficient, N = number of cases, \* Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

## DISSCATION

Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in Western countries[16]. Nevertheless, chemical toxins (including acetaminophen, carbon tetrachloride, galactosamine and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both in vivo and in vitro conditions [17]. Pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective drugs. [18].

The focus of the present study was to assess the potential role of propolis in amelioration of CCl<sub>4</sub>-induced hepatotoxicity in rat model. In the present study, bilirubin is an important degradation product of hemoglobin and is normally excreted into the bile. If hepatic parenchymal damage is severe, less bilirubin will be excreted and hyperbilirubinemia is observed [19]. The elevation of serum bilirubin indicated defect in hepatic biotransformation, and the significant decline in serum albumin indicated the toxicant-induced change in protein biosynthesis via a substantial deficit in ribosomal RNA methylation and a decrease in polyamine synthesis [20]. Albumin binds and transports metal ions, bilirubin, drugs etc. Its levels may be used to assess the synthetic function of the liver [21]. Serum bilirubin was considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease [22]. The bilirubin levels, were improved after treatment with propolis as well as the level of albumin. It is known that bilirubin, a hydrophobic and potentially toxic substance, circulates through the plasma bound to albumin [23]. Hypoalbuminemia can be deemed as a useful index of severity of



hepatocellular damage, the serum albumin level is reduced due to protein synthesis disruption in the liver, [24]. Glutathione system including GP<sub>x</sub>, GR, GST, as well as SOD and CAT represents a mutually loyal team of defense against reactive oxygen species (ROS), nitric oxide could protect the liver from lipid peroxidation by interacting with superoxide anion and other free radical to produce less toxic species [25].

In the present study, administration of propolis improved the activities of antioxidant enzymatic (NO, GST). In recent years, there has been a considerable rising in scientific researches about natural antioxidant agents and their potential protective effects, propolis is one of these natural antioxidant agents [26]. The aqueous propolis extract was shown to have a protective effect on hepatocytes against carbon tetrachloride (CCl<sub>4</sub>)-induced injury in vitro and in vivo [27]. The antitumor activity of propolis has been recently reviewed [28]. Anti-inflammatory and immunomodulatory effects of propolis have been shown in many experimental models [29].

### Conclusion and recommendation

This paper revealed the protective effect of propolis on the liver of rats against carbon tetrachloride toxicity. This was concluded from the improvement in all biochemical tests compared with the carbon tetrachloride supplemented rats. This protective effect of propolis may be attributed to the biologically active compounds such as vitamins, flavonoids, and antioxidants that work together to scavenge free radicals. Therefore, bees' propolis can be used to protect animals and humans against the adverse effects of carbon tetrachloride toxicity.

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