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# Protective Effects of Honey on Carbon Tetrachloride Induced Hepatotoxicity in Rats

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## Abstract

The present paper aimed to evaluate the hepatotoxic effects of carbon tetrachloride and the hepatoprotective effects of bees' honey on rats, the experiment was carried out on four groups of female Wister rats. Hepatic lesions were induced by intraperitoneal (i.p) injection of carbon tetrachloride (0.5 ml/Kg body weight (b.wt)) diluted in olive oil (0.5 ml/Kg with 1:1 ratio), twice a week a period of for four weeks. Biochemical parameters of hepatic damage such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and catalase (CAT) activity were determined. Four weeks treatment using Carbon tetrachloride increased the serum AST, ALT and LDH levels significantly compared to control group (**G1**), while these enzymes were significantly reduced by the effect of honey (10%) plus $CCl_4$ (0.5ml/Kg (b.wt) (i.p.)) for 4 weeks compared to $CCl_4$ group (**G3**) ($P<0.05$), but there were significant decreases in catalase (CAT) values of $CCl_4$ group (**G3**) compared to the control group (**G1**) ($P<0.05$).
**Keywords**: Liver, hepatotoxic, Free radical, Antioxidation.

## Introduction

Liver is the first organ to be exposed to the damaging effects of the newly formed toxic substances. There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independent of the origin agent [1, 2]. Liver injury induced by carbon tetrachloride is the bestcharacterized system of xenobiotics induced hepatotoxicity [3, 4]. Liver damage induced by $CCl_4$ is the most widely used model to study hepatoprotective activity of drugs. It is one of the powerful hepatotoxins which cause liver tissue necrosis leading to biochemical changes [5, 6]. $CCl_4$ is commonly used for free radical induced liver injury [7]. Studies have demonstrated that $CCl_4$ can cause generation of reactive oxygen species (ROS) in tissues other than liver, such as kidneys, heart, lung, testis, brain, and blood [8, 9]. Oxidative stress resulting from increased free radical production after $CCl_4$-induced toxicity may play an important role in acute and chronic liver injury. To prevent the damage caused by ROS, living organisms have developed an antioxidant defense system that includes the presence of nonenzymatic antioxidants (e.g., glutathione, uric acid, bilirubin, and vitamins C, E) and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [10]. Honey is a natural product produced from the nectar and exudation of plants by the honeybees [11]. The natural honey has been reported to contain about 200 substances, which consist of not only highly concentrated

solution of sugars, but also the complex mixture of other saccharides, amino acids, peptides, enzymes, proteins, organic acids, polyphenols, carotenoidlike substances, vitamins, and minerals [12, 13]. Honey has a long history of use as an effective medicine since ancient civilization for a wide range of disease conditions [14]. The physiological property of honey has been attributed to production of hydrogen peroxide formed by the enzyme glucose oxidase; antioxidant content, low pH value; osmotic action, and a variety of enzymes [15]. Honey contains a significantly high level of antioxidants, both enzymatic and nonenzymatic, including catalase, phenolic acids, flavonoids, carotenoids, organic acids, ascorbic acid, amino acids, proteins and Maillard reaction products [16, 12, 17, 18, and 19]. Chemically, a free radical is any atom such as oxygen or nitrogen with at least one unpaired electron present, and is able to exist independently [20]. Free radicals can easily be formed in three ways: 1) by the homolytic cleavage of a covalent bond, generally incurring by high energy input; 2) by the loss of a single electron from a normal molecule; 3) by addition of a single electron to a normal molecule [21]. These free radicals that are highly reactive molecules can be extremely damaging to the lipids, proteins and cellular deoxyribonucleic acid (DNA) [22]. Which may lead to many biological complications, including carcinogenesis, mutagenesis, aging, and atherosclerosis [23]. Natural products have been used for many years in folk medicine. Apitherapy, or therapy with bee products (e.g., honey, pollen, propolis, fortified honey, etc.) is an old tradition that has been revitalized in recent research [24]. Natural products with biological activity are the main sources for new chemical structures and are useful in the development of molecules with potential utilization in pharmacology, agronomy and other areas. Increasing interest is being focused on secondary metabolites with putative roles in chemical communication between organisms [25].

## MATERIALS AND METHODS

**Chemicals used:** All chemicals were of the highest commercially available purity. The kits for all biochemical estimation (ALT, AST, LDH and CAT) were purchased from Biodiagnostics Co. (Cairo, Egypt). Activities of enzymes were determined using commercial kit. $CCl_4$ was purchased from Research labs of faculty of science, Elmergib University while olive oil and honey used were of herbal markets Liby.

**Experimental animals:** Forty female Wister albino rats weighing 120-150 g were purchased from the animal house of Biochemistry Department of faculty of medicine. 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± 2°C) All animals received humane care in compliance with the guidelines of the Ethics committee.

**Experimental design:** Hepatotoxicity was induced by $CCl_4$ (0.5 ml/kg body Weight (b.wt) in olive oil (0.5 ml/kg b.wt) (1:1 v/v), injected intraperitoneally (i.p) twice a week for 4 weeks according to the method described by Akram et al., (2012) [26]. The present study was designated to determine the toxic effects of $CCl_4$ on rats and the possible protective role of honey bee. Therefore, rats were

randomly divided into 4 groups each group containing of 10 female rats treated as follows:

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

**Group (2):** Olive oil group: Rats were injected interperitoneally (i.p) twice a week with olive oil (0.5 ml/kg b.wt) for 4 weeks.

**Group (3):** $CCl_4$ group: Rats were injected twice a week with $CCl_4$ (0.5 ml/kg b.w) in Olive oil (0.5 ml/kg b.w) ( 1:1) (i.p) for 4 weeks.

**Group (4):** Honey group: Rats were injected with $CCl_4$ (0.5 ml/kg b.w) in olive oil (0.5 ml/kg b.w) ( 1:1) (i.p) and honeybee (10%) daily in drinking water for 4 weeks.

At the end of 4$^{th}$ week, rats were anaesthetized by Chloroform, and each blood sample was withdrawn directly from heart. Blood samples were obtained by centrifugation for 10 minutes at 4000 rmp. Serum was used for further biochemical parameters estimation. These samples were kept in clean well-Stoppard plastic vials at – 20 $^{\circ}$C until assayed.

**Statistical analysis:** All statistical analyses were done by a statistical software package (SPSS 15.0 for Microsoft, SPSS Inc.) The results are expressed as means ± standard deviation (S.D). The difference between experimental groups were compared by one-way ANOVA (Newman's Keuls test) followed by Student's $t$-test and were considered statistically significant when P< 0.05, and were considered statistically significant when P< 0.05.

**RESULTS**

The results in **Table 1.** Indicated that animals treated with $CCl_4$ showed a significant increase in ALT, AST and LDH compared to normal animals' group (P<0.05), while prophylactic administration of honey showed a significant decrease in the activity of LAT and LDH enzymes (P<0.05), in addition, there was a non-significant improvement in AST compared with $CCl_4$-treated group (P>0.05). Also, table 1. Indicates that, there is a non-significant decrease of (ALT, AST) and a non-significant increase of (LDH) between olive oil group (G2) compared with the control group (G1) (P>0.05).

**Table 1.** Effects of honey on $CCl_4$-induced liver damage in rats

| **Serum Enzymes** | | | |
|---|---|---|---|
| Groups | ALT (U/L) | AST (U/L) | LDH (U/L) |
| Control | 43.6±10.9 | 243.2±113.1 | 425.1±113.1 |
| Olive oil | 43.3±14.2 | 214.3±68.8 | 513.3±142.2 |
| $CCl_4$ | 72.5±13.6[a] | 350.5±74.4[a] | 871.6±96.3[a] |
| Honey+$CCl_4$ | 53.3±15.1[ab] | 304.0±62.9[a] | 565.5±184.6[ab] |

*Note: Values were expressed as mean±SD (n=10); [a]P < 0.05 versus group1, [b]P < 0.05 versus group 3.*

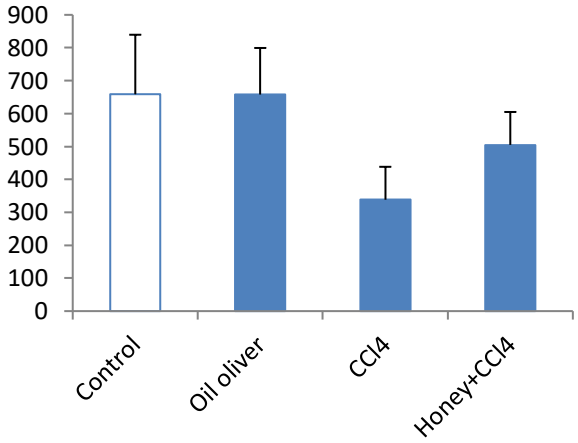


**Figure 1.** Effect of bees' honey on catalase levels of rats liver treated with carbon tetrachloride.

**Figure 1.** Show a significant decrease (P<0.05) in activity of CAT in $CCl_4$-treated group compared control group. The results also indicated that treatment with honey resulted in a significant increase (improvement) in CAT compared to $CCl_4$-treated group, while there is a non-significant decrease in activity of CAT between olive oil group (G2) compared with control group (G1) (P>0.05).

**Table 2.** Correlation between (LDH, CAT) and (ALT, AST)

| **Enzymes** | | **ALT** | **AST** |
|---|---|---|---|
| **LDH** | r | .704** | .645** |
| | P value | .000 | .000 |
| | N | 35 | 36 |
| **CAT** | r | -.343* | -.301 |
| | P value | .041 | .066 |
| | N | 36 | 38 |

*r = 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).*

**Table 2.** A significant negative correlation was found between CAT, and ALT (r = - 0.343, P = 0.41). In addition, a significant positive correlation was between LDH, and ALT, AST (r = 0.704, P = 0.000& r = 0.645, P = 0.000) respectively. However, a non-significant correlation was recorded between CAT and AST.

**Discussion**

Carbon tetrachloride ($CCl_4$) is one of the most extensively studied hepatotoxicants and the mechanism by which $CCl_4$ causes hepatotoxicity is well documented [27]. The hepatotoxicity induced by $CCl_4$ is due to its metabolite trichloromethyl free radicals ($CCl_3^*$), a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum which causes changes in the physical and chemical properties of cellular membranes, thus effecting their fluidity and permeability for ion exchange, resulting in leakage of enzymes in blood and finally results in swelling, cytolysis, and cell death [28].

Hepatic cells contain high concentrations of hepatic enzymes in the cytoplasm, and AST particularly exists in the mitochondria. Due to the damage caused to hepatic cells, the leakage of cytosol increases the levels of these hepatospecific enzymes in the serum. The elevated serum enzyme levels such as AST and ALT are indicative of cellular leakage and functional integrity of cell membrane in the liver [29]. Measurement of the activities of marker enzymes, like AST and ALT can be used in the assessment of liver function. The enzymes are of major importance in assessing and monitoring liver cytolysis. Their presence in the serum may give information on organ dysfunction [30]. In the present study, there is a significant increase in serum ALT, AST and LDH. These results may indicate degenerative changes and hypofunction of liver [31]. Which increase the release of ALT and AST in the blood stream [32]. Liver function tests showed that honey leads to some improvement in rats treated with honey (10%). These results are in agreement with Resende et al., (2003) [33]. CAT is a key component of the antioxidant defense system.

In the present study, honey treatment has been found that the enzymes ALT, AST were significantly decreased compared $CCl_4$ group. On the contrary, treatment with honey significantly increased the antioxidant enzymes levels such as CAT. This increase is due to the ability of honey to prevent the formation of free radicals, enhance the endogenous antioxidant activity beyond its free radical scavenging property and the reduction of hepatic lipoperoxide formation [34].

**Conclusion:**

This work revealed the protective effect of honey to 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 honey may be attributed to the biologically active compounds such as vitamins, flavonoids, and antioxidants that work together to scavenge free radicals. Therefore, bees' honey can be used to protect animals and humans against the adverse effects of carbon tetrachloride toxicity.

**References**

[1]. Loguercio C, Federico A (2003) Oxidative stress in viral and alcoholic hepatitis. Free Radical Biol Med. 34:1–10.

[2]. Vitaglione P, Morisco F, Caporaso N, Fogliano V (2004) Dietary antioxidant compounds and liver health. Crit Rev Food Sci Nutr 44:575–586.

[3]. Nan JX, Jiang YZ, Park EJ, Ko G, Kim YC, Sohm DH (2003) Protective effect of Rhodiola sachalinesis extract on carbon tetrachloride induced liver injury in rats. J Ethnopharmacol. 84:143–148.
[4]. Lee TY, Mai LM, Wang GJ, Chiu JH, Lin YL, Lin HC (2003) Protective mechanism of Salvia miltiorrhiza on carbon tetrachloride induced hepatotoxicity in rats. J Pharmacol Sci. 91:202–210.
[5]. Pradeep HA (2009) Hepatoprotective evaluation of Anogeissus latifolia : In vitro and in vivo studies. World J. Gastroenterol. 15(38):4816-22.
[6]. Wagner HK, Wolff PM (2011) New Natural Products and Plant Drugs with Pharmacological, Biological Or Therapeutical Activity: Proceedings of the First International Congress on Medicinal Plant Research, Section A, Held at the University of Munich, Germany, September 6-10, 1976. Springer London, Limited.
[7]. Kim H.Y, Kim J.K, Choi J.H, Jung J.Y, Oh W.Y, Kim D.C, Lee H.S, Kim Y.S, Kang S.S, Lee S.H, and Lee S.M (2010) Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damage in mice. J. Pharmacol. Sci. 112(1):105-112.
[8]. Ohta Y, Nishida K, Sasaki E, Kongo M, Ishiguro I (1999) Attenuation of disrupted hepatic active oxygen metabolism with the recovery of acute liver injury in rats intoxicated with carbon tetrachloride. Res Commun Mol Pathol Pharmacol. 95:191–207.
[9]. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K (2003) Carbon tetra-chloride induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology. 62:353–356.
[10]. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidantsin normal physiological functions and human disease. Int J Biochem Cell Biol. 39:44–84.
[11]. Alvarez-Suarez, J. M., Tulipani, S., Bertoli, S. R.-E., & Battino, M (2010) Contribution of honey in nutrition and human health: a review. Mediterranean Journal of Nutrition and Metabolism. 3, 15-23.
[12]. Gheldof, N., & Engeseth, N. J (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. Journal of Agricultural and Food Chemistry. 50, 3050-3055.
[13]. Sato, T., & Miyata, G (2000) The Nutraceutical Benefit, Part III: Honey. Nutrition 16, 468-469.
[14]. Molan, P. C (2001) Why honey is effective as a medicine. 2. The scientific explanation of its effects. Bee World. 82(1), 22-40.
[15]. Molan, P. C (2009) Why honey works. In R. Cooper, P. Molan & R. White (Eds.), Honey in modern wound management. Aberdeen: Wounds UK Ltd.
[16]. Aljadi, A., & Kamaruddin, M (2004) Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry. 85(4), 513-518.
[17]. Nasuti, C., Gabbianelli, R., Falcioni, G., & Cantalamess, F (2006) Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. Nutrition Research. 26, 130-137.

[18]. Schramm, D. D., Karim, M., Schrader, H. R., Holt, R. R., Cardetti, M., & Keen, C. L (2003) Honey with high level of antioxidants can provide protection to healthy human subjects. Journal of Agricultural and Food Chemistry. 51, 1732-1735.
[19]. Vela, L., de Lorenzo, C., & Pérez, R. A (2007) Antioxidant capacity of Spanish
honeys and its correlation with polyphenol content and other physicochemical properties. Journal of the Science of Food and Agriculture. 87(6), 1069-1075.
[20]. Karlsson, J (1997) Introduction to Nutraology and Radical Formation. In J. Karlsson (Eds), Antioxidants and Exercise (pp. 1-143). Illinois: Human Kinetics Press.
[21]. Cheeseman, K. H., & Slater, T. F (1993) An introduction to free radical biochemistry. British Medical Bulletin. 49, 481-493.
[22]. Cochrane, C. G (1991) Cellular injury by oxidants. American Journal of Medicine. 91(Suppl. 3c), 23S-30S.
[23]. Halliwell, B., & Gutteridge, J (1989) Free Radicals in Biology and Medicine.
Oxford, UK: Calendon Press.
[24]. Banskota A.H, Tezuka Y, and Kadota S (2001) Recent progress in pharmacological research of propolis. Phytotherapy Res.15:561-571.
[25]. Macías F.A, Oliveiros-Bastidas A, Marín D, Carrera C, Chinchilla N, and Molinillo J.M.G ( 2008) Plant biocommunicators: their phytotoxicity, degradation studies and potencial use as herbicides models. Phytochem. Rev. 7: 179–194.
[26]. Akram, E; Pejman, M; Mayam, B; Jalal, Z (2012) Hepatoprotective activity of cinnamon ethanolic extract against $CCl_4$-induced liver injury in rats. EXCLI journal.11: 495-507.
[27]. Hong R .T, Xu J.M, and Mei Q (2009) Melatonin ameliorates experimental hepatic fibrosis induced by carbon tetrachloride in rats. World J. Gastroenterol. 15(12): 1452-1458.
[28]. Fang Y-Z, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition.18(10):872–9.
[29]. Almalki A. L (2013) "Oat attenuation of hyperglycemia-induced retinal oxidative stress and NF-$\kappa$B activation in streptozotocininduced diabetic rats," Evidence-Based Complementary and AlternativeMedicine, vol. 2013, Article ID 983923. 8 pages.
[30]. Ulican O, Greksak M, Vancova O, Zlatos L, Galbavy S, Bozek P, Nakano M (2003) Hepatoprotective effect of Rooibos tea (Aspalathus linearis) on CCl4 – induced liver damage in rats. Physiol. Res. 52: 461-466.
[31]. Adebajo A.C, Iwalewa E.O, Obuotor E.M, Ibikunle G.F, Omisore N.O, and Adewunmi C.O (2009) Pharmacological properties of the extract and some isolated compounds of Clausena lansium stem bark: anti-trichomonal, antidiabetic, anti-inflammatory, hepatoprotective and antioxidant effects. J Ethnopharmacol.122 (1):10–9.
[32]. Jaramillo-Juarez F, Rodriguez-Vazquez M.L, Rincon-Sanchez A.R, Consolacion M, Martinez G.G, Ortiz J, and Reyes JL (2008) Caffeic acid

phenethyl ester against carbon tetrachloride toxicity in rats. Ann Hepatol. 7:331–338.

[33]. Resende R.J, Beletti M.E, Machado E.R, Fonseca N.M and Mundim A.V (2003) Effect of honey and amino acids containing either levulose or liver proteolysates against carbon tetrachloride-induced acute hepatotoxicity in mice. Arch. Velerinaria. 19: 104-109.

[34]. Beretta G, Granata P, Ferrero M, Orioli M, and Facino M.R (2005) Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal Chim Acta.533:185–91.