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Protective Effect of Honey and Propolis against Carbon Tetrachloride (CCl4)-Induced Hepatotoxicity in Rats

KEYWORDS: hepatotoxicity, oxidative stress, propolis and honeybees

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ABSTRACT *The aim of the current study was to evaluate the protective effect of honey or propolis against carbon tetrachloride (CCl4) - induced hepatotoxicity in rats. Fifty female Albino Wister rats were divided into five different groups each of 10 rats. Normal control group, Oil group: rats injected with i.p. 0.5ml/kg olive oil. CCl4 group: rats i.p. injected with CCl4 0.5 ml/kg body weight dissolved in olive oil (v/v) for three times per week for 6 weeks. CCl4 &honey group: rats injected with CCl4 and orally administered with 10% honey in drinking water once daily for 6 weeks. CCl4 & propolis group: injected with CCl4 and orally administered with propolis 200 mg/kg body weight once daily for 6 weeks. At the end of 6th week, rats were sacrificed and whole blood, serum samples and liver tissues were collected. Biomedical and histopathological evaluations were done and CCl4-treated groups were compared with Normal control group and with CCl4 and honey or propolis groups. The results indicated that i.p. administration of CCl4 induced severe hepatic injury associated with oxidative stress. The treatment with honey or propolis resulted in a significant improvement in all evaluated parameters. It could be concluded that honey and propolis protect rats against the severe CCl4-induced hepatic toxic effects. Our results suggest that the protective activity of honey and propolis may have been related to their antioxidant properties.*

Introduction

Carbon tetrachloride (CCl4) has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain **(Preethi and Kaur., 2009; Khan et al., 2010),** in addition to liver pathogenesis **(Murugesan et al., 2009)**. Liver is particularly susceptible to oxidative stress due to the direct release of CCl4 metabolites and cytokines, which propagate inflammatory response **(Werner et al., 1995)**, CCl4 is one of the xenobiotics that has been reported to induce acute and chronic tissue injuries **(Ogeturk et al., 2005; Jaramillo-Juarez et al., 2008)** through bioactivation of the phase I cytochrome P450 system to form reactive metabolic trichloromethyl radicals (•CCl3) and peroxy trichloromethyl radicals (•OOCCl3). These free radicals can covalently bind to macromolecules such as proteins, lipids and nucleic acids.

Oxidative Stress is a general term used to describe the effect of oxidation in which an abnormal level of reactive oxygen species (ROS), such as the free radicals (e.g. hydroxyl, nitric acid, superoxide) or the non-radicals (e.g. hydrogen peroxide (H_2O_2), lipid peroxide) lead of damage called oxidative damage to specific molecules with consequential injury to cells or tissue **(Atessahoiu et al., 2005)**. Moreover, these radicals can damage cell membranes inducing lipid peroxidation of polyunsaturated fatty acids in the cell membranes and other complexes **(Fang et al., 2002)**. Malondialdehyde (MDA) is one of the final products of lipid peroxidation. The concentration of MDA is the direct evidence of toxic processes caused by free radicals. Damaged lipids lead to rigid cell membranes; oxidized cholesterol often leads to hardening of the arteries and poorly repaired DNA chains lead to cell mutation (future generation of cells) as implicated in cancer and aging **(Talas and Gulhan., 2009; Tatli Seven et al., 2009)**.

The increase of antioxidant enzyme activities and GSH may be considered as a protective mechanism against heat-induced free radical production and lipid peroxidation **(Tatli Seven et al., 2009).** Moreover, a similar response has been reported in many human diseases, in which MDA concentrations increased concomitantly with an increase in antioxidant enzyme activities**.** These increases in antioxidant enzyme activities have been considered as a protective response against oxidative stress **(Altan et al., 2003).**

Propolis is a multifunctional material used by honeybees in construction and maintenance of beehives **(Banskota, 2000).** Biological activities of propolis mainly depend upon the presence of more than 300 compounds including flavonoids, phenolics and their esters in particular **(Seven et al., 2010; Sforcin and Bankova, 2011).** Chyrisin is one of the propolis compounds which is has hepatoprotective and antioxidant activities in rats **(Sathiavelu et al., 2009).** Benzoic acid derivate exhibits antioxidant effects using inhibition assays of luminol luminescence, 2, 2-diphenyl-1-picrylhydrazyl, and lipoperoxidation. Particularly caffeic acid, caffeoylquinic acid and cinnamic acid are effective $O2^{-}$ scavenging activity **(Christov et al., 2006; Nakajima et al., 2007).** Ethanolic propolis extract shows free radical scavenging activity and used as a source of natural antioxidant **(Mohammadzaden et al., 2007).** Dietary propolis decreased lipid peroxidation and regulated antioxidant enzymes activities in the broilers exposed to heat stress **(Tatli seven et al., 2009).** Researchers suggest that propolis and especially propolis in dose supplemented 3 mg/kg diet might be considered to prevent oxidative stress in the broilers exposed to heat stress **(Tatli Seven et al., 2009).**

Honey is composed of a complex mixture of carbohydrates, proteins, enzymes , amino and organic acids ,lipids, vitamins, volatile chemicals, phenolic acids, flavonoids,

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carotenoid-like substances and minerals**)** all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals **(Blasa et al., 2006 ; Johnston et al., 2005).** Its protective role against the kidney dysfunctions induced by sodium nitrite, a known food additives, hepatoprotective, hypoglycemic, reproductive, antihypertensive, and of course antioxidant effects has also been reported (**Hassan, 2007 ; Erejuwa et al., 2012).**

The main goal of the study was to explore the hepatoprotective effects of honey and propolis on carbon tetrachloride (CCl_4) hepatptoxic model and understand the mechanism of this protection.

Material and Methods

The present study was conducted on fifty female albino Wistar rats, obtained from Helwan animal station, Ministry of Health, Egypt. Animals were allowed to adapt for two weeks and housed in animal house of Zoology Department, Damietta faculty of Science, Damietta University, Egypt.

Rats were divided into 5 groups each of 10 rats. **Control group:** not injected with CCl_4 and considered normal control group. **Oil group:** injected with 0.5 ml/kg olive oil. **CCl_4 group:** rats intraperitoneal injected with CCl_4 0.5 ml/kg body weight (5 mmol/kg body mass) mixed with same volume of olive oil for three doses per week for 6 weeks. **CCl_4 &honey:** group injected with CCl_4 and orally administered with honey 10% in drinking water once daily for 6 weeks. **CCl_4 & propolis**: group injected with CCl_4 and orally administered with propolis 200 mg/kg body weight once daily for 6 weeks. At the end of 6th week, rats were sacrificed and whole blood, serum samples and liver tissues were collected.

Biochemical parameters:

Determination of ALT, AST, GGT enzymes, albumin and total proteins kits were purchased from Diamond Co. (Cairo, Egypt).Glutathione reduced (GSH), Glutathione peroxidase (GP_X) and malondialdyhide (MDA) kits were purchased from Biodiagnostics Co. (Cairo, Egypt).

Histopathological examination:

Liver tissues were collected and passed in ascending serial dilutions of alcohol for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees blocks were prepared for sectioning at 4 microns by microtome. The obtained tissue sections were collected on glass slides deparaffinized and stained by hematoxylin and eosin (H&E) stains for histopathological examination by light microscope **(Banchroft *et al.*, 1996)**.

Statistical analysis:

Student t-test was performed using the statistical program package, SPSS, version 14. Degrees of significance were as follow P< 0.05 significant, P< 0.01 highly significant and P < 0.001 extremely significant.

RESULTS

Serum activities of liver enzymes:

Table (1), showed that, the level of ALT and AST enzyme in rats injected with CCl4 and treated with Honey and propolis. The levels were increased significantly after injection of rats with CCl_4. After treatment with honey and propolis, the levels were decreased significantly (p<0.05). The level of GGT enzyme activity was increased in rats injected with CCl_4 in comparing with normal rats. On the other hand, the activity of GGT enzyme was decreased in rats injected with CCl_4 and treated with Honey and propolis compared with rats injected CCl_4 (P< 0.0001).

Serum levels of bilirubin, albumin and total proteins:

Table (1), showed Serum levels of bilirubin, albumin and total proteins in rats injected with CCl_4 and treated with honey and propolis compared with normal control (P< 0.0001). The level of bilirubin was increase while, albumin and total proteins were decreased significantly after injection with CCl_4 but after treatment with honey and propolis, the level increased again significantly (P< 0.0001).

Table (1): Effects of honey and propolis on biochemical indicators of some liver functions (Mean ± SD).

Groups / Parameters	Control group	Oil group	CCl_4 group	CCl_4& honey	CCl_4& propolis	P value
ALT(U/l)	43.7±10.5	43.3±14.3	72.5±13.7	68.5±15.0	53.3±15.3	< 0.0001
AST(U/l)	234.3±82.8	214.3±68.8	350.5±74.7	304.0±60	234.3±63.3	0.033
GGT(U/l)	10.6±2.0	10.8±0.8	33.0±4.0	22.4±5.5	24.0±7.8	<0.0001
T.Blirubin(mg/d)	0.64±0.10	0.67±0.14	0.93±0.7	0.71±0.1	0.69±0.12	< 0.0001
Albumin(g/dl)	7.0±1.6	7.4±1.0	4.1±0.3	4.7±0.4	4.5±0.1	< 0.0001
T.Protein(g/l)	8.6±1.2	9.1±0.8	6.6±1.0	7.0±0.7	7.3±0.5	< 0.0001

P< 0.05 significant, P< 0.01 highly significant and P < 0.001 extremely significant.

Serum levels of MDA and GSH

Table (2) showed Malondialdhyde level was increased significantly in rats injected with CCl4 (P<0.05). After treatment with Honey and Propolis, the level was decreased compared with those rats injected with CCl4 (P<0.05). ANOVA test showed extremely significant difference between groups under study (P< 0.0001). On the other hand, the concentration of glutathione reduced (GSH) was reduced significatly after injection of rats with CCl_4 compared with that of the normal rats. The level was inceased after treatment of honey and propolis. ANOVA test showed extremely significant difference between groups under study (P< 0.0001).

Serum level of GSH-P_x

Table (2) showed Serum levels of glutathione peroxidase in rats injected with CCl_4 and treated with honey and propolis compared with normal control. The level of glutathione peroxidase was decreased significantly after injection with CCl_4. However, no significant difference was observed after treatment with honey and propolis (P= 0.130).

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Table (2): Effects of honey and propolis on blood MDA, GPx and GSH levels (Mean±SD).

Groups / Parameters	Control group	Oil group	CCl_4 Group	CCl_4& Honey	CCl_4& propolis	P value
GP_X (mU/ml)	248.5±70.1	251.5±71.2	194.5±26.5	207.5±26.0	194.5±23.0	N.S.
GSH (mg/dl)	18.6±6.0	24.7±7.3	11.0±1.7	13.8±1.4	14.0±2.2	< 0.0001
MDA(nmol/ml)	22.0±4.3	24.8±4.4	41.8±2.9	31.8±4.0	29.1±2.2	< 0.0001

P< 0.05 significant, P< 0.01 highly significant and P < 0.001 extremely significant.
N.S.: Non-significant.

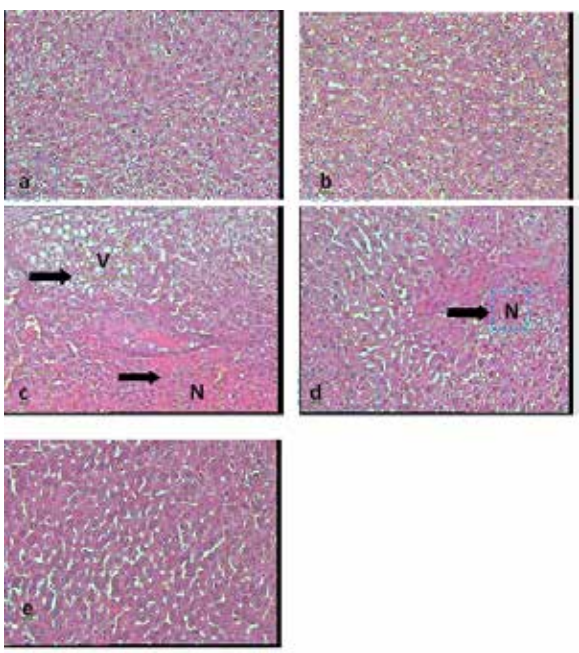

Figure (1): (a) and (b) Hepatic tissues without treatment and treated with olive oil respectively: Normal hepatic with no histopathological changes. (c) Hepatic tissues treated with CCl_4 highly degree of coagulative necrosis (N) with vacuolization (V). (d) Hepatic tissue of rats treated with honey showing less necrosis and vacuolization compared to (c). (e) Hepatic tissues of rats treated with propolis show marked improvement than (d) ×200 (H&E stains).

Discussion

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 **(Andritoin et al., 2014).**

The focus of our interest was to assess the potential role of honey and propolis in amelioration of CCl_4-induced hepatotoxicity in rat model. In the present study, there is a significant increase in serum ALT, AST, total blirubin and MDA levels accompanied with a significant decrease in total protein, GSH and GSH-P_X blood were found in rats treated with CCl_4. These results may indicate degenerative changes and hypo function of liver **(Abdel-Wahhab and Aly., 2005; Adebajo et al., 2009)** as well as hepatic cell necrosis **(Singh et al., 2005; Song et al., 2007)** which increase the release of ALT and AST in the blood stream **(Jaramillo-Juárez et al., 2008).**

Furthermore, GSH-P_X converts hydrogen peroxide or other lipid hydroperoxides to water or hydroxyl lipids, and in this process, GSH is converted to GSSG. To recycle GSSG, the cell utilizes the enzyme GSH-Rd **(Mate´s, 2000).** Our results confirm this hypothesis through consumption of GSH-P_X after CCl_4 injection and re increased after administration of honey and propolis.

In the present study, injection of CCl_4 significantly increased MDA, product of lipid peroxidation, injection that CCl_4 preferentially affects cell membrane **(Abdel-Wahhab et al., 2006).** These results clearly showed that CCl_4 has a harmful and stressful influence on the hepatic tissues consistent with those reported in the previous literature **(Chandan et al., 2007; Song et al., 2007; Bhattacharjee and Sil, 2007).**

A previous study of **Okutan et al., (2005),** investigated the effects of caffeic acid phenethyl ester (CAPE) which is a component of propolis on lipid peroxidation and antioxidant enzymes in diabetic rat heart. It's founded that in untreated diabetic group, the SOD activities and CAT levels have significantly decreased, while GSH-P_X and GSH activities was increased in the CAPE-treated diabetic rats compared to those observed in untreated diabetic rats. Moreover, The GSH-P_X activities in blood, liver and kidneys of heat stressed birds were significantly reduced, while SOD, CAT and GSH were increased in blood. Also, this is confirmed by **Nakazawa et al., (1996)** who postulate that propolis supports liver metabolism under oxidative stress. This may be explained by GSH-P_X inhibition at increased free radical levels in tissues causing liver damage**.** Also, on the level liver tissues our results show marked regression of coagulative necrosis and vacuolization after propolis administrations more than after honey treatment.

It is well documented that honey contains a variety of phenolics and represents a good source of antioxidants, which makes it a good antioxidant additive and increases its usability potential in ethno medicine **(Beretta et al., 2005).** However, the phenolic contents of honey are well known to be affected by the botanical origin **(Martos et al., 2000; Almaraz-Abarca and Campos, 2004, 2007).** The antioxidant activities of phenolics are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl. A direct relationship has been found between the phenolic content and antioxidant capacity of plants **(Robards et al., 1999; Al-Mamary et al., 2002).**

Conclusion

It could be concluded from the present results that honey and propolis had a protective effects against hepatotoxicity. These protective effects may have been related to their antioxidant properties.

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REFERENCE

Abdel-Wahhab MA, Ahmed HH, Hagazi MM. Prevention of aflatoxin B1 -initiated | hepatotoxicity in rat by marine algae extracts. J Appl Toxicol (2006);26:229–38. | Abdel-Wahhab MA, Aly SE. Antioxidant property of Nigella sativa (black cumin) | and Syzygium aromaticum (clove) in rats during aflatoxicosis. J Appl Toxicol | (2005);25(3):218–23. | Adebajo AC, Iwalewa EO, Obuotor EM, Ibikunle GF, Omisore NO, Adewunmi CO, et al. 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 (2009);122(1):10–9. | Al-Mamary M, Al-Meeri A, Al-Habori M. Antioxidant activities and total phenolics | of different types of honey. Nutr Res (2002);22:1041–7. | Almaraz-Abarca N, Campos MG, Ávila-Reyes JA, Naranjo-Jiménez N, Herrera-Corral J, González-Valdez LS. Variability of antioxidant activity among honey beecollected pollen of different botanical origin. Interciencia (2004);29:574–8. | Almaraz-Abarca N, Campos MG, Ávila-Reyes JA, Naranjo-Jiménez N, Herrera-Corral J, González-Valdez LS. Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (Prosopis juliflora, Leguminosae). J Food Compos Anal (2007);20:119–24. | Altan, O.; Pabuccuoglu, A.; Altan, A.; Konyalioglu, S. & Bayraktar, H. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br Poult Sci (2003); 44. 545–550. | | Andritoiu Calin Vasile, Lacramioara Ochiuz, Vasile Andritoiu and Marcel Popa. Effect of Apitherapy Formulations against Carbon Tetrachloride- Induced Toxicity Wistar Rats after Three Weeks of Treatment. Molecules (2014) ; 19:13374- 13391. | Atessahin, A.; Yilmaz, S.; Karahan, I.; Ceribasi, A.O. & Karaoglu, A. Effects of lycopene against cisplatin-induced nephrotoxicity and oxidative stress in rats.Toxicology (2005); 212, 116–123. | Banchroft, J., Stevens, A., Turner, D. Theory and Practice of Histological | Techniques, fourth ed. Churchil Livingstone, New York, London, San Francisco, | Tokyo., (1996). | Banskota, A.H.; Tezuka, Y.; Adnyana, I.K.; Midorikawa, K.; Matsushige, K.; Message, D.; Huertas, A.A.G. & Kadota, S. Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. Journal of Ethnopharmacology, (2000);72, 239–246. | Beretta G, Granata P, Ferrero M, Orioli M, Facino MR. Standardization of antioxidant | properties of honey by a combination of spectrophotometric/fluorimetric assays | and chemometrics. Anal Chim Acta (2005);533:185–91. | Bhattacharjee R, Sil PC. Protein isolate from the herb, Phyllanthus niruri L. (Euphorbiaceae), plays hepatoprotective role against carbon tetrachloride induced liver damage via its antioxidant properties. Food Chem Toxicol (2007);45(5):817–26. | Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Suri KA, et al., Bhadauria M. Hepatoprotective potential of Aloe barbadensis Mill against carbon tetrachloride induced hepatotoxicity. J Ethnopharmacol (2007);111(3):560–6. | Christov, R.; Trusheva, B.; Popova, M.; Bankova, V. & Bertrand, M . Chemical composition of propolis from Canada, its antiradical activity and plant origin. Nat Prod Res (2006); 20, 531-536. | Erejuwa, O.O. Management of diabetes mellitus: Could simultaneous targeting of hyperglycemia and oxidative stress be a better panacea? Int. J. Mol. Sci. (2012); 13, 2965–2972. | Fang, Y.Z.; Yang, S. & Wu, G. Free radicals, antioxidants and nutrition. Nutrition (2002);18,872–879. | Hassan , HN,H.A."The possible protective role of bees honey against hazard effects of some synthetic food additives on the kidney functions of male rats," Journal of the Egyptian Society of Toxicology, (2007); vol. 36, pp. 13–21. | Jaramillo-Juárez F, Rodríguez-Vázquez ML, Rincón-Sánchez AR, Consolación | Martínez M, Ortiz GG, Llamas J, et al. Acute renal failure induced by carbon | tetrachloride in rats with hepatic cirrhosis. Ann Hepatol (2008);7(4):331–8. | Johnston JE, Sepe HA, Miano CL, Brannan RG, Alderton AL. "Honey inhibits lipid oxidation in ready-to-eat ground beef patties," Meat Science, (2005); vol. 70, no. 4, pp. 627–631. | Khan RA, Khan MR, Sahreen S: Evaluation of Launaea procumbens use in renal disorders: A rat model. J. Ethanopharmacol. (2010); 128:452–461. | Martos I, Ferreres F, Tomás-Barberán FA. Identification of flavonoid markers for the | botanical origin of Eucalyptus honey. J Agric Food Chem (2000);48:1498–502. | Manuela Blasa, Manila Candiracci, Augusto Accorsi, Maria Piera Piacentini, Maria Cristina Albertini, Elena "Raw Millefiri honey is packed full of antioxidants," Food Chemistry, (2006); vol. 97, no. 2, pp. 217–222. | Mate´s JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology (2000);153(1-3): 83–104. | | Mohammadzadeh, S.; Sharriatpanahi, M.; Hamedi, M.; Amanzadeh, Y.; Ebrahimi, S.E.S. & Ostad, S.N. Antioxidant power of Iranian propolis extract. Food Chemistry (2007); 103, 729–733. | Murugesan GS, Sathishkumar M, Jayabalan R, Binupriya AR, Swaminathan K,Yun SEZ: Hepatoprotective and curative properties of Kombucha tea against carbon tetrachloride-induced toxicity. J Microbiol Biotechnol (2009);19:397–402. | Nakajima, Y.; Shimazawa, M.; Mishima, S. & Hara, H. Water extract of propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. Life Sci, (2007); 80, 370-377. | Nakazawa, H.; Genka, C. & Fujishima, M. Pathological aspect of active oxygens/free radicals. Jpn J Physiol (1996); 46, 15-32. | Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M: Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats J. Ethnopharmacol. (2005) ;97:273–280. | Okutan, H.; Ozcelik, N.; Yilmaz, H.R. & Uz, E. Effects of caffeic acid phenethyl ester on lipid | peroxidation and antioxidant enzymes in diabetic rat heart. Clin Biochem (2005); 38, 191- | 196. | Preethi KC, Kuttan R: Hepato and reno protective action of Calendula officinalis L. flower extract. Indian J. Exp. Biol (2009); 47:163–168. | Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem (1999);66:401–36. | | Sathiavelu, J.; Senapathy, G.J.; Devaraj, R. & Namasivayam, N. Hepatoprotective effect of chrysin on prooxidant-antioxidant status during ethanol-induced toxicity in female albino rats. J Pharm Pharmacol, (2009); 61, 809-817. | Seven, İ.; Aksu, T. & Tatli Seven, P. The effects of propolis on biochemical parameters and activity of antioxidant enzymes in broilers exposed to lead-induced oxidative stress. AJAS, (2010); 23, 1482-1489. | Sforcin, J.M. & Bankova, V. Propolis: Is there a potential for the development of new drugs? (Review). Journal of Ethnopharmacology, (2011);133, 253–260. | Singh VK, Dixit P, Saxena PN. Cybil induced hepatobiochemical changes in wistar | rats. J Environ Biol (2005);26(4):725–7. | Song JY, Li L, Ahn JB, Park JG, Jo JS, Park DH, et al. Acute liver toxicity by carbon | tetrachloride in HSP70 knockout mice. Exp Toxicol Pathol (2007);59(1):29–34. | Talas, Z.S. & Gulhan, M.F. Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss). Ecotoxicology Environmental Saffet, (2009); 72, 1994–1998. | Tatli Seven, P.; Yılmaz, S.; Seven, İ.; Çerçi, İ.H.; Azman, M.A. & Yılmaz, M. The effect of propolis on selected blood indicators and antioxidant enzyme activities inbroilers under heat stress. Acta Vet Brno, (2009); 78, 75-83. | Werner M, Costa MJ, Mitchell LG, Nayar R: Nephrotoxicity of xenobiotics. Review Clin. Chim. Acta.(1995); 237:107–154. |