Evaluation of hepatoprotective potential of propolis extract in carbon tetrachloride induced liver injury in rats

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Propolis (bee glue), a resinous wax-like beehive product has been used since ancient times for its pharmaceutical properties. In the present study, the ethanolic extract of propolis (50, 100, 200 and 400 mg/kg, p.o.) was studied for its hepatoprotective activity against carbon tetrachloride (CCL₄, 1.5 ml/kg, i.p.) induced liver damage in rats. Administration of CCL₄ caused a sharp elevation in the activity of serum transaminases, serum alkaline phosphatase, acid phosphatase and hepatic lipid peroxidation (LPO) levels, and a significant decrease in the ATPase, alkaline phosphatase and succinic dehydrogenase activities in the liver and kidney and hepatic GSH level. The treatment with propolis extract at the doses of 200 and 400 mg/kg significantly reversed the various biochemical alterations in blood, liver and kidney induced by CCL₄ intoxication. The hepatoprotective property of propolis may be due to its antioxidant activity.

Keywords: Propolis, hepatoprotective activity, rat, carbon tetrachloride, oxidative stress, liver function

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Propolis, a natural product derived from plant resins collected by honeybees is used in folk medicine, all over the world and as a constituent of ‘bio-cosmetics’ and ‘health foods’. Many medicinal properties, such as bacteriostatic, antibacterial, anti-inflammatory, antiprotozoan, antiviral, spasmolytic, astringent, immuno-stimulatory and free radical scavenging have been ascribed to propolis. It contains several constituents, such as polyphenols (flavonoids, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), coumarins, steroids, amino acids and inorganic compounds, but the composition differs greatly due to variation in its geographical and botanical origin. The biological activities of propolis may be due to the presence of a large number of flavonoids. Propolis is relatively safe, with no observed effect level (NOEL) of 1400 mg/kg body wt/day in mice. No untoward effects or mortality were observed in mice at doses up to 2 g/kg, po. In the present study, an attempt has been made to validate its hepatoprotective activity against CCL₄-induced liver injury in rats.

Materials and Methods
Preparation of extract and hepatotoxicant
Propolis from the hive of Apis mellifera was collected from BSF Academy, Tekanpur (Gwalior, M.P.) and its identity was confirmed by Prof. O.P. Agrawal, Senior Entomologist, Department of Zoology, Jiwaji University, Gwalior. The extract from propolis had a concentration of 68.4% (w/v) in 95% ethanol and stored at 4°C. Aqueous suspension of ethanolic extract of propolis (EEP) was prepared in gum acacia and administered (50, 100, 200 and 400 mg/kg) to the animals orally. Control animals received equal amount of vehicle only.
Carbon tetrachloride (CCL₄, 1.5 ml/kg) was used as hepatotoxicant and administered i.p. after mixing with equal amount of liquid paraffin and control animals received equal amount of liquid paraffin.

Animals and treatment
Female albino rats of Sprague Dawley strain (130±10 g body wt) were used in the study. Animals were housed under standard husbandry conditions (25±2°C temp, 60-70% relative humidity and 12 hr photoperiod) and had access to food and water ad libitum. They were divided into 6 groups of five animals each. Group 1 served as normal control and treated with vehicle only. Groups 2-6 were administered CCL₄ (1.5 ml/kg, i.p.) and group 2 treated as experimental control. Groups 3-6 were administered ethanolic extract of propolis (50, 100,
200 and 400 mg/kg, orally) after 24 hr of CCl₄ administration. Animals were sacrificed after 24 hr of the last treatment. Immediately after necropsy, liver and kidney were excised and blotted free of fluids. Experiments were carried out in accordance with the CPCSEA.

Assessment of liver functions and oxidative stress

Blood samples were withdrawn by puncturing the retro-orbital venous sinus, centrifuged and serum was used for the estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT)²⁰ and serum alkaline phosphatase (SALP)²¹. Liver of each rat was promptly removed to determine the level of lipid peroxidation (LPO)²². The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and was used as an index of lipid peroxidation. Reduced glutathione (GSH) was estimated in the liver homogenate using dithio nitrobenzoic acid (DTNB)²³. Homogenates were prepared in KCI and sucrose solution for LPO and GSH, respectively.

Biochemical parameters

Tissue homogenates of liver and kidney were prepared in chilled hypotonic solution for estimation of alkaline (ALP) and acid phosphatase (ACP)²¹, adenosine triphosphatase (ATPase)²⁴ and succinic dehydrogenase (SDH²⁵).

Histopathological study

For histopathological study, the liver tissues were fixed in Bouin’s solution and photomicrographs of haematoxylin-eosin stained slides were taken.

Statistical analysis

Data were subjected to statistical analysis using student’s ‘t’ test and one way analysis of variance (ANOVA)²⁶.

Results

CCl₄ (1.5 ml/kg body wt) administration caused a sharp elevation in the activity of transaminases and serum ALP at 24 hr duration (Table 1, P≤ 0.001). Significant increase in hepatic LPO and a fall in GSH level were also observed (P≤ 0.001). Administration of CCl₄ also caused a significant increase in ACP activity, and a marked fall in ALP, ATPase and SDH activities in liver and kidney. Propolis extract at all four doses (50, 100, 200 and 400 mg/kg) prevented leakage of transaminases and serum ALP, thus restoring the enzymatic activity towards normal; significant recoupment was observed at the doses of 200 and 400 mg/kg (P≤ 0.001). Analysis of variance showed significant reversal in the level in LPO and GSH after propolis administration; restoration of normalcy in the levels was observed at 200 and 400 mg/kg doses (P≤0.001, Table 1). Significant restoration of the other enzymatic activities (Table 2) was also observed with propolis administration.

The normal histoarchitecture of liver is shown in Fig. 1a. Extensive degenerative lesions were seen on administration of CCl₄ (Fig. 1b) at a dose of 1.5 ml/kg. Besides, vacuolization of hepatocytes was very common, perportal fibrosis and fatty degeneration were present, leucocytic infiltration was common and the central sinus showed congestion. Administration of propolis at 50 mg/kg maintained chord arrangement with perinuclear vacuolation. Clear sinusoidal spaces were found and debris was present in canal. Cuboidal hepatocytes were also observed (Fig. 1d). Propolis extract at 50 and 100 mg/kg did not show any significant recoupment. However, at 200 and 400 mg/kg, it showed well-maintained histoarchitecture,

<table>
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<tr>
<th>Table 1—Effect of propolis extract against CCl₄-induced liver injury on markers of serum enzymes and oxidative stress</th>
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<tbody>
<tr>
<td>[Values are mean ± S.E., n=5]</td>
</tr>
<tr>
<td><strong>AST</strong> (IU/L)</td>
</tr>
<tr>
<td>Group 1 65.5±3.92</td>
</tr>
<tr>
<td>Group 2 200.0±12.54c</td>
</tr>
<tr>
<td>Group 3 127.0±6.83d</td>
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<tr>
<td>Group 4 107.0±5.93e</td>
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<tr>
<td>Group 5 68.8±4.49f</td>
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<tr>
<td>Group 6 69.6±3.91f</td>
</tr>
<tr>
<td>F Variance 33.170*</td>
</tr>
</tbody>
</table>

*Significant at 5% level

P value: CCl₄ vs control at *<0.05, **<0.01, ***<0.001, P value: drugs vs CCl₄ at *<0.05, **<0.01, ***<0.001.
in caused a increase in GSH and SDH activities at all doses of propolis extract at all levels of variance (LPO and perinuclear vacuolation was recouped in the hepatocytes, however, Kupffer cells were observed at some places (Fig. 1e & f).

Discussion
The study demonstrated that various biochemical alterations induced by CCl₄ intoxication in blood, liver and kidney were significantly reversed with the treatment of ethanolic extract of propolis at doses of 200 and 400 mg/kg. The toxicity due to CCl₄ depends upon the cleavage of the C-Cl bond to generate a trichloromethyl and trichloromethylperoxy radicals, which may contribute to hepatotoxicity and subsequent perturbation in hepatic enzymes. Increased serum transaminases level in severe acute liver damage indicates that both cellular and mitochondrial

Table 2—Effect of propolis extract on the activity of acid phosphatase, alkaline phosphatase, ATPase and succinic dehydrogenase against CCl₄ intoxication

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Kidney</th>
<th>Liver</th>
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<th>Liver</th>
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<tbody>
<tr>
<td>Acid phosphatase</td>
<td>242.0±13.28</td>
<td>270.0±15.21</td>
<td>74.0±2.87</td>
<td>2635±156.21</td>
<td>1989±118.72</td>
<td>2478±137.31</td>
</tr>
<tr>
<td>(mgPi/100g/hr)</td>
<td></td>
<td></td>
<td>1050±54.06</td>
<td>909±50.50</td>
<td>30.00±1.80</td>
<td>32.00±1.69</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>364.0±18.76</td>
<td>359.0±18.83</td>
<td>42.0±2.42</td>
<td>1852±101.16</td>
<td>1192±63.18</td>
<td>1003±71.75</td>
</tr>
<tr>
<td>(mgPi/100g/hr)</td>
<td></td>
<td></td>
<td>43.0±2.62</td>
<td>1898±101.61</td>
<td>31.0±1.59</td>
<td>32.80±1.74</td>
</tr>
<tr>
<td>ATPase</td>
<td>302.0±16.06</td>
<td>314.0±16.56</td>
<td>55.0±2.91</td>
<td>2116±139.04</td>
<td>1414±80.59</td>
<td>1328±77.33</td>
</tr>
<tr>
<td>(mgPi/100g/hr)</td>
<td></td>
<td></td>
<td>70.0±3.88</td>
<td>2472±143.86</td>
<td>1784±98.90</td>
<td>1998±101.32</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>280.0±15.03</td>
<td>289.0±15.77</td>
<td>71.0±3.98</td>
<td>2474±144.24</td>
<td>1807±109.07</td>
<td>2182±111.65</td>
</tr>
<tr>
<td>(n moles K₃Fe(CN)₆ red/mg protein)</td>
<td>253.0±14.15</td>
<td>285.0±14.79</td>
<td></td>
<td></td>
<td>21.637*</td>
<td>58.650*</td>
</tr>
</tbody>
</table>


*Significant at 5% level; ns, non significant
P value: CCl₄ vs control at *<0.05, *<0.01, *<0.001, P value: drugs vs CCl₄ at *<0.05, *<0.01, *<0.001

Fig. 1—Histoarchitecture of liver (a): Normal chord arrangement well developed hepatocytes with Kupffer cells (140x, H & E); (b): CCl₄-induced hypertrophy in hepatocytes (140x, H & E); (c): propolis treatment at 50 mg/kg showed maintained chord arrangement (140x, H & E); (d): maintained portal tried seen with at 100 mg/kg propolis treatment (140x, H & E); (e): propolis treatment at 200 mg/kg shows hexagonal hepatocytes (140x, H & E); (f): and propolis treatment at 400 mg/kg recouped the structure, but hypertrophy of nucleus was observed at some places (140x, H & E).
membranes have been damaged. Due to liver injury, the transport function of the hepatocytes gets disturbed, resulting in the leakage of SALP in plasma membrane, thereby causing an elevation. Propolis treatment attenuated the increase in the activity of AST, ALT and SALP, indicating its protective effect in the liver injury.

Administration of CCl₄ leads to the assimilation of fat in the liver and kidney and a continuous process of autophagy by lysosomes leads to the increased activity of ACP. Increased ACP activity may also be due to the lysosomal imbalance, resulting in the destruction of the intact membranes. It has been suggested to increase the tissue catabolism and autophagy. Recoupment observed with the administration of propolis extract was similar to the observation made earlier.

ATPase is lipid-dependent membrane-bound enzyme. Any alteration in membrane lipids leads to change in membrane fluidity, which in turn alters the ATPase-mediated cellular functions. A significant decrease in the ATPase activity was observed after CCl₄ administration. Peroxidative damage to cellular membrane lipids and fatty acids due to CCl₄ intoxication may result membrane fragility and permeability. Propolis treatment resulted in recovery of depleted ATPase. Succinic dehydrogenase (SDH), a mitochondrial enzyme that tightly binds to the inner mitochondrial membrane plays an important role in energy conversion. A significant fall in its activity could result in serious impairment of mitochondrial function and metabolic turnover. This may be due to the structural and functional disorganization of the mitochondrial assembly. Propolis possibly plays a role in preventing the impairment of mitochondrial function.

One of the principal causes of CCl₄-induced liver injury is lipid peroxidation by free radical derivatives of CCl₄. In oxidative stress, GSH is converted to GSSG and its depleted amount leads to lipid peroxidation. Decrease in GSH level observed may be due to increased utilization by the hepatocytes, as GSH seems to act as scavenger for toxic chemical agents. Propolis significantly inhibited lipid peroxidation and countered the decrease in hepatic GSH level, which in turn helped in maintaining the liver tissue damage. These findings are also supported by earlier studies. Histopathological studies demonstrated that CCl₄-induced extensive degenerative lesions, vacuolization of hepatocytes, periportal fibrosis and fatty degeneration. These findings are further supported by earlier reports. Significant recoupment in histarchitecture was seen with propolis treatment.

Some flavonoids are reported to react with peroxy radicals of polyunsaturated fatty acids, thereby break the chain reaction and inhibit lipid peroxidation. Propolis possesses scavenging action against oxygen radicals as well as hepatoprotective effect. Its extract may interfere the formation of hepatotoxic CCl₄. Thus, it is suggested that the hepatoprotective potential of propolis may be due to its strong free radical scavenging activity.

Acknowledgement
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References
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Chem 
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aker M & 
ateno T, 
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A (1995) 
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56

NOTES
325

22 Sharma S K & Krishnamurthy C R (1968) J Neurochem 15, 147-149
24 Seth P K & Tangari K K (1966) J Pharm Pharmacol 18, 831
25 Slatter E C & Bonner W D (1952) J Biochem 82, 185-191