Evaluation of protective effects of *Apis mellifica* against CCl₄ induced toxicity in rabbits

Mehjabeen¹, Farah Saeed², Mansoor Ahmed⁴, Noor Jahan³

¹Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University of Arts Science and Technology, Karachi-Pakistan
²Department of Pharmacognosy, Dow College of Pharmacy, Dow University of Health and Sciences, Karachi-Pakistan
³Research Institute of Pharmaceutical Sciences, University of Karachi-Pakistan
⁴Department of Pharmacology, Dow College of Pharmacy, Dow University of Health and Sciences, Karachi-Pakistan

**ABSTRACT**

To investigate the effect of CCl₄ in rabbits pretreated with low dose of *Apis mellifica* extract by carrying out lipid profile and histopathology. The rabbits treated with *A. mellifica* extract for three months were administered carbon tetrachloride 1.5ml five hours before drawing of blood via cardiac puncture for LFT and carrying out histopathology of heart, stomach, liver and kidney tissues of the control and *A. mellifica* treated rabbits. Liver function test revealed following results: Total bilirubin (0.033±0.0046), direct bilirubin (0.023±0.0046) and gamma GT (9±0.632) levels were found lower, and SGPT (240±0.632) and alkaline phosphatase (126.83±0.658) levels were found elevated as compared to the control group. Histopathological changes in test group were compared with control group. In test group heart tissues focal myocytolysis of right ventricular wall was seen. In liver tissues of test animals, mild portal inflammation and periportal fibrosis with Centri-lobular hepatocytic degeneration was noted. No significant pathology was seen in stomach and kidney tissues. Our research work revealed that pretreatment with *A. mellifica* significantly inhibited the CCl₄ induced toxicity in test group as compared to control group.

**Key words:** *Apis mellifica*, carbon tetrachloride, histopathology, liver function tests, traditional medicine

**INTRODUCTION**

*Apis mellifica* is an insect drug and belongs to family Apidae. It is reported that *A. mellifica* extract consists of enzymes (protein in nature), phospholipase A₂, phospholipase B, Hyaluronidase, Phosphatase, α-glucosidase, peptides, Melittin, Apanime, MCD, Secapine, Pamine, Minimine, Adolapine, Protease inhibitor, Tertiapinecardiopep, melittin F; Phospholipids, Biogenic amine, Histamine, Dopamine, Acetylcholine, Noradrenalain, Aminobuycrider acid, α-amino acids; sugar, glucose, fructose; volatiles (Pheromones) complex ethers; minerals, phosphorus (P), calcium (Ca) & magnesium. *A. mellifica* extract is made from whole insect (female bee) which contains bee venom, sting and honey³. It is used in bites, stings, sore throat, urine retention, pain and headaches⁵. There are no side effects associated with its extract⁶.⁷. Carbon tetrachloride induced oxidative stress due to which injury to different organs can be produced by the formation of free radicals⁸. Carbon tetrachloride may cause toxic effects on liver, kidney, heart, lung, brain tissues and blood⁸. Inflammation is also a part of liver damage. Whereas swelling and tenderness is also reported in mild cases of carbon tetrachloride induced toxicity. The kidneys are affected by carbon tetrachloride and may lead to less urine formation. Kidney failure had been the main cause of fatalities after excessive exposure to carbon tetrachloride. Blood parameters are affected even on mild to moderate exposure to CCl₄, whereas, lungs and brain tissues may be affected on exposure to high level of carbon tetrachloride⁹.

**MATERIAL & METHOD**

*A. mellifica* mother tincture was purchased from William Schwae homeopathic drug suppliers. The extracts obtained were stored in cool, dry place for further studies. All the chemicals and reagents were procured from authorized dealers. Eighteen female rabbits weighing between 1000 and 1200 g were purchased from Animal House of Dow University of Health Sciences, (DUHS), Karachi and kept in animal house for a period 15 days to acclimatize. They were divided in to three groups (n = 6) each. Group 1 was positive control group and group 2 was negative control group. Both were fed commercial feed and water *ad libitum* for 3 months. Whereas, Group 3 was test group treated with 25 mg of *A. mellifica* for a period of 3 months along with its normal feed and water. Their weights were checked on random basis. Blood (6 ml) was collected from rabbits for lipid profile analyses by cardiac puncture at the end of three month with 10 ml sterile syringe using 1mg/ml EDTA as anticoagulant. The rabbits were sacrificed and its heart, stomach, kidneys and

*Author for Correspondence*
liver were carefully dissected and removed from abdominal region and were immediately fixed in 10% neutral buffered formalin.

Table 1: Shows the effect on Carbon tetrachloride on Liver Enzymes of Rabbits with and without A. mellifica extract

<table>
<thead>
<tr>
<th>Liver Function Test Parameters</th>
<th>Control (M±SEM)</th>
<th>Test (M±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin</td>
<td>0.3±0.0063</td>
<td>0.033±0.0046</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0.068±0.0065</td>
<td>0.023±0.0046</td>
</tr>
<tr>
<td>SGPT</td>
<td>18.17±0.658</td>
<td>240±0.632</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>49.5±0.836</td>
<td>126.83±0.658</td>
</tr>
<tr>
<td>Gamma GT</td>
<td>11±0.632</td>
<td>9±0.632</td>
</tr>
</tbody>
</table>

Fixed samples were trimmed and processed for paraffin embedding. Sections (5–7 μm) were cut and the tissues were dehydrated with alcohol of graded concentrations and allowed to dry. The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded11–13.

Statistical Analysis
All results were presented as a mean plus or minus standard error of mean (M ± SEM). Differences between control and treatment groups were analyzed by student “t” test14.

RESULTS
The rabbits treated with A. mellifica extract for three months were administered carbon tetrachloride 1.5ml, 5 hours before taking out blood via cardiac puncture for LFT. Total bilirubin (0.033±0.0046), direct bilirubin (0.023±0.0046) and gamma GT (9±0.632) levels were found lowered. SGPT (240±0.632) and alkaline phosphatase (126.83±0.658) levels were found elevated in comparison to control group (Table 1; Graph 1).

Histopathological tissues of the test groups were compared with positive and negative control groups. In heart tissues of the test group rabbits treated with A. mellifica extract, focal myocytolysis of right ventricular wall was seen, while in liver tissues, mild portal inflammation and periportal fibrosis with centrilobular hepatocytic degeneration was observed. No significant pathology was seen in stomach and kidney tissues (Figures 1-12).

DISCUSSION
Reported components of this insect (Honey bee, A. mellifica) drug are extensively studied by different researchers14,15. The active components (pharmacologically active) have been reported to be useful for the treatment of different diseases, for example Melittin is reported to produce following effects: increases capillary permeability, increasing blood circulation and lowering the blood pressure, lowers blood coagulation, immune-stimulatory and immune-suppressive, radiation protective, influences the central nervous system, anti-cancer, anti-bacterial, anti-fungal and anti-viral; Phospholipase A, destroys phospholipids and dissolves the cell membrane of blood bodies; lowers the blood coagulation and blood pressure, prevents neuronal cell death caused by prion peptides; Phospholipase B has detoxicating activity; Hyaluronidase catalyses the hydrolysis of protein, dilates blood vessels, increases their...
permeability, causing an increase of blood circulation; Apamine is anti-inflammatory, increases the defense capability, immune-suppressor and stimulates the CNS; MCD has anti-inflammatory effect, stimulates CNS, lyses mast cells, releasing histamine, serotonin and heparine, melittin-like effect increasing capillary permeability; Adolapin inhibits the specific brain enzymes, cyclooxygenase and lipoxygenase, anti-rheumatic, decreases pain, anti-pyretic, inhibits the aggregation of erythrocytes; Protease inhibitor inhibits the activity of different proteases, thereby reducing inflammation, anti-rheumatic; Cardiopep and Minimin possess anti-radiation effects; Procamine has anti-arrhythmic effects; Histamine dilates blood vessels, increasing the permeability of blood capillaries and increases blood circulation, stimulates smooth muscles1,2,7,16-18.

It is used for following therapeutic purposes because of the above mentioned main biological components and effects: arthritis1,2,7,18,19, diseases of the central and peripheral nervous system, heart and blood system, skin diseases, other disease concerning ophthalmology, gastroenterology, pulmonology, endocrinology, urology and gynecology18,19,20,21. Polypeptide melittin, carbohydrates, amino acid, enzymes present in A. mellifica extract protected the body tissues against CCl4 induced toxicity due to potent anti-oxidant activity of A. mellifica at low dose22.
CONCLUSION
Our research work revealed prominent anti-oxidant, anti-inflammatory and healing properties of A. mellifica extract that protected the tissues against carbon tetrachloride induced toxicity.

REFERENCES


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