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Research Article

Evaluation of protective effects of *Apis mellifica* against CCl_4 induced toxicity in rabbits

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ABSTRACT
To investigate the effect of CCl_4 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 tissuesfocal myocytolysis of right ventricular wall was seen. In liver tissuesof 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 CCl_4 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, Apamine, MCD, Secapine, Pamine, Minimine, Adolapine, Protease inhibitor, Tertiapinecardiopep, melittin F; Phospholipids, Biogenic amine, Histamine, Dopamine, Acetylcholine, Noradrenalin; Aminobuyric acid, α-amino acids; sugar, glucose, fructose; volatiles (Pheromones) complex ethers; minerals, phosphorus (P), calcium (Ca) & magnesium[1,2].*A. mellifica* extract is made from whole insect (female bee) which contains bee venom, sting and honey[3]. It is used in bites, stings, sore throat, urine retention, pain and headaches[4,5]. There are no side effects associated with its extract[6,7].

Carbon tetrachloride induced oxidative stress due to which injury to different organs can be produced by the formation of free radicals[8]. Carbon tetrachloride may cause toxic effects on liver, kidney, heart, lung, brain tissues and blood[9]. 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 CCl_4, whereas, lungs and brain tissues may be affected on exposure to high level of carbon tetrachloride[10].

MATERIAL & METHOD

A. mellifica mother tincture was purchased from William Schwabe 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 1,200 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/1ml EDTA as anticoagulant. The rabbits were sacrificed and its heart, stomach, kidneys and

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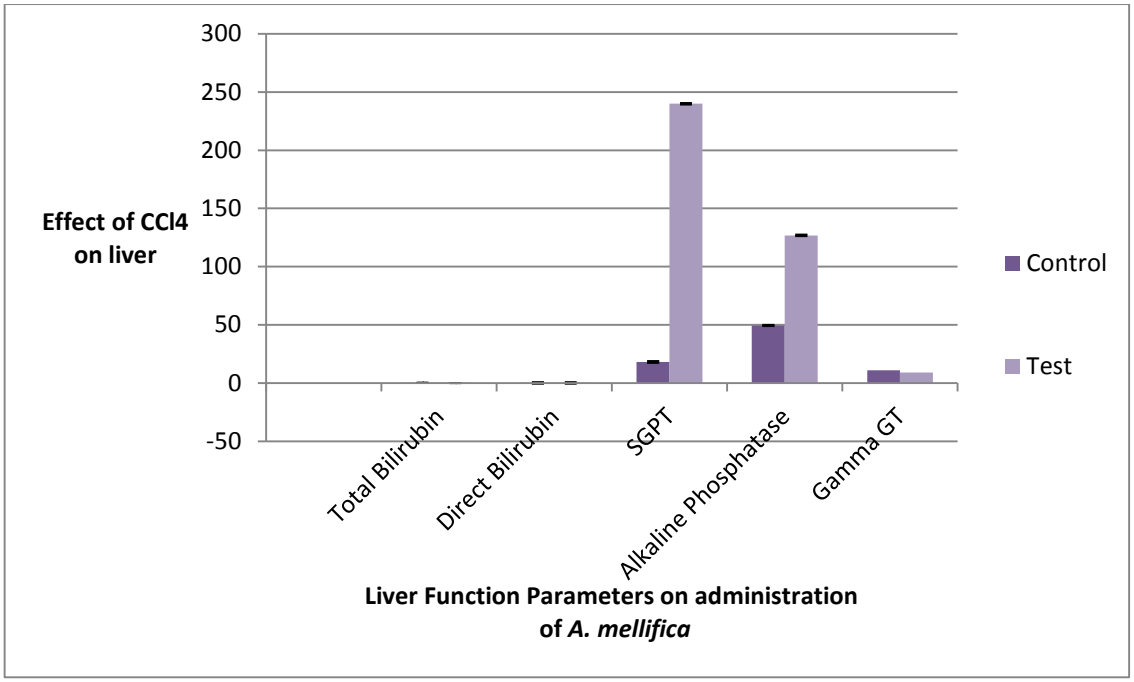

Graph 1: Shows the effect of *A. mellifica* extract on liver function parameters of rabbit in comparison with the control. *A. mellifica* extract was administered in the dose of 25mg for 3 months. Blood for liver function test was taken 1 hour after the IM injection of carbon tetrachloride prior to dissection

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

Liver Function Test Parameters	Control (M±SEM)	Test (M±SEM)
Total Bilirubin	0.3±0.0063	0.033±0.0046
Direct Bilirubin	0.068±0.0065	0.023±0.0046
SGPT	18.17±0.658	240±0.632
Alkaline Phosphatase	49.5±0.836	126.83±0.658
Gamma GT	11±0.632	9±0.632

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 recorded[11-13].

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" test[14].

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 peri-portal 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 researchers[14,15]. 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

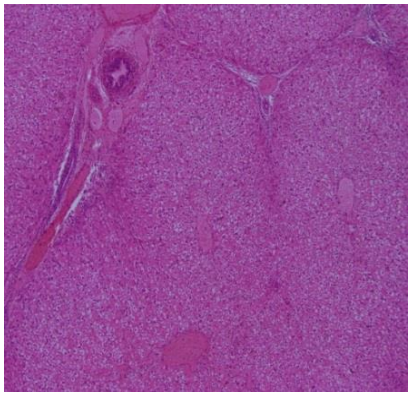

Figure 1: Liver (Positive Control): No significant pathology is seen. Sections show liver tissue with overall preserved lobular architecture. Portal tracts are within normal limits, containing portal triad and scanty fibrous tissue. No significant portal or lobular inflammation seen. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen

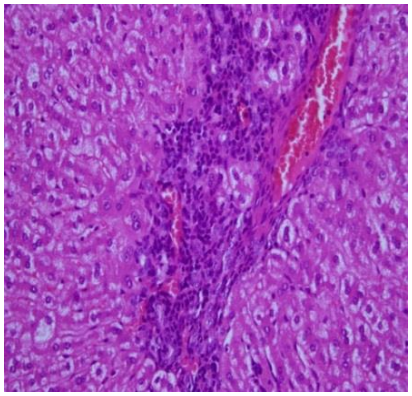

Figure 2: Liver (Negative control): Sections show liver tissue with overall preserved lobular architecture. Portal tracts are mildly dilated with lymphocytic infiltrate and minimal fibrosis. Centrilobular hepatocytic degeneration also noted. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen

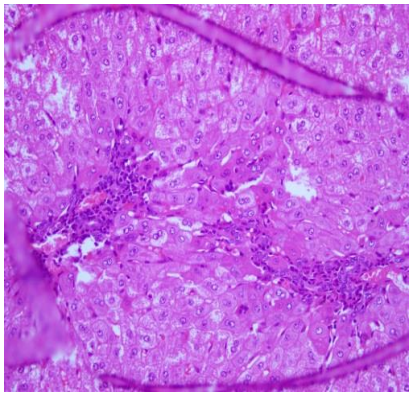

Figure 3: Liver (AMF – Test group): Sections show liver tissue with overall preserved lobular architecture. Portal tracts are mildly dilated with lymphocytic inflammatory infiltrate and mild fibrosis. Centrilobular degeneration of hepatocytes is seen. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen

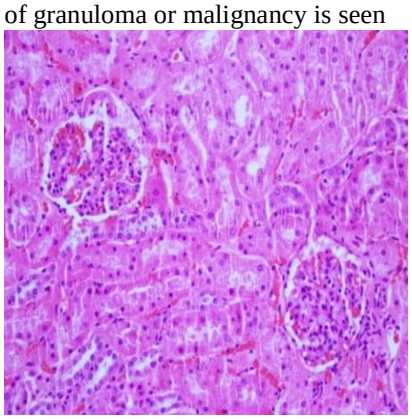

Figure 4: Kidney (Positive Control): No significant pathology is seen. Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows no significant pathology. Vascular structures are distributed evenly. No significant pathology is seen in any of the sections examined

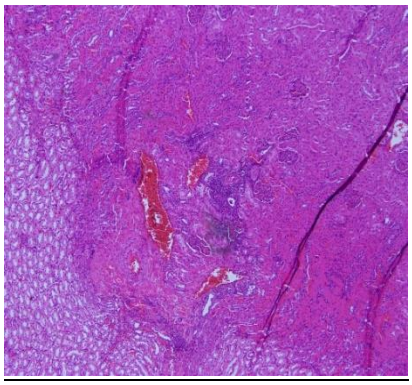

Figure 5: Kidney (Negative Control): Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows focal lymphocytic infiltrate. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined

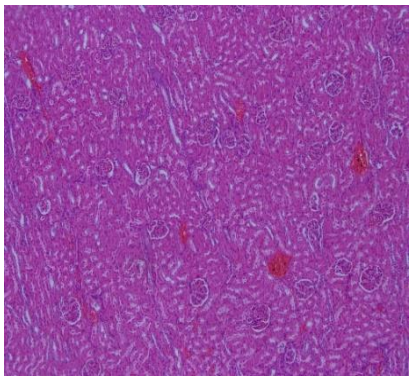

Figure 6: Kidney (AMF – Test group): Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows no significant pathology. Vascular structures are distributed evenly. No significant pathology is seen in any of the sections examined.

permeability, causing an increase of blood circulation; Apamine is anti-inflammatory, increases the defense capability, immune-supressor and stimulates the CNS; MCD has anti-inflammatory effect, stimulates CNS, lyses mast cells, releasing histamine, serotonine and heparine, melittin-like effect increasing capillary permeability; Adolapin inhibits the specific brain enzymes, cyclooxygenase and lipooxygenase, 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 muscles[1,2,7,16-18].

It is used for following therapeutic purposes because of the above mentioned main biological components and effects: arthritis[1,2,7,18,19], diseases of the central and peripheral nervous system, heart and blood system, skin diseases, other disease concerning ophthalmology, gastroenterology, pulmonology, endocrinology, urology and gynecology[18,19,20,21].

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 dose[22].

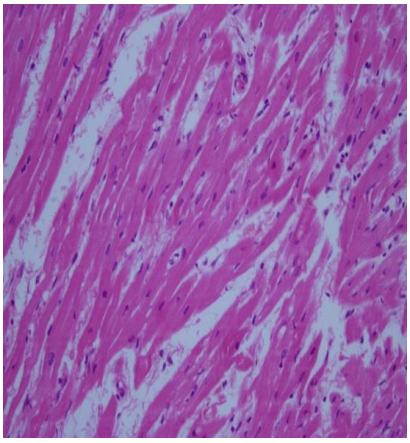

Figure 7: (Positive Control): Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined

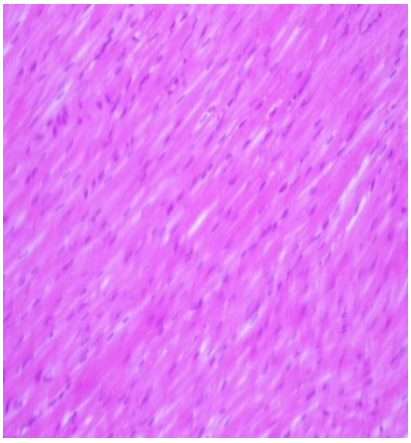

Figure 8: Heart (Negative Control): Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined

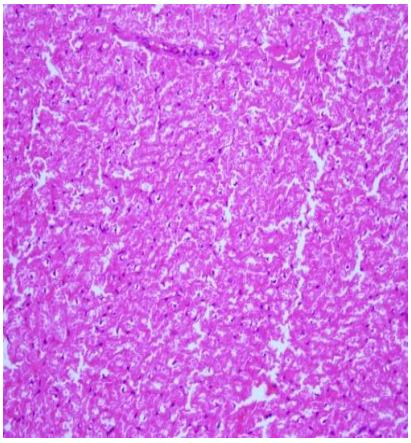

Figure 9: Heat (AMF – Test group): Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Focal myocytolysis is seen in the right ventricular wall. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane

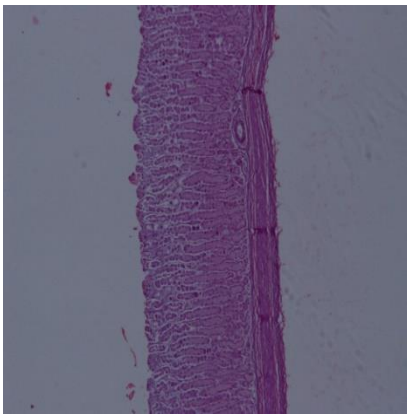

Figure 10: Stomach (Positive Control): Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined

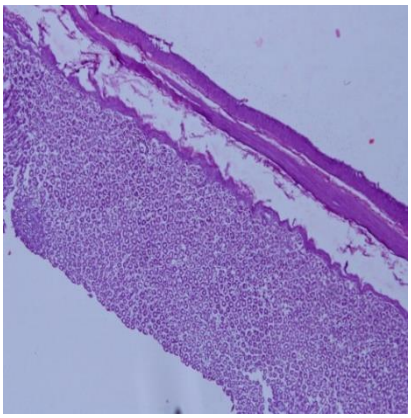

Figure 11: Stomach (Negative Control): Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined

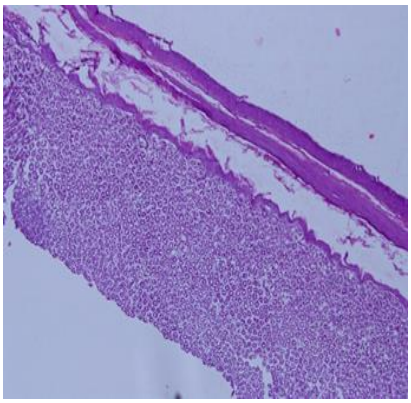

Figure 12: Stomach (AMF – Test group): Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined

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.

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