EFFECTS OF A. MELLIFICA EXTRACT ON THE HEMATOLOGICAL, BIOCHEMICAL, URINE AND HISTO-PATHOLOGICAL PARAMETERS OF WHITE ALBINO RABBITS

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ABSTRACT

Objective: To observe the effects of Apis mellifica extract on different hematologist, biochemistry and histopathology parameters in rabbits for determining its safety and efficacy.

Method: Hematological, biochemical investigations, urine analysis and histopathological studies were carried out on rabbits after the administration of the low dose of A. mellifica extract for a period of three months.

Results: Our studies revealed gender wise difference in the result of hematology, lipid profile, liver enzymes and kidney function test except for the raised white blood cells, platelet levels, serum calcium, CK, CK-MB, triglycerides, VLDL, direct bilirubin in both groups. Histopathology of stomach and kidney tissues revealed no toxicity as compared to the control group whereas slight toxicity was observed in liver and heart tissues.

Conclusion: Our research work revealed the significance of carrying out blood, biochemical and histo-pathological investigations to study the effects produced by low dose of A. mellifica administered for the long time. Partial protective effects of A. mellifica extract observed may be due to potent anti-oxidant and anti-inflammatory properties of its active constituents.

Keywords: Blood parameters, Cardiac enzymes, Liver function test, Lipid profile, Kidney function test, Urine analysis, Histopathology.

INTRODUCTION

The entire body of the Apis mellifica (Apidaeae) is used in the preparation of allopathic and homeopathic medicines. The reported chemical constituents of A. mellifica extract are Melittin, Hyaaluronidase, Apamine, Adolapine, Secapine, Minimine, Phospholipase A2 (PLA2), Histamine, Glycosidase, Tertiapin, Dopamine, Phospholipase B, Phosphatase α-glucosidase, MCD peptide, Pamine, Procamine A, Protease inhibitor, Noradrenalin, γ-amino butyric acid, α-amino acids, glucose, fructose, complex ethers, P, Ca, Mg. A. mellifica extract is used in the treatment of arthritis, diseases of the central and peripheral nervous system, cardiac and haematological disorders, skin diseases, eye problems, gastrointestinal disorders and endocrine diseases. In homeopathic system of treatment A. mellifica may be used for the treatment of inflammation with a burning sensation in all parts of the body, stinging pain, itchy skin, swollen and sensitive skin, red, flushed, hot face and hive-like welts on the skin [1-4].

This research work revealed the safe and efficacious utilization of A. mellifica extract for the cure of various symptoms associated with different pathologies by treatment of rabbits with A. mellifica extract (25 mg*/kg) orally daily for three months and then carried out haematological, biochemical and histo-pathological examination.

MATERIALS AND METHODS

Chemicals

Ethanol, acetic acid, formalin, Diagnostic kits (SGPT, SGOT, ALP, and TB), Xylene, Paraffin wax, Eosin, Hematoxylin and Canada balsam were purchased from Merck, Germany. All the chemicals were of analytical grade.

Insect extract

Apis mellifica mother tincture was purchased from Bioron (France). Extracts was concentrated under reduce pressure on a rota evaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40 °C. The extracts obtained were stored in cool, dry place for further studies.

Experimental animals

Twenty-four male 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 acclamized in separate cages. They were fed commercial feed and water ad libitum. Their weights were checked on the random basis. Blood (6 ml) was collected from rabbits for analyses of hematological and biochemical parameters by cardiac puncture at the end of three month. Blood samples collected into clean non-heparinised bottles were allowed to clot and serum, was separated from the clot and centrifuged according to groups into clean bottles for the biochemical analyses. After the collection of blood samples, urine analysis and histopathology were carried out. Animal studies were carried out according to Ethical Principles and Guidelines for Experiments on Animals formulated jointly by the Swiss Academy of Medical Sciences and the Swiss academy of sciences.

Drug dosing

Animals were divided into four groups, viz male control group (6 rabbits), female control group (6 rabbits), male test group – AMF (6 rabbits). Male and female control groups were given distilled water, while test groups AMG and AMF were given 25 mg*/kg A. mellifica extract. All the drug doses were administered orally. The treatment continued for 90 days. Blood (6 ml) was collected by cardiac puncture with 10 ml sterile syringe using 1 mg*/1 ml EDTA as anticoagulant for the determination of blood and biochemical parameters.

Hematological evaluation

Hematological examination of the collected blood samples was performed according to standard procedures listed as follow. Total erythrocyte counts were counted using a Neubauer chamber under a light microscope at 40 x 10 magnifications. Blood samples were
diluted to 200 times by Hayem’s reagent before counting. Blood hemoglobin concentration was determined using a Sahli’s hemometer. Micro Wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the (PCV). Total leucocyte counts were detected using a Neubar chamber under a light microscope at 10 x 10 magnification after diluting blood samples to 10 times with Turk’s solution. Mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for particular blood samples was also calculated [5-9].

Biochemical evaluation

Serum samples were obtained by centrifugation of blood at 1300 rpm for 15 min. The Menarini Classic Chemistry Analyzer was used to determine the calcium (Ca), phosphorus (P), blood urea, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), cholesterol, glucose, amylase, and gamma-glutamyltransferase (GGT). The globulin concentration was determined by subtracting the albumin concentration from the total protein concentration. Kits were purchased from Diagnostica Merck (Germany). Spectrophotometer U – 2000 (Hitachi) was used to measure the absorbance of light [10, 11].

Urinalysis

Voided sample of urine was collected by placing a clean, empty litter box in the site where the animals usually urinates. The samples were analyzed on automatic urine analyzer (Japan) [12].

Histo-pathological of organs

After blood collection, the liver, kidney heart and stomach of the male control and test group were carefully dissected from the abdominal region and were immediately fixed in 10% neutral buffered formalin. Fixed samples were trimmed and processed for paraffin embedding. Sections (5-7 μm) were cut with Leica RM 2145 – Rotation microtome 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 through Nikon advance microphotography system; model UFX-DX-35 and phase contrast N plan [13-15].

Statistical analysis

All the results are presented as a mean plus or minus standard error of mean (M ± SEM). Differences between control and treatment groups are analyzed by student "t" test [16].

RESULTS AND DISCUSSION

In the male test group treated with *A. mellifica* extract, slight elevation in hemoglobin (10.41±0.0658), total protein (5.4±2.5±0.0836), creatinine (0.925±0.0083), serum calcium (14.45±0.014), albumin (5.035±0.0083), A/G ratio (2.205±0.012) levels were found to be elevated, whereas, phosphorus (4.275±0.0083), uric acid (0.045±0.0083), total proteins (7.5±2±0.01) and globulin (3.2±0.012) were reduced in male test group treated with *A. mellifica* extract as compare to respective male control group (table 2, graph 2).

In female test group treated with *A. mellifica* extract a decrease in hemoglobin (10.41±0.0658), total protein (5.4±2.5±0.0836), creatinine (0.925±0.0083), serum calcium (14.45±0.014), albumin (5.035±0.0083), A/G ratio (2.205±0.012) levels were reduced, whereas, phosphorus (4.275±0.0083), uric acid (0.045±0.0083) and globulin (3.2±0.012) were raised in female group treated with *A. mellifica* extract in comparison to its female control group (table 2, graph 2).

LDH (205.5±0.03) level was found lowered; CPK (1758.83±1.036) and CK-MB (851.16±1.036) enzymes were raised in male control group treated with *A. mellifica* extract in comparison with its respective male control group (table 3, graph 3).

The cardiac enzymes; LDH (312.5±0.03), CPK (842.16±1.036) and CK-MB (888.67±0.096) were found raised in female test group treated with *A. mellifica* extract as compare to its female control group (table 3, graph 3).

Triglycerides (287.16±1.18) and VLDL (57.83±1.11) were raised; however, cholesterol (40.5±1.009), HDL (5.5±0.83) and LDL (3.6±0.83) levels were lowered in male test group treated with *A. mellifica* extract as compare to respective male control group (table 4, graph 4).

Cholesterol (161.16±1.036), triglycerides (45.5±0.83), HDL (32.3±0.96), LDL (123.5±0.83), VLDL (9.42±1.034) levels were raised in female test group treated with *A. mellifica* extract in comparison to its respective control group (table 4, graph 4).

Direct bilirubin (0.12±0.017) was raised, while the rest of the liver enzymes, that is, SGOT (38.83±1.036), total bilirubin (0.25±0.0083), SGPT (28.3±1.15), alkaline phosphatase (88.5±0.036) and gamma GT (12.5±0.083) were reduced in male test group treated with *A. mellifica* extract as compare to the male control group (table 5, graph 5).

Total bilirubin (0.245±0.0083) was slightly reduced, while the other liver enzymes; direct bilirubin (0.065±0.0083), SGOT (51.6±1.154), SGPT (110.67±0.96), alkaline phosphatase (101.83±1.036) and gamma GT (9.5±0.836) were found raised in female test group treated with *A. mellifica* extract in comparison to the respective female control group (table 5, graph 5).

The urine of the male group treated with *A. mellifica* extract was yellow in color and turbid, like that of its respective male control group. The pH was 9.05, slightly raised as compare to that of its respective control group. While the rest of the urine parameters of the test group was alike that of control group (Table 6). The urine parameters of the female test group were parallel to that of its respective female control group (table 6).

Old healed myocardial infarction in the wall of left ventricle and inter-ventricular septum was found in the heart tissues of the male group treated with *A. mellifica* extract. No significant pathology was seen in stomach tissues. In liver tissues mild portal inflamation and peri-portal fibrosis with loci of lobulitis were observed. Chronic nonspecific pyelonephritis was found in kidney tissues (table 7; fig. 1 - 6).

The chemical constituents and pharmacological effects of *A. mellifica* are studied in detail by different researchers. Many of the therapeutic effects produced by *A. mellifica* are due to its pronounced anti-oxidant, analgesic, anti-inflammatory and anti-microbial effects. Its active constituents melittin, phospholipase A, phospholipase B, apamine , adolapin, procamine, enzymes, histamine, dopamine, acetylcholine, noradrenaline and minerals are found effective for the treatment of diseases of the central and peripheral nervous system, heart and blood system, skin diseases, other disease concerning ophthalmology, gastroenterology, pulmonology, otornolaringology, endocrinology, urology and gynecology [17-23].

Increased white blood cells and platelet count reveals healing potential of *A. mellifica*. Apart from that variations were observed in hematomal, and biochemical parameters of the male and female rabbits. No toxic effects were observed in stomach and kidneys tissues while as compared to control group, heart and liver tissues were observed to be affected in test group on low dose administration of *A. mellifica* for a period of three months.

CONCLUSION

It is a safe drug but on prolong use may produce toxic effects on different organs of the body like on liver and kidneys.

CONFLICT OF INTERESTS

Authors have no conflict of interest.
REFERENCES


