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EVALUATION, HEPATOPROTECTIVE EFFECTS AND THE DETERMINATION OF SAFTY AND EFFICACY OF ZOSIMA ABSINTHIFOLIA

Javed Ul Haq[1], Shafi Muhammad[1] , Almas Kanwal[2], Gul Muhammad[2] ,Mohammad Arsalan[1] , Muhammad Ishaque Mujeeb Rehman[3] , Nagina Soomer Khan[3] and Saeeda Baloch[2] .

[1]Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan.

[3]Department of Eastern Medicine, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta-Pakistan.

Abstract:

Zosima absinthifolia (family Apiaceae) is a traditional medicinal plant of Balochistan, Pakistan, Used to reduce bowel disorders, to treat cough, stomach gas and indigestion. Present study was performed to determine the Hepatoprotective activity of Z. absinthifolia. For chronic toxicity the test animals were rabbits were administered Z. absinthifolia 300mg/kg/ given orally for 3 months The biochemical parameters (blood glucose, Kidney function test, hematological parameters, cardiac enzymes, liver function test, urea, lipid profile, and serum calcium) were determined. Current study reveals that the Z. absinthifolia methanol extract showed non significant changes in cardiac enzymes (LDH), lipid profile (Triglycerides and Cholesterol level) and hematological profile while significant increase platelets count was observed. Result of present study shows that Z. absinthifolia's methanol extract posses Hepatoprotective effect with non toxic profile.

Corresponding author:

Gul Muhammad,

Department of Pharmaceutical Chemistry,
Faculty of Pharmacy and Health Sciences, University of Balochistan,
Quetta-Pakistan.
(gul-e-balochistan@hotmail.com and gulm1427@gmail.com.

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INTRODUCTION:

Natural products show remarkable significance as medicines in preventing and treating different human diseases for more than thousands of years. Plants play important role in the development of synthetic and semi-synthetic medicines (1, 2, 3, and 4). From plants the discovery of drugs can be carried out by determining their biological activity (5). In current years, the studies of ethno-medical include major consideration in medicinal plants as well as in other natural products to discovery of new and active compounds. In all over the world, about thirty five thousand (35,000) plant species are known to be used for therapeutic purposes (6).

Pakistan has a long history of traditional medicine. About 6,000 plants in Pakistan are used as traditional folk and herbal medicine (7). Northern Areas, Azad Kashmir, Murree hills, Kurram agency, Malakand, Hazara and Balochistan are rich habitats of medicinal plants etc. Medicinal plants from these areas, are collected from the wild, and sold in the local market (8).

Balochistan is native home of medicinal plants. Scientific data available in these plants is very limited (7).

The genus *Zosima* (Apiaceae) consists of six biennial or perennial herbs. (9). *Z. absinthifolia* specie is native to Iran. It is distributed from Central Asia to Afghanistan, Iran and Turkey and cultivated on the lime stone slopes and steppe fields at a height of 400-2000 meters. Aeiel parts of this plant have a number of medicinal uses in folk medicine of Pakistan to reduce bowel disorders, to treat cough, stomach gas and indigestion (10). Previously antibacterial (11) ant proliferative and antioxidant effects (12) have been reported (13).

MATERIAL AND METHODOLOGY:

Plant Parts

Plant parts were collected from the Tehsil Punjpae (Quetta). Plant parts were identified and voucher specimen (Voucher # P-112) in Department of Pharmacognosy, FOPHS (Faculty of Pharmacy & Health Sciences), Balochistan University, Quetta.

Experimental Animals

For Hepatoprotective studies both gender rabbits (weighing about 1000 -1400 g) were used. Humidity as well as temperature (Standard) were kept for all day dark / light cycle (14). Standard procedures (SOP) of Good Laboratory Practice (GLP) were pursued (15).

Hepatoprotective Activity Study Design

The total experimental animals comprising of eighteen (18) rabbits was divided into three (3) groups, each group contain six (6) animals. All three (3) groups were treated as follows:

I. Group I: Control (Non treated)
II. Group II: Group treated with CCI4
III. Group III: CCI4 + *Z. absinthifolia* Crude extract (300 mg/kg orally)

Liver function test:

For liver function test the blood of experimental animal were collected in a tube containing anti-coagulant. For determining the liver function Following tests were performed i.e. SGPT, total Bilirubin, and Alkaline Phosphatase, these tests were determined by using standard reagent kits obtained from Merck, Germany at 37 ^{o}c on automatic analyzer (16)

Chronic Toxicity test

The chronic toxicity test was performed following the protocol described by the OECD guideline 408 for testing chemicals (17). Rabbits of both sexes were randomly assigned into 2 groups: a control group and drug 300mg/kg treatment groups. Methanol extracts of *Z. absinthifolia* were dissolved in 10% Tween 20 and administered orally on daily basis for 90 days at single doses of 300 mg/kg, while the control group received only 10% Tween 20 in distilled water. The extract was freshly prepared with vehicle on daily basis. The rabbits were weighed and visual observations for mortality, behavioral pattern (Salivation, fur, lethargy, and sleep), changes in physical appearance, injury, pain and signs of illness were conducted once daily during that period (16).

Biochemical test

The blood was collected by using technique of cardiac puncture with the help of 22 gauge sterile needle. The blood is than transferred in a clean and dry test tube and allowed for coagulation. By the help of centrifuge Serum was separated. For Biological test standard method (18) was used. For determining the different organ functions following tests were performed, i.e Total proteins, Lipid Profile (Triglycerides & Cholesterol), Albumin, Globulin. Liver function test (LFT) such as SGPT, Direct Bilirubin, Alkaline Phosphatase and Total Bilirubin. Cardiac enzymes test (SGOT, CK-MB and LDH). Renal profiles (Blood Glucose, Creatinine and Urea) (19).

Analysis of Hematological Profile
The blood was collected by using technique of cardiac puncture with the help of 22 gauge sterile needle. The blood is than transferred in a clean and dry test tube and allowed for coagulation (15). Parameter were WBC, RBC and platelet count, mean corpuscular hemoglobin (MCH), hemoglobin (Hb), Hematocrit (HCT), Pack cell volume(PVC) count, MCHC, MCV (mean corpuscular volume) were determined (19).

STATISTICAL ANALYSIS
The mean±S.E.M were used to calculate Data. For biochemical parameter analysis One way ANOVA and Dunnets t-test were used, *Z. absinthifolia* treated group were compared with control group and the highly significant value was measured at ($P<0.01$), while ($P<0.05$) value measured significant value (19-26).

RESULTS:

Hepatoprotective activity
Liver function test
Total Bilirubin (mg/dL) was 0.23±0.02 for control, 0.12±0.1 for CCl4 treated group and 0.68±0.06 for *Z. absinthifolia* treated group. Alkaline Phospatase (U/L) was 51.8±3.42 for control, 22.2±19.50 for CCl4 treated group and 113.4±22.11 for *Z. absinthifolia* treated group. SGPT (U/L) was 80.2±3.79 for control, 24.7±22.38 for CCl4 treated group and 166.8±85.83 for *Z. absinthifolia* treated group Results shown in Table 1 and Figure No 1.

Renal function test
Concentration of Urea in mg/dl was 77.0 ± 2.92 and 75.33 ± 3.73 for control and drug treated rabbits respectively & Concentration of Creatinine was 1.2 ± 019 and 1.18±0.15 for control and drug treated rabbits respectively Results shown in Table No 2 and figure No 2.

Random Blood Glucose
Concentration of Random Blood Glucose was 100.01 ± 9.45 and 105.4 ± 1.54 for control and for plant extracts treated animals respectively Results shown in Table No 3 and figure No 3.

Cardiac Enzymes Test
Level of LDH in µ/l was 720±98.31 and 730.5±19.29 for control and for plant extracts treated animals respectively. CKMB in µ/l level was 30±0.80 and 24±0.82 for control and for plant extracts treated animals respectively. SGOT in µ/l level was 94.2±3.79 and 96±3.55 for control and for plant extracts treated animals respectively Results shown in Table No 4 and Figure No 4.

Serum Concentration of Calcium and Uric acid
Serum concentration of Calcium in mg/dl is 13.3 ± 0.51 and 13±0.3 for control and for plant extracts treated animals respectively. Serum concentration of Uric acid in mg/dl level was 00.2 ± 00.054 and 00.1±00.01 for control and for plant extracts treated animals respectively Results shown in Table No 5 and Figure No 5.

Hematological profile
Concentration of Hemoglobin in g/dl was 11.22 ± 0.57 and 12.7 ± 0.93 for control and for plant extracts treated animals respectively. RBC Count in million/µl was 5.95 ± 0.33 and 7.01 ± 0.55 for control and for plant extracts treated animals respectively. Hematocrit (HCT/PCV) in % was 40.7 ± 2.29 and 44.48 ± 2.55 for control and for plant extracts treated animals respectively. MCV in fl was 67.90 ± 0.88 & 63.42 ± 1.74 for control and for plant extracts treated animals respectively. MCH in pg was 20.46 ± 0.39 and 19.16 ± 0.64 for control and for plant extracts treated animals respectively. MCHC in g/l was 30.14 ± 0.69 and 30.4 ± 0.58 for control and for plant extracts treated animals respectively. Total WBC Count in $\times 10^9$/l was 9.08 ± 0.77 and 7.96 ± 1.44 for control and for plant extracts treated animals respectively. Platelet Count in $\times 10^9$/l was 324.4 ± 34.22 and 395.6 ± 38.36 for control and for plant extracts treated animals respectively Results shown in Table 6 and Figure No 6.

Total protein
Total proteins in g/dl were 6.64 ± 0.12 and 7.00 ± 0.15 for control group and for drug treated group respectively. Globulin in g/dl level was 2.54 ± 0.13 and 2.70 ± 0.11 for control and for plant extracts treated animals respectively. Albumin in g/dl level was 4.10 ± 0.14 and 4.20 ± 0.08 for control and for plant extracts treated animals respectively. A/G ratio was 1.63 ± 0.13 and 1.60 ± 0.02 for control and for plant extracts treated animals respectively Results shown in Table No 7 and Figure No 7.

Lipid profile
Cholesterol in mg/dl was 34.20 ± 4.03 and 34.2 ± 4.21 for control and for plant extracts treated animals respectively. Triglyceride in mg/dl were 49.2 ± 4.62 and 89.0 ± 7.45 for control and for plant extracts treated animals respectively Results shown in Table No 8 and Figure No 8.

DISCUSSION:

The more commonly used model for hepatoprotective

drug screening is CCl4 induced liver damaged process. The increase in the ALT, Cholesterol and AST levels in serum shows that the structural integrity of the liver is damaged, they are present in hepatic cell cytoplasm so after hepatic cell damage they release in system circulation. CCl_4 treated Rabbit stimulates hepatotoxicity by activation of metabolism owing to induces selective toxicity in liver cells but sustaining semi-normal metabolic function. Trichloromethyl free radical (CCl3) is constituted in endoplasmic reticulum by metabolic conversion of Carbon tetrachloride by the enzymatic action of cytochrome P-450 dependent mixed oxidase, subsequently free radical combined with cellular protein and lipid in the presence of oxygen to stimulate lipid peroxidation. All this phenomena results in structural alteration of membranes and endoplasmic reticulum, failure of glucose 6-phasphate induction, protein synthesis reduction and failure of metabolic enzyme activation predominantly leading to liver injury (27,28).no considerable alteration comparative to control animals has been reported. Observed result indicates the absence of any reported toxicological effect.

The *Z. absinthifolia* administration leads to decrease serum concentration of both the urea and serum creatinine significantly, due to the effects of extracts on the liver function (21). Such effect lead to protect the liver is due to the ant oxidation properties of plant extract (22). According to the recent study the plant extract of zosima absinthifolia is safe in kidneys (23). The administration of *Z. absinthifolia* does not show significant change in blood glucose concentration. The analyses of cardiac enzymes are very mush necessary. The myocardial ischemia is best diagnosed by the Serum concentration of CKMB activity, the LDH concentration raise also show proportionality to the myocardial tissue injury extent (23). There is non significant change in the concentration of LDH and SGOT in serum after the Administration of *Z. absinthifolia*. On other hand there was significant decrease in the concentration of CKMB. So this major decrease in the concentration of CKMB indicates that the plant extracts were safe on heart muscles (24). Therefore according to recent studies the plant extracts of Z. absinthifolia are protective on heart. There was no significant change in the serum concentration of calcium and uric acid after the administration of plant extracts. In hematological test non significant change in the concentration of RBC Count, Hb, MCV, Hematocrit (HCT/PCV) %, MCHC, MCH & WBCs count. But the platelets count increased slightly. Increase in PTAFR and ALOX 12 gene responsible to enhance the creation of mega karyocytes & platelets, current study reveals that the *Z. absinthifolia* extract increased the production of the platelet which may be due to elevation in the concentration of PTFAR and ALOX 12 action (25). In LFT the administration of *Z. absinthifolia* significant change the serum concentration of the SGPT, Alkaline Phospatase, and Total Bilirubin was observed. The plant extracts of Z. absinthifolia showed non-significant effects on the serum concentration of A/G ratio, Albumin, & Total proteins. In lipid profile test non significant change in the serum concentration of cholesterol, whereas the serum concentration of TAG increased significantly, results of lipid profile test *Z. absinthifolia* have non toxic effects on the lipid profile.

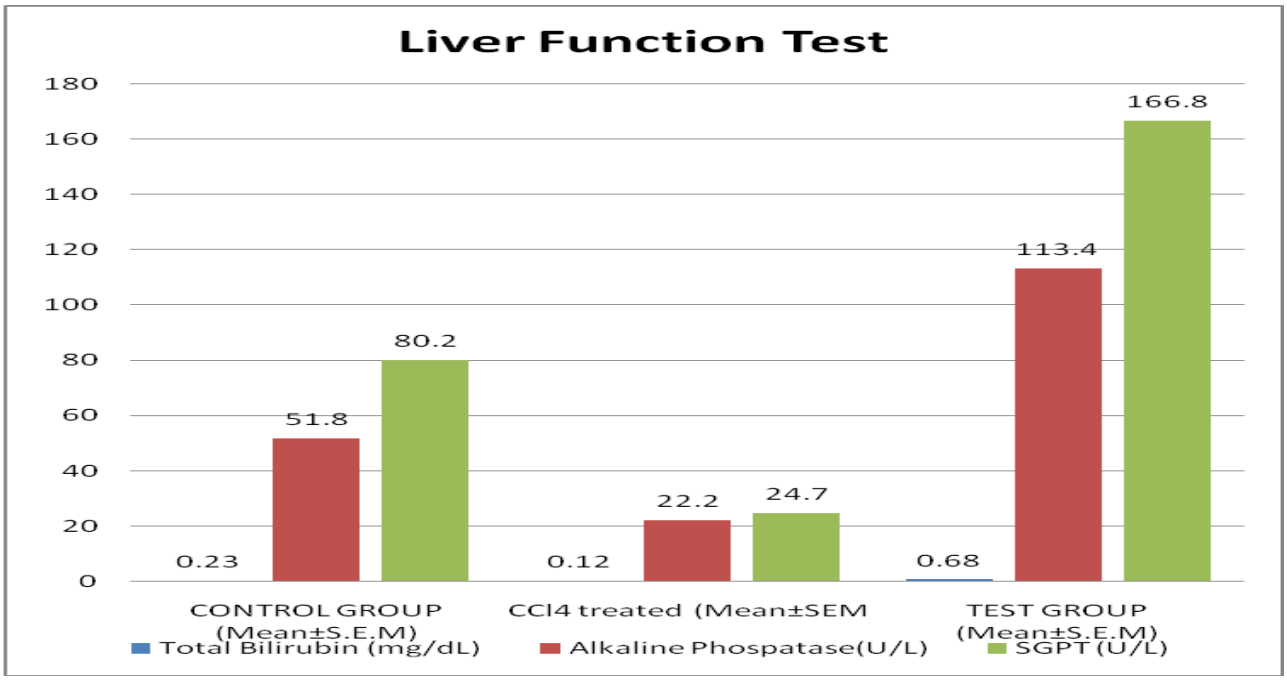

Figure No.1: Impact of *Z. Absinthifolia* on Liver function test of rabbits.

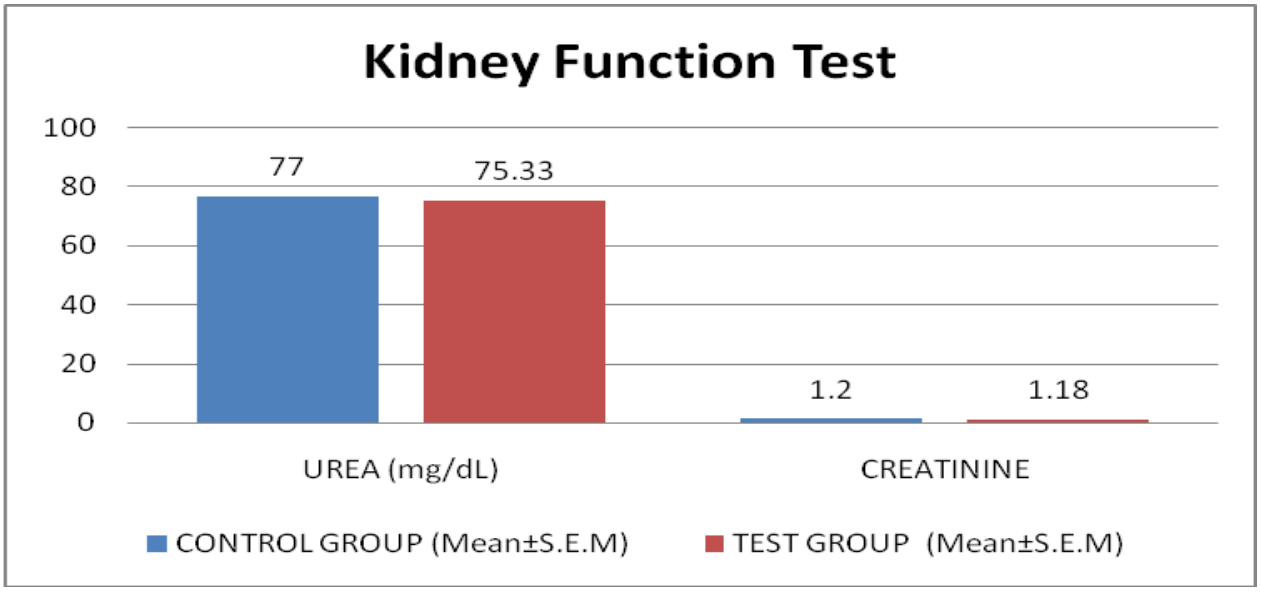

Figure No.2: Impact of *Z. Absinthifolia* on function test of rabbits's Kidney

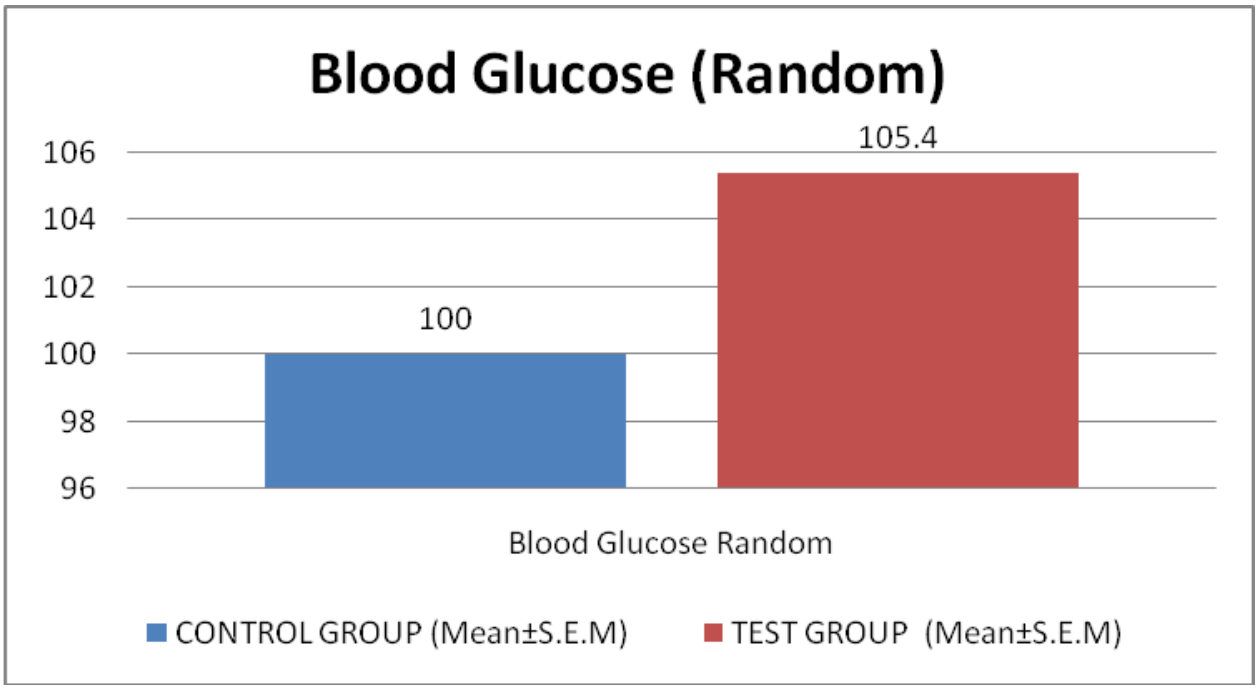

Figure No.3: Impact of *Z. Absinthifolia* on Blood Glucose (random) of rabbits

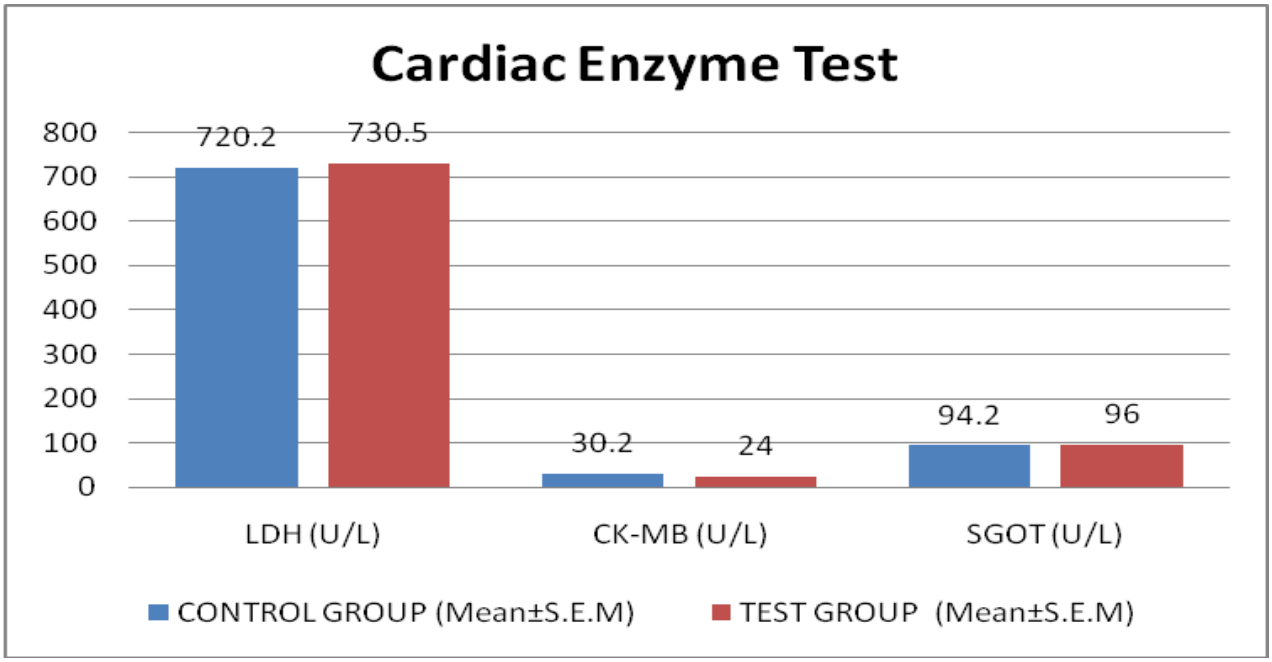

Figure No.4: Impact of *Z. Absinthifolia* on Cardiac Enzymes of rabbits

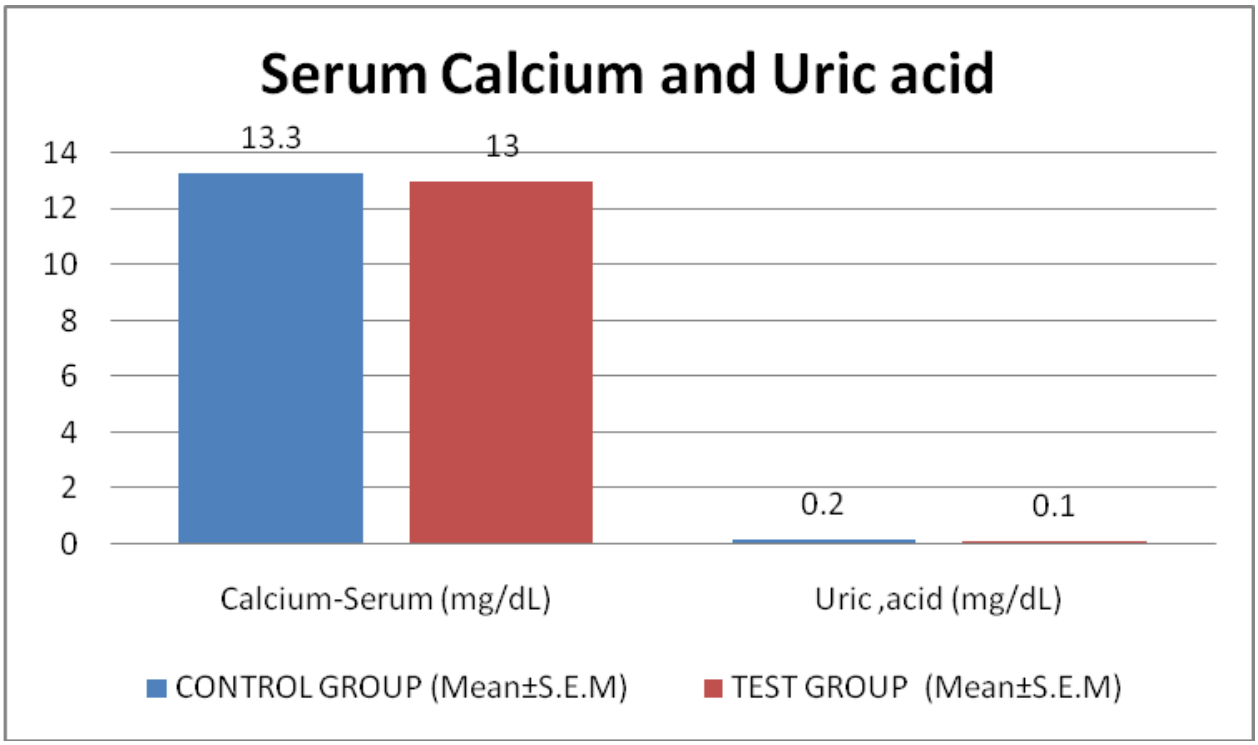

Figure No.5: Impact of *Z. Absinthifolia* on Serum Calcium and Uric acid

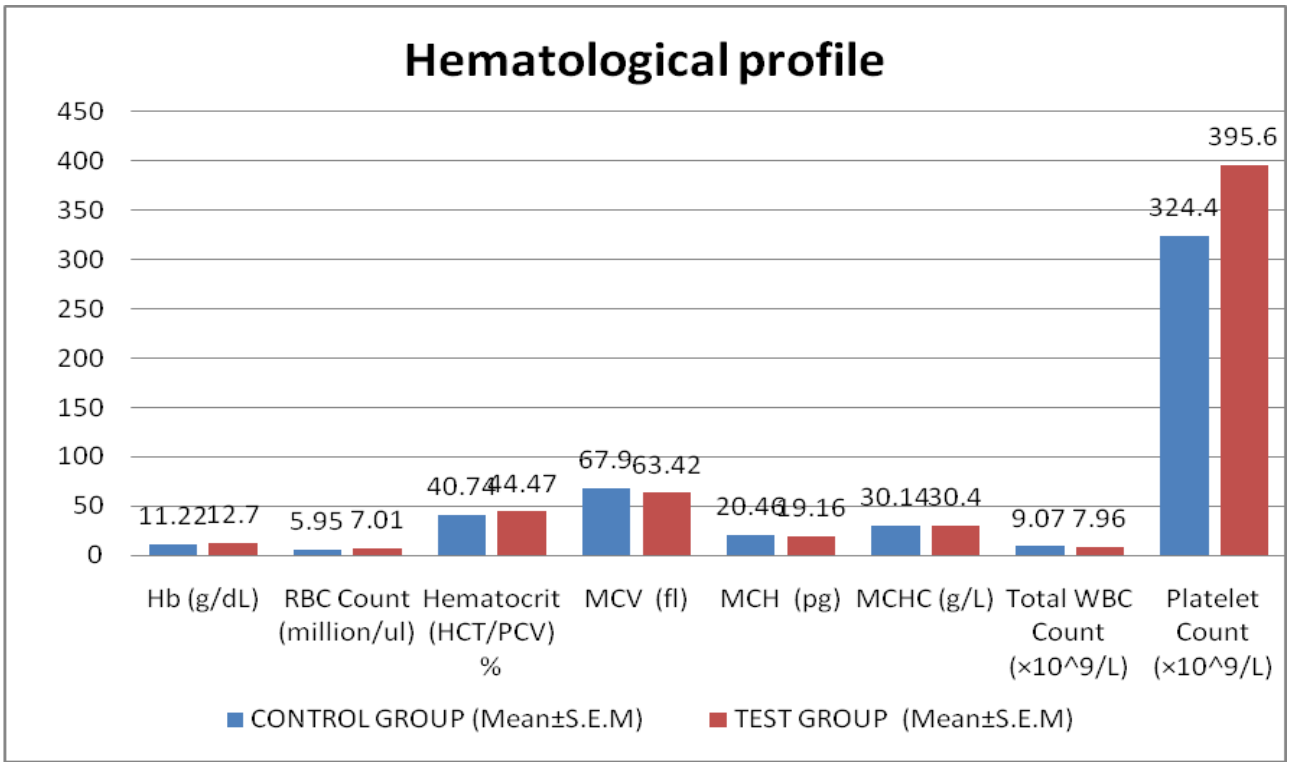

Figure No.6: Impact of *Z. Absinthifolia* on rabbit's Hematological profile

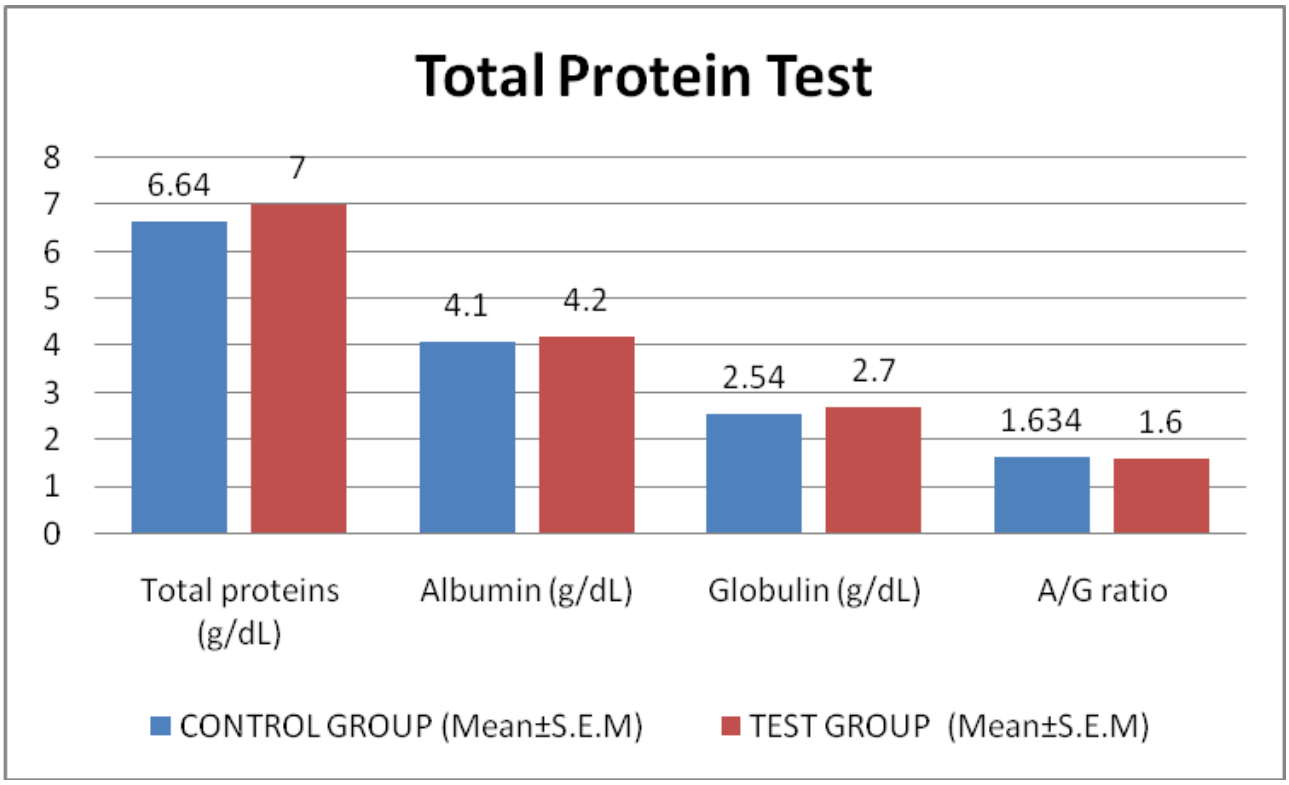

Figure No.7: Impact of *Z. Absinthifolia* on Total Protein test of rabbits

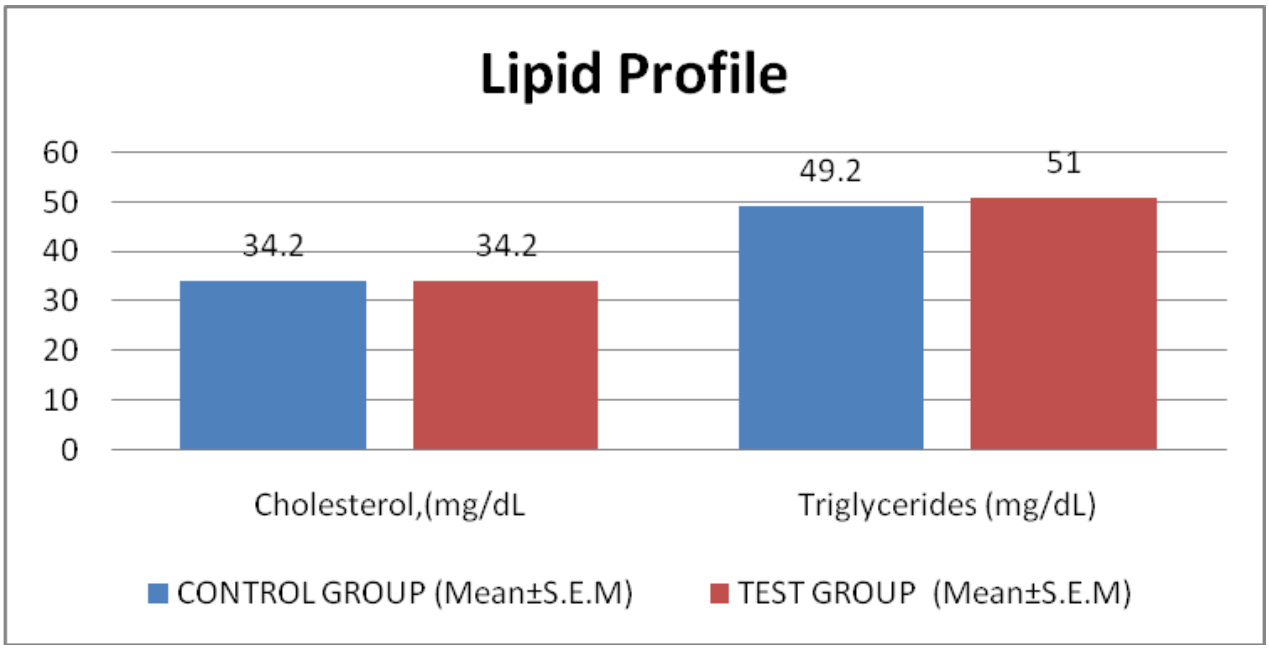

Figure No.8: Impact of *Z. Absinthifolia* on Lipid profile of rabbits

CONCLUSION:

Z. absinthifolia crude methanolic extracts produced significant heptoprotective activity with non toxic profile, however further studies are required to isolate the compounds responsible for activity.

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