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Research Article

Hematological, Biochemical and Histopathological Studies of *Cicuta virosa* Linn. Extract on Wistar Rabbits

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Abstract

Complete blood count, biochemistry of the blood, urine analysis and histo-pathological effects of extract of *Cicuta virosa* L. on heart, liver, kidney and stomach tissues of rabbits were investigated to find out the effects of low dose administration of *C. virosa* for three months. Four groups of animals were made, male control group, male test, female control and female test group (6 animals in each group). Blood samples were obtained through cardiac puncture for complete blood count and blood biochemistry. Histo-pathological study of heart, liver, kidney and stomach tissues was carried out after administration of 25 mg/kg of extract of *C. virosa* for a period of three months. There were significant differences ($p< 0.05$) in hematological and biochemical parameters of the control and the test groups. No significant pathology was observed in male heart and stomach tissues. Moderate degree of siderosis was found in test liver tissue as compared to control tissue. Patchy areas of cortical necrosis were seen in male test kidney tissues. No malignancy or mortality was observed on administration of daily oral dose of 25 mg/ kg for 3 months. Our research work supports the use of low dose of *C. virosa* extract in various neuropathies and skin diseases.

1. INTRODUCTION

Cicuta virosa Linn. commonly known as Water Hemlock, has umbrella shaped white or green colour flower, grows in marshy areas, belonging to family Apiaceae. It contains coumarins, polyacetylenes like cicutoxin, and essential oils, mostly *γ*-terpinene, *p*-cymene and cumin aldehyde.[1] It contains poisonous principle, cicutoxin that is present in all stages of growth and in all parts of plant of *C. virosa* which is a non-competitive GABA receptor antagonist.[2] The reported pharmacologically active compounds are: 3β-acetyloxy-16-hydroxy-olean-12-en-28-oic acid; 9 (11), 12-dieneoleana-3β-ol ; 9, 19-cyclolanaost-24-en-3-one; 9, 19-cycloergost-23-en-3, 25-diol ; stigmasterol; falcarindiol; 1, 2-benzenedicarboxylic acid; his (2-ethylhexyl) ester; stigmast-5-en-3β-ol; β-daucosterol.

Hematological and biochemical investigations facilitate the identification of causative factor of any pathology due to environmental factors, stress, and improper nutrition.[3]

2. MATERIALS AND METHOD

2.1 Experimental Animals

The rabbits of both sexes, having 1.5kg weight were purchased from Animal House of Dow University of Health Sciences (DUHS), Karachi and kept in animal house for a period of 15 days to acclamized. Male and female rabbits were kept in separate cages and fed with their normal diet and water. Their weights were checked on random basis. The drug was administered at the interval of 24 hours for a period of 3 months. The blood of the rabbits was taken by cardiac puncture at the end of 3 month.[4] 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 2005.

2.2 Animal grouping and drug dosing for hematological and biochemical evaluation

Four groups were made (male control – 6 rabbits), (female control – 6 rabbits), (male test (CM) – 6 rabbits) and (female control (CF) – 6 rabbits). Male and female control groups were given distil water, while test groups CM and CF were given 25mg/kg *C. virosa* extract. All the administrations were given orally. The treatment continued for 90 days. Blood (6 ml) was collected by cardiac puncture with 10 ml sterile syringe using 1mg/1ml EDTA as anticoagulant for the determination of blood and biochemical parameters.

2.3 Animal grouping and drug dosing for histopathological examination

Two groups were made namely group I (male control), group II (male test):

Group 1 (male control): Six animals (male) were kept as control. Water and food was provided to the animals during the whole period of experiment.

Group 2 (male test): Six animals were administered 25mg of Test drug extract, water and food was provided to the animals during the whole period of experiment.

The animals were sacrificed at the end of 90 days after taking out blood through cardiac puncture technique for the above mentioned tests.

2.4 Hematological evaluation

Total erythrocyte counts were counted using a Neubar 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'shemometer. 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

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hemoglobin concentration (MCHC) for particular blood samples were calculated using hematological data as mentioned by Burnett *et al.* (2006)[5]; differentiation of leucocytes was carried out according to Ivanova 1983.[6] Determination of the relative abundance of all the cell types was carried out by counting total of 200 blood cells.[7]

2.5 Biochemical evaluation

Serum samples were obtained by centrifugation of blood at 1300 x g 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.[8]

2.6 Urine Analysis

Voided sample of urine was collected by placing a clean, empty litter box in the site where the animals usually urinates.[9]

2.7 Histopathological Analysis

The liver, kidney, heart and stomach tissues were dehydrated separately with ethanol of graded concentrations. The tissues were passed through xylene solution to clear the ethanol and facilitate molten paraffin wax infiltration (55°C). After that, they were treated with paraffin wax and cast into blocks; sections of 5 µm thickness were cut with microtome. These were later placed on clean glass slide. The sample slides were subsequently stained in haematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded.[10-11]

2.8 Statistical analysis

Results of the study were presented as a mean plus or minus standard error of mean (Mean ± SEM). Differences between control and treatment groups were analyzed by student t- test.[12]

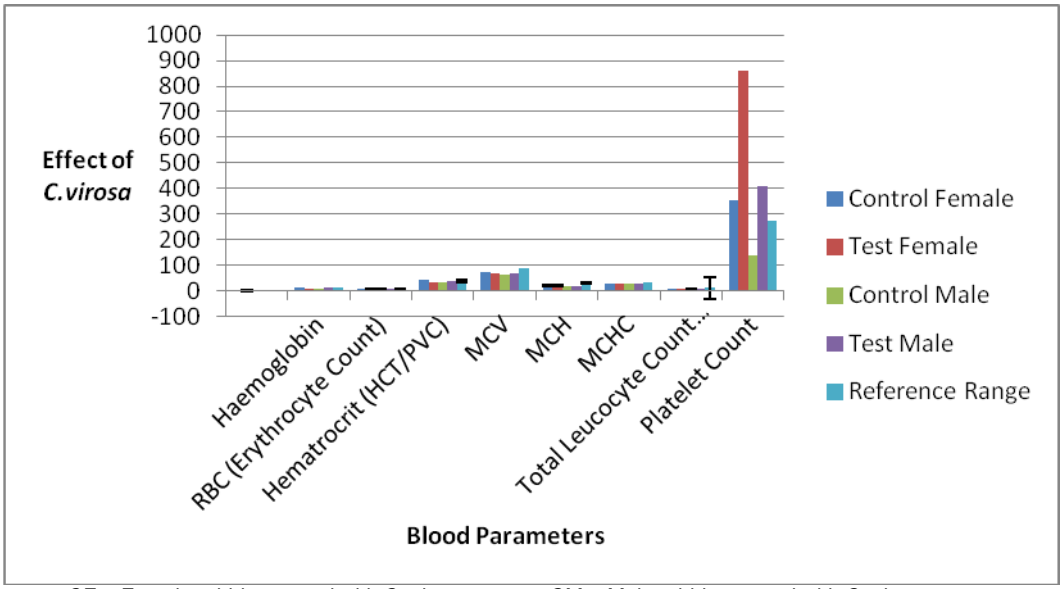
3. RESULTS

Haematological effects on male and female rabbits' blood were observed (Graph 1). Biochemical changes were observed in rabbits of both sexes as compared to their respective control groups (Graphs 2, 3, 4 & 5). Urine analysis of test group was compared with the control group (Table 1). No histo-pathological changes were observed in heart and stomach tissues while slight changes were observed in liver and kidney tissues (Figures 1-8).

Table 1: Shows the effect on Urine Parameters of Rabbits with and without *C. virosa* extract.
A dose of 25 mg/kg was given each day for 1.5 months.

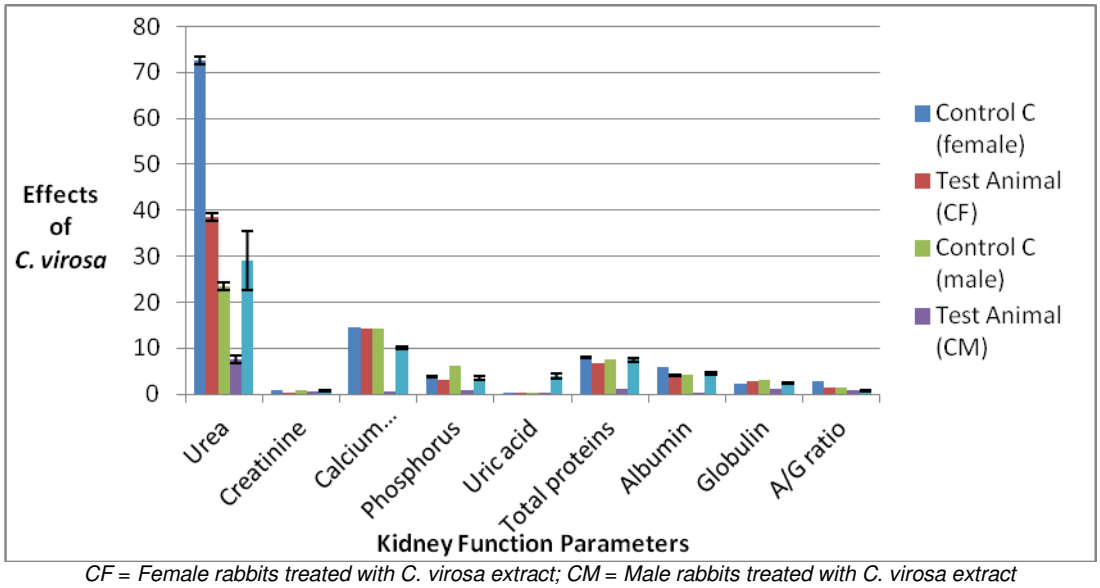
Urine Parameters	Control Animal C (female)	Test Animal (CF)	Control Animal C (Male)	Test Animal (CM)	Reference Range
Urine Physical					
Volume	30.08±0.11	15±0.632	25.01±0.136	30.5±0.836	179.17±61.81
Colour	Yellow	Yellow	Yellow	Yellow	Pale yellow-red brown
Appearance	Turbid	Turbid	Turbid	Turbid	Clear
Sp. Gravity	1.0045±0.00037	1.0045±0.00046	1.0045±0.00037	1.0045±0.00046	1.019±0.007
pH	9±0.063	9±0.063	9±0.063	9±0.063	8.53±0.195
Urine Chemical					
Protein	Nil	Nil	+1 (30 mg/dL)	Nil	Negative
Glucose	Nil	Nil	Nil	Nil	Negative
Ketone Bodies	Negative	Negative	Negative	Negative	Negative
Urobilinogen	Normal	Normal	Normal	Normal	Negative -weak positive
Blood	Negative	Positive (+)	Negative	Negative	Negative
Bilirubin	Nil	Nil	Nil	Nil	Negative
Urine Microscopy					
RBC	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF
WBC	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF
Epithelial Cell	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF	Nil/ HPF

CF = Female rabbits treated with drug; CM = Male rabbits treated with drug



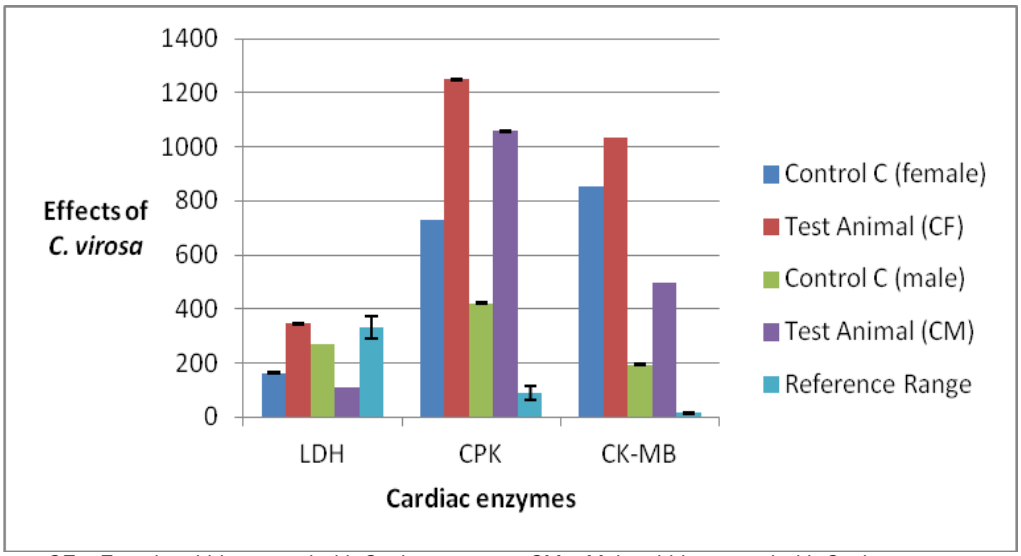
CF = Female rabbits treated with C. virosa extract; CM = Male rabbits treated with C. virosa extract

Graph 1: Shows the effect of *C. virosa* extract on the blood parameters of rabbit in comparison with the control.



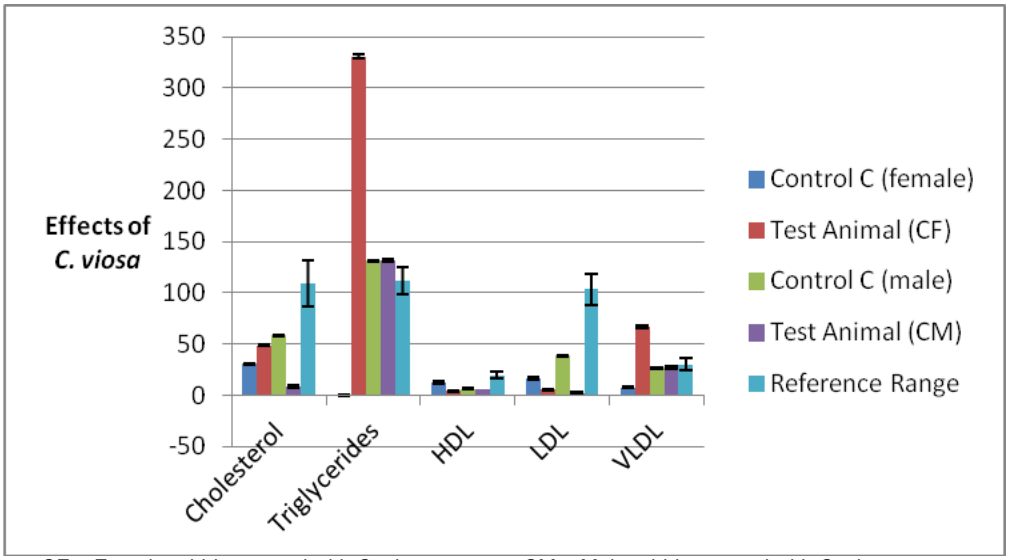
CF = Female rabbits treated with C. virosa extract; CM = Male rabbits treated with C. virosa extract

Graph 2: Shows the effect of *C. virosa* extract on the Kidney function parameters of rabbit in comparison with the control.



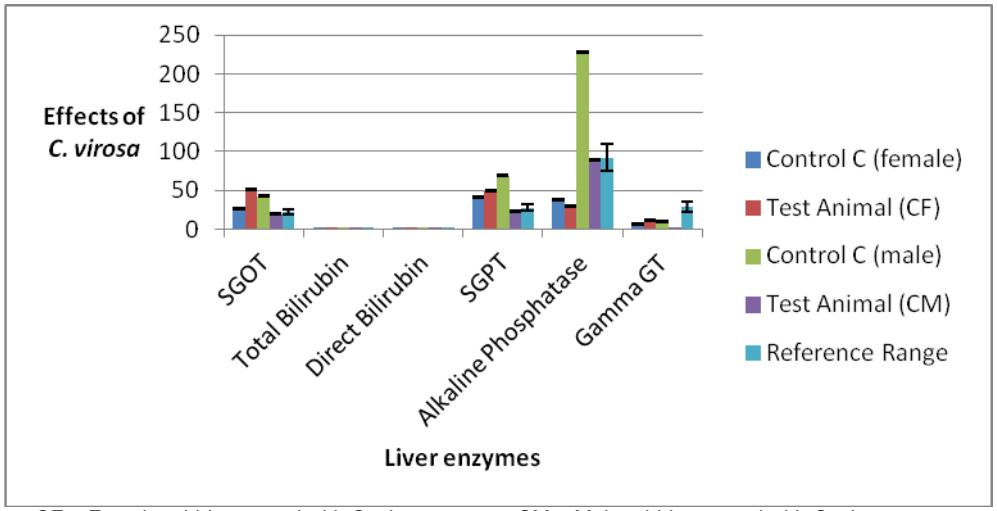
CF = Female rabbits treated with C. virosa extract; CM = Male rabbits treated with C. virosa extract

Graph 3: Shows the effect of *C. virosa* extract on the Cardiac enzymes of rabbit in comparison with the control.



CF = Female rabbits treated with C. virosa extract; CM = Male rabbits treated with C. virosa extract

Graph 4: Shows the effect of *C. virosa* extract on the Lipid profile parameters of rabbit in comparison with the control.



CF = Female rabbits treated with C. virosa extract; CM = Male rabbits treated with C. virosa extract

Graph 5: Shows the effect of *C. virosa* extract on the Liver enzymes of rabbit in comparison with the control.

4. DISCUSSION

Increase in the levels of all the blood parameters; Haemoglobin (11.25±0.0836); RBC count (5.725±0.00836); Hematrocrit (39.05±0.0836); MCV (68.25±0.0836); MCH (19.85±0.0836); MCHC (29.25±0.0836); WBC count (7.85±0.0836); Platelet Count (410±0.632) were found in the male test group treated with *C. virosa* in comparison to the control male rabbit group (Table 1; Graph 1).

Decrease in hemoglobin (8.25±1.58), RBC count (5.33±0.562), and hematocrit (32.48±0.78), MCV (68.85±0.0836), MCH (20.05±0.0836) and MCHC (28.5±0.836) observed, whereas, total leucocyte count (6.25±0.0836) was slightly increased, while, platelet count (861±0.632) was significantly elevated (Table 1; Graph 1).

All biochemical parameters, i.e., urea (7.5±0.836), creatinine (0.67±0.009), serum calcium (0.721±0.01), phosphorus (0.818±0.01), uric acid (0.028±0.0065), total protein (1.106±0.0096), albumin (0.045±0.0083), globulin (1.06±0.0063), A/G ratio (0.995±0.025) were found lowered in male test group treated with *C. virosa* in comparison to its respective male control group (Table 2; Graph 2).

The levels of uric acid (0.176±0.009) and globulin (2.75±0.015) were slightly elevated while the rest of the parameters, urea (38.5±0.836), creatinine (0.44±0.01), serum calcium (14.35±0.01), phosphorus (3.075±0.031), total protein (6.86±0.014), albumin (4.106±0.012) and A/G ratio (1.505±0.014) were found reduced as compare to female control group (Table 2; Graph 2).

CPK (1057.33±0.96) and CK-MB (497.83±1.11) enzymes were found to be significantly raised; whereas LDH (111±1.166) levels were lowered in *C. virosa* treated test animals in comparison with the respective male control group (Table 3; Graph 3).

All the cardiac enzymes; CPK (1249.67±1.78), CK-MB (1032.67±1.154) and LDH (344.83±1.036) were found raised in test group treated with *C. virosa* when compared with its female control group (Table 3; Graph 3).

Triglycerides (131.83±1.036) and VLDL (27±1.058) levels were slightly raised. However, cholesterol (8.5±0.836), HDL (6±0.632) and LDL (2.167±0.52) levels were lowered in the test group treated with *C. virosa* as compared to the male control group (Table 4; Graph 4).

Cholesterol (48.67±0.96), triglycerides (330.83±1.58) and VLDL (67.16±1.18) levels were raised, while HDL (4±0.63) and LDL (5.83±0.65) levels were reduced in test group treated with *C. virosa* when compared with its female control group (Table 4; Graph 4).

Direct bilirubin (0.103±0.013) was found slightly raised; whereas, the rest of the liver enzymes; SGOT (19.5±0.836); total bilirubin (0.24±0.0096); SGPT (22.5±0.836); alkaline phosphatase (88.5±0.836) and Gamma GT (0.978±0.34) were lowered in test group treated with *C. virosa* in comparison to the respective male control group (Table 5; Graph 5).

SGOT (51.5±0.836), direct bilirubin (0.03±0.0063), SGPT (48.83±1.036), and Gamma GT (11.83±1.03) were found to be elevated whereas, the levels of total bilirubin (0.186±0.0096) and alkaline phosphatase (29.5±0.836) were diminished in the test group treated with *C. virosa* as compared to the female control group (Table 5; Graph 5).

The urine parameters of the male test group were similar to that of its respective male control group (Table 6).

The urine parameters of the female test group were comparable to that of its respective female control group; except for the presence of blood in the urine of the test group (Table 6).

No gross pathology was observed in *C. virosa* male treated group heart (Figure 1 and 2) and stomach tissues (3 & 4). Moderate portal inflammation and Peri-portal fibrosis with moderate siderosis was seen in liver tissues (Figure 6) as compared to its respective control group (Figure 5), whereas, patchy areas of cortical necrosis were observed in kidney tissues (Figure 8) as compared to the control group (Figure 7).

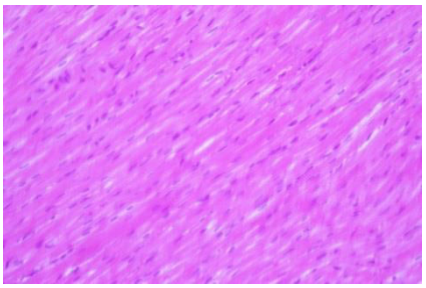


Figure 1: Male Control Heart Tissue: Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined. Male Control Heart Tissue: 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.

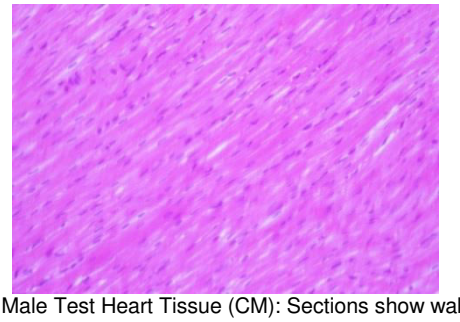


Figure 2: Male Test Heart Tissue (CM): Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

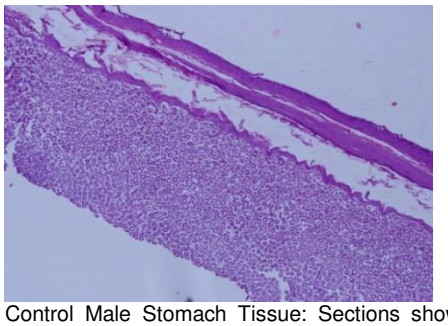


Figure 3: Control Male Stomach Tissue: Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layer is seen beneath,lined externally by serosa. No significant pathology is seen in any of the sections examined.

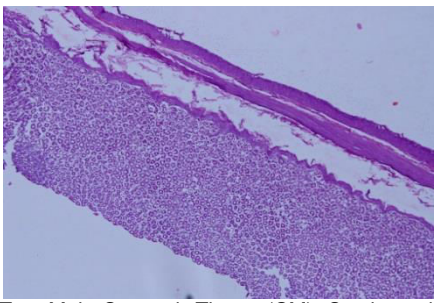


Figure 4: Test Male Stomach Tissue (CM): Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and in unremarkable. Well organized muscular layeris seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

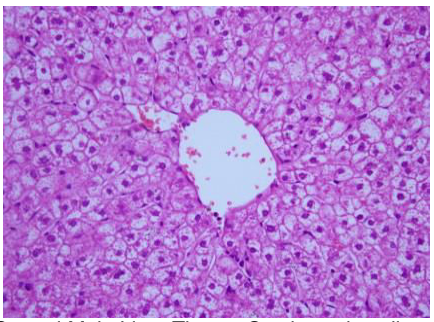


Figure 5: Control Male Liver Tissue: Sections show liver tissue with overall preserved lobular architecture. Portal tracts aremildly dilated with lymphocytic infiltrate and minimal fibrosis. Centrilobularhepatocytic degeneration also noted. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen.

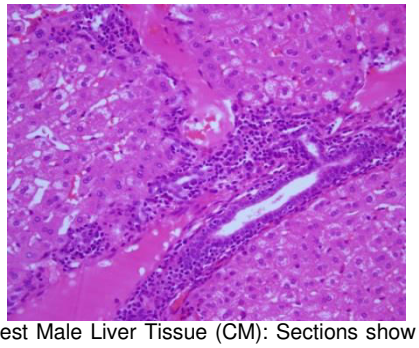


Figure 6: Test Male Liver Tissue (CM): Sections show liver tissue with overall preserved lobular architecture. Portal tracts are moderately expanded with lymphocytic infiltrate and fibrosis. No significant lobular inflammation seen. Moderate degree of siderosis is seen. No cholestasis. No evidence of granuloma or malignancy is seen.

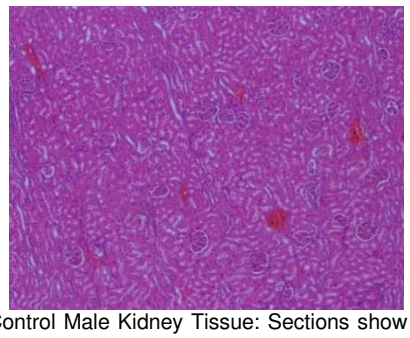


Figure 7: Control Male Kidney Tissue: Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows focal lymphocytic infiltrate. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

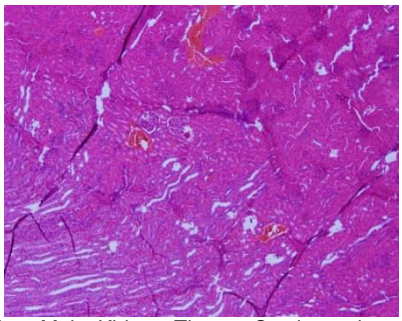


Figure 8: Test Male Kidney Tissue: Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Patchy areas of cortical necrosis are seen. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen in any of the sections examined.

C. virosa contains cicutoxin, isocicutoxin, Virol A, Virol B, Virol C, furocoumarins and essential oils. These components are responsible for the potent activity and toxicity of *C. virosa*.[13-14]Its strong effects on the nervous system may be due to the presence of triple bond and epoxide group in its structure.[15]

Elevated complete blood count values were observed in male test group treated with *C. virosa* in comparison with control group. Raised white blood cells may indicate the presence of infection, inflammation and damage to body tissues. Raised red blood cells may be due to polycythemia, long-term lung, kidney, heart or liver disease. High platelet values may be seen with iron deficiency, some diseases like cancer, or problems with the bone marrow.[16] In female group treated with *C. virosa* decrease in hemoglobin, RBC count, and hematocrit, MCV, MCH and MCHC was observed. Anemia lowers RBC values. Anemia can be caused by stomach ulcers, colon cancer, and inflammatory bowel disease. Total leucocyte count was slightly increased while platelet count was significantly elevated in the female treated group. Conditions that cause high WBC values may include infection, inflammation, and damage to body tissues, kidney failure, lupus, tuberculosis,

rheumatoid arthritis, malnutrition, leukemia, and diseases such as cancer. High platelet values may be seen with iron deficiency, some diseases like cancer, or problems with the bone marrow. Kidney function parameters were found lowered in male test group treated with *C. virosa*. Lowered urea level maybe due to malabsorption or liver damage. Low levels of creatinine may indicate kidney or liver damage. Lowered uric acid level may be an indicator of kidney disease, malabsorption, liver damage or an overly acid kidney. Inadequate intake,primary hyperparathyroidism, hypercalcaemia of malignancy, diabetes mellitus, renal tubular defects and eclampsia maybe the causes of hypophosphotaemia. Hypoalbuminaemia, renal secondary hyperparathyroidism, eclampsia, pancreatitis with fat necrosis, hypoparathyroidism, excessive phosphate intake, intestinalmalabsorption might be the leading cause of hypocalcaemia. Hypoalbuminaemia in male group may be due to any of the following reasons; primary or secondary intestinal malabsorption, exocrine pancreatic insufficiency, malnutrition, dietary or parasitism, chronic liver disease, glomerulonephropathy resulting in proteinuria, acute inflammation. In female group treated with *C. virosa* elevated levels of globulin might be due to monoclonal gammopathy, polyclonal gammopathies, multiple myeloma, mass inflammation, infection, neoplasia, ehrlichiosis, leishmaniasis, chronic liver disease, systemic lupus erythromatasus. Raised level of uric acid might be due to kidney disease, malabsorption, liver damage or an overly acid kidney.Hypophosphotaemia, hypoalbuminaemia, hypocalcaemia were also observed in female test group. Cardiac enzymes were found raised in both male and female test groups may be indicative of skeletal muscle disease, acute MI, cerebral vascular disease, electrolyte imbalance or hypokalemia. Increased triglycerides level in male group may indicate atherosclerosis, fatty liver disease, pancreatitis, and diabetes or kidney disease. Hypo-cholesterolaemia was present, may be due to intestinal malabsorption or advanced liver disease. HDL and LDL levels were found to be lowered in both the male and female test group. Whereas in female test group, cholesterol and triglycerides levels were found raised.[17] Raised cholesterol may be indicative of increased risk of heart disease and stroke. Elevated triglyceride levels are a risk factor for atherosclerosis, fatty liver disease, and pancreatitis; or may be associated with diabetes, kidney disease. In male test group, elevations of direct bilirubin typically may result from obstruction either within the liver or a source outside the liver (example gallstones or a tumor blocking the bile ducts). Rest of the liver function enzymes were found to be lowered that do not indicates any clinical significance. In female treated group elevated SGOT, direct bilirubin, SGPT, and gamma GT levels. The cause of elevated direct bilirubin is usually outside the liver. These types of causes are typically gallstones. SGPT is an enzyme produced in liver cells is elevated when cells are excessively damaged or die secondary to hepatitis. SGOT may be raised due to liver disease. While gamma GT may be elevated with bile duct disease, any liver disease. The presence of blood in the female urine sample may be indicative of UTIs, kidney infection or disease, bladder or kidney stones or kidney injury.[18] In male group treated with *C. virosa*, moderate portal inflammation and periportal fibrosis with moderate siderosis was seen in liver tissues and patchy areas of cortical necrosis were observed in kidney tissues.

In low dose in homeopathy *C. virosa* is used for the treatment of spasms, epilepsy, seizure and convulsions.[19]

5. CONCLUSION

Effects of *C. virosa* on hematological and biochemical parameters and its histopathological studies on heart, liver, kidney and stomach tissues revealed the effectiveness of its extract used for the treatment of various pathologies associated with spasm and convulsions.

6. CONFLICTS OF INTEREST

The authors report no conflict of interests.

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