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ANTI-HYPERLIPIDEMIC ACTIVITY OF ALCOHOLIC AND METHONOLIC EXTRACTS OF *CROTOLARIA JUNCEA* IN TRITON-WR 1339 INDUCED HYPERLIPIDEMIA

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available. Literature claims that Saponins are able to reduce hyperlipidemia. Based on high saponin content in herbal plants, *Crotolaria juncea* (CJ) was selected and the present study focus on the antihyperlipidemic activity of alcoholic and methanol extract of leaves of CJ against Triton induced hyperlipidemia in mice. CJ was administered at a dose of 100 and 200mg/kg (p.o) to Triton induced hyperlipidemic mice. Atorvastatin was used as reference standard. The statistical analyses were carried out using one way ANOVA followed by bonferroni test using computer based fitting program (Prism, Graph pad.). CJ shows a significant decrease in the levels of serum total cholesterol, triglyceride, LDL, VLDL and significant increase in the level of serum HDL at the dose of 100 and 200mg/kg (p.o) against Triton induced hyperlipidemia in mice. Methanol and alcoholic extracts of CJ showed significant anti-hyperlipidemic effect and this study provides the scientific proof for their traditional claims. Therefore it effectively suppressed the Triton induced hyperlipidemia in mice, suggesting the potential protective role in Coronary heart disease.

Key Words: *Crotolaria juncea*, Hyperlipidemia, Triglycerides, Saponin, Lipoproteins, Triton-WR 1339, Atorvastatin.

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (Grundy, 1986). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Davey Smith, 1993). Hyperlipidemia is characterized by elevated serum total cholesterol, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan *et al*., 2003). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Kaesancini *et al.*, 1994*)*. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurence of further cardiovascular disease or cerebrovascular disease (Smith *et al.*, 1992). Currently available drugs have been associated with number of side effects (Brown, 1996). The consumption of synthetic drugs leads to hyperuricemic, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Ecobichon, 1997). Certainly, a food, a nutritional supplement or a drug that has documented anti-viral activity as well as an ability to positively affect the immune response would be of considerable clinical interest. Since, there is a high incidence of mortality for type 2 diabetics with their first myocardial infarction,

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aggressive therapy for treating diabetic dyslipidemia is recommended (Gramza *et al., 2005*). As currently available hypolipidemic agents lack desired properties of an ideal drug in combating the disease, there is an ongoing need for additional gents. Thus, researchers are involved to find out an effective, safe, and less expensive drug from natural origin to treat dyslipidemia associated with hyperglycemia. Many indigenous Indian medicinal plants have been found to be useful in successful management of diabetes.

Crotalaria juncea L. *Family: Fabaceae* is an annual herb, native to tropical America and found as a weed in cultivated lands as well as follow lands throughout India. it is being considered useful for treating antihelmenthiasis, sedative, anti-inflammatory, nematocidal and worm infection. Flowers, Fruits and Stem are used in anti-helmenthiasis and Skin diseases. The presence of active constituent's viz. Alkaloids, Glycosides, Phenyl coumarin 5-6 have been reported from the leaves. It may have a beneficial effect in the prevention of cardiovascular diseases. Decreases in blood pressure and plasma lipid concentrations, especially triacylglycerols and low density lipoprotein-cholesterol have been demonstrated as a result of oral consumption of *CJ*. It has also been shown to indirectly modify the total cholesterol and high density lipoprotein cholesterol values (Harborne, 1998). As the antihyperlipidemic activity of *CJ* had not been elucidated in an exclusive Triton induced hyperlipidemic animal model, the present study has been designed to evaluate the lipid controlling activity of *CJ* in Triton induced hyperlipidemia in Swiss albino Mice.

MATERIALS AND METHODS

Chemicals

Triton WR-1339 (Hi-Media), Atorvastatin standard drug (TG-TOR*10), Ethanol, Methanol, Carboxy Methyl Cellulose, 0.9% Normal Saline all other chemicals used were of analytical grade.

Collection of Plant Material and Extraction

Crotolaria juncea was collected in the month of December 2011 from tirupati, Andhra Pradesh. The plant authentification was done by Mr. Madhava Chetty, Assistant Professor, Botany Department, College Of Sciences, S.V.University, Tirupati, Andhra Pradesh.

The fresh and mature leaves of *crotolaria juncea* was collected and dried under shade, made in to coarse powder which is being used for preparation of Alcoholic and Methanolic extracts. To prepare extracts 50g of plant leaf powder in 250 ml of ethanol and methanol was performed by soxhlet apparatus for 8h at room temperature for 15 days. The residue was removed by filtration. The filtrate was evaporated to dryness at 40-50ºc under reduced pressure in a rotary evaporator. The yield of both ethanolic and methanolic extracts was approximately 10%. The extract was suspended in carboxy methyl cellulose and used for oral administration.

Preliminary Phytochemical Analysis

The methanol and alcoholic extracts of CJ were screened for the presence of various Phytoconstituents (PHS, 1996).

Experimental Animals

Swiss albino mice weighing 25-30 gm of male were used. Mice were maintained on a standard diet and water ad libitum. All animals were housed at ambient temperature (21 ± 10 °C) and relative humidity (55 ± 5%) with fixed 12h/12h light/dark cycle. Animals had free access to standard pellet diet and water given ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (TM Speight *et al.*, 1987 & JA Berliner, 1996).

Acute Toxicity Studies

Acute oral toxicity study was performed as OECD – 423 guidelines (acute toxic class method), (Ecobichon 1997). Swiss albino mice (n=6) of female selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for providing only water, after which the extracts were, administered orally at the dose levels of 2000 mg/kg body weight by oral feeding needle and observed for 24 hours. If mortality was observed in 2 out 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in animal, the same dose was repeated again to confirm the toxic dose. If mortality was not observed the procedure was repeated for further higher dose such as 3000 mg/kg of body weight. The Ethanolic and Methanolic extracts of *Crotolaria juncea* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals.

Antihyperlipidemic Studies

Induction of Hyperlipidemia

Hyperlipidemia was induced in male Swiss albino mice by intraperitoneal administration of Triton WR 1339 (400 mg/kg body weight) which is dissolved in 0.9% normal saline, twelve hours following the triton was injected after overnight fasting for 8 hours. Triton is capable to produce hypercholesterolemia by accelerating hepatic cholesterol synthesis. Hyperlipidemia was confirmed by the elevated blood cholesterol levels, determined by after administration of 24 hours (Vogel WH *et al.*, 2002).

Blood Sample Collection

Blood samples were collected by retro-orbital plexus puncture method and blood serum total cholesterol, HDL, triglyceride levels were estimated by using Cholesterol kit, span diagnostics Ltd, Ind and Triglyceride kit, span diagnostics Ltd, Ind.

Liver Lipid Extraction

The liver was homogenized in cold 0.15M KCl and extracted with CHCl3 CH3OH (2% v/v). This lipid extract was used for the estimation of lipid parameters (Ding *et al.,* 1992).

Biochemical Analysis

The serum and liver extract were assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods (Moss JN *et al.,* 1971).

Statistical Analysis

All the data expressed as Mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by bonferroni test using computer based fitting program (prism, graph pad.). Statistical significance was determined at $p< 0.05$.

RESULT AND DISCUSSION

Administration of triton in mice show significant ($p<0.001$) increase in the cholesterol levels in blood serum compared to respective normal group. Treatment with alcoholic, ethanolic extracts of *crotolaria juncea* and atorvastatin show significant ($p<0.001$) reduction in the test 1, test 2 groups and standard group compared to respective induction group. There was a significant ($p<0.001$) decrease in the HDL cholesterol levels in blood serum of induction group compared to normal group. There was a significant ($p<0.001$) increase in the amount of HDL cholesterol levels in test1, test2 and standard groups compared to respective induced group.

There was a significant ($p<0.001$) increase in the triglyceride levels in blood serum of induction group compared to normal group. There was a significant ($p<0.001$) decrease in the amount of triglyceride levels in test1, test 2 and standard groups compared to respective induced group. Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting into increased blood lipid concentration. The biphasic nature of triton - induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemia agents. Drugs interfering with lipid biosynthesis or uptake will be active in the synthesis phase and metabolism will be active in the excretory phase. In the present study, the methanol extracts of CJ reduced the cholesterol and triglycerides in a manner similar to the reduction facilitated by atorvastatin. The hypolipidemic activities of atorvastatin and ethanolic and methanol extract of CJ were evident in both synthesis and excretory phases of triton-induced hyperlipidemia in mice. Triton induces hyperlipidemia by increasing the hepatic synthesis of cholesterol and triglycerides (Vogel HG *et al.,* 1997; Kumar S *et al.,* 2011). So, it can be assumed that CJ inhibits the biosynthesis of cholesterol and triglycerides and therefore can be used for the prevention (prophylactic) of hyperlipidemia. The results were shown in Graph: 1 to 10.

Despite considerable effort on the part of a number of investigators, there has been only a limited success in developing an idea animal model of lipidemic disease that faithfully mimics human hyperlipidemia. Various procedures have been reported by numerous investigators from time to time such as selective diets, high fat diets, and drug induced hyperlipidemia in experimental animals. In the animal model, hyperlipidemia induces fat deposition. The experimental model selected for the present study is administration triton 400 mg/kg b.w. The present study on hyperlipidemia was carried out on the male swiss albino mice strain. The project was aimed to study the antihyperlipidemia and protective role of *crotolaria juncea* (leaf extracts) in hyperlipidemia. Bio-chemical parameters of hyperlipidemia were analyzed from blood serum. Histopathological study was carried out to confirm the biological changes.

In the present study, administration of triton to mice caused increased deposition of fat in liver and various blood vessels. Treatment of *Crotolaria juncea* ethanolic and methanolic leaf extracts protected against triton induced hyperlipidemia. When compare to ethanolic extract, methanolic extract shows significant decrease in blood serum total, HDL, LDL, VLDL cholesterol levels and triglyceride levels. The results are also in concurrence with histopathological studies.

Paoletti et al., suggested the use of triton WR 1339 (a non ionic detergent, oxythylated tertiary octyl phenol formaldehyde polymer) induced hypercholesterolemia as a model to screen antihyperlipidemic agents. Intraperitoneal injection of triton in experimental animal results in the progressive increase in the concentration of blood lipids. The action is believed to be due, at least in part, to the capacity of the detergents to associate with triglycerides in the plasma in such a way as to reduce their rate of hydrolysis by the enzyme, clearing factor lipase or lipoprotein lipase, and so to interfere with their uptake from circulation by the extra hepatic tissues.

Increase in the serum lipid profile with triton treatment was found to be significantly decreasing with test drugs and atorvastatin. The activity of test drugs over hyperlipidemia may due to presence of saponins because; saponins are acts as bile acid sequestrants. so,

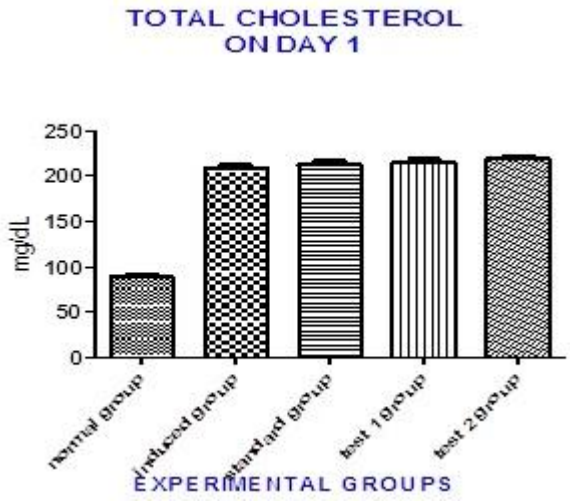
administration of *crotolaria juncea* leaf extracts significantly reduce both fat deposition and reduced in serum cholesterol and increased serum HDL levels in leaf extracts treated groups when compare to their respective induction group. This indicates that leaf extracts of *crotolaria juncea* has beneficial effect in preventing hyperlipidemia.

In addition the body weights also increased in induction group due to the fat deposition in liver and various organs. The body weight of the animals treated with leaf extracts of *crotolaria juncea* showed decrease when compare with that of in induction group.

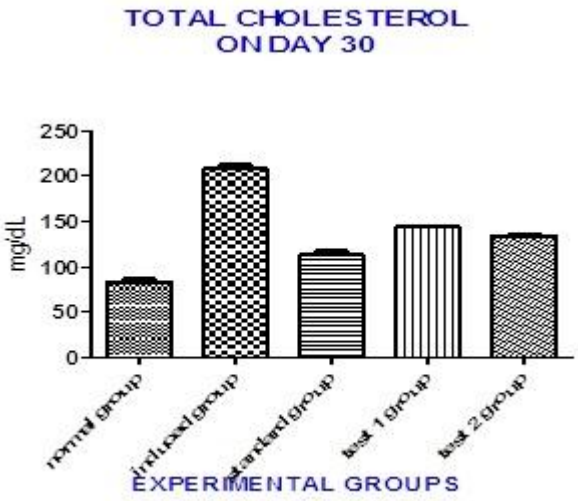
On histopathological examination induction group showed fat deposition in liver and hemorrhage. These histological observations support the presence of fat deposition in liver cells in human hyperlipidemia. On administration of leaf extracts of *crotolaria juncea* significantly decrease the fat deposition. The present study indicates the ability of leaf extracts of *crotolaria juncea* decrease the accumulation of fat. These histological studies support the fat deposition data in liver. Thus, the ethanolic and methanolic leaf extracts of *crotolaria juncea* showed significant antihyperlipidemic activity.

The results are also in concurrence with histopathological studies.The histopathological results of ethanolic and methanolic leaf extracts of *crotolaria juncea* were shown in figure. 1, 2, 3, 4 & 5.

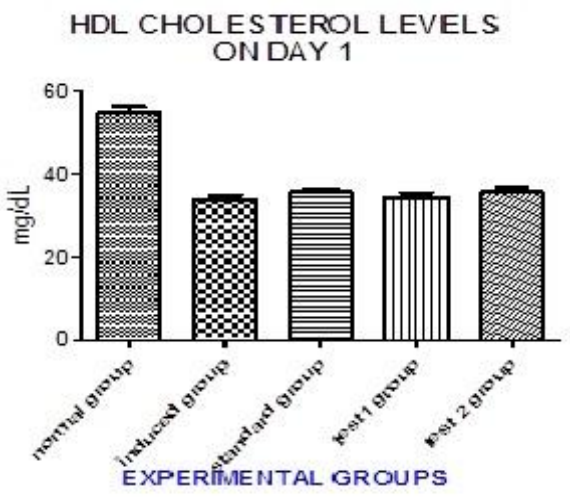
Graph 1. Total cholesterol Levels (Day 1)



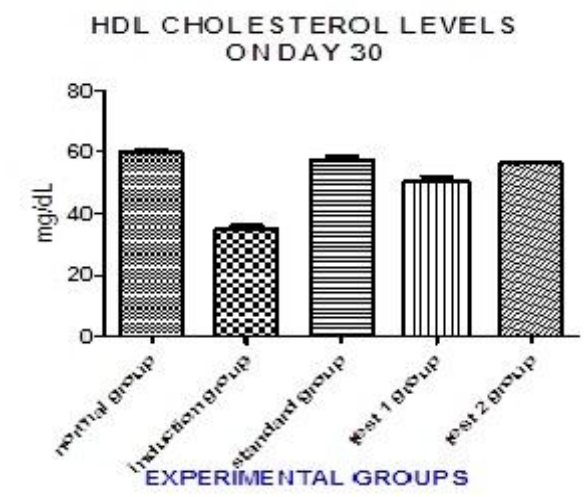
Graph 2. Total cholesterol Levels (Day 30)



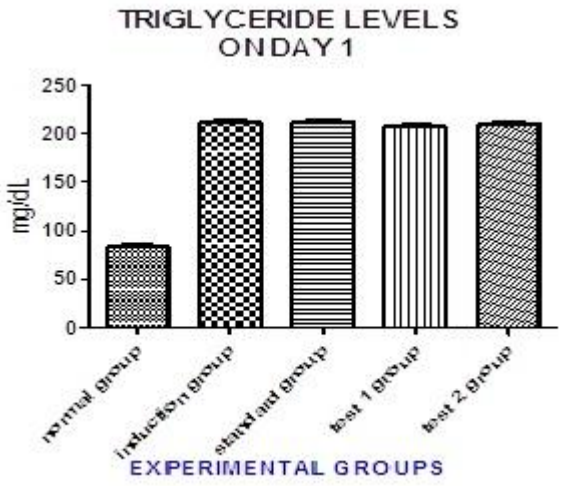
Graph 3. HDL Cholesterol Levels (Day 1)



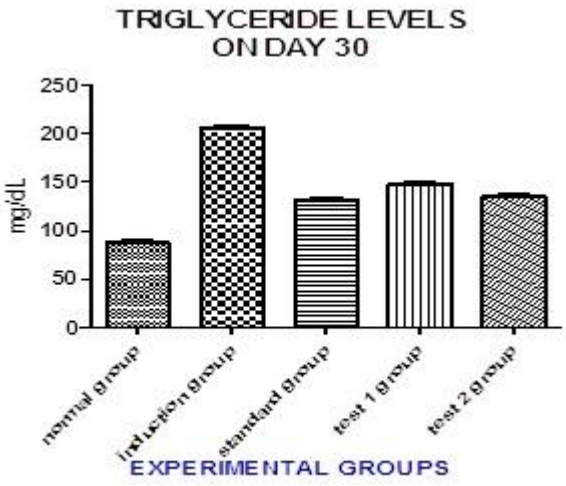
Graph 4. HDL Cholesterol Levels (Day 30)



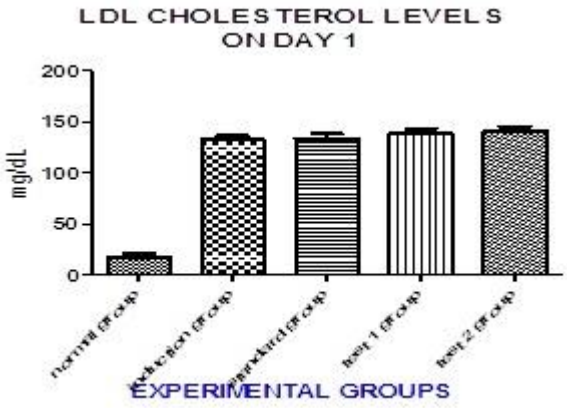
Graph 5. Triglyceride Levels (Day 1)



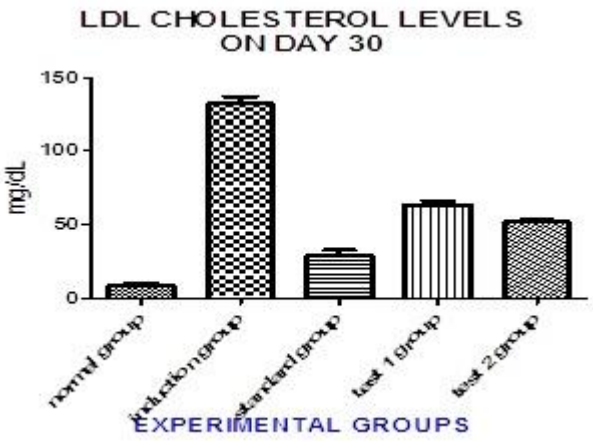
Graph 6. Triglyceride Levels (Day 30)



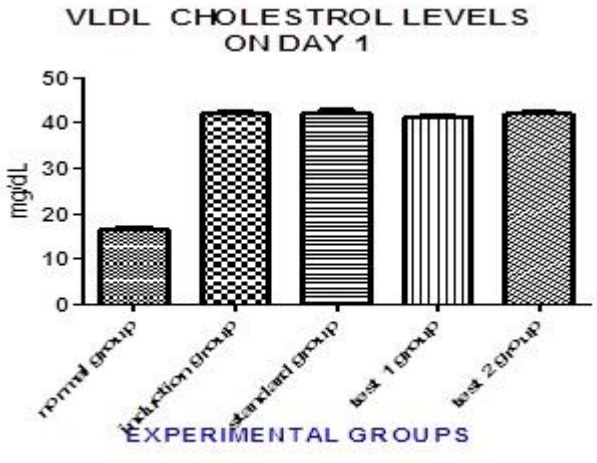
Graph 7. LDL Cholesterol Levels (Day 1)



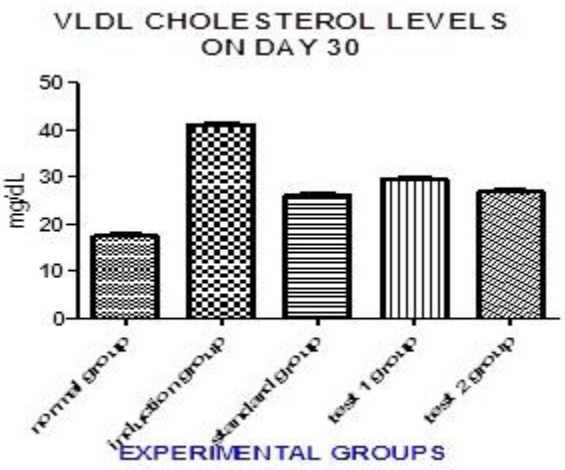
Graph 8. LDL Cholesterol Levels (Day 30)



Graph 9. VLDL Cholesterol Levels (Day 1)



Graph 10. VLDL Cholesterol Levels (Day 30)



All values shown are mean ± SEM and n = 7*** P < 0.001 compared to induction group

Histopathology

Figure 1. Induction group: The degenerative changes in liver tissue show congestion with hemorrhages with lower magnification (10X) and higher magnification (40X).

INDUCTION GROUP 10X **INDUCTION GROUP 40X**

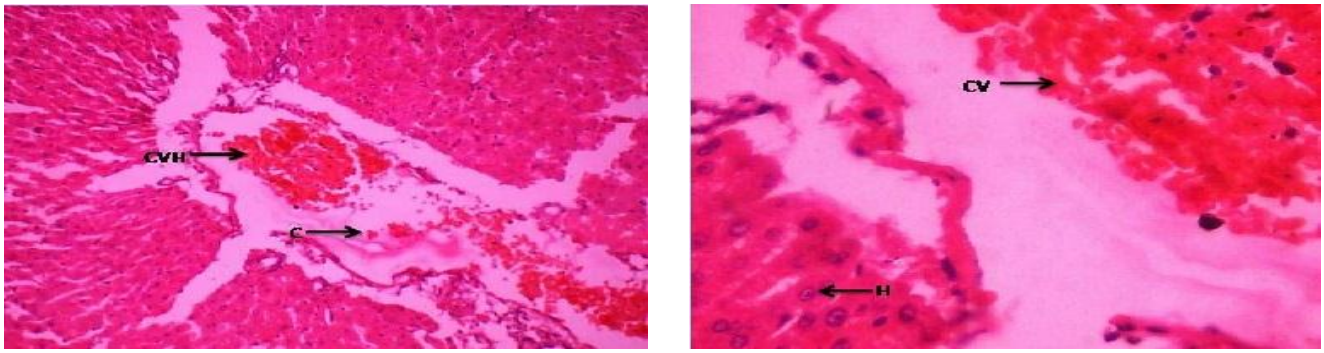


Figure 2. Normal group: The normal architecture of the Liver tissue with lower magnification (10X) and higher magnification (40X).

NORMAL GROUP 10X **NORMAL GROUP 40X**

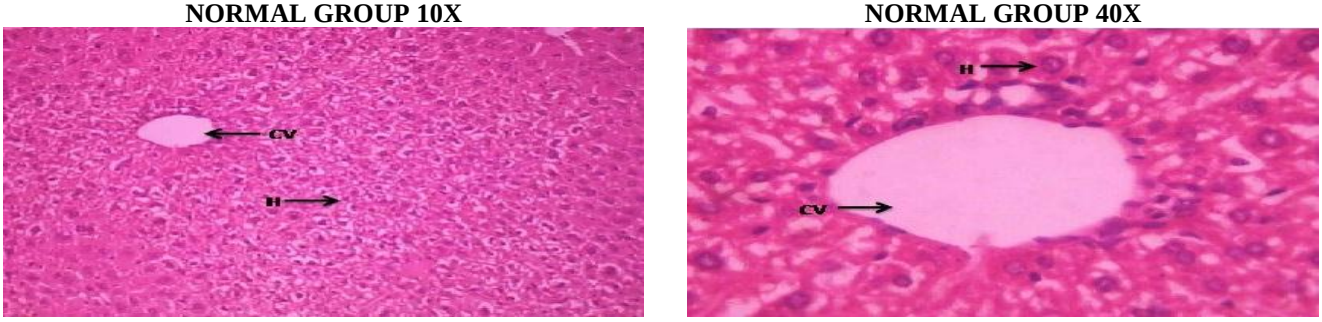


Figure 3. Standard group: The Regeneration changes in the Liver tissue with lower magnifications (10X) and higher Magnification (40X).

STANDARD GROUP 10X **STANDARD GROUP 40X**

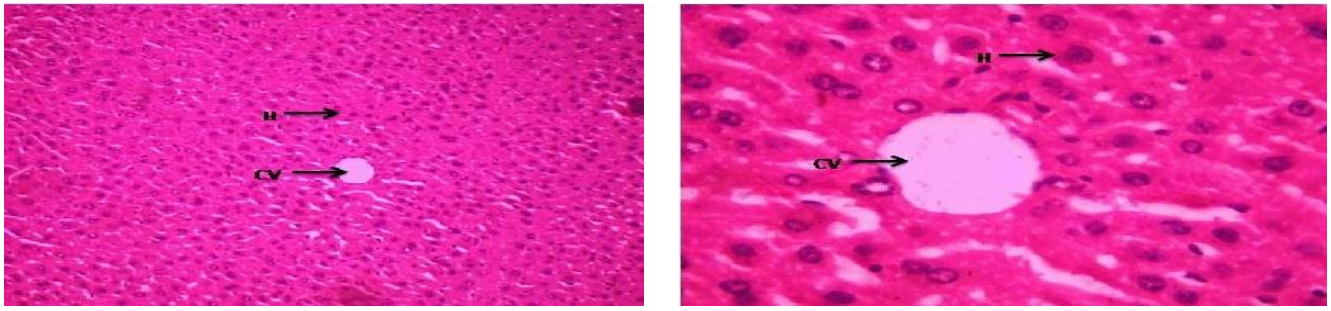


Figure 4. Test 1 group: The regenerative changes shows recovery of the tissue to normal in mild manner with lower magnifications (10X) and higher Magnification (40X).

TEST 1 GROUP 10X **TEST 1 GROUP 40X**

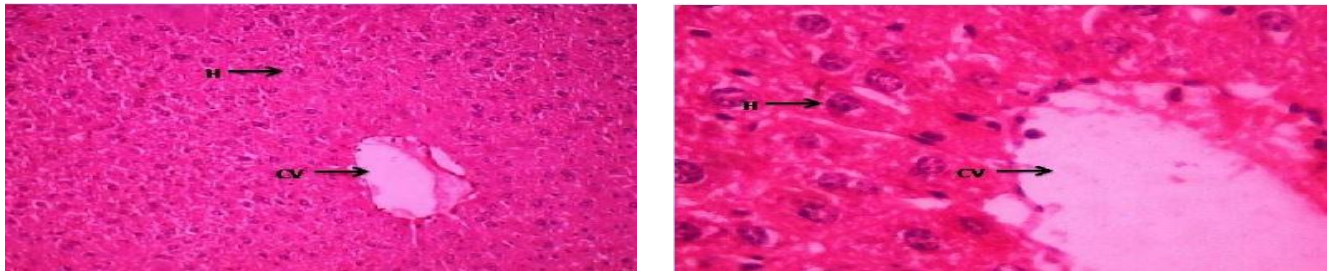
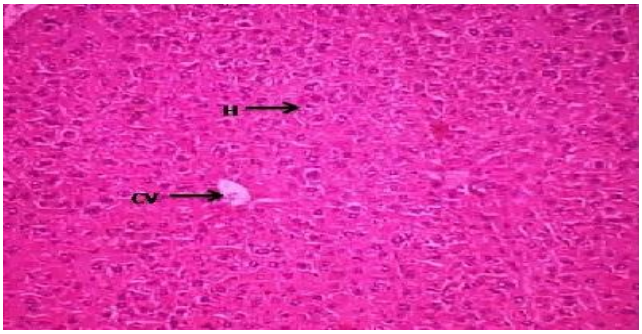
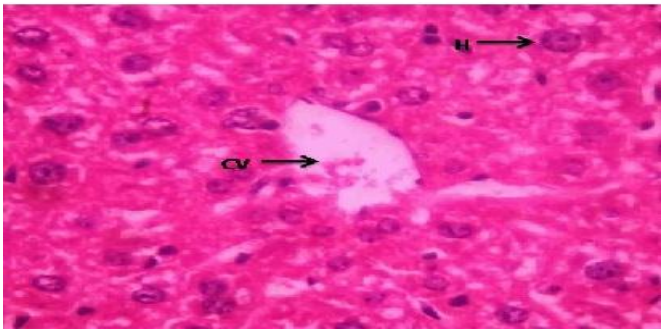


Figure 5. Test 2 group: The regenerative changes in the tissue show similar to the normal architecture with lower magnifications (10X) and higher Magnification (40X).

TEST 2 GROUP 10X



TEST 2 GROUP 40X



CONCLUSION

Hence treatment of methanolic and alcoholic leaf extracts CJ significantly decreases the Total cholesterol, Triglyceride, VLDL-C, LDL-C, Atherogenic index and a significantly increase in HDL-C in serum and various tissue homogenate like Aorta, Liver & Heart. These results were further substantiated with the histopathological results Anti-hyperlipidemic activity of methanolic and alcoholic leaf extracts of CJ may be due to presence of tannins, anthraquinones, chebulinic acids, chebulic acid, ellagic acid and gallic acid and this requires further investigation. Methanolic and alcoholic leaf extracts CJ showed significant anti-hyperlipidemic effect and this study provides the scientific proof for their traditional claims.

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