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Evaluation of Antihyperlipidemic Activity of Ethanolic Extract of *Crossandra infundibuliformis* Leaves and Stems on High Fat Diet Induced Hyperlipidemia

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ABSTRACT

Hypercholesterolaemia is the state been depicted by an expanded greasy substance called lipids, a rise in plasma treacher collins syndrome (TCs), and Triglycerides (TGs) levels; it is additionally called hyperlipoproteinemia. The present study was designed to investigate the hypolipidemic effect of ethanolic extract of *Crossandra infundibuliformis* leaves and stems (ECILS) in the high-fat diet (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal laboratory feed). The rats feed with high-fat diet containing (cholesterol 2%, sodium cholate 1%, sucrose 48%, peanut oil, methionine 4%, and 47% normal standard laboratory feed) for 1-month. Then they are checked for the blood parameter levels like TC, TGs, low-density lipoproteins (LDL), very-low-density lipoprotein (VLDL), and low-density lipoproteins (HDL). Ethanolic extracts at a low dose (100 mg/kg), significantly reduced the levels of TC, TGL, LDL, VLDL, and increased the levels of HDL and reduced the body weights on 30th day, and at medium, high doses it reduced the levels of TC, TGL, LDL, VLDL and increased the levels of HDL and reduced the body weights.

INTRODUCTION

Hyperlipidemia is the state of increased levels of TC, TG, LDLs, and VLDL.^[1] Hyperlipidemia is a human lifestyle disease. It seriously affects the human health due to its various complications like cardiovascular disorders such as myocardial infarction (MI), Congestive heart failure (CHF), hypertension and atherosclerosis. Hyperlipidemia also is correlative with diabetics mellitus (DM). Atherosclerosis is the most common cause of mortality and morbidity worldwide. Although several factors, such as a diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle, play a significant role in causing heart failure, the high levels of cholesterol, particularly TC, TG, and LDL cholesterol is

mainly responsible for the onset of CHDs. A 20% reduction of blood cholesterol level can decrease by about 31% of CHD incidence, and 33% of its mortality rate.^[2-6]

Lipids play out some significant capacities in the body. Also, hyperlipidemia is induced by the secondary effect of diabetes. Therefore, the agents having some antioxidant and anti-diabetic effect also shows a favorable effect on hyperlipidemia. β -Hydroxy β -methylglutaryl-CoA (HMG CoA) reductase inhibitor has been used in the treatment of hyperlipidemia, and Atorvastatin is one of the most prevalently used HMG CoA, reductase inhibitors.^[7-10]

A literature survey explains that flavonoid-rich sources are potential for the treatment of hyperlipidemia.^[11,12] Based on the extensive literature survey, *Crossandra*

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infundibuliformis is found to be a good source of flavonoids. Therefore, we have selected the plant *Crossandra infundibuliformis* leaves and stems for a screening of hypolipidemic activity against high-fat diet-induced hyperlipidemia in male rats.^[11,12]

Crossandra infundibuliformis (Acanthaceae) is an enduring herb local to India and Sri Lanka and other Asian nations and it is ordinarily developed as a blooming plant it is the evergreen sub bush with polished and wavy margined leaves and fan-molded blossoms which may show up whenever consistently.^[13,14] This bloom hue runs from the regular orange shading to a salmon-orange coral to the red shading and even green turquoise. These plant parts are utilized since old times in treating different sorts of turmoil like aggravation, and it is likewise known for its injury mending action. This plant is additionally notable for its sexual enhancer action, and for its mitigating movement, it is otherwise called sparkler since when the dried case of the plant interacts with water, it tears open as a sparkler subsequently these plants were granted with this name.^[15,16] The present investigation is directed to the exploration of the antihyperlipidemic activity of the ethanol extract of leaves and stems of *Crossandra infundibuliformis*.

MATERIALS AND METHODS

Procurement and Authentication of Plant

The leaves and stems of *Crossandra infundibuliformis* were collected from Guntur, Andhra Pradesh. The plant material was identified and authenticated by Dr. P.V. Prasanna, Plant Taxologist, Scientist-F and HOD, Professor, Ministry of Environment, Botanical Survey of India, Hyderabad, Telangana.

Preparation of Plants Extract

The leaves and stems of *Crossandra infundibuliformis* were collected and washed thoroughly with distilled water to make sure the leaves and stem are free of dust

and are shade-dried. The dried leaves and stems are then powdered finely using a mechanical grinder. And then, the required quantity of the powder is subjected to solvent extraction with ethanol. The obtained extract is dried completely using desiccators.^[17-19]

Animals and Treatment

Animals

Healthy male Sprague Dawley rats weighing between 250 and 300 g were procured and maintained in polypropylene cages at an ambient temperature of $22 \pm 1^\circ\text{C}$ and relative humidity of 50–60% with a 12 hours light/dark cycle in registered animal house. The animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals approved under the number (1048/a/07/CPCSEA). India and approved by the Institutional Animal Ethics Committee (IAEC). Throughout the experimental period, the animals were fed with standard pellet diet and water *ad libitum*.

Experimental Design

Animals were randomly divided into six groups containing six ($n = 6$) animals each and treated as explained in Table 1.

Body weights were recorded once a week throughout the experimental study. At the end of each week, blood samples were collected from the retro-orbital plexus. Blood samples were kept aside for approximately 1-hour at room temperature and centrifuged at 2500 rpm at 4°C for 15 minutes to separate the serum from blood. The serum samples were used for the estimation of biochemical parameters such as TC, HDL, LDL, VLDL, and TGs.

At the end of the study, animals were sacrificed by cervical dislocation and cut open to isolate the liver and weighed immediately. Then the liver was fixed in 10% formalin and was used for histopathological study.

Table 1: Treatment Groups

S. No.	Name of the group	Treatment
1	Normal	Received standard pellet diet for 30 days.
2	HFD	Received high-fat diet for 30 days
3	HFD + Standard atorvastatin	Received high fat diet + Standard Atorvastatin (20 mg/kg b.wt, p.o) for 30 days.
4	HFD + Test dose 100 mg/kg	Received high fat diet + 100 mg mg/kg b. wt , p.o of plant extract for 30 days.
5	HFD + Test dose 200 mg/kg	Received high fat diet + 200 mg mg/kg b. wt , p.o of plant extract for 30 days.
6	HFD + Test dose 400 mg/kg	Received high fat diet + 400 mg mg/kg b. wt , p.o of plant extract for 30 days.

Table 2: Blood parameters (TC, TGs, HDL, LDL, and VLDL), Bodyweight, before disease-induced hyperlipidemia in experimental rats

S. No.	Group	Body weight	TC	TG	HDL	LDL	VLDL
1	Normal	152 $\pm$ 0.12	61.12 $\pm$ 2.8	72.25 $\pm$ 1.9	29.65 $\pm$ 1.6	17.02 $\pm$ 2.4	16.08 $\pm$ 3.7
2	HFD	150 $\pm$ 1.12**	65.59 $\pm$ 1.5**	72.12 $\pm$ 1.0**	32.30 $\pm$ 1.3**	18.88 $\pm$ 3.2**	16.12 $\pm$ 1.2**
3	HFD $\pm$ Atorvastatin 20 mg/kg	156 $\pm$ 0.36*	62.32 $\pm$ 3.8*	72.12 $\pm$ 1.9*	45.25 $\pm$ 2.3*	30.36 $\pm$ 1.5*	24.23 $\pm$ 1.5*
4	HFD $\pm$ Test dose 100 mg/kg	160 $\pm$ 2.35*	62.32 $\pm$ 1.6*	84.32 $\pm$ 3.5*	29.68 $\pm$ 1.9*	15.77 $\pm$ 2.3	23.03 $\pm$ 2.6**
5	HFD $\pm$ Test dose 200 mg/kg	158 $\pm$ 1.22*	72.23 $\pm$ 1.5*	70.1 $\pm$ 2.3*	30.25 $\pm$ 1.5*	27.96 $\pm$ 2.9	22.6 $\pm$ 2.06*
6	HFD $\pm$ Test dose 400 mg/kg	162 $\pm$ 0.66*	65.75 $\pm$ 1.2*	72.8 $\pm$ 2.6*	36.2 $\pm$ 2.1*	15.31 $\pm$ 1.8*	27.1 $\pm$ 2.3*

Statistical Analysis

The results were expressed as mean $\pm$ S.D. [Standard error of the mean (SEM)]. Statistical analysis was calculated using one-way ANOVA followed by post hoc Dunnett's test for multiple comparisons, and statistical significance was set at $p < 0.05$. Values are represented as mean $\pm$ SEM; a (***) - $p < 0.0001$, b (**) - $p < 0.01$, c (*) - $p < 0.05$.

RESULTS

Estimation of Serum Biochemical Parameters

The estimated values of biochemical parameters in all the experimental rats before and after the treatment period are summarized in Tables 2 and 3, Figs. 1 and 2.

Histopathological Findings

Histopathological changes in the liver of high-fat diet-induced hyperlipidemia after 30 days treatment is shown in Fig. 3.

DISCUSSION

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis. In our study, we choose cholesterol diet, which contains the common ingredients in our daily food. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess the

hypercholesterolemia related metabolic disturbances in animals. Cholesterol feeding alone, however, does not affect the serum TG level. It is assumed that a high level of saturated fat, in addition to cholesterol, is required to elevate serum TG levels in a rat model significantly.^[20-25]

The extract exhibited a significant control of serum lipid profiles in rats. From the obtained result, it was observed that keeping the animal on HFD significantly increased the TC, TG, LDL-C level in serum ($p < 0.05$) as compared to rats on normal diet. When high functioning depression HFD was co-administered with ECILS, the elevated levels of TC, TG, and LDL-C conditions have shown a considerable decline. It was noted that TC, TG, and LDL-C lowering activity of ECILS (400 mg/kg) was more significant as compared to the other two lower doses. There was a significant elevation in plasma HDL-C in ECILS treated rats as compared to HFD rats, thus indicating the efficacy of ECILS in preventing the elevation seen in various components of lipid profile under experimentally-induced hyperlipidemia. Ample of evidence exists with respect to the fact that HDL cholesterol is inversely related to the total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall.^[26-29] Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats.^[30, 31] Flavonoids and polyphenols found in our ECILS

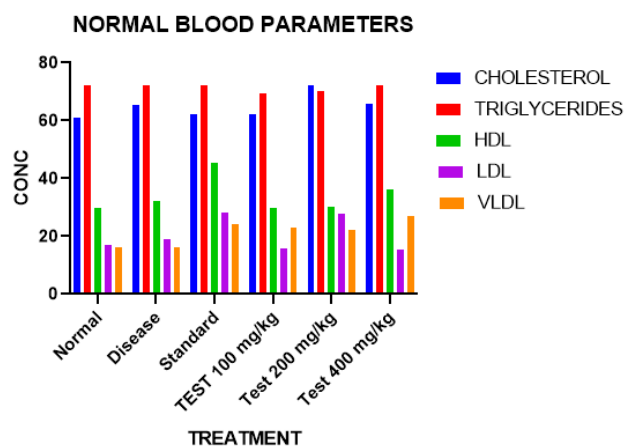


Fig. 1: Blood parameters (TC, TGs, HDL, LDL, and VLDL), body weight, before disease-induced hyperlipidemia

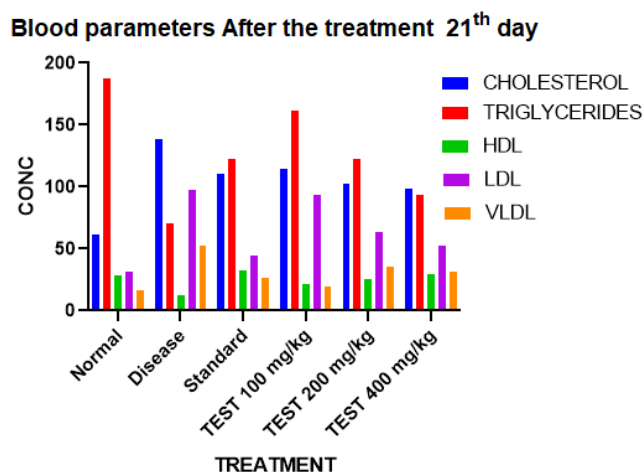


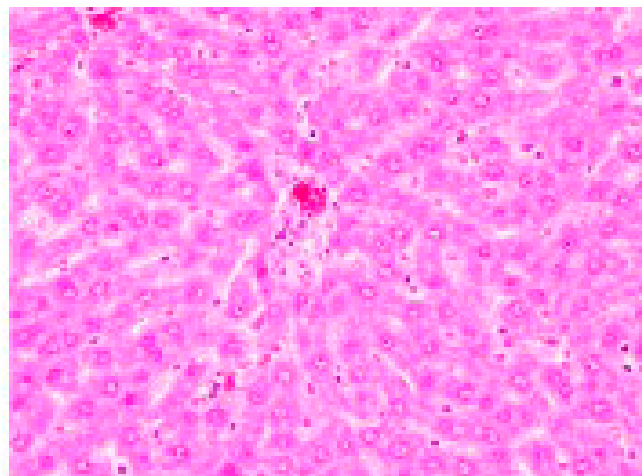
Fig. 2: Effect of ethanolic extracts of *Crossandra infundibuliformis* on various lipid parameters after 30th day of treatment.

Table 3: Effect of ethanolic extracts of *Crossandra infundibuliformis* on various blood parameters (TC, TGs, HDL, LDL and VLDL), after 30th day of treatment

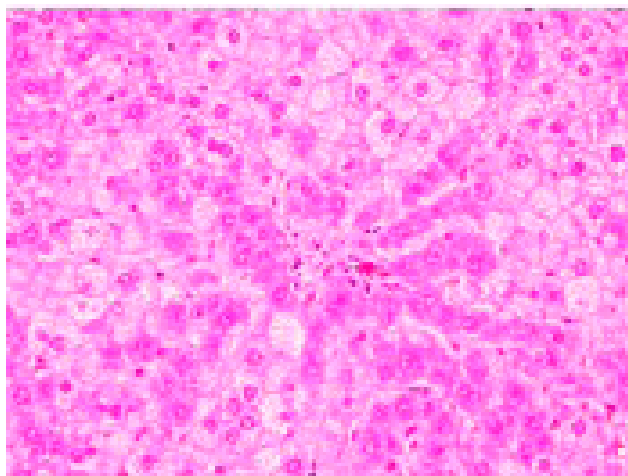
S.NO	Group	TC	TG	HDL	LDL	VLDL
1	Normal	61.09 $\pm$ 1.0	187.02 $\pm$ 2.4	28.03 $\pm$ 1.6	31.23 $\pm$ 1.8	16.09 $\pm$ 1.2
2	HFD	138.01 $\pm$ 01***	70.03 $\pm$ 2.6***	12.11 $\pm$ 0.6***	97.32 $\pm$ 3.4***	52.09 $\pm$ 0.3***
3	HFD + Atorvastatin (20 mg/kg)	110.0 $\pm$ 6.3**	122.0 $\pm$ 0.9**	32.08 $\pm$ 3.0**	44.24 $\pm$ 1.2**	26.0 $\pm$ 2.4**
4	HFD + Test dose 100 mg/kg	114.03 $\pm$ 0.2*	161.08 $\pm$ 0.9*	21.06 $\pm$ 1.6*	93.33 $\pm$ 1.8	19.04 $\pm$ 1.6*
5	HFD + Test dose 200 mg/kg	102.04 $\pm$ 2.4*	122.04 $\pm$ 2.8*	25.52 $\pm$ 1.8*	63.01 $\pm$ 1.4	35.02 $\pm$ 0.8*
6	HFD + Test dose 400 mg/kg	98.02 $\pm$ 1.0*	93.03 $\pm$ 2.1*	29.04 $\pm$ 0.6*	52.23 $\pm$ 1.0	31.06 $\pm$ 1.0*

Values are mean $\pm$ SEM, n = 6 ** $p < 0.01$, when compared with control Group* $p < 0.01$, when compared with standard group

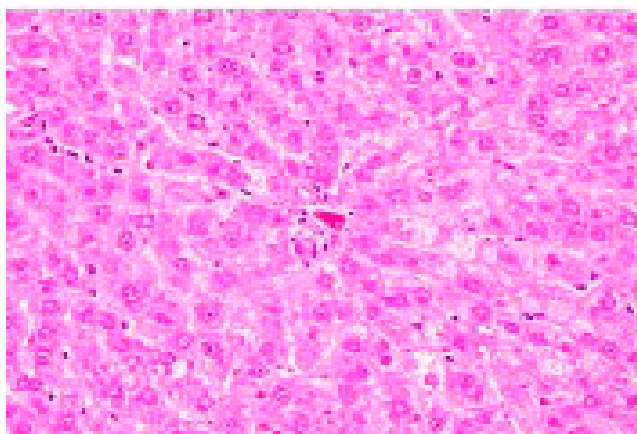




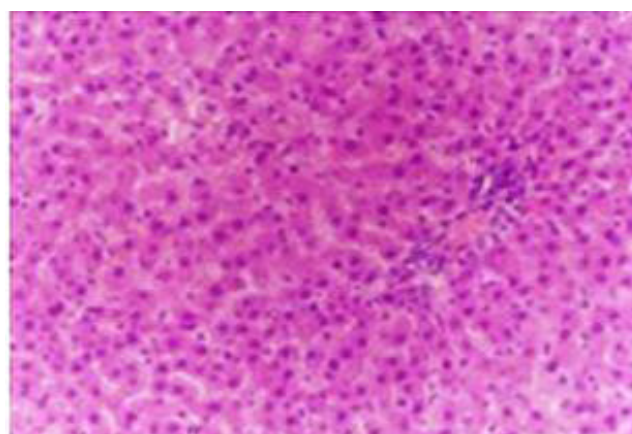
Liver of rat from normal control group showing the normal histopathological structure of hepatocytes (H & E x 200)



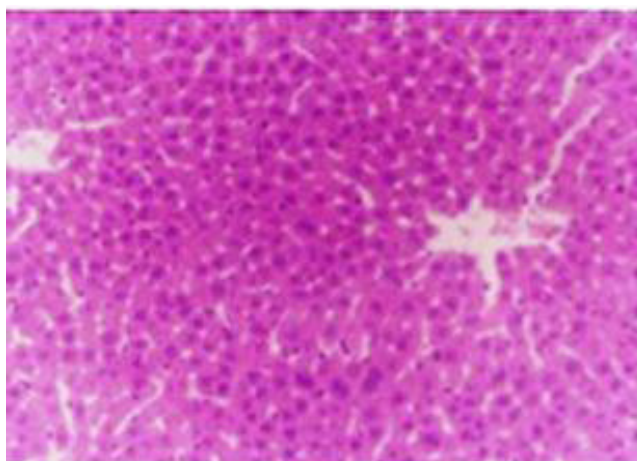
Liver of rat from disease control group showing the fat deposits in histopathological structure hepatocytes (H & E x 200)



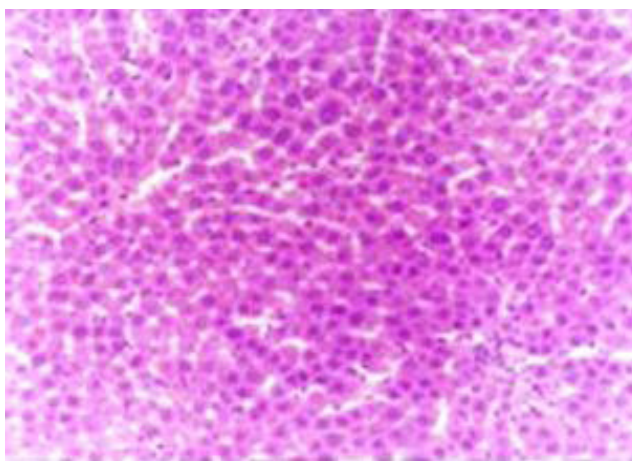
Liver of rat from standard control group showing an almost normal histopathological structure of hepatocytes treated with standard drug Atorvastatin (20 mg/kg)(H & E x 200)



Photomicrograph of a liver section of rat subjected showing the hyperlipidemia and treated with low dose (100 mg/kg) of ECILS for 30 days showing necrosis & fat deposits (100 mg/kg)(H & E x 200)



Photomicrograph of a liver section of rat subjected to hyperlipidemia and treated with medium dose (200 mg/kg) of ECILS for 30 days showing very few effects of necrosis and fat deposits.



Photomicrograph of a liver section of rat subjected to hyperlipidemia and treated with high dose (400 mg/kg) of ECILS for 30 days showing significant effect with almost normal hepatocytes but with necrosis.

Fig. 3: Effect of ECILS and other treatment groups on histopathology of the liver after 30th day of treatment

could, therefore, be considered favorable in increasing HDL and decreasing LDL and VLDL in ECILS treated rats. The findings in the study in this context with the previous studies are quite comparable.^[30,31]

CONCLUSION

The result of this present study revealed that the ethanolic extract of *Crossandra infundibuliformis* leaves and stems improved the serum lipid profile in rats by decreasing serum TC, TG, LDL-C, and increasing serum HDL-C. This finding provides some biochemical basis for the use of leaves and stems extract of *Crossandra infundibuliformis* as an antihyperlipidemic agent having a preventive and curative effect against hyperlipidemia. Further, studies are required to gain more insight into the possible mechanism of action.

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