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Evaluation of antihyperlipidemic and antioxidant activity of *Rubia Cordifolia Linn*.

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ABSTRACT

Hyperlipidemia is major problem among population those, who have sedentary life style. The effects of ethanolic and aqueous root extract of Rubiacordifoliain experimentally induced hyperlipidemic rats were investigated. 42 wistar rats of both sexes were used for the study. The animals were completely randomized into seven groups comprising 6 animals each. The groups were treated as follows: Group I: normal diet (ND); Group II: HFD (Vanaspati ghee + coconut oil mixture in ratio of 3:2 at 10 ml/kg/day); Group III: HFD + Atorvastatin (10 mg/kg/day); Group IV: HFD+ ethanolic extract of roots of *Rubia cordifolia* low dose(200 mg/kg/day); Group V: HFD + ethanolic extract of roots of *Rubia cordifolia* high dose (400 mg/kg/day); Group VI: HFD + aqueous extract of roots of *Rubia cordifolia* low dose (200 mg/kg/day); Group VII: HFD + aqueous extract of roots of *Rubia cordifolia* high dose (400 mg/kg/day). Hypercholesterolemia was induced by feeding the animals with high fat diet for 21 days before administration of the extract. After 21thday of feeding, administration of extract lasted for 14 days. Preliminary phytochemical screening revealed that the ethanolic and aqueous root extract of *Rubia cordifolia* contains glycosides, triterpenoids, saponins, alkaloids and flavonoids. Overall, findings from the present study suggest that the antihyperlipidemic activity observed with Atorvastatin (10mg/kg oral) and the ethanolic extracts of *Rubia cordifolia* (400mg/kg) showed better activity thanaqueous extracts of *Rubia cordifolia* (200mg/kg).

Keywords: *Rubia cordifolia*, body weight, high fat diet, serum lipid, anti-hyperlipidemic activity.

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1. INTRODUCTION

Hyperlipidemia is caused by overabundance of lipids or fatty substances in the blood and is an important risk factor in development of atherosclerosis and heart diseases.[1] Hyperlipidemia may be caused by genetic factors or by generalized metabolic disorders like diabetes mellitus, excessive alcohol intake, hypothyroidism or primary biliary cirrhosis. Alteration in cholesterol [2] triglyceride very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and intermediate density lipoproteins (IDL), which are different forms of lipids, responsible for possible complications in human body such as acute pancreatitis, occlusion of blood vessels and reduced elasticity of the lumen of the artery.[3] Moreover risk increases with diabetes mellitus, hypothyroidism, nephrosis, alcoholism, of oral contraceptives, family history of hyperlipidemia,[4,5] and improper diet that is high in fat and cholesterol. Though drugs therapies available for the treatment of hyperlipidemia includes use of drugs like Niacin, Fibrates (Clofibrate, Gemfibrozil), HMG-CoA reductase inhibitors (Lovastatin, Pravastatin, Simvastatin,[6] and Fluvastatin), Bile acid binding resins (Cholestyramine and Cholestipol) and Probucol but associated with lots of side effects.[7,8] Therefore, herbal treatment for hyperlipidemia has been appreciated because of no side effects, economic and easy availablility. Herbal drugs involved in the treatment of hyperlipidemia are *Allium sativum*, *Allium cepa*, *Bosswellia serrata*, *Brassica vercapitata*, *Commiphora mukul*, *Garcinia cambogia*, *Glycine max*, *Phyllanthus niruri*, *Moringa olifera*,[9,10] *Saururu schinensis*, *Curcuma loga*, *Terminalia arjuna*, *Acorus calamus*, *Liriopeplatyphylla*, *Citrus*, *Hibiscuss abdariffa*, *Schisandrin B*, *ShanZha*, *Ulva Pertusa*, *Picrorrhiza rhizoma*.[4,11,12]

2. MATERILS AND METHODS

2.1. Material

2.1.1. Plant materials and authentication

Roots of *Rubia cordifolia* Linn, (2kg) were collected from local area supplier of Delhi, during the month of October 2015. Sample of plant material was sent to the Department

of Botany, National Institute of Science Communication and Information Resources, (NISCAIR) Delhi-110011, for identification and taxonomic authentication

2.2.2 Experimental Animals

Wistar rat of either sex, weighing 150-250g purchased from All India Institute of Medical Sciences animal house, New Delhi. All test animals were allowed free access to food and water *ad libitum*, both being withdrawn just prior to experimentation.

2.2 Methods

2.2.1. Preparation of plant extract of *Rubia cordifolia*

The authenticated roots of *Rubia cordifolia* was collected and subjected to shade drying. The size were reduced and made to coarse powder and then further passed through the appropriate sieve no. to obtain uniform particle size. The powdered root was extracted with ethanol and water by using soxhlet apparatus. The root extracts were filtered, collected, and concentrated by using Rotatory Flash Evaporator. The extracts were used for the further experimental models.

2.2.2. Determination of Body Weight

The weight of individual rat of each group was measured 'only' before and 'after' administration of extract.

2.3. Animal grouping, feeding and extract administration

2.3.1. Experimental design:

Animals were fasted for 24 hours before the experiment with free access to water.

2.3.2. Preparation of drugs

The ethanolic extract of root of *Rubia cordifolia* was suspended in 2% Tween-80 and used for oral administration. Each time fresh preparations of the extracts were prepared.

2.3.3. Assessment of antihyperlipidemic activity

MODEL- High Fat Diet (HFD) induced hyperlipidemic model used for study.

Preparation of Feed

Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 2% , cholic acid 1% , sucrose 40% , and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixed with water without the other excipients. This preparation of feed was done once in three days for all the animals. 42 Wistar rats were randomly divided into seven groups each group containing six animals. The chronic hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats were then given ethanolic and aqueous root extract of *Rubia cordifolia*(200 and 400 mg/kg, oral) and Atorvastatin (10 mg/kg, oral) once daily in the morning.

Table 1: Classification of groups according to their doses

Group	N	Treatment	Dose
Group I	6	Normal saline	10ml/kg body weight
Group II	6	High fat diet	
Group III	6	High fat diet+ Atorvastatin	10 mg/kg body weight
Group IV	6	*Rubia cordifolia* ethanolic root extract of low dose+HFD	200 mg/kg body weight
Group V	6	*Rubia cordifolia* ethanolic root extract of high dose+HFD	400 mg/kg body weight
Group VI	6	*Rubia cordifolia* aqueous root extract of low dose+ HFD	200 mg/kg body weight
Group VII	6	*Rubia cordifolia* aqueous root extract of high dose+ HFD	400 mg/kg body weight

N= Number of animals in each group HFD= High fat diet

On the day 21th, animals were anaesthetized with chloroform and blood was collected by tail vein. The blood samples were centrifuged for 15 min at 2500rpm to obtain serum. The collected serum was analyzed for serum Total Cholesterol, Triglycerides, High Density Lipoprotein Cholesterol, Low Density Lipoprotein Cholesterol and Very Low Density Lipoprotein Cholesterol.

2.4. Evaluation of antioxidant activity

The antioxidant property of *Rubia cordifolia* roots extracts were studied by the following methods

- Determination of total phenolic content

Determination of the total antioxidant capacity

Table 2: Total phenol content of roots extract of *Rubia cordifolia* plant

Sample	Total Phenol Contents (mg/g)
Aqueous extract of *Rubia cordifolia* root	380.2
Ethanolic extract of *Rubia cordifolia* root	413.6

3. RESULTS

3.1. Antihyperlipidemic activity of ethanolic and aqueous root extracts of *Rubia cordifolia*

Table 3: Effects of ethanolic and aqueous extracts of roots of *Rubia cordifolia* on body weight HFD induced hyperlipidemic rats.

Day	Mean Body weight (gm)(% change in body weight)						
	Normal	**HCD**	**STD**	**Eth.200 EERC mg/kg**	**Eth.400 EERC mg/kg**	**Aqu.200 AERC mg/kg**	**Aqu.400 AERC mg/kg**
0th day	148.33	138.66	146.33	144.83	144.44	145.00	146.66
5th day	161.33 (↑8.76)	164.66 (↑18.75)	165.33 (↑12.98)	166.50 (↑14.96)	167.44 (↑15.92)	170.50 (↑17.58)	169.66 (↑15.68)
10th day	169.10 (↑14.00)	179.66 (↑29.56)	181.66 (↑24.14)	181.00 (↑24.97)	180.00 (↑24.61)	182.66 (↑25.97)	179.00 (↑22.05)
15th day	178.33 (↑20.22)	187.33 (↑35.10)	189.53 (↑29.72)	188.00 (↑29.80)	185.33 (↑28.30)	190.00 (↑31.03)	183.53 (↑25.13)
20th day	183.66 (↑23.81)	199.33 (↑44.09)	192.33 (↑31.43)	192.66 (↑33.02)	190.33 (↑31.77)	195.33 (↑34.71)	194.66 (↑32.72)

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05, using one- way ANOVA followed by Dunnett's t test. **(EERC)** Ethanolic extracts of roots of *Rubiacordifolia* **(AERC)** Aqueous extracts of roots of *Rubiacordifolia*

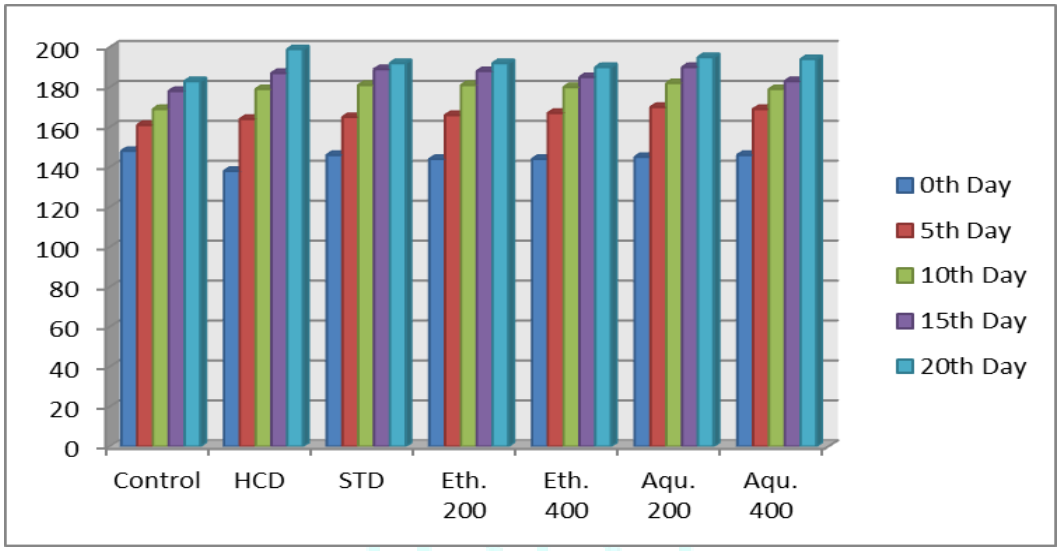

Figure 1: Effect of the ethanolic and aqueous root extract of *Rubiacordifolia* on serum lipid profile levels (mg/dL) at 0th, 5th, 10th, 15thand 20th day in HFD induced wistar rats.

Table 4: Various parameters of antihyperlipidemic activity in wistar rats, using ethanolic and aqueous root extracts of *Rubia cordifolia*.

Group No	Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
I	**Normal**	77.77± 4.05	70.15± 6.16	34.81± 1.58	14.03± 1.23	27.08± 2.91
II	**HCD**	183.33 ±6.67 (↑ 135.73)	148.92± 6.53 (↑112.28)	18.51± 1.36 ↓46.82	29.77± 1.30 (↑112.18)	135.04± 5.47 (↑1041.40)
III	**HCD+Atorva. (10mg/kg)**	103.33±7.13* (↓43.64)	97.22± 5.28* (↓34.71)	31.47± 2.54* (↑70.01)	19.60±1.03* (↓34.16)	51.80± 3.97* (↓61.64)
IV	**HCD + Eth.(200mg/kg)**	150.0±10.33* (↓18.19)	121.54±3.29* (↓18.38)	25.92± 1.58* (↑40.03)	24.41±0.75* (↓18.00)	99.77± 9.85* (↓26.57)
V	**HCD + Eth.(400mg/kg)**	128.33± 6.01* (↓30.00)	112.74±5.56* (↓24.29)	28.10± 2.19* (↑51.80)	22.51±1.12* (↓24.38)	77.67± 3.97* (↓42.48)
VI	**HCD + Aqu.(200mg/kg)**	153.33±7.60* (↓16.37)	124.36±6.05* (↓16.49)	22.58± 1.66 (↑21.98)	24.86±1.21* (↓16.49)	105.96 ±6.06 (↓21.53)
VII	**HCD + Aqu.(400mg/kg)**	146.66± 6.15* (↓20.01)	119.60±7.54* (↓19.68)	26.65± 1.72* (↑43.97)	23.86±1.52* (↓19.85)	96.15± 4.88* (↓28.79)

All values were expressed as Mean ± SEM, n.s. (non significant) as compared to vehicle control group.
 ** p<0.01, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.
 *p<0.05, when compared with normal control group (i.e., group I), ANOVA followed by Dunnett's t-test.
 p>0.05, ns (not significant) when compared with the group I.

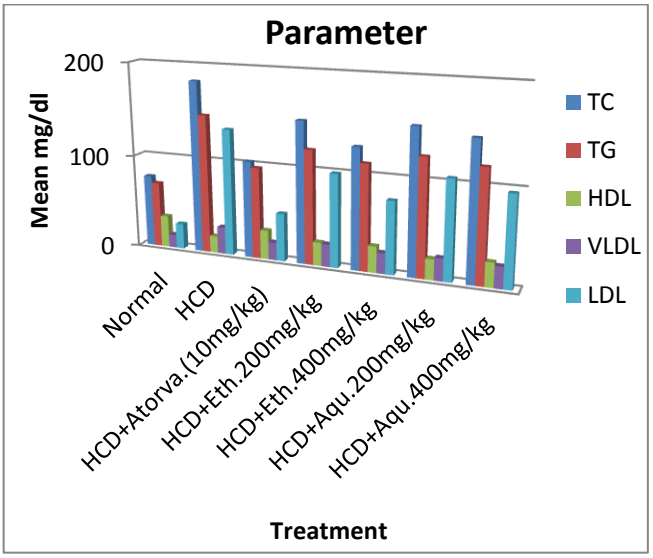

Figure 2: Effect of ethanolic and aqueous extract of *Rubia cordifolia*on concentrations of TC, TG, HDL, VLDL, and LDL, of rats fed high fat containing diet. The values were are mean ± S.E.M. for six rats. Groups' I with II and III, IV, V, VI, VII were are compared with Group II, ** p<0.01, * p<0.05.

Statistical Analysis

Results were expressed as mean ± SEM. Difference among data were determined using statistics express lite software. The data obtained from antihyperlipidemic study was subjected to one-way ANOVA, followed by Dunnett's t test for statistical significance P<0.001,P<0.05 and P<0.01 is considered to be statically significant.

4. DISCUSSION

Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health problems. Plant have been used as medicines for thousands of years and are used today in their natural form as well as processed from many medicinal plants. The importance of plants has been forgotten by modern man as a result of his dependence on the quick result of allopathic medicines. Plant drugs are being rediscovered because of growing awareness of unwanted side effects. The plant has been chosen for this study as it was easily available and has various pharmacological activities with their scientific evidences.

42 Wistar rats were randomly divided into 7 groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats were then given test plant extracts i.e., *Rubia cordifolia* ethanolic and aqueous root extracts (200 and 400 mg/kg, oral) and Atorvastatin (10 mg/kg, oral) once daily in the morning. The result have revealed 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 HFD was co-administered with *Rubia cordifolia* extracts, the elevated levels of TC, TG and LDL-C condition have been shown considerable decline. It was noted that TC, TG and LDL-C lowering activity of ethanolic root extract (400mg/kg) of *Rubia cordifolia* was more significant as compared to aqueous root extract of *Rubia cordifolia*. There was significant elevation in plasma HDL-C in *Rubia cordifolia* treated rats as compared to HFD rats, the efficacy of *Rubia cordifolia* extract in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats. Studies have shown that increased formation of free radical reactive oxygen species contribute to the progression of cardiovascular disease, reactive oxygen species induced cardiac disfunction and cardiac apoptosis. It was noted that different extract of *Rubia cordifolia* produce significantly higher total phenolic content. The high amount of phenols in extracts may explain their high antioxidant activities. Several phytoconstituents like glycosides, triterpenoids, saponins, alkaloids and flavonoids are known to have anti-hyperlipidemic properties. High fat diet In chosen in the present study, contain the common ingredients in our daily food. The high fat diet used this study contain saturated fatty acids which increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis. This may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate. Antihyperlipidemic activity observed with Atorvastatin (10mg/kg oral) and the *Rubia cordifolia* ethanolic extracts (400mg/kg) showed better activity than*Rubia cordifolia* aqueous extracts (200mg/kg).

5. CONCLUSION

The roots of *Rubia cordifolia* plants subjected to extractions in ethanol extract 14.7 % w/w and water extract 11.8 % w/w yielded respectively. Phytochemical analysis of the plant extracts showed different phytoconstituents viz. glycosides, phytosterols, triterpinoids, alkaloids and flavonoids. Several phytoconstituents like glycosides, triterpinoids, saponins, alkaloids and flavonoids are known to have anti-hyperlipidemic properties. This study revealed that tested root extracts of *Rubiacordifolia* have significant antioxidant and antihyperlipidemic activity. In the present study, the pharmacological screening has led to the conclusion that, ethanolic and aqueous roots extracts of *Rubia cordifolia* have significant antihyperlipidemic activity. Hence it can be exploited as an antihyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipidemia. This study reveals that tested root extracts of *Rubia cordifolia* have significant free radical scavenging activity. The result of the present study suggests that the roots of *Rubia cordifolia* can be used as a source of antioxidants for the management of different diseases. This plant is an effective potential source of natural antioxidants. It has been concluded that the etanolic and aqueous root extracts of *Rubia cordifolia* have antioxidant activity in dose dependent manner.The broad spectrum antihyperlipidemic and antioxidant activities of the plant extracts may be due to their active constituents like tannins, alkaloids, flavonoids, glycosides, phytosterols, and triterpinoids.

This study has demonstrated the antioxidant and antihyperlipidemic activities of roots extract from *Rubia cordifolia* plant. The antioxidant and antihyperlipidemic activity of the plant extract of *Rubia cordifolia* may play a significant role in the prevention and treatment of atherosclerosis and other cardiovascular diseases. Thus, *Rubia cordifolia* plant can be considered as an easily accessible source of natural antihyperlipidemic and antioxidant agents.

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