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Antihyperlipidemic effect of hydroalcoholic extract of Kenaf (*Hibiscus cannabinus* L.) leaves in high fat diet fed rats

Shivali[1], N. Mahadevan[2] and Pradeep Kamboj[3]

[1]Rayat and Bahra Institute of Pharmacy, Sahauran, Chandigarh
[2]Rajendra Institute Of Technology and Sciences, Sirsa, Haryana
[3]ISF College of Pharmacy, GT Road, Moga, Punjab

ABSTRACT

The 50% hydroalcoholic extract of Hibiscus cannabinus leaves was examined for its antihyperlipidemic effect in in-vivo high fat diet fed model. The activity was assessed by estimation of serum lipid profile viz. total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), stress (TBARS) and liver histopathological studies of control and drug-treated animals. The extract exhibited a strong dose dependent antihyperlipidemic activity and at dose level 400mg/kg p.o. the extract showed a significant decrease in the levels of serum TC, TG, LDL-C, VLDL-C and TBARS. In addition, the extract markedly prevented the liver microvesicular steatosis in hyperlipidemic rats. The present study demonstrated that the extract exhibits a potent lipid lowering activity in diet induced hyperlipidemia which account for some of the medical claims attributed to this plant.

Keywords: Antihyperlipidemic, Atherosclerosis, Atorvastatin, *Hibiscus cannabinus*, Kenaf, Microvesicular steatosis

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INTRODUCTION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases [1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death [2]. Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high-

density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease [3]. Atherosclerosis (Sclrero-hardening) of arteries is a generalized disease of arterial network known as a progressive and silent killer disease characterized by the formation of lesions called atherosclerosis plaques in the walls of large and or medium sized coronary arteries and reduces blood flow to the myocardium called coronary artery diseases (CAD) [4]. Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated using anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosisorhypothyroidism demands the treatment of the original disease rather than hyperlipidemia [5]. Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [6].

The present study was conducted to evaluate the beneficial effects of the oral administration of the 50% ethanol extract of *Hibiscus cannabinus* leaves (HC) on diet induced hyperlipidemia in rats. *Hibiscus cannabinus* L. (Malvaceae) is a woody to herbaceous annual plant producing large cream coloured flowers characterised by a reddish purple or scarlet throat, popular in the western world as “Kenaf” and widely grown as a fibre crop. The plant is a hermaphrodite mostly un-branched, fast-growing with prickly stems [7]. This plant was traditionally prescribed in traditional folk medicine in Africa and India; reported to contain several active components as tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids. For centuries, plant was used, as antidote to chemicals (acid, alkali, pesticides) poisoning and venomous mushrooms [8], to treat bruises, bilious conditions, fever and puerperium. The stem peelings were applicable in treating dysentery and blood and throat disorders. The seeds were used externally to treat aches and bruises. In addition, this plant has been reported to be an anodyne, aperitif, aphrodisiac, as well as fattening, purgative and stomachic [9]. Flower consists mostly of the glucoside cannabiscitrin, cannabiscetin and anthocyanin glycoside cannabinidin [10] , lignans boehmenan K (0.10%), boehmenan H (0.11%), threo-carolignan K (0.21%) and threocarolignan H (0.05%) were isolated from the acetone extract of core and grosamide K (0.25%) and erythro-canabisine H (0.42%) from the acetone extract of bark [8]. Few pharmacological studies have been reported; essential oil fraction showed phytotoxic and fungitoxic activities [8,9], aqueous extract demonstrated haematinically active and anti-oxidative potential, aqueous leaf extract displays hepatoprotective activity against carbon tetrachloride and paracetamol-induced liver damage in rats. In addition, 80% ethanol leaves extract showed significant immunomodulatory effect in activated macrophages [9]. Lignans isolated are active against HeLa, Hep-2 and A-549 cell lines and possessed significant cytotoxic activity [8].

MATERIALS AND METHODS

2.1. Plant material

Fresh, mature leaves of *Hibiscus cannabinus* L. were collected in the month of July from Salem, Tamil Nadu, India. The plant was identified and authenticated at the Botanical Survey of India, Coimbatore, India. The leaves were shade dried and powdered.

1.2. Preparation of plant extract

Powdered leaves of *Hibiscus cannabinus* L. was extracted with 50% hydroalcohol by maceration in a round bottom flask for 24hrs. The solvent was filtered and the marc was re-extracted for another two times. The combined filtrate was concentrated *in-vacuo* using a rotary evaporator and kept in desiccator to obtain the dried extract (HC).

2.3. Phytochemical screening

Screening of phytoconstituents present in the ethanol extract and its ethyl acetate fraction were carried out by using respective testing reagents [11].

2.4. Chemicals and Reagents

The high fat diet components such as cholesterol, cholic acid, casein, choline, sucrose were purchased from Himedia Laboratories Pvt. Ltd., Mumbai and multivitamin multimineral capsules Becadexamin was procured from Galaxo SmithKline Pharmaceutical Ltd., Banglore. Diagnostic kits used for the estimation of TC, triglyceride and HDL-C were obtained from Coral Ltd., Goa. Standard drug Atorvastatin was purchased from Unichem chemicals, Baddi, India. Animal feed was supplied by Ashirwad industries Ropar, India. All other chemicals and reagents were of highest commercial grade.

2.5. Animals

Albino Wistar male rats, weighing 150-200g were obtained from the institutional animal house for the present investigations. The animals were housed at a room temperature of 25 ± 2^0C, relative humidity of 75±5% and 12hrs dark-light cycle, animals were provided basal standard rat diet supplied by Ashirwad Industries., Ropar, India and water *ad libitum*. The experiments were conducted according to the ethical norms and Institutional Animal Ethics Committee Guidelines.

2.6. High fat diet-induced hypercholesterolemia

The rats were fed with hypercholesterolemia diet for 28days to induce hyperlipidemic except normal control group rats which received normal diet. The composition of hypercholesterolemia diet was cholesterol (1%), cholic acid (0.5%), casein (20%), choline (0.25%), d-*l*-methionine (0.4%), coconut oil (25%), multi vitamin mix (3.5%) and sucrose (48.4%) [12]. Growth rate was monitored during the treatment.

2.7. Experimental design

The rats were divided into six groups comprising of six animals each (n=6). The standard drug atorvastatin and extracts were suspended in 0.3%w/v carboxymethyl cellulose (CMC) for oral administration.

Group I. Normal control group (NC); rats received 0.3% CMC.

Group II. Hyperlipidemic control group (HL); rats were administered with high fat diet for 28days.

Group III. Test group A (HL+HC 100mg/kg); rats were administered with high fat diet and treated with extract 100mg/kg/day p.o for 28days.

Group IV. Test group B (HL+HC 200mg/kg); rats were administered with high fat diet and treated with extract 200mg/kg/day p.o for 28days.

Group V. Test group C (HL+HC 400mg/kg); rats were administered with high fat diet and treated with extract 400mg/kg/day p.o for 28days.

Group VI. Standard drug group D (HL+ATOR 30mg/kg); rats were administered with high fat diet and treated with atorvastatin 30mg/kg/day p.o for 28days.

The blood samples were collected from rats for biochemical estimations by retro orbital sinus puncture at the end of 28th day of the experimental protocol, samples were immediately centrifuged and assayed. Serum TC, triglycerides and HDL-C were estimated using commercially available kits [13,14,15]. VLDL-C was calculated using Friedewald equation. Lipid peroxidation in serum was estimated colorimetrically by measuring TBARS [16]. The liver from each group was excised and subjected for histopathological studies [17,18].

2.8. Statistical analysis

All the results were expressed as mean ±S.E.M. Statistical significance was calculated using Student's t test [19]. Significance was accepted at $p \leq 0.05$.

RESULTS

3.1. Effects of HC on serum lipids profile

The oral administration of high fat diet for 28days to rats produced a significant ($p<0.01$) increase in serum TC, LDL-C, VLDL-C and triglycerides as compared to normal control rats. These significant rises were accompanied by significant ($p<0.01$) decline of serum HDL-C as compared to normal control rats. The treatment with HC (400mg/kg) and standard drug atorvastatin (30mg/kg/day p.o.) to high fat rats resulted in significant ($p<0.01$) decline in serum TC, LDL-C, VLDL-C and triglycerides as compared to hyperlipidemic control rats (Fig. 1). Further, atorvastatin treated group significantly increased the serum HDL-C level in high fat-induced rats (Fig. 1). Whereas, treatment with HC extract did not modulate the reduced serum HDL-C level.

3.2. Effects of HC on serum lipid peroxidation

The oxidative stress was assessed in terms of measurement of TBARS, which is an index of lipid peroxidation. Administration of high fat diet for 28days significantly ($p<0.01$) increased the serum TBARS level as compared to normal control. The hyperlipidemic induced increase serum TBARS was significantly reduced in rats treated with HC at dose levels 200 & 400mg/kg and standard drug atorvastatin (Fig. 2).

3.3. Effects of HC on Liver microvesicular steatosis

Administration of high fat diet for 28days markedly increased the deposition of fat and showed microvesicular steatosis in the liver of hyperlipidemic control rats. Treatment with HC at dose level 400mg/kg and standard drug atorvastatin (30mg/kg) markedly prevented the microvesicular steatosis (Fig 3).

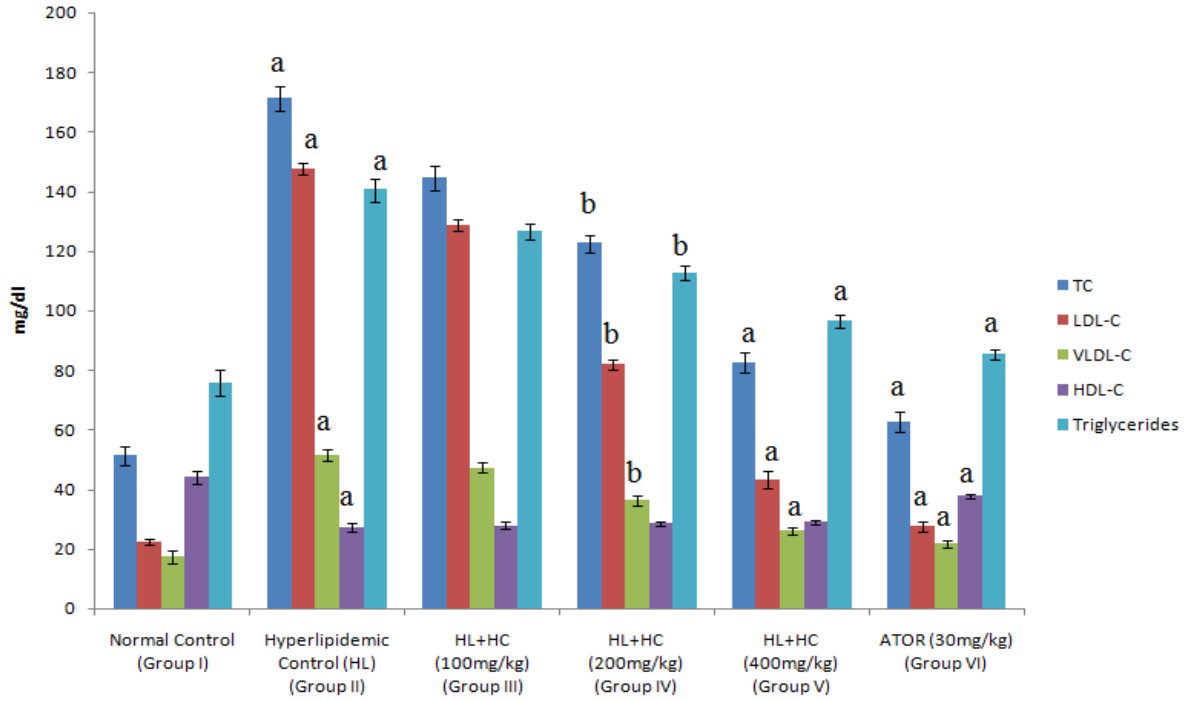

Figure 1: Effect of hydroalcoholic extract of Hibiscus cannabinus on concentrations of TC, LDL, VLDL, HDL and triglycerides, of rats fed high fat containing diet. The values are mean±S.E.M. for six rats. Groups' I with II and III, IV, V, VI are compared with Group II. a: P<0.01 and b: P<0.05.

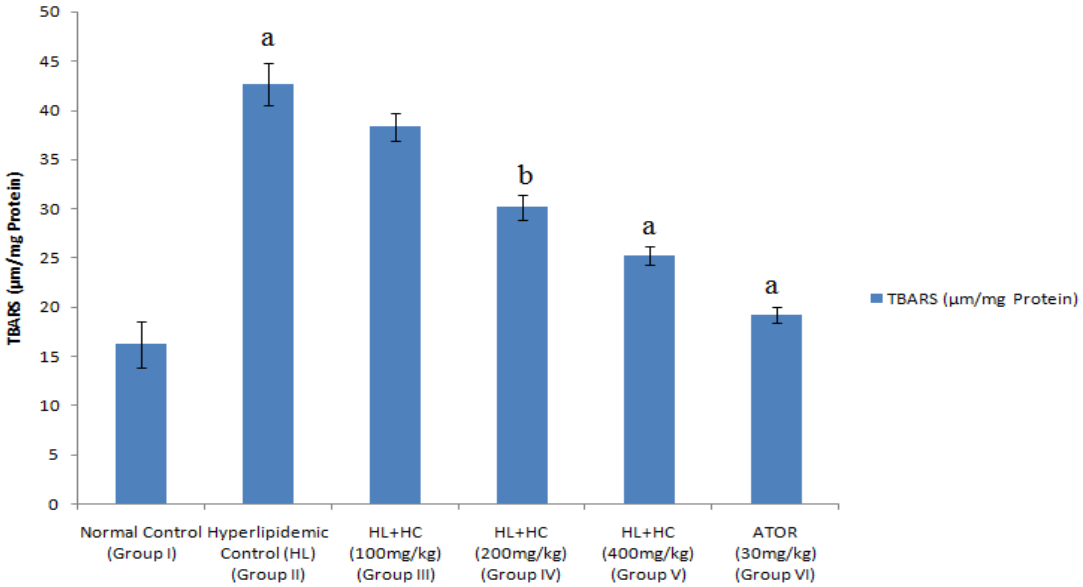

Figure 2: Effect of hydroalcoholic extract of Hibiscus cannabinus on thiobarbituric acid reactive substances (TBARS) level of rats fed high fat containing diet. The values are mean±S.E.M. for six rats. Groups' I with II and III, IV, V, VI are compared with Group II. a: P<0.01 and b: P<0.05.

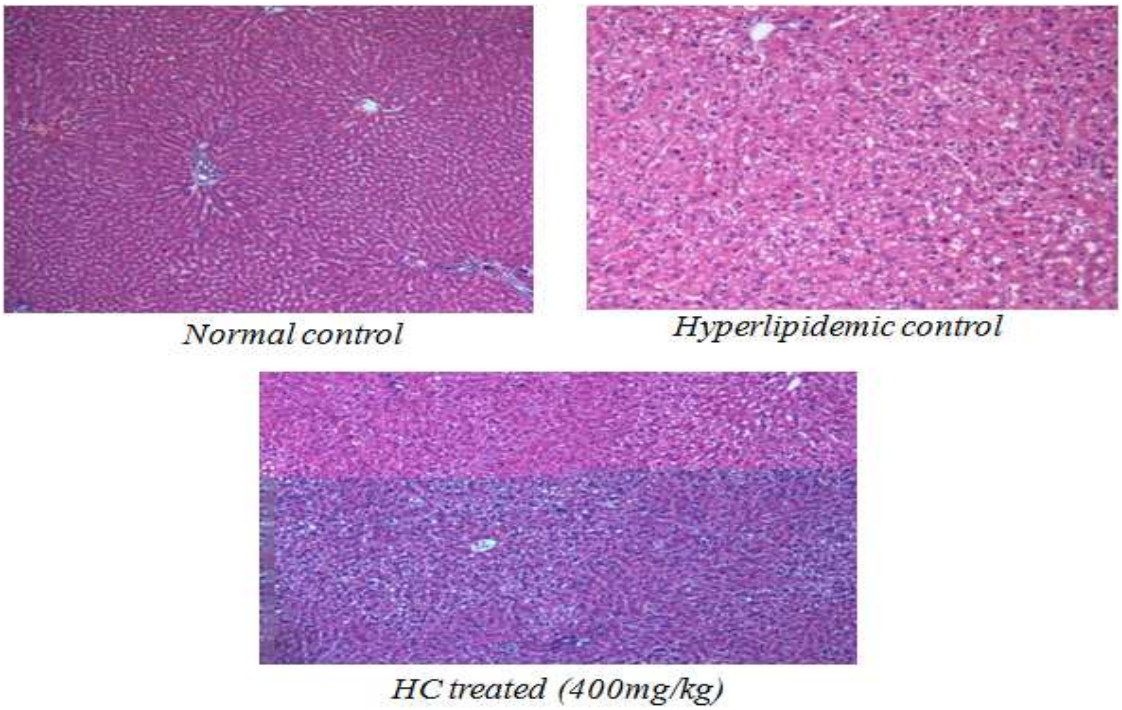

Figure 3: Effect of hydroalcoholic extract of Hibiscus cannabinus on histopathology of liver section of normal control, hyperlipidemic control and HC treated (400mg/kg) rats.

DISCUSSION & CONCLUSION

Recently, a number of clinical studies suggest that the increased risk of coronary heart disease is associated with a high serum concentration of TC, LDL-C and triglyceride. The abnormally high concentration of serum lipids is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots [20]. On the other hand, low serum concentration of HDL-C is also responsible for coronary heart disease [21]. Preclinical observations demonstrate that hyperlipidemia promotes accumulation of oxidatively modified low density lipoproteins (OX-LDL) in the arterial wall, promoting endothelial dysfunction and development of atherosclerosis and congestive heart diseases [22,23]. The physiological effect of flavonoids include possible antioxidant activity, therefore suggestion their role in prevention of coronary heart disease including atherosclerosis [24]. Flavonoids may also work by making liver cells more efficient to remove LDL-C from blood by increasing the LDL-C receptor densities in liver and by binding to apolipoprotein B [25].

For *Hibiscus cannabinus* L., scientific data for efficacy as antihyperlipidemic effect was scarce. In the present study, the effect of oral administration of 50% hydroalcoholic extract of *Hibiscus cannabinus* L. leaves (HC) on experimentally induced hyperlipidemia in rats was investigated. The HC showed protective action at a dose of 200 & 400mg/kg and demonstrated a significant decrease in the raised diet-induced levels of serum TC, VLDL-C and triglycerides. At a dose of 400mg/kg, effects were comparable with that of the standard drug atorvastatin and results indicate that antihyperlipidemic effect of HC is dose depended. The HC however, failed to show a effect on serum HDL-C level.

Further, treatment with HC 200 & 400mg/kg dose levels showed significant reduction in serum TBARS level, these results were comparable with that of the standard drug, studies also showed that there was microvesicular steatosis in the liver of hyperlipidemic rats. Treatment with HC at dose levels 100 & 200mg/kg slightly prevented the microvesicular steatosis in the liver of hyperlipidemic rats. However, treatment with HC at dose level 400mg/kg markedly prevented the microvesicular steatosis.

Chemical studies on *H. cannabinus* have reported the presence of lignans, alkaloids and flavonoids as main chemicals constituents and were confirmed by phytochemical screening. One or more of these pharmacology active compounds is/are likely to have contributed for the observed hypolipidemic activity of HC. A myriad of nutritional benefits has been attributed to these phytochemicals. It has been reported that administration of these compounds to hypercholesterolemic and hypertriglyceridemic rats evokes a significant lipid lowering activity and improves dyslipidemia [26,27].

Thus to conclude, the study showed that administration of HC at dose level 400mg/kg is effective as hypolipidemic agent. The active ingredient present in plant may recover the disorders in lipid metabolism noted in hyperlipidemic state and further work would be necessary to evaluate the active constituents responsible for the activity and mechanisms of these effects.

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Abbreviations
ATOR= Atorvastatin, CAD= Coronary artery diseases, CMC= Carboxymethyl cellulose, HC= 50% hydroalcoholic *Hibiscus cannabinus* L. leaves extract, HDL-C= High density lipoproteins cholesterol, HL= Hyperlipidemic control group, LDL-C= low density lipoproteins cholesterol, NC= Normal control group, OX-LDL = Oxidatively modified low density lipoprotein, p.o.= Per-oral, S.D= Standard deviation, TBARS= Thiobarbituric acid reactive substances, TC= Serum total cholesterol, TG= Triglycerides, VLDL-C= Very low density lipoproteins cholesterol

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