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Antioxidant and hypolipidemic activities of ethanolic leaves extract of *Hibiscus cannabinus*

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ABSTRACT

Background: To main aim of the study is to investigate the vitro antioxidant and hypolipidemic activity of *Hibiscus cannabinus* against high cholesterol diet induced hyperlipidemia in rats for 30 days.

Material and methods: Rats were fed with ethanolic leaves extract of *Hibiscus cannabinus* (250 mg/kg and 250 mg/kg p.o.) and atorvastatin (30 mg/kg, p.o.) along with hyperlipidemic diet for 30 days.

Results: Ethanolic leaves extract of *Hibiscus cannabinus* and atorvastatin were found to lower the serum cholesterol, triglyceride, VLDL, LDL levels and increase the HDL leaves as compared to the corresponding high fed cholesterol diet group. The hypolipidemic activity of *Hibiscus cannabinus* can be ascribed to its inhibitory effect on the liver HMG CoA reductase activity.

Conclusion: The study demonstrates that *Hibiscus cannabinus* possesses a hypolipidemic effect.

Keywords: *Hibiscus cannabinus,* HMG Co-A reductase, hypolipidemic effect, lipid profile.

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INTRODUCTION

Lipids and lipoproteins abnormalities are preceding risk factor for cardiovascular diseases and prevalence of this in general population has increased considerably in last few decades. Hyperlipidemia contributes significantly in the prevalence and severity of atherosclerosis and coronary heart diseases (Grundy 1986). Cardiovascular diseases are the primary cause of mortality and morbidity worldwide. Numerous factors, such as diet rich in saturated fats and cholesterol, age, family history, hypertension and life style play an important role in the development of high cholesterol and LDL levels, which are primarily responsible for the onset of atherosclerosis and coronary heart diseases (Farias et al., 1996). Lowering of lipids levels, by a drug or diet management could reduce the risk of cardiovascular diseases. Current awareness of medicinal plants in the management of cardiovascular diseases has encouraged the researchers for exploring novel lipid lowering pharmaceuticals (Lozoya 1980).

Hibiscus cannabinus (Malvaceae) is an annual or perennial herbaceous bush and has several forms with varying colors of flowers. It is native to China and grown widely as an ornamental plant throughout India. The flowers are considered emollient, and an infusion of the petals is used as a demulcent. Its decotion is given in bronchial catarrh in India. Previous studies show that the plant possesses anticomplimentary, antidiarrhetic and antiphologistic activities (Reddy 1997). The leaves and flowers have been found to be effective in the treatment of heart disorders. No reports are available on the antidiabetic activity of *Hibiscus cannabinus* leaves. Hence, the present study focuses on the scientific investigation of antidiabetic activity of *Hibiscus cannabinus* leaves (Kasture et al., 2002 and Nadkarni 1954).

MATERIALS AND METHODS

Preparation of plant material and ethanolic extract

The leaves were dried under shade at room temperature for seven days and powdered by the means of grinder and were sieved through sieve no.40 to get the coarse powder

(750 g) and was extracted with ethanol by Soxhlet apparatus and obtained extract was concentrated and stored in vacuum desiccator. The obtained yield was calculated. Then the ethanolic extract of *Hibiscus cannabinus* was subjected to qualitative and phytochemical analysis.

Preliminary phytochemical screening

The ethanolic extracts of *Hibiscus cannabinus* were subjected to preliminary phytochemical screening for their presence or absence of active phytochemical constituents by the following methods (Khandelwal 2004 and Kokate, 2007).

Experimental animals

Wistar albino rats (150-200 g) of both sexes were obtained from the animal house. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

In-*Vitro* evaluation of antioxidant activity by DPPH method (1, 1 diphenyl 2, picryl hydrazyl) (Gayatri et al., 2010**)**

To 1 ml of DPPH dissolved in methanol (0.33%), 1 ml of (1.25-10 µl/ml) essential oil/ascorbic acid was added. After the incubation for 30 min, at 37°C, the absorbance at 517 nm was measured using UV-spectrophotometer. Corresponding blanks were taken for the same. The experiment was performed in triplicate. The absorbance of DPPH as control was obtained at 518 nm. Lower absorbance of the reaction mixture was an indication of higher radical scavenging activity of essential oil/standard antioxidant. DPPH become a stable diamagnetic molecule by accepting an electron. The methanolic solution of DPPH (violet colour) has got a strong UV absorbance at 517 nm. The presence of a reducing environment in the solution pairs the odd electrons of DPPH radical and the solution in turn losses its colour stoichiometrically and also decreases the absorbance at 517 nm. The DPPH scavenging activity (%) was measured using the following formula: DPPH radical scavenging activity (%)

DPPH radical scavenging activity (%) =
$\mathbf{[(Abs_{control}-Abs_{sample})Abs_{control}] \times 100}$

Where,
$Abs_{control}$ is the absorbance of DPPH radical + methanol
Abs_{sample} is the absorbance of DPPH radical + essential oil/standard

Acute toxicity studies

The acute oral toxicity of ethanolic extract of leaves of the *Hibiscus cannabinus* was carried out as per OECD 423 – guidelines

Evaluation of antihyperlipidemic activity (Jamuna et al., 2013**)**

Animals were divided into 5 groups containing 6 in each:

Group I	Administered with normal saline along with normal diet
Group II	Administered with normal saline along with cholesterol diet (2% cholesterol, 1% sodium cholate and 2% arachis oil) for 30 days
Group III	Administered with ethanolic extract of *Hibiscus cannabinus* (250 mg/kg, p.o) along with high cholesterol diet for 30 days.
Group IV	Administered with a different effective dose of ethanolic extract of *Hibiscus cannabinus* (500 mg/kg, p.o.) along with high cholesterol diet for 30 days.
Group V	Administered with atorvastatin (30 mg/kg, p.o.) along with high cholesterol diet for 30 days.

On the 31st day, blood samples were collected from the retro orbital sinus and serum samples were analyzed for serum total cholesterol (TC), triglyceride (TG), High density lipoprotein cholesterol (HDL-C), Very low density lipoprotein (VLDL), Low density lipoprotein (LDL) was analyzed using diagnostic.

Statistical analysis

Results are expressed as mean ± SEM (standard error mean) and subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test and values with $p<0.05$ were considered to be statistically different.

RESULTS

Preliminary phytochemical studies of ethanolic leaves extract *of Hibiscus cannabinus* revealed the presence of flavanoids, triterpenoids, tannins, saponins, steroids and carbohydrates etc.

Acute toxicity studies

The acute toxicity studies were conducted according to OECD 423 guidelines. The ethanolic extract *of Hibiscus cannabinus* found to be non toxic up to 2000 mg/kg.

In-vitro antioxidant activity

The in vitro antioxidant activity of ethanolic extract of *Hibiscus cannabinus* were also studied by DPPH methods. In ethanolic leaves extract *of Hibiscus cannabinus*, the evaluation of antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method showed significant results. DPPH is one of the stable organic nitrogen free radicals, which is widely used for testing preliminary radical scavenging activity of a compound or a plant extract. It has a maximum absorbance at 517 nm. Absorbance decreases when antioxidants donate protons to DPPH, thereby reducing the latter. The % scavenging of *Hibiscus cannabinus* was found to be 71.27±0.26% at a dose of 100

µg mL^{-1} as compared to the standard ascorbic acid (82.24±0.25%) in case of DPPH free radical scavenging activity (Table 1).

Table 1. Percentage scavenging of DPPH radical

Concentration (µg mL^{-1})	Scavenging of DPPH (%)	Ascorbic Acid
25	46.54±1.02	55.43±1.48
50	60.26±2.92	68.26±4.22
100	71.27±0.26	82.24±0.25

Effect of ethanolic extract of *Hibiscus cannabinus* on TC , TGs , HDL-C , VLDL and LDL in rats

The effect of ethanolic extract of *Hibiscus cannabinus* on TC , TGs , HDL-C , VLDL and LDL in rats are summarized in Table 2. There was a significant increase in TC , TGs , VLDL and LDL in Cholesterol diet group II rats, when compared to the normal control group. The HDL-C levels were significantly decreased to 16.31 mg/dl in Cholesterol diet rats from the level of 42.38 mg/dl in normal group. On the other hand the group with received both leaves extract 250mg/kg and 500 mg/kg + Cholesterol diet (Group III and Group IV) and cholesterol + atorvastatin (Group V) showed significantly decreased the elevated TC , TGs , VLDL and LDL when given orally and reversed the altered HDL-C to almost normal level (Table 2).

Table 2. The effect of *Hibiscus cannabinus* on TC, TGs, HDL-C, VLDL and LDL.

Groups	TC (mg/dl)	TGs (mg/dl)	HDL-C (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal	76.46 ±2.26	36.43± 6.72	42.38 ±1.28	7.86± 0.11	32.52 ±1.64
Cholesterol diet	240.22 ±6.34	145.29 ±2.35	16.31 ±1.47	26.29 ±1.24	198.83±2.3
Cholesterol+ *Hibiscus cannabinus* 250 mg/kg	190.47 ±3.51	102.51 ±1.11	23.56 ±1.61	21.94 ±2.18	152.47±6.5
Cholesterol+ *Hibiscus cannabinus* 500 mg/kg	105.24 ±2.28	64.66± 3.91	36.25 ±2.50	14.63 ±1.12	61.52 ±4.01
Cholesterol+ atorvastatin 30 mg/kg	82.36± 3.82	52.23± 1.20	40.86 ±3.90	9.50± 0.66	48.26 ±4.03

DISCUSSION

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 (Ahmed et al., 2001). Previous works on *Hibiscus cannabinus* were carried out by many researchers and significance of *Hibiscus cannabinus* in various disease treatments was illustrated through various animal models. The present work were carrying out on antioxidant and anti hyperlipidemic activity of *Hibiscus cannabinus,* a direct approach of treating hypercholesterolemia in animal model is shown with various parameters. The previous work done on hyperlipidemia only show that the relation between blood glucose and lipid profile modifications. But from this research project this has been illustrated that *Hibiscus cannabinus* could directly be effective in treating hyperlipidemia.

In the present preliminary phytochemical studies of ethanolic leaves extract *of Hibiscus cannabinus* revealed the presence of tannins, saponins, alkaloids, flavanoids, triterpenoids and steroids. The acute toxicity studies *of Hibiscus cannabinus* leaves found to be non toxic up to 2000 mg/kg.

The possible mechanism involved in the atherogenesis in rat may be due to enhance cholesterol biosynthesis by increasing activity of HMGCoA reductase. In addition, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet (Bradley et al., 1995). The biochemical estimations shown that the extracts MEMD & FFMD increased the protective HDL-C level and decreased the atherogenic LDL and VLDL levels. The possible mechanism of test drug may involve increase of HDL-C, which can lead to the mobilization of cholesterol from peripheral cells to the liver (Khanna et al., 2002).

Flavonoids activate multi enzyme systems, such as cytocrome P450 and b5(Hodek et al., 2009) and this action affects the whole metabolism, as these systems are involved in the metabolism of xenobiotics, including drugs, insecticides, and pollutants, that have great importance on pharmacology and toxicology. Due to this effect, flavonoids act on body lipid constituents like steroids and bile acids, and influence lipid metabolism. They increase bile acid excretion because cytocrome P-450 enzymes bind some compounds to the bile acids and therefore reduce cholesterol level in the body (Hofmann et al., 1964). The physiological effect of flavonoids include possible antioxidant activity, therefore suggestion their role in prevention of coronary heart disease including atherosclerosis (Beshbishy et al., 2006). 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 (Haslam 1981 and Baum et al., 1998).

In the present study, the in vitro antioxidant activity of *Hibiscus cannabinus* by DPPH methods showed significant results when compared to Vitamin C. The results shown suggest that the study carried out on *Hibiscus cannabinus* (500 mg/kg) is found to more effective than *Hibiscus cannabinus* (250 mg/kg) against hyperlipidemia in reducing the levels of low density and very low density lipoproteins and increase in the HDL levels in the present model of research.

CONCLUSION

Chronic hyperlipidemia was induced by feeding male rats with high cholesterol diet for 30 days. Administration of ethanolic leaves extract of *Hibiscus cannabinus* (250/500 mg/kg) for 30 days in high cholesterol diet successfully

prevented the elevation of TG, TC, LDL-C and VLDL levels. While administration of *Hibiscus cannabinus* (250 mg/kg and 500 mg/kg) for 30 days successfully prevented the decrease of serum HDL-C in high cholesterol diet model rats. Antihyperlipidemic activity was observed with atorvastatin (30 mg/kg, p.o.) but *Hibiscus cannabinus* (250 mg/kg and 500 mg/kg) extracts also showed better results. Ethanolic leaves extract of *Hibiscus cannabinus* also exhibited good antioxidant effect in DPPH in vitro method. In conclusion, the In-vitro antioxidant and antihyperlipidemic activity of *Hibiscus cannabinus* might be due to flavonoids present in ethanolic leaves extract.

CONFLICT OF INTEREST

None declared.

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