ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antihyperlipidemic effect of ethanol extract of whole plant of *Canscora perfoliata* Lam in Triton X-100 induced hyperlipidemic rats

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Manuscript Info

Manuscript History:

Received: 12 June 2013
Final Accepted: 24 June 2013
Published Online: July 2013

Key words:
Lipid profile,
Athrogenic index,
Canscora perfoliata

Abstract

The present study is designed to evaluate the effect of ethanol extract of *Canscora perfoliata* whole plant on lipid profiles in Triton X-100 induced hyperlipidemia in male Wistar albino rats. The Triton X-100 induced hyperlipidemic rats treated with whole plant extract of *C. perfoliata* showed a significant decrease in the serum TC, TG, LDL-C, VLDL-C and PL values along with an increase in serum HDL-C levels. The extract treated groups also showed significant decrease in the atherogenic index and LDL-C: HDL-C risk ratio compared to Triton X-100 induced hyperlipidemic rats. Significant antihyperlipidemic activity was shown by ethanol extract of whole plant of *C. perfoliata*.

Introduction

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases (Oehami and Mello,2009). World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year (Third report). Hyperlipidemia is a metabolic disorder, specifically characterized by alternations occurring in serum lipid and lipid protein profile due to increased concentration of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) with a concomitant decrease in the concentration of high density lipoprotein cholesterol (HDL-C) in the blood circulation (Duhley *et al*.,1999). Therefore it is required to develop drugs for treatment of hyperlipidemia reducing their side effects.

Currently, the use of complimentary / alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long term use (Kaliora *et al*.,2006)

Canscora perfoliata Lam is one of the medicinally important plant belonging to Gentianaceae. The juice prepared from the plant is given to treat any poisonous bites by Palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu (Muthukumarasamy *et al.*, 2003). Survey of the literatures revealed that the antihyperlipidemic activity of *Canscora perfoliata* has not been clinically evaluated so far. In view of this, the present study was aimed at evaluating the antihyperlipidemic activity of the ethanol extract of *Canscora perfoliata* whole plant in rats.

Materials and Methods

Plant material

The whole plant of *Canscora perfoliata* Lam were collected from the natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu and were identified by the Botanical Survey of India, Coimbatore. A voucher specimen was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin for further reference.

Preparation of plant extract

The dried whole plant materials of *Canscora perfoliata* were powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a

Soxhlet apparatus and extracted with ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures (Murugan, M., and Mohan V.R. 2011 and Packia lincy *et al.*,2013.). The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for antihypertlipidemic studies.

Animals

Wistar albino adult male rats weighing 200 – 220 g were selected and housed in polypropylene cages in a room where the congenial temperature was maintained at 27°C ± 1°C and the rats were given 12 h light and 12 h dark cycles. The animals were allowed to acclimatize to the environment for 7 days and were fed with standard pellet diet (Goldmohar brand, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Hyperlipidaemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/Kg) in physiological saline after overnight fasting for 18 h.

Acute toxicity study

Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD.2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 and 2000 mg/kg body weight.

Antihyperlipidemic studies

The rats were divided into five groups of five rats in each group of which, four groups contained hyperlipidemia induced rats by Triton X-100 and the remaining one group contained the normal rats. The hyperlipidemia induced rats were treated with the standard drug atorvastatin, or with the whole plant extract of *C. perfoliata*, a single dose/day for 14 days orally, by IGC.

CCl_4 hepatic toxicity induced rats such as,

Group I : Rats received normal saline for 14 days orally, by IGC, and served as

Normal control.

Group II : Hyperlipidemia induced rats by Triton X-100 (100 mg/Kg, i. p)

received normal saline for 14 days orally, by IGC, and served as

Hyperlipidemic control.

Group III : Hyperlipidemia induced rats by Triton X-100 (100 mg/Kg, i. p)

received whole plant extract of *C. perfoliata* (150 mg/Kg) for 14 days orally, by IGC.

Group IV : Hyperlipidemia induced rats by Triton X-100 (100 mg/Kg, i. p)

received whole plant extract of *C. perfoliata* (300 mg/Kg) for 14 days orally, by IGC.

Group V :Hyperlipidemia induced rats by Triton X-100 (100 mg/Kg, i. p)

received atorvastatin (AT) (10 mg/Kg) for 14 days orally, by IGC.

Collection of blood samples

This study was carried out for 14 days. On 15^{th} day of treatment, the blood was collected by retro-orbital sinus puncture, under mild ether anesthesia in heparinized tubes. Serum obtained by immediate centrifugation of blood samples using Remi ultra cooling centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum lipid profiles. Liver was removed, cleaned and stored at 4°C until analysis.

Faecal matters of all the rats which received various treatments were collected during the last five days of treatment. The faecal matters were dried at 40ºC and used for analysis of total lipid, total cholesterol and triglycerides.

Biochemical analysis

Plasma lipid levels including total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL-C) were estimated as per the procedure given by Gulati *et al*. (2003) and were estimated using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India. Low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) were estimated as per the procedure formulated by Friedwald *et al*. (1972). Fecal total lipids were extracted and estimated gravimetrically (Folch *et al*., 1957). Total cholesterol (Zlatkis *et al*., 1953) and

triglycerides (Gottfried and Rosenberg, 1973) were also determined.

Results

Acute oral toxicity studies following OECD guidelines 420 fixed dose procedure, showed that ethanol extract upto 200mg/kg are non-toxic and sage.

Table 1 shows that in group II, Triton X-100 induced a significant increase ($p < 0.05$) in serum total cholesterol (TC), glycerides (TG), low density lipoprotein- cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and phospholipids (PL) and significant decrease in the high density lipoprotein-cholesterol (HDL-C) levels in comparison with the control (Group-I). Thus injection of Triton X-100 (100mg/kg) (Group II) has successfully induced hyperlipidemic in rats. Treatment with ethanol extracts whole plant of *C. perfoliata* at the doses of 150mg/kg and 300 mg/kg (Group III and Group IV) significantly reduced the serum TC, TG, LDL-C, VLDL-C and PL levels and significantly increased the serum HDL-C levels when compared to the hyperlipidemic control group. The changes in lipid profiles levels in group III and IV were comparable with group V of Atorvastatin treated rats.

Table-1. Effect of whole plant extracts of *Canscora perfoliata* on the TC, TG, LDL - C and PL in the serum of normal and hyperlipidemic induced rats.

Treatment Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	PL (mg/dl)
Group - I	102.42±4.18	129.56± 3.23	21.42± 2.44	25.91± 1.46	56.09±2.45	159.15 ± 5.27
Group - II	168.53 ±.23**	243.32 6.24**	14.33± 5.21	48.66± 1.21*	95.34±5.82**	217.99±8.47**
Group - III	128.56± 2.12a	183.21 ±3.56a	32.14±3.08a	36.64±1.74ns	59.78±2.19aa	182.41±3.56aa
Group - IV	104.71±3.43aa	156.38±3.28aa	30.21±2.52NS	31.27±1.33a	43.23±1.67aa	161.19±5.62aa
Group - V	106.73±2.48aa	122.79±3.25aa	38.43±2.82a	24.55±1.91aa	43.75±1.21aa	162.98±5.88aa

Each Value is SEM of 5 individual observations * $P < 0.05$; ** $P<0.01$ Compared Normal Control vs - Hyperlipidemic Control rats a - $P < 0.05$; aa - $P<0.01$ Compared - Hyperlipidemic Control rats vs Drug treated

Table-2 Effect of whole plant extracts of *Canscora perfoliata* on the body weight, faecal lipid profile of normal and hyperlipidemic and Drug treated rats.

Treatment Groups	Body Weight			TL (mg/dl)	TC (mg/dl)	TGL (mg/dl)
	0 day	7th day	14th day			
Group - I	138.45±6.8	146.28±5.12	149.28±4.95	49.94±1.84	10.14.±1.04	14.32± 1.42
Group - II	163.54±6.2	178.74±4.*	186.56±4.68**	93.76±1.87*	21.18±1.93*	26.23 ± 1.84*
Group - III	154.33±5.3	163.48±3.*	160.62±5.22b	63.83±2.51a	14.25±1.23a	18.36±1.27a
Group - IV	161.79±4.9	165.57±4.*	166.51±3.26ns	51.36±2.73a	11.33±0.93a	15.72±1.36aa
Group - V	156.37±4.1	163.26±4.73	154.26±4.81ns	50.16±2.37a	9.24± 0.31a	10.19±1.03aa

The hyperlipdemic rats treated with the extracts of *C. perfoliata* showed decreased in body weight (Table 2) when compared to Triton X 100 induced hyperlipidemic control rats.

Total lipid, total cholesterol and triglycerides levels of faecal matter showed a significant increase in Triton X-100 treated rats (Table -2). There were significant reduction in faecal total lipids, total cholesterol and triglycerides levels at the dose of 300 mg/kg of whole plant extract of *C. perfoliata* respectively.

Each Value is SEM of 5 individual observations * P < 0.05; ** P<0.01 Compared Normal Control vs - Hyperlipidaemic Control rats a - P < 0.05; aa - P<0.01 Compared - Hyperlipidaemic Control rats vs Drug treated; ns – Not significant

The Triton X 100 groups treated with extracts of *C. perfoliata* and atorvastain demonstrated decrease in the atherogenic index and LDL-C: HDL-C risk rats. (Table-3)

Table-3 Effect of *Canscora perfoliata* extracts on various biological parameters like atherogenic index and LDL-C/HDL-C ratio in hyperlipidemic rats.

Treatment Groups	Atherogenic Index	LDL/HDL-C
Group - I	4.80	2.62
Group - II	12.84	7.22
Group - III	3.90	1.86
Group - IV	3.47	1.44
Group - V	2.77	1.13

Discussion

Lipid is an important part of a healthy body because it is used to form cell membranes, sexual hormones and is necessary for other cellular functions. The various forms of lipid cannot dissolve in the blood and must be transported to and from the cells by low density and high density lipoproteins. High density lipoprotein cholesterol (HDL-C) tends to carry cholesterol away from arteries back to the river. Therefore, high serum cholesterol level can be due to hepatic dysfunction (Gupta *et al*., 2008). Triton X-100 induced rise in serum triglyceride is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up triglyceride. The high TG level along with decreased absorption of fatty acids by adipose is associated with a low level of HDL-C, insulin resistance and increased risk of atherosclerosis (Terasawa *et al*., 2000). In the present study, high cholesterol level in Triton X-100 intoxication may also be due to decreased activity of cytochrome P450 enzymes (Witmer *et al*.,1994). The rise in serum lipid profiles may also be attributed to increased lipolysis, medicated by increased nonepinopterine release which act through interface with the intracellular fraction of Ca^{+2} in the cytoplasm (Liu and Lin, 1997).

Administration of whole plant of *C. perfoliata* extracts caused a significant decrease in serum TC, LDL-C, VLDL-C and PL suggesting beneficial modulatory influence on cholesterol metabolism and turn over. Elevated serum triglyceride is considered as independent risk factor for cardiovascular disease (Asia Pacific Cohort Studies Collaboration, 2004). A significant decline in the triglyceride level observed in *C. perfoliata* whole plant extract treated rats support the cardiovascular protective influence.

The cholesterol lowering effects of the *C. perfoliata* whole plant extract is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in faecal excretion of neutral lipids (Purohit and Vyas, 2006).

The atherogenic index (AI) was significantly increased in Triton X-100 hyperlipidemic rats compared with normal group and these elevated AI were returned to near normal levels in groups of rats treated with ethanol extracts of *C. perfoliata* and atorvastation. The rise in AI in hyperlipidemic rats enhances the probability of cardiovascular pathogenesis and endothelial dysfunction (Keshethy *et al*.,2009) A significant decrease in AI value was observed in plant extract supplemented rats, suggests the atheroprotective, cardioprotective potential of this plant.

From these results it can be concluded that, ethanol extract of whole plant of *C. perfoliata* contains active compounds which decreases serum lipid profiles and lowers the risk of atherosclerosis in hyperlipidemic rats.

Acknowledgement

The Authors wishes to thank Dr. R. Sampatharaj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur, for their assistance in animal studies.

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