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Antihyperlipidaemic Activity of *Spermacoce Hispida* Ethanolic Extract in Triton WR-1339 Induced Hyperlipidaemic Rats

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ABSTRACT

Hyperlipidaemia is the greatest risk factor of coronary heart disease. Currently available hypolipidaemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidaemia has no side effects and is relatively cheap and locally available. Literature claims that Saponins are able to reduce hyperlipidemia. Based on high saponin content in herbal plants, *Spermacoce hispida* (*S. hispida*) was selected and the present study focus on the antihyperlipidaemic activity of ethanolic seed extract of *S. hispida* against triton-WR-1339 induced hyperlipidaemia in rats. Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. *S. hispida* was administered orally at a dose of 200 mg/kg to triton WR-1339 induced hyperlipidaemic rats. After administration of *S. hispida* shows a significant decrease in the levels of cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of HDL in serum and liver tissues against triton induced hyperlipidaemic in rats. Therefore it effectively suppressed the triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

Keywords: Hyperlipidaemia, Triton WR-1339, *S. hispida*, Cholesterol.

INTRODUCTION

Hyperlipidaemia mainly increased level of cholesterol or low-density lipoprotein cholesterol (LDL-C) contributes significantly to the manifestation and development of atherosclerosis and coronary heart diseases (CHDs). Cardiovascular diseases, including atherosclerosis, are the most common causes of mortality and morbidity worldwide (Yokozawa *et al.*, 2003). Approximately 12 million people reportedly die of cardiovascular disease each year worldwide. Although several factors such as diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle play a significant role in causing heart failure, the high level of cholesterol, particularly LDL-C is mainly responsible for the onset of CHDs (Yokozawa *et al.*, 2003, Farias *et al.*, 1996). The lowering of lipids and cholesterol levels by drug or dietary interventions could reduce the risk of CHDs. The known lipid-lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but they also have many side effects (Chattopadhyaya *et al.*, 1996). Therefore, the development of lipid-lowering drugs from natural sources is the best option and is in great demand. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems.

Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of hyperlipidaemias in many parts of the world. There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine. Recently, the search for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs (Newman *et al.*, 2004). Plants that were once considered of no value are now being investigated, evaluated and developed in to drugs with no side effects. One of such plant is *S. hispida*. Linn commonly known as 'Shaggi button weed' belongs to the family *Rubiaceae* and is widely distributed throughout the world as a useful medicinal plant (Narayan *et al.*, 2003). The seeds of plants as confection are cooling demulcent and given in diarrhea and dysentery. Seeds have been recommended as a substitute for coffee. Seeds are crushed in to paste and taken orally to treat stomach problems (Chellaiah *et al.*, 1999). According to some studies, *S. hispida*. Linn has also anti hypertensive activity (Arnold and Schmidt, 2003). The plant has been extensively studied for its phytochemical composition and a large number of active ingredients such as, Borrelin, β -sitosterol, Ursolic acid and Isorhamnetin. Recently, pharmacological studies have shown that *S. hispida* seeds exhibit antidiabetic properties in rats (Kaviarasan *et al.*, 2008). Hence, in the present study, the ethanolic extract of *S. hispida* seeds was investigated for Antihyperlipidaemic activity in triton WR-1339 induced hyperlipidaemic rats.

MATERIALS AND METHODS

Collection of plant material and extraction

The seeds of *S. hispida* were collected from Gobichettipalayam. The plant was identified by the voucher specimen was deposited at the Herbarium of Botany department, PSG college of Arts and science, Coimbatore. The dried seeds were made into fine powder with an auto-mix blender and were kept separately in an airtight container until use. The powder was exhaustively extracted with ethanol in the ratio of 1:5 (w/v) for 24 hr by using soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash. This extract was dissolved in 5% carboxy methyl cellulose (CMC) solution and used in the study.

Animals

Eight week old adult male albino rats of *Wistar* strain, weighing approximately 150 to 200 g, were acclimatized for 7 days at room temperature ($22\pm 2^\circ\text{C}$) and humidity of 45-64% in a 12-hour light/dark cycle in a room under hygienic condition (Kumaravel *et al.*, 2010). They were given access to water and a commercial diet *ad libitum*. The experiments were carried out in the Department of Pharmacology, Periyar college of Pharmaceutical science for girls, Trichy, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC).

Chemicals

Triton WR-1339 (A non-ionic detergent, Isooctyl poly oxyethylene phenol) was obtained from Sigma Chemicals Co, Mumbai.

Induction of hyperlipidaemia

Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. After 72 hours of triton injection received a daily dose of 5% CMC in 5ml/kg body weight for 7 days (Sundarrajan *et al.*, 2010).

Experimental design

In the experiment, the rats were divided into three groups of eight rats each. Group I rats received 5% CMC and considered as controls, Group II rats were treated with Triton WR-1339 (400 mg/kg body weight; i.p.) (Sundarrajan *et al.*, 2010) and Group III rats were treated with Triton WR-1339 (400 mg/kg body weight) and ethanolic extract of *S. hispida* seed (200mg/kg body weight) (Rathi *et al.*, 2010).

At the end of 8th day, rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected, and serum were separated by centrifugation. Liver tissues were excised immediately and rinsed in ice-chilled normal saline, 500mg of the tissues were homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH, 7.4). Biochemical estimations were carried out in serum and liver tissues, parameters such as cholesterol (Zak's, 1977), phospholipids (Rouser *et al.*, 1970), triglycerides (Rice, 1970), LDL (Friedwald Levy and Frederickson, 1972), VLDL (Henry *et al.*, 1998) and HDL (Varley *et al.*, 1980) were analyzed.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean \pm SD from 8 rats in each group. *P* values <0.05 were considered as significant.

RESULT

The results were discussed under the following headings. Lipid profile in serum and liver tissues. Table 1 shows the levels of cholesterol and phospholipids were found to be significantly increased in serum and liver tissues of triton induced rats when compared to control rats. These levels were found to be significantly reduced in hyperlipidaemic rats treated with ethanolic seed extract of *S. hispida*. In triton induced rats the levels of triglycerides, LDL and VLDL were significantly higher in serum and liver tissue when compared to those in control rats, while the HDL levels were significantly decreased when compared to control rats. After the treatment with ethanolic seed extract of *S. hispida* at the doses 200 mg/kg in the Triton induced rats, a significant reduction in LDL, VLDL and significant increase in HDL were observed when compared to control rats were shown in the table 2 & 3, respectively.

Table.1: Effect of *S. hispidia* on changes in the levels of cholesterol and phospholipids in serum and liver tissue of control and experimental rats.

Groups	Cholesterol		Phospholipids	
	Serum	Liver	Serum	Liver
Control	078.84±06.16 ^a	063.68±04.69 ^a	051.32 ±04.75 ^a	086.02±03.58 ^a
Triton WR-1339(400 mg/kg)	206.53±12.38 ^b	265.80±13.70 ^b	144.50 ±09.55 ^b	146.00±06.08 ^b
Triton WR-1339+ <i>S. hispidia</i> (200mg/kg)	097.30±07.18 ^c	099.68±07.23 ^c	064.30±03.96 ^c	092.30±02.36 ^c

Each value is mean ± SD for eight rats in each group. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$.

Table. 2: Effect of *S. hispidia* on changes in the levels of triglycerides and LDL in serum and liver tissue of control and experimental rats.

Groups	Triglycerides		LDL	
	Serum	Liver	Serum	Liver
Control	076.42±04.55 ^a	060.50±02.76 ^a	039.12 ±04.76 ^a	020.90±01.64 ^a
Triton WR-1339(400 mg/kg)	124.22±08.04 ^b	110.40±05.83 ^b	187.90 ±09.34 ^b	177.68±07.67 ^b
Triton WR-1339+ <i>S. hispidia</i> (200 mg/kg)	083.54±05.92 ^c	092.04±03.78 ^c	063.06±02.88 ^c	039.54±04.07 ^c

Each value is mean ± SD for eight rats in each group. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$.

Table. 3: Effect of *S. hispidia* on changes in the levels of VLDL and HDL in serum and liver tissue of control and experimental rats.

Groups	VLDL		HDL	
	Serum	Liver	Serum	Liver
Control	15.22±0.90 ^a	12.10±0.71 ^a	51.50±2.21 ^a	29.60±1.85 ^a
Triton WR-1339(400 mg/kg)	24.82±1.23 ^b	21.87±1.93 ^b	38.10±3.20 ^b	18.45±3.77 ^b
Triton WR-1339+ <i>S. hispidia</i> (200 mg/kg)	16.70±1.29 ^c	16.40±1.01 ^c	47.30±5.92 ^c	26.54±4.07 ^c

Each value is mean ± SD for eight rats in each group. ANOVA followed by Duncan's multiple range test. Values not sharing a common superscript (a, b, c) differ significantly at $P \leq 0.05$.

DISCUSSION

Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values have been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. Several studies reveal that an increase in HDL cholesterol and decrease in cholesterol, LDL cholesterol and triglycerides is associated with a decrease in the risk of ischemic heart diseases (Kellner *et al.*, 1951). Triton WR-1339 has been widely used to block clearance of triglyceride rich lipoproteins to induce acute hyperlipidaemia in several animals (Schurr *et al.*, 1972).

The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglycerides, phospholipids and the reduction in the HDL level. Most of the antihyperlipidemic drugs are causing significant reduction in both total cholesterol and increase in HDL cholesterol levels (Fiser *et al.*, 1951). This model is widely used for a number of different aims (Ye- Yuli *et al.*, 2005) particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs (Harbowy and Balentine, 1997). In fact, flavonoids, steroids, saponins and anthocyanin, a heterogeneous group of ubiquitous plant polyphenols have exhibited a variety of pharmacological activities including the anti-atherogenesis. The plant steroids reduce the absorption of cholesterol and thus increase faecal excretion of cholesterol (Del *et al.*, 2005). Lupeol, a triterpenoid was reported for its cardioprotective activity against experimental hypercholesterolemia (Sudhakar *et al.*, 2007). Interestingly, the results of the present study show that ethanolic extract of *S. hispidia* seeds reduces the level of cholesterol, triglycerides, phospholipids and increase the level of HDL, which may probably be due to the presence of steroids, flavonoids and triterpenoids.

Schurr *et al.*, (1972) demonstrated that a parenteral administration of a dose of TritonWR-1339 to adult rats induced hyperlipidemia. The large increase in cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (Otway and Robinson, 1967). The reduction of total cholesterol by the *S. hispidia* extract was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol lowering activity of the herb extract can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids as demonstrated by Khanna *et al* (2002). The *S. hispidia* markedly lowers the levels of serum and liver tissue of VLDL and LDL due to the presence of large number of active ingredients in seeds of plant such as Borrelina, Ursolic acid and Isorhamnetin. In conclusion, the present study demonstrated that the ethanolic extract of *S. hispidia* seeds exhibited maximum efficacy in lowering the elevated lipid levels.

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