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Evaluation of antihyperlipidemic activity of *Himalrandia tetrasperma* in Triton WR-1339 (Tyloxapol) induced hyperlipidemia in rat model

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Abstract

Pants are used by human beings since prehistoric times for therapeutic purposes. Statistical records revealed that more than 250,000 higher plants are found on the globe. Among these a large number are practicing for the management of various pathological disorders. *Himalrandia tetrasperma* (*H. tetrasperma*) also carries immense importance medicinal properties such as abortifacient, anthelmintic, antidysentric, antidiabetic, antifungal and antibacterial. The aim of the current study was to evaluate the antihyperlipidemic activity of Methanolic extract of *Himalrandia tetrasperma* (MEHT) in Tyloxapol (Triton WR-1339) induced hyperlipidemia in rat model. The administration of Tyloxapol prominently increased the level of lipid parameters such as total cholesterol (TC), triglycerides (TG), Low density lipoprotein (LDL), Very low density lipoprotein high density lipoprotein (VLDL) and reduced the level of high density lipoprotein (HDL). Treatment with *H. tetrasperma* caused reduction in the level of TC, TG, LDL and VLDL while increase in the level of HDL in a dose reliant manner. It is concluded that in Tyloxapol induced hyperlipidemia, MEHT decreases the levels of TC, TG, LDL, VLDL and increases the level of HDL and could be used be used to develop a natural drug to treat the hyperlipidemia.

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Introduction

Hyperlipidemia has been reported as one of the key risk factors involved in the development of cardiovascular ailments (Rajani and Ashok, 2009). Pathological disorders such as diabetes mellitus, obesity, primary hyperlipoproteiamia and chronic renal disease related with elevated triglycerides (TG) carry high risk of cardiovascular diseases (Lamarche *et al.*, 2001). Hyperlipidemia is metabolic complication of obesity (both clinical and experimental obesity) (Avogaro and de Kreutzenberg, 2005). Hyperlipidemia is caused by high level of TC, TG, LDL and VLDL, and low level of HDL. It is the cause of obesity in the majority of patients and have 2-5 folds the risk of cardiac ailments (Naderali, 2009). Utilization of high amount of fat may cause the production of extra VLDL, resulting in the formation high amount of LDL which may stick to the wall of circulatory system if the amount of the HDL is inadequate, leading to the blockages of normal blood flow in circulatory system. Finally, this causes coronary atherosclerotic heart diseases (Pascot *et al.*, 2001).

A large variety of synthetic antihyperlipidemic drugs are available in the market for hyperlipidemia treatment, but these drugs have been associated with a number of adverse effects. The consequences of utilization of these synthetic drugs are diarrhea, nausea, hyperuricemia, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Speight and Avery, 1987). New medicines are obtained from natural resources i.e. plants, fungi etc. which have low adverse effects and are more effective for treatment of various diseases (Idrees *et al.*, 2018). Medicinally valuable plants are used for different research purposes. It has been reported that conventional systems have immune potential against different pathological conditions. Approximately 13,000 plants have been investigated for different therapeutic properties. A conventional mode of therapy for hyperlipidemia has no adverse effects and is comparatively safe, effective, cheap and locally available (Pandit *et al.*, 2011, Raj *et al.*, 2014).

The selected medicinal plant, *H. tetrasperma*, is a member of Rubiaceae family. According to literature review there is no scientific basis for the antihyperlipidemic activity of this plant, therefore, the current study was planned to evaluate the antihyperlipidemic activity of MEHT in Tyloxapol (Triton WR-1339) induced hyperlipidemic rats model.

Materials and methods

Animals

Albino rats of either sex, weighing (100-150g) were used in this scheme of study. The animals were kept under standard laboratory condition i.e. 25°C and 12 hours light and dark cycle. They were allowed to free access with standard laboratory diet and water *ad libitum*. Animals were acclimatized to experimental conditions seven days prior to experiment.

Chemicals

Tyloxapol (Triton WR-1339) and Simvastatin were purchased from Sigma Chemical Co. USA. Enzymatic kits were procured from Merck. All other solvents and chemicals used in this study were of analytical grade.

Plant materials

The stems of *H. tetrasperma* were collected in May, 2017 from district Mansehra, Khyber Pakhtunkhwa, Pakistan. The plant material was authenticated and identified by a Taxonomist at the Department of Botany, University of Peshawar, Peshawar, Khyber Pakhtunkhwa, Pakistan. At herbarium of university of Peshawar, a voucher specimen was deposited.

Plant extract preparation

Stems of the selected plant were washed several times with water to remove the impurities, dried under shade at room temperature and grinded with the help of mechanical grinder. The powdered material was extracted with methanol. The extract was filtered and concentrated under vacuum at low temperature (40-45°C) with the help of rotary evaporator. As a result, semi-solid extract was obtained. The MEHT was stored at low temperature in refrigerator. MEHT at dose levels of 250, 500 and 750mg/kg body weight to be administered to animals were selected.

Induction of hyperlipidemia

For the induction of hyperlipidemia, a single dose (300mg/kg body weight i.p) of Tyloxapol (Triton WR-

1339) was administered in the rats. Hyperlipidemia was confirmed after 24 hours of Tyloxapol injection by determining the blood cholesterol concentration. The results were compared with of the standard drug simvastatin at dose level of 80mg/kg body weight (Pandit *et al.*, 2011).

Experimental design and protocol

The animals were distributed in six groups containing six rats in each group. Group 1 was treated with normal saline (normal control / negative control). Animals of group II to VI were treated with Tyloxapol (Triton WR-1339) at a dose level of 300mg/kg (i.p.) to induce hyperlipidemia. Twelve hours following the Tyloxapol treatment, groups IV, V and VI were treated with MEHT at dose levels of 250, 500 and 750mg/kg b.w. (p.o.) respectively and group 111 was treated with the standard drug Simvastatin (Positive control) at the dose level of 80mg/kg b.w. (p.o.) (Pandit *et al.*, 2011).

Blood collection

At the end of the experimental period, after 24 hours, the animals were anesthetized by chloroform vapour before dissection. Through cardiac puncture blood was collected into plasma separator tubes containing anticoagulant.

Plasma sample preparation

The blood was allowed to standing at room temperature for 30 minutes and then refrigerated for another 30 minutes. The resultant clear portion was centrifuged at 3500rpm for 10 minutes. Then the supernatant also called plasma was isolated and refrigerated for further analyses.

Biochemical analysis

The amount of total cholesterol, Triglycerides, HDL-C, LDL-C and VLDL-C were estimated by using the standard methods (Pandit *et al.*, 2011, Raj *et al.*, 2014).

Statistical analysis

All the values obtained were represented as mean ± SEM for six animals in each group (n=6). Graph Pad Prism 06 version (San Diego, CA, USA) was used for the statistical analysis of data. One-way analysis of variance (ANOVA), followed by Tukey, s test for multiple comparisons was used for statistical significant differences between mean values. The value of $p < 0.05$ was considered to be statistically significant in all cases.

Results and discussion

In Tyloxapol induced hyperlipidemia, the effect of the MEHT on the level of plasma lipid parameters is reflected in Table 1 and, 2. Tyloxapol induced an increase in TC, TG, LDL and VLDL and induced a fall in HDL-cholesterol plasma level when compared with normal control group. The treatment of 250, 500, 750mg/kg b.w. doses of MEHT in Tyloxapol administered rats decrease the plasma TC, TG, LDL and VLDL and increase in HDL-cholesterol with a dose reliant manner. The reduction of TC at dose levels of 250, 500 and 750mg/kg b.w. was 23.78%, 28.31% and 38.49% respectively. The reduction of TG at dose levels of 250, 500 and 750mg/kg b.w. was 27.65%, 35.11% and 41.70% respectively. The reduction of LDL at dose levels of 250, 500 and 750mg/kg b.w. was 31.60%, 39.63% and 66.93% respectively. The reduction of VLDL at dose levels of 250, 500 and 750mg/kg b.w. was 27.65%, 35.11% and 41.70% respectively. The increment of HDL at dose levels of 250, 500 and 750mg/kg b.w. was 8.59%, 23.78% and 31.61% respectively. The MEHT showed prominent ($P<0.01$) effect at the dose levels of 500mg and 750mg/kg b.w. in Tyloxapol induced hyperlipidemia.

Table 1. Effect of MEHT on TC, TG and HDL in Tyloxapol (Triton WR-1339) induced hyperlipidemic rats.

Group	Treatment	Dose /kg	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)
I	Saline (Normal control)	10 ml	83.52±2.92	54.01 ±2.26	56.64±2.76
II	Tyloxapol (Triton WR-1339)	300mg	231.85±21.09**	690.78±44.82***	31.54±0.21**
III	Simvastatin (Positive control)	80mg	163.13±13.81**	464.59±15.05**	40.58±1.58**
IV	MEHT	250mg	176.72±13.99**	499.78±15.16**	34.25±1.03
V	MEHT	500mg	166.21±13.93**	448.25±14.95**	39.04±1.38**
VI	MEHT	750mg	142.61±12.56**	402.72±14.89**	41.51±1.60**

Where MEHT = Methanolic extract of *Himalrandia tetrasperma* TC = Total cholesterol, TG = Triglyceride, HDL = High density lipoprotein. Tyloxapol-treated control group (II) was compared with normal control group (I), while drug and ME treated groups (III-VI) were compared with Tyloxapol-treated control group (II). **P < 0.01 and ***P < 0.001.

Table 2. Effect of MEHT on LDL and VLDL in Tyloxapol (Triton WR-1339) induced hyperlipidemic rats.

Group	Treatment	Dose/kg	LDL (mg/dl)	VLDL (mg/dl)
I	Saline (Normal control)	10 ml	16.08±0.92	10.80±0.45
II	Tyloxapol (Triton WR-1339)	300mg	62.15±6.65**	138.16±8.96***
III	Simvastatin (Positive control)	80mg	29.63±3.31**	92.94±3.01**
IV	MEHT	250mg	42.51±3.98**	99.96±3.03**
V	MEHT	500mg	37.52±3.81**	89.65±2.99**
VI	MEHT	750mg	20.56±2.46**	80.54±2.98**

Where MEHT = Methanolic extract of *Himalrandia tetrasperma,* LDL = Low density lipoprotein, VLDL = Very low density lipoprotein. Tyloxapol-treated control group (II) was compared with normal control group (I), while drug and ME treated groups (III-VI) were compared with Tyloxapol-treated control group (II). **P < 0.01 and ***P < 0.001.

The simvastatin caused prominent (P<0.01) reduction in the level of TC, TG, LDL and VLDL-cholesterol levels and prominently (P<0.01) increased in the level of HDL-cholesterol level compared to Tyloxapol-treated control group. The reduction for TC, TG, LDL and VLDL was 29.64%, 32.73%, 52.32% and 32.73% respectively. The increment for HDL was 28.66%.

The current scheme of study was planned to evaluate the antihyperlipidemic effect of MEHT at dose levels ranging from 250mg to 750mg/kg body weight in Tyloxapol induced hyperlipidemia in rats and its possible mechanism(s) of action. Tyloxapol has been widely used for a number of aims particularly, it has been used for screening natural and chemicals hypolipidemic drugs because it is convenient in terms of length of treatment period and handling and also studying lipid metabolism and for investigating interrelationship between plasma lipoproteins (Kellner *et al.*, 1951, Banerjee *et al.*, 2006).

Tyloxapol induced hyperlipidemia is known to be bi-phasic: The first phase is thought to be due to elevated hepatic cholesterol biosynthesis through interference of Tyloxapol with the tissue uptake of plasma lipid in which Tyloxapol acts as surfactant which blocks the uptake of lipoproteins from the blood circulation by extra hepatic tissues, as a result the level of circulatory lipoproteins is increased (Holmes, 1964, Tamasi *et al.*, 1968, Schurr *et al.*, 1972) while the second phase involves interference of Tyloxapol with the excretion and metabolism of cholesterol (Garattini *et al.*, 1959, Raj *et al.*, 2014). Moreover, Tyloxapol physically raises VLDL-cholesterol level by rendering them refractive to the metabolic action of plasma and tissue lipolytic enzymes, hence preventing or delaying their plasma clearance (Friedman and Byers, 1957). Therefore, the overall effect of the Tyloxapol is elevated hepatic triglyceride (TG) and cholesterol biosynthesis which in turn result in increased plasma level of TG, TC, LDL and VLDL while causing the reverse (decrease) in the plasma level of HDL (Banerjee *et al.*, 2006).

The current studies reflect that MEHT decreased TC, TG, LDL and VLDL prominently at dose levels of 250mg, 500mg and 750mg/kg body weight in Tyloxapol induced hyperlipidemia. The large increase in the plasma level of cholesterol and triglyceride due to Tyloxapol treatment results from an increase of VLDL secretion by the liver, accompanied by a strong reduction of VLDL and LDL catabolism (Otway and Robinson, 1967). In the current investigation the reduction of plasma total cholesterol level in response to administration of MEHT was associated with a prominent decrease in LDL fraction, which, in fact, is the goal of several hypolipidemic drugs. This result suggests that antihyperlipidemic activity of MEHT, can results from the rapid catabolism LDL through its hepatic receptors for its final elimination in the form of bile acids (Khanna *et al.*, 2002).

It is an established fact that HDL-C levels have a protective role in cardiovascular disease. Similarly, an increased risk for the development of atherosclerosis is strongly associated with increased level of LDL (Wilson *et al.*, 1988). The increased level of HDL and decreased level of cholesterol along with its LDL fraction which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption from gut. *H. tetrasperma* contains flavonoids, saponin, tannins, and phenolic compounds (Salman *et al.*, 2015, Ajaib *et al.*, 2016) which could be attributed to

hypolipidemic activity of *H. tetrasperma*. These compounds are known to exhibit hypolipidemic activity (Mukherjee, 2003).

Conclusion

The study in reference reflects that prominent attenuation in the level of lipid profile towards the control level resulted by MEHT in Tyloxapol induced hyperlipidemia in rats, which supports the antihyperlipidemic effect of the extract. The effect was comparable to the simvastatin. The result strongly suggests that the antihyperlipidemic activity of MEHT could be attributed to the presence of valuable phytoconstituents such as flavonoids and saponins in the extract. The results are encouraging enough for further studies with respect to the exact mechanism(s) of action, the role of specific extract's component as well as bioassay-guided isolation of the pharmacologically active phytochemicals of the plant.

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