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ANTIHYPERLIPIDEMIC EFFECT OF REOSTO, A HERBOMINERAL FORMULATION IN TRITON INDUCED HYPERLIPIDAEMIC RATS

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Abbreviations:

CG- Control Group; FF- Fenofibrate; HDL-C- High Density Lipoprotein Cholesterol; HG- Hyperlipidemic Group; LDL-C- Low Density Lipoprotein Cholesterol; TG- Triglycerides; VLDL- Very Low Density Lipoprotein

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ABSTRACT

In the present study, an aqueous extract from a marketed mineralo-herbal preparation Reosto was evaluated for its hypocholesterolaemic and hypertriglyceridemic activities using Triton WR-1339 induced hyper-lipidemic rats as experimental model. Hyperlipidemia was developed by intraperitoneal injection of Triton (200 mg/kg body weight). The animals were divided into control (CG), hyperlipidemic (HG), hyperlipidemic plus Reosto extract (200 mg/kg body weight) (HG + ReA), hyperlipidemic plus Reosto extract (400 mg/kg body weight) (HG + ReB) and hyperlipidemic plus Fenofibrate (HG + FF) treated groups. Intragastric administration of Reosto extract to the rats caused a significant decrease on their plasma lipid levels (quantified using enzymatic kits). In Fenofibrate treated rats, lipid parameters were significantly changed indicating that this aqueous extract may contain products that lower plasma lipid concentrations and may be beneficial in treatment of hyperlipidemia.

INTRODUCTION: Human health is of prime importance for a country's development and progress. Herbal preparations/medicaments have been used since ancient times in many parts of the world including India. In recent years, their use as a popular alternative to modern medicine has increased considerably even in developed countries [1-2]. Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic lesions [3]. The beneficial effect of lowering elevated serum cholesterol level in the prevention of coronary heart disease is well established [4, 5]. Generally the therapeutic purpose of using hypolipidemic drugs is to reduce the elevated levels of plasma lipids, notably cholesterol [6].

In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual. Various herbal formulations such as Diamed, Coagent db, Diasulin, Hyponidd and Garlip are well known for their effects [7].

The Reosto is a herbomineral preparation which contains *Terminalia arjuna, Withania somnifera, Commiphora wightii, Sida cordifolia*, *Vanda roxburghii*, Godanti bhasma, and Kukkutandatvak bhasma that slows bone loss and strengthens bone [8]. Some of its constituents are known medicines and reported as atherosclerosis, hypercholesterolemia, rheumatism, and obesity (*Commiphora wightii*) [9]; antioxidant, immunomodulatory, hypocholesteremic (Withania

somnifera) [10], cardioprotective, ischaemic heart diseases, hypocholesterolaemic and antioxidant (*Terminalia arjuna*) [11].

With this background, the present investigation was undertaken to study the effect of the potential herbomineral formulation, Reosto on tissue lipid profile in triton induced hyperlipidemic rats as experimental model. The effects produced by this drug on different parameters were compared with Fenofibrate, a reference drug.

MATERIAL AND METHODS:

Animals: Male Wistar albino rats (weighing 160-180g) were procured from the Animal house, under standard environmental conditions (12 h light/dark cycles at 25-28°C, 60-80% RH) and kept in clean and dry cages and maintained in well-ventilated animal house. Animals were divided into 5 groups of five each and were fed with standard diet and water *ad libitum*. All studies were conducted in accordance with the CPCSEA guidelines with due permission from institutional animal ethical committee (Registration No. 778/03/C/CPCSEA).

Preparation of drug: Reosto was suspended in 10 ml distilled water kept for 3-4 hours at room temperature with intermittent shaking. The contents were centrifuged at 3000 rpm for 5 minutes to remove the particulate matter, supernatant was then collected and filter sterilized. The suspension was given orally with the help of intragastric tube to animals. The required amount of the suspension was made fresh daily.

Chemicals: Triton WR 1339 (isooctyl-polyoxy- ethylene phenol) was obtained from Sigma Chemicals Company Inc., St. Louis, Mo, and USA. Diagnostic kits for estimation of cholesterol, triglyceride and high density lipoprotein (Accurex Biomedical) were purchased from Science House, Bhopal, India. Fenofibrate (Cipla Ltd.) was procured from local medical store. All other chemicals of analytical reagent grade were used.

Drug administration: Reosto was suspended in distilled water and administered orally through intragastric tube at the following doses of 200 and 400 mg/kg body weight.

Triton WR-1339-induced Hyperlipidemia: Rats were made hyperlipidemic by single administration of Triton WR-1339 dissolved in dose of 200 mg/kg in normal saline and gavaged with distilled water.

Experimental protocol: Overnight fast rats were divided into five groups of five rats each. The first group served as control (CG), received intraperitoneal administration of normal saline and water by gavage the second, hyperlipidemic group (HG), was treated with intraperitoneal injection of Triton WR-1339 at a dose of 200 mg/kg in normal saline and gavaged with distilled water; in the third group (HG + ReA), the animals were also treated with intraperitoneal injection of Triton (200 mg/kg BW) followed by intragastric administration of Reosto extract (200mg/kg); the forth group in (HG +ReB), the animals were also treated with intraperitoneal injection of Triton (200 mg/kg BW) followed by intragastric administration of Reosto extract (400mg/kg); (HG + FF) received intraperitoneal injection of Triton (200 mg/kg) plus fenofibrate (65 mg/kg BW) by gavage.

In the following period of study (24 h), animals had access only to water. After 24 h from treatments, animals were anaesthetized with diethyl ether and blood was withdrawn from the plexus of eye vein using heparinized capillary. The blood samples were immediately centrifuged (2500 rpm/10 min) and plasma used for lipid analysis.

Estimation of Serum Lipid Profile:

Estimation of Serum Total Cholesterol (TC): CHOD-POD method [12] was used for the estimation of serum cholesterol. For this method the following were pipetted into the reaction vessels using a micropipette. Test sample (T): 0.01 ml serum, 1.00 ml reaction solution; the standard sample (S): 0.01 ml standard and 1.00 ml reaction solution, while for the blank sample (B): 0.01 ml DW and 1.00 ml reaction solution. The mixture were mixed well and incubated for 5 min at 37 ◦C. The absorbance was read at 510 nm against the reagent blank in Biochemistry Auto analyzer (STAR 21 plus, RAPID Diagnostic Pvt. Ltd.).

Estimation of Serum Triglycerides (TG): GPO-POD method [13] was used to estimate the serum triglycerides. For this 0.01 ml of serum were taken in a test tube (T) in which 1ml reaction solution were

added. In an another test tube (S) 0.01 ml standard and 1ml reaction solution were added. The solutions were mixed well and incubated at 37°C for 10 min. The absorbance of the sample and standard were measured against reaction solution of same amount within 30 min at 510 nm in Biochemistry Auto analyzer (STAR 21 plus, RAPID Diagnostic Pvt. Ltd.).

Estimation of HDL-cholesterol: Phosphotungstate method [12] was used to estimate the serum HDL cholesterol level. For this 0.5ml of serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaked thoroughly and left to stand for 10 min at 25 to 30 °C and then centrifuged for 10 min at 4000 rpm. Within 2 h after centrifugation, the clear supernatant was used for the determination of HDL-C. 0.05 ml of the supernatant was taken in a test tube (T) and 1ml reaction solution was added to it. In another test tube 0.1 ml DW was taken and 1ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10 min at 15-25^{o}C or for 5 min at 37^{o}C and measured the absorbance of the sample against reagent blank at 510 nm in Biochemistry Auto analyzer (STAR 21 plus, RAPID Diagnostic Pvt. Ltd.).

Estimation of LDL cholesterol: LDL cholesterol was estimated by using Friedwald's [14] formula as follows:

LDL in mg% = total cholesterol − (HDL-C + VLDL)

Estimation of VLDL-Cholesterol: Very Low density lipoprotein was estimated by using Friedwald's [14] formula as follows:

VLDL in mg% = T.G /5

Statistical Analysis: The blood lipid level data of different dose level was tabulated and analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test for comparisons with control. The data are expressed as mean ± standard error mean. The Dunnet's test was employed to analyze the result and values were considered statistically significant when at $p < 0.05$. [15].

RESULTS: There were no differences in weight gain, food intake and water intake pattern in rats between the control and treated groups. There was no mortality in any group during experimental period.

Effect on Serum Total Cholesterol (TC) level: In the group administered with only Triton WR-1339, the TC value gradually increased (**Table 1**) up to 20 post-treatment hours (194.62±0.72 mg/dl). The groups administered with Reosto (200 & 400 mg/kg), and Fenofibrate showed significant decreases in TC compared with the Triton WR-1339 administered group (ReA: 162.7±1.09 mg/dl; ReB: 155.68±0.79 mg/dl; Fenofibrate group: 141.5±0.80 mg/dl) at 24 post-treatment hour.

Effect on Serum Triglycerides (TG) level: In the group administered with only Triton WR-1339, the TG value gradually increased up to 24 post-treatment hours (118.04 ± 0.58 mg/dl). The groups administered with Reosto (200 & 400 mg/kg), and Fenofibrate showed significant decreases in TG compared with the Triton WR-1339 administered group (ReA: 108.94±0.74 mg/dl; ReB: 96.6±0.35 mg/dl; Fenofibrate group: 80.03 ± 0.79 mg/dl) at 24 post-treatment hour. The serum TG levels were decreased significantly ($P < 0.01$) from their control level after treating with Reosto and FF. (Table 1)

TABLE 1: EFFECT OF AQUEOUS EXTRACT OF REOSTO ON CHOLESTEROL, TG, HDL, LDL, VLDL IN SERUM OF CONTROL AND EXPERIMENTAL RATS

Parameter	CG	HG	HG + ReA	HG + ReB	HG + FF
TC	94.02±1.45	194.62±0.72$^{\#\#}$	162.7±1.09**	155.68±0.79**	141.5±0.80**
TG	73.64±0.81	118.04±0.58$^{\#\#}$	108.94±0.74**	96.6±0.35**	80.03±0.79**
HDL	28.72±0.32	23.74±0.45$^{\#\#}$	24.05±0.28^{NS}	25.82±0.36**	30.15±0.42**
LDL	50.57±1.45	147.27±0.45$^{\#\#}$	116.86±1.27**	110.54±0.92**	95.23±0.70**
VLDL	14.73±0.16	23.61±0.12$^{\#\#}$	21.79±0.15**	19.32±0.07**	16±0.16**

Values are mean ± S.E.M, n=6. CG: Where, control group; HG: hyperlipidemic group; HG + ReA: hyperlipidemic group + Reosto 200 mg/kg extract; HG + ReB: hyperlipidemic group + Reosto 400 mg/kg extract; HG+ FF: hyperlipidemic group + fenofibrate. TC: total cholesterol; TG: triglycerides. Hyperlipidemic group is compared with control group. HG + ReA, HG + ReB and HG + FF are compared with HG.
NS: no significant. ** $p < 0.01$, ## $p < 0.01$.

Effect on Serum High Density Lipoprotein (HDL) level: The HDL value increased in Reosto and FF group at 24 post-treatment hour (Triton only: 23.74 ± 0.45 mg/dl; ReA: 24.05±0.28 mg/dl; ReB: 25.82±0.36 mg/dl; Fenofibrate group: 30.15 ± 0.42 mg/dl). Initial TC/HDL ratio was not different among groups, but significantly decreased in ReB and FF groups, compared with Triton WR-1339-treated group from post-treatment hours (post-treatment hour: Triton only TC/HDL ratio: 8.2; ReA: 6.77; ReB: 6.03 mg/dl; Fenofibrate group: 4.69). (Table 1-2)

Effect on Serum Low Density Lipoprotein (LDL): Table 1 show that lowering of LDL was significant ($P < 0.01$) in case of Reosto and FF Groups at the end of the experiment (Triton only: 147.27±0.45 mg/dl; ReA: 116.86±1.27mg/dl; ReB: 110.54±0.92 mg/dl; Fenofibrate group: 95.23±0.70 mg/dl). As summarized in table. 2 initial LDL/HDL ratio was not different among groups, but significantly decreased in Reosto and FF groups, compared with Triton WR-1339-treated group from post-treatment hours (post-treatment hour: Triton only LDL/HDL ratio: 6.2; ReA: 4.86; ReB: 4.28 mg/dl; Fenofibrate group: 3.16).

Effect on Serum Very Low Density Lipoprotein (VLDL): VLDL after administration of triton increased significantly compares to control group. The lowering of VLDL was significant ($P < 0.01$) in case of Reosto and FF Groups at the end of the experiment. (Triton: 23.61±0.12 mg/dl; ReA: 21.79±0.15 mg/dl; ReB: 19.32±0.07 mg/dl; Fenofibrate group: 16±0.16 mg/dl). (Table 1-2)

RESULTS & DISCUSSION: Triton WR-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [16]. This model is widely used for a number of different aims [17-18] and, in particular, in rats it has been used for screening natural or chemical hypolipidemic drugs [19] because it is convenient in terms of length of treatment period and handling. In this aim, many plants such as *Vaccinum myrtillus* [20] and *Phyllanthus niruri* [21] have been investigated for their acute hypolipidemic activity in Triton WR-1339 induced hyperlipidemic animals. Schurr *et al*., (1972) demonstrated that a parenteral administration of a dose of Triton WR-1339 to adult rats induced hyperlipidemia. The maximum plasma triglycerides and total cholesterol were reached at 20 h, followed by a decline to normal values [19]. Similar results were described by Lauk *et al*., (1989) and by Khanna *et al.,* (1992) when investigating with the same model, the hypolipidemic effect of *Mucuna pruriens* and *Achyranthus aspera*, respectively [21-22]. Total cholesterol/HDL cholesterol and LDL: HDL cholesterol ratios are also predictors of coronary risk [23].

In this study, these ratios are markedly reduced by Reosto (**Table 2**).

The data presented in this report shows that the extract of Reosto in doses of 200 mg/kg and 400 mg/kg significantly reduced total and LDL cholesterol levels in hyperlipidemic rats. At 400 mg/kg it also reduced total HDL and LDL: HDL ratios. Our study is unique as we used two dosage regimens. It is seen that while both the dosage forms were effective in decreasing VLDL, total and LDL cholesterol, the TC: HDL and LDL: HDL ratios are influenced by higher dose of 400 mg/kg only (Table 2). The results found demonstrate that the bioactive compound(s) contained in this plant have a polar character since they are soluble in water. This finding is in agreement with previous reports showing that plant methanol, ethyl acetate and water soluble extracts possess cholesterol-suppressive capacities and ability to attenuate the accelerated development of atherosclerosis in hypercholesterolemia models.

On the basis of above results, it could be concluded that Reosto, a combination of herbal plants exert a significant antihyperlipidemic effect. This could be due to different types of active principles, each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present armamentarium of Antihyperlipidemic drug. Hence the antihyperlipidemic effect of Reosto in particular could be considered as of possible therapeutic value.

TABLE 2: RATIO OF THE MEAN VALUES OF THE DIFFERENT LIPID FRACTIONS IN CONTROL AND EXPERIMENTAL RATS IN EXPERIMENTATION

Lipid	CG	HG	HG + ReA	HG + ReB	HG + FF
TC: HDL	3.27	8.20	6.77	6.03	4.69
LDL: HDL	1.76	6.20	4.86	4.28	3.16

CG: control group; HG: hyperlipidemic group; HG + ReA: hyperlipidemic group + Reosto 200 mg/kg extract; HG + ReB: hyperlipidemic group + Reosto 400 mg/kg extract; HG + FF: hyperlipidemic group + fenofibrate. TC: total cholesterol; LDL: low density lipoprotein and HDL: high density lipoprotein.

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