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RESEARCH ARTICLE

Anti-Atherosclerosis Activity of Seed oil of *Punica Granatum* Linn in Triton X-100 induced hyperlipidemic rats.

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Abstract

The present study is designed to evaluate the effect of pomegranate seeds oil on lipid profiles in Triton X-100 induced hyperlipidemia in ratus norvegicus rats. The solution of triton-X 100 has successfully been used to induce hyperlipidemia in rats in triton X-100 induced hyperlipidemic model. Treatment with pomegranate seeds oil at a 750 mg/kg dose significantly reduced the serum TC, TG, & LDL-C levels & increased the serum HDL-C levels when compared to hyperlipidemic control. It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. Increased levels of HDL after administration of pomegranate seeds oil concluded that the extract is a potent cardio-protective agent & this effect due to super CLA i.e. Punicic acid present in pomegranate seeds oil. Pomegranate seeds oil contains about 70-75 % punicic acid.

INTRODUCTION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery disease, & cerebral vascular disease. It is defined as an elevation of one or more plasma lipids, including cholesterol, triglycerides & phospholipids. Triton X-100 acts as surfactant & suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra-hepatic tissue resulting in to increased blood lipid concentration.

Pomegranate is used as fruit & medicinal shrub. Treatment with pomegranate seeds oil at 750 mg/kg dose significantly reduced the serum TC, TG, & LDL-C levels & increased the serum HDL-C levels when compared to hyperlipidemic control.

Material & methods:

The fresh fruits of *Punica granatum* Linn (pomegranate) were obtained from the local market in Pune, India. Seeds are separated out, washed carefully 3 to 4 times with water to remove sugars and other adhering material. Separated seeds were dried at 35-37 ° C until a constant weight was reached. Dried pomegranate seeds were pulverized & particles with size distribution of less than 40 meshe were used for the extraction. Fifty grams of the crushed seeds were refluxed using two different organic solvents (Benzene & hexane: 255 ml) in a soxhlet apparatus. The solvents were then evaporated in a vacuum oven at 35 ° C. The obtained PSO stored at 20 ° C. The extraction yield of pomegranate seeds oil was 19.542 % w/w. (Abbasi, H., *et al 2008*)

Pomegranate fruits are purches from local market, Pune and authenticated by Mr. T. Chakraborty (Scientist-D For joint director) Botanical Survey of India, Pune as *Punica granatum* Linn (Family- Punicaceae) with a voucher specimen no: **RASUKA 1.** Despite the availability of literature on the medicinal properties of pomegranate seed oil & its chemical constituents, no reports exit on its hypolipidemic activity. We, therefore attempt to investigate the effect of PSO on the lipid in rats.

Chemicals:

All chemicals used were analytical grade from SD fine chem., India. Cholesterol kit (Enzymatic method), Triglyceride kit & HDL- C kit were procured from Med source Ozone Biomedicals Pvt. Ltd Company, India.

Animals & experimental design:

Ratus norvegicus rats of either sex weighing 150-200 gm inbreed in Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune.

They were kept in departmental animal house in well cross ventilated room at 27±2 ◦C, light and dark cycles of 12 h, respectively, for 1 week before and during the experiments. All the animals of either sex were housed in groups of five under standard laboratory conditions with free access to standard pellet diet and water ad libitum. All experiments were conducted in accordance with guidelines of local animal ethical committee (DYPIPSR/ IAEC/P-14).

Anti-hyperlipidemic studies: (Venkatesham, A., *et al*, 2009; Ansarullah, R. N., *et al*, 2009; Vogal H.G., *et al*, 2002)

The rats were divided into five groups of six rats in each group & were treated with single dose/ day (p.o.) of standard drug or test drug (PSO).

Group – I: Normal control.
Group – II: Hyperlipidemic control [triton X- 100 (100 mg / kg)] i.p.
Group –III: Standard (Atorvastatin 10 mg / kg) p.o. (Sahu, D. R., *et al*, 2000)
Group – IV: Test 1 (250 mg / kg) p.o. (Meerts, I. A. T. M., *et al*, 2009)
Group – V: Test 2 (500 mg / kg) p.o.
Group – VI: Test 3 (750 mg / kg) p.o.

Hyperlipidemia was induced by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/ kg) in physiological solution after overnight fasting for 18 hrs.

This study was carried out for 7 days & the protocol of the present study was carried out for 7 days 7 protocol of the present study was approved by the Institutional Animal Ethics Committee.

Collection of blood samples:

After 8th day of treatment (after 18 hrs injection of triton X- 100), the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. Serum obtained by immediate centrifugation of blood samples using ultra cooling centrifuge at 3000 rpm for 15 min at room temperature. Plasma was quantified using enzymatic kit.

Biochemical analysis:

Plasma lipids levels include TC, TG & HDL-C was carried out using respective diagnostic commercial kits from Med source Ozone Biomedicals Pvt. Ltd Company. According to that result LDL-C, VLDL-C & atherogenic index were calculated by using following formulas:

For **LDL-C**: (Rajani, G.P., *et al*, 2009)
Formula-
LDL = TC- HDL - Triglyceride/5.
For **VLDL-C:**
Formula-
VLDL= Triglycerides / 5
For **Atherogenic index:**
Formula-
Total cholesterol / HDL cholesterol.

Statistical analysis:

The results were presented as mean ± S.E.M. All the groups are compared with control. Statistical analysis was performed by one way ANOVA followed by Dennett's multiple comparison using INTA software. The level of significance was set as $p < 0.05$ and $p < 0.01$.

RESULTS

Synthetic chemicals have potential effect on biological system but prolonged use may cause many adverse reactions. Considering the fact, people are looking for biofriendly drugs, which may be safe and effective. As reported earlier, Injection of Triton X-100 has successfully induced hyperlipidemia in rats by increasing the serum TC, TG & LDL-C levels. Triton X-100 acts as surfactant & suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissue resulting in to increased blood lipid concentration. Induction of hyperlipidemia with triton X-100: the levels of plasma total cholesterol, triglycerides, phospholipids, HDL-C, assayed after respective treatment. Treatment with pomegranate seed oil at the doses of test 1, test 2, test 3 i.e. (250 mg, 500 mg, 750 mg / kg) reduced the serum TC, TG & LDL-C levels & increased the serum HDL-C levels when compared to the hyperlipidemic (HL) control group. It is widely accepted that reduction in plasma HDL-C levels is a risk factor for developing atherosclerosis. The change in lipid levels in groups of test 1, test 2, and test 3 were comparable with group of atorvastatin treated rats. Among three fractions, test 3 reduced the elevated lipid levels more significantly than the others.

Hyperlipidemia is the major cause of atherosclerosis. Hyperlipidemia is define as an elevation of one or more of the plasma lipids, including cholesterol, cholesterol esters, triglyceride, & phospholipids. From these investigations, it may be concluded that the studies have shown an inverse correlation between consumption of CLA & cardiovascular events. Pomegranate seeds oil which is rich in Punicic acid significantly reduced oxidative stress by inhibiting the formation of oxidized LDL lipoproteins & macrophage lipid peroxidation & this mechanism of hyperlipidemia is reduced. Also it decreases LDL susceptibility to aggregation & retention, increase the activity of serum paraxonase a HDL associated esterase that can protect against lipid peroxidation by 20 %.

The results of present study with *Punica granatum* Linn have good antihyperlipidemic activity due to presence of the Poly Unsaturated Fatty Acid in seed oil.

Table 1: Effect of pomegranate seed oil on serum lipid levels in triton induced hyperlipidemic rats

Groups	Control	HD	Standard	Test 1	Test 2	Test 3
TC (mg/dl)	83.38± 0.047	158.90 ± 0.25	111.47± 0.35 **	129.62± 0.26 **	121.89 ± 0.30 **	117.08 ± 0.39 **
TG (mg/dl)	73.25± 0.029	140.03 0.10	92.13 ± 0.38 **	116.96 ± 0.54 **	103.24 ± 0.31 **	98.13± 0.28 **
HDL (mg/dl)	39.20± 0.11	35.33 ± 0.20	54.71± 0.30 **	43.39± 0.095 **	46.61± 1.68 **	50.66 ± 0.14 **
LDL (mg/dl)	29.53± 0.10	95.55 ± 0.22	36.99 ± 1.42 **	62.75 ± 0.31 **	54.62± 1.04 **	46.76± 0.33 **
VLDL(mg/dl)	14.65± 0.006	28.01 ± 0.019	18.43 ± 0.076 **	23.39± 0.076 **	20.64 ± 0.06 **	19.63 ± 0.056 **
A.I.	2.07	4.49	2.03 **	2.98 **	2.61 **	2.31 **

Standard: Atorvastatin (10 mg / kg) p.o.

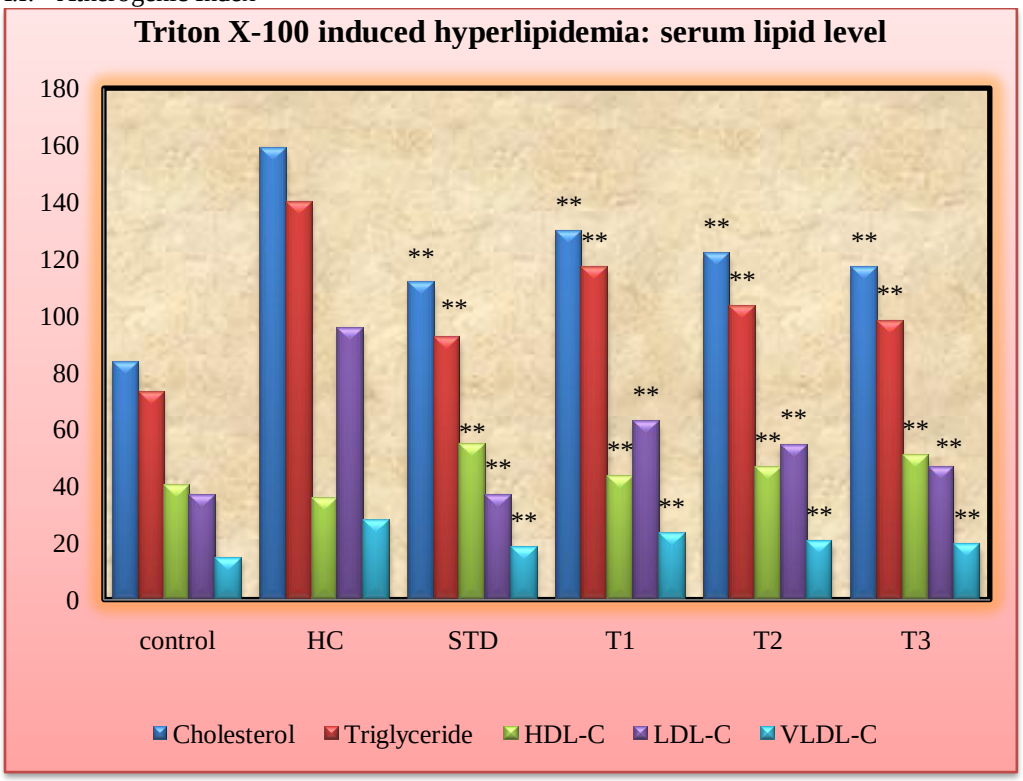
Test 1: Pomegranate seeds oil (250 mg / kg) p.o.

Test 2: Pomegranate seeds oil (500 mg / kg) p.o.

Test 3: Pomegranate seeds oil (750 mg / kg) p.o

Abbreviations:
TC- Total Cholesterol
TG- Triglyceride
LDL- Low Density Lipoprotein
HDL- High Density Lipoprotein

VLDL- Very Low Density Lipoprotein
A.I. - Atherogenic Index



Graph 1:

Effect of triton X-100 on blood serum lipid level of rat
Where, N=6.
Values are expressed as Mean ± SEM = Mean ± Std. error of mean.
*p ≤ 0.05
**p ≤ 0.01
***p ≤ 0.001
Hyperlipidemic control: Triton X- 100 (100 mg / kg) i.p.

Standard: Atorvastatin (10 mg / kg) p.o.

Test 1: Pomegranate seeds oil (250 mg / kg) p.o.

Test 2: Pomegranate seeds oil (500 mg / kg) p.o.

Test 3: Pomegranate seeds oil (750 mg / kg) p.o

Abbreviations:
TC- Total Cholesterol
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LDL- Low Density Lipoprotein
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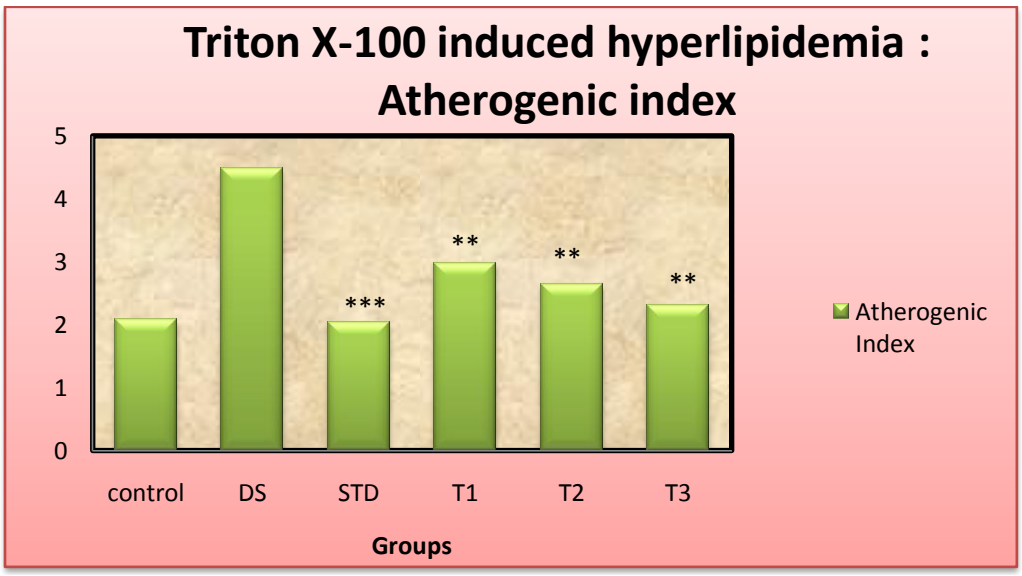

Graph 2: Atherogenic index of serum lipid level in triton X-100 induced hyperlipidemia

Discussion:

Triton X-100 acts as surfactant & suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissue resulting in to increased blood lipid concentration. Induction of hyperlipidemia with triton X-100: the levels of plasma total cholesterol, triglycerides, phospholipids, HDL-C, assayed after respective treatment. In the PSO treated groups of animals; moderate decrease was seen in the levels of plasma TC, TG, LDL-C, VLDL-C. In present study tritonised animals have been used to test the anti-atherosclerotic & anti hyperlipidemic activity. Pomegranate seeds oil exert significant anti hyperlipidemic effect marked by significantly lower plasma TC & TG levels. The anti hyperlipidemic drugs atorvastatin seems more potent in preventing the elevation in plasma cholesterol levels.(Mohale, D. S.,2008)

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