



Protective effect of methanolic extract of *Trianthema portulacastrum* in atherosclerotic diet induced renal and hepatic changes in rats

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Abstract

The aim of the present study was to investigate the protective effect of Methanolic Extract of *Trianthema portulacastrum* plant (METP) in atherosclerotic diet induced renal and hepatic changes in rats. Atherosclerotic diet or CCT diet was successfully induces atherosclerosis/hyperlipidemia in rats by elevating the serum lipid levels and also produced glomerulosclerosis / nephropathy and early fatty changes in the liver cells. The treatment with METP extract at doses of 100 & 200 mg/kg, b.w produced a marked reduction in these elevated serum lipid levels and protected against the glomerulosclerosis or fatty changes in the hepatocytes induced by the atherosclerotic diet. The protective role of METP was confirmed by the significant reduction in the elevated serum LDH, AST, ALT, ALP, bilirubin and creatinine levels when compared with CCT diet fed control group and also by the histopathological evaluations of glomeruli, renal tubules and liver sections.

Keywords: *Trianthema portulacastrum*; atherosclerotic diet; CCT diet; glomerulosclerosis; protective effect; AST; ALT.

Introduction

Trianthema portulacastrum Linn is a plant belongs to the family Aizoaceae, found almost throughout the India as a weed in cultivated and wastelands. The plant is bitter and used as analgesic, stomachic, laxative and serves as alterative cure for bronchitis, heart disease, anemia and inflammation. The root applied to the eye to treat corneal ulcers, itching, dimness of sight and night blindness [1]. A decoction of the herb is used as a vermifuge and is useful in rheumatitis. The plant has a remarkable protection against the hepatotoxicity [2] and

hepatocarcinogenesis [3, 4] induced by the various chemical methods. It has also been reported that the atherogenic diet produces foam cells in the blood vessels [5] and high plasma cholesterol concentrations have been associated with liver and renal lesions in the rats. [6]. In the present work, an atherogenic diet protocol has been followed for the two weeks, to study the detrimental changes in the liver and kidney tissues in experimental hypercholesterolemic rats. The present study was investigated the protective effect of methanolic extract of *Trianthema portulacastrum* in atherosclerotic diet induced renal and hepatic changes in rats.

Materials and Methods

Experimental

Extraction of plant material

The plant *Trianthema portulacastrum* was obtained from the Government Siddha Medical College, Chennai (Tamil Nadu), India. The plant was taxonomically identified and authenticated as *Trianthema portulacastrum* Linn. by Prof. Chelladurai, Research Botanist, Palayamkottai, Tamil Nadu, India. The whole plant was dried under shade and ground to a fine powder in a mechanical blender. The powdered plant material was defatted with petroleum ether (60-80⁰) and then extracted with ethanol (95%) in a *Soxhlet apparatus*. The solvent was removed under reduced pressure to yield a greenish-black sticky residue. (Yield: 4.5%w/w with respect to dried plant material) and this methanolic extract was stored at 2-8°C and used for subsequent experiments.

Photochemical screening

The methanolic extract was screened for the presence of various phyto-constituents like steroids, alkaloids, terpenoids, flavonoids, phenolic compounds and carbohydrates [7].

Chemicals

Atorvastatin pure drug was a kind gift from Dr. Reddy's Pharmaceutical Ltd, Hyderabad. All other chemical used were analytical grade from SD fine chemicals, India. Cholesterol kit (Enzymatic Method) and Triglycerides kit were procured from Qualigens Diagnostics and E-Merck Limited, Mumbai, India respectively.

Animals

Wistar albino adult male rats weighing 200-250g were selected and housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and 12hrs dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. Before induction of hyperlipidemia, the weight of the individual animals and plasma cholesterol levels were estimated.

The animals were divided into five groups of six rats each. Group I served as the control. Rats in groups II, III, IV and V were fed with an atherogenic diet comprising of the normal rat chow supplemented with 4% cholesterol, 1% cholic acid and 0.5% thiouracil (CCT diet) for 2 weeks. However, the groups III and IV also received METP extract at 100 and 200mg/kg treatment respectively commencing 1 week after the start of the experimental period, whereas group V rats received the standard atorvastatin (20mg/kg). At the end of the 2 week experimental period, the

blood samples were collected and serum was separated for biochemical estimations and enzyme assays. Then the rats were sacrificed by decapitation and the liver, kidney tissues were quickly excised, washed with saline, blotted with a piece of filter paper and a 10% (w/v) buffered homogenate was prepared for biochemical assays. Sections of liver and kidney tissues were set aside for histological processing. This study was carried out for 14 days and the protocol of the present study was approved by the Ethics Committee of Rajah Muthiah Medical College and Hospital (RMMCH), Annamalai University, Tamil Nadu.

Analysis of serum lipids

Serum total cholesterol and triglycerides levels were estimated [8] using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India.

Enzymic indices of cellular integrity

Activities of lactate dehydrogenase (LDH), aminotransferases (aspartate and alanine transaminases, AST and ALT respectively), alkaline phosphatase [9 a, b, c], creatinine [10] and albumin [11] were assayed in the serum.

Histopathological studies

A portion of the liver and kidney tissue were fixed in 10% formalin immediately after scarification of rats. The washed tissue was dehydrated in the descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax and thin sections were made, stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopathological changes.

Statistical Analysis

The results were expressed as mean \pm SD. Statistical analysis were carried out using Paired t-test and one-way ANOVA followed by Bonferroni's test. Differences below $P < 0.05$ implied statistically significance.

Results and Discussion

In the present study, CCT diet feeding for 2 weeks was chosen as the experimental model of early phase atherogenesis. The role of METP extract in combating the hepatic and renal aberrations accompanying diet-induced hypercholesterolemia has also been investigated here.

Figure.1 shows the abnormally increased serum cholesterol and triglyceride concentrations in the CCT diet fed rats and the normalizing effect of METP. Total cholesterol and triglycerides concentrations in METP treated groups of III and IV was significantly reduced when compared with the untreated group II ($p < 0.001$).

Table. 1 presents the changes in serum biochemical variables in the atherogenic diet fed rats and the protective role of METP. Lowered serum albumin concentrations in the CCT diet fed rats ($p < 0.01$) may imply hepatic damage as well as abnormal renal glomerular function as a result of severe hyperlipidemia induced by the diet.

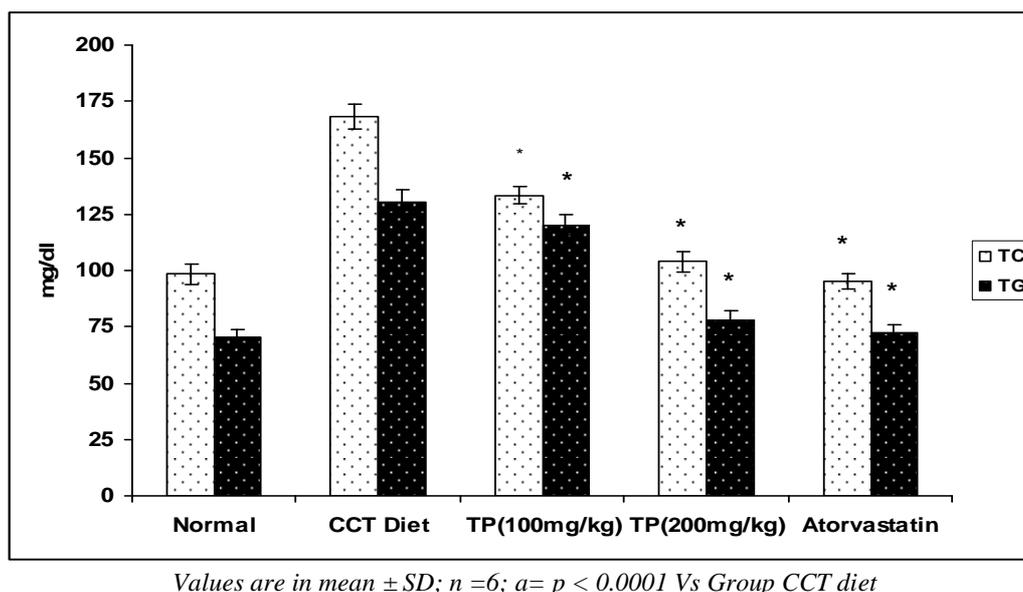


Figure 1: Effect of methanolic extract of TP on serum lipid profile in CCT fed rats

Further, increased serum creatinine concentrations ($p < 0.01$) may serve to indicate developing glomerulopathy. Group III, IV and V shows the concentrations of these parameters to be comparable with the control group. Table 1 also reveals the abnormal concentrations of serum enzymes that indicate cellular damage caused by the atherogenic diet. Increase in serum LDH, AST and ALT was recorded in group II against the controls; however, treatment with METP reversed these values to normal ($p < 0.01$).

Table 1: Effect of methanolic extract of TP on serum biochemical parameters in rats fed atherogenic diet (compared with control groups)

Group	LDH	AST	ALT	ALP	Creatinine	Albumin
Normal	6.2 \pm 1.2	1.1 \pm 0.3	1.3 \pm 0.04	2.3 \pm 0.9	1.1 \pm 0.08	3.3 \pm 1.1
CCT Diet	12.1 \pm 2.6	3.1 \pm 1.1	3.8 \pm 0.2	4.1 \pm 1.2	1.5 \pm 0.09	2.1 \pm 0.8
TP(100mg/kg)	9.4 \pm 2.4 ^a	2.6 \pm 0.9 ^a	2.9 \pm 0.9 ^a	3.2 \pm 1.1 ^a	1.4 \pm 0.07 ^a	2.5 \pm 0.9 ^a
TP(200mg/kg)	8.3 \pm 1.9 ^a	2.2 \pm 1.1 ^a	2.4 \pm 0.8 ^a	2.8 \pm 1.0 ^a	1.3 \pm 0.05 ^a	2.8 \pm 1.1 ^a
Atorvastatin	7.4 \pm 1.6 ^a	1.6 \pm 0.7 ^a	1.9 \pm 0.3 ^a	2.5 \pm 1.3 ^a	1.2 \pm 0.03	3.01 \pm 0.8 ^a

Values are in mean \pm SD; n = 6; a = p < 0.01 Vs Group CCT diet

Fig. 2(A) and (C) shows glomerulosclerosis / nephropathy and mild tubular damage induced respectively by the untreated CCT diet fed group II, while Fig. 2(B) and (D) presents normal renal histology corresponding to the METP treated groups. Rats maintained on CCT atherogenic diet for 2 weeks has been reported to result in significant mononuclear cell adhesion to the vessel wall, followed by their emigration into the intima where they accumulate lipid and become foam cells [12]. In the present study, we observed substantial protection rendered by METP against the renal changes in early stages of atherogenesis. It has been suggested that diet-induced hypercholesterolemia leads to changes in both the endothelium and circulating monocytes that precipitate these early events of lesion formation [13].

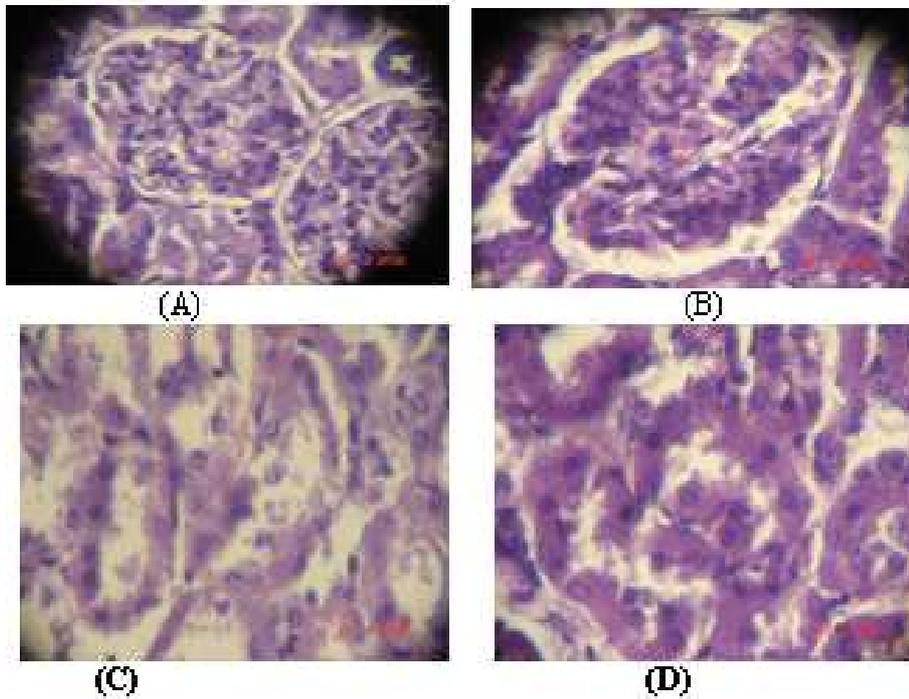


Figure 2: (A)- shows the glomerulosclerosis / nephropathy induced by CCT Diet; (B)- METP treatment shows almost normal glomeruli; (C)-shows the mild renal tubular damage in CCT diet fed group; (D)-shows near normal renal histology in METP treated group.

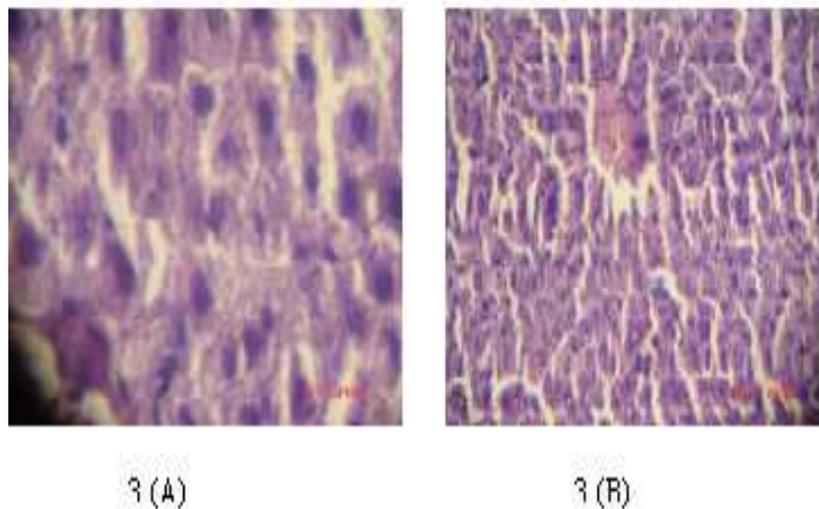


Figure.3 (A) liver sections shows the fatty changes induced by the atherogenic diet in group II and fig. 3(B) depicts a more or less normal hepatic architecture with the parenchyma structure preserved and occasional fat cells in the METP treated CCT diet fed groups.

In recent times, there has been renewed interest in the toxic potential of lipids for both the glomeruli and tubules of the kidney. It has been noted that the hallmark histological lesions in the kidney in chronic renal failure, namely glomerulosclerosis has many analogies to atherosclerosis. There is experimental evidence that an increase in dietary cholesterol favors the development of glomerulosclerosis in rats [6]. The glomerulus has many structural features that resemble arteries commonly involved in atherosclerosis. Glomerular mesangial cells are structurally similar to arterial smooth muscle cells, known to be important in the pathogenesis of atherosclerosis. Lipid-laden macrophages or foam cells frequently found in early atherosclerotic lesions are also found in glomeruli of human and experimental focal glomerulosclerosis [14]. These inflammatory cells are early and significant components in the initiation of atherosclerosis. Hyperlipidemia may also induce vascular smooth muscle and mesangial cell proliferation [15].

In conclusion, the methanolic extract of TP produced a protection against CCT diet induced glomerulosclerosis and hepatic damage by reducing serum lipid levels, AST, ALT & creatinine levels in rats.

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