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Anti-Atherosclerotic Activity of Para Methoxy Cinnamic Acid in High Fat Diet Induced Hyperlipidemia Model Rats

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ABSTRACT

Hypercholesterolemia is a well-known etiological feature for cardiovascular diseases and a common indication of maximum categories of metabolic disorders. Para methoxy cinnamic acid is one of the cinnamic acid derivatives as a natural product obtained from the rice bran oil as an active constituent and has the antioxidant property. The present study was designed to evaluate the hypolipedemic activity of p-methoxy cinnamic acid against high fat diet induced hyperlipidemia in experimental rats. Male Wistar albino rats were divided into five groups (n=6) and high fat diet was used to induce the hyperlipidemia for 28 days. P-methoxy cinnamic acid was used in two different doses (40 and 80 mg/kg Body weight) and they were administered orally to the rats for 28 days during high fat diet. Atorvastatin (5 mg/kg) was used as reference standard. A significant elevated level of lipids abnormalities and tissue antioxidant parameters were reversed from normal level by the treatment of P-methoxy cinnamic acid in both the doses. Histopathological evidence further supported the protective action. Based on the initial findings, it was concluded that P-methoxy cinnamic acid was able to offer significant protection against high fat diet induced atherosclerosis. Future studies were recommended to identify the molecular mechanism of P-methoxy cinnamic acid against atherosclerosis protection.

Keywords: Atherosclerosis, Hyperlipidemia, Lipoprotein, Cinnamic acid, Natural products.

INTRODUCTION

High lipid level and oxidative stress are reported to be responsible for the development of atherosclerosis related cardiovascular risk. Hyperlipidemia is most common 'metabolic disorder which is characterized by an increase in the level of cholesterol, low density lipoprotein, triglyceride and low level of high density lipoprotein [1-4].

Hypercholesterolemia and hyperlipidemia have been carefully studied in the development of coronary artery disease. Hypercholesterolemia means a high blood level of cholesterol hastens the oxidation of serum lipids and is known to subsidize to the disturbance of the cardiovascular system homeostasis by a diversity of biological and physical processes. Several factors like genetic disposition, diet practice, insufficient physical activity and obesity influence the development of hypercholesterolemia. As per the previous report major adult populations from developed countries were affected by atherosclerosis disease [5]. PMCA brings about reduction in cholesterol by bringing about its effect on the plasma glucose and insulin concentrations and also bringing about its effect on various types of glucose-reducing enzymes [6]. Medicinal plants are used to treat different types of diseases. Most of the medicinal plants are used for their antioxidant, hypolipidemic and hypoglycemic activity [1]. Cinnamic acid derivatives are naturally occurring substance found in fruits, vegetables, flowers and are utilized as dietary compounds; these play an important role in the development of commercially intermediate molecules which are necessary for the production of different pharmaceutical ingredient.

Cinnamic acid derivatives show diversity of pharmacological activities along with their slighter to moderate side effect. P-methoxy cinnamic acid (PMCA) is one of the cinnamic acid derivatives obtained as a natural product from the rice bran oil as an active constituent having antioxidant property [7].

Earlier, it was stated that PMCA owns antioxidant and antilipidperoxidative effects on 1, 2-dimethylhyrdrazine (DMH)-induced colon carcinogenesis. Anti-inflammatory and anticancer effects of PMCA are also documented [8]. Earlier work from some research group discovered significant hypolipidemic and hypoglycemic effects of PMCA diesters from Carnauba-derived wax powder [9, 10]. It has been observed that PMCA play an important role in the mechanism of reducing cholesterol in comparison to the other derivatives of cinnamic acid [11]. Recently, the hypocholesterolemic effect of PMCA diester was documented by using high fat diet fed mice model also [12]. Hence, the present study was intended to evaluate the hypolipedemic activity of PMCA against high fat diet induced hyperlipidemia in experimental rats.

MATERIALS AND METHODS

Drugs and chemicals

P-Methoxy cinnamic acid was purchased from Sigma Aldrich, Bangalore India. Atorvastatin was obtained from Dr. Reddy's laboratories Hyderabad as a gifted sample. All the biochemical kits were procured from Span diagnostics, Surat, India. All the chemicals were used for this experimental research was research grade and procured from Sigma Aldrich, Bangalore, India.

Experimental animals

Thirty healthy male albino Wistar rats (3 months old) weighing between 180–200 g were used for the study. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding in an air conditioned room and allowed access to pellet diet and water ad libitum (sainath agency, Hyderabad). They were maintained under standard conditions (temperature 22 ± 2 ° C, relative humidity 50 ± 5% and 12 h light/dark cycle). All the animals are acclimatized for seven days before the actual study. The animals were randomized into experimental, normal and control groups (each group with 6 animals). Animals were habituated to laboratory condition for 48 hrs prior to experimental protocol to minimize if any specific stress.

All the studies conducted were approved by the Institutional Animal Ethics Committee with the approval number IAEC/ANCP/2018-19/18.

Induction of atherosclerosis

The research was conducted for 28 days, in which rats n=30 are randomly divided into five groups (6 rat in each group) and fed with modified self-made diet containing 20% ground nut oil, 0.5% cholesterol, 1% cholic acid and followed by treatment with Atorvastatin 5 mg/kg, p-methoxy cinnamic acid (PMCA) 40 and 80 mg/kg (The dose of the PMCA was selected based on previous report by Gunasekaran et al., [13] and periodic blood samplings for the estimation of biochemical parameters.

Treatment protocol:

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days

Group 4: Test group received PMCA 40 mg/kg BW orally, along with the high fat diet daily for 28 days

Group 5: Test group received PMCA 80 mg/kg BW orally, along with the high fat diet daily for 28 days

The blood samples were withdrawn on 0 th, 7 th, 14 th, 21 st and 28 th day from the retro orbital venous plexus of rats deprived of any coagulant for the parting of serum. After collecting the blood in eppendroff tubes kept for 1 hour at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored analyzed for many bio chemical parameters. The rats were sacrificed by an overdose of pentobarbital (60 mg/kg BW i.p) and the liver were removed and weighed from each group.

Biochemical analysis

Biochemical parameter estimated in serum: Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL), and total protein were estimated by using standard research kits according to the manufacturer protocol. The instrument used for the estimation is EPOCH microplate spectrophotometer, BioTek Instruments, USA.

Biochemical parameter estimated in liver homogenate: Liver from experimental animals was homogenized in 0.1 mM phosphate buffer and the homogenate was used to estimate various biochemical parameters as follows.

Estimation of myocardial Thio Barbituric Acid Reactive Substances (TBARS)

TBARS levels in the myocardium were measured using the method described by Ohkawa et al. [14]. Briefly, 0.2 ml of homogenate was pipetted out, followed by the addition of 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA). Boiling of the tubes was done for 60 min at 95 ° C and then was cooled on ice. In the tubes double distilled water (1.0 ml) and 5.0 ml of n-butanol–pyridine (15:1 v/v) mixture were added and centrifuged at 4000 ᵡ g for 10 min.

The absorbance of the colour which was developed in the organic layer was measured at 532 nm. Data are expressed as n mol of TBARS/ g wet weight.

Myocardial reduced glutathione (GSH): Myocardial reduced glutathione (GSH) was estimated by the method of Ellman et al. [15]. Briefly, the reaction mixture contains 0.1 mL of supernatant, 2.0 ml of 0.3 M phosphate buffer (pH - 8.4), 0.4 ml of double-distilled water and 0.5 ml of 5, 5 dithio bis 2-nitrobenzoic acid (DTNB). Incubation of reaction mixture was done for 10 minutes and then absorbance was measured at 412 nm. Data are expressed as mole per gram wet weight.

Myocardial superoxide dismutase (SOD): Superoxide dismutase (SOD) levels in the hearts were determined by the modified method described by Kakkar et al [16]. Briefly, the homogenate (0.6 ml) was added to sodium pyrophosphate buffer (pH -8.3), followed by the addition of 0. 1 ml of 186 M phenazine methosulfate, 0.3 ml of 300 mM nitro blue tetrazolium and 0.2 ml of 780 M NADH. For 90 seconds the reaction mixture was incubated at 30°C and then the reaction was stopped by adding 1.0 ml of acetic acid, further 4.0 ml of n-butanol was added and then the reaction mixture was centrifuged at 3000 ᵡ g for 10 min. The absorbance of the organic layer was measured at 560 nm. Data are expressed as units per mg protein.

Myocardial catalase: Catalase was estimated by the method described by Aebi and Bergmeyer [17]. Briefly, homogenate was added to a 3.0-ml cuvette containing 1.95 ml of 50 mM phosphate buffer (pH 7.0). Then after adding 1.0 ml of 30 mM hydrogen peroxide, changes in absorbance were followed for 30 s at 240 nm at an interval of 15 s. Catalase levels are expressed as units per mg protein.

Protein estimation for the tissue sample of SOD and CAT were done by the method of Bradford, [18]. A sample was added up to 20 µ l with double-distilled water, 50 µ l in sodium hydroxide and 1 ml of Bradford reagent and kept aside for 10 min after vortexing. The absorbance was measured at 595 nm.

Histological examination: Once the rats were sacrificed, the liver tissues were removed, washed instantly with saline, and then fixed at 10% buffered formalin. After fixation, the liver tissues were embedded in paraffin and these paraffin blocks were then cut into 5 µ m thick sections. These sections were stained with hematoxylineosin and then examined under the light microscope for histological changes.

Statistical analysis: All values are expressed as mean ± standard error of the mean (SEM). All the data obtained for various biochemical parameters were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (GraphPad Version 5.0, La Jolla, CA, USA). $P<0.05$ was considered as statistically significant.

RESULTS

Effect of PMCA on Body Weight Changes

There was a significant rise in body weight of the disease control group (Group 2) administered with high fat diet, compared to the normal group (group 1). Also, it was originated that group 3 rats received atorvastatin 5 mg/kg, there was a significant decrease in body weight. Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively) showed significant reduction in body weight compared to disease control group (Group 2) (Table 1).

Treatment Groups	**Body weight (g)**	
	0 day	28th day
Group 1	188 ± 16.47	190 ± 17.42
Group 2	192.6 ± 12.82	268 ± 14.27^{a}
Group 3	190.1 ± 10.39	209.4 ± 15.18^{b}
Group 4	188.4 ± 17.24	214.8 ± 11.58^{b}
Group 5	193 ± 15.94	200 ± 11.26^{b}

Table 1: Effect of PMCA against body weight changes in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); a=$p<0.01$ When compared to normal (Group 1); b=$p<0.05$ When compared to control (Group 2)

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days.

Effect of PMCA on serum lipid levels

Animals administered with high fat diet (group 2) show a significant rise in serum cholesterol on 14th, 21stand 28th day, when compared to normal animals (group 1). This shows that administration of high fat diet induces hyperlipidemia for the present study. Group 3 that received the standard drug (atorvastatin, 5 mg/kg) showed a significant decrease of serum cholesterol on 14th, 21st and 28th day, when compared to high fat diet treated group (group 2). Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively) showed a significant reduction in serum cholesterol on 14th, 21st and 28th day.

There were significant reductions in serum HDL cholesterol levels in the control group (group 2), on 28th day when compared to the normal group (group 1). The group 3 treated with atorvastatin exhibited a significant rise in serum HDL cholesterol levels, when compared to control group (group 2). The effect was significant from 28th day onwards. Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively) showed a significant increase in serum HDL levels when compared with control group (group 2) on the 28th day.

It was detected that there was a significant rise in serum LDL cholesterol levels in rats treated with a high fat diet group (group 2). The group 3 treated with standard drug atorvastatin 5 mg/kg shows a reduction in LDL cholesterol levels on 14th, 21st, 28th day when compared to control group (group 2). Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively) exhibited significant reduction in serum LDL cholesterol levels on 14th , 21st and 28th day in comparison with control group (group 2).

Group 2 received high fat diets displays a significant rise in serum triglyceride levels on 14th, 21st and 28th day, when compared to normal group 1. The group 3 treated with standard drug atorvastatin 5 mg/kg had significantly lowered triglyceride levels on 14th, 21st and 28th day, when compared with the control group. Reduction in serum triglyceride levels was detected in groups 4 and 5 and this decrease was significant on 14th, 21st and 28th day.

Administration of high fat diet in group 2 animals exhibited a significant reduction in total proteins on 14th day onwards when compared to the normal group (group 1). A significant rise in serum total proteins was found in animals treated with atorvastatin from 14th day onwards. Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively) showed a significant rise in serum total proteins on 14th, 21st and 28th day (Table 2).

Treatment Groups	Total cholesterol (mg/dl)				
	0 day	**7th day**	**14th day**	**21st day**	**28th day**
Group 1	216.4 ± 14.34	215.4 ± 12.04	214.8 ± 12.61	215.0 ± 5.06	218.3 ± 12.64
Group 2	215.1 ± 8.28	246.7 ± 8.47	328.0 ± 13.52[a]	349.0 ± 14.28[a]	360.0 ± 8.06[a]
Group 3	218.1 ± 12.63	213.4 ± 9.81	236.0 ± 12.83[b]	262.7 ± 13.53[c]	254.2 ± 12.87[d]
Group 4	217.0 ± 7.04	220.0 ± 12.61	274.0 ± 14.47[b]	268.0 ± 12.61[c]	260.0 ± 11.71[d]
Group 5	216.2 ± 10.49	210.2 ± 14.72	215.8 ± 12.92[b]	257.0 ± 8.04[c]	243.2 ± 12.06[d]

Table 2: Effect of PMCA against Total cholesterol in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6), a=p<0.001 When compared to normal (Group 1); b=p<0.05 When compared to control (Group 2); c=p<0.01 When compared to control (Group 2)

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days (Table 3).

Treatment Groups	HDL cholesterol (mg/dl)				
	0 day	**7th day**	**14th day**	**21st day**	**28th day**
Group 1	48.37 ± 4.92	49.28 ± 3.81	50.00 ± 5.45	50.47 ± 6.14	49.87 ± 5.47
Group 2	36.28 ± 4.83	36.14 ± 3.63	24.18 ± 4.57	29.28 ± 4.74	28.29 ± 0.90[a]
Group 3	42.25 ± 3.44	37.29 ± 4.72	39.14 ± 5.89	38.42 ± 5.91	39.91 ± 2.56[b]
Group 4	49.64 ± 5.49	36.73 ± 4.86	38.26 ± 5.77	39.19 ± 4.80	38.46 ± 3.23[b]
Group 5	42.62 ± 3.17	37.15 ± 4.44	39.28 ± 5.65	40.16 ± 5.30	39.58 ± 7.17[c]

Table 3: Effect of PMCA against HDL cholesterol in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); a=p<0.001 when compared to Group 1 b=p<0.05 when compared to Group 2; c=p<0.01 when compared to Group 2

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days (Table 4).

Treatment Groups	LDL cholesterol (mg/dl)				
	0 day	**7th day**	**14th day**	**21st day**	**28th day**
Group 1	126 ± 14.47	127 ± 14.91	126 ± 14.03	125 ± 15.19	129 ± 14.11
Group 2	138 ± 12.91	194 ± 12.29	257 ± 19.64[a]	271.0 ± 17.08[a]	315 ± 15.9[a]
Group 3	139.6 ± 17.26	152.4 ± 14.83	160.8 ± 14.48[b]	171.4 ± 14.24[d]	175.8 ± 11.32[d]
Group 4	136.4 ± 13.15	165.4 ± 15.61	164.9 ± 14.92[b]	174.8 ± 13.42[c]	187.9 ± 16.31[d]
Group 5	134.8 ± 16.65	148.7 ± 14.87	158.2 ± 12.08[c]	163.9 ± 13.95[d]	182.1 ± 13.90[d]

Table 4: Effect of PMCA against LDL cholesterol in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); a=p<0.001 When compared to normal (Group 1); b=p<0.05 When compared to control (Group 2); c=p<0.01 When compared to control (Group 2); d=p<0.001 When compared to control (Group 2).

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days (Table 5).

Treatment Groups	Triglyceride (mg/dl)				
	0 day	**7th day**	**14th day**	**21st day**	**28th day**
Group 1	116.8 ± 15.35	114.8 ± 12.87	111.0 ± 12.12	112.8 ± 13.19	117.2 ± 13.04
Group 2	118.4 ± 17.61	154.0 ± 15.95	250.0 ± 15.71[a]	271.9 ± 15.71[a]	278.2 ± 14.23[a]
Group 3	118.9 ± 12.15	116.7 ± 13.18	136.7 ± 15.09[b]	148.6 ± 12.18[c]	170.4 ± 17.56[d]
Group 4	115.2 ± 12.24	119.3 ± 16.52	154.7 ± 15.12[b]	167.4 ± 15.44[d]	178.2 ± 13.36[c]
Group 5	113.7 ± 13.40	111.3 ± 12.77	134.2 ± 14.13[c]	142.5 ± 21.57[c]	163.7 ± 16.81[d]

Table 5: Effect of PMCA against Triglyceride in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); a=p<0.001 When compared to normal (Group 1); b=p<0.05 When compared to control (Group 2); c=p<0.01 When compared to control (Group 2); d=p<0.001 When compared to control (Group 2)

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days (Table 6).

Treatment Groups	Total protein (mg/dl)				
	0 day	**7th day**	**14th day**	**21st day**	**28th day**
Group 1	9.4 ± 0.92	9.2 ± 0.49	9.5 ± 0.94	9.1 ± 0.82	9.1 ± 0.45
Group 2	8.4 ± 0.41	6.1 ± 0.47	5.7 ± 0.84[a]	5.3 ± 0.92[a]	4.9. ± 0.67[a]
Group 3	8.8 ± 0.62	8.4 ± 0.28	7.3 ± 0.64[c]	7.1 ± 0.71[c]	7.1 ± 0.84[c]
Group 4	8.7 ± 0.49	7.1 ± 0.38	6.9 ± 0.73[b]	6.2 ± 0.92[c]	6.2 ± 0.96[c]
Group 5	8.9 ± 0.73	8.2 ± 0.41	7.8 ± 0.92[c]	7.8 ± 0.95[c]	7.5 ± 0.93[c]

Table 6: Effect of PMCA against total protein in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); a=p<0.001 When compared to normal (Group 1); b=p<0.05 When compared to control (Group 2); c=p<0.01 When compared to control (Group 2); d=p<0.001 When compared to control (Group 2).

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days.

Effect of PMCA against tissue antioxidant parameters

Numerous antioxidant parameters were assessed in the liver at the end of the treatment schedule i.e. on 28th day. High fat diet induces significant oxidative stress in experimental animals as denoted by the result of the present study. A significant reduction in liver superoxide dismutase (SOD), Catalase, GSH and significant increase in liver TBARS were detected in disease control group 2, when compared to the normal group (group 1). Treatment with atorvastatin (Group 3) displays a significant increase in SOD, Catalase, and GSH along with a significant reduction in TBARS of the liver, when compared with control group (Group 2). Group 4 and 5 treated by test drug PMCA in two different doses (40 and 80 mg/ kg, respectively). exhibited significant protection against high fat diet induced oxidative stress. These can be supported by an increase in SOD, Catalase, reduced glutathione (GSH) and reduction in TBARS in group 4 and 5 respectively, when compared to control group (Group 2) (Table 7).

Treatment Groups	Tissue antioxidants			
	TBARS (n mol/gm)	**GSH (µ g/ gm)**	**SOD (IU / dl)**	**CATALASE (IU / dl)**
Group 1	153.6 ± 8.226	29.23 ± 2.665	368.9 ± 91.83	221.4 ± 6.495[c]
Group 2	233.2 ± 4.227[b]	90.32 ± 5645[b]	97.29 ± 19.3[a]	677.5 ± 162.3
Group 3	140.8 ± 19.33[a]	97.95 ± 20.78	49.21 ± 4.093	400.5 ± 85.33
Group 4	157.4 ± 12.85[b]	120.0 ± 4.175	47.46 ± 6.062	568.5 ± 65.55
Group 5	160.7 ± 18.38[b]	60.32 ± 6.349	32.65 ± 0.693	437.7 ± 114.6

Table 7: Effect of PMCA against tissue antioxidant parameters in high fat diet induced hyperlipidemia.

All the values are expressed as Mean ± SEM (n=6); b P<0.0001 (Group 2) vs. control (Group 1) and Bp<0.0001 (Group 2) vs. Treatment groups (Group 3, 4, 5).

Group 1: Normal control group received distilled water 10 ml/kg along with normal regular rat diet for 28 days.

Group 2: Disease control group received distilled water 10 ml/kg along with high fat diet daily for 28 days.

Group 3: Standard group received Atorvastatin 5 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 4: Test group received P-methoxy cinnamic acid 40 mg/kg BW orally, along with the high fat diet daily for 28 days.

Group 5: Test group received P-methoxy cinnamic acid 80 mg/kg BW orally, along with the high fat diet daily for 28 days.

Histopathological Analysis

Transverse section of liver of high fat diet treated group (Group 2) showed a marked deposition of fat, dilation of sinusoids and disruption of hepatocytes in comparison with normal liver histopathology. Treatment with atorvastatin (Group 3) showed a notable decrease in lipid accumulation, normal structure of hepatocytes and sinusoids, when compared with the disease control group.

Administration of PMCA (Group 4 and 5) in two different doses (40 and 80 mg/ kg, respectively) exhibits only a slight difference in morphology of hepatocytes and were observed to be similar to that of the normal group. Moreover, it also shows decreased fat accumulation in adipocyte cells of the liver (Figure 1).

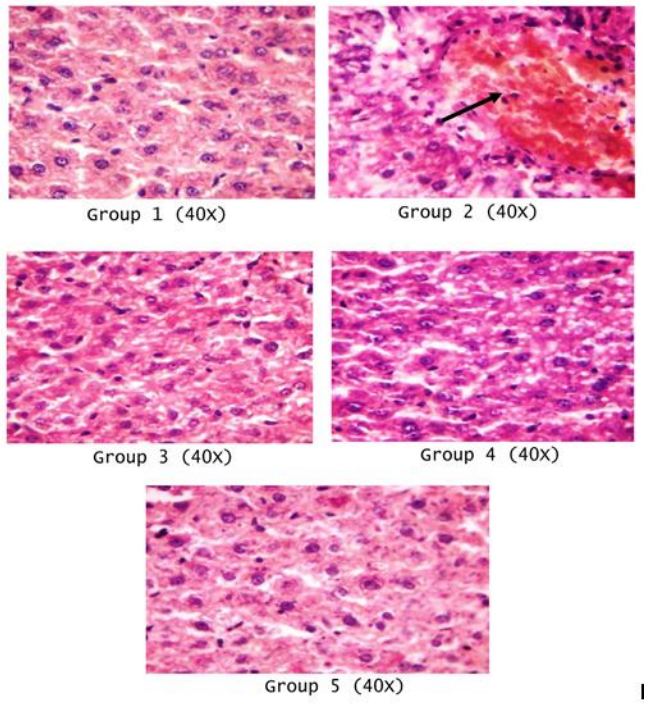

Figure 1: Effect of PMCA against high fat diet induced histopathological changes in liver tissue (Light microscopically analysis by eosin stain with 40 ϰ magnification study).

The action of atorvastatin showed a notable decrease in the lipid content of the liver which was similar to the addition of PMCA with slight change in the morphology of the hepatocytes. (Light microscopically analysis by eosin stain with 40 ϰ magnification study)

DISCUSSION

The main risk factor for coronary artery disease is Atherosclerosis. Accumulation of lipids in the walls of the artery causes lesion along with complex inflammatory conditions. Elevated level of TC, TG and LDL contribute specific risk for human to develop hypercholesterolemia, along with other contributors like insulin resistance, inflammation and oxidative stress [19-21]. Foods rich in saturated fat and cholesterol have been connected to increase in circulating cholesterol levels [22].

The present research was planned to evaluate the hypolipedemic activity of p-methoxy cinnamic acid against high fat diet induced hyperlipidemia in experimental rats. Biochemical parameters of serum cholesterol, HDL, LDL, triglycerides, total proteins and tissue antioxidant parameters were analyzed from liver homogenate as a part of experimental design, along with a note on body weight.

In the current study administration of high fat diet to rats causes significant upsurges in serum cholesterol, LDL, triglycerides on 14th, 21st, 28th day, with a reduction in protective HDL total proteins. The result on HDL was important on 28th day, when compared to the normal group (Group 1). Significant decrease of total cholesterol, LDL, triglyceride levels, with a rise in HDL, total protein levels were observed in the standard group (Group 3) treated with atorvastatin 5 mg/kg.

The improvement in the serum lipid profile with selective mechanism is, that atorvastatin is a lipid lowering agent comes below a class of HMG- CoA reductase inhibitor. Statins inhibit the HMG-CoA reductase competitively in the liver, which is more important for the synthesis of cholesterol in liver. Furthermore, statins escalate the expression of LDL receptors in the liver. These LDL receptors rise uptake and subsequent elimination of LDL, VLDL and ILDL and thus reestablish cholesterol homeostasis. Atorvastatin is seemed to decrease triglyceride levels by growing LDL clearance and by inhibiting triglyceride synthesis. It has also surged HDL cholesterol levels [23].

It is also clear from the present study that treatment of PMCA to high fat diet rats of the test groups (Group 4 and 5) revealed a significant reduction in serum cholesterol. LDL, triglyceride and development in HDL, total protein levels, when compared to control group (Group 2).

The concentration of VLDL, LDL cholesterol was decreased and an increase in the level of HDL cholesterol was observed due to polyunsaturated fatty acids. Also, they develop protective result against oxidation, which can be enlightened by their double bonds. 40% of both oleic acid and linoleic acid are hard to oxidize and this encompasses in the fluidity of lipoproteins and a significant amount of HDL formation [24]. More newly linoleic acid derivatives mainly gamma linoleic acid were originated to be stronger in reducing blood cholesterol [25].

Intake of food and body weight is connected to the metabolism of macronutrients and they can be used as subtle markers of overall health prominence [26]. A decrease in body weight of animals treated with PMCA was observed in the present study. This decrease in body weight may owe to useful effects of PMCA on serum lipid profile.

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Treatment of high fat diet of rats of disease control group (Group 2) causes significant increase in oxidative stress. These can be evidenced by reduction in levels of SOD, Catalase, GSH and an increase in lipid peroxidation. Hyperlipidemia is well known to increase to reactive oxygen species generation and subsequent lipid peroxidation. Hypertriglyceridemia is another factor that could enhance the reactive oxygen formation. The increases in plasma lipid peroxides could also have decline non enzymatic and enzymatic antioxidant potential in high fat diet rats. Free radical damage decrease the actions of antioxidant enzymes like as SOD, Catalase, GSH with elevated lipid peroxidation. These findings suggest a higher susceptibility to oxidative stress in high fat diet rats. Also, oxidative modification of LDL cholesterol leads to generation of cellular free radicals causing endothelial dysfunction and atherosclerosis [27].

Free radicals are accomplished of inducing cell injury by modifying proteins, lipids, nucleic acids. These might be one of the reasons for the decrease in total proteins in the disease control group (Group 2) [28].

PMCA significantly defends against high fat diet and cholesterol (0.5%) brought oxidative stress in rats which was reproduced by amending antioxidant parameters like increases in SOD, Catalase, GSH levels and reduced Lipid Per Oxidation (LPO).

Recovery from liver injury usually correlates with antioxidant ability to prevent lipid peroxidation [29]. Histopathology of livers of control groups administered with high fat diet exhibited rise in fat deposition in the liver along with sinusoids and altered morphology of liver cells, when compared to the normal group (group 1).

Whereas groups treated with PMCA showed protective effect on liver. These can be proved by decreased deposition of fat in the liver with a slight change in morphology of hepatocytes when compared to control group (Group 2).

CONCLUSION

PMCA offers significant protection against high fat diet induced hyperlipidemia. The study clearly reveals the antioxidant property of PMCA by decreasing lipid peroxidation and enhancing protective antioxidant enzyme levels. Histopathological findings of liver add an additional note for the protective effect. The limitation of the study was, the protective role of PMCA not established in related to histopathological section of the aorta due to lack of time and planned to do in future studies. More studies are acceptable to expound possible mechanisms to control the hyperlipidemia by using PMCA.

CONFLICT OF INTEREST

The author declares that there was no conflict of interest.

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