Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

ANTI-ATHEROGENIC ACTIVITY OF METHANOLIC EXTRACT OF *GARDENIA GUMMIFERA* LINN.F. ON HIGH FAT DIET INDUCED ATHEROSCLEROSIS IN RATS

S.P PRABHA[1], P.N ANSIL[1], A. NITHA[1], P.J WILLS[2], M.S LATHA[1]*

[1]Biochemistry and Pharmacognosy Research Laboratory, School of Biosciences, Mahatma Gandhi University, P. D. Hills. P. O, Kottayam 686560, Kerala, India, [2]MIMS Research Foundation, Malabar Institute of Medical Sciences, Calicut 673007, Kerala, India.
Email: mslathasbs@yahoo.com

Received: 27 Jan 2013, Revised and Accepted: 25 Mar 2013

ABSTRACT

Objective: To investigate the anti-atherogenic effect of methanolic extract of *Gardenia gummifera* root (MEGG) against high fat diet induced atherosclerosis in male Wistar rats. Methods: Rats were divided in to six groups, each having six animals. Group I served as normal control, group II received atherogenic diet (AD) (10g cholesterol, 5g sodium cholate, and 100g hydrogenated vegetable oil in 1000g standard laboratory chow), group III served as drug control (MEGG 250 mg/kg b.w p.o) and the remaining three groups IV, V and VI received AD along with standard drug atorvastatin (5mg/kg b.w p.o) and MEGG (125 and 250 mg/kg b.w p.o) respectively for 90 days. Anti-atherogenic activity was assessed by quantifying the serum levels of cardiac marker enzymes (LDH, AST, ALT and CPK), the concentration of serum total cholesterol (TC), triglyceride (TG), low desity lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, very low density lipoprotein (VLDL) cholesterol and phospholipids (PL). Tissue levels of TC, TG and PL were also determined. Antioxidants such as catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) and reduced glutathione (GSH) were determined in heart tissue. The level of lipid peroxidation, malondialdehyde (MDA) and histopathological analysis of the heart tissue supported the dose dependent anti-atherogenic effects of MEGG.

Result: MEGG significantly ($p \leq 0.05$) protected the above mentioned parameters to fall from the normal levels.

Conclusion: These observations suggest that the MEGG has a dose dependent anti-atherogenic effect against high fat diet induced atherosclerosis.

Keywords: Atherosclerosis; Antioxidants; Atorvastatin; Cholesterol; *Gardenia gummifera;* High fat diet; Hperlipidemic effect.

INTRODUCTION

Atherosclerosis and its thrombotic complications remain by far the most common cause of morbidity and mortality in the developed countries. Atherosclerosis is an oxidative, inflammatory and thrombotic disease of the arterial wall that is precipitated by elevated levels of low-density lipoprotein (LDL) cholesterol in the blood [1].Several lines of evidences support the hypothesis that the oxidation of LDL may play a critical role in early state of atherosclerosis, while thrombosis is one of the latest fatal clinical consequences of this disease [2]. Hyperlipidemia is one of the important factors associated with atherosclerosis, others being hypertension, smoking, diabetes mellitus and other factors. Diet is an important tool for the prevention of cardiovascular diseases [3].

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase are currently extensively used to lower serum cholesterol levels and improve the survival of individuals at risk of atherosclerotic vascular disease [4]. The most effective and widely used drugs for the treatment of hyperlipidemia are the statins. Their primary site of action is in the liver where they inhibit HMG-CoA reductase, the metabolic pathway that produces cholesterol and isoprenoids [5]. Plants have a significant role in maintaining human health and improving the quality of human life for thousands of years and served humans as valuable components of medicine.

Gardenia gummifera Linn.f. belonging to the family Rubiaceae is a large medicinal shrub with resinous buds. The resin is acrid, bitter, thermogenic, cardiotonic, carminative, antispasmodic, stimulant, diaphoretic, antihelmintic, antiseptic and expectorant. It is traditionally used in conditions of cardiac debility, obesity, lipolytic disorders, bronchitis, neuropathy and splenomegaly and is given to children in nervous disorders and diarrhoea due to dentition [6]. The gum yielded flavones, including gardenin, de-Me-tangeretin and nevadensin, wogonins, isoscutellarein, apigenin and de-MeO-sudachitin [7,8]. Oleanolic aldehyde, sitosterol, D-mannitol, erythrodiol and 19α-hydroxyerythrodiol were isolated and characterized from *G. gummifera* stem bark [9]. Previously we have reported the antioxidant and antihepatotoxic activity of MEGG against thioacetamide induced oxidative stress in rats. The present investigation was undertaken to evaluate the anti-atherosclerotic effect of MEGG on high cholesterol diet induced atherosclerosis in experimental rats.

MATERIALS AND METHODS

Chemicals

Cholesterol, sodium cholate and atorvastatin were purchased from Sisco Research Laboratories (SRL), Mumbai, India and Ranbaxy (New Delhi and Goa, India). Assay kits for serum Total Cholesterol (TC), Triglyceride (TG), High density lipoprotein cholesterol (HDL-C), Lactate dehydrogenase (LDH), Serum transaminases (ALT, AST), Creatine phosphokinase (CPK) were purchased from Agappe Diagnostic Ltd., India. All other chemicals were of analytical grade.

Collection of plant material and preparation of plant extracts

Gardenia gummifera Linn. f. were collected from its natural habitat (Idukki, Kerala, India) and authenticated. A voucher specimen (SBSBRL.05) is maintained in School of Biosciences, M.G University, Kottayam. Roots were cleaned, chopped, shade-dried and powdered. A 50 g of dried powder was soxhlet extracted with 400 mL of methanol for 48 h. The extracts were concentrated under reduced pressure using a rotary evaporator and were kept under refrigeration. The yield of methanolic extract was 10.3 % (w/w). The concentrate was suspended in 5% Tween 80 for *in vivo* studies.

Animals and diets

Adult male wistar rats weighing 150 – 200 g were used in this study. The rats were fed with standard laboratory chow (Hindustan Lever Foods, Bangalore, India) and provided with water ad libitum. The *Animals* were maintained at a controlled condition of temperature of 26 – 28^{0}C with a 12 h light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics Committee (IAEC) regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg.No. B2442009/3) and conducted humanely.

Dosage fixation

MEGG showed no lethal effect at least up to a dose of 5g/kg body weight indicating that LD_{50} if any should be higher than this dose.

The effective doses of MEGG (125 and 250mg/kg body weight.) was fixed based on the activities of serum aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH) and levels of antioxidant enzymes in the preliminary study, preventive and curative effect of methanolic extract of *Gardenia gummifera* Linn. f. on thioacetamide induced oxidative stress in rats.

Experimental Protocol

The animals were divided in to 6 groups (Six rats/group).

Group I - Normal control (Animals fed on normal laboratory feed and 5% Tween 80 instead of MEGG).

Group II -Atherogenic control: Animals fed on Atherogenic Diet (AD) [10g cholesterol, 5g sodium cholate and 100g hydrogenated vegetable oil in 1000g diet].

Group III - Drug control: Animals were given MEGG (250mg/kg body weight p.o).

Group IV- Animals fed on AD + Atorvastatin (5mg/kg body weight p.o).

Group V- Animals fed on AD + MEGG (125mg/kg body weight p.o).

Group VI- Animals fed on AD + MEGG (250 mg/kg body weight p.o).

The animals were fed for a period of 90 days. At the end of the experiment, the animals were sacrificed under mild ether anesthesia and the blood was collected from the neck blood vessels and serum was separated by centrifugation.

Phytochemical screening

Phytochemical screening of MEGG was carried out for the detection of phytoconstituents using standard conventional protocols [11].

Biochemical analysis

Serum analysis

Atherosclerosis was assessed by quantifying the serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), phospholipid (PL), AST (EC 2.6.1.1), ALT (EC 2.6.1.2), LDH (EC 1.1.1.27) and CPK (EC 2.7.3.2) by using the kit of Agappe Diagnostic Ltd., India. Activities of these serum parameters were measured using semi autoanalyzer (RMS, India). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated as per the standard methods [12].

Tissue analysis

Heart was excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1M Tris HCl buffer (pH – 7.4). The homogenate was centrifuged at 3000 rpm for 20 min at 4°C and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH), lipid peroxidation product (Thiobarbituric Acid Reactive Substances – TBARS) and total protein.

Tissue CAT (EC 1.11.1.6) activity was determined from the rate of decomposition of H_2O_2 [13]. GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [14]. GR (EC 1.6.4.2) activity was assayed at 37°C and 340 nm by following the oxidation of NADPH by GSSG [15]. GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB [16]. GSH was determined based on the formation of an yellow colored complex with DTNB [17]. The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1'1'3'3' tetramethoxypropane as standard [18]. Protein content in the tissue was determined using bovine serum albumin (BSA) as the standard [19]. The tissue lipids were extracted according to the method described by Folch et al [20]. The lipid parameters including total cholesterol [21], triglycerides [22] and phospholipids [23] were estimated.

Histopathological studies

Small pieces of heart fixed in 10% buffered formalin were processed for embedding in paraffin [24]. Sections (5-6 µm) were cut and stained with haematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam 1000 camera at original magnification of 100x.

Statistical Analysis

Results were expressed as mean + S.D and all statistical comparisons were made by means of one way ANOVA test followed by Tukey's post hoc analysis and P – Values less than or equal to 0.05 were considered significant.

RESULTS

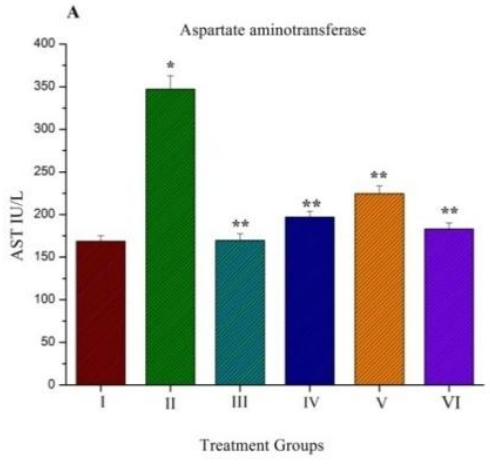
Phytochemical analysis

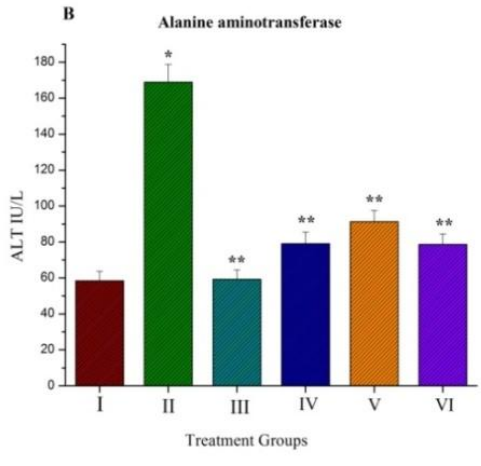
Phytochemical analysis of MEGG revealed the presence of phytochemical constituents such as alkaloids, triterpenes, steroids, glycosides, phenols, flavonoids, saponins and carbohydrates.

Serum Analysis

Effects of MEGG on serum enzyme levels

The serum levels of AST, ALT, CPK and LDH in group II were significantly ($p \leq 0.05$) elevated by the administration of AD, when compared to normal control. The treatment of MEGG at a dose of 125 and 250 mg/kg showed a significant decrease ($p \leq 0.05$) of AST, ALT, CPK and LDH. Standard control drug, atorvastatin at a dose of 5 mg/kg also prevented the elevation of serum enzymes (Fig.1). Treatment with 250 mg/kg MEGG and atorvastatin exhibited a protection of 91.84 and 84.09% in AST levels, 81.32 and 81.72% in ALT levels, 87.30 and 88.32% in CPK levels and 84.35 and 84.54% in LDH levels respectively. The protective effect of the extract in decreasing the elevated levels of serum enzymes was in a dose dependent manner.





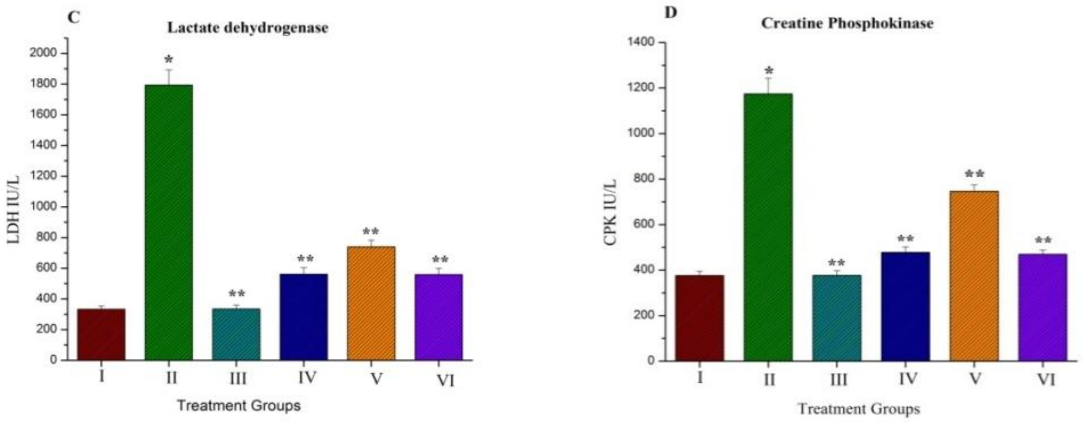


Fig. 1: Effects of MEGG on changes in serum enzyme levels of rats treated with AD. (A) Aspartate aminotransferase, (B) Alanine aminotransferase, (C) Lactate dehydrogenase (D) Creatine phosphokinase. I - Normal control, II –Atherogenic control, III MEGG alone treated (250 mg/kg), IV - AD + Atorvastatin (5mg/kg– 250 mg/kg), V- AD + MEGG (125mg/kg), VI- AD + MEGG (250 mg/kg). Values are mean ± S.D, error bar indicating the standard deviation, n = 6 animals. * $p \leq 0.05$ vs. normal control. **$p \leq 0.05$ vs. atherogenic control.

Effect of MEGG on Serum Lipid profile and Phospholipid

AD fed rats produced significant increase ($p \leq 0.05$) in serum cholesterol, triglyceride, LDL-C, VLDL-C and PL levels, while HDL-C decreased significantly ($p \leq 0.05$) as compared to normal animals (Table: 1). Treatment with MEGG at a dose of 125 and 250 mg/kg showed a significant decrease ($p \leq 0.05$) of TC, TG, LDL-C, VLDL-C and PL. HDL-C was increased significantly ($p \leq 0.05$) in MEGG treated groups (125 and 250 mg/kg). Treatment with atorvastatin (5mg/ kg) also prevented the elevation of TC, TG, LDL-C, VLDL-C, PL levels. HDL-C is increased significantly in atorvastatin treatment. MEGG (250mg/kg) and atorvastatin (5mg/kg) exhibited a protection of 79.70 and 75.13% in TC, 85.03 and 77.65% in TG, 83.76 and 76.07% in LDL-C, 67.20 and 60.20% in VLDL-C, 80.80 and 77.07% in PL and 74.83 and 61.4% in HDL-C. MEGG alone treated (Group: III) rats showed non-significant changes in all these parameters as compared with control group. The protective effect of the extract was in a dose dependent manner.

Table 1: Effect of MEGG on serum lipid profile (TC,TG, HDL-C, LDL-C, VLDL-C) and phospholipid (PL) in control and experimental animals

Treatment Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	PL (mg/dl)
Normal control	58.16±2.60	75.36±2.17	44.24±4.68	27.82±1.35	13.27±0.31	142.03±4.28
AD control	216.37±8.63^a	168.32±7.8^a	24.85±2.30^a	75.92±3.5^a	28.85±0.7^a	279.87±8.92^a
MEGG (250mg/kg)	57.10±3.11	73.98±3.12	44.95±3.68	28.10±1.20	14.53±0.29	140.95±5.24
Atorvastatin (5mg/kg) +AD	97.50±4.83^b	96.13±4.62^b	36.76±3.71^b	39.33±2.4^b	19.47±0.3^b	173.63±6.37^b
MEGG (125mg/kg) +AD	128.17±5.16^b	103.52±4.7^b	31.12±2.90^b	43.02±3.2^b	21.05±0.3^b	181.92±6.06^b
MEGG (250mg/kg) +AD	94.25±5.12^b	89.27±3.78^b	39.36±3.8^b	35.63±2.3^b	18.38±0.2^b	168.49±5.72^b

Values are mean ± S.D., n = 6. Statistical significance: ^{a} $p \leq 0.05$ - AD group differs significantly from normal control group. ^{b} $p \leq 0.05$ - Atorvastatin 5mg/kg +AD, 125mg/kg MEGG+AD and 250 mg/kg MEGG+AD groups differs significantly from AD control group

Tissue analysis

The antioxidant enzyme activities such as GST, GPX, GR and CAT were found to be decreased significantly (P≤0.05) in AD administered rats when compared to control rats (Group: I) (Table.2). The reduced glutathione (GSH) level was decreased significantly (P≤0.05) in AD administered rats (Group: II). Treatment with MEGG (125 and 250 mg/kg) and atorvastatin significantly elevated (P≤0.05) all these parameters. The significant increase (P≤0.05) in tissue MDA levels was observed in AD treated rats.

However AD induced elevation of MDA concentration was lowered significantly (P≤0.05) by the treatment with MEGG at a dose of 125 and 250mg/kg and atorvastatin (5mg/kg). MEGG alone treated (Group: III) rats showed non-significant changes in all these parameters as compared with control group.

Table 2: Effect of MEGG on the levels of MDA, GSH and activities of antioxidant enzymes (GST, GR, GPx, and CAT) in the heart of control and experimental rats.

Treatment Groups	GSH (nmol/mg protein)	GST (mmol CDNB-GSH conjugate formed/min/mg protein)	GR (nmol of GSSG utilized/min/mg protein)	GPx (nmol of GSH oxidized/min/mg protein)	CAT (U/mg protein)	MDA (nmol/g tissue)
Normal control	10.57 ±0.62	24.96 ± 0.45	8.16 ± 0.53	6.64 ± 0.51	16.91± 0.72	1.47 ± 0.28
AD control	2.41 ± 0.18^a	9.30 ± 0.13^a	1.7 ± 0.15^a	1.2 ± 0.24^a	5.23 ± 0.24^a	5.28 ±0.59^a
MEGG (250mg/kg)	9.95 ± 0.63	25.11 ± 0.40	7.37 ± 0.48	7.78 ± 0.46	17.18 ±0.65	1.20 ± 0.31
Atorvastatin (5mg/kg)+ AD	7.61 ±0.52^b	18.10±0.38^b	6.32 ±0.50^b	4.92 ±0.53^b	13.64±0.57^b	2.26 ±0.26^b
MEGG (125mg/kg) +AD	5.16 ±0.49^b	16.71±0.42^b	5.89 ±0.47^b	4.51 ±0.48^b	11.28±0.51^b	3.11 ±0.25^b
MEGG(250mg/kg)+AD	9.05±0.61^b	20.71±0.36^b	6.85±0.57^b	5.83±0.57^b	16.18±0.62^b	1.73±0.24^b

Values are mean ± S.D., n = 6. Statistical significance: ^{a}$p \leq 0.05$ - AD group differs significantly from normal control group. ^{b} $p \leq 0.05$ – Atorvastatin 5mg/kg +AD, 125mg/kg MEGG+AD and 250 mg/kg MEGG+AD groups differs significantly from AD control group.

AD fed rats produced significant (P≤0.05) increase in tissue cholesterol, TG and PL. treatment with MEGG (125 and 250 mg/kg) and atorvastatin (5mg/kg) showed significant reduction (P≤0.05) in TC, TG and PL. MEGG alone treated (Group: III) rats showed non-significant changes in all these parameters as compared with control group (Table:3).

Table 3: Effect of MEGG on tissue total cholesterol (TC), triglyceride (TG) and phospholipid (PL) in control and experimental animals

Treatment Groups	TC (mg/100g wt. tissue)	TG (mg/100g wt. tissue)	PL (mg/100gm wt. tissue)
Normal control			
Liver	329.21± 10.24	416.36 ± 12.19	1826.15± 68.14
Heart	241.52 ± 7.18	165.50 ± 6.75	872.17± 23.38
Kidney	319.38 ± 9.68	251.39 ± 9.67	970.83± 24.68
AD control			
Liver	592.94 ± 18.12[a]	820.53 ± 26.23[a]	3431.63 ± 128.36[a]
Heart	477.58 ± 12.46[a]	373.30 ± 10.53[a]	1562.62 ± 63.19[a]
Kidney	564.26 ± 16.17[a]	518.96 ±17.53[a]	1749.97± 88.23[a]
MEGG (250mg/kg)			
Liver	328.29 ± 9.98	418.09 ± 14.38	1822.61±66.84
Heart	238.61± 7.35	163.44 ± 5.84	876.14 ± 23.72
Kidney	314.38 ± 10.21	253.23 ± 6.86	966.68 ± 25.61
Atorvastatin (5mg/kg) +AD			
Liver	381.52 ± 11.23[b]	510.75 ± 15.14[b]	1967.49 ± 74.05[b]
Heart	286.95± 8.56[b]	189.72 ± 7.51[b]	1148.92 ± 47.25[b]
Kidney	366.51± 11.52[b]	327.62 ± 8.13[b]	1268.75 ± 67.19[b]
MEGG (125mg/kg)+AD			
Liver	405.18 ± 11.90[b]	568.14 ± 15.78[b]	2034.62 ± 83.56[b]
Heart	308.75 ± 9.82[b]	208.17 ± 8.32[b]	1186.30 ± 48.45[b]
Kidney	397.86 ± 11.31[b]	358.39 ± 9.11[b]	1315.62 ± 74.28[b]
MEGG (250mg/kg)+AD			
Liver	378.61± 10.63[b]	502.72 ± 13.62[b]	1959.41± 75.19[b]
Heart	282.54±7.89[b]	182.51 ± 6.93[b]	1132.74 ± 45.91[b]
Kidney	359.50 ± 10.22[b]	323.84 ± 8.27[b]	1262.15 ±65.32[b]

Values are mean ± S.D., n = 6. Statistical significance: [a]$p \leq 0.05$ - AD group differs significantly from normal control. group [b] $p \leq 0.05$ - Atorvastatin 5mg/kg + AD, 125mg/kg MEGG+AD and 250 mg/kg MEGG+AD groups differs significantly from AD control group.

Effect of MEGG treatment on histopathological changes of rat myocardium

The microscopic observations of myocardial histoarchitecture were qualitatively graded on the basis of myonecrosis, inflammatory cells and edema. The myocardium of control group showed a normal histoarchitecture (Fig.2A). Myocardium of AD control rats showed massive necrosis of myofibers with cell infiltration, edema and increased connective tissue among myocardial fibers along with extra vasation of red blood cells (Fig.2B). Rats received only MEGG (250mg/kg) did not showed any adverse effect on myocardial histology (Fig.2C).

On the other hand, treatment with atorvastatin (5mg/kg b.w) and MEGG (125mg/kg) in AD treated rats showed mild edema and inflammatory cells as compared to AD control group (Fig:2E). MEGG treated at 250mg/kg showed almost the absence of myonecrosis and sparse focal edema and inflammatory cells (Fig. 2F).

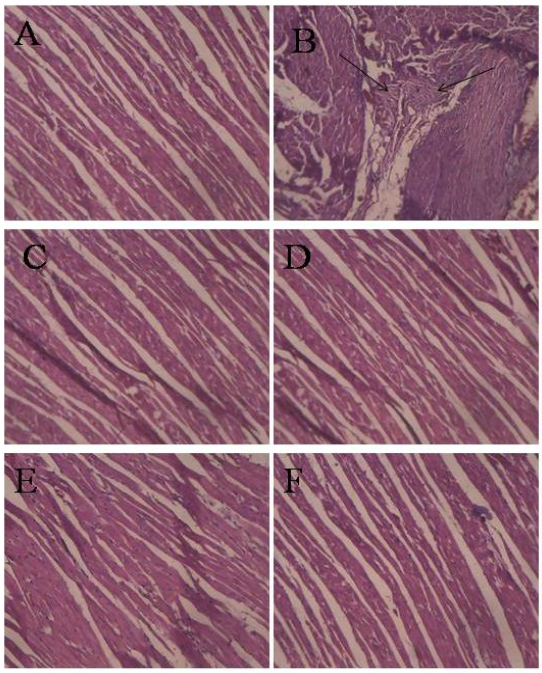


Fig. 2: Effect of MEGG on AD induced histopathological changes in heart tissue. (Hematoxylin and eosin, 100×). (A) Normal control (B) AD control (C) Drug control MEGG (250mg/kg b.w) alone treated (D) AD + Atorvastatin (5mg/kg b.w) (E) AD + MEGG (125mg/kg b.w) (F) AD + MEGG (250 mg/kg b.w)

DISCUSSION

Hyperlipidemia has been documented as one of the major causative factor for atherosclerosis, resulting in coronary heart diseases (CHD). Elevated cholesterol particularly LDL are the major reasons attributed to cardiovascular diseases. The imbalance in the lipid metabolism plays a role in aggravating the lipid peroxidation [25,26].

The results of the present study showed a significant increase in serum AST, ALT, LDH and CPK levels in atherogenic control when compared to normal group. There was a significant ($p \leq 0.05$) restoration of these enzyme levels on administration of the MEGG in a dose dependent manner and also by atorvastatin at a dose of 5mg/kg. Higher activities of these enzymes in serum have been found in response to oxidative stress induced by high fat diets [27,28]. Marked decrease in serum transaminases, CKP and LDH levels demonstrate the cardioprotective effect of MEGG.

Cholesterol-enriched diet resulted in a significant increase in total cholesterol, triacylglycerol and phospholipids in plasma which is accompanied by increased serum LDL-C level, with decreased circulating HDL-C, thus providing a model for dietary hyperlipidemia [29]. There was significant increase in the levels of serum and tissue TC, TG, PL in AD fed rats. Serum LDL-C and VLDL-C were elevated significantly in AD fed rats and also there was a significant reduction in HDL-C levels in these animals. Treatment with MEGG showed a marked reduction in TC, TG, PL and LDL-C levels. But there was a significant rise in HDL cholesterol levels in all the groups. Atorvastatin also produced significant reduction in serum TC, TG, PL, LDL-C levels and a rise in HDL-C levels (Table1). The proposed mechanism of action for the improvement in serum lipid profile is, that atorvastatin is a lipid lowering agent comes under a class of HMG- CoA reductase inhibitor. Statins competitively inhibit enzyme HMG-CoA reductase in liver that converts HMG-CoA to mevalonate an early precursor for cholesterol synthesis. More over statins increaseas the expression of LDL receptors in liver. These LDL receptors increases uptake and subsequent removal of LDL, VLDL and ILDL and thus restore cholesterol homeostasis. Atorvastatin appeared to reduce triglyceride levels by increasing LDL clearance and by inhibiting triglyceride synthesis. It also increases HDL cholesterol levels [30]. The increase of lipid parameters has been shown to be a strong risk factor for coronary heart diseases in many populations.The high cholesterol level in tissue and serum may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids. The metabolism of free and ester cholesterol are impaired in liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats. Lipid deposition is a major clinical complication of hyperlipidemia [31].

In the present study, we measured the amount of MDA in cardiac tissue of AD fed rats to get an indication of the amount of *in vivo* oxidative stress [31]. The measurement results reveal that MDA levels were significantly higher in cardiac tissues of rats fed AD diet compared with those fed a normal diet, which may indeed indicate an increased amount of oxidative stress in the AD fed rats. The results of the present study indicate that treatment with MEGG inhibits AD induced MDA accumulation in heart. This demonstrates the anti- lipid peroxidative effect of the extract. Atorvastatin also prevented the elevated level of MDA. The biological antioxidant defense system is an integrated array of enzymes and antioxidants. GSH, a substrate for GSH-peroxidase, CAT, GST, GPx, and GR constitute the first line of cellular antioxidant defense enzymes. Catalase and GPx that catalyze the conversion of hydrogen peroxide to water [33]. GST offers protection against lipid peroxidation by promoting the conjugation of toxic electrophiles with GSH [34]. GR is also essential for the maintenance of GSH levels *in vivo* [35]. GSH is required to maintain the normal reduced state and to counteract the deleterious effects of oxidative stress. During the reduction of hydrogen peroxide, GSH is oxidized to GSSG. When GSSG levels increased, the GSH-reductase activity was activated to convert GSSG in GSH [36]. Different doses (125mg/kg and 250mg/kg) of MEGG treated groups have shown significant and dose dependently elevated levels of CAT, GST, GPx, GR and GSH. This evidently shows the antioxidant property of the extract against oxygen free radicals. Atorvastatin also produced significant rise in CAT, GST, GPx, GR and GSH levels in cardiac tissue. Histopathological studies also provide supportive evidence for biochemical analysis. Therapy of MEGG significantly improved cellular morphology in a dose dependent manner.

CONCLUSION

The result of serum and tissue biochemical parameters, level of cardiac lipid peroxides, tissue antioxidants and histopathological studies together support the highly potent anti-atherogenic and antioxidant activity of MEGG. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenolics, triterpenes, glycosides and steroids in MEGG [37]. β - sitosterol, oleanolic aldehyde, erythrodiol etc reported from *Gardenia gummifera* [9]. The LCMS analysis of MEGG revealed the chemical composition of the extract and constituents with potent antioxidant, anti inflammatory and anti hypercholesterolemic effect. The major compounds identified in MEGG with the potential to improve the cardiac health includes erythrodiol, lupeol, epicatechin, β- sitosterol, asiatic acid and myricetin. Erythrodiol is a triterpene, exhibits both antioxidant and antithrombotic properties [38]. Sudhakar *et al* [39] demonstrated lupeol's triterpene's mode of action by a restoration of several transmembrane enzymes, total cholesterol, triglyceride and phospholipids to normal levels, preventing hypertrophic cardiac histology. Epicatechin reduces blood pressure and limits infarct size in animal models of myocardial ischemia reperfusion injury [40]. β-sitosterol reduces the blood levels of cholesterol and was used in treating hypercholesterolemia [41]. Asiatic acid was reported to possess a wide spectrum of biological activities including antioxidation, anti-inflammation, antitumor, antidepression, anti-Alzheimers disease, cardiovascular protection, and hepatoprotective effect [42]. Myricetin could be of benefit in the prevention of atherosclerotic lesions because it was a potent inhibitor of oxidative modification of LDL by macrophages [43]. The identified class of components in single or in combination with other components present in the extract might be responsible for the anti-atherogenic and antioxidant activity in the treatment groups. Nevertheless, more efforts are still required for the isolation, characterization and biological evaluation of the active principle(s) of the methanolic extract of *Gardenia gummifera root*.

ACKNOWLEDGMENT

Financial support from the Mahatma Gandhi University Kerala, India as Junior Research Fellowship to Prabha S.P [U.O. No. 4851/AcAVI/2008/Acad.] was greatfully acknowledged.

REFERENCES

1. Allouche Y, Beltran G, Gaforio JJ, Uceda M, Mesa MD. Antioxidant and anti atherogenic activities of pentacyclic triterpenic diols and acids. Food and Chemical Toxicology 2010;48:2885-2890.
2. Lusis AJ. Atherosclerosis. Nature 2000;407:233-241.
3. Ferdowsian HR, Barnard ND. Effect of plant- based diets on plasma lipids. Am.J.Cardiol 2009;104:947-956.
4. Endo A. The discovery and development of HMG CoA reductase inhibitors. J. Lipid Res 1992;33:1569–1582.
5. Adnan F, Sadiq M, Jehangir A. Anti-hyperlipidemic effect of Acacia Honey (Desi Kikar) in cholesterol- diet induced hyperlipidemia in rats. Biomedica 2011;27:62-67.
6. Varier PS. Indian medicinal plants-a compendium of 500 species. Madras: Orient Longman Publications 1995.p.65-68.
7. Khare CP. Indian Medicinal Plants. An Illustrated Dictionary. New York; Springer 2007.p.45.
8. Mir FA, Yadav AS, Wankhede S. Efficiency of media's for propagation of medicinal tree *Gardenia gummifera* Linn.f.-An endangered medicinal plant. J Phytol 2010; 2:47-51.
9. Reddy GC, Rangaswami S, Sunder R. Triterpenoids of the stem bark of Gardenia gummifera. Planta Med 1977;32:206-211.
10. Prabha SP, Ansil PN, Nitha A, Wills PJ, Latha MS. Preventive and curative effect of methanolic extract of Gardenia gummifera

Linn. f. on thioacetamide induced oxidative stress in rats. Asian Pac J Trop Dis 2012;2:90-98.
11. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis. Springer Verlag, Berlin-Heidelberg; 1984.
12. Dabhi JK, Solanki JK, Mehta A. Antiatherogenic activity of ibruprofen, a non selective COX inhibitor- an animal study. Indian J Exp Biol 2008;46:476-481.
13. El-Demerdash FM, Yousef MI, Radwan FM. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. Food Chem Toxicol 2009;47:249-254.
14. Ajith TA, Hema U, Aswathy, MS. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food Chem Toxicol 2007;45:2267-72.
15. Beutler E. Red cell metabolism: A manual of biochemical methods. 3rd ed. New York: Grune and Startton. 1984.
16. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130 - 39.
17. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82:70-77.
18. Hwang YP, Choi JH, Jeong HG. Protective effect of the Aralia continentalis root extract against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol 2009; 47:75-81.
19. Lowry OH, Rosebrough, NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. J Biol Chem 1951;193:265-75.
20. Folch J, Lees N, Solane S. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem. 1957;226:497-509.
21. Zaltkis A, Zak B, Boyel AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med. 1953;41:486-492.
22. Foster LB, Dunn RT. Stable reagent for determination of serum triglyceride by calorimetric Hantzsch condensation method. Clin Chem 1973;19:338-340.
23. Zilversmith BB, Davis AK. Micro-determination of plasma phospholipid by TCA precipitation. J Lab Clin Med 1950;35:155-161.
24. Maheshkumar K, Nelsonkumar S, Rajaram C, Rupesh SK, Ravindrareddy K. Evaluation of Cardio protective effect of methanolic extract of *Ventilago maderaspatana* against Isoproterenol induced myocardial infarction in experimental rats. *IJAPR.* 2012; 3:1167-1176.
25. Saikrishna N, Ranganayakulu D, Gopisudheerkumar J, Vijaykumar G, Anusha M. Anti atherosclerotic activity of the ethanolic extract of the *Butea monosperma* leaves as alone and in combination with the Atorvastatin. J Adv Drug Res 2010;1:20-26.
26. Satish kumar D, Muthu KA. Ondium Suffruticosum (Ging.) (Family: Violaceae) in rat fed with high fat diet. Int J Pharm Pharm Sci 2012; 4(4); 69-73.
27. Yang JJ, Li GL, Liu HR., Ren BB. Effect of eveningrose oil on activities of oxygen free radical scavenging-related enzymes and hepatic morphosis in rats on high lipid diet. J. NingXia Med Coll 2003;25:244-246.
28. Demori I, Voci A, Fugassa E, Burlando B. Combined effects of high-fat diet and ethanol induce oxidative stress in rat liver. Alcohol 2006;40:185-191.
29. Cohen S, Moore A, Ward W. Flaxseed oil and inflammation–associated bone abnormalities in interleukin 10 Knockout mice. J Nutr Biochem 2005;16:368-374.
30. Ray SK, Rege NN. Atorvastatin in the management of hyperlipidemia. Journal of post graduate medicine 2000;46:242-243.
31. Venu P, Rema R, Hariprasad M.G. Evaluation of the antihyperlipidemic, cardioprotective avtivity of a polyherbal formulation. Int J Pharm Pharm Sci 2010;2:86-91.
32. Ansil PN, Nitha A, Prabha SP, Wills PJ, Jazaira V, Latha MS. Protective effect of *Amorphophallus campanulatus* (Roxb.) Blume. tuber against thioacetamide induced oxidative stress in rats. Asian Pac J Trop Med 2011;4:870-7.
33. Rocha KKR, Souza GA, Ebaid GX, Seiva FRF, Cataneo AC, Novelli ELB. Resveratrol toxicity: Effects on risk factors for atherosclerosis and hepatic oxidative stress in standard and high-fat diets. Food Chem. Toxicol 2009;47:1362-1367.
34. Jakoby WB. Detoxification, conjugation and hydrolysis in liver biology and pathology. In: Arias IM, Jakoby WB, editors. Raven Press, New York*;* 1988.p 375-385.
35. Carlberg I, Mannervik B. Glutathione Reductase. Methods Enzymol 1985;113:484-490.
36. Cui BK, Liu S, Lin XJ, Wang J, Li SH, Wang QB, Li SP. Effects of *Lycium Barbarum* Aqueous and Ethanol Extracts on High-Fat-Diet Induced Oxidative Stress in Rat Liver Tissue. Molecules 2011;16:9116-9128.
37. Tambekar DH, Khante BS, Chandak BR, Tltare AS, Boralkar SS, Aghadte SN. Screening of antibacterial potentials of some medicinal plants from Melghat forest in India. Afr J Trade CAM 2009;6:228-232.
38. Whellan DJ. Heart failure disease management: implementation and outcomes. Cardiol Rev 2005;13:231-9
39. Sudhakar V, Kumar SA, Sudharsan PT, Varalakshmi P. Protective effect of lupeol and its ester on Cardiac abnormalities in experimental hyper cholesterolemia. Vascular Pharmacology 2007;46:412-418.
40. Yamazaki KG, Taub PR, Barraza-Hidalgo M, Rivas MM, Zambon AC, Ceballos G, Villarreal FJ. Effects of (-)-epicatechin on myocardial infarct size and left ventricular remodeling after permanent coronary occlusion.. J. Am. Coll. Cardiol 2010;55:2869-76.
41. Matsuoka K, Nakazawa T, Nakamura A, Honda C, Endo K, Tsukada M. "Study of Thermodynamic Parameters for Solubilization of Plant Sterol and Stanol in Bile Salt Micelles". Chem. Phys. Lipids 2008;154:87-93.
42. Zhang L, Chen J, Gang Y, Liu J, Zhang L, Hua W, Sun H. Synthesis and biological evaluation of asiatic acid derivatives as inhibitors of glycogen phosphorylase. Chemistry and Biodiversity 2009;6:864-874.
43. de Whalley CV, Rankin SM, Hoult JR, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem. Pharmac 1990;39:1743-1750.