SUMMARY AND CONCLUSION
*Gardenia gummifera* Linn. f. belongs to the family Rubiaceae, is one of the endangered plant species of India. The plant is about 3-7m tall and is found in dry forests of Kerala, Tamil Nadu, Andra Pradesh and Karnadaka. It contain resin, volatile oil and a coloring matter gardenin-A. This plant is traditionally used in conditions of cardiac debility, obesity and lipolytic disorders. Resin from the leaf buds is also traditionally used in cardiac problems, wound healing, indigestion, gas trouble and ulcer. Oleanolic aldehyde, sitosterol, D-mannitol, erythrodiol and 19α-hydroxyerythrodiol were isolated and characterized from *G. gummifera* stem bark. There was no data available regarding the cardioprotective and antioxidant activity of *G. gummifera* to verify the traditional claims. Hence the present study was undertaken with the following objectives. To evaluate the antioxidant potential, antiatherogenic effect on high fat diet induced atherosclerosis, cardioprotective efficacy on isoproterenol (ISO) induced myocardial infarction (MI) and to identify the phytochemical constituents responsible for cardioprotection.

Preliminary phytochemical screening of the different solvent extracts of *Gardenia gummifera* Linn.f. root such as petroleum ether (PEGG), chloroform (CHGG), acetone (ACGG), ethanol (ETGG) and methanol (MEGG) revealed the presence of alkaloids, flavonoids, terpenoids, phenols, tannins, glycosides and saponins. Of these, the methanolic extract of *G. gummifera* root (MEGG) was abundant in phytochemical constituents such as alkaloids, flavonoids, steroids, terpenoids, phenols, tannins, glycosides, saponins, carbohydrates and proteins. *In vitro* antioxidant studies of the different extracts of *G. gummifera* root revealed that MEGG has higher antioxidant and radical scavenging activity than the other extracts, which may be attributed to its phytochemical constituents. The antioxidant
activity of the extracts were compared with that of standard compounds viz. ascorbic acid and quercetin. As MEGG has proven a promising in vitro antioxidant, it was selected for further in vivo studies.

Herbal products when ingested into the body may be toxic to important organs such as kidney, liver, spleen, stomach, lungs and heart because of their diverse roles in the human body. So the MEGG was subjected to efficacy and safety tests. Acute and subacute toxicity study was conducted in male Wistar rats. Analysis of mortality rate, biochemical parameters and histopathological changes revealed the nontoxic nature of the drug. Liquid chromatography mass spectrometry (LCMS) analysis of MEGG was done. The results showed that MEGG contain compounds that possess potent antioxidant and cardioprotective properties.

The cardioprotective compounds identified using LCMS analysis includes erythodiol, epicatechin, carotenoids, lupeol, asiatic acid, β-sitosterol, oleanolic aldehyde, vernolic acid and myricetin. In vivo antioxidant potential of MEGG was evaluated in thioacetamide (TAA) intoxicated male Wistar rats in preventive and curative models. Single dose of TAA (100mg/kg; b.w.) suspended in normal saline was administered subcutaneously to induce oxidative stress in rats. In pre-treatment groups, rats were treated with daily dose of MEGG (125 and 250mg/kg; p.o.) for 9 days prior to TAA administration. In post-treatment evaluation, TAA was administered on the first day of the experiment and MEGG treatment were started at 2h, 24h and 48h after TAA intoxication. Pre and post-treatment with MEGG at a dose of 250mg/kg b.w. significantly (p≤0.05) prevented and reversed the elevation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and tissue
malondialdehyde (MDA) levels in both the experimental models. Hepatic and renal GSH, GST, GR, GPx and catalase levels were remarkably increased by the administration of MEGG in both the treatment regiments. Quantification of histopathological changes also supported the preventive and curative effects of the extract and the results were comparable with silymarin, the standard drug. Further, in both the experimental model, the extract exhibited its antioxidant potential in a dose dependent manner. Many antioxidant substances have cardioprotective properties. Since MEGG exhibited potential antioxidant activity in both in vitro and in vivo experimental models, further studies were conducted to exploit its cardioprotective efficacy against atherosclerosis and myocardial infarction.

Atherosclerosis and its thrombotic complications remain by far the most common cause of morbidity and mortality in the developed countries. To investigate the anti-atherogenic effect of MEGG against high fat diet induced atherosclerosis in male Wistar rats, the rats were divided in to six groups, each having six animals. Group I served as normal control, group II received atherogenic diet (AD). 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. MEGG at a dose of 250mg/kg b.w. significantly decreased the body weight and liver weight, the serum marker enzymes such as AST, ALT, CPK and LDH. Total cholesterol, triglycerides and phospholipids in serum and tissues, the serum LDL-C and atherogenic ratio were decreased and HDL-C was increased in MEGG (250mg/kg b.w) treated rats. Treatment with MEGG prevented the depletion of tissue GSH, GST, GR, GPx, CAT and increase in MDA. Increased excretion of bile acids and
neutral sterols were observed in MEGG treated rats. MEGG-treated rats (250mg/kg) showed decrease in the HMG CoA reductase activity, glucose-6-phosphate dehydrogenase and malic enzymes, indicated the decreased lipogenesis. Histopathological alterations caused by atherogenic diet were also returned to normal by the administration of MEGG. MEGG at a dose of 250mg/kg evoked more efficacy than 125mg/kg body weight. The results were comparable with atorvastatin, the standard drug.

MI is the major form of ischemic heart diseases (IHD) and is characterized by an imbalance of coronary blood supply and myocardial oxygen demand which results in ischemia and myocardial death. Cardioprotective effect of MEGG on ISO induced MI was evaluated in Wistar rats. MI was induced by the subcutaneous injection of ISO (6mg/100g body weight) at an interval of 24h for 2 days. MEGG (125 and 250 mg/kg b.w, p.o) was given to rats once daily for 45 days prior to the ISO challenge. Administration of ISO to Group II animals resulted in the induction of myocardial infarction, was evident from the increased levels of marker enzymes namely AST, ALT, LDH and CK-MB. The prior administration of MEGG (250 mg/kg b.w) decreased the activities of these marker enzymes. Treatment with MEGG 250mg/kg b.w decreased the serum uric acid and increased the ceruloplasmin levels. Upon MEGG (250mg/kg b.w) pretreatment, the free iron concentration was decreased with a significant increase in serum iron binding capacity. It also prevented the depletion of tissue GSH, GST, GR, GPx, CAT and increase in MDA levels in tissues. Pretreatment with MEGG (250mg/kg b.w) elicited more significant lowering of TC, TG, LDL-C, VLDL-C and phospholipids. The HDL-C was found to be significantly increased in the case of rats treated with
MEGG. In the TTC (Triphenyl Tetrazoleum Chloride) macroscopic enzyme mapping assay, MEGG pretreated rats (250mg/kg b.w) showed results almost similar to that of normal rats. Histopathological changes also supported the cardioprotective efficacy of MEGG.

MEGG was further fractionated using solvents of increasing polarity viz. petroleum ether, chloroform, ethyl acetate and methanol and the fractions were collected as petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (ETF) and methanol fraction (MEF) respectively.

PEF showed maximum in vitro antioxidant activity and hence the cardioprotective activity of the PEF was further examined by using ISO induced myocardial infarction models. The animals were divided in to 5 groups (Six rats/group). Group I vehicle control (5% tween 80 and normal saline instead of MEGG and ISO respectively). Group II toxic control animals were given subcutaneous injection of ISO (6mg/100g) dissolved in 0.1ml normal saline at an interval of 24hrs for 2 days. Group III animals were given PEF (10 mg/kg body weight p.o.), Group IV rats pretreated with PEF (20 mg/kg body weight p.o.) and Group V animals were given PEF (30mg/kg body weight p.o.) for 15 days and on the 16th and 17th days, received ISO (6 mg /100g b.w) subcutaneously. Twenty four hours after the second dose of ISO injection, the animals were sacrificed. PEF pretreated groups (10mg, 20mg and 30mg/kg) showed cardioprotective effect in a dose dependent manner as evidenced by reduced histological changes, compared to ISO myocardial infarcted rats. The effective dose of PEF was 20mg/kg, which showed maximum cardioprotective effect by increasing the antioxidant status, decreasing the serum cardiac marker enzymes, serum cholesterol and triglycerides,
lipid peroxidation and also by the absence of myonecrosis, edema and inflammation in histopathology. LCMS analysis of the petroleum ether fraction of MEGG revealed the presence of oleanolic aldehyde and vernolic acid as active ingredients which in turn may be responsible for its cardioprotective efficacy. Thus, the present study conclude that the root of *G. gummifera* Linn. f. possess excellent antioxidant and cardioprotective properties. These medicinal properties attributed to this root may be due to the combined activity of the identified class of phytochemicals especially oleanolic aldehyde and vernolic acid. The isolation of the active phytochemical constituents from *G.gummifera* root and the determination of their individual antioxidant and cardioprotective activity will be further performed.