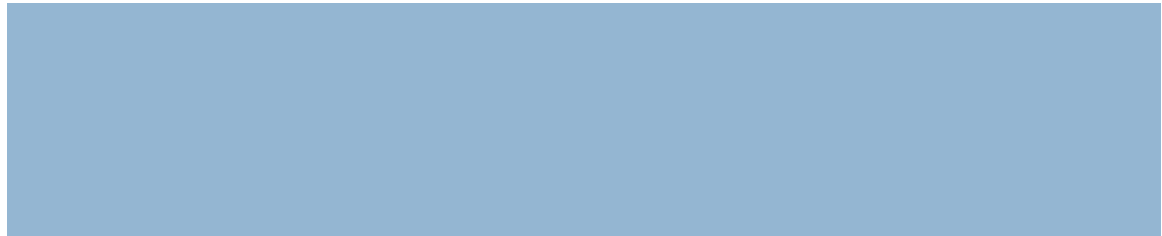


CHAPTER 2

*Cardio Protective effect of
Anethum graveolens L. on
isoproterenol induced
myocardial infarction*



CHAPTER 2**Cardio Protective Effect of *Anethum graveolens* L. on Isoproterenol Induced Myocardial Infarction****INTRODUCTION**

Isoproterenol (ISO) [1-(3, 40-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], a synthetic catecholamine and β -adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscles (Wexler, 1978; Manglik *et al.*, 2015). Some of the mechanisms proposed to explain ISO induced damage to cardiac myocytes includes hypoxia due to myocardial hyperactivity, calcium overload, depletion of energy reserve and excessive production of free radicals resulting from oxidative metabolism of catecholamine (Mohanty *et al.*, 2004 and Hassan *et al.*, 2016).

The pathophysiological and morphological alterations in heart of non-coronary myocardial necrotic rat model are comparable with those taking place in human myocardial infarction (Anandan *et al.*, 2007; Panda and Naik, 2008; Zhou *et al.*, 2008).

Recently, there has been a growing interest in establishing therapeutic potentials of medicinal plants against various diseases. The use of plant extracts for medicinal purposes seems to be more natural, less expensive and without side effects. The biological activities of these plants are due to the presence of various biologically active compounds like vitamins, flavonoids and polyphenols. Hyperlipidemia and oxidative stress has been implicated in the pathogenesis of myocardial infarction also. Therefore therapeutic interventions having hypolipidemic and antioxidant activity may exert beneficial effects against various cardiovascular diseases including

ischemic heart diseases (Bandyopadhyay *et al.*, 2004; Kasa *et al.*, 2015; Khalil *et al.*, 2015; Young and Woodside, 2001).

Anethum graveolens L. (AG; family Apiaceae or Umbelliferae) is an annual aromatic herb that grows in the Mediterranean region, Europe, central and southern Asia and is widely cultivated in southeastern region of Iran (Yazdanparast and Bahramikia, 2008). In India, it is known as 'dill or sowa' and is grown chiefly in the states of Punjab, Uttar Pradesh, Gujarat, Maharashtra, Assam and West Bengal. It is a popular Indian culinary item and traditionally known as a carminative, antispasmodic, sedative, lactagogue and diuretic agent besides serving as a home remedy against hyperlipidemia (Jana and Shekhawa, 2010). Pharmacological properties such as antibacterial (Singh *et al.*, 2001, Lopez *et al.*, 2005, Stavri and Gibbons 2005), antifungal (Stavri and Gibbons, 2005), antioxidant (Singh *et al.*, 2005, Taher *et al.*, 2007, Bahramikia and Yazdanparast, 2008), anti-ulcer (Hosseinzadeh *et al.*, 2002), anticancer (Zheng *et al.*, 1992), anti-diabetic (Panda, 2008), chemopreventive (Zheng *et al.*, 1992), hypoglycaemic (Mishra, 2013) and diuretic (Mahran *et al.*, 1992) have all been accredited to dill in recent times. AG extract has been reported to be rich in flavonoids, phenolic compounds, alkaloids, tannins, saponins and cardiac glycosides (Kaur and arora, 2009). Aqueous extract and essential oils present in AG have been reported to possess hypolipidemic and cardioprotective potentials (Hajhashemi and Abbasi, 2008). Studies on a detailed phytochemical analysis have established the presence of flavonoids (rutin, quercetin), hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid), coumarins (scopoletin), sterols (beta sitosterol/stigmasterol) and mucilages.

In the present study, we evaluated the preventive effect of AG on lipid peroxides, enzymatic, nonenzymatic antioxidants and histopathological findings in normal and ISO-induced myocardial infarction (MI) in male albino Wistar rats.

MATERIAL AND METHODS

Collection and Identification of Plant

As per chapter 1.

Preparation of Extract

As per chapter 1. The extract was re-suspended in 0.5% Carboxy Methyl Cellulose (CMC) and was orally administered to the experimental animals. Control animals were administered 0.5% CMC only.

Experimental Animals

Adult male *Wistar* rats weighing between 150-200 gm were obtained from Zyodus Cadila Research Centre, Ahmedabad, Gujarat, India. They were housed and maintained in clean poly-propylene cages and placed in animal house conditions (temperature: 23±2°C; photoperiod: 12 h light: dark; humidity: 45-50%). They were fed with standard laboratory pellets (M/S Pranav agro, Ltd., Baroda, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India and approved by the animal ethical committee of Department of Zoology, The M. S. University of Baroda, Vadodara (Approval No.827/ac/04/CPCSEA).

Experiment Design

Thirty animals were randomly divided into four groups of six animals in each. Group I (NC) served as control and received 0.5% Carboxy Methyl Cellulose (CMC; p.o.) for 28 days and normal saline (s.c.) on 29th and 30th day. Group II (ISO) served as

disease control and received 0.5 CMC (p.o.) for 28 days and isoproterenol (85 mg/kg body weight, s.c.) on 29th and 30th day. Whereas, the remaining groups (Group IV (ISO+AG200) and group V (ISO+AG400) received 200 and 400 mg/kg body weight of AG extract daily for 28 days (p.o.) respectively and ISO (85 mg/kg, s.c.) on 29th and 30th day. Food and water intake were recorded daily, whereas body weight was recorded every week throughout the study period.

At the end of 30 days, blood samples were collected in ethylene diamine tetra acetic acid (EDTA) coated vials from overnight fasted animals through retro-orbital sinus puncture and plasma was separated by cold centrifugation (Plasto Crafts Superspin-R centrifuge) at 3000 rpm for 10 min. The heart tissue was excised immediately from the animals, rinsed with ice-chilled physiological saline, blotted and weighted. A piece of each tissue was fixed in 10% paraformaldehyde for paraffin histology.

Then, the fresh heart tissues were homogenized in 0.25 M ice-cold sucrose solution at 4 °C. Homogenate was subjected to centrifugation at 600×g for 10 min. Pellet contained structural proteins, nucleus, and cell debris. Then the supernatant was re-centrifuged at 5000×g for 10 min to obtain mitochondrial fraction. After centrifugation pellet was suspended in 0.25M sucrose solution.

Assay of Cardiac Marker Enzymes

Activity levels of creatine phosphokinase- MB (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and uric acid levels were determined by using commercially available kits (Reckon Diagnostic Ltd., Vadodara, India).

Plasma Lipid Profile

Triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL-C) content were assayed by using commercially available kits (Recon Diagnostic, Ltd., Vadodara, India). Very low-density lipoprotein (VLDL-C) and low-density lipoprotein (LDL-C) were calculated by Friedewald's formula.

Cardiac Antioxidants and Lipid Peroxidation

Enzymatic anti-oxidants such as super oxide dismutase (SOD; Markund and Marklund, 1974), catalase (CAT; Aebi *et al.*, 1983) and glutathione peroxidase (Gpx; Rotruck *et al.*, 1979) were assessed. Also, contents of non-enzymatic anti-oxidants, reduced glutathione (GSH; Beutler, 1963) total protein (Lowry *et al.*, 1951) and lipid peroxidation levels (LPO; Buege and Aust, 1978) were assayed in the supernatant of control and treated cardiac tissue homogenate. The ascorbic acid (AA; Roe *et al.*, 1943) content was estimated using 6% trichloro acetic acid of control and treated rats.

Cardiac ATPases

The pellet after centrifugation of tissue homogenates were re-suspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of Na⁺ K⁺ ATPase (Bonting, 1970), Ca²⁺ ATPase (Hjerken and Pan, 1983)

and Mg^{2+} ATPase (Ohnishi *et al.*, 1982). Protein was estimated according to the method of Lowry *et al.*, (1951).

Macroscopic and Microscopic Evaluation of Cardiac Tissue

Fresh cardiac tissue slices (approx. 2-3 mm thick) were transversely cut across the ventricle, kept in a covered glass dish containing of 1% TTC (2, 3, 5-triphenyltetrazolium chloride; Sigma, St. Louis, MO) solution and incubated at 37°C for 20 min. Viable tissue was stained brick red due to the formation of a formazan precipitates whereas, infarcted area was pale in colour (Lie *et al.*, 1975).

Statistical Analysis

Statistical analysis of data was done by one way ANOVA followed by Bonferroni's multiple comparison test and results were expressed as mean \pm S.E.M (Using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA).

RESULTS

Plasma Markers of Cardiac Damage

ISO treated rats recorded significant ($p<0.001$) increment in the plasma CK-MB, LDH, AST, ALT and uric acid levels compared to NC rats. Whereas, the rats pretreated with AG (200 and 400 mg/kg body weight) prevented the ISO induced increase in the serum levels of these parameters in a dose dependent manner (Table 2.1 and Figure 2.1).

Plasma Lipid Profile

ISO treated group recorded significant ($p<0.001$) increase in plasma TG, TC, LDL, and VLDL and decrement in HDL levels compared to the NC group. Whereas, AG pretreated groups showed dose dependent decrement in TC, TG, LDL, VLDL and significant increment in HDL levels compared to ISO treated rats (Table 2.2 and Figure 2.2).

Cardiac Tissue Anti-oxidants and LPO

ISO treated group recorded significant ($p<0.001$) increment in LPO level, as well as a significant ($p<0.001$) decrement in the activities of enzymatic antioxidants (SOD, CAT, GPx and GST) and non-enzymatic antioxidants (GSH and AA) in cardiac tissue as compared to NC rats. Whereas, the rats pretreated with AG seed extract (200 and 400 mg/kg body weight) markedly prevented all the alterations of antioxidants and LPO levels in ISO treated rats and maintained them to the near normal levels (Table 2.3 and Figure 2.3).

Cardiac ATPase

Rats treated with ISO showed significant ($p < 0.001$) decrement in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in the heart tissue homogenate compared to NC rats. Whereas, ISO+AG groups recorded significant increment in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase compared to ISO rats (Table 2.4 and Figure 2.4).

TTC staining of cardiac tissue

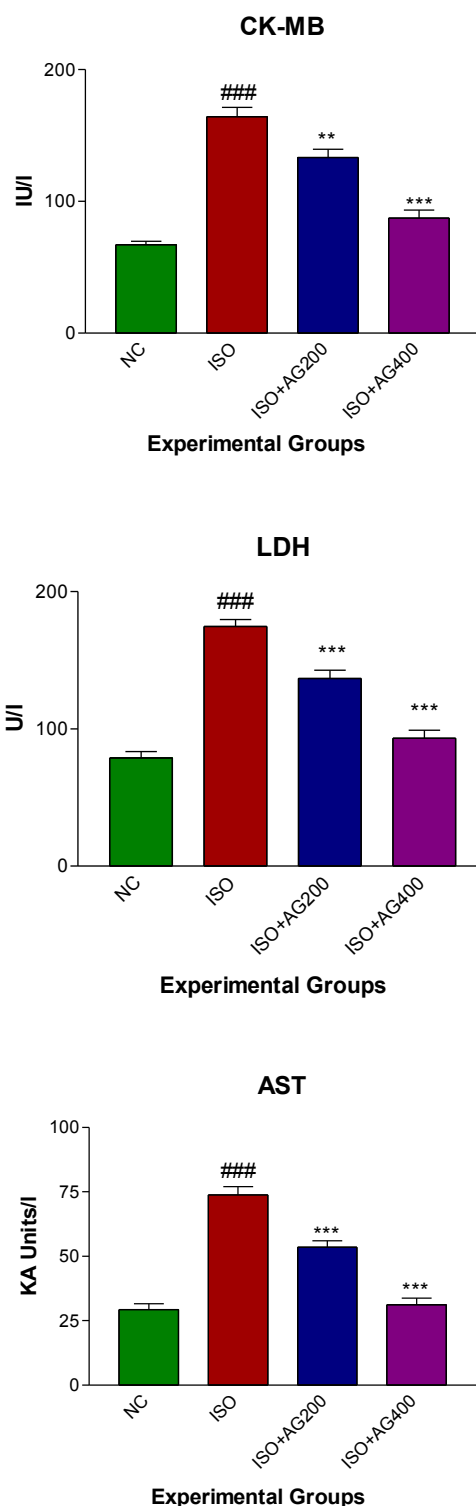
TTC staining of heart of control and treated rats showed brick red coloration in control rats indicating more number of viable cells and a pale yellow coloration in ISO treated cardiac slices suggestive of infarction. However, pretreatment of ISO rats with AG showed a decrement in pale yellow coloration (Figure 2.5).

TABLES AND FIGURES**Table 2.1: Effect of *Anethum graveolens* L. on Plasma Markers of Cardiac Damage**

Parameters	NC	ISO	ISO + AG200	ISO + AG400
CkMB^s	67.16 ± 2.39	164.2 ± 7.01 ^{###}	133.2 ± 6.32 ^{**}	87.23 ± 6.15 ^{***}
LDH^a	78.83 ± 4.51	174.5 ± 5.26 ^{###}	136.8 ± 5.95 ^{***}	93.33 ± 5.70 ^{***}
AST^b	29.33 ± 2.20	73.83 ± 3.17 ^{###}	53.50 ± 2.54 ^{***}	31.17 ± 2.62 ^{***}
ALT^b	20.17 ± 2.28	56.50 ± 4.03 ^{###}	36.83 ± 2.00 ^{***}	25.17 ± 2.63 ^{***}
Uric acid[@]	2.012 ± 0.22	6.501 ± 0.47 ^{###}	4.346 ± 0.36 ^{**}	3.358 ± 0.25 ^{***}

Where, \$=IU/l, a=U/l, b= Unit/L, @=mg/dl. n=6. Data were expressed as mean ± S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), * (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

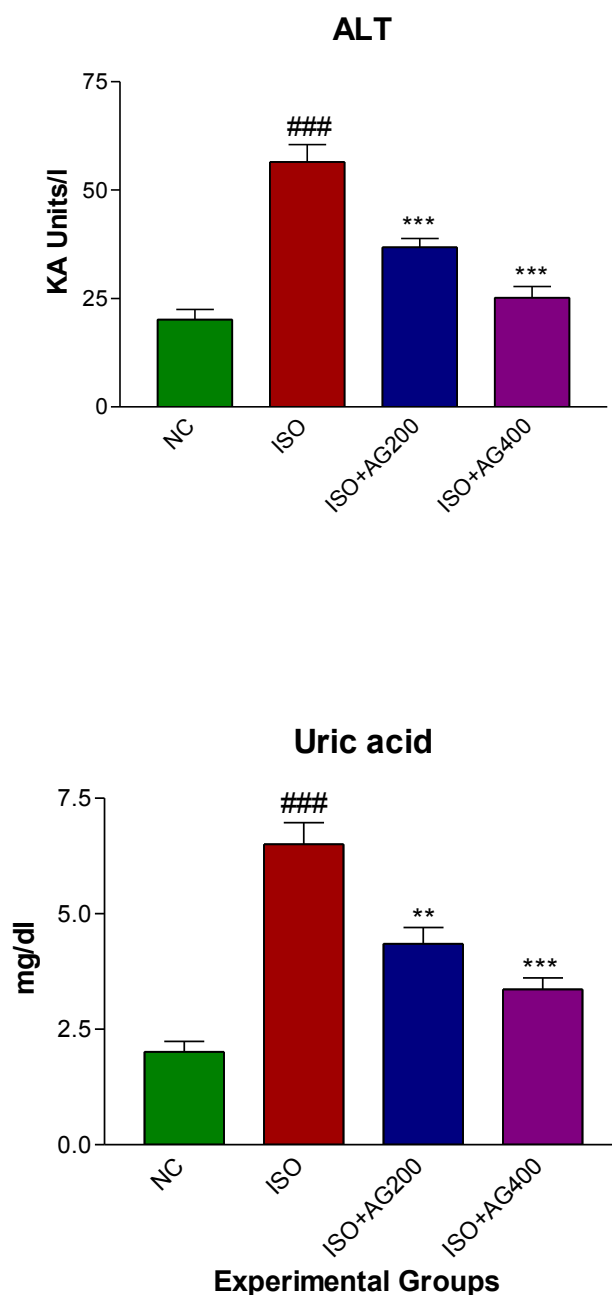
Figure 2.1: Effect of *Anethum graveolens* L. on Plasma Markers of Cardiac Damage



Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body

weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.1 (Continued)

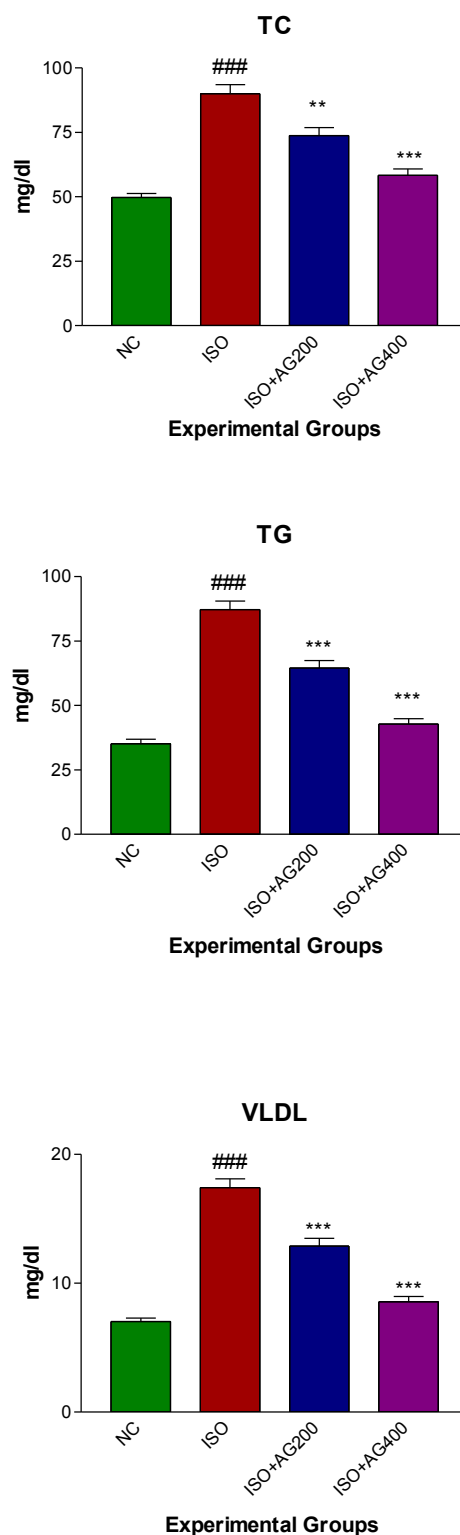


Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Table 2.2: Effect of *Anethum graveolens* L. on Plasma Lipid Profile

Parameters	NC	ISO	ISO + AG200	ISO + AG400
TC[@]	49.80 ± 1.46	90.00 ± 3.57 ^{###}	73.80 ± 3.07 ^{**}	58.40 ± 2.42 ^{***}
TG[@]	35.17 ± 1.80	87.17 ± 3.39 ^{###}	64.50 ± 2.97 ^{***}	42.83 ± 2.05 ^{***}
VLDL[@]	7.03 ± 0.26	17.43 ± 0.67 ^{###}	12.90 ± 0.59 ^{***}	8.56 ± 0.41 ^{***}
LDL[@]	1.84 ± 0.05	3.34 ± 0.13 ^{###}	2.25 ± 0.09 ^{***}	1.91 ± 0.07 ^{***}
HDL[@]	20.00 ± 1.26	9.50 ± 1.17 ^{###}	15.00 ± 0.73 ^{**}	17.33 ± 1.33 ^{**}

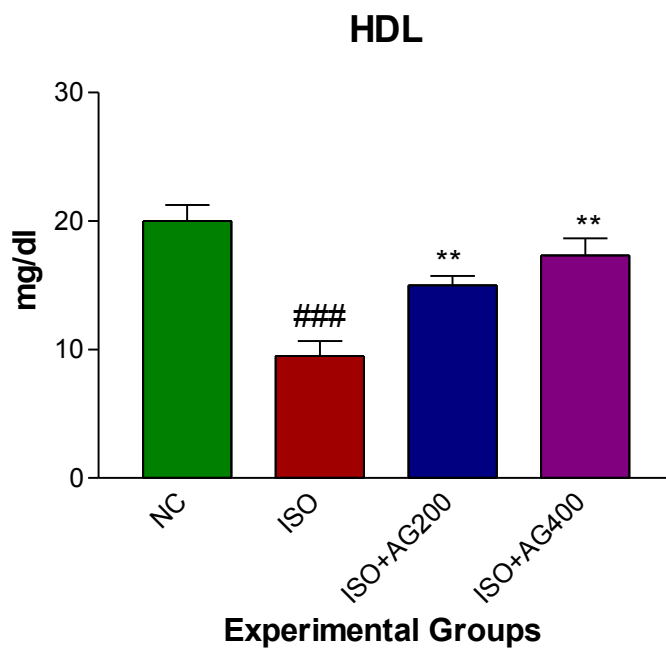
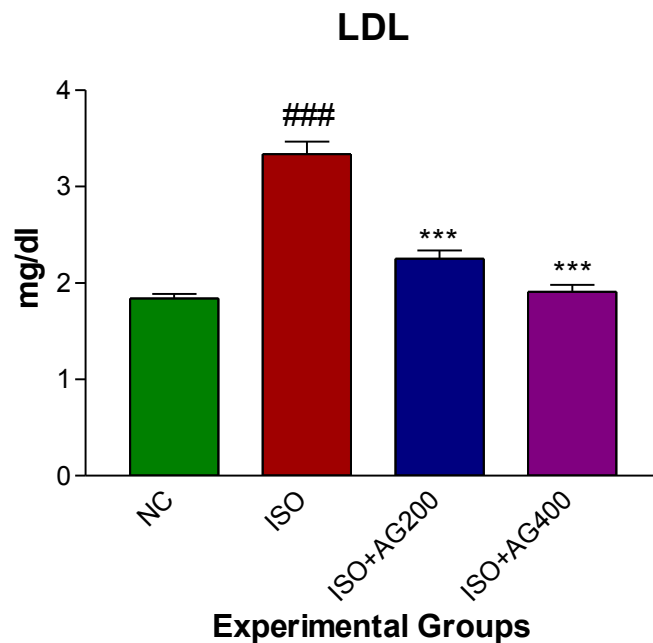
Where, @=mg/dl. n=6. Data were expressed as mean ± S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.2: Effect of *Anethum graveolens* L. on Plasma Lipid Profile

Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body

weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.2 (Continued)



Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body

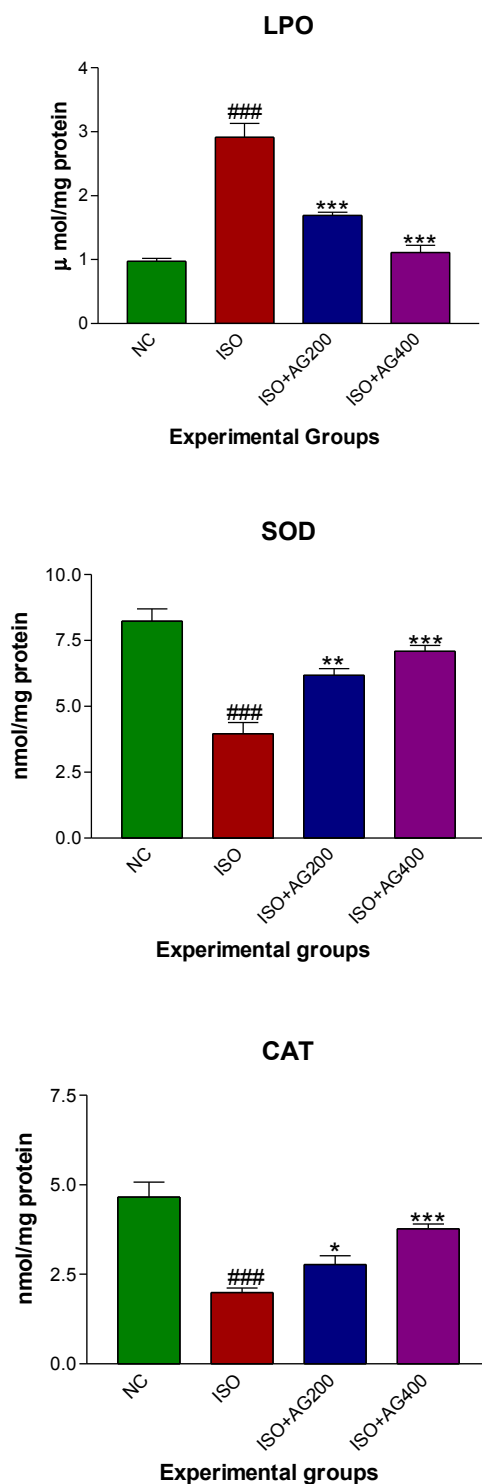
weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Table 2.3: Effect of *Anethum graveolens* L. on Cardiac LPO Levels and Enzymatic and Non-enzymatic Anti-oxidant

Parameters	NC	ISO	ISO + AG200	ISO + AG400
LPO^a	0.97 ± 0.05	2.91 ± 0.22 ^{###}	1.69 ± 0.05 ^{***}	1.11 ± 0.11 ^{***}
SOD[@]	8.23 ± 0.47	3.95 ± 0.44 ^{###}	6.18 ± 0.25 ^{**}	7.09 ± 0.22 ^{***}
CAT[@]	4.66 ± 0.41	1.99 ± 0.13 ^{###}	2.77 ± 0.25 [*]	3.77 ± 0.13 ^{***}
GPx^{\$}	3.33 ± 0.12	1.35 ± 0.21 ^{###}	2.01 ± 0.13 [*]	3.07 ± 0.17 ^{***}
GST^b	791.2 ± 27.09	470.5 ± 37.46 ^{###}	664.5 ± 28.61 ^{**}	776.7 ± 28.06 ^{***}
GSH[@]	10.05 ± 0.75	4.77 ± 0.28 ^{###}	6.27 ± 0.21 ^{**}	8.09 ± 0.15 ^{***}
AA[€]	212.2 ± 6.48	122.5 ± 5.24 ^{###}	160.3 ± 4.07 ^{***}	196.0 ± 4.92 ^{***}

^a=μmol/mg protein, [@]=nmol/mg protein, ^{\$}=unit/mg protein, ^b=μmol/min/mg protein, [€]= mg/100 g tissue Where, n=6. Data were expressed as mean ± S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

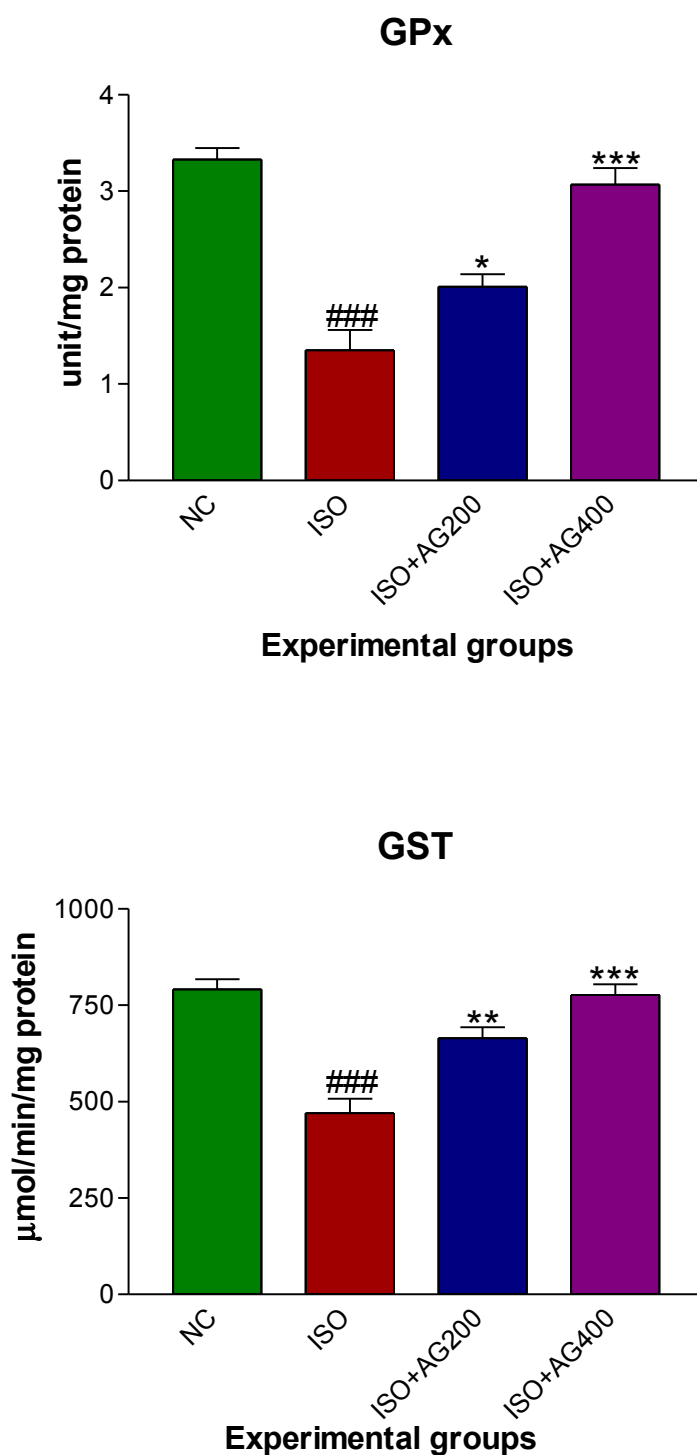
Figure 2.3: Effect of *Anethum graveolens* L. on Cardiac LPO Levels and Enzymatic and Non-enzymatic Anti-oxidant



Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body

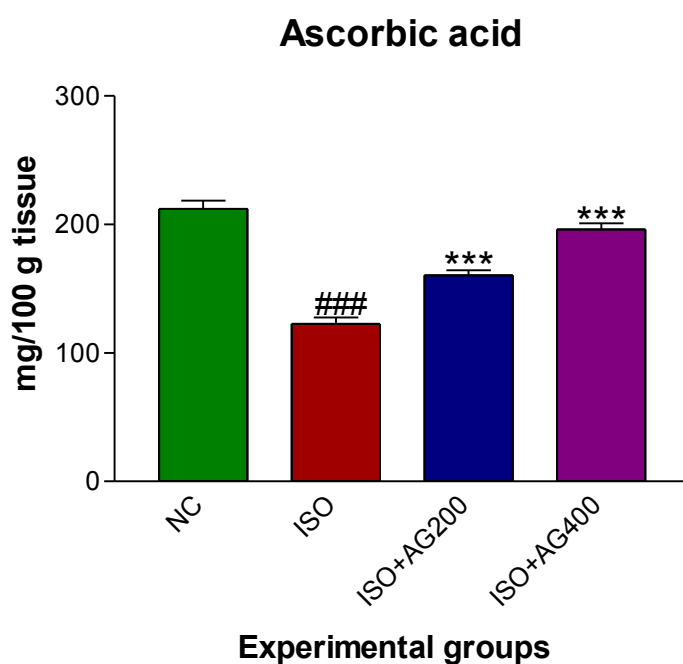
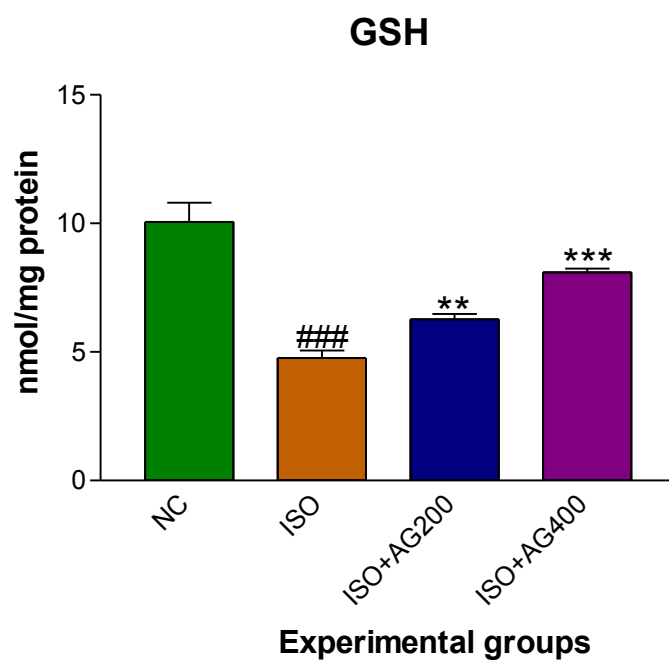
weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.3 (Continued)



Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.3 (Continued)

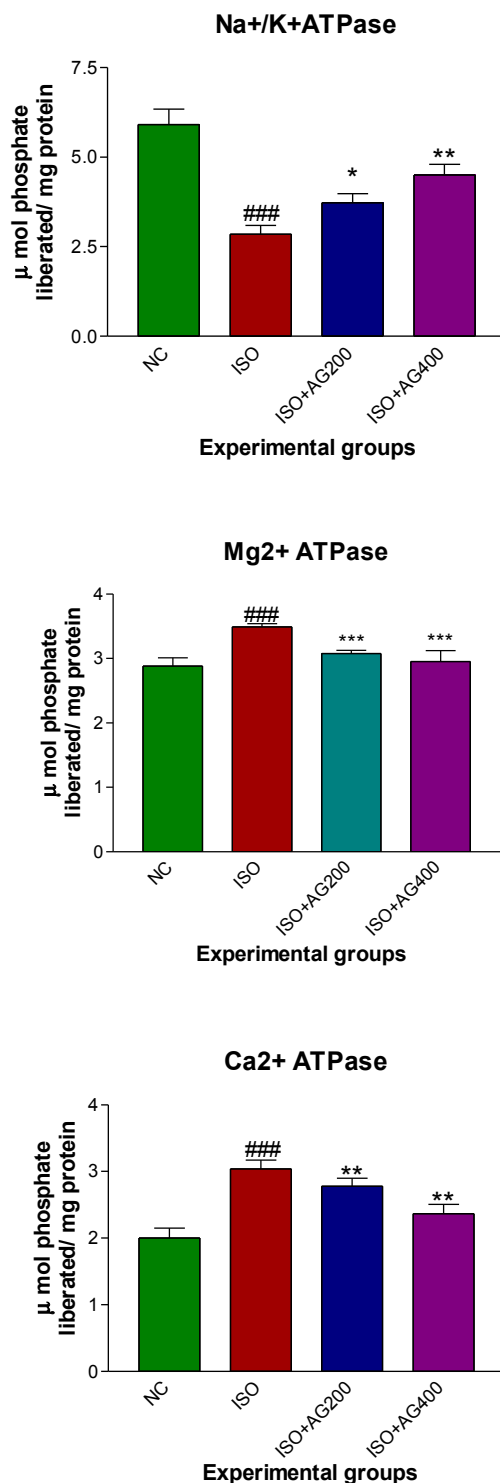


Where, n = 6, Data were expressed as mean \pm S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Table 2.4: Effect of *Anethum graveolens* L. on Cardiac ATPases

Parameters	NC	ISO	ISO + AG200	ISO + AG400
Na⁺/K⁺ATPase[@]	5.91 ± 0.43	2.85 ± 0.25 ^{###}	3.73 ± 0.25 [*]	4.50 ± 0.30 ^{**}
Mg²⁺ ATPase[@]	2.88 ± 0.13	3.49 ± 0.052 ^{###}	3.08 ± 0.048 ^{***}	2.95 ± 0.17 ^{***}
Ca²⁺ ATPase[@]	2.00 ± 0.15	3.04 ± 0.13 ^{###}	2.78 ± 0.12 ^{**}	2.37 ± 0.14 ^{**}

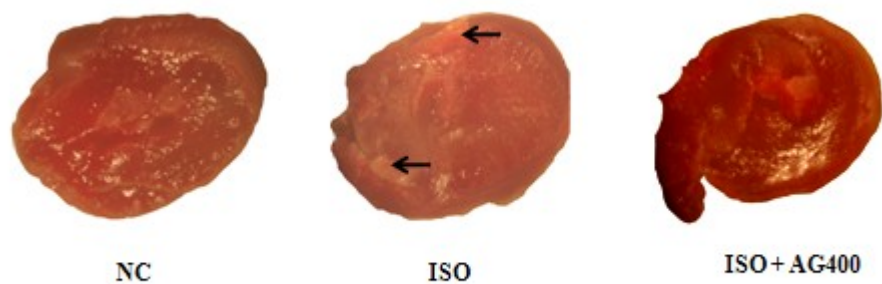
Where, @ = μmol phosphate liberated/ mg protein, n=6. Data were expressed as mean ± S.E.M. # (p<0.05), ## (p<0.01), ### (p<0.001) when NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG (Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.4: Effect of *Anethum graveolens* L. on Cardiac ATPases

Where n=6. Data were expressed as mean \pm S.E.M. ### (p<0.001) NC (Normal Control) vs. ISO (Isoproterenol, 85 mg/kg body weight) and * (p<0.05), ** (p<0.01), *** (p<0.001) when ISO (Isoproterenol, 85 mg/kg body weight) vs. ISO + AG

(Isoproterenol, 85 mg/kg body weight + *Anethum graveolens*, 200 and 400 mg/kg body weight).

Figure 2.5: Effect of *Anethum graveolens* L. on Cardiac Tissue



Cardiac tissue slices were stained with triphenyltetrazolium chloride (TTC). Arrows indicate necrotic tissue

DISCUSSION

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reactions in cellular mechanisms during myocardial ischemia (Singal *et al.*, 1983). In the present study, injection of ISO induced a significant increase in lipid peroxidation in group ISO which has been suggested to be due to an enhanced oxidative stress in experimentally induced myocardial injury. Lipid peroxidation of membranes is regulated by the availability of substrate in the form of poly unsaturated fatty acid (PUFA), the availability of inducers, such as free radicals and excited state molecules, to initiate propagation, the antioxidant defence status of environment, and the physical status of the membrane lipids (Anandan *et al.*, 1998).

When myocardial cells are damaged or destroyed, the cell membrane becomes permeable or may rupture and result in the leakage of enzymes. Although the diagnostic marker enzymes of MI are CK, LDH, AST and ALT (Khalil *et al.*, 2015 and Farvin *et al.*, 2004), levels of CK-MB has been the gold standard for diagnosis of MI recently due to being more sensitive and specific markers in the determination of myocardial cell injury (Geetha and Sanker, 1990). We have observed a significant increase in the activities of ALT, AST, LDH, CK and CK-MB in plasma after ISO-induction in rats when compared with that of intact control group. The release of cellular enzymes reflects the alterations in plasma membrane integrity and/or permeability as a response to β -adrenergic stimulation. This might be due to the damage caused to the sarcolemma by the β -agonist that has rendered it leaky. ISO-induction produces free radicals through β -adrenoceptor mechanism, which affects the cell metabolism to such a degree that cytotoxic free radicals are formed, producing myocardial necrosis (Priscilla and Prince, 2009). Elevated activities of the cardiac

marker enzymes and TnI levels predict the risk of both cardiac death and subsequent infarction. Our results are consistent with the previous reports by Suchalatha and Shyamaladevi (2004) and Acikel *et al.* (2005). Pre-treatment treatment with AG decreased the activities of these enzymes in serum which increased as a result of ISO-application to rats. This could be due to the protective effect of AG on the myocardium, reducing the cardiac damage, thereby restricting the leakage of these enzymes. While the protective effects of AG groups were significant when compared to the control group.

Alterations in lipid profile following ISO treatment were significant as evidenced by high levels of TG, TC, LDL and VLDL. However, ISO+AG200 and ISO+AG400 treatment groups accounted for decrement in the said parameters with the lipid profile of the latter being comparable to that of the control group. Significant increment in circulating levels of HDL was recorded in ISO+AG400 treated group. Since hypocholesteremic agents cause reduction of both HDL and LDL levels (Guo *et al.*, 2015) significant reduction in LDL and significant increment in HDL levels observed in ISO+AG400 treated group are of considerable relevance in management of lipids based myocardial damage. These observations are in agreement with Anosike and Cajetan (2015) who had demonstrated cardio protective potential of polyphenol rich extract in preventing ISO mediated perturbations in lipid profile and related enzymes (HMG CoA and LCAT).

Cells or tissues oxidative stress results in the generation of reactive oxygen species (ROS) and/or depletion of the antioxidants in the defence system, thereby causing an imbalance between the pro-oxidants and antioxidants. The enzymatic and non-enzymatic antioxidant system scavenges ROS generation in cells and tissues. Free radical scavenging enzymes SOD, catalase, GPx and GST make primary defence

system against oxidative stress. SOD plays an important role in protecting the cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized by Catalase to molecular oxygen and water. Superoxide radicals generated at the site of damage modulates SOD and Catalase resulting in the decreased activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium (Rahman and MacNee, 1999 and Erejuwa *et al.*, 2011). AG pre-treatment significantly increased the activities of SOD and catalase in the cardiac tissue of ISO treated rats. In our study, we recorded decreased concentration of GSH in heart and decreased activities levels of glutathione dependent enzymes such as GPx and GST in the heart of ISO treated rats. GSH is an abundant and ubiquitous antioxidant, a tripeptide and essential biofactor synthesized in all living cells. It functions mainly as an effective intracellular reducing agent (Rahman and MacNee, 1999 and Erejuwa *et al.*, 2011). It protects the cells from free radical mediated damage caused by drugs and ionizing radiation. It forms an important substrate for GPx, GST and several other enzymes, which is involved in the free radical scavenging action (Jagetia *et al.*, 2004). In cardiac tissue, prolonged depressions in the activity of GPx may lead to the intracellular peroxide accumulation. GST acts like peroxidase and removes the stable peroxides from the system, resulting in the reduction of peroxide-induced damage (Jagetia *et al.*, 2004). The unavailability of GSH may decrease the activity levels of GPx and GST in ISO-treated rats. Other research groups have shown that, pre-treatment with naringin significantly improved GSH, GPx and GST in the cardiac of ISO-induced rats (Jeond *et al.*, 2002). Kim *et al.*, (2009) had reported that the antioxidant effect of naringin is similar to that of GSH, and furthermore, it inhibits the hydrogen peroxide-induced lipid peroxidation. Our findings are in agreement and comparable with these studies and hence it can be

summarised that AG mediated prevention of oxidative damage and depletion of intracellular antioxidants is attributable to its reported free radical scavenging potential.

ISO treated rats showed a significant decrement in the activity of Na^+/K^+ ATPase and significant increment of Mg^{2+} ATPase and Ca^{2+} ATPase activities in the cardiac tissue. Decreased activity of Na^+/K^+ ATPase could be due to ISO induced enhanced production of free radicals and subsequent lipid peroxidation. Na^+/K^+ ATPase is a 'SH' group containing enzyme and is lipid dependent (Govindan *et al.*, 2016). Decreased activity of Na^+/K^+ ATPase can lead to a decrease in sodium efflux, thereby altering membrane permeability (Finotti and Palatini, 1986). Also, the calcium pump activity is regulated by Ca^{2+} ATPase (Lam *et al.*, 1994) and hence, enhanced Ca^{2+} ATPase activity was observed in ISO treated rats. Calcium overload in the myocardial cells during ischemia activates the Ca^{2+} -dependent ATPase of the membrane depleting high energy phosphate stores, thereby indirectly inhibiting Na^+ and K^+ transport and inactivation of Na^+/K^+ ATPase (Ithayarasi and Devi 1997). Mg^{2+} ATPase activity is involved in other energy requiring process in the cell and its activity is sensitive to lipid peroxidation. AG pre-treatment normalized the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in ISO treated rats. Restoration of Na^+/K^+ ATPase activity due to AG pre-treatment in ISO treated rats was instrumental in regulating the intracellular Ca^{2+} levels, thereby protecting the myocardium from excess damage. Hence, protective effect of AG could be due to its ability to inhibit oxidation of SH group of an enzyme and peroxidation of membrane lipid. Thus, protective effect of AG on these channels may be due to membrane stabilizing properties, as reported by Saffari and Sadrzadeh (2004).

Triphenyl tetrazolium Chloride (TTC) is a redox indicator that is commonly used to differentiate between metabolically active and inactive cells and tissues. Appearance of patches of pale white colour in cardiac tissue slices of ISO treated rats indicates areas of focal necrosis due to non-reduction of TTC as observed in the present study. However AG (400mg/ml) pre-treatment showed brick red coloration which was significantly comparable to control group. These observations are in agreement with other data envisaged herein and provide conclusive evidence on cardioprotective potential of AG in ISO induced experimental model of myocardial infarction. Hence, it can be concluded that AG showed cardioprotective potential by reducing lipid peroxidation, strengthening endogenous antioxidant defence system and preventing histopathological damages.

SUMMARY

Isoproterenol (ISO) a synthetic catecholamine and β -adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscles. In the present study, we evaluated the preventive effect of AG on lipid peroxides, enzymatic, nonenzymatic antioxidants and histopathological findings in normal and ISO-induced myocardial infarction (MI) in male albino Wistar rats. AG pretreatment prevented the ISO induced increase in the serum levels of CK-MB, LDH, AST, ALT and uric acid levels in a dose dependent manner. AG pretreatment also improved lipid profile and prevented decrement in enzymatic (SOD, CAT, GPx and GST) and non-enzymatic (GSH and AA) antioxidants in cardiac tissue. AG extract decrease lipid peroxidation levels of cardiac tissues. Increment in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{+2} ATPase of cardiac tissues indicated the protective effects of AG extract on ISO treatment. Based on the results of present study it can be concluded that the presence of antioxidants in AG extract prevented myocardial damage.