Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(12):720-727

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Ocimum sanctum Linn. leaves ameliorates cardiac toxicity by enhancing paraoxonase 1 activity and expression of heart type fatty acid binding protein

Kavitha S., Febi John and M. Indira*

Department of Biochemistry, University of Kerala, Thiruvanathapuram, Kerala, India

---

ABSTRACT

Ocimum sanctum (OS) Linn.herb, commonly known as 'Tulsi' has been recommended for the treatment many disorders, because of its antioxidant, hypolipidemic, anti-inflammatory and various other pharmacologic effects. The present study was focussed on the effect of methanol extract of OS leaves (MOS), on the changes in lipid profile, activity of paraoxonase 1 (PON1) and mRNA expression of heart type fatty acid binding protein (H-FABP) in isoproterenol (ISP) induced cardiac damage. Rats were grouped as follows: 1) Control, 2) MOS (15mg/100g b.wt.), 3) ISP (10mg/100g b.wt.), 4) MOS(15mg/100g b.wt.) +ISP (10mg/100g b.wt.). MOS was administered every day for 30 days. ISP was administered at 29th and 30th day to induce myocardial damage. At the 31st day, all animals were sacrificed and various parameters were studied. ISP treatment caused a significant elevation in the activities of serum cardiac markers and reduction in the expression of H-FABP in heart tissue, which was reversed on MOS pre-treatment. MOS pre-treatment also caused reduction in the infarct size in the myocardium after ISP induction. The serum and tissue lipid profiles were reduced, while the serum high density lipoprotein cholesterol was increased by the pre-treatment of MOS. The activity of cardioprotective enzyme PON1 was also increased on MOS pre-treatment. MOS administration caused a reduction in the activities of the enzymes HMG CoA reductase and phospholipases. Hence it can be concluded that MOS possesses significant hypolipidemic and cardioprotective effect.

Key words: Cardiovascular diseases; lipid profile; PON 1; H-FABP; Infarct size.

INTRODUCTION

Cardiovascular disease (CVD), a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects[1].Lipids play an important role in CVD, by modifying the composition, structure and stability of cell membranes. An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death that follows coronary artery occlusion[2]. Studies have shown that high levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and low levels of high density lipoproteins cholesterol (HDL-C) are the risk factors of CVD [3].

Paraoxonase-1 (PON1) belongs to a family of high-density lipoprotein (HDL)associated enzymes that show hydrolytic activity towards a variety of substrates, including oxidized lipids in the body [4].PON1 is synthesized in the liver and bound to plasma HDL in the circulation. PON1 contributes to the anti-inflammatory and anti-oxidant properties of HDL-C [5]. PON1 reduces inflammatory plaque formation and protects against macrophage mediated LDL oxidation[6].PON1 activity is decreased in coronary artery disease [7].

Heart-type specific fatty acid binding protein (H-FABP) is a potential marker for the early diagnosis of acute MI [8]. H-FABPs are relatively small cytoplasmic proteins(12–15 KDa) which are found abundantly in heart, where the active fatty acid metabolism occurs and it is involved in the intracellular transport of insoluble fatty acids [9].

Isoproterenol (ISP) is a synthetic catecholamine and β-adrenergic agonist which has been found to cause severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle [10]. Studies demonstrated that isoproterenol can cause alterations in the serum and tissue lipid profile in experimental animals[2, 11]. The free radicals generated by ISP are a causative factor for irreversible damage to the myocardial membrane [12]. ISP induced cardiac necrosis include increased oxygen consumption, insufficient oxygen utilization, increased calcium overload and accumulation, changes in myocardial cell metabolism, increased myocardial cAMP levels, deranged electrolyte milieu, alterations of membrane permeability, intracellular acidosis and increase in lipid profile [13].

Ocimum sanctum Linn. (belonging to the family *Labiatae*), commonly known as 'Tulsi' is a traditional medicinal plant widely used in ayurvedic medicines [14]. Pharmacological effects of extracts of various parts of tulsi plant was studied on immune system, reproductive system, central nervous system, cardiovascular system, gastric system, urinary system and blood biochemistry, which revealed the therapeutic significance of tulsi in the management of various ailments[15]. *Ocimum sanctum* (OS) contain potent antioxidants, flavonoids (orientin, vicenin) and phenolic compounds (eugenol, cirsilineol, apigenin) [16]. The leaves of the plant have been shown to possess good antioxidant as well as anti-stress potentials in experimental animals [17].An earlier study by Suanarunsawat reported the lipid lowering effect of essential oils extracted from OS leaves in high fat diet fed rats [18]. But there are no reports on the mechanism of action of OS leaves. Hence our objective of the study was to evaluate the efficacy of methanol extract of OS leaves on ISP induced changes in lipid profile, activity of PON 1 and the expression of H-FABP.

EXPERIMENTAL SECTION

Preparation of 50% methanol extract of *Ocimum sanctum* leaves (MOS)

The leaves of *Ocimum sanctum* were collected from Trivandrum, India. The plant was authenticated by Dr. Valsaladevi, Curator, Department of Botany, Kerala University. The identified and authenticated specimen was deposited in the herbarium of the Department of Botany, University of Kerala (Voucher No: KUBH5840). Fresh leaves were collected, washed and dried in shade. Dried powdered leaves (10 g) were mixed with 100 ml of 50% methanol. This was refluxed in a water bath for 1½ h at 60-65°C. The whole extract was filtered and defatted using petroleum ether and it was concentrated using a rotary flash evaporator. The yield of MOS was 15.16%.

Experimental design

Male albino rats (Sprague Dawley strain) weighing between 200-250 g bred and reared in our animal house were used for the experiment. A total of 24 rats were divided into 4 groups of 6 rats each.

Group I (CON) : Control
Group II (MOS) : MOS (15 mg/100 g b.wt.)
Group III (ISP) : ISP control (10 mg/100 g b.wt.)
Group IV (MOS+ISP) : MOS (15 mg/100 g b.wt.) + ISP (10 mg/100 g b.wt.)

Animals were housed in polypropylene cages kept in a room maintained at 28-32°C. The light cycle was 12 h light and dark. The animals were handled using laboratory animal welfare guidelines [19]. Rats were fed with standard laboratory diet supplied by Ashirwad Pvt Ltd., India and water was given *ad libitum*. MOS at a dose of 15 mg/kg b.wt. suspended in distilled water was given by gastric intubation for 30 days. ISP was purchased from Sigma Aldrich, India. The dose of ISP was selected from a previous study [15]. ISP at a dose of 10 mg/100 g b.wt. in physiological saline was injected subcutaneously on the 29^{th} and 30^{th} day at an interval of 24 h. On the 31^{st} day animals were sacrificed. Heart was dissected out and blood was collected for the analysis of various parameters. Animal experiments were approved by the Institutional Animal Ethics Committee [IAEC No-KU-13-2011-BC-MI (30)].

Determination of the area at risk and myocardial infarct size

After the induction of myocardial infarction, the area at risk (AAR) and the size of the infarct were determined using a staining technique. A previously described double-staining technique with Evans blue and 2, 3, 5-triphenyl-tetrazolium chloride (TTC) was used [20, 21]. At first, the AAR was determined by retrograde injection of 2 ml of 0.1% Evans blue dye into the aorta. Thus all myocardial tissue was stained blue, except the AAR. Briefly, a plastic catheter filled with heparinized normal saline was surgically inserted into the abdominal aorta. It is critical for this step to avoid air bubbles within the catheter, as they would be injected into the coronary circulation and prevent Evans blue staining. Evans blue solution was then injected retrogradely into the aorta [21]. The heart was excised and washed in ice-cold 0.9% saline. The Evans blue-stained hearts were then frozen (-20°C), cut into approximately 3 mm thick slices, incubated in 1% TTC, dissolved in Krebs buffer for 10 min and then in 3% formaldehyde for 15 min. Slices were then scanned between glass plates. The AAR and the infarct size were quantified by planimetry

(Image J, National Institutes of Health, Bethesda, MA, USA) [22]. Infarct size was expressed as the percentage of infarcted tissue within the AAR zone (infarct size as % AAR zone).

Biochemical Analysis

The activities of cardiac markers creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) were estimated by using kits from Reckon diagnostics Pvt Ltd., India. Serum total cholesterol (TC), triglycerides and HDL-cholesterol (HDL-C) were estimated by using kits from Agappe diagnostics, India. Concentration of LDL-cholesterol (LDL-C) was calculated using the formula [LDL =TC –(TG/5 +HDL)][23].PON1 activity was measured by the method of Mackness[24].3-hydroxy-3-methyl glutaryl CoA(HMG CoA) reductase activity in liver tissue was analysed by the method of Rao and Ramakrishnan [25]. The heart tissue was extracted for lipid estimation according to the procedure of Folch et al [26]. The cholesterol was estimated by the method of Abellet al [27]. Triacylglycerolwas estimated by the method of Van Handel and Zilversmith [28]. Free fatty acids (FFA)were estimated according to the procedure of Falholtet al [29]. The activity of phospholipase A (PLA) was assayed by the method of Rimon and Shapiro [30]. Phospholipase C (PLC) was assayed by the method of Kleiman and Lands [31] and phospholipase D (PLD) by the method Mollerung and Bergmayer [32].

Total RNA isolation

Total RNA was isolated from the heart using TRI Reagent (Sigma Aldrich) by the method described by Chomczynski and Sacchi [33].

Reverse Transcription PCR

The isolated RNA was used for reverse transcriptase-polymerase chain reaction (RT-PCR) to quantify the expression. Total RNA was reverse transcribed and PCR was performed using Eppendorff RT-PCR kit with gene specific primers. Primer sequences for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), H-FABP are given in table 1. The PCR mixture contained 10 mM Tris (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, dNTP at 20mM each and gene specific primers at 0.5mM each and 0.025 units/µl Taq polymerase. After an initial denaturation step at 94°C, 35 amplification cycles were performed. A final extension step of 5 minutes at 72°C was performed in order to complete the PCR reaction.

PCR mixture was resolved on 2% agarose gel containing ethidium bromide. Then the gels were subjected to densitometric scanning (Bio-Rad Gel Doc, California, USA) to determine the OD of each and then normalized against GAPDH (internal control) using quantity One imaging software.

Table 1: Primer sequences for RT-PCR analysis

Genes	Primer sequences	Accession number
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)	Forward 5' TGACAACTCCCTCAAGATTGTCA 3' Reverse 5' GGCATGGACTGTGGTCATGA 3'	NM 017008.4
Heart type fatty acid binding protein (H-FABP)	Forward 5' GACAGGTGGCTAGCATGACC 3' Reverse 5' GTCCCACTTCTGCACATGGA 3'	NM 024162.1

Statistical analysis

The results were analysed using a statistical programme SPSS/PC+, Version 17 (SPAA Inc., Chicago, IL, USA). One way ANOVA was employed for comparison among the groups. Duncan's post-hoc multiple comparison tests of significant differences among groups were determined, $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Table 2: Activities of LDH, CK-MB in serum

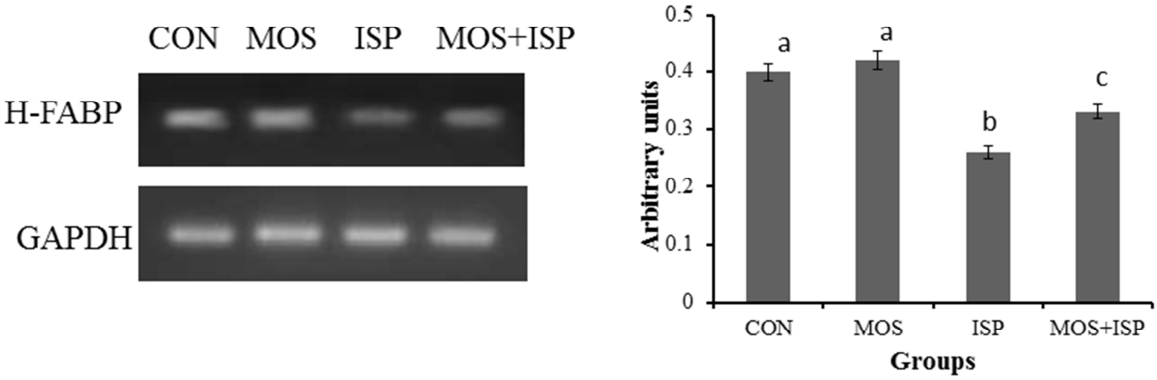
Groups	LDH (IU/L)	CK-MB (IU/L)
CON	152.40±5.97 [a]	175.10±6.52[a]
MOS	149.61±5.86 [a]	172.61±6.43[a]
ISP	229.62±8.99 [b]	351.40±13.09[b]
MOS+ISP	179.55±7.03 [c]	280.62±10.46[c]

Values are expressed as mean ±SEM.
Mean values with same superscript do not differ significantly, p<0.05

Cytosolic enzymes CK-MB, LDH, which serve as the diagnostic markers of myocardial damage, leak out from the damaged tissue to blood stream when cell membrane becomes permeable or rupture [34]. In accordance with this the serum activities these cardiac markers were significantly elevated in ISP treated rats compared to the control (table 2). This indicated the cardiac damage caused by ISP. MOS pre-treatment significantly reduced the activity of these enzymes in the serum compared to ISP group, which revealed the lesser damage of the myocardium in MOS+ISP

group. Previous study by Panda et al. also reported the effect of hydro alcoholic extract of OS leaves in reducing the serum levels of cardiac markers [35].

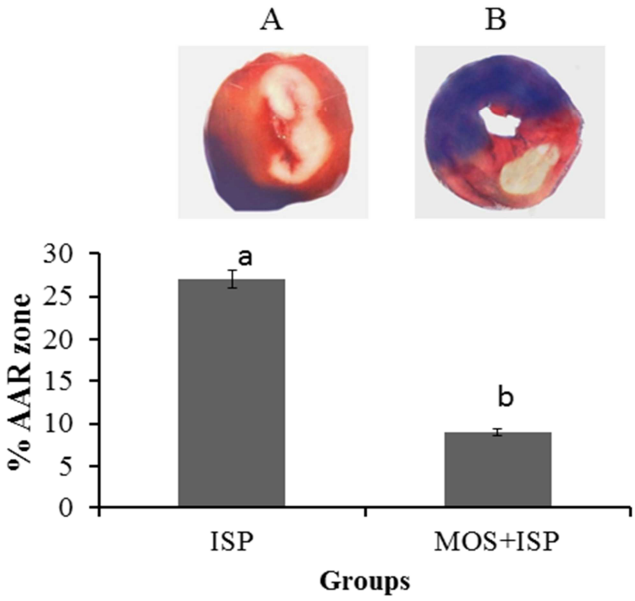
Figure 1: mRNA expression of H-FABP in heart



The relative amount of H-FABP mRNA was estimated by semi-quantitative RT-PCR. The PCR products were quantified by densitometry and standardized to their respective GAPDH controls. Results are expressed as average of quadruplicate experiments ± SEM. Different superscripts indicate values statistically significant at $p<0.05$.

The cardiac damage in the myocardium by ISP treatment was further confirmed by the mRNA expression of H-FABP in the heart tissue. The mRNA expression of H-FABP decreased significantly in the heart tissue of ISP treated rats as compared to control (figure 1). Being a small protein H-FABP diffuses more rapidly through the interstitial space and appears in the circulation as early as 90 min, following the myocardial cell damage [9]. Since it is released into the circulation, the level of FABP in the heart tissue will be decreased during cardiac damage. In agreement with that Wang et al [36] reported that the mRNA expression of H-FABP in the infarcted myocardium. The significant increase in the mRNA expression of H-FABP in MOS pre-treated rats indicated the potency of MOS in protecting the myocardium from cell membrane damage.

Figure 2: Measurement of infarct size



Myocardial infarct size was measured by Evans blue-TTC staining. The Evans blue stained (dark/blue stained area) indicates the normal myocytes which are at the non-ischemic area. TTC stained (red stained area) indicated the myocyes at the ischemic area but are viable (area at risk of infarction-AAR). The unstained (white) area indicates the area of infarction.

A) The photography of ISP induced and rat heart section of Evans blue-TTC staining. This showed more infract size and AAR and less viable tissue.

B) The photography of MOS+ ISP induced rat heart section of Evans blue-TTC staining showed more viable area and less AAR and infarct size compared to ISP group.

The measurement of infarct size was performed by Evans blue-TTC staining (figure 2). Evans blue staining has been widely used for the viability assays. TTC dye forms a red formazan precipitate with LDH of the viable myocardial tissue, and there is a failure of infarcted myocardium to stain with TTC because of the leakage of LDH. The measurement of infarct size of ISP and MOS+ISP groups was performed by Evans blue-TTC staining. ISP treated rat heart showed more infarct size and AAR as showed in figure 2. The MOS pre-treated rat showed less infarct size and AAR compared to ISP. The MOS pre-treated rat also showed more viable tissue compared to ISP. This also confirmed the protective role of MOS in preventing the myocardial damage.

Table 3: Concentration of serum Total cholesterol (TC), HDL cholesterol (HDL) and Triglycerides (TG) in serum

Groups	TC (mg/dl)	TG(mg/dl)	HDL-C(mg/dl)	LDL-C(mg/dl)
CON	90.19±3.54 [a]	48.15±1.89 [a]	36.13±1.35 [a]	35.86±1.34 [a]
MOS	85.12±3.29 [a]	45.34±1.78 [a]	37.55±1.39 [a]	33.26±1.24 [a]
ISP	138.50±5.42 [b]	74.23±2.91 [b]	22.10±0.82 [b]	61.46±2.29 [b]
MOS+ISP	109.42±4.26 [c]	58.58±2.91 [c]	29.27±1.09 [c]	46.26±1.72 [c]

Values are expressed as mean ±SEM.
Mean values with same superscript do not differ significantly, p<0.05

Table 4: Concentration of cholesterol, triglycerides, free fatty acids and phospholipids in the heart

Groups	Cholesterol (mg/100g wet tissue)	Triglycerides (mg/100g wet tissue)	Free fatty acids (mg/100g wet tissue)	Phospholipids (mg/100g wet tissue)
CON	283.90±11.12 [a]	121.55±4.76 [a]	572.59±21.33 [a]	3.55±0.13 [a]
MOS	263.82±10.33 [a]	118.18±4.63 [a]	560.02±20.86 [a]	3.43±0.13 [a]
ISP	340.05±12.23 [b]	206.40±8.08 [b]	689.95±25.71 [b]	2.64±0.10 [b]
MOS+ISP	306.84±12.02 [c]	174.38±6.83 [c]	627.34±23.37 [a]	3.17±0.12 [c]

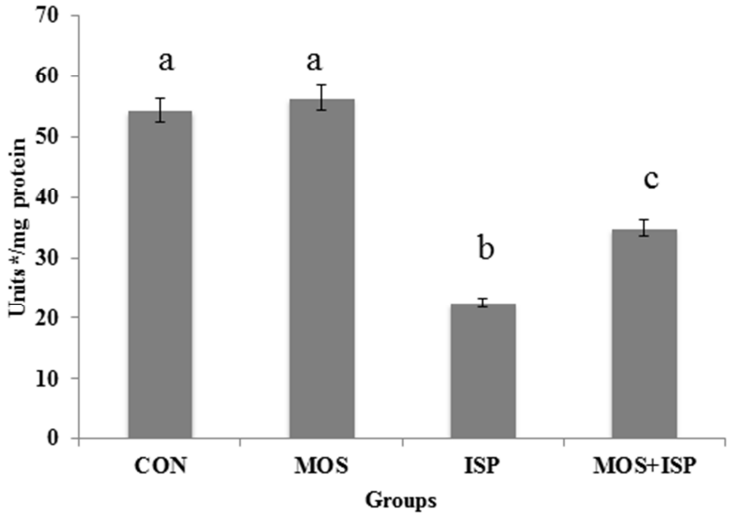
Values are expressed as mean ±SEM.
Mean values with same superscript do not differ significantly, p<0.05.

CVD is associated with altered lipid metabolism. Hypercholesterolemia and hypertriglyceridemia are the risk factors for the development of CVD. In the serum, total cholesterol, triglycerides and LDL-C levels were found to be increased and HDL-C level was decreased significantly in ISP treated rats (table 3). This is in accordance with the previous studies [2, 15]. The increased concentration of cholesterol could be due to the decreased HDL-C, since it is involved in the transport of cholesterol from tissue to the liver for its catabolism [37]. But LDL-C delivers cholesterol to peripheral tissues, which would accumulate if supply exceeded demand. Studies have shown that high levels of HDL-C have a negative correlation with CVD, while high levels of LDL-C show a positive correlation [15]. ISP treatment also caused significant increase in the levels of cholesterol, FFA and TG in the heart tissue (table 4). The enhanced cholesterol levels may be due to the increased biosynthesis since HMG CoA reductase activity is enhanced in the ISP group. ISP administration has been reported to increase the adenylatecyclase activity resulting in enhanced cAMP formation. Cardiac cAMP mediated lipid biosynthesis is the underlying mechanism for the increased lipid profile ISP administration [38] and promotes higher lipid accumulation in the myocardium [39]. Changes in membrane cholesterol content affect its fluidity, permeability to ions, activities of membrane-bound enzymes [40]. The excess free fatty acid may be used for the synthesis of triglycerides, resulting in hypertriglyceridemia [38]. The MOS pre-treatment caused alterations to the lipid profile both in the serum and heart tissue. A study by Dahia*et al* also reported that OS can decrease the hyperlipidemia [41].

Phospholipids are essential components for the integrity of cellular membrane and subcellular organelles [38]. A significant decrease in the phospholipid content was observed in the heart tissue of ISP treated rats compared to control (table 4). The decreased phospholipid content may be due to its greater degradation which could cause membrane dysfunction. Alterations in tissue cholesterol content can also cause increased degradation of phospholipids [40]. The rats pre-treated with MOS showed a significant increase in phospholipid content in heart tissue, which may account for its membrane stabilizing activity.

Probing into the detailed mechanism of hypolipidemic effect of MOS revealed that, MOS caused significant enhancement of PON1 activity in the serum (figure 3). This may be due to the potent antioxidant activity of OS. The beneficial effect of HDL-C is attributed to its associated protein PON1. Its activity decreases systemic oxidative stress and is also associated with lower incidence of cardiovascular diseases [42]. There is a significant reduction in the activity of PON1 in ISP induced rats. This can be correlated with the decreased levels of HDL-C in serum. Reduction in the PON1 activity on ISP induced cardiac damage has been observed earlier also [43].

Figure 3: Activity of PON 1 in serum



*Values are expressed as mean ±SEM. Mean values with same superscript do not differ significantly, p<0.05. *µmoles of p-nitrophenol liberated per minute.*

Table 5: Activities of HMG CoA reductase in liver and phospholipases in heart

Groups	HMG CoA reductase[¥]	PLA*	PLC[#]	PLD[$]
CON	4.62±0.18[a]	0.919±0.036 [a]	0.307±0.012[a]	0.49±0.019[a]
MOS	4.75±0.19 [a]	0.901±0.035[a]	0.301±0.012[a]	0.48±0.019[a]
ISP	1.90±0.07 [b]	2.24±0.088[b]	0.692±0.027[b]	1.46±0.057[b]
MOS+ISP	2.89±0.11 [c]	1.56±0.061[c]	0.459±0.018[c]	0.82±0.032[c]

*Values are expressed as mean ±SEM of 6 animals in each group. Mean values with same superscript do not differ significantly, P<0.05.[¥]HMG CoA/mevalonate, *milliequivalents of ester hydrolyzed/min/mg protein,#millimoles of phosphoryl formed/min/mg protein, $millimoles of choline formed/min/mg protein.*

HMG CoA reductase, which is a rate limiting enzyme in the pathway of cholesterol biosynthesis, plays a major role in the regulation of cholesterol metabolism [44]. The activity of HMG CoA reductase was measured as the ratio of HMG CoA to mevalonate. Lower the ratio, higher will be the activity of the enzyme. There was an upregulation in the cholesterol biosynthesis, as evident from the increased activity of HMG CoA reductase in the liver tissue of ISP treated rats (table 5). But the pre-treatment of rats with MOS resulted in a decrease in the activity this key regulatory enzyme compared to ISP group.

Phospholipases are the enzymes involved in the hydrolysis of phospholipids. ISP treatment in rats caused a significant elevation in the activities if PLA, PLC and PLD. Previous study in our laboratory also showed the elevated levels of these enzymes in ISP treated rats [45]. The observed decrease in the phospholipid content in the heart tissue of ISP treated rats (table 5) might be due its increased degradation by these phospholipases. But MOS pre-treated rats showed reduced activities of these three enzymes as compared to ISP group. This may account for the increased content of phospholipids and decreased content of FFA in the heart tissue of MOS pre-treated rats.

The reports suggests that leaves of OS contain a volatile oil composed of limonene, borneol,copaene, caryophyllene, and elemol, phenolic compounds (rosmarinic acid, apigenin, cirsimaritin, and isothymusin), flavonoids (orientin and vicenin), and aromatic compounds (methyl chavicol and methyl eugenol) [46]. These active components are shown to possess potent antioxidant as well as other pharmacological properties. The synergistic effect of these components may be the underlying mechanism of cardioprotective and hypolipidemic effect of MOS.

CONCLUSION

Administration of MOS attenuated the changes in lipid metabolism and lipid profile caused by the treatment with ISP. The mechanism is by reducing the lipid biosynthesis and increasing HDL-C and PON1 activity. The increased mRNA level of H-FABP in the heart tissue by MOS pre-treatment revealed the efficacy of MOS in protecting the myocardium from severe damage. Hence this study shows the lipid lowering and cardioprotective effect of MOS.

Acknowledgement
I would like to acknowledge INSPIRE Fellowship from Department of Science and Technology for their financial support.

REFERENCES

[1] M Padmanabhan; M Rajadurai; PS Prince. *Basic Clin. Pharmacol. Toxicol*.,**2008**,103(6),507-513.
[2] AUpaganlawar;R Balaraman.*Sci. Pharm*.,**2009**, 77, 791–803.
[3] PS Nair;CSS Devi.*Toxicology*,**2006**, 228(2-3), 135–139.
[4] DM Shih;L Gu;YR Xia;M Navab;WF Li;S Hama;LW Castellani;CE Furlong;LG Costa;AM Fogelman;AJ Lusis.*Nature*,**1998**, 394(6690), 284–287.
[5] M Mackness; Mackness B.*Free. Radic. Biol. Med*.,**2004**, 37(9), 1317–1323.
[6] NS Nagane;PE Jagtap;SP Dhonde;GJBelwalkar. *Journal of Advance Researches in Biological Sciences*, **2013**, 5(2), 131-134.
[7] A Ayub;MI Mackness;S Arrol;B Mackness;J Patel;PN Durrington.*Arterioscler. Thromb. Vasc. Biol*.,**1999**, 19(2), 330–335.
[8] L Figiel; JD Kasprzak; J Peruga; P Lipiec; J Drozdz; M Krzemińska-Pakuła; J Smigielski. *Kardiol. Pol*., **2008**, 66(3), 253-9.
[9] P Gururajan; P Gurumurthy; P Nayar; G SrinivasaNageswaraRao; S Babu; KM Cherian. *Heart Lung Circ*., **2010**, 19(11), 660-4.
[10] BC Wexler; BP Greenberg. *Atherosclerosis*, **1978**, 29(3), 373–395.
[11] KV Sailaja; V LeelaShivaranjani; H Poornima; SBMdRahamathulla; K Lakshmi Devi. *EXCLI. J*.,**2013**, 12, 373-383.
[12] P Chatelain; M Gremel; R Brotelle. *Eur. J. Pharmacol*.,**1987**, 144(1), 83–90
[13] S Bloom; PA Cancilla. *Am. J. Pathol*., **1969**, 54(3), 373–391.
[14] KRKiritikar,BD Basu. Indian Medicinal Plants, Periodical Experts Book Agency, New Delhi, Vol3,**1975**;1965.
[15] P Prakash;N Gupta.*Indian J. Physiol. Pharmacol*.,**2005**, 49(2), 125–131.
[16] DS Arya;M Nandave;SK Ojha;S Kumari;S Joshi;I Mohanty.*Curr. Sci*.,**2006**, 91(5), 667-672.
[17] J Sethi;S Sood;S Seth;AThakur.*Indian J. Physiol. Pharmacol*.,**2003**, 47(1)**,** 115-119.
[18] T Suanarunsawat; W Devakul Na Ayutthaya; T Songsak; S Thirawarapan; S Poungshompoo.*J. Clin. Biochem. Nutr*.,**2010**, 46(1), 52-9.
[19] CW Hume. The UFAW Handbook on the Care and Management of Laboratory Animals. Edinburgh/London: Churchill Livingstone, **1972**.
[20] O Hartmut;K Franz;K Holger. *Am. J. Physiol. Heart. Circ. Physiol.*,**2006**, 291(5), H2533-40.
[21] U Schwanke;I Konietzka;A Duschin;X Li;R Schulz;G Heusch. *Am. J. Physiol. Heart. Circ. Physiol*.,**2002**, 283(4), H1740-2.
[22] A Giricz;JP Gorbe;DS Burley;P Ferdinandy;GF Baxter. *Br. J.Pharmacol*.,**2009**, 158(6), 1495-1502.
[23] WT Friedewald;RI Levy;DS Fredrickson. *Clin. Chem*.,**1972**, 18(6), 499-502.
[24] MI Mackness;D Harty;D Bhatnagar;PH Winocour;S Arrol;M Ishola;PN Durrington.*Atherosclerosis*,**1991**, 86(2-3), 193–198.
[25] AV Rao;S Ramakrishnan.*Clin. Chem*.,**1975**, 21(10), 1523-1525.
[26] J Folch;M Less;GHS Stanley.*J. Biol. Chem*., **1957**, 226(1), 497-509.
[27] LL Abell;BB Levy;BB Brodie;RB Kendal.*J. Biol. Chem*., **1952,** 195(1), 357-366.
[28] L Van Handel;DB Zilver smith.*J. Lab. Clin. Med*., **1957**, 1550, 152-162.
[29] K Falholt;B Lund;W Falholt. *Cin. Chem. Acta*. **1973**, 46, 105-110.
[30] A Rimon;B Shapiro.*Biochem. J*.,**1959,** 71(4), 620-623.
[31] JH Kleiman;WEM Lands.*Biochem. Biophys. Acta*.,**1969**, 187(4), 477-85.
[32] H Mollerung,HU Bergmeyer, Methods of Enzyme Analysis, Bergmeyer H J (eds), Academic Press, Newyork, **1964**, 415.
[33] P Chomczynski;N Sacchi. *Anal.Biochem.*,**1995**, 162(1), 156-159.
[34] AS Wakade;AS Sha;MP Kulkarni;AR Juvekar.*Indian J. Exp. Biol*., **2008**, 46(7), 528-33.
[35] VS Panda; SR Naik. *Altern. Med. Rev*., **2009**, 14(2), 161-171.
[36] HY Wang; XY Zhao; FZ Yang; HY Xing; X Guo. *Zhongguo. Ying. Yong. Sheng. Li.Xue.Za.Zhi*., **2009**, 25(3), 312-315.
[37] S Sushamakumari;AVarghese;D Muraleedharan;VP Menon.*Indian. J. Exp. Biol*. **1990**, 28(5), 480-485.
[38] M Vijayakumar;V Selvi;SKrishnakumari.*Int. J. Pharma. Bio. Sci.,***2010**, 1, 295-300.
[39] S Dhawan;NK Kapoor;S Nityanand. *Indian. J. Exp. Biol*. **1978**, 16(3), 376-378.
[40] PL Yeagle. *Biochim. Biophys. Acta*.,**1985,** 822(3-4), 267–287.
[41] K Dahiya; J Sethi; R Dhankhar; V Singh ; SB Singh; M Yadav; S Sood; A Sachdeva.*Arch. Physiol. Biochem*., **2011**, 117(1), 8-11.

[42] B Mackness, P Durrington, P McElduff, J Yarnell, N Azam, M Watt, M Mackness.,*Circulation*, **2003**, 107(22), 2775–2779.
[43] F John;S Kavitha; M Indira. *International Journal of Advances in Pharmacy, Biology and Chemistry*,**2014**, 3(2), 473-480.
[44] P Prince;B Sathya.*Eur. J. Pharmacol*.,**2010**, 635, 142-8.
[45] S Panicker;SS Swathy;F John; M I.*Inflammation*, **2012**, 35(1), 74-80.
[46] P Pattanayak; P Behera; D Das; S Panda. *Pharmacogn. Rev*., **2010**, 4(7), 95–105.