Evaluation of *avena sativa seeds extract* as cardioprotective agent in rats

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Abstract
The present study was undertaken to evaluate the cardioprotective effect of *Avena Sativa* Linn. seeds in rats. Isoproterenol at a dose level 5.25 on 11th day and 8.5 mg/kg s.c. on 12th day had been utilized to induce Myocardial infarction in Rats. The animals were pretreated with ASSAE (125, 250 and 500 mg/kg/day, oral) for period of 10 days and Isoproterenol (ISO) was injected on 11th and 12th day. The blood was collected after 24 hours through retero-orbital plexus for the estimation of biochemical parameters. The histopathological studies were also performed. The infarct size in heart was evaluated using TTC staining. The administration of ASSAE significantly increased GSH level and lowered the level of the alkaline phosphatase (ALP), lactate dehydrogenase (LDH), TBARS levels, creatinine phosphokinase (CK-MB) as well as significantly changed haemodynamic parameters in the Isoproterenol Induced myocardial necrosis. The integrity of the myocardial cell membrane and inflammatory cell infiltration and reduced muscle separation were promptly improved in the histopathological studies of the ISO-induced myocardium. These results presented here clearly demonstrated the cardioprotective activity of the ASSAE against Isoproterenol induced myocardial infarction in rats. The effect of climatic conditions on the chemical constituents responsible for the cardioprotective activity can also be explored.

Keywords: *Avena sativa,* Cardioprotective agents, Isoprotenenol, Myocardial infarction.

Introduction
Cardiovascular disease (CVD) remains the predominant cause of death in the whole world, claiming 17.1 million lives a year. World Health Organisation (WHO) predicted that CVD will be the most important source of mortality in India by 2020.[1] Cardiovascular diseases (CVD) is the name for the group of disorders of the heart and blood vessels and includes hypertension (high blood pressure), coronary heart disease (heart attack), peripheral vascular disease, heart failure and cerebrovascular disease (stroke) etc.[2] Apart from other cardiovascular diseases myocardial infarction (MI) is becoming worldwide health issue. Along with western countries developing countries are also facing this major public health problem, such as India and makes remarkable augmentation to the mortality census.[3,4] The necrosis of the myocardium can be the outcome of a clinical ailment arising from sudden and persistent reduction of myocardium blood supply, known as Myocardial infarction (MI).[5] MI is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between myocardial blood supply and coronary blood demand.[6] MI increases the generation of the reactive oxygen species in ischemic tissue, with this oxidation of membrane lipids, proteins, carbohydrates and DNA increases, resulting in the changes of biochemical properties, structural, electrical and mechanical of the heart.[7]

Isoproterenol (ISO) induced MI is extensively used as experimental model because of its uncountable reasons. This model possesses extraordinary technical simplicity, an acceptable low mortality as well as an exceptional reproducibility. ISO induced MI has been reported to exhibit many similar morphologic and metabolic aberrations in the heart tissue of both the experimental animals as well as the human MI.[8] ISO is a sympathomimetic β-adenergic receptor agonist (molecular formula $C_{11}H_{17}NO_3$. HCl) causing severe stress to the myocardium producing an infarct like necrosis of myocardium.[9] Catecholamines administration at high doses or excess release of it from the endogenous stores may deplete the energy reserve of cardiomyocytes and thus may result in biochemical and structural changes which are responsible for the development of the irreversible damage.[10] The generation of free radicals results in oxidative stress.[11] These radicals can bind to the macromolecules covalently and provoke lipid peroxidative degradation of membrane lipids resulting in the formation of lipid peroxides chased by multiple pathological changes.[12]

Avena sativa with common name Oats, Avena, Straw, Biova, Jai and javi (Indian subcontinent) and Oatmeal, belongs to Family Poaceae or Graminaceae. The Avena is a Sanskrit word "avi," meaning "sheep" or "avasa," meaning "foodstuff." Avena is originally a secondary crop derived from a weed of barley and wheat.[13] Oat is recognized to be better suited for production under scantly climate, including cool wet environment and low fertility soil.[14] The seeds are antispasmodic, stimulant and nerve tonic, exerting a very healthful action upon the cardiac muscles.[15] Oats is an inexpensive, nutritious and edible cereal. It possesses a high concentration proteins and soluble fibers[16] with abundant antioxidants such as β-carotene, polyphenols, anthocyanins, flavonoids, and Nitrous oxide (NO).[17-19] Oat antioxidants have been reported to promote scavenging of reactive oxygen species by inhibiting the low-density lipoprotein (LDL) oxidation.[20]

Materials and Methods
Plant Collection and Authentication
Avena sativa seeds were purchased from the local market of Ropar. Plant was authenticated by Dr. A. S. Sandhu from National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar (Mohali) Pb.

Drugs and Chemicals

Isoproterenol was purchased from Sigma-aldrich, U.S.A. LDH and CK-MB kits for enzyme estimation were purchased from Reckon Diagnostics, Chandigarh and Span Diagnotics Ltd., Surat. All chemicals used in the present study were of analytical grade.

Experimental Animals

Albino Wistar rats weighing 200-250 gm were used. The research protocol of this study has been approved by Institutional animal ethics committee (IAEC) of Amar Shaheed Baba Ajit Singh Jhujhar Singh Memorial College of Pharmacy, Bela (Ropar) Pb. *vide* approval no. ASCB/1ACE/05/12/069. The animals were maintained under controlled condition of temperature (23 ± 2ºC), humidity (50 ±5%) and 12 hour light and dark cycles. The animals were randomized into experimental and control group and housed in sanitized polypropylene cage containing sterile paddy husk as bedding. They were fed with commercial pelleted rat chow and given water *ad libitum*. All the experimental procedures were carried out in accordance with IAEC for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental protocols were duly approved by the institutional animal ethical committee (IAEC).

Selection of Dose (Test drug)

The study was carried out using three dose levels of alcoholic extract of *Avena sativa* linn. (ASSAE) at three dose level, *i.e.*125, 250 and 500 mg/kg/p.o. The selection of dose was based upon the research findings of Chen CY *et al.*, 2004.[20]

Preparation of Test Drug

Seeds of *Avena sativa* were grinded for separation of bran from seed. Bran was crushed into small pieces. Defatting was done with petroleum ether and then extraction was done with 75% ethanol. Three different concentration of alcoholic extract of *Avena sativa* seeds (ASSAE) were prepared, 125 mg/kg, 250 mg/kg and 500 mg/kg doses were prepared corresponding to body weight of animal. Plant extract was dissolved in distilled water and given for 12 days.

Experimental Protocol

The animals were divided into 8 groups. Group I (control), Group II (ISO with dose 5.25 on 11th and 8.5 mg/kg s.c. on 12th day), Group III (125 mg/kg), Group IV (250 mg/kg), Group V (500 mg/kg), Group VI (125 mg/ kg + ISO), Group VII (250 mg/ kg + ISO) and Group VIII (500 mg/ kg + ISO). ASSAE was given orally and ISO was given by s.c. route.

Estimation of Serum Markers

On 13th day, blood was withdrawn from retero orbital vein under anesthesia. Blood was centrifuged to separate the serum. Biological markers lactate dehydrogenase (LDH), creatinine phosphokinase (CK-MB) and alkaline phosphatase (ALP) were estimated. The heart was isolated from animals and homogenate was prepared with help of tissue homogenizer.

Estimation of Hemodynamic Parameters

Rats were anesthetized with 25% urethane (1 g/kg, i.p). The body temp of the animals was maintained at 37 ºC throughout the experimental protocol. To perform tracheostomy, the neck was opened with a ventral midline incision. The left carotid artery was cannulated with polyethylene tube (internal diameter 0.30 mm; outer diameter 0.40 mm) attached to a three-way cannula. The cannula was heparinized (Heparin 300 IU/ml) and connected to POWER LAB 4/30 (AD Instruments, NSW, Australia) system using a pressure transducer for the measurement of mean arterial, systolic, diastolic, pressures and heart rate.

Estimation of Antioxidant Enzymes

Animals were sacrificed by cervical dislocation and the heart tissues were removed, washed with the cold isotonic saline, and dried with filter paper. After this hearts were diced and homogenized in 0.05M ice cold phosphate buffer. After centrifugation supernatant was used for analysis of antioxidant enzymes GSH and TBARS.

Histopathological Study

The animals were sacrificed and the hearts were taken out. The excised heart was fixed in buffered formalin solution (10%). Histopathology was done by Sandhu Pathology Labs, Sec-34A, and Chandigarh. Pictures are obtained from photomicroscope from Department of Pharmacognosy, ASBASJSM College of Pharmacy, Bela (Ropar).

Statistical Analysis

The data were expressed as Mean ± SEM. One-way ANOVA followed by Turkey multiple comparison tests (using Graph Pad Prism software package) were analyzed on the statistical differences at $p<0.05$ between the all the eight groups. The IC_{50} values were calculated graphically utilizing linear regression (LR) analysis.

Results

Effect of ASSAE on Serum Markers

Effect of ASSAE on Alkaline Phosphatase (ALP)

In case of ALP, on 13th day, a highly significant ($p<0.001$) and significant ($p<0.01$) elevation was observed in group II (ISO treated) and in group VI (125 mg/kg + ISO), respectively when compared to group I (control group). In comparison to group I (control group), the result of serum ALP level of group III (125 mg/kg), group IV (250 mg/kg), group V (500 mg/kg), group VII (250 mg/kg + ISO) and group VIII (500 mg/kg + ISO) were non-significant (Table 1 and Fig. 1).

Effect of ASSAE on Creatine Kinase (CK-MB)

A highly significant ($p<0.001$) elevation in level of serum CK-MB was observed in group II (ISO treated) and in group VI (125mg/kg + ISO), when compared to group I (control group) (Table 1 and Fig. 2).

Effect of ASSAE on Lactate dehydrogenase (LDH)

A significant ($p<0.05$) elevation was observed in group VII (250mg/kg + ISO), when compared to group I (control group). The result of serum LDH level of groups III, IV, V

and VIII was non-significant, when compared to group I (control group).

During 12 days of pretreatment with ASSAE, the serum level of LDH was highly significantly ($p<0.001$) decreased in group I, III, IV, V, VI, VII and VIII when compared to group II (ISO treated) (Table 1 and Fig. 3).

Effect of ASSAE on levels of TBARS/MDA

A highly significant (*p<0.001*) elevation in MDA level was observed in Group II (ISO treated at dose 5.25 and 8.5 mg/kg s.c. for two consecutive days), when compared to group I (control group) on 13th day.

Animals of Group VI (125 mg/kg + ISO) were pre-treated with ASSAE for 12 days, showed a significant (*p<0.01*) elevation in level of MDA, when compared to group I (control group). Animals of group VII (250 mg/kg + ISO) showed significant (*p<0.05*) elevation in level of MDA, when compared to group I (control group). The animals of group I, III, IV, V, VI, VII and VIII showed highly significant (*p<0.001*) reduction in MDA level, when compared with group II (ISO treated), after pre-treatment with ASSAE for 12 days of (Table 3 and Fig. 4).

The result of MDA level of group III, IV, V and VIII was non-significant, when compared to group I (control group).

Effect of ASSAE on Glutathione (GSH)

A highly significant (*p<0.001*) reduction in GSH level was observed in group II (ISO treated) and in group VI (125 mg/kg + ISO), when compared with group I (control group).

Within 12 days of pre-treatment with ASSAE, a significant (*p<0.01*) decreased in level of GSH was observed in group VII (250 mg/kg + ISO), when compared with group I (control group). The result of GSH level of group III (125 mg/kg), group IV (250 mg/kg), group V (500 mg/kg) and group VIII (500 mg/kg + ISO) animals was non-significant, when compared to group I (control group).

The animals of group I, III, IV, V, VI, VII and VIII showed highly significant (*p<0.001*) reduction in LPO level, when compared with group II (ISO treated), after pre-treatment with ASSAE for 12 days (Table 4 and Fig. 5).

Table 1: Effect of ASSAE on various serum markers in ISO induced myocardial infarction.

S. No.	Groups	Treatment	ALP (IU/L)	CK-MB (IU/L)	LDH (IU/L)
1.	Group I	Control (Saline)	$88.7 \pm 0.882^{\gamma}$	$61.47 \pm 0.4619^{\gamma}$	$458 \pm 4.87^{\gamma}$
2.	Group II	ISO treated	164 ± 2.35^{c}	127.4 ± 0.7879^{c}	1380 ± 13.4^{c}
3.	Group III	ASSAE 125 mg/kg	$89.2 \pm 2.43^{\gamma}$	$62.95 \pm 0.2104^{\gamma}$	$456 \pm 5.39^{\gamma}$
4.	Group IV	ASSAE 250 mg/kg	$91.5 \pm 0.764^{\gamma}$	$63.01 \pm 0.3530^{\gamma}$	$461 \pm 5.94^{\gamma}$
5.	Group V	ASSAE 500 mg/kg	$90.0 \pm 1.32^{\gamma}$	$63.36 \pm 0.4470^{\gamma}$	$466 \pm 5.70^{\gamma}$
6.	Group VI	ASSAE 125 mg/kg + ISO	$96.7 \pm 0.955^{b\gamma}$	$68.33 \pm 0.6247^{c\gamma}$	$549 \pm 5.88^{c\gamma}$
7.	Group VII	ASSAE 250 mg/kg + ISO	$95.0 \pm 0.447^{\gamma}$	$64.20 \pm 0.5239^{a\gamma}$	$495 \pm 4.36^{a\gamma}$
8.	Group VIII	ASSAE 500 mg/kg + ISO	$90.7 \pm 0.333^{\gamma}$	$62.49 \pm 0.5180^{\gamma}$	$454 \pm 4.14^{\gamma}$

Isoproterenol treated animals (5.25 and 8.5 mg/kg s.c. for two consecutive days on 11th and 12th day.

Data expressed as Mean ± S.E.M (n=6).

$^{c}p<0.001$, $^{b}p<0.01$ and $^{a}p<0.05$ when compared to Group I, $^{\gamma}p<0.001$ when compared to Group II.

One way ANOVA followed by Tukey's multiple Comparison Test;

ALP: Alkaline Phosphatase, **CK-MB:** Creatine Kinase, **LDH:** Lactate Dehydrogenase, **ISO:** Isoproterenol and **ASSAE:** *Avena sativa seeds* alcoholic extract

Effect of ASSAE on hemodynamic parameters

Table 2: Effect of ASSAE on hemodynamic parameters in ISO induced MI in rats.

S. No.	Groups	Treatment	HR (BPM)	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	AP (mmHg)
1.	Group I	Control (Saline)	$355 \pm 2.59^{\gamma}$	$126 \pm 0.575^{\gamma}$	$98.1 \pm 1.04^{\gamma}$	$108 \pm 1.02^{\gamma}$	$113 \pm 0.665^{\delta}$
2.	Group II	ISO treated	408 ± 4.91^{c}	136 ± 2.91^{c}	81.4 ± 0.819^{c}	98.3 ± 1.96^{c}	107 ± 1.31^{a}
3.	Group III	ASSAE 125 mg/kg	$357 \pm 2.46^{\gamma}$	$125 \pm 0.413^{\gamma}$	$98.0 \pm 0.885^{\gamma}$	$107 \pm 0.546^{\gamma}$	111 ± 0.857
4.	Group IV	ASSAE 250 mg/kg	$356 \pm 1.38^{\gamma}$	$125 \pm 0.419^{\gamma}$	$97.7 \pm 1.00^{\gamma}$	$107 \pm 0.736^{\gamma}$	110 ± 1.33
5.	Group V	ASSAE 500 mg/kg	$359 \pm 3.48^{\gamma}$	$126 \pm 0.983^{\gamma}$	$97.8 \pm 0.740^{\gamma}$	$107 \pm 0.405^{\gamma}$	111 ± 1.62
6.	Group VI	ASSAE 125 mg/kg + ISO	$373 \pm 3.04^{b\gamma}$	135 ± 0.309^{c}	85.2 ± 0.823^{c}	102 ± 0.607^{c}	110 ± 0.938
7.	Group VII	ASSAE 250 mg/kg + ISO	$370 \pm 2.73^{a\gamma}$	$130 \pm 0.602^{\beta}$	$93.1 \pm 0.906^{a\gamma}$	$104 \pm 0.576^{\beta}$	111 ± 1.05
8.	Group VIII	ASSAE 500 mg/kg + ISO	$359 \pm 2.42^{\gamma}$	$127 \pm 0.840^{\gamma}$	$97.1 \pm 1.18^{\gamma}$	$109 \pm 0.838^{\gamma}$	111 ± 1.32

ISO treated (5.25 and 8.5 mg/kg s.c. for two consecutive days) on 11th and 12th day.

Data expressed as ± S.E.M (n=6), $^{c}p<0.001$, $^{b}p<0.01$ and $^{a}p<0.05$, when compared with Group I (control group), $^{\gamma}p<0.001$, $^{\delta}p<0.05$ and $^{\beta}p<0.01$, when compared with Group II (ISO treated group). One way ANOVA followed by Tukey's Multiple Comparison Test.

HR: Heart rate, **SAP:** Systolic arterial pressure, **DAP:** Diastolic arterial pressure, **AP:** Arterial pressure, **MAP:** Mean arterial pressure. **ISO:** Isoproterenol and **ASSAE:** *Avena sativa seeds* alcoholic extract.

Table 3: Effect of ASSAE on TBARS/MDA level in ISO induced MI in rats

S. No.	Groups	Treatment	MDA (nmol MDA/g wet tissue)
1.	Group I	Control (Saline)	$2.67 \pm 0.305^{\gamma}$
2.	Group II	ISO treated	11.1 ± 0.393^{c}
3.	Group III	ASSAE 125 mg/kg	$2.56 \pm 0.234^{\gamma}$
4.	Group IV	ASSAE 250 mg/kg	$2.13 \pm 0.135^{\gamma}$
5.	Group V	ASSAE 500 mg/kg	$1.57 \pm 0.277^{\gamma}$
6.	Group VI	ASSAE 125 mg/kg + ISO	$4.61 \pm 0.375^{b\gamma}$
7.	Group VII	ASSAE 250 mg/kg + ISO	$4.22 \pm 0.419^{a\gamma}$
8.	Group VIII	ASSAE 500 mg/kg + ISO	$2.67 \pm 0.197^{\gamma}$

ISO treated (5.25 and 8.5 mg/kg s.c. for two consecutive days on 11th and 12th)
Data expressed as ± S.E.M (n=6),
$^{c}p<0.001$,$^{b}p<0.01$ and $^{a}p<0.05$ when compared with Group I (control group), $^{\gamma}p<0.001$ when compared with Group II (ISO treated group).
One way ANOVA followed by Tukey's Multiple Comparison Test.
TBARS: Thiobarbituric Acid Reactive Substances (nmol MDA/g wet tissue), **LPO:** Lipid peroxides, **ISO:** Isoproterenol and **ASSAE:** *Avena sativa seeds* alcoholic extract.

Table 4: Effect of ASSAE on GSH level in ISO induced myocardial infarction in rat.

S. No.	Groups	Treatment	GSH (µmol/g wet tissue)
1.	Group I	Control (Saline)	47.8 ± 1.73^{x}
2.	Group II	ISO treated	9.75 ± 0.738^{a}
3.	Group III	ASSAE 125 mg/kg	47.4 ± 0.928^{x}
4.	Group IV	ASSAE 250 mg/kg	48.1 ± 2.25^{x}
5.	Group V	ASSAE 500 mg/kg	55.3 ± 1.17^{x}
6.	Group VI	ASSAE 125 mg/kg + ISO	$26.4 \pm 0.357^{a\,x}$
7.	Group VII	ASSAE 250 mg/kg + ISO	$33.9 \pm 0.798^{b\,x}$
8.	Group VIII	ASSAE 500 mg/kg + ISO	44.6 ± 1.37^{x}

ISO treated (5.25 and 8.5 mg/kg s.c. for two consecutive days on 11th and 12th days),
Data expressed as ± S.E.M (n=6),
$^{a}p<0.001$ and $^{b}p<0.01$ when compared with Group I (control group), $^{x}p<0.001$ when compared with Group II (ISO treated group).
One way ANOVA followed by Tukey's Multiple Comparison Test..
GSH: Glutathione (µmol/g wet tissue). **ISO:** Isoproterenol and **ASSAE:** *Avena sativa seeds* alcoholic extract

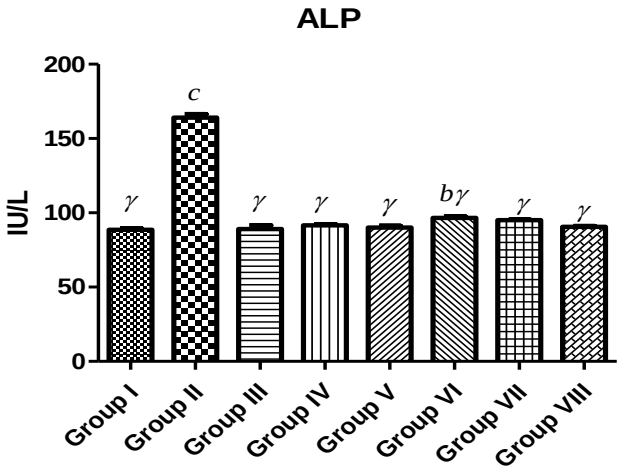

Fig. 1: Effect of ASSAE treatment on level of ALP (IU/L) in rats

Data expressed as Mean ± S.E.M (n=6); $^{c}p<0.001$ and $^{b}p<0.01$ when compared to Group I, $^{\gamma}p<0.001$ when compared to Group II.
One way ANOVA followed by Tukey's multiple Comparison Test;

ALP: Alkaline Phosphatase.
Within 12 days of ASSAE pre-treatment, the level of serum ALP was highly significant ($p<0.001$) decreased in groups I, III, IV, V, VI, VII and VIII, when compared to group II (ISO treated) (Table 1 and Fig. 1).

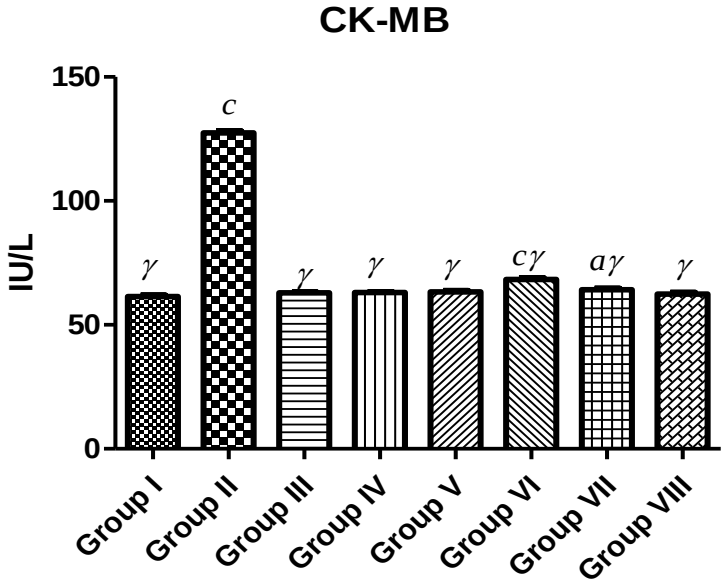

Fig. 2: Effect of ASSAE treatment on level of CK-MB(IU/L) in rats.

Data expressed as Mean ± S.E.M (n=6)
$^{c}p<0.001$ and $^{a}p<0.05$ when compared to Group-I, $^{\gamma}p<0.001$ when compared to Group-II.
One way ANOVA followed by Tukey's multiple Comparison Test;
CK-MB: Creatine Kinase
A significant ($p<0.05$) elevation was observed in group VII (250mg/kg + ISO) when compared to group I (control group). The result of serum CK-MB level of groups III, IV V and VIII was non-significant, when compared to group I (control group).
During 12 days of pretreatment with ASSAE, the serum level of CK-MB was highly significantly ($p<0.001$) decreased in group I, III, IV, V, VI, VII and VIII, when compared to group II(ISO treated) (Table 1 and Fig. 2).

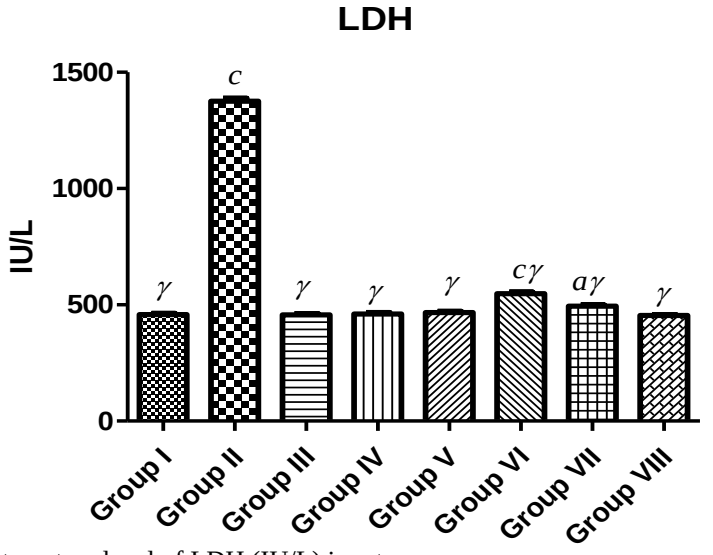

Fig. 3: Effect of ASSAE treatment on level of LDH (IU/L) in rats.

Data expressed as Mean ± S.E.M (n=6)
$^{c}p<0.001$ and $^{a}p<0.05$ when compared to Group I, $^{\gamma}p<0.001$ when compared to Group II.
One way ANOVA followed by Tukey's multiple Comparison Test;
LDH: Lactate Dehydrogenase
A highly significant ($p<0.001$) elevation in level of serum LDH was observed in group II (ISO treated) and in group VI (125mg/kg + ISO), when compared to group I (control group).

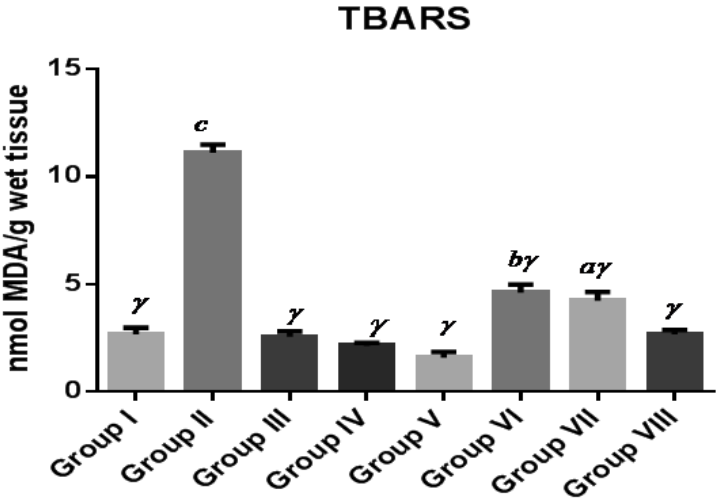

Fig. 4: Effect of ASSAE treatment on levels of MDA (nmol MDA/g wet tissue) in rats.

Data expressed as ± S.E.M (n=6),
$^{c}p<0.001$,$^{b}p<0.01$ and $^{a}p<0.05$ when compared with Group I (control group), $^{\gamma}p<0.001$ when compared with Group II (ISO treated group).
One way ANOVA followed by Tukey's Multiple Comparison Test.
TBARS: Thiobarbituric Acid Reactive Substances (nmol MDA/g wet tissue),

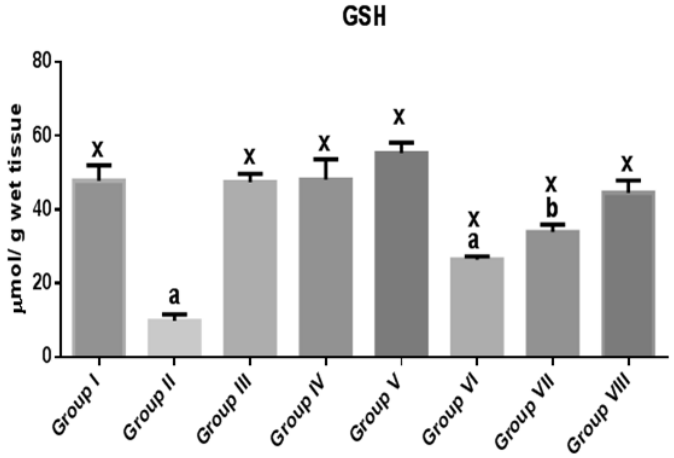

Fig. 5: Effect of ASSAE treatment on levels of GSH (µmol/g wet tissue) in rats.
Data expressed as ± S.E.M (n=6),
$^{a}p<0.001$ and $^{b}p<0.01$ when compared with Group I (control group), $^{x}p<0.001$ when compared with Group II (ISO treated group).
One way ANOVA followed by Tukey's Multiple Comparison Test..
GSH: Glutathione (µmol/g wet tissue).

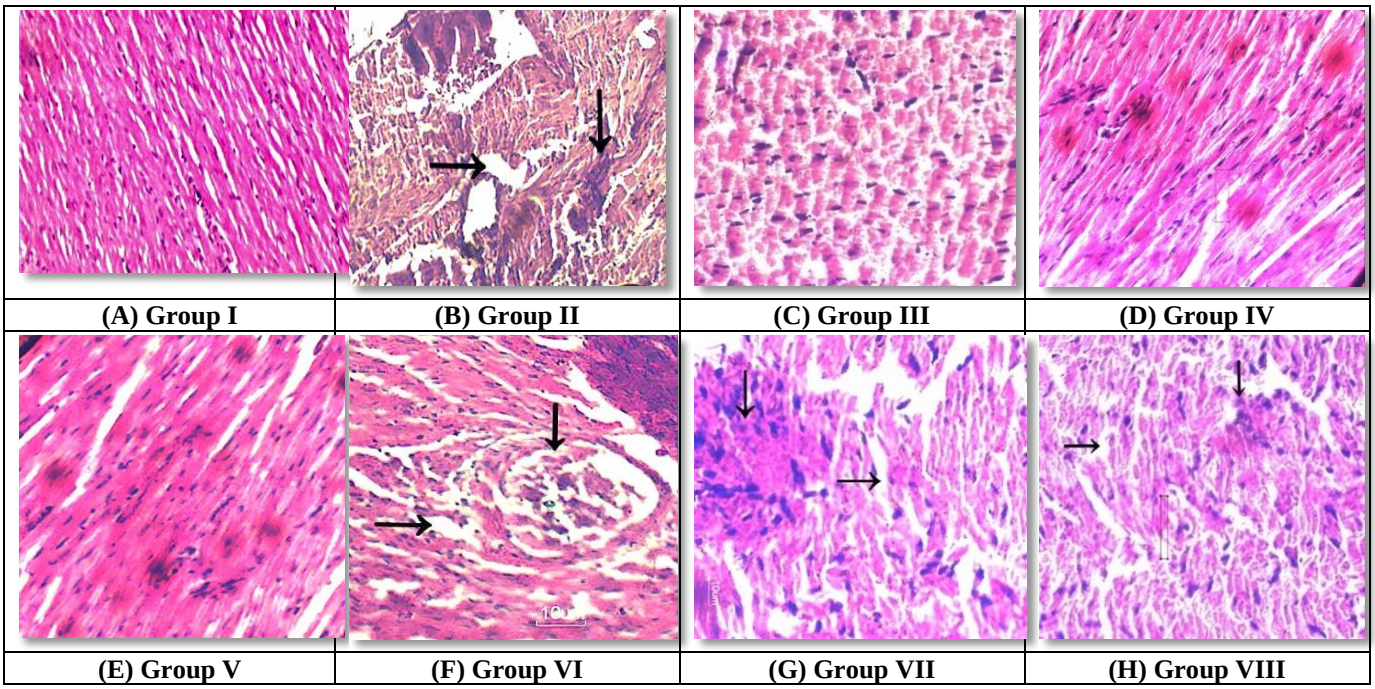

Fig. 6: Effect of Avena sativa on the histological morphology of rat heart: (A) Control (B) ISO treated (C) ASSAE(125mg/kg) (D) ASSAE (250 mg/kg) (E) ASSAE (500mg/kg) (F) ASSAE(125mg/kg)+ ISO (G) ASSAE (250mg/kg) + ISO (H)ASSAE(500 mg/kg) +ISO

TTC staining

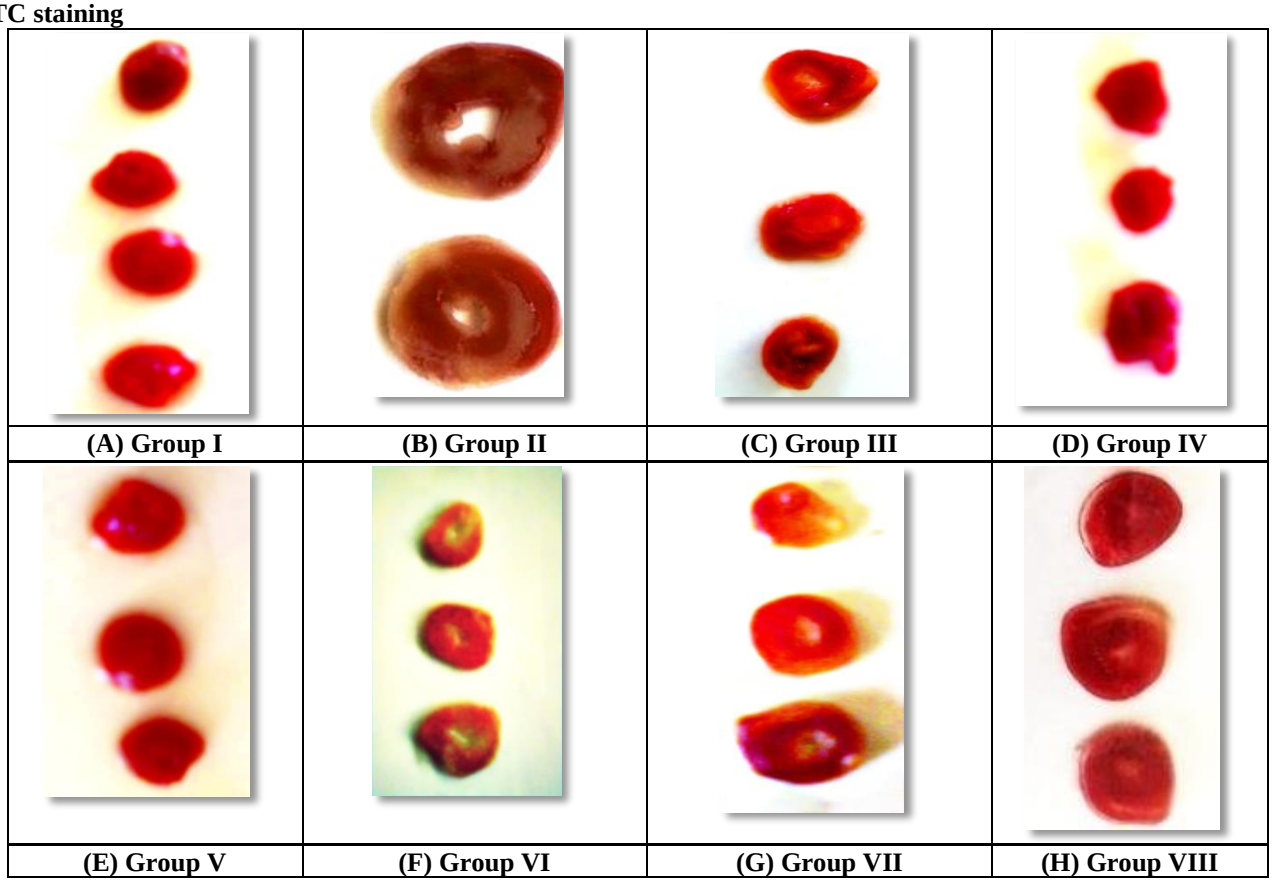

Fig. 7: Effect of Avena sativa on the rat heart shown by TTC staining: (A) Control (B) ISO treated (C) ASSAE(125mg/kg) (D) ASSAE (250 mg/kg) (E) ASSAE (500mg/kg) (F) ASSAE(125mg/kg)+ ISO (G) ASSAE (250mg/kg) + ISO (H)ASSAE(500 mg/kg) +ISO

Discussion

The present study exhibits cardioprotective effect of *Avena sativa* Linn. in isoproterenol-induced cardiotoxicity as validated by improved antioxidant defence system, attenuation of haemodynamic impairment as well as inhibition of lipid peroxidation and prevention of leakage of myocytes injury marker enzymes from heart.

Catecholamines are important regulators of myocardial contractility and metabolism. However, it has been found from various studies that excess levels of catecholamines are responsible for cellular damages in various cardiac diseases such as transient myocardial hypoxia, acute coronary insufficiency, angina, and subendocardial infarct. ISO is a potent catcholamine which has effects on mitochondrial LPO, antioxidants and many other cardiac enzymes as well as infarct like lesions develop in animals after administration of ISO.[21]

It has been found from previous studies that *Avena sativa* contains number of chemical constituents comprising of antioxidants like tocols[18,19,22] and various polyphenolic compounds**.** *Avena sativa* antioxidants have been reported to promote scavenging of reactive oxygen species and inhibiting low-density lipoprotein oxidation.[20] Antioxidant works synergistically with Vitamin E, in the formation of antibodies, maintaining a healthy heart and liver. It protects the immune system by preventing the formation of free radical. It helps to regulate the effects of thyroid hormones on fat metabolism.[23]

Along with these properties *Avena sativa* also possesses anti-hyperlipidemic property attributed to the presence of an organic acid β-glucan by lowering lipid absorption and in turn decreases the risk of cardiovascular diseases.[24] The impaired nitric oxide (NO) production and proliferation of vascular smooth muscle cells (SMC) are both crucial pathophysiological processes in the endothelial dysfunctioning which leads to formation of atherosclerosis lesion and other cardiological disease events like myocardial infarction. A phenol named as avenathramides of *Avena sativa* increase NO production and endothelial NO synthase expression by both endothelial cells and VSMC.[25]

In present study, ISO has been shown to induce myocardial stress by the generation of free radicals and depletion of endogenous antioxidant network in heart.[26,27] Free radicals are basically responsible for initiating lipid peroxidation result into the alteration of membrane integrity, fluidity and permeability.[28] Isoproterenol significantly increased MDA level with reduced activity of myocardial LDH enzyme, ALP and CK-MB. Elevated MDA level reflects an increase in membrane permeability, which could be responsible for leakage of myocardial enzymes (CK-MB and LDH) from cardiomyocytes. LDH, CK-MB and ALP, localized in myocytes, are released during isoproterenol-induced irreversible myocardial injury and are considered as characteristic of cardiac muscle injury.[29] The reduction in the leakage of CK-MB and LDH enzymes from heart as validated by increased levels of CK-MB and LDH in cardiac tissue, suggest the cardioprotective effect of *Avena sativa* pretreatment.

The glutathion (GSH) mechanism plays a central role in antioxidant defense system. A higher concentration of GSH is associated with good health status.[30] Lipid peroxidation elevation results into reduction of GSH level.[31] ISO generally leads to decrease in level of antioxidant enzymes *e.g.* GSH. The reduction of the antioxidant enzymes can result in an excess availability of H_2O_2 and superoxide ion (O_2^-) in biological medium, which in turn generates hydroxyl ions resulting in initiation and propagation of lipid peroxidation. The GSH level was significantly raised in animals after pretreatment with ASSAE for 12 days.

The histopathological findings of myocardial tissue in control illustrated clear integrity of the myocardial cell membrane and no inflammatory cell infiltration was observed. The ISO-induced myocardium exhibited disruption of cardiac myofibers, degeneration and marked necrosis in the ventricular region, inflammatory cells, infracted zone with oedema, and separation of heart muscle fibers. Pretreatment of ASSAE exhibited coagulative necrosis with inflammatory cells, moderate oedema in myocardium and reduced area of infarction. The protective effect might have been mediated through *Avena sativa* induced activity.

In the present study, the observed changes in hemodynamic parameters after administration of ISO in control animals were comparable to earlier reported ones.[32] ISO injection on 11th and 12th day significantly increased the heart rate in ISO treated animals. Elevation in myocardial oxygen demand resulted in ischaemic necrosis of the myocardium in animals. A significant decline in MAP, DAP, AP and a significant elevation in SAP in the heart of ISO treated animals was observed. HR and SAP was significantly decline, DAP and MAP was significantly increased after ASSAE pretreatment for 12 days. An increase in heart rate is responsible for increased oxygen consumption that leads to accelerated myocardial necrosis. Along with these all findings TTC staining of heart tissue also confirmed these results of study.

Conclusion

Hence it can be summarized, that pretreatment with ASSAE in ISO treated rats possess cardioprotective effect in dose dependent manner by inhibiting generation of highly cytotoxic free radicals, myocardial hyperactivity and coronary hypotension, lipid peroxidation of membrane lipids and leakage of enzymes into blood.

Conflict of Interest: None.

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