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THE CARDIOPROTECTIVE EFFECT OF *ARGEMONE MEXICANA* ON ISOPROTERENOL INDUCED CARDIAC TOXICITY IN RATS

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ABSTRACT

Cardiovascular diseases comprise the most prevalent serious disorders in the developed nations. Cardiotoxicity occurs during therapy with several drugs and may be the dose limiting factor in the treatment. In similar to, Isoprenalin (a synthetic catecholamine and beta adrenergic receptor) has been found to cause severe cardiac damage. Clinical and experimental investigations suggested that increased oxidative stress associated with an impaired antioxidant defence status initiates a cascade of reactions responsible for Drugs induced cardio toxicity. The interest to undertake this investigation is due to Argemone mexicana could be a potential source of natural antioxidant, that could have greater importance as medicinal agent in blocking or slowing oxidative stress related degenerative diseases. Argemone mexicana have been reported for in vitro oxidant activity. The present study aimed to evaluate cardioprotective potential screened in Isoproterenol induced cardiac stress in which Ethanolic Extract of Argemone Mexicana Leaves (EAML 200 mg/kg,400mg/kg) were administered & Isoproterenol (85mg/kg s.c.) administered groups respectively. The present study concludes that restoration of Hemodynamic Parameters(BP,ECG),Biochemical Parameters-cardiac markers(CK-MB,LDH,SGOT), antioxidant markers(MDA,CAT,SOD,GTH)) Histopahological indications

KEYWORDS: Isoproterenol, Antioxidant, ECG, blood pressure, cardiotoxicity.

INTRODUCTION

Cardiovascular diseases comprise the most prevalent serious disorders in the developed nations. The prevalence rises progressively with age from 5% at age 20 to 75% at age ≥ 75 years.[1] The use of higher doses of anthracyclines and their combined use with other agents, the incidence of cardiomyopathy have greatly increased.[2]

Isoproterenol (ISO) induced myocardial cell death is well known standard drugs model to study the advantageous effect of many drugs on cardiac dysfunction. Extreme stress in myocardium and necrotic lesions in the heart muscles is caused by ISO which is a β-adrenergic agonist. ISO causes myocardial injury and cause of that membrane permeability changes take place, which brings about the loss of activity and integrity of myocardial membranes. Myocardial Infarction caused by ISO in rats has been shown to be accompanied by hyperglycemia and lactate dehydrogenase activities. Isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been seen as one of the causative factor.[3]

Argemone mexicana is an local herb commonly known as Prickly poppy. It belongs to the family Papaveraceae. Argemone mexicana is noted to possess medicinal uses in traditional system of medicine. During last few years, there has been growing interest in the study of medicinal properties of this plant and it is reported for Antimicrobial, Antidiabetic, Antioxidant and Hepatoprotective activity. The plant was also reported for other activities like Larvicidal activity, Wound healing activity, Cancer activity, Anthelmintic activity, Anti-inflammatory and analgesic, Neuropharmacological studies. In light of these medicinal properties, this plant can be represented as a valuable source of medicinal compound.[5]

Argemone Mexicana leaves has been reported for different chemical constituents like Alkaloids, Amino acids, flavonoids, Phenolics, and fatty acids as a major phytochemical groups.[7]

The interest to undertake this investigation is due to the fact that no study regarding the cardioprotective effect have been reported in literature. Argemone mexicana have been reported for in vitro oxidant activity. In proposed investigation its cardioprotective potential will screen in Isoproterenol induced cardiac stress which might be reduced due to its antioxidant property.

MATERIALS AND METHODS

1. Drugs/inducers used

Sr. No.	List of Drugs used	Procured from
1	Isoprenaline hydrochloride	Research-lab Fine Chem Industries (Mumbai)

2. Animals - Experimental Animals

Swiss Albino rat of either sex weighing 200±20 gm, procured from animal house of Appasaheb Birnale College of Pharmacy, Sangli, were used for the study. Form B protocol were prepared and submitted to Institutional Animal Ethics committee (IAEC). Approval of animal use was obtained from IAEC prior to experimental study. The experimental protocol (IAEC/ABCP/04/2016-17) was approved by the IAEC.

3. PLANT MATERIAL PROCESSING

3. 1. Procurement and Authentication of plant

The aerial parts of plant *Argemone mexicana* were collected from the local area of Sangli in the month of October and November. The plant was authenticated by Dr. S.S. Sathe, Asso.Prof. Department Of Botany, Rajeramrao College, Jath, Dist: Sangli.

3. 2. Preparation of Ethanolic extract of aerial parts of *Argemone mexicana* plant

The aerial parts of *Argemone mexicana* were dried in a shade. Dried material was powdered coarsely using mixture grinder and passed through sieve no. 40. Powdered plant material was extracted by using soxhlet apparatus with 90% ethanol as a solvent for 48 hours at 60^0C. Extract was cooled at room temperature and evaporated to dryness under reduced pressure in Rotary Vacuum Evaporator. Extract was dissolved in a water just before oral administration.

3.3. Preliminary Phytochemical investigation

The extract was subjected to chemical tests qualitatively for the identification of different phytoconstituents like glycosides, saponins, carbohydrates, sterols, alkaloids, flavonoids, tannins, proteins and triterpenoids etc.

4. Thin layer chromatography[8]

TLC plates were prepared using standard grade silica gel G.

Solvent system used for TLC

Extracts	Solvent system	Composition
EAML(Flavonoids)	Ethyl acetate:formic acid:glacial acetic acid:water	100:11:11:26
EAML(Alkaloids)	n-propranol:Formic acid:water	90:1:9

The plates were run and then dried, R_f values were measured by using the formula as given below.

$$R_{f=}\ \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

5.Acute oral Toxicity (Limit Test)- OECD guideline 423

After administration of drug orally animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

EXPERIMENTAL DESIGN

Groups of 6 animals each was assessed the cardioprotective activity by Isoproterenol induced cardiac toxicity. The group were as follows.

1.Isoproterenol induced Cardiac Stress(dose and route of administration)

Group1- Normal (Normal saline)

Group 2 -Control –Normal saline (upto 28 days)+ (Isoproterenol 85 mg/kg s.c, on 29^{th}, 30^{th}day)

Group 3-Test(EEAM of leaves, dose -II of 200 mg/kg daily for 30 days)+ (Isoproterenol 85 mg/kg s.c, on 29^{th},30^{th} day).

Group 4- Test(EEAM of leaves, dose -II of 400 mg/kg daily for 30 days+Isoproterenol 85 mg/kg s.c, on 29^{th},30^{th} day.).

2.Evaluating Parameters

After completion of experimental period, BP was recording and animals were anesthetized for ECG, Blood was collected to estimate level of cardiac markers, animals were sacrificed to isolate heart. Heart tissue homogenate was prepared to determine level of different antioxidant markers, tissue specimen was preserved in 10% buffered formalin for Histopathology Study. The various parameters were carried out as described below.

2.1. Hemodynamic Parameters

2.2.1. Blood Pressure Hemodynamic Parameter

Blood pressure was determined by Non- Invasive Tail cuff method CODA-KENT scientific. Here, various parameters such as Systolic, Diastolic, Mean BP and Heart rate were determined.

2.2.2.Electrocardiography (ECG)

ECG was recorded at the end of the treatment after the last dosing of Isoproterenol. Biopac MP -35 instrument was used to record and monitor ECG tracings. Rats from each group were anesthetized with

Urethane(1.25g/kg), a needle electrodes were inserted under the skin for the limb lead at position II. For each ECG tracing P wave, QRS complex, QT interval, RR interval and Cardiac Cycle were measured.

3.1. Biochemical study

3.1.1Serum Biomarkers

Estimation of Creatine Phosphokinase – MB (CK- MB) by Immunoinhibition method.,Estimation of Lactate Dehydrogenase (LDH) by Modified IFCC,Estimation of Serum Glutamate Oxaloacetate Transaminase (SGOT) by 2,4-DNPH method. All the kit procedures were used according to manufacturers instruction on semi-autoanalyser.

3.1.2.Tissue Antioxidant Biomarkers

Determination of Malondialdehyde (MDA)[product of lipid peroxidation] level from heart tissue homogenate by Ohkawa et.al.[11] Determination of Superoxide dismutase (SOD) enzyme activity from heart tissue homogenate by the method of Marklund et al.[11] Determination of Catalase (CAT) enzyme activity from heart tissue homogenate by the method of Aebi et al.[7] Determination of Reduced Glutathione (GSH) enzyme activity from heart homogenate by the method of Ellmans et al.[11]

2.3.Histopathological study

At the end of study, the heart was isolated, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope (10 x).

3. Statistical analysis

Values are expressed as Mean ± SEM for six rats in each group, statistical analysis was performed using one way ANOVA followed by Dunnett t test (Graph Pad Instat 7.00, USA)$p<0.05$ was taken as the criterion of statistical significance.

RESULT

1 Phytochemical screening: Phytochemical screening of ethanolic extract of plant was carried out. It showed the presence of alkaloids, glycosides, flavonoids, steroids, tannins and proteins.

2. Thin layer chromatography

Table No.1: Rf values of various spots

Phytoconstituents	Solvent system	R_f value of EEAM	R_f values
Flavonoids:	Ethyl acetate:formic acid:glacial acetic acid:water(100:11:11:26)	0.79	0.75-0.85
Alkaloid:	n-propranol:Formic acid:water(90:1:9)	0.2	0.15-0.20

3.Toxicity studies and behavior changes

Acute toxicity studies Ethanolic extract of *Argemone mexicana* (EEAM) Leaves was performed by using OECD 425 guideline(limit test) and it was found to be safe at 2000 mg /kg dose which indicated that its LD50 is more than 2000 mg /kg.None of the animals showed any toxic signs or death which indicated that LD 50 is more than 2000 mg/kg.

4. Pharmacological Screening: ISO induced Cardiotoxicity

4.1.Hemodynamic parameter

4.1.2. Blood Pressure determination

Table no.1 shows effect of EEAM on the animals treated with Isoproterenol showed significant ($p<0.001$) in the systolic, diastolic, mean BP and the heart rate when compared with the normal, treatment with EEAM 200mg/kg and 400 mg/kg showed a dose dependent, significant increase in the systolic BP (***P<0.0002), diastolic BP (****P<0.0001), mean BP (***P<0.0001) and the heart rate (***P<0.0002) respectively, when compared with the control group.

Table No.2: Effect of Ethanolic extracts of *Argemone mexicana* leaves on hemodynamic parameter (Blood Pressure) in ISO induced cardiotoxicity.

Groups	Systolic BP mmHg	Diastolic BP mmHg	Mean BP mmHg	Heart Rate (bpm)
Normal(Normal saline)	125.7612 ± 0.7292	118.6231± 0.2078	113.6142± 0.4912	273.8215± 0.7311
Control (ISO 85mg/kg)	74.1780± 0.0591### (↓41.02%)	69.3503±0.6029#### (↓41.52%)	72.098± 0.0143### (↓36.28%)	220.2490± 0.3017### (↓24.09%)
EEAM DOSE- I+ISO (200 mg/kg)	101.1123± 0.3001*** (↑36.48%)	93.4412± 0.039112**** (↑34.78%)	101.0281± 0.1772*** (↑40.27%)	251.2356± 0.3865*** (↑14.09%)
EEAM DOSE- II+ISO (400 mg/kg)	112.9120± 0.1035*** (↑51.35%)	98.3421± 0.1205**** (↑42.02%)	105.9578± 0.7611*** (↑45.83%)	265.2656± 0.4578*** (↑20.4%5)

ECG parameters were expressed in seconds (sec.). values were expressed as Mean ±SEM and n = 6,

****P<0.0001, ***P<0.0002 using one way ANOVA coupled with "Dunnett t test".****P<0.0001 is considered as significant. # indicate control group compared with normal (####P<0.0001, ###P<0.0002) and * indicate other groups compared with control group. The values in bracket indicates % increase↑ or decrease↓.

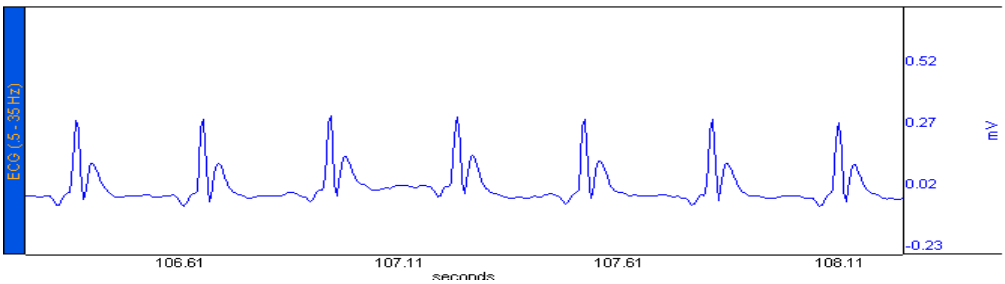
4.1.2. ECG recordings

Normal group showed a normal ECG pattern, where control animals treated with ISO showed significant, elevation in ST segment, prolongation in P wave, QRS complex and R-R interval. In addition there was a decreased in cardiac cycles and prolongation of QT interval as compared to normal rats. These changes were restored to near normal in EEAM 400 mg/kg. Pretreated ISO induced rats when compared to ISO control group, The groups-II which received EEAM 200mg/kg, in same schedule was not produced much alteration in ST-elevation.

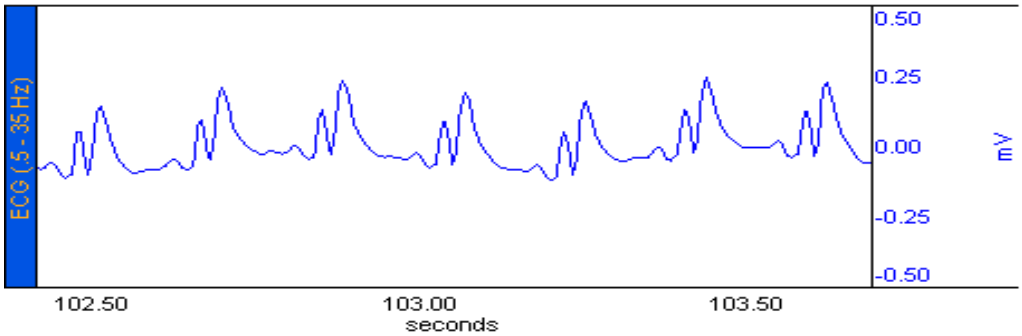
Table No.3: Effect of Ethanolic extracts of *Argemone mexicana* leaves (EEAM) on hemodynamic parameter(ECG)) in ISO induced cardiotoxicity.

Groups	P wave (sec.)	QRS Complex (sec.)	QT Interval (sec.)	RR Interval (sec.)	Cardiac Cycle (sec.)	ST Segment (mv)
Normal (Normal saline)	0.0267 ±0.0255	0.0333 ±.0025	0.0685 ±0.012	0.1995 ±0.130	0.1658 ±0.0.0029	0.176 ± 0.0034
Control (ISO 85mg/kg)	0.0425 ±0.019#### (↑59.17%)	0.0433 ±0.015### (↑30.07%)	0.1092 ±0.0012### (↑59.14%)	0.3305 0.089### (↑65.66%)	0.1442 ±0.0189### (↓13.02%)	0.332 ± 0.0058### (↑88.18%)
EEAM DOSE I+ISO(200 mg/kg)	0.0346 ±0.033**** (↓18.58%)	0.0325 ±0.029*** (↓24.94%)	0.0867 ±0.0091*** (↓20.60%)	0.2775 0.0080*** (↓16.03%)	0.1632 ±0.0451*** (↑13.17%)	0.236 ± 0.0043*** (↓18.07.%)
EEAM DOSE II+ISO(400 mg/kg)	0.0301 ±0.029**** (↓29.17%)	0.0316 ±0.039**** (↓27.02%)	0.0833 ±0.0081*** (↓23.71%)	0.2325 0.0171*** (↓29.65%)	0.1770 ±0.041*** (↑22.74%)	0.180 ± 0.0031*** (↓45.78.%)

ECG parameters were expressed in seconds (sec.) values were expressed as Mean ±SEM and n = 6, ****P<0.0001, ***P<0.0002 using one way ANOVA coupled with "Dunnett t test".****P<0.0001 is considered as significant. # indicate control group compared with normal (####P<0.0oo1, ###P<0.0002) and * indicate other groups compared with control group. The values in bracket indicates % increase↑ or decrease↓.



1)Normal (Normal saline)



2)Control (ISO 85mg/kg)

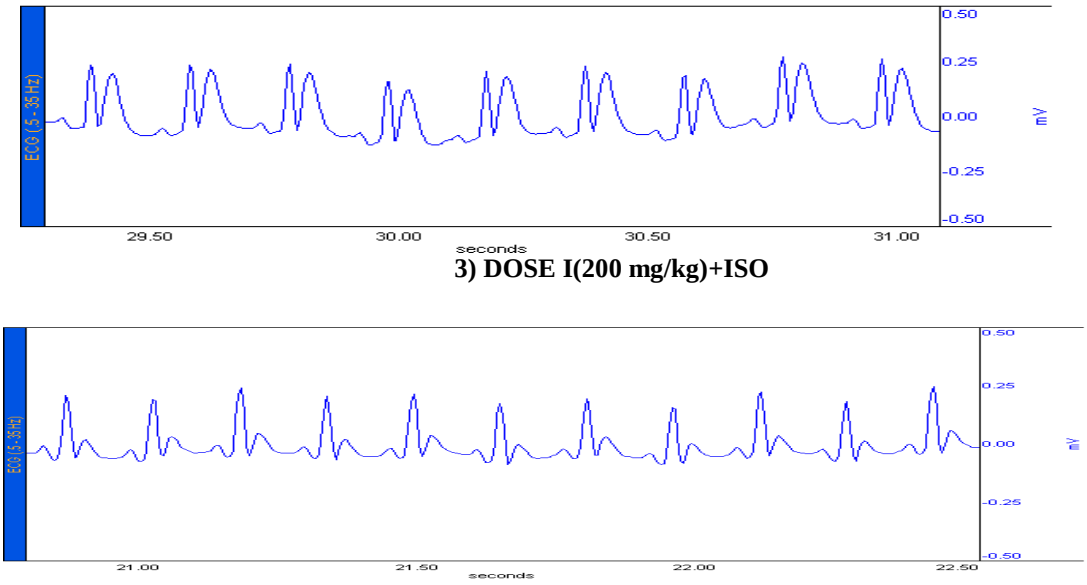


Fig.No.1: Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves (EEAM) on hemodynamic parameter(ECG)) in ISO induced cardiotoxicity.

4.2. Biochemical study

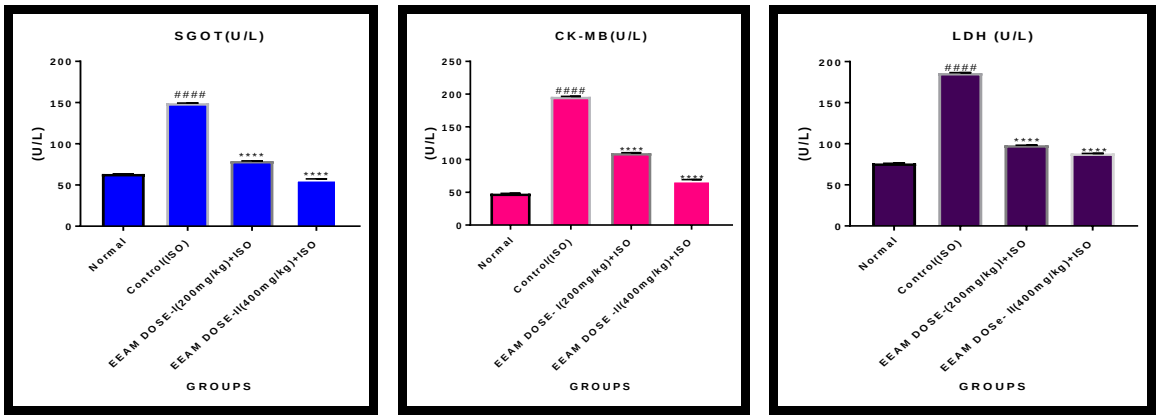
4.2.1 Serum Markers: CK – MB, LDH, SGOT

Table 4, shows the ISO induction caused significant increase in the activities of acute serum myocardial marker enzymes viz. LDH, CK-MB,SGOT when compared to control rats. Pretreatment with ethanolic extract of *Argemone mexicana* (EEAM) 200 mg and EEAM 400 mg/kg, significant decrease in CK-MB (****P<0.0001), LDH (****P<0.0001), and SGOT (****P<0.0001) when compared with the Iso control group.

Table No.4: Effect of Ethanolic extracts of *Argemone mexicana* leaves on Serum Markers.

GROUPS	CK-MB(U/L)	LDH(U/L)	SGOT(U/L)
Normal (Normal saline)	48.21±.0267	76.11±.1953	63.36±.097
Control (ISO 85mg/kg)	196.0±.2865#### (↑306.55%)	186.1±..1096#### (↑144.51%)	149.3±.067#### (↑135.63%)
EEAM DOSE I (200 mg/kg)+ISO	109.9±.2329**** (↓43.92%)	98.2±.0048**** (↓47.23%)	79.15±.0519**** (↓46.98%)
EEAM DOSE II(400 mg/kg)+ISO	68.78±.3107**** (↓64.90%)	88.08±.046**** (↓52.67%)	57.17±.0728**** (↓61.70%)

values were expressed as Mean ±SEM and n = 6, ****P<0.0001, using one way ANOVA coupled with "Dunnett t test".****P<0.0001 is considered as significant. # indicate control group compared with normal (####P<0.0oo1) and * indicate other groups compared with control group. The values in bracket indicates % increase↑ or decrease↓.



(SGOT) (CK-MB) (LDH)

Fig. No. 2: Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves (EEAM) of *Serum Markers.*

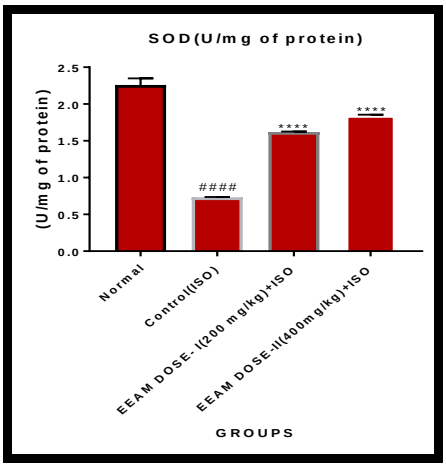
4.2.2. Tissue antioxidant markers and lipid peroxidation of heart tissue homogenate

Table no.5 shows a significant (P<0.0001) increase in content of MDA as well as significant decline in activity of SOD, CAT, & Glutathione was observed in heart tissue homogenate of isoprenaline treated rat as compared to normal. Administration of EEAM leaves markedly prevented all changes produced by ISO, significantly restore these levels near normal. Much significant effect was observed with EEAM 400mg/kg dose.

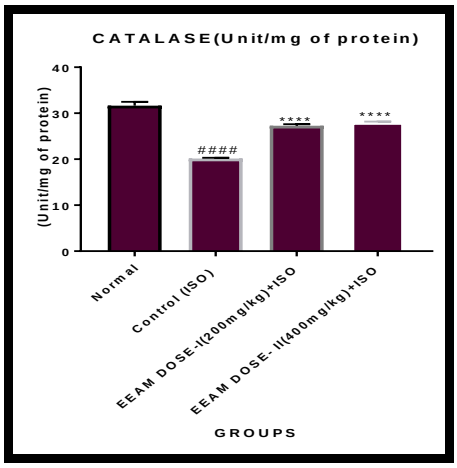
Table no.5: Effect of Ethanolic extracts of *Argemone mexicana* leaves on antioxidant Markers in ISO induced cardiotoxicity.

Groups	SOD(Unit/mg of protein	CAT(Unit/mg of protein)	MDA(µmoles/mg protein)	GSH (µmoles/L)
Normal (Normal saline)	2.26±0.03661	31.64±.3379	0.8815±.0027	33.25±0.1142
Control (ISO 85mg/kg)	0.7328±0.00166#### (↓67.57%)	20.16±.0584#### (↓35.48%)	1.905±.0042#### (↑113.44%)	16.44±0.1127#### (↓50.55%)
EEAM DOSE I+ISO(200 mg/kg)	1.619±.00263**** (↑220.93%)	27.28±.132**** (↑35.31%)	1.218±.0060**** (↓36.06%)	29.26±0.09315**** (↑77.98%)
EEAM DOSE II+ISO(400 mg/kg)	1.851±.001453**** (↑152.59%)	28.12±.0368**** (↑39.48%)	1.115±.00142**** (↓41.46%)	30.36±0.1159**** (↑84.67%)

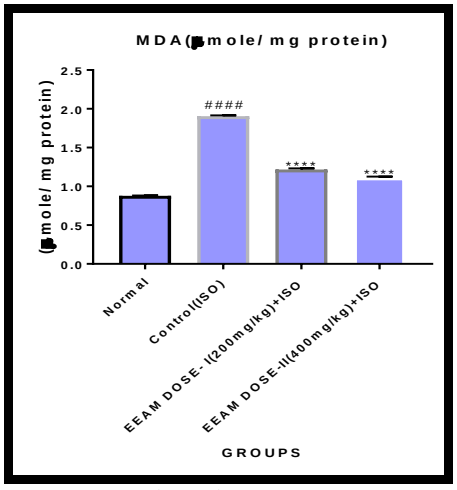
values were expressed as Mean ±SEM and n = 6, ****P<0.0001, using one way ANOVA coupled with "Dunnett t test".****P<0.0001 is considered as significant. # indicate control group compared with normal (####P<0.0oo1) and * indicate other groups compared with control group. The values in bracket indicates % increase↑ or decrease↓.



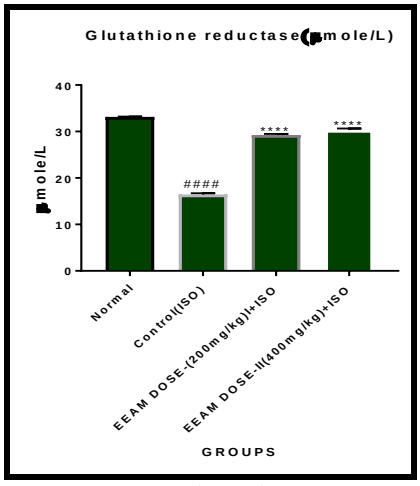
(SOD)



(MDA)



(LDH)



(GRH)

Fig. No. 3: Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves on antioxidant markers in ISO induced cardiotoxicity.

4.3. Histopathological changes on ISO-induced toxicity on organ: Histopathology of Heart:
Histopathological examination of myocardial tissue obtained from normal animals exhibited clear integrity of myocardial membrane. Normal rats showed normal cardiac fibers without any infarction. The heart sections obtained from ISO treated animals showed abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular muscle fiber. Animals pretreated with EEAM 200mg/kg & 400 mg/kg showed improvement in the cell integrity evidenced absence of necrosis, marked decrease in infiltration of inflammatory cells and maintenance of normal integrity of the cardiac muscles. (fig.no.4)

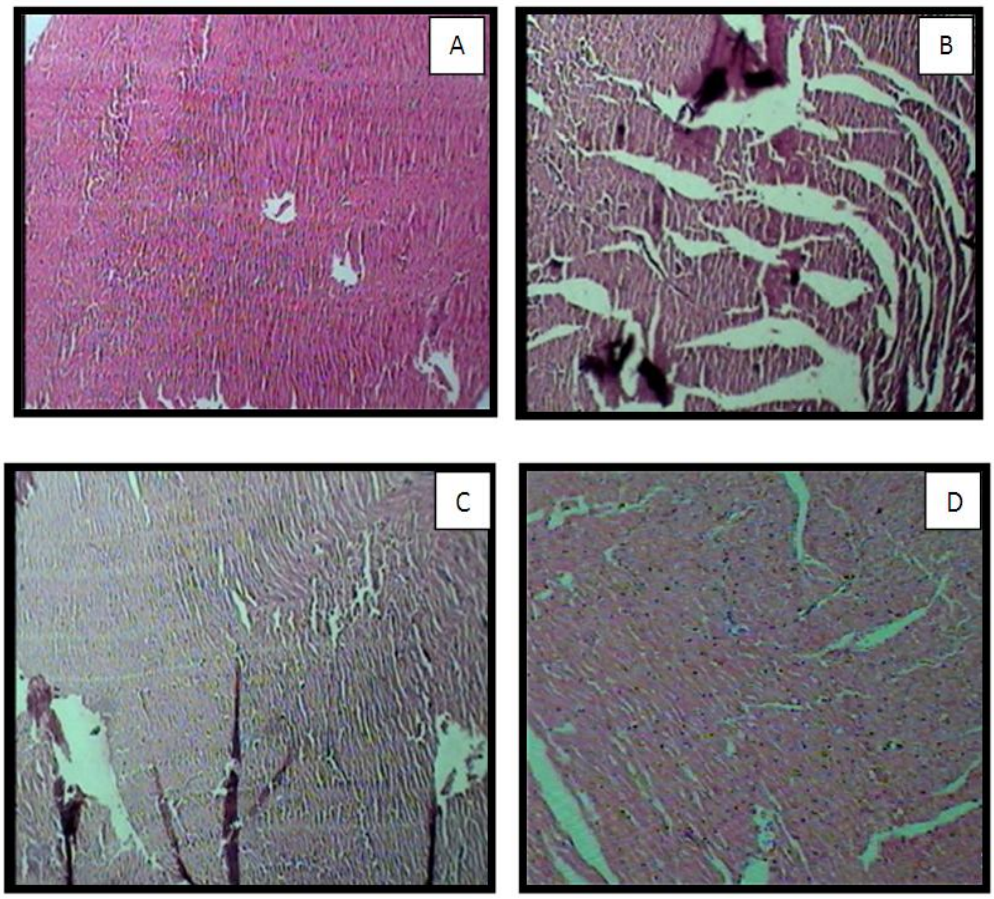


Fig. No.4: Histopathological images of heart pretreated with EEAM by Isoproterenol induced cardiac toxicity. A – Normal, B – Control (ISO), C -EEAM Dose-I+ 200mg/Kg+(ISO), D Dose-I+ 400mg/Kg+(ISO).

DISCUSSION
In present investigation effects of plant Ethanolic Extract of *Argemone mexicana* (EEAM) leaves was studied to establish its effect on ISO induced changes in hemodynamics, biochemical and histology of heart.

The Argemone Mexicana (AM) is one of the plant reported for having Antimicrobial, Antidiabetic, Antioxidant, Hepatoprotective, Larvicidal activity, Wound healing activity, Cancer activity, Anti helminthic activity, Anti-inflammatory and analgesic, Neuropharmacological activities. The research article by Joginder Singh Duhan et.al., have reported in-vitro antioxidant activity of AM. AM. Leaves has been also reported for its hepatoprotective, antipyretic action[6], probably due to inhibition of lipid peroxidation.

Argemone mexicana (AM) consist of phytochemicals like, alkaloids, glycosides, flavonoids, steroids, tannins and proteins.[5] The phytochemical investigation of Ethanolic Extract of *Argemone mexicana* (EEAM) leaves showed presence of alkaloids, glycosides, flavonoids, steroids, tannins and proteins.

Acute toxicity studies Ethanolic extract of *Argemone mexicana* (EEAM) Leaves was also performed by using OECD 423 guideline (limit test) and it found safe at 2000 mg /kg dose which indicate that its LD50 is more than 2000 mg /kg.

ISOPROTERENOL INDUCED CARDIOTOXICITY
Isoproterenol (ISO) induced myocardial cell death is well known standard drugs model to study the advantageous effect of many drugs on cardiac dysfunction. Extreme stress in myocardium and necrotic lesions in the heart muscles is caused by ISO which is a β-adrenergic agonist. ISO causes myocardial injury and cause of that membrane permeability changes take place,which brings

about the loss of activity and integrity of myocardial membranes.[1]

Table no.2, ISO cause a decrease in the systolic, diastolic, mean BP and heart rate this is probable due to effect of ISO on the myofibrils, causes its disruption hence the systolic, diastolic, mean BP and heart rate decreases. Our study demonstrated an increase in the systolic, diastolic, mean BP and the heart rate when compared with the control. Stabilization of the myocardium due to extract, causes the decrease in the myofibrils disruption and shifts the blood pressure close to normal as shown in previous studies reported by Chander Hass Yadav & et.al,.

Electrocardiograph-abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. ST-segment elevation was observed either in patient with acute myocardial ischemia or in isoproterenol-induced myocardial infarction in rat. The study shows significant alterations of ECG patterns were observed in ISO administered rats as compared to normal control rats as shown in previous studies reported by Chander Hass Yadav & et.al,. The characteristic findings were reductions in the P wave intensity, QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle. We also observed a significant elevation in the ST segment and increase in heart rate. These alterations could be due to the consecutive loss of cell membrane in injured myocardium. In the present study (Table no.3), we observed an elevation of ST-segments in isoproterenol-induced rat and pretreatment with EAML (200mg/kg &400 mg/kg) not significantly inhibited isoproterenol-induced ST-segment elevation suggestive of its cell membrane protecting effects. The appearance of Q wave and ST segment elevation are some of the indicative signs of ischemia. In the present study we did not observe pathological Q wave due to conditions of ischemia. The prominent Q wave were seen only on severe ischemia, infarction and in patients with severe heart diseases. The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation.

Control group (Table no. 4) shown ↑ 306.55% in CK-MB, ↑ 144.51% in LDH, ↑135.63% in SGOT. The groups treated with EEAM doses has shown significant decline in level these cardiac markers indicating their ability to reduces leakages of this enzymes from cardiomyocytes. Among the groups treated EEAM at dose 400 mg/kg has showed to significant deduction ($p<0.0001$).

As Oxidative stress is responsible for ISO related cardiotoxicity, hence in study also investigate the level of different antioxidant markers from heart tissue homogenate hence in current study MDA,SOD,CAT and GSH levels were analysed. The control group (Table no.5) have shown significant in ↑ level of MDA ($p<0.0001$) while ↓ in SOD, CAT, GSH which is in correlation with previous studies reported by P.R. Deepa et.al.[13]

Isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been seen as one of the causative factor.[1]

Isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been seen as one of the causative factor.[1] Isoproterenol is well known cardiotoxic agent due to its ability it will destruct myocardial cells and hence diagnosis of cardiac enzyme is prerequisite in case of ISO induced cardiac stress. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability.[10]

The free radical chain reaction of auto-oxidation are also inhibited by antioxidants by donating the hydrogen of the phenolic hydroxyl group and thereby, giving rise to a stable end product which does not initiate the further oxidation of lipids.[10] The data showed that, these extracts are free radical scavenger and may act as primary antioxidants, which may react with the free radical by donating hydrogen.

The Histopathological studies of the heart tissue evident in myocardial edema and separation of fibres with loss of striation as mark of myocardial injury in ISO control.[10] Fig no.4 Shows Animals pretreated with EEAM 200 mg /kg & 400 mg /kg demonstrated less myocardial edema and separation of fibres.

The observed myocardial protective effect of title plant could be due to the free radical scavenging activity of the extract in the presence of phytochemicals. Presence of constituents like Alkaloids and Flavonoids might be responsible for having cardioprotective actiity.which are also present it *Cucumis trigonus* Roxb, *Terminalia paniculata* plant which has alredy proved to have cardioprotective.[3] These data further confirmed the cardioprotective action of EEAM.

CONCLUSION

In current investigation has elaborated cardio protective effect of *Argemone mexicana* leaves in ISO induced cardiac stress. The administration of EEAM leaves 200mg/kg & 400mg/kg restore heamo-dynaamic alteration in both models. The study also provided experimental evidence that EEAM leaves maintained antioxidant enzyme levels even after exposed to the agent responsible for causing cardiac damage.

The restoration of defined cardiac marker like LDH, CK-MB, SGOT suggest EEAM has ability to protect cardiomyocytes from damage. These finding suggest possible usefulness of EEAM leaves as a cardio-protective agent. Further investigation are needed to find

the exact phytoconstituent responsible for cardioprotective activity of *Argemone mexicana* (AM) leaves.

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