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THE CARDIOPROTECTIVE EFFECT OF ARGEMONE MEXICANA ON DOXORUBICIN INDUCED CARDIOTOXICITY 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 Doxorubicin (antitumor) 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 Doxorubicin induced cardiac stress in which Ethanolic Extract of Argemone Mexicana Leaves(EAML 200 mg/kg,400mg/kg) were administered to both Doxorubicin(15mg/kg i.p.) administered groups respectively. The present study concludes that restoration of Haemodynamic Parameters(BP,ECG),Biochemical parametsrs-cardiac markers(CK-MB,LDH,SGOT), antioxidant markers(MDA,CAT,SOD,GTH)) Histopahological indications.

Keywords: *Doxorubicin, Antioxidant, ECG, Blood pressure, Cardiotoxicity*

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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].

Adriamycin, also called Doxorubicin, is an antibiotic anthracycline that was isolated from a pigment Streptomyces peacetius in the early 1960's but which is now chemically synthesized. Have been employed for more than 30 years in the battle against cancer. Doxorubicin is essential in treating breast and esophageal carcinomas, solid tumors in childhood, osteosarcomas, Kaposi's sarcoma, soft tissue sarcomas, and Hodgkin and non-Hodgkin lymphomas. Doxorubicin has acute and chronic toxic effects. Acute side effects related to intravenous injection of Doxorubicin appear within minutes after infusion, including nausea, vomiting, myelosuppression and arrhythmia. On the other hand, chronic effects may develop several weeks or even months after the recurrent administration of drug, provoking heart, liver and brain injury [3].

Although a close examination of this list indicates that Adriamycin-induced injury may be multifactorial and complex, one mechanism common to most of these suggestions is the increased oxidative stress. Because of the presence of semi Quinone in the tetracyclic aglycone molecule of Adriamycin, the drug is reported to increase the oxygen radical activity as well as peroxidation of polyunsaturated fatty acids within the membrane phase. This may also explain Adriamycin induced defects in membrane function caused by this drug [4].

Drugs used in doxorubicin induced cardio toxicity are MPZ (mercaptopropionyl glycine), Probucol, Dexrazoxane, Amlodipine, Carvedilol, Sildenafil, Superoxide dismutase, Endothelin receptor antagonist, Granulocyte colony stimulating factor [5].

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 [6].

Argemone Mexicana leaves has been reported for different chemical constituents like Alkaloids, Amino acids, flavonoids, Phenolics, and fatty acids as a major phytochemical groups [6].

MATERIALS AND METHODS:

1.Drugs/inducers used:

Sr. No.	List of Drugs used	Procured from
1.	Doxorubicin hydrochloride	Life sciences Ltd.,Ankleshwar (Gujrat)

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 fanimal 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 Wagner et .,1996) [7]:

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 Doxorubicin induced cardiac toxicity .The group were as follows:

1 .Doxorubicin induced cardiac Stress (dose and route of administration)

Group1- Normal (Normal saline)

Group 2 –Control-(Normal saline upto 7 days+ Doxorubicin i.e. DXR of 2.5mg/kg i.p. injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg.)

Group 3-Test -(EEAM of leaves, dose–I of 200 mg/kg ,daily for 3 weeks)+

(DXR of 2.5mg/kg i.p. injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg.)

Group 4- Test-*(*EEAM of leaves, dose -II of 400 mg/kg , daily for 3 weeks)+

(DXR of 2.5mg/kg i.p. injected on 8th, 10th, 14th, 16th, 18th and 21st day to reach total cumulative dose of 15 mg/kg.)

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

2.1.1. 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.

2.1.2. Electrocardiography (ECG):

ECG was recorded at the end of the treatment after the last dosing of DOX. 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.

2.2. Biochemical study

2.2.1Serum 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.

2.2.2.Tissue Antioxidant Biomarkers:

Determination of Malondialdehyde (MDA)[product of lipid peroxidation] level from heart tissue homogenate[8]. Determination of Superoxide dismutase (SOD) enzyme activity from heart tissue homogenate by the method of Marklund et al. (Mahammad et al., 2013) [8]. Determination of Catalase (CAT) enzyme activity from heart tissue homogenate by the method of Aebi et al. [9]. Determination of Reduced Glutathione (GSH) enzyme activity from heart homogenate by the method of Ellmans et al. (Mahammad et al., 2013)

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) and SAGLO research software and equipment.

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:

- **DXR induced cardiotoxicity:**

4.1.Hemodynamic Parameter:

4.1.1. Blood Pressure determination:

Table no.2 shows effect of EEAM on the animals treated with DXR showed significant ($p<0.001$) in the systolic, diastolic, mean BP and the heart rate when compared with the normal, treatment with EAML 200 mg 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<0.0002) 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 (EEAM) hemodynamic parameter (Blood pressure) in DXR 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 (DXR 15mg/kg)	78.5610 ± 0.2591### (↓37.53%)	68.2625 ±0.3629#### (↓42.25%)	73.8213 ± 0.2196### (↓35.02%)	211.2621 ± 0.3023### (↓22.84%)
DXR+EEAM DOS-I(200 mg/kg)	104.1023 ± 0.3681*** (↑32.51%)	92.5415 ± 0.3996**** (↑35.56%)	99.6206 ± 0.2892*** (↑34.94%)	244.8982 ±0 .1438*** (↑15.62%)
DXR+ EEAM DOSE-II(400 mg/kg)	110.9912 ± 0.3935*** (↑41.28%)	94.2627 ± 0.4605**** (↑40.46%)	103.6498 ± 0.3611*** (↑40.46%)	252.6137 ±0 .2314*** (↑19.53%)

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:

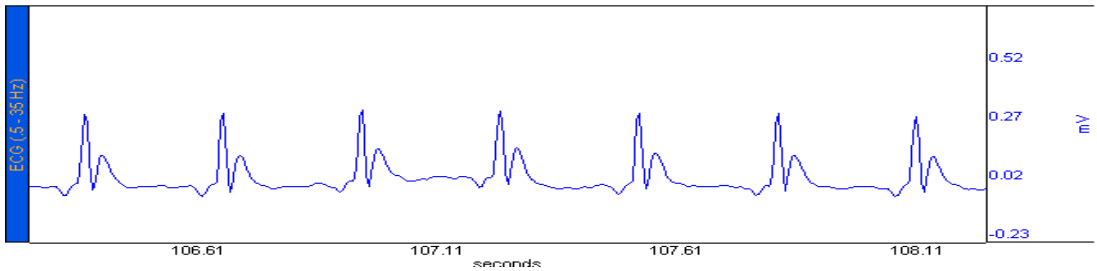
Normal group showed a normal ECG pattern, where as animals treated with Dox alone showed significant elevation in ST segment, prolongation in P wave, QRS complex and R-R interval. In addition there was a decreased in cardiac cycles and prolongation of QT interval as compared to normal rats. Group treated with ethanolic extract of *Argemone mexicana* (EEAM) 200 mg/kg and 400 mg/kg reduces duration of P-wave, QRS complex, QT-inetrval as well as suppression of ST-segment. From graph we can observe clearly the restoration of ST- elevation near normal due to treatment with EEAM 400 mg/kg.

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

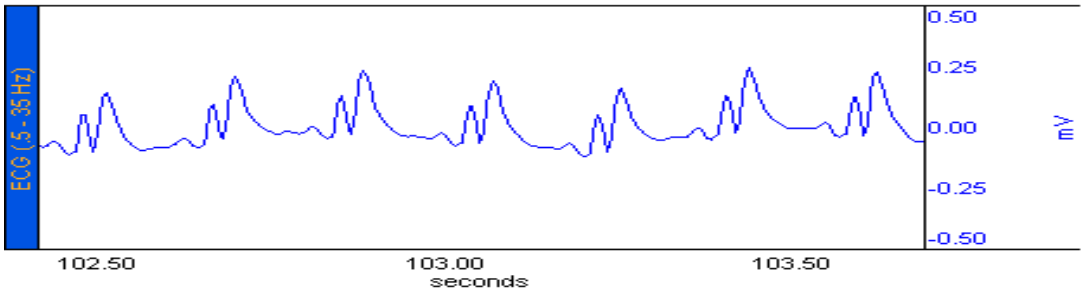
Groups	P-wave (sec.)	QRS Complex (sec.)	RR Interval (sec.)	Cardiac Cycle (sec.)	ST Segment (mv)
Normal (Normal saline)	0.0267 ±0.0255	0.0333 ±.0025	0.0685 ±0.012	0.1658 ±0.0.0029	0.176 ± 0.0034
Control(DXR-15mg/kg)	0.0417 ±0.0011#### (↑56.17%)	0.0426 ±0.0017### (↑27.92%)	0.1086 ±0.011#### (↑58.54%)	0.1245 ±0.0012### (↓24.90%)	0.292 ± 0.0058### (↑54.54%)
DXR+EEAM DOSE -I(200 mg/kg)	0.0356 ±0.0020**** (↓14.62%)	0.0398 ±0.0016*** (↓6.52%)	0.0903 ±0.033**** (↓16.85%)	0.1632 ±0.0019*** (↑31.08%)	0.256 ± 0.0043*** (↓12.32%)
DXR+EEAM DOSE -II(400 mg/kg)	0.0342 ±0.0018**** (↓20.18%)	0.0372 ±0.0033*** (↓12.38%)	0.0874 ±0.023**** (↓19.52%)	0.1648 ±0.0034*** (↑32.36%)	0.232 ± 0.0031*** (↓20.05%)

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↓.

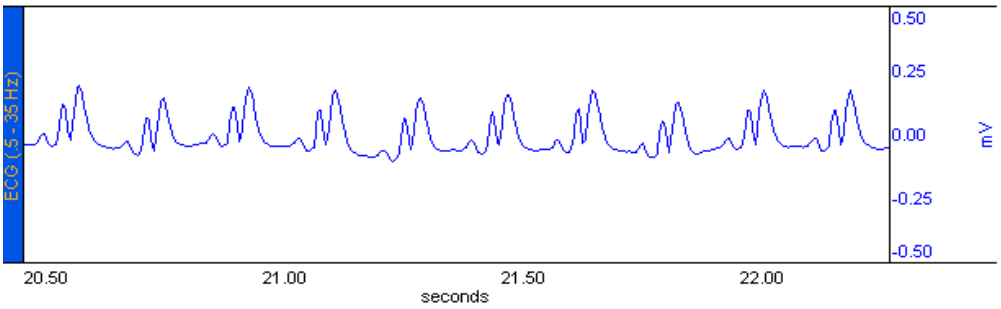
Fig. No. 1: Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves (EEAM) on hemodynamic parameter (ECG)) in DXR induced cardiotoxicity



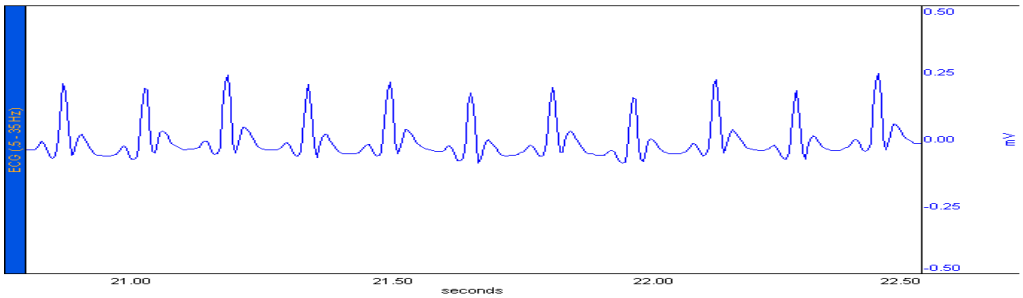
1)Normal (Normal saline)



2)Control (DXR 15mg/kg)



3)DXR+DOSE I(200 mg/kg)



4)DXR+DOSE II(400 mg/kg)

4.2.Biochemical study:

4.2.1. Serum Markers: CK – MB, LDH, SGOT .

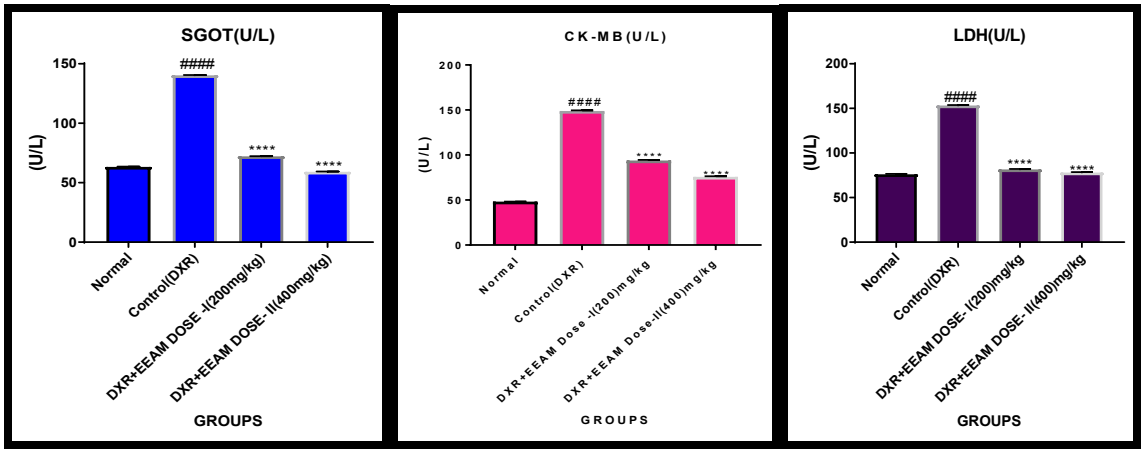
Table no.4 shows effect of EEAM on biochemical parameters Treatment with doxorubicin causes ant significant(P<0.001) elevation in level of CK-MB, LDH, SGOT which are considered as the selective biomarkers of myocardial damage when compared with the normal. Our. Oral Pretreatment with ethanolic extract of *Argemone mexicana* (EEAM) 200 mg/kg and 400 mg/kg for 30 days showed a dose dependent, significant decrease in CK-MB (****P<0.0001), LDH (****P<0.0001), and SGOT (****P<0.0001) when compared with the DOX control group.

Table No. 4: Effect of Ethanolic extracts of *Argemone mexicana leaves* (EEAM) on cardiac serum markers) in DXR induced cardiotoxicity.

Groups	CK-MB(U/L)	LDH(U/L)	SGOT(U/L)
Normal (Normal saline)	48.21 ±.0267	76.11 ±.1953	63.36 ±.097
Control (DXR 1 5mg/kg)	149 ±.2491#### (↑209.06%)	157.2 ±.2056#### (↑106.28%)	140.3 ±.1094#### (↑121.62%)
DXR+EEAM DOSE-I(200 mg/kg)	93.91 ±.2545**** (↓36.97%)	81.58 ±.2274**** (↓48.09%)	72.16 ±.0549**** (↓48.56%)
DXR+EEAM DOSE-II(400 mg/kg)	75.76 ±.2471**** (↓49.15%)	78.08 ±.2353**** (↓50.31%)	59.2 ±.08552**** (↓57.80%)

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<0.0001) and * indicate other groups compared with control group. The values in bracket indicates % increase↑ or decrease↓.

Fig. No.2 Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves on serum marker SGOT, CK-MB, LDH Heart tissue homogenate) in DXR induced cardiotoxicity



(SGOT) (CK-MB) (LDH)

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

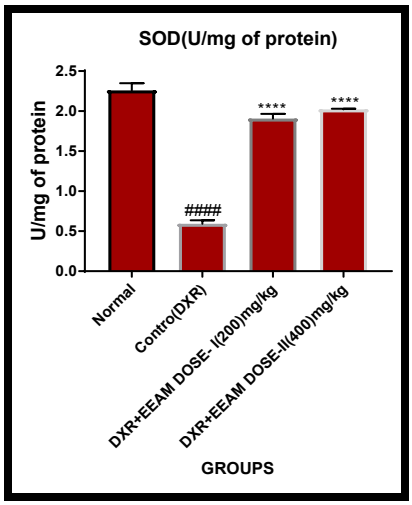
Table no.5 Shows Doxorubicin cause a decrease in the level of endogenous antioxidant reserves viz. SOD and CAT and shows significant(P<0.0001) increase in the lipid peroxidation of the heart when compared with the normal. Pretreatment with EAML 200 and 400 mg/kg showed a significant increase in the level of enzyme SOD (****P<0.0001),CAT (****P<0.0001) and GSH(****P<0.0001)While significantly decrease the level of MDA (****P<0.0001) in a dose dependent manner, when compared with the control.

Table No.5: Effect of Ethanolic extracts of *Argemone mexicana* leaves on (antioxidant markers) in DXR induced cardiotoxicity.

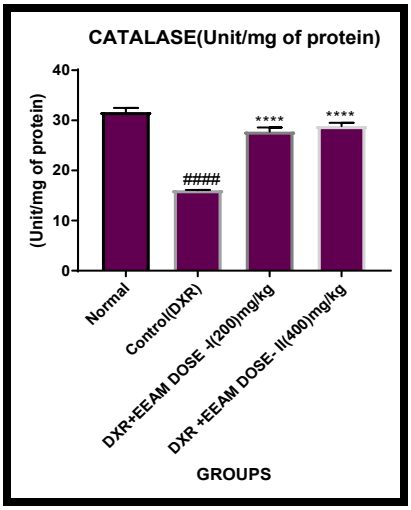
Groups	SOD(Unit/mg of protein	CAT(Unit/mg of protein)	MDA(µmoles/mg protein)	GSH (µmoles/L)
Normal (Normal saline)	2.26 ±0.03661	31.64 ±.3379	0.8815 ±.0027	33.25 ±0.1142
Control (DXR 15mg/kg)	0.5933 ±0.01764#### (↓73.48%)	16.06 ±.024#### (↓49.24%)	2.325 ±.0076#### (↑163.75%)	19.23 ±0.08#### (↓42.16%)
DXR+EEAM DOSE-I(200 mg/kg)	1.907 ±0.02418**** (↑221.42%)	27.75 ±.3394**** (↑68.75%)	1.341 ±.0086**** (↓42.32%)	22.41 ±0.167**** (↑15.13%)
DXR+EEAM DOSE-II(400 mg/kg)	2.022 ±0.0030**** (↑240.80%)	28.84 ±.272**** (↑75.11%)	1.025 ±.0076**** (↓55.91%)	25.29 ±0.109**** (↑31.15%)

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↓.

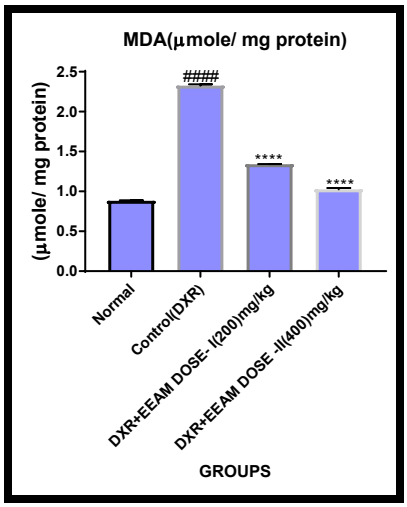
Fig. No 3:.Graphical representation of Effect of Ethanolic extracts of *Argemone mexicana* leaves on antioxidant markers) in DXR induced cardiotoxicity.



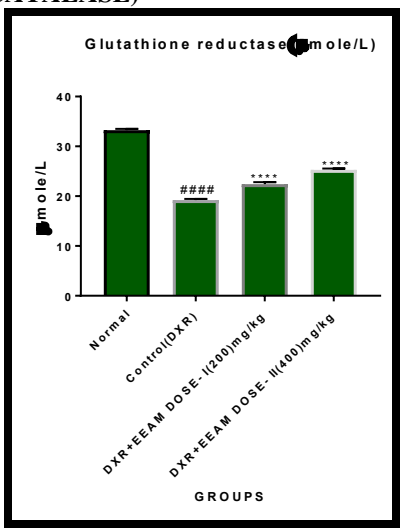
(SOD)



(CATALASE)



(MDA)



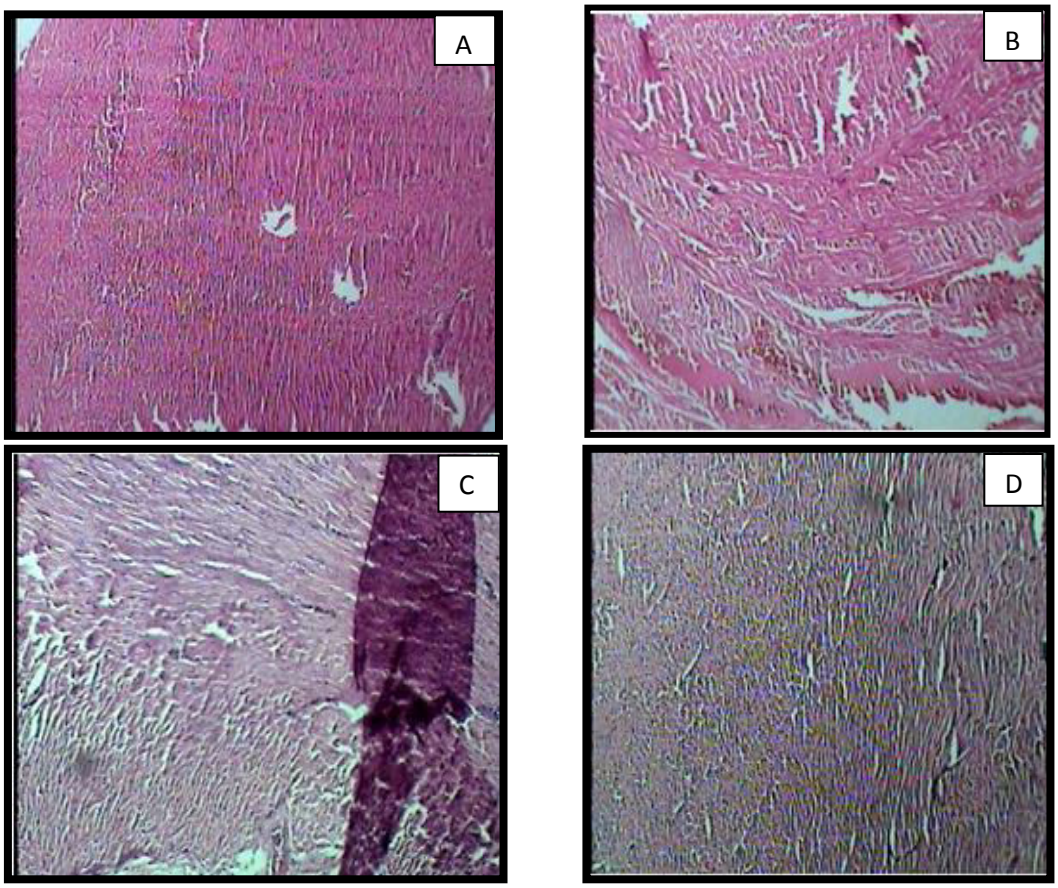
(GRH)

4.3. Histopathological changes on DOX-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 DOX treated animals showed abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular muscle fiber. Animals pretreated with EEAM 200 mg/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 Doxorubicin induced cardiac toxicity. A – Normal, B – Control(DXR), C -(DXR)+EEAM Dose-I 200mg/Kg, D -(DXR)+EEAM Dose-II 400mg/Kg



DISCUSSION:

In present investigation effects of plant Ethanolic Extract of *Argemone mexicana* (EEAM) leaves was studied to establish its effect on DOX 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,(Sourabie et al.,2012) probably due to inhibition of lipid peroxidation.

Argemone mexicana (AM) consist of phytochemicals like, alkaloids, glycosides, flavonoids, steroids, tannins and proteins (Chrles et al.,2014). 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.

DOXORUBICIN INDUCED CARDIOTOXICITY

Repeated administration of adriamycin beyond a certain dose has been shown to cause cardiomyopathic changes in patients' as well as in a variety of animal species. The rat model is considered to be a good, reproducible, and cost-effective system for testing beneficial effects of different drugs.

Clinical and experimental investigations suggested

that increased oxidative stress plays a critical role in subsequent cardiomyopathy and heart failure associated with DOX treatment. These finding are in line with those observed by previous investigators Davey M.S.& et.al.

The present investigation was designed to evaluate protective effect of EEAM leaves against DOX induced cardiotoxicity in rats. DOX induced cardiotoxicity and oxidative stress has been confirmed in many experimental models.

Table no.2 shows, DOX cause a decrease in the systolic, diastolic, mean BP and heart rate, this is probable due to effect of DOX on the myofibrils, causes its disruption hence the systolic, diastolic, mean BP and heart rate decreases. Our study demonstrated the raise in the systolic, diastolic, mean BP and heart rate in treated groups as compared with the control as shown in previous studies reported by Sourabie et al.,2012. Stabilization of the myocardium due to EEAM, causes the decrease in the myofibrils disruption and may be the reason for maintaining B.P. near normal. Among groups treated EEAM at dose 400 mg/kg has shown significant restoration of levels near normal.

Table no.3 shows the influence of EEAM on DOX treatment on Rat ECG parameters, At the end of treatment animals were anesthetized and ECG were recorded. DOX treatment showed significant changes in repolarisation phase of ECG (ST elevation) which clearly observed from graph as shown in previous studies reported by Holland R.P & et.al. DOX produces significant prolongation in ST segment; QT interval; QRS complex; RR interval. Pretreatment with both doses of EEAM significantly altered these changes in ST & QT interval. Therefore EEAM prevented various alteration in ECG parameters produced by DOX treatment.

After completion of Experimental period various parameters were checked. The control group treated with DOX has shows significant alteration in the level of different cardiac markers. The LDH, CK-MB and the cardiac markers which get raised in circulation as a result of cardiomyocyte damage and release of enzymes from mitochondria. Table no.4 shows Control group ↑ 209.06% in CK-MB, ↑ 106.28% in LDH, ↑121.62% in SGOT. This increase in CK-MB, LDH activities demonstrate DOX induced cardiotoxicity.

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 cornerstone in DOX related cardiotoxicity, it is important to investigate the level of different antioxidant markers from heart tissue homogenate hence in current evaluation 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 N. Silveski-Iliskovic et al.,2007 These finding suggest that DOX is responsible for causing cell damage and increase oxidative stress.

Lipid peroxidation plays a major role in the myocardial cell damage and the accumulated lipid peroxides reflect the various stages of diseases and its complications. Significant elevation observed in the level of GSH with a antiperoxidative enzymes activity (CAT, and SOD) in heart of negative control group. Depletion of glutathione is known to result in enhanced LPO and excessive LPO can cause increased glutathione consumption. Inhibition in the activities of antioxidant enzymes may lead to the increased generation of O_2^- and H_2O_2, which in turn can form hydroxyl radical (OH) and bring about a number of reactions harmful to structural and functional intigrity .

Pretreatment of rats with EEAM significance restore level of MDA(↓42.32%,↓55.91%),CAT((↑68.75%,↑75.11%), SOD(↑221.42%,↑240.80%),GSH((↑15.13%,↑31.15) respectively by EEAM 200 mg /kg & 400 mg /kg compared to control (Table no.5) which suggest its beneficially cardioprotective ability to reduce oxidative stress either by reducing generation ROS or free radical scavenging effect of EEAM.

Histopathological examination of myocardial tissue obtained from normal control animal exhibited clear integrity of myocardial membrane. Normal rats showed cardiac fibers without any infarction. The heart sections obtained from DOX treated animals showed disruption of several subcellular elements including loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm by Young and Myers et al.

Fig no.5 Shows Animals pretreated with EEAM 200 mg /kg & 400 mg /kg demonstrated less disruption of the myofibrils and less vacuolization of the cytoplasm. This further confirms the membrane stabilizing effect of the EEAM.

CONCLUSION:

In current investigation has elaborated cardio protective effect of *Argemone mexicana* leaves in DOX induced cardiac stress. The administration of EEAM leaves 200mg/kg & 400mg/kg restore heamo-dynaamic alteration in model. 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. It may contribute for safer use of DOX in patient subjected to cancer chemotherapy as well as oxidative stress related cardiac damage (viz ZIR injury, CAD etc.) Further investigations are needed to find the exact phytoconstituent responsible for cardioprotective activity of *Argemone mexicana* (AM) leaves.

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