World Journal of Pharmacy and Biotechnology
ISSN: 2349-9087
Journal Home Page: www.pharmaresearchlibrary.com/wjpbt
W. J. Pharm. Biotech., 2020, 7(2): 22-32
DOI: https://doi.org/10.30904/j.wjpbt.2020.4288

Investigation of Cardio Protective Activity of Ethanolic Extracts of *Euryale Ferox* Seeds in Rats

Dhanaboina Mahesh, Murali Sollu*, Gampa Vijay Kumar
KGR Institute of Technology and Management, Rampally Vill, Kesara Mdl, Medchal-501301, Telangana, India

A B S T R A C T
Medicinal plants and plant derived products are used as medicines in a large group of world population. Euryale ferox. (Family - Nymphaeaceae) is used in Indian Ayurvedic medicine for the treatment of various diseases. As Euryale ferox seeds have the native habitat the production is more so it is locally available cost effective with no side effects. Doxorubicin forms an iron anthracyclin complex that generates free radicals, which in turn causes severe damage to the plasma membrane that results in further increase in LDH, CK-MB and SGOT levels in blood. Pretreatment with Euryale Ferox seeds Ethanolic extract produced significant decrease in LDH, CK-MB and SGOT levels indicating the protective effect of cardiac tissue. Heart tissue is especially susceptible to free radical injury because of low levels of free radical detoxifying enzymes like CAT and GSH. On Doxorubicin treatment a dose dependent decrease in CAT and GSH levels were observed due to its accumulation in the heart tissue. Pretreatment with *Euryale Ferox seeds* Ethanolic extract produced significant (<0.01) increase in CAT and GSH levels in dose dependent manner, thus indicating that the antioxidant status is improved on extract treatment. The Histopathological report of hearts treated with Doxorubicin (15mg/kg, i.p) produced focal cardiocyte degeneration with patch inflammation of lymphocytes and mild interstitial fibrosis. But, pretreatment with 150mg/kg, 300mg/kg and 600mg/kg doses plant extract showed mild or no degenerative changes when compared with Doxorubicin control group from this experimental and histopathological results indicate that treatments with the *Euryale Ferox* seeds possess significant cardio protective activity in experiment animals.
Keywords: Euryale Ferox, Myocardial Infarction, Doxorubicin-induced cardio toxicity.

AR T I C L E I N F O

Corresponding Author
Murali Sollu
KGR Institute of Technology and Management
Rampally Vill, Kesara Mdl
Medchal-501301, Telangana, India

Article History
Received : 10 Feb 2020
Revised : 07 Mar 2020
Accepted : 05 April 2020
Published : 29 June 2020

Citation: Murali Sollu, *et al*. Investigation of Cardio Protective Activity of Ethanolic Extracts of Euryale Ferox Seeds in Rats. W. J. Pharm. Biotech.., 2020; 7(2): 22-32.

C O N T E N T S

1. Introduction

The cardiovascular system consists of heart & blood vessels which circulate blood throughout the body. It is responsible for transporting oxygen, nutrients, and hormones to body and removes cellular waste products from the body. The term cardiovascular disease [CVD] is very much familiar which commonly refers to a group of diseases that affects heart and its parts, whereas the term CVD mostly refers to MI [Myocardial infarction], angina pectoris, hypertension, stroke and other circulatory diseases. The common heart diseases that have been reported are coronary artery diseases, congestive heart failure, cardiac arrest, arrhythmias, and peripheral artery diseases. Makhana

(Euryale ferox Salisbury) grows as an exclusive aquatic cash crop in shallow water bodies in north Bihar and lower Assam regions of India. It has nutritional and medicinal properties and supports cottage industry. It is a monotypic genus and the available genetic variability is limited.

2. Materials and Methods

Euryale ferox was collected and the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber of the Soxhlet apparatus. Doxorubicin was gifted by Aurobindo Pharma, Hyderabad.

Experimental procedure

Study Design for Cardioprotective Activity

Animals were grouped into 6 groups, 6 rats in each group

Group 1: Vehicle control group - Administration of distilled water orally.

Group 2: Cardiac control group - Doxorubicin with cumulative dose of 15 mg/kg i.p (Dose as per standardized marketed formulation converted in to animal ratio) for 2 weeks alternatively.

Group 3: First dose treated group - Pretreated with grass extract of 150 mg/Kg, i.p, b.wt (1/20 of the maximum safe dose given in the acute toxicity studies) along with doxorubicin.

Group 4: Second dose treated group - Pretreated with grass extract of 300mg/Kg, i.p, b,wt(1/10 of the maximum safe dose given in the acute toxicity studies) along with doxorubicin.

Group 5: Third dose treated group - Pretreated with grass extract of 600 mg/Kg, b.wt i.p,(1/5 of the maximum safe dose given in the acute toxicity studies) along with doxorubicin.

Group 6: Atorvastatin was given orally (10 mg / kg b.wt) for 30 days.

Doxorubicin was given by s.c injection on 17th day. Atorvastatin was used as a positive control. Group 2, 3 and 4 ,5 received the treatment of doxorubicin at alternate days for a period of 2 weeks. At the end of treatment blood samples were collected and assayed for LDH, CK-MB, SGOT levels.

3.2.8 Collection of blood samples and organs

Blood samples were collected by retro orbital puncture of rats' eye using a capillary tube about 2-3 inches. Sodium citrate (3.8% w/v) is used as an anti-coagulant to collect the blood 1:9 ratio of sodium citrate to blood was used. The serum was separated by centrifugation at 3000rpm for 15min to collect the serum, were analyzed for various cardiac biomarkers.

Rats were sacrificed by cervical decapitation and hearts were removed rapidly one heart from each group kept in formalin solution to carry out histopathalogical examination remaining was used to estimate the levels antioxidant enzymes.

Analysis of serum sample

The serum samples were analyzed for various biomarkers that represent cardiotoxicity.

Estimation of lactate dehydrogenase (LDH) (Mod. IFCC method):

Principle

Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to from NAD. The rate of oxidation of NADH to NAD is measure as a decrease in absorbance and it is proportion to the LDH activity in the sample.

Pyruvate+ NADH + H^+ ^{LDH} Lactate + NAD^+

Procedure

- Blood was collected in a clean dry open droff tube. Use of plastic or siliconized container was avoided since it may prolong clotting time.
- For plasma separation, sodium citrate (4:1 ratio) was used as anticoagulant.
- 20micro grams of sample & 1000 micrograms of working reagent were pipette out and mixed well & then aspirated. Then absorbance was read out at 340nm against a blank taken waters

Wave length/filter: 340 nm

Temperature: 37^0C or 25^0

Light path: 1 cm

Table-1 Sample start assay: Pipette in to clean and dry test tubes labeled as test.

Addition sequence	T (ml) 25^0C	T (ml) 37^0C
Working reagent (ml)	1.0	1.0
Incubate at the assay temperature for 1 minute and add		
Sample (ml)	0.05	0.02

Mix well and read the initial absorbance A_0 & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA/min).

Calculation

LDH activity in U/L: 25^0 = ΔA/min 3333

37^0 = ΔA/min 8095

Estimation of creatine kinase- mb activity (Neumeir, 1981)

Principle:

The sample is incubated in the CK-MB reagent which includes the anti-CK-M antibody. The activity of the non-inhibite CK-B is then determined using the following series of reactions:

CK ADP + Creatine Phosphate ———————— Creatinine + ATP HK

ATP + Glucose ————————> ADP + Glucose-6-phosphate

G6PDH G-6-P + NAD^+ ————————> 6-Phosphogluconate + NADH + $_H$+

CK-B catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP format, to produce ADP and glucoses-phosphate (G-6-P) is oxidized to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK-MB activity.

Reagent

CK-MB Reagent: Creatine Phosphate 30mM Adenosine-5-Phosphate 2mM NAD 2mM Hexokinase (Yeast) > 3000U/L G-6-PDH (Bacterial) > 2000U/L Anti Human CK-M antibody (Goat) - sufficient amount to inhibit up to 1500 U/L of CK-MM.

Procedure

To the test tubes added lOOOml of the reagent and 50fil of the sample. The mixture was mixed and incubated at 37 C. The absorbance was measured after 300 seconds. Two additional absorbance was taken at 1 minute interval. The mean absorbance change/minutes (AA/min) was calculated. The change in absorbance/ minute was multiplied by factor 3376 that is equal to CK-MB.

Estimation of serum glutamate oxaloacetate transaminase (SGOT) (IFCC without P-5[1]-P method)

Clinical significance

Serum glutamic oxaloacetic transaminase (SGT) also called as Aspertate transaminase (AST), catalyses the transfer of the amino group of L-aspartate to a ketoglutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscle and kidneys. Damage to cell of this tissue induces AST increase in serum. In case of fluminant forms of hepatitis, especially viral hepatitis the enzyme level is markedly elevated. In case of myocardial infarction.

Principle

Kinetic determination of the aspartate aminotransferase (AST) activity:

L-Aspartate + α-ketoglutarate AST Oxaloacetate + L- Glutamate

Oxaloacetate + NADH + H^+ MDH L-Malate + NAD^+

Reagents: enzyme reagent, buffer solution.

Sample: serum, heparin or EDTA plasma

Normal: < 40 U/L

Preparation and stability of working reagent

Reconstitute one viral of enzyme reagent with 10 ML buffer solution, this working reagent is stable up to 30 days at 2-8°c.

Procedure

Working reagent	1ml
Sample	0.1ml

T.3

Mix and after a 1 minute incubation, measure the change of optical density per minute (ΔOD/min.) during 3 minute.

Calculation

Activity (U/L) = ΔOD/min. × 1768.

Serum Glutamate Pyruvate Transaminase (SGPT)

Clinical significance: Alanine transaminase present large amounts in liver, kidney, heart and skeletal tissues. It is also present in spleen, lungs, pancreas, brain and erythrocytes at lower concentration. Primary to liver damages and secondary to other causes result in elevated levels of GPT.

Principle:

SGPT converts L- Alanine and α- ketoglutarate to pyruvate and Glutamate. The pyruvate formed reacts with 2,4, Dinitrophenyl hydrazine to procedure a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured. The reaction does not obey Beer's law and hence a calibration curve is plotted using a pyruvate standard. The activity of SGPT (ALAT) is read off this calibration curve *(*Excel diagnostics Pvt, Ltd, Hyd, India).

L-Alanine SGPT Pyruvate + pH 7.4 + α -Ketoglutarate L-Glutamate

Pyruvate Alkaline 2,4, Dinitrophenyl Hydrazone + Medium (Brown coloured complex) 2,4DNPH

Reagents:

- Enzyme Reagent
- Buffer Solution

Preparation and stability of working reagent: Reconstitute one vial of Enzyme reagent with 10 ML Buffer solution, this working reagent is stable upto 30 days at 2-8°C.

Sample: Serum free hemolysis. SGPT is reported to be stable in serum for 3days at 2-8°C

Procedure:

Table 2: Assay procedure for SGPT

Working Reagent	1ml
Sample	0.1ml

T.4

Mix and after a 1minute incubation, measure the change of optical dencity per minute (ΔOD/min). During 3 minutes.

Normal range: <40U/L.

Wave length: 340nm.

Calculation: Activity (U/L) = ΔOD/min × 1768

Estimation of catalase

Biopsy was washed with phosphate buffer, pH 7.4, to remove red blood cells. The tissue was then blotted dry, weighted followed by homogenization in 1.5 ml cold buffer (50 mM potassium phosphate and 1 mM EDTA, pH 7) and centrifugation at 10,000g for 15 minutes at 4°C was done. The supernatant was used for the assay. Catalase assay kit provided by Cayman Chemical Company, USA. CAT is a ubiquitous antioxidant enzyme that is present in most aerobic cells. CAT is involved in the detoxification of H2O2. This enzyme catalyzes the conversion of two molecules of H2O2 to molecular oxygen and two molecules of water (catalytic activity). CAT also demonstrates peroxidatic activity, in which low-molecular-weight alcohols can serve as electron donors, while the aliphatic alcohols serve as specific substrates. In human beings, the highest levels of CAT are found in the liver, kidney and erythrocytes, where it is believed to account for the majority of H2O2 decomposition. The Cayman Chemical Catalase Assay Kit utilizes the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with ethanol, in the presence of an optimal concentration of H2O2 .The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-trizazole as the chromogen. The assay can be used to measure CAT activity in plasma, serum, erythrocyte lysates, tissue homogenates and cell lysates

Glutathione peroxidase assay (GSH-Px)

The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 mol; pH 7.4), 0.1 ml EDTA (1 mmol), 0.1 ml sodium

azide (1 mmol), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mmol), 0.1 ml NADPH (0.2 mmol), 0.01 ml H2O2 (0.25 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of 6.22 × 103 M-1 cm-1.

Measurement of plasma MDA

The principle of the test is based on the derivation of MDA with 2,4-dinitrophenylhydrazine and its subsequent conversion into pyrazole and hydrazone derivatives, which are then separated using high-performance liquid chromatography (HPLC). This method allows for a more specific estimation of MDA compared to the thiobarbituric acid reactive substances (TBARS) method. HPLC analyses were performed on a LC-10 AT VP Shidmadzu (Kyoto, Japan) liquid chromatography system equipped with a diode array detector and an auto injection valve. An α-bond C18 125A column (3.9×150 mm) with a 5 µm particle size (Alltech, Deerfield, Illinois, USA) was used. A Shidmadzu Class-VP software system controlled the equipment and was utilized for data processing. Elution was performed isocratically with a mixture of 0.2% (v/v) acetic acid in MilliQ water and acetonitrile (62:38) (v/v) at a flow rate of 0.6 ml/min at room temperature. The chromatograms were acquired at 310 nm.

Standard and sample preparation for MDA

A standard of 1,1,3,3-tetraetoxypropane (TEP) was used. A stock solution of MDA was obtained as follows: 25 µl TEP was dissolved in 100 ml of deionized water to yield a 1 mmol/l stock solution. The MDA was prepared by hydrolysis of 1 ml TEP stock solution in 50 ml of 1% sulfuric acid and was incubated for 2 h at room temperature. The solution was stored at 40°C and used within four weeks. The resulting MDA standard of 20 nmol/ml was further diluted with 1% sulfuric acid to yield different concentrations from 1 to 20 nmol/ml of MDA. An aliquot of 250 µl diluted standard or plasma was placed in a 1.5-ml Eppendorf tube, and 50 µl of 6 M aqueous sodium hydroxide was added. This mixture was incubated in a 60°C water bath for 30 min to achieve the alkaline hydrolysis of the protein-bound MDA. The protein was precipitated by adding 125 µl of 35% (v/v) perchloric acid, and the mixture was centrifuged at 6000×g for 10 min. The entire volume of the supernatant was transferred to an Eppendorf vial and mixed with 50 µl 2,4-dinitrophenylhydrazine prepared as a 5 mM solution in 2 M hydrochloric acid. Finally, this reaction mixture was incubated for 30 min at room temperature and was protected from light. An aliquot of 40µl of this reaction mixture was injected into the HPLC system. The retention time for the MDA was detected at the 8th minute, and the area under the peak represented the amount of MDA in 40 µl of the reaction mixture. A serial concentration of the standard was generated as a reference curve.

Histopathological examination

At the end of the study, the abdomen was cut open to isolate heart from each animal. Isolated hearts were washed with cold saline and cleaned off extraneous tissue and kept in 10% neutral formalin solution, tissues were embedded in paraffin wax and 5µm thick sections was cut and stained with eosin and hematoxylin. Then the sections were observed under light microscope and photo micrographs were taken.

Statistical analysis

The data are expressed as Mean ± SEM and Statistical analysis was performed using one way ANOVA followed by Dunnett t- test for cardioprotective activity. (P<0.05) was regarded as statistically significant.

3. Results and Discussion

Phytochemical Studies

Table 3 Preliminary phytochemical test of *Euryale ferox* Ethanolic extract

Phytochemical test	Results
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Steroids	_
Glycosides	+
Proteins	_
Phenols	+
Saponins	_
Tannins	+

(+) indicates presence, (-) indicates absence

Lactate dehydrogenase (LDH)

LDH is a cardiac biomarker. Elevated levels are observed in toxic conditions. Table and figure show the effect of extract on LDH levels. The values are compared with Doxorubicin control group. Values are expressed in mean ± standard deviation.

Table 4 Effect of Euryale Ferox seeds extract on LDH levels in rats treated with doxorubicin

Group Name	Concentration levels in Mean ± S.D(IU/L)
Normal Control	67.70±5.0
Doxorubicin control (20mg/kg b,wt)	141.10±12.80
Extract (150mg/kg) +Doxorubicin	1119.5±11.8
Extract (300mg/kg) + Doxorubicin	92.60±12.60
Extract (600mg/kg) + Doxorubicin	100.2±9.3
Atorvostatin (10mg/kg) + Doxorubicin	75.5±6.82

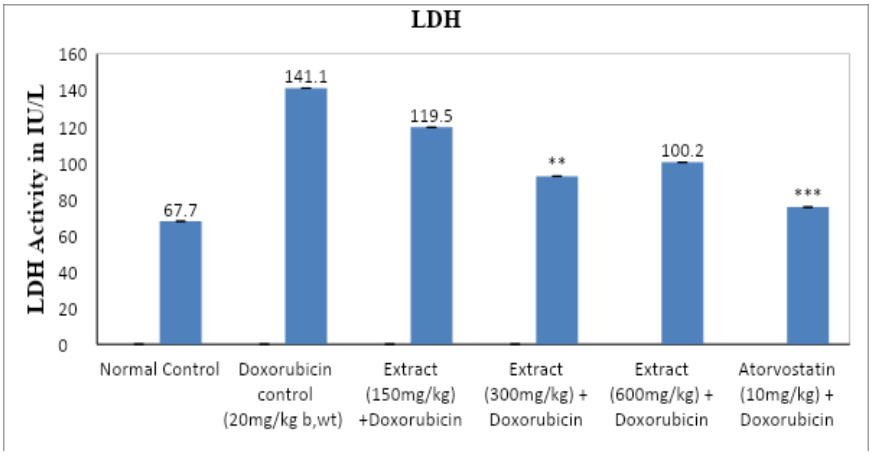


Fig.1 Effect of Euryale Ferox seeds extract on plasma LDH levels in rats treated with doxorubicin.
* p<0.05, ***p<0.001 vs. DOX control

Table 5 Effect of *Euryale Ferox* seeds extract on CK-MB levels in rats treated with Doxorubicin.

Group Name	Concentration levels in Mean ±S.D(IU/L)
Normal Control	42.50±5.20
Doxorubicin control(20mg/kg)	137.40±9.0
Extract (150mg/kg)+ Doxorubicin	109.30±11.0
Extract (300mg/kg)+ Doxorubicin	92.40±9.90
Extract (600mg/kg) + Doxorubicin	90.2±2
Atorvostatin (10mg/kg) + Doxorubicin	55.3±2.3

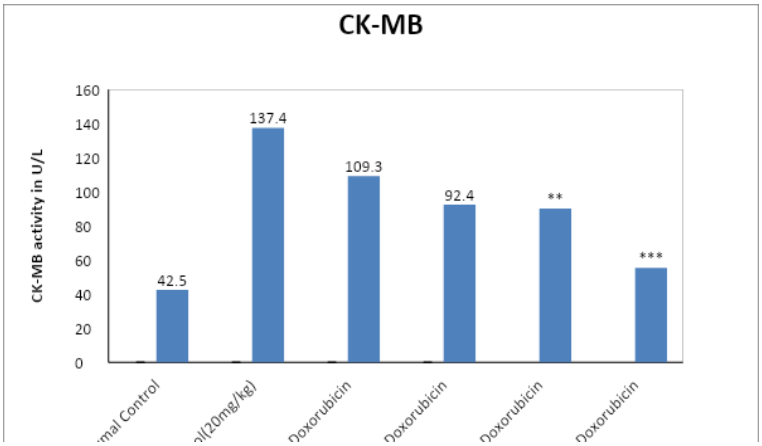


Fig 2 Effect of *Euryale Ferox* seeds extract on CK-MB levels in rats treated with Doxorubicin

Serum Glutamate Oxalate Transaminase (SGOT)

Table 6: Standard Values of SGOT

Concentration	Absorbance
0	0
24	0.1
61	0.218
114	0.359
190	0.601

From the above standard values graph was plotted and it shown as in below.

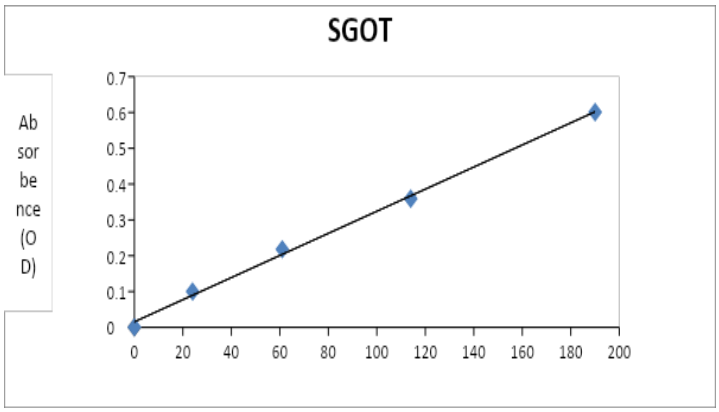


Fig 3 Standard Graph for SGOT

Table 7 Effect of Euryale Ferox seeds extract on SGOT levels in Doxorubicin treated Rats.

Group Name	Concentration level in Mean±SD(IU/L)
Normal Control	42.5±7.32
Doxorubicin control(20mg/kg)	111.0±15.12
Extract (150mg/kg)+Doxorubicin	93.4±12.51
Extract (300mg/kg)+ Doxorubicin	70.4±10.34
Extract (600mg/kg) + Doxorubicin	68.2±5.02
Atorvostatin (10mg/kg) + Doxorubicin	55.5±4.8

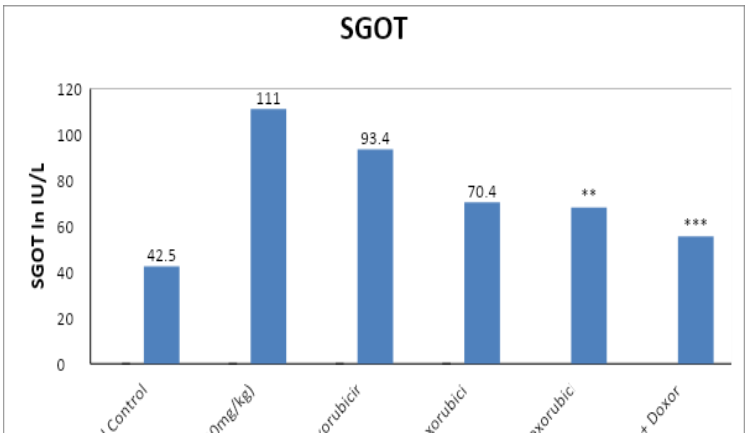


Fig 4 Effect of *Euryale Ferox* seeds extract on plasma SGOT levels in rats treated with Doxorubicin * p<0.05, ***p<0.001 vs. DOX control

4.1.5 Serum Glutamate Pyruvate Transaminase (SGPT)

Table 8 Standard Values of SGPT

Concentration	Absorbance
0	0
24	0.11
61	0.22
114	0.36
190	0.59

From the above standard values graph was plotted and it shown as in below.

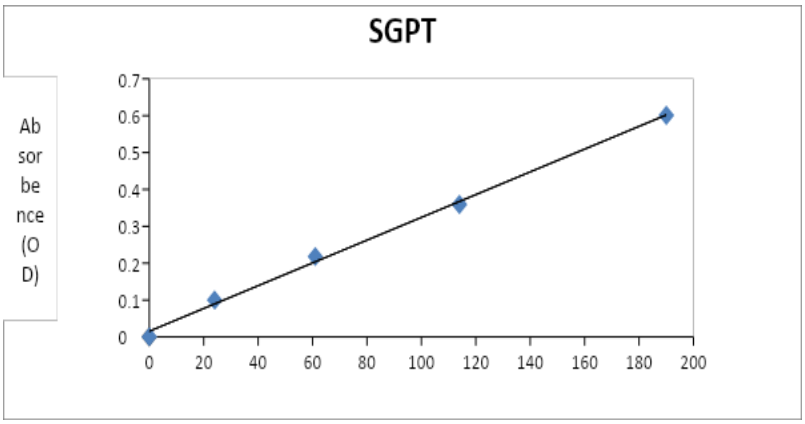


Fig 5 Standard Graph for SGPT

Table 9 Effect of Euryale Ferox seeds extract on SGPT levels in Doxorubicin treated Rats.

Group Name	Concentration level in Mean± SD(IU/L)
Normal Control	38.5±2.82
Doxorubicin control(20mg/kg)	100.8±9.12
Extract (150mg/kg)+Doxorubicin	83.4±7.51
Extract (300mg/kg)+ Doxorubicin	68.4±10.34
Extract (600mg/kg) + Doxorubicin	57.2±4.02
Atorvostatin (10mg/kg) + Doxorubicin	50.5±3.8

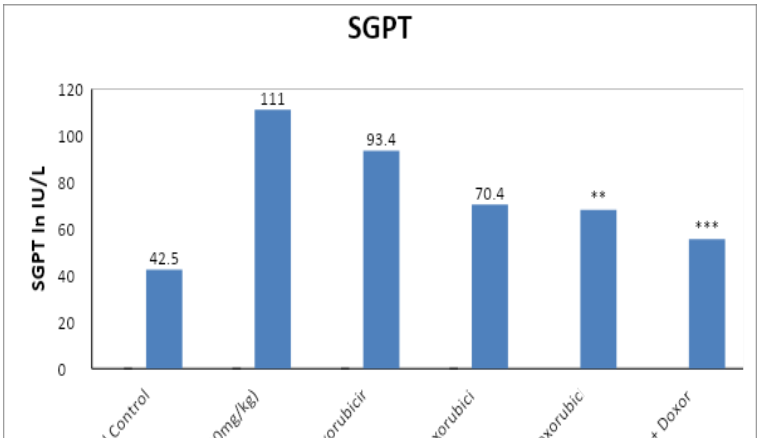


Fig 6 Effect of *Euryale Ferox* seeds extract on plasma SGPT levels in rats treated with Doxorubicin.
* p<0.05, ***p<0.001 vs. doxorubicin control

4.1.6 Serum Catalase (CAT)

Table 10 Standard graph values of H_2O_2

Concentration µm	Absorbance
700	0.004
1000	0.008
3000	0.024
7500	0.062

From the above standard values was plotted and it shown as in below

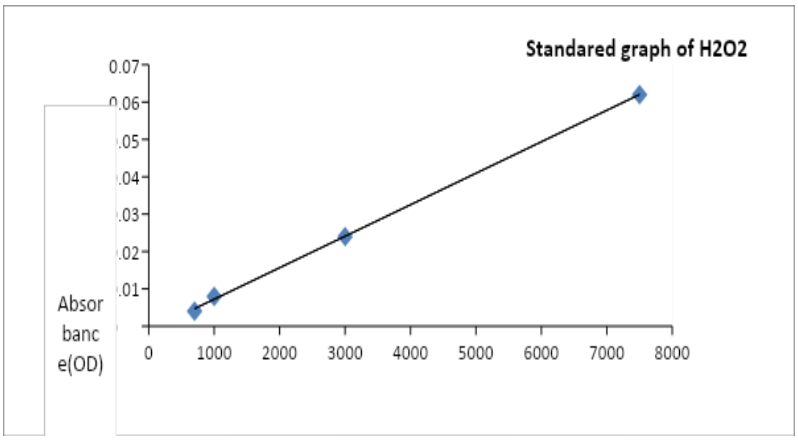


Fig 7 Standard graph for Catalase Activity

Table 11 Effect of *Euryale Ferox* seeds extract on Catalase levels in rats treated with Doxorubicin.

Group Name	Catalase Concentrations in Mean±SD (IU/ml)
Normal Control	2.50±0.03
Doxorubicin control	0.57±0.02
Extract (150mg/kg)+ Doxorubicin	1.07±0.11
Extract (300mg/kg)+ Doxorubicin	1.41±0.05
Extract (600mg/kg) + Doxorubicin	1.92±0.02
Atorvostatin (10mg/kg) + Doxorubicin	2.0±0.08

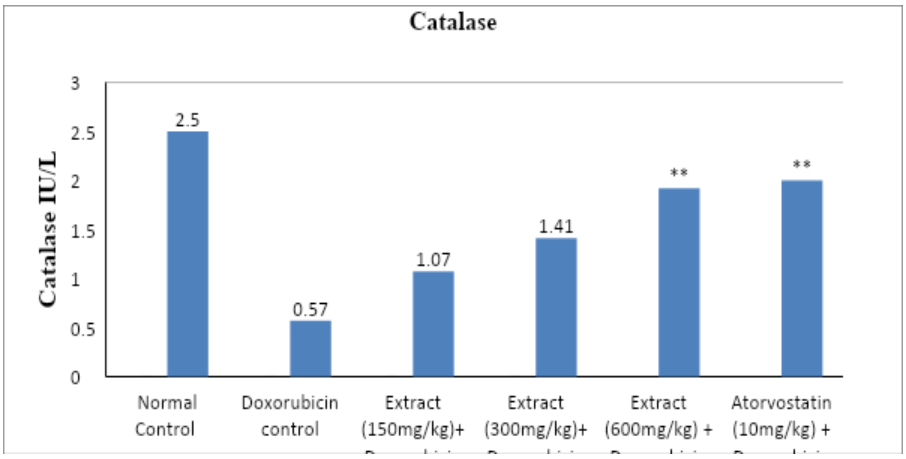


Fig 8 Effect of *Euryale Ferox* seeds extract on tissue catalase levels in rats treated with Doxorubicin.
** p<0.01 vs. DOX control

Table 12 Standard graph values for Glutathione

Concentration µm	Absorbance
30	0.141
50	0.232
100	0.27
200	0.321
400	0.47
600	0.6
800	0.714

From the above standard values graph was plotted and it shown as in below.

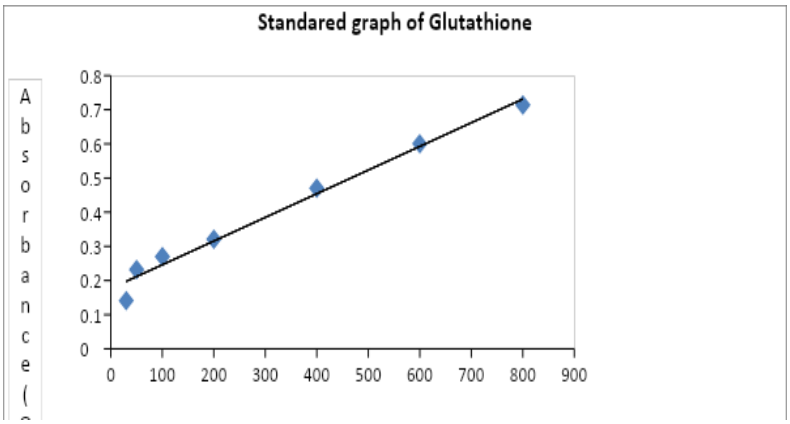


Fig 9 Standard Graph for Glutathone

Table 13 Effect of *Euryale Ferox* seeds extract on Glutathione levels in rats treated with Doxorubicin.

Group Name	Glutathione Concentrations in Mean±SD (IU/L)
Normal Control	50.63±1.70
Doxorubicin control	17.96±1.97
Extract (150mg/kg)+ Doxorubicin	24.50±0.66
Extract (300mg/kg)+ Doxorubicin	37.39±1.03
Extract (600mg/kg) + Doxorubicin	41.52±2.02
Atorvostatin (10mg/kg) + Doxorubicin	48.0±2.8

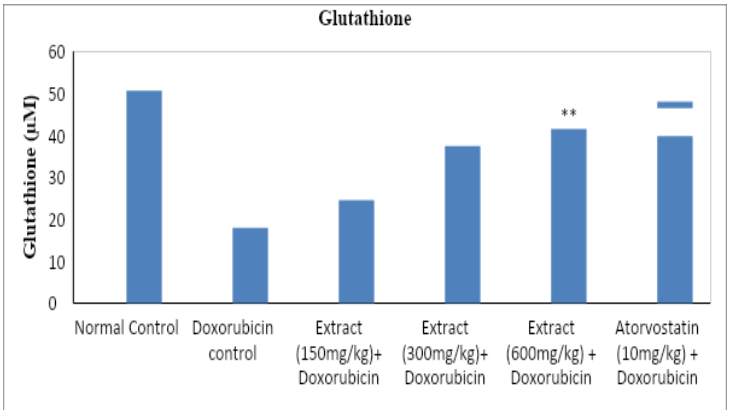


Fig 10 Effect of *Euryale Ferox* seeds extract on tissue Glutathione levels in rats treated with Doxorubicin.
** p<0.01 vs. DOX control

Malondialdehyde

Table 14 Effect of *Euryale Ferox* seeds extract on Malondialdehyde levels in rats treated with Doxorubicin

Group Name	Malondialdehyde Concentrations in Mean±SD (IU/L).
Normal Control	13.35±1.58
Doxorubicin control	87.81 ± 3.81
Extract (150mg/kg)+ Doxorubicin	61.1 ± 2.6
Extract (300mg/kg)+ Doxorubicin	48.06 ± 3.2
Extract (600mg/kg) + Doxorubicin	36.37±2.08
Atorvostatin (10mg/kg) + Doxorubicin	30.79 ± 2.9

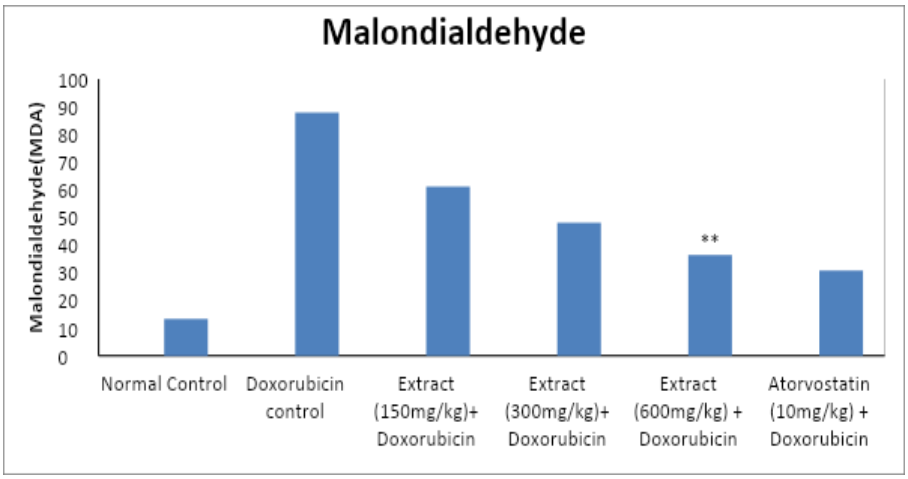
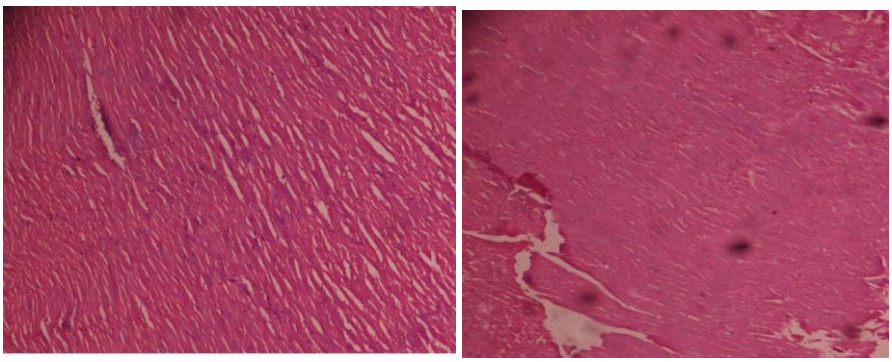


Fig 11 Effect of *Euryale Ferox* seeds extract on tissue Malondialdehyde levels in rats treated with Doxorubicin. ** p<0.01 vs. DOX control

Histpathology



(a) **(b)**

Fig 12 Histopathology images

On Histopathological examination, normal control group heart section (Fig-10.A) shows the cardiocyte cellular and architectural pattern maintained. There is no evidence of fibrosis (or) inflammation (or) degenerative changes.In pretreated rats shows normal cellular and architectural pattern with no degeneration. Myofibrils and mononuclear cells appeared normal. Thus histopathological results indicates that the pretreatment with extract at doses has less degeneration showing protective effect over doxorubicin control group .

Discussion

In this study Doxorubicin –Induced Cardio toxicity model was used. Doxorubicin forms an iron anthracyclin complex that generates free radicals, which in turn causes severe damage to the plasma membrane that results in further increase in LDH, CK-MB and SGOT levels in blood. Pretreatment with *Euryale Ferox* aqueous methanolic extract produced significant decrease in LDH, CK-MB and SGOT levels indicating the protective effect of cardiac tissue. Heart tissue is especially susceptible to free radical injury because of low levels of free radical detoxifying enzymes like CAT and GSH. On Doxorubicin treatment a dose dependent decrease in CAT and GSH levels were observed due to its accumulation in the heart tissue. Pretreatment with *Euryale Ferox* aqueous methanolic extract produced significant (<0.01) increase in CAT and GSH levels in dose dependent manner, thus indicating that the antioxidant status is improved on extract treatment. The Histopathological report of hearts treated with Doxorubicin (15mg/kg, i.p) produced focal cardiocyte degeneration with patch inflammation of lymphocytes and mild interstitial fibrosis. But, pretreatment with 150mg/kg, 300mg/kg and 600mg/kg doses plant extract showed mild or no degenerative changes when compared with Doxorubicin control group. So, it shows that the *Euryale Ferox* plant extract may have some protective effect against Doxorubicin induced cardiotoxicity.

4. Conclusion

In summary, it has been concluded from the experimental studies carried out on Ethanolic extract of *Euryale Ferox* seeds at three different doses (150mg/kg, 300mg/kg, 600mg/kg) showed dose dependent cardio protective activity with Doxorubicin-induced cardio toxicity. The higher dose 600mg/kg showed significant protective activity compared to low dose 300mg/kg and 150 mg/kg. Furthers studies should be carried out to isolate the potential chemical constituents of Ethanolic extract of *Euryale Ferox* seeds and to find its mechanism of action in the treatment.

5. References

[1] Tunyu Jian, Bingru Ren, Chen Yu, Xiaoqin Ding, Jian Chen, Jiawei Li, Yuanyuan Zuo, Han Lv, and Weilin Li. Hepatoprotective Effect of Seed Coat of Euryale ferox Extract in Non-alcoholic Fatty Liver Disease Induced by High-fat Diet in Mice by Increasing IRs-1 and Inhibiting CYP2E1. Journal of Oleo Science. J. Oleo Sci. 2019, 68(6): 581-589.

[2] Ahmed D, Kumar V, Verma A, Shukla GS, Sharma M. Antidiabetic, antioxidant, antihyperlipidemic effect of extract of Euryale ferox salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. Springerplus. 2015;4:315.

[3] Ahmed D, Sharma M, Kumar V, Bajaj HK, Verma A. 2β-hydroxybetulinic acid 3β-caprylate: an active principle from Euryale Ferox Salisb. seeds with antidiabetic, antioxidant, pancreas & hepatoprotective potential in streptozotocin induced diabetic rats. J Food Sci Technol. 2015;52(9):5427–5441.

[4] Cheng Ying Wu, Antioxidant and Anti-Fatigue Activities of Phenolic Extract from the Seed Coat of Euryale ferox Salisb. and Identification of Three Phenolic Compounds by LC-ESI-MS/MS, Molecules 2013, 18(9), 11003-11021;

[5] Samarjit Das Peter Der Utpal Raychaudhuri Nilanjana Maulik Dipak K. Das. The Effect of Euryale Ferox (Makhana), an Herb of Aquatic Origin, on Myocardial Ischemic Reperfusion Injury, Molecular and Cellular Biochemistry. September 2006, Volume 289, Issue 1–2, pp 55–63.

[6] Mallapu koshma , V. jaya sankar reddy, t.naga aditya, s. Jilani basha, Y. Sudha rani, p.swaroopa, cardioprotective activity of medicinal plants: a review, int. Res. J. Pharm. 2017, 8 (12).

[7] Eman Taha Mohamed, Ghada Mohamed Safwat, Evaluation of cardioprotective activity of Lepidium sativum seed powder in albino rats treated with 5-fluorouracil, Beni-Suef University Journal of Basic and Applied Sciences, Volume 5, Issue 2, June 2016, Pages 208-215

[8] Kharadi GB, Patel KJ, Purohit BM, Baxi SN, Tripathi CB. Evaluation of cardioprotective effect of aqueous extract of Allium cepa Linn. bulb on isoprenaline-induced myocardial injury in Wistar albino rats. Research in Pharmaceutical Sciences. 2016;11(5):419-427.

[9] Fatiqa Zafar, Nazish Jahan, Khalil-Ur-Rahman, Ahrar Khan, and Waseem Akram 4, Cardioprotective Potential of Polyphenolic Rich Green Combination in Catecholamine Induced Myocardial Necrosis in Rabbits, Evid Based Complement Alternat Med. 2015; 2015: 734903.

[10] Patel jignasa s, settee seema k, chakraborty manodeep, kamath jagadish v, evaluation of cardioprotective activity of medohar vati by isoproterenol induced myocardial damage in rats, irjp 2012, 3 (8).

[11] Bhatia Nitish, Maiti Partha Pratim, Kumar Abhinit, Tuli Atul, Ara Tasneem, Khan Masih Uzzaman, "Evaluation of Cardioprotective Activity of Ethanolic Extract Of Solanum Nigrum Linn. In Rats". Int. J. Drug Dev. & Res., 2011, 3(3):139-147

[12] Padhye S, Ahmad A, Oswal N, Sarkar FH. Emerging role of Garcinol, the antioxidant chalcone from *Euryale ferox* Choisy and its synthetic analogs. Journal of Hematology and Oncology. 2009; 2(38):1-13.