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"Evaluation of Cardioprotective activity of Canna indica flower extract on rats"

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ABSTRACT

Objective: The present study was designed to evaluate the cardioprotective activity of flowers extract of Canna indica L in rats.

Methods: Canna indica flowers extract (CIFE) was evaluated for protection against isoproterenol (200 mg/kg body wt., s.c.) and doxorubicin (10 mg/kg body wt., i.p) induced cardiotoxicity in albino rats. Biomarkers like ALT, AST, CPK and LDH along with heart weight index was considered to determine the cardioprotective property. Histopathological studies were also carried out on heart of experimental animals.

Result: The Canna indica flowers extract found to contain cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. The extract to protect the cardiotoxicity produced by isoproterenol (ISO) and doxorubicin (DOX) in experimental animals. The histopathological studies on heart were in agreement with biomarkers estimation. The Cardioprotective action may be due to antioxidant action of the extract.

Conclusion: The Canna indica flowers extract has significant cardioprotective activity when tested in albino rats.

I. INTRODUCTION:

Individual cardiac cells undergo depolarization and repolarization to form cardiac action potentials about sixty times per minute. Each heart beat is the result of the highly integrated electrophysiological behavior of multiple gene products on multiple cardiac cells [1].

Cardiovascular disease is a group of problems that occur when the heart and blood vessels are not working the way they should. And especially myocardial infarction (MI) is a complex phenomenon affecting the mechanical, electrical, structural, and biochemical properties of the heart. The heart failure is one of the most common causes of death and disability in industrialized nations and is among the syndromes most commonly encountered in clinical practice. Over 4.6 million patients in the united states alone carry this diagnosis, and it is the cause of death in several hundred thousand patients each year[1,2.]

According to world health organization (WHO) cardiovascular disease (CVD) is now more numerous in India and China by the year 2022 and India will be the largest CVD burden in the world than in all economically developed countries [3]. The various risk factors associated with CVD like alcohol use, high cholesterol, obesity, high blood pressure (hypertension), physical inactivity, unhealthy diets, tobacco use, chemotherapy treatment [4]. Adverse effects of heavy metals intake leads to morbity and mortality world-wide [5].

Currently available management of cardiovascular disorders like arrhythmia, hypertension, angina pectoris, congestive heart failure, myocardial infarction are problematic. In addition, conventional drugs used are sometimes inadequate and may lead to serious adverse effects. Abciximab causes arrhythmia and haemorrhage; Dipyridamole causes risk of stroke in transient ischemia attacks. Streptokinase itself causes hypotension, hypersensitivity reaction, arrhythmia; It is therefore imperative to search alternative drugs for treatment of cardiovascular disorders to replace the currently available drugs with known efficacies and safety [6]. A number of medicinal plants have been evaluated for cardiovascular disorders in India and various parts of the world using cardio toxic models.

Isoproterenol (ISO) and induced cardio toxicity are well known standard models to study the beneficial effect of many drugs on cardiac dysfunction. Isoproterenol is a synthetic catecholamine and β-adrenoceptor agonist, on administration of this drug in larger doses produces myocardial infarction because, generation of highly cytotoxic free radicals through its auto-oxidation and it has been suggested that the oxidative products of catechol amines are responsible for changes in the myocardium that is, resulting in infarction like necrosis of heart muscles. And it has

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been implicated as a causatie factor [7,8]. Whereas therapeutic use is limited by late-onset, acute and chronic cardio toxicities, it is believed to be mediated through different mechanisms like inducing semiquinone free radical formation, ion dependent oxidative damage to biological macromolecules as well as induces the peroxidation of unsaturated lipids within the membranes.

There are many drugs available for Cardioprotective activities. Flavonoids and phenolic compound (Antioxidants) are one of the agents which are extensively used for Cardioprotective activities. Antioxidants are the substances which chemically react with free radicals and render them harmless and at the same time break the viscous circle, which involve in the decomposition of fatty acids and proteins, the creation of new free radicals and leads to eventual cell death [12].The antioxidant defense system includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants[14].Antioxidants may play an important role in the chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital molecule such as DNA, lipids and proteins[15].

However, for a number of reasons, complimentary medicine has grown popularity in recent years. Dietary measures and traditional plant therapies as prescribed by Ayurvedic and other indigenous systems of medicine are commonly used in India. Many indigenous Indian medicinal plants have been found to be useful to successfully manage Cardioprotection and some of them have been tested and their active ingredients isolated. The World Health Organization (WHO) has also recommended the evaluation of the plants.

Digitalis purpurea, Cheiranthus cheiri, Apocynum cannabinum plants have evaluated and reported that they have significant cardiac and nephoroprotective activity which contain cardiac glycoside, carbohydrate, proteins, flavonoids, terpenoids, alkaloids, steroids, tannins and saponis.

Canna indica flower extract also containing cardic glycosides, carbohydrate, proteins, flavonoids, terpenoids, alkaloids, steroids , tannins, saponins, and phylobatinins. So, I intended to caary out cardioprotective activity of ethanolic extract of canna indica flower againt Isoproterenol and doxorubicin induced toxicities.

II. MATERIALS AND METHODS

2.1. Material

2.1.1 Experimental animals

Species: Albino rats
Strain: Wistar
Sex: Male
Source: Invivo Biosciences, Kamath Layout, Bengaluru-91, Karnataka
Body weight: 150-200 g
Identification: By cage card and body markings.
Number of animals: 6 in each group.
Acclimation: One week in experimental room.

2.1.2 Selection of animals:

After acclimatization the animals were subjected to a gross observation to ensure that the selected rats were in good state of health. Rats were randomly selected for final allotment to the study.

2.1.3. Environmental condition:

Air conditioned rooms with optimal air changes per hour, relative humidity, temperature and elimination cycle set to 12 hour light and 12 hour in dark. The animals were maintained under standard condition in an animal house approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Mallige College Of Pharmacy, Bangalore. Reg No.1432/ PO/ RE/S/11/CPCSEA & 27/05/2017.

2.1.4 Accommodation :

Group housed in polypropylene cages with stainless steel grill top. Facilities for food and water bottle and bedding of clean paddy husk. The husks in the cages were renewed thrice a week to ensure hygiene and maximum comfort for animals.

- **Diet:** "Amrut" brand pelleted feed was provided ad libitum.
- **Water:** UV purified and filtered water was provided ad libitum in polypropylene bottles with stainless steel sipper tubes.

2.2.4 Determination of acute toxicity of various fractions : [16]

Acute toxicity study will be conducted to determine median lethal dose (LD50) of the methanol extract. Acute toxicity study of extract. Acute toxicity study will be carried out in albino Mice by "Up and Down method" (OECD guidelines 425).

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Different dose levels (Up to 2000 mg/kg body weight) of the extract will be administered orally to overnight fasted Mice to different groups consisting of three animals in each group. Following the administration of the extract, animals will be observed continuously for 2-3 h.
Hence in the present study 125 mg/kg and 250 mg /kg was taken as dose for cardioprotective and nephroprotective activity.

2.2.5 Evaluation of Cardioprotective activity of Canna indica flower extract on rats.

Animals:

Healthy male albino rats of weighing 180-220g will be included for the study. The animals will be obtained from animal house of Mallige College of Pharmacy. The protocol for animal clearance is approved from Institutional Animal Ethical Committee for experimental purpose. They will be maintained under laboratory condition with controlled environment of temperature, humidity as per Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. They will be provided with standard diet and water ad libitum.

1 Evaluation of Cardioprotective activity

1. Isoproterenol induced cardiotoxicity:

Male Albino Wistar rats (150-200g) was used to evaluate the cardioprotective activity. Rats will be treated with canna indica flower extract daily for 28 days. On 28th day, myocardial injury was induced in experimental rats by injection of Isoproterenol (ISO) (200 mg/kg, s.c.) twice at an interval of 24 hr. (i.e., on 28th and 29th day of canna indica flower extract treatment), while normal control and drug control rats was given an equivalent volume of the vehicle.
Treatment protocol: The experimental rats will be divided into 4 groups of 6 animals each and treated as follows: The doses are selected as per pervious literature survey.

Table 3: Effect of Canna indica flower against Isoproterenol induced cardiotoxicity

Sl.No.	Group	Treatment	Duration of treatment
1.	Normal Control	Normal Saline	Daily for 28 days
2.	Positive control	ISO (200mg/kg s.c)	28th and 29th day
3.	Canna indica flower extract Control	Canna indica flower extract 125mg/kg, p.o+ ISO (200mg/kg s.c)	Daily for 28 days+28th and 29th day
4.	Canna indica flower extract Pretreated	Canna indica flower extract 250mg/kg, p.o + ISO (200mg/kg s.c.)	Daily for 28 days+28th and 29th day
ISO: Isoproterenol; s.c: Subcutaneous; p.o: oral administration			

2. Doxorubicin induced Cardiotoxicity

Table 4: Effect of Canna indica flower against Doxorubicin induced cardiotoxicity

Sl. No.	Group	Treatment	Duration of treatment
1.	Normal control	Normal saline	Daily for 28 days
2.	Positive control	DOX (10mg/kg)	28th and 29th days
3.	Canna indica flower	Canna indica flower extract	Daily for 28 days + 28th

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	extract control	(125mg/kg p.o)+ DOX (10mg/kg)	and 29th days
4.	Canna indica flower extract pretreated	Canna indica flower extract 250mg/kg p.o+ DOX (10mg/kg i.p)	Daily for 28 days + 28th and 29th days

Biochemical analysis:

After 24 hour treatment period on 30th day blood was collected from retro-orbital plexus, serum will be separated by centrifuging 10000rpm for 15min. The Separated liquid was subjected for biochemical estimation of cardiac marker enzymes, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH) and Creatine Phosphokinase (CPK) using standard procedure.

Estimation of Cardiac markers enzymes

A. Estimation of serum CK-MB: [17]

Clinical significance: Creatinine Kinase is diametric enzyme composed of two types of monomer subunits, M (muscular) and B (brain). The subunits combine to form three distinct CK so enzymes, CK-BB(CK-1), CK-MB(CK-2), and CK-MM(CK-3). CK-MM is the predominate from of CK in skeletal muscle, CK-BB is found in brain and smooth muscle. CK-BB is found in brain and smooth muscle. CK-MB is found in a high concentration in the myocardium (between 14 and 42%) and to a lesser extent skeletal muscle. In the absence of disease, most CK activity in serum is due to the CK-MM iso form.

Increases: Levels increases in myocardial infarction, acute cerebro vascular disease, muscular dystrophy or injury. Following a myocardial infarction, CK activity begins to rise within 4-6 hours, peak between 18 and 30 hours and returns to normal by the third day.

Methodology: International Federation of Clinical Chemistry (IFCC). In this method serum is added to a modified CK-NAC reagent which contains the anti M antibody. The activity of CK-B is determined using the following reaction sequence:

Principle:

Creatine Phosphate + ADP $\xrightarrow{\text{Creatine Kinase}}$ Creatine + ATP

D-Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ ADP + Glucose-6-Phosphate

Glucose-6-phosphate + NADP+ $\xrightarrow{\text{G6PDH}}$ 6-Phospo gluconate + NADPH

G6PDH: Glucose-6-phophate dehydrogenase.

The rate of increase in absorbance is measured and directly proportional to enzyme activity.

Assay procedure:

Pipette	Volume
Working Reagent	500 µl
Sample	20 µl

Mix well and aspire.

Calculation: The general formula for converting absorbance change into international units (IU) of activity is :

$$(CK\text{-}B)\ IU/L = \frac{\text{---/Min} \times T.V \times 100}{S.V \times \text{Absorptivity} \times P}$$

TV = Total reduction volume in 1µl

SV = Sample volume in µl

Absorptivity = Milimolar absorption coefficient of NADH at 340 nm.

= 6-22

p = path length in cm.

$$\%\ \text{of CK – MB activity} = \frac{\text{CK-MB (IU/L)} \times 100}{\text{Total CK-MB (IU/L)}}$$

B. Estimation of serum LDH: [18]

Clinical significance: The enzyme LDH is concentrated in heart, Kidney, liver musule and other body tissues.

Increase: LDH levels increase in case of myocardial infarction, renal damge, hepatitis, anemia, malignancies and other muscular disease or injury.

Principle:

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Pyruvate + NADH + H^+ LDH Lactate + NAD^+
LDH: Lactate dehydrogenase

Assay procedure:

Pipette	Volumes
Working Reagent	1000 µl
Test	20 µl

Mix well and aspirate.
Decrease in absorbance due to oxidation of NADH is monitored at 340 nm and directly proportional to LDH activity.
Calculation: Determine the absorbance change/ min (--A/ Min.) for every reading, find the /mean value. The general formula for converting absorbance change into international units (IU) of activity is :

$$IU/L = \frac{---/Min \times T.V \times 100}{S.V \times Absorptivity \times P}$$

C. **Estimation of serum AST:** [19]
Clinical Significance: AST occurs in all human tissues and present in large amounts in liver, renal, cardiac and skeletal muscle tissue. AST Level increased in liver disease, myocardial infarction, muscular renal dialysis and those with B_6 deficiency.
Increases: Increased levels are associated with liver diseases or damage, myocardial infarction, muscular dystrophy and cholecystitis.
Methodology: International Federation of Clinical Chemistry (IFCC).
Principle:
L-Aspartate + 2-Oxoglutarate AST Oxaloacetate + L-Glutamate
Oxaloacetate +NADH MDH Malate + NAD
Sample pyruvate + NADH LDH Lactate + NAD
AST: Aspartate aminiotransferase
MDH: Malate dehydrogenase
LDH: Lactate dehydrogenase

Assay procedure:

Pipette	Volumes
Working reagent	1000 µl
Test	100 µl

The working reagent was allowed to attain 370C before performing the test. 1 ml of working reagent was mixed with 100 µl of test solution and the absorbance was recorded.

D. **Estimation of serum ALT:** [20]
Intended use: This reagent kit was intended for in-vitro quantitative determination of SGOT, AST activity in serum/ plasma.
Clinical significance: ALT is present in high concentration in liver and to a lesser extent in kidney, skeletal muscle, pancreas, spleen and lungs.
Increases: Increased levels are generally a result of primary liver disease such cirrhosis, carcinoma, viral or toxicity hepatitis and obstructive jaundice.
Decreases: Decrease levels may be observed in renal dialysis patients and those with vitamin B_6 deficiency.
Methodology: International Federation of Clinical Chemistry (IFCC)
Principle:
L-Alanine + 2-Oxoglutarate ALT Pyruvate + L-Glutamate
Pyruvate + NAD LDH L-Lactate + NAD
ALT: Alanine aminotrasferase
LDH: Lactate dehydrogenase

Assay procedure:

Pipette	Volumes
Working reagent	1000 µl
Test	100 µl

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The working reagent was allowed to attain 37^0C before performing the test. 1 ml of working reagent was mixed with 100 µl of test solution and the absorbance was recorded.

II. Heart weight index (HWI)

After blood withdrawal, all the rats were sacrificed by cervical dislocation; the hearts were dissected out, washed in ice cold saline, weighed after blotting with filter paper and heart weight index (HWI) was computed in table 6 and graphically presented.

$$\text{Heart weight index (HWI)} = \frac{\text{Heart weight (mg)}}{\text{Body weight (g)}}$$

Then myocardial tissue was immediately fixed in 10% buffered neutral formalin solution and processed for histopathological studies.

III. STATISTICAL ANALYSIS

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Bunferroni comparison test by using Graph Pad Instat Software Version 6. P value less than 0.05 was considered to be statistically significant.

IV. HISTOPATHOLOGICAL STUDIES [21]

Processing of isolated myocardial tissue

The animals were sacrificed and heart was isolated. The isolated heart was cut into small pieces and preserved in 10% formalin for two days. Then the myocardial pieces were washed in running water for about 12 hours. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each and the final dehydration was done using absolute alcohol for 12 hours.

Clearing was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit.

Embedding in paraffin by vaccum: Hard paraffin was melted and poured into blocks. The myocardial piece was then dropped into molten paraffin quickly and allowed to cool.

Sectioning: The blocks were cut using microtome blade to get sections of thickness of 5µ. The sections were taken on a microslide on which egg albumin (sticking substance) was applied. The sections were then allowed to remain in an oven at 60^0C for 1 hour. Paraffin melts and egg albumin denatures, thereby tissues were fixed to the slide.

Staining: Eosin is an acid stain, and it stains all the cell constituents which were basic in nature to pink colour. eg. (RNA, Cytoplasm). Haematoxylin is a basic stain which stains all the acidic cell components blue e.g.: DNA in the nucleus.

Procedure

1. The sections were deparaffinized by washing with chloroform for about 15 minutes.
2. Hydrated the sections by washing in isopropyl alcohol of decreasing strength (100%, 90%, 80% and 70%).
3. Finally washed with water.
4. Stained with haematoxylin for 15 minutes.
5. Rinsed under tap water.
6. Differentiated in 1% acid alcohol by 3 to 10 quick dips checked the differentiation with a microscope. Nuclei were distinct and the back ground was very light (or colourless).
7. Washed with tap water.
8. Dipped in lithium carbonate until sections become bright blue (3-5 dips).
9. Washed under running tap water for 10-20 minutes. (if washing is inadequate eosin will not stain evenly).
10. Stained with eosin for 15 seconds-2 minutes depending on the age of eosin and depth of the counter stain desired. For even staining results, dip slides several times before allowing them to set in eosin for the desired time.
11. Dehydrated in 95% isopropyl alcohol and absolute isopropyl alcohol until excess eosin was removed, two changes of 2 minutes each were done and checked under microscope.
12. Absolute isopropyl alcohol, two changes of 3 minute each.
13. Chloroform, two changes of 2 minutes each.
14. Mounted in DPX (Desterene dibutyl phthalate xylene).

The photographs were taken under 450X magnification and presented at figure

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V. RESULTS

Table 7: Chemical constituents of Canna indica flowers extract:

Sl. No.	Particulars	Results
1.	Carbohydrates	+
2.	Cholesterol	+
3.	Proteins	+
4.	Amino Acids	+
5.	Alkaloids	+
6.	Flavonoids	+
7.	Terpenoids	+
8.	Cardiac Glycosides	+
9.	Steroids	+
10.	Tannins	+
11.	Sapponins	+
12.	Phlobatininis	+

Note: Present (+), Absent (-)

Table 8: Effect of Canna indica L. flowers extract on cardiac markers enzymes in the serum of control and isoproterenol (ISO)-induced oxidative stress and cardiotoxicity in rats.

Group	AST (IU/L)	ALT (IU/L)	CPK (IU/L)	LDH (IU/L)
I. Healthy control	58.7±0.67	46.3±1.28	375±1.99	519±1.45
II. ISO	128±8.33	153.0±1.61	2006±99.3	1470±128
III. CIFE125mg+ISO	175±6.99$^{\#}$	161.0±4.26	2477±89.9$^{\#}$	1615±74.2
IV. CIFE250mg+ISO	75.5±2.46*	57.0±2.13*	1711±81.5**	1241±37.5**

N=6, values are expressed as mean ± SEM, *P<0.01, **P<0.001 in comparison with healthy control, $^{\#}$p<0.01 in comparison with ISO.

Table 9: Effect of Canna indica flowers extract on heart weight index (HWI) of control and isoproterenol (ISO)-induced oxidative stress and cardiotoxicity in rats.

Group	HWI(mg/g)
I. Healthy control	2.97±0.0244
II. Isoproterenol	3.47±0.0521*
III. CIFE125mg+ISO	3.67±0.137**
IV. CIFE250mg+ISO	3.06±0.108

N=6, values are expressed as mean ± SEM, *P<0.01 and **P<0.001 in comparison with healthy control.

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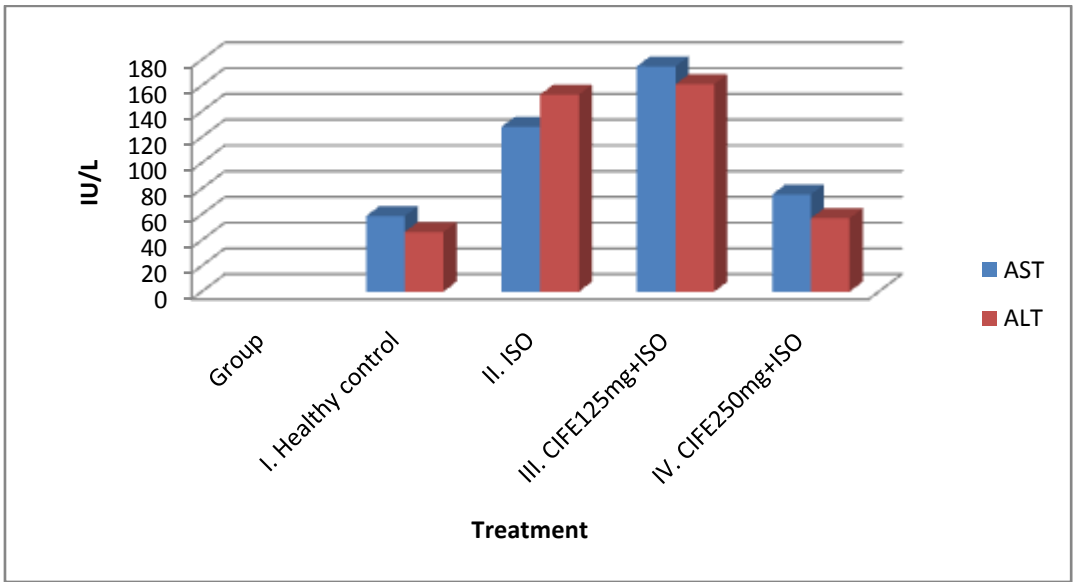

Figure 15: Effect of Canna indica flowers extract on cardiac markers enzymes, AST and ALT in the serum of control and isoproterenol(ISO)-induced oxidative stress and cardiotoxicity in rats.

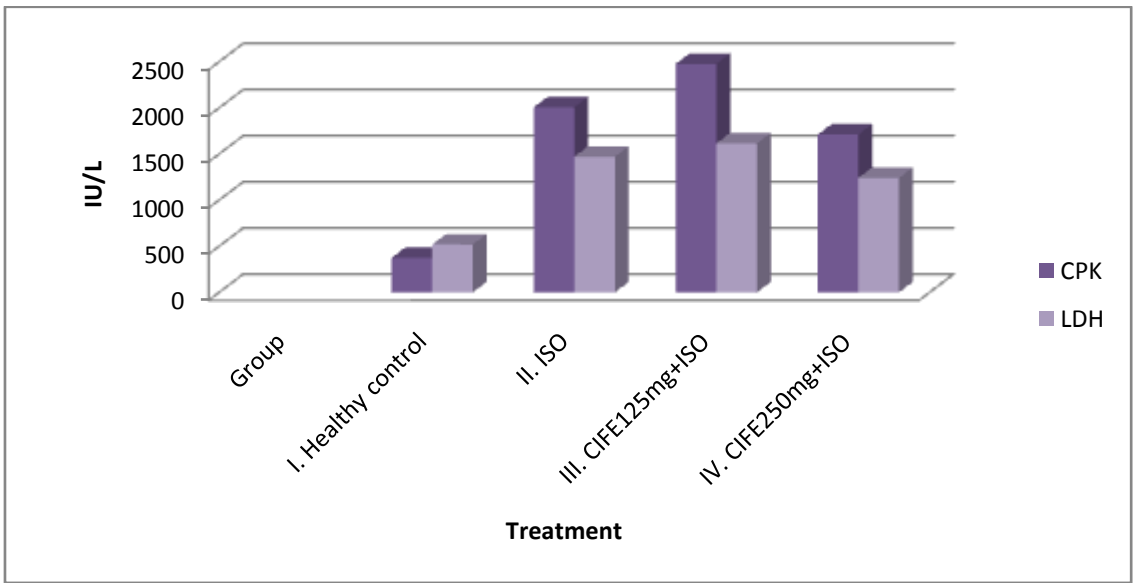

Figure 16: Effect of Canna indica flowers extract on cardiac markers enzymes, CPK and LDH in the serum of control and isoproterenol(ISO)-induced oxidative stress and cardiotoxicity in rats.

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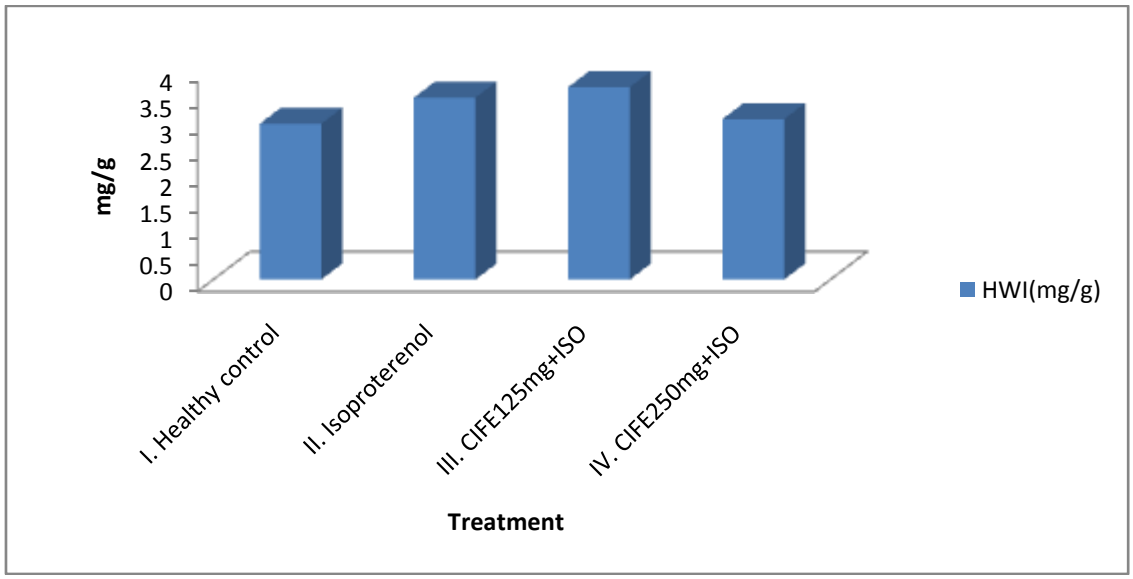


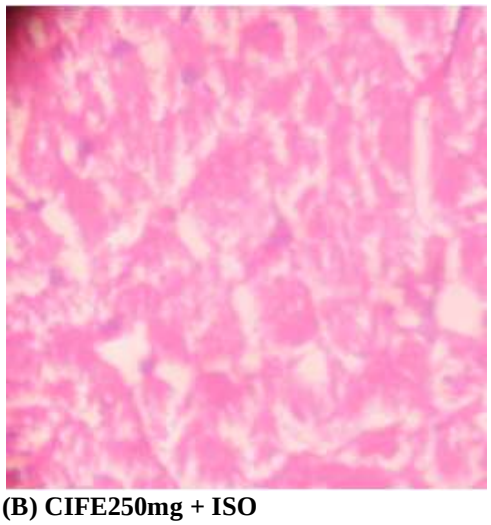
Figure 17: Effect of Canna indica flowers extract on heart weight indices (HWI) of control and isoproterenol(ISO)-induced oxidative stress and cardiotoxicity in rats.

Table 10: Effect of Canna indica flowers extract on heart architectures of control and isoproterenol(ISO)-induced oxidative stress and cardiotoxicity in rats.

Observation	Normal control	ISO	CIFE125mg+ ISO	CIFE250mg+ ISO
Necrosis of myocytes	-	+ + +	+ + +	+ +
Hemorrhage	-	+ +	+ + +	+
Infilteration of inflammatory cells	-	+ + +	+ + +	+ +
Fibroblastic proliferation	-	+	+	-
Hyaline necrosis	-	+ +	+ +	-
Inflamation	-	+ +	+ + +	+ +

Note: Nil (-), Present (+), Moderate (+ +), Severe (+ + +)

A) Normal control



(B) CIFE250mg + ISO

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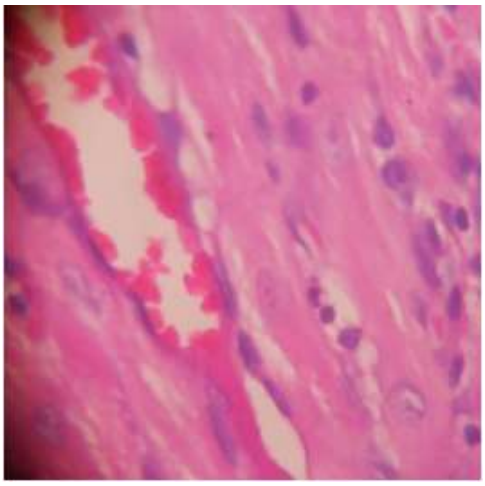
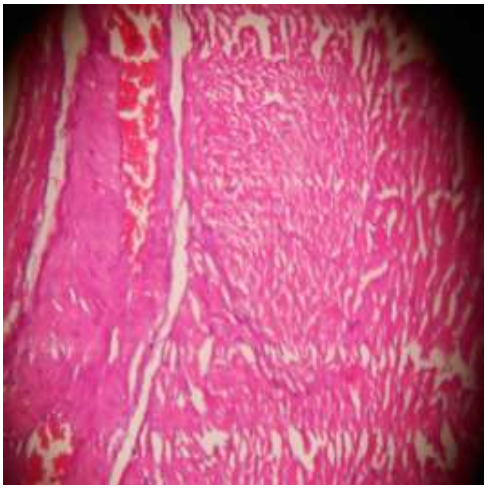


(C) ISO



(D) CIFE125mg+ ISO

Figure 18: Photographs of myocardial tissues of various treatment groups.

Table 11: Effect of Canna indica L. flowers extract on cardiac markers enzymes in the serum of control and Doxorubicin (Dox)-induced oxidative stress and cardiotoxicity in rats.

Group	AST (IU/L)	ALT (IU/L)	CPK (IU/L)	LDH (IU/L)
I.Normal control	56.7±0.67	43.3±1.28	365±1.99	509±1.45
II. DOX	118±8.33	143.0±1.61	2006±99.3	1470±128
III.CIFE 125mg+ DOX	72.5±2.46*	54.0±2.13*	1701±81.5**	1221±37.5**
IV. CIFE 250mg+ DOX	172±6.99$^{\#}$	151.0±4.26	2457±89.9$^{\#}$	1605±74.2

N=6, values are expressed as mean ± SEM, *P<0.01, **P<0.001 in comparison with healthy control, $^{\#}$p<0.01 in comparison with ISO.

Table 12: Effect of Canna indica flowers extract on heart weight index (HWI) of control and Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats.

Group	HWI(mg/g)
I.Normal control	2.97±0.0244
II DOX	3.47±0.0521*
III. CIFE125mg+DOX	3.67±0.137**

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IV. CIFE250mg+DOX	3.06±0.108

N=6, values are expressed as mean ± SEM, *P<0.01 and **P<0.001 in comparison with healthy control.

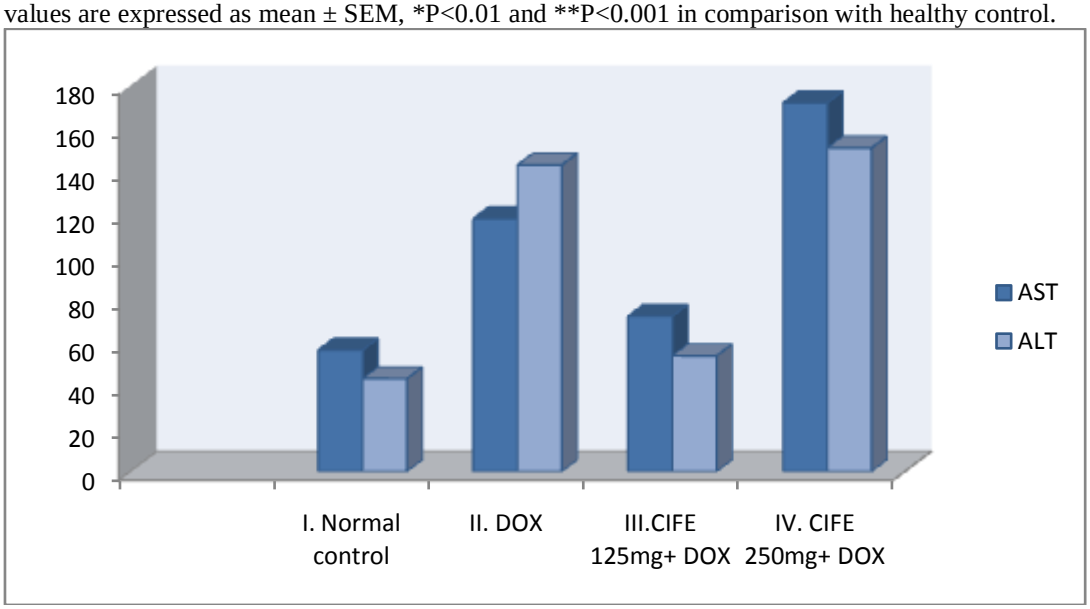

Figure 19: Effect of Canna indica flowers extract on cardiac markers enzymes, AST and ALT in the serum of control and Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats.

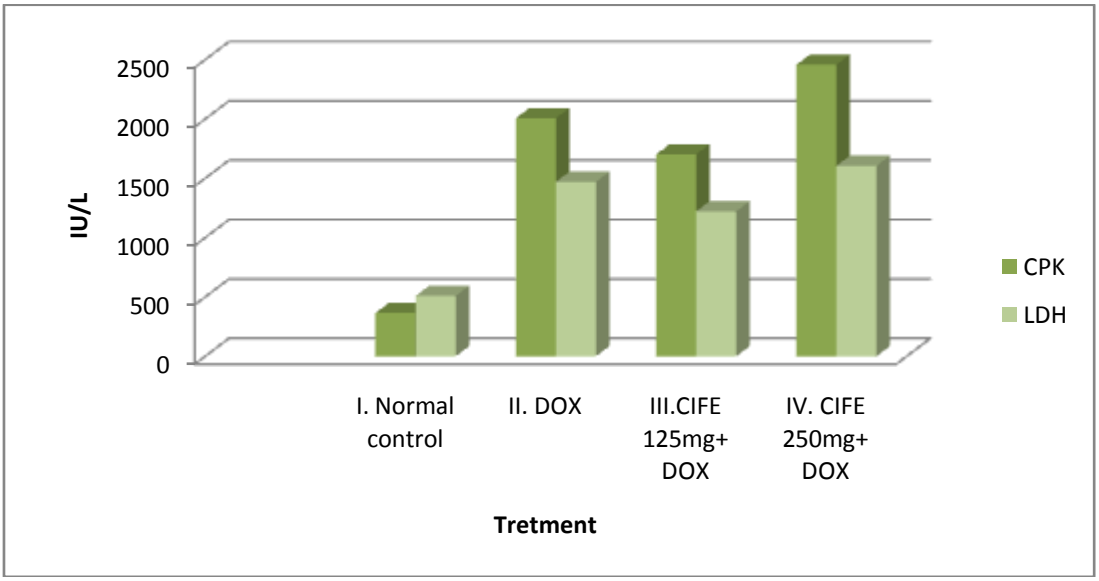

Figure 20: Effect of Canna indica flowers extract on cardiac markers enzymes, CPK and LDH in the serum of control and Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats.

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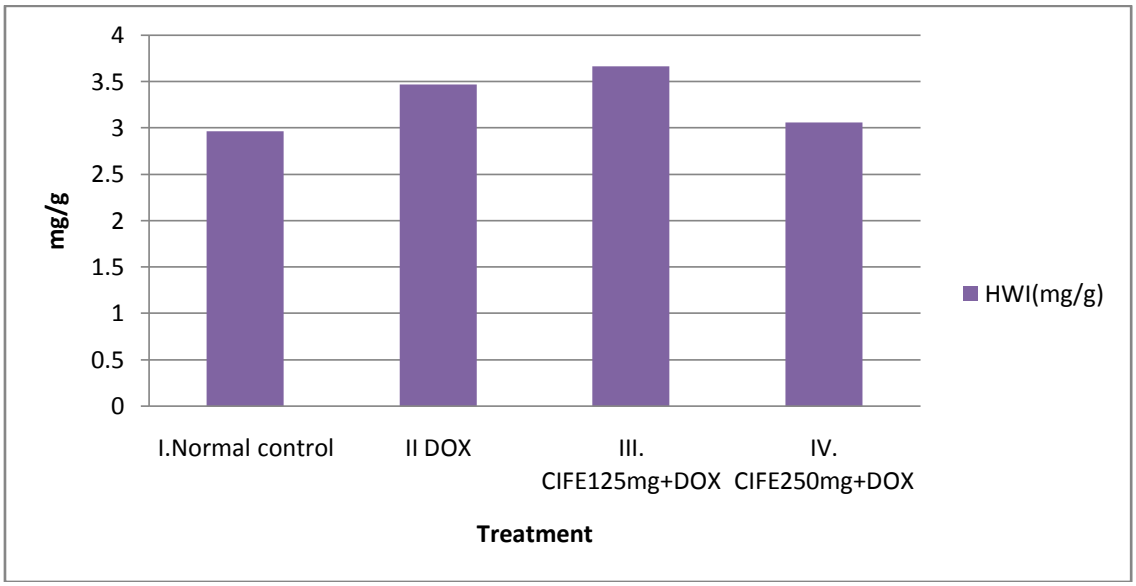


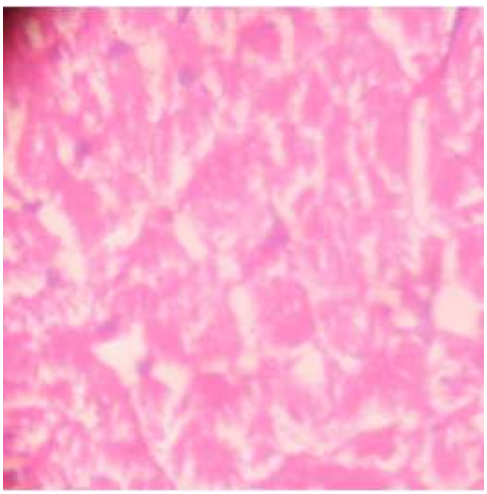
Figure 21: Effect of Canna indica flowers extract on heart weight indices (HWI) of control and Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats.

Table 13: Effect of Canna indica flowers extract on heart architectures of control and Doxorubicin (DOX))-induced oxidative stress and cardiotoxicity in rats.

Observation	Normal control	DOX	CIFE125mg+ DOX	CIFE250mg+ DOX
Necrosis of myocytes	-	+ + +	+ + +	+ +
Hemorrhage	-	+ +	+ + +	+
Infilteration of inflammatory cells	-	+ + +	+ + +	+ +
Fibroblastic proliferation	-	+	+	-
Hyaline necrosis	-	+ +	+ +	-
Inflamation	-	+ +	+ + +	+ +

Note: Nil (-), Present (+), Moderate (+ +), Severe (+ + +)

A) Normal control



(B) CIFE250mg + DOX

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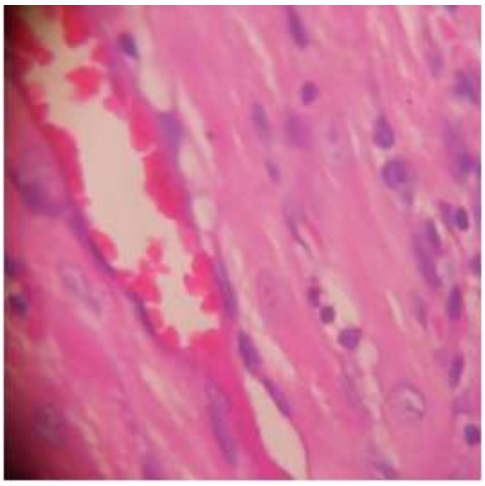
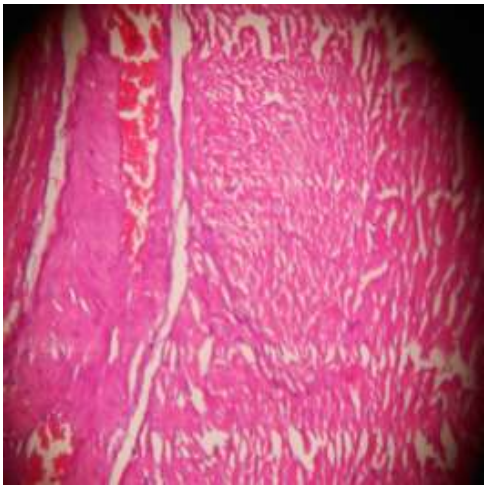


(C) DOX



(D) CIFE125mg+ DOX

Figure 22: Photographs of myocardial tissues of various treatment groups.

5.1 Extraction and phytochemical analysis of Canna indica L. flowers extract

Successive soxhlet extraction process of Canna indica L. flowersyielded 9.8% of extract. The extract powder was deep brown in colour and hygroscopic in nature. The extract wassubjected to qualitative test to identify the presence of phytoconstituents. The result is depicted in Table 7. It was observed from the preliminary phytochemical screening of ethanolic extract ofCanna indica L. flowers, cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. However the principal components like flavonoids, phenols, glycosides were present in the extract.

5.2 Cardioprotective activity of Canna indica L. flowers extract in albino rats

5.2.1 Cardioprotective activity of Canna indica L. flowers extract against ISO-induced cardiotoxicity in albino rats.

The cardiac activity was evaluated by measuring four cardiac marker enzymes in serum of albino rats and the result are presented in table 8. Rats subjected to the isoproterenol (ISO) challenge alone for two days developed heart injury as evident from a significant elevation in the biochemical marker like AST, ALT, LDH and CPK when compared with healthy control. The AST level has been increased from 58.7 to 128, ALT 46.3 to 153, CPK from 375 to 2006 and LDH from 519 to 1470 IU/L. In group, where rats were treated with CIFE 125 mg/kg p.o. for 28 days followed by ISO for two days, surprisingly biomarkers increased significantly when compared to control. However biomarkers level were found lower than level observed in group two animals where ISO was used to module cardiotoxicity. The biomarkers levels in animals treated with CIFE 250 mg/kg p.o. for 28 days flowed by ISO for two days were found significantly decreased. To our surprise the biomarker levels were lower than the level observed in ISO induced toxic group.

The increase in heart weight index (HWI) indicates cardiac injury. In the study as shown in table 9 and figure 17, the HWI of the group two animals treated with isoproterenol increased significantly confirming the cardiotoxicity. Treatment with CIFE 250mg/ kg for 28 days flowed by two days ISO did not increase HWI significantly. However in the group four, where in animals are treated with CIFE 250 mg /kg and ISO, the HWI increased significantly when compared with group two animals. Cardiac injury induced group i.e. rats treated with ISO alone revealed the rise in HWI in compared to healthy control. Group received CIFE 250mg/kg p.o. and ISO have also shown significant rise in HWI compared to healthy animals. Increase of HWI from 2.97 mg/g to 3.06 mg/g in rats received RSLE 250mg/kg has add-on to the cardio toxicity of the extract.

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Further, histopathological findings revealed the induction of cardiopathy by the extract. The severity of cardiotoxicity is cited in table 10 and microscopical photographs are shown in figure 18. Necrosis of myocytes, and infilteration of inflammatory cells are moderate in rats which received ISO alone, where as it was found to be severe in group treated with ISO and CIFE 125mg/kg. Intensity of hemorrhage and inflammation is more in the group treated with CIFE 259mg/kg and ISO than as injected with ISO alone. In group received CIFE 125mg/kg alone, has also shown necrosis of myocytes, infilteration of inflammatory cells and inflammation moderately moreover mild hemorrhage was also observed in the group. Fibroblastic proliferation was present in group II and group III where as absent in group I and group IV.

Hence, from the above observed parameters and histopathological studies, the Canna idica L. flowers extract is found to be cardioprotective activity in the albino rats.

5.2.2 Cardioprotective activity of Canna indica L. flowers extract against DOX-induced cardiotoxicity in albino rats.

The cardiac activity was evaluated by measuring four cardiac marker enzymes in serum of albino rats and the result are presented in table 11. Rats subjected to the doxorubicin (DOX) challenge alone for two days developed heart injury as evident from a significant elevation in the biochemical marker like AST, ALT, LDH and CPK when compared with healthy control. The AST level has been increased from 56.7 to 118, ALT 43.3 to 143, CPK from 365 to 2006 and LDH from 509 to 1470 IU/L. In group, where rats were treated with CIFE 125 mg/kg p.o. for 28 days followed by DOX for two days, surprisingly biomarkers increased significantly when compared to control. However biomarkers level were found lower than level observed in group two animals where DOX was used to module cardiotoxicity. The biomarkers levels in animals treated with CIFE 250 mg/kg p.o. for 28 days flowed by DOX for two days were found significantly decreased. To our surprise the biomarker levels were lower than the level observed in DOX induced toxic group.

The increase in heart weight index (HWI) indicates cardiac injury. In the study as shown in table 12 and figure 21, the HWI of the group two animals treated with doxorubicin increased significantly confirming the cardiotoxicity. Treatment with CIFE 250mg/ kg for 28 days flowed by two days DOX did not increase HWI significantly. However in the group four, where in animals are treated with CIFE 250 mg /kg and ISO, the HWI increased significantly when compared with group two animals. Cardiac injury induced group i.e. rats treated with DOX alone revealed the rise in HWI in compared to healthy control. Group received CIFE 250mg/kg p.o. and DOX have also shown significant rise in HWI compared to healthy animals. Increase of HWI from 2.97 mg/g to 3.06 mg/g in rats received CIFE 250mg/kg has add-on to the cardio toxicity of the extract.

Further, histopathological findings revealed the induction of cardiopathy by the extract. The severity of cardiotoxicity is cited in table 10 and microscopical photographs are shown in figure 22. Necrosis of myocytes, and infilteration of inflammatory cells are moderate in rats which received DOX alone, where as it was found to be severe in group treated with DOX and CIFE 125mg/kg. Intensity of hemorrhage and inflammation is more in the group treated with CIFE 250mg/kg and DOX than as injected with DOX alone. In group received CIFE 125mg/kg alone, has also shown necrosis of myocytes, infilteration of inflammatory cells and inflammation moderately moreover mild hemorrhage was also observed in the group. Fibroblastic proliferation was present in group II and group III where as absent in group I and group IV.

Hence, from the above observed parameters and histopathological studies, the Canna idica L. flowers extract is found to be cardioprotective activity in the albino rats.

VI. DISCUSSION

The leaf extract of Canna indica flowers yielded the good percentage of yield of 9.8%. The alcoholic extract contained chemical constituent such as cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents. These results are in agreement with results published in standard books and research papers.

Myocardial infarction is one of the most common manifestations of cardiovascular disease. The pathogenesis of acute myocardial infarction has not yet been fully understood, but studies on ISO or DOX induced cardio-toxicity provide a good insight into this pathology and clearly indicate the involvement of oxidative stress 94, 95.

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In the present study, we found that canna indica a strong cardio-protective effect against ISO and DOX – induced myocardial necrosis in rats.

Myocardium contains n abundant amount of diagnostic marker enzymes for MI and once metabolically damaged, it released its intercellular contents into the extracellular fluid 96. Hence the serum levels of these marker enzymes reflect the alterations in membrane integrity and/or permeability. Cytosolic enzymes CPK, LDH, AST, ALT and SGPT which serve as the diagnostic markers, leak out from the damaged tissue to blood stream when cell membrane becomes permeable or rupture 97,98. Assay of the activity of CPK in serum in an important diagnostic, because of the marked abundance of this enzyme in myocardial tissue and virtual absence from most of other tissues and its consequent sensitivity. CPK iso-enzyme activity is useful not only as an index of early diagnosis of MI, but also any type of myocardial injury. ISO or DOX administration to rats showed elevated levels of serum CPK 99, which was found to be significantly low in the bioactive fractions pretreated rats. Lactate dehydrogenase (LDH) is an intracellular enzymes, which catalyzes the readily reversible reaction involving the oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide (NAD) serving as coenzyme. This clinically significant enzyme rises within 24-48 hours after a heart attack and peaks in two to three days in the serum 100. Consistent with the above clinical observations, in the present study we observed a significant rise in the LDH levels of rats treated with DOX or ISO after 48 or 72h of the respective treatment. Canna indica pretreated significantly reduced the elevated levels of LDH indicating the reduction in the severity of MI.

It has been reported that infarction of as little as 10% of the total myocardium produces a significant rise in the serum levels of AST, ALT and is linear with the amount of infarction 101. In accordance to the above reports, ISO or DOX treatment elevated these enzyme levels in serum to a significant extent. Pretreatment with Canna indica flower extract (125and 250 mg/kg) significantly lowered the ISO or DOX-induced elevation of serum levels of these diagnostic marker enzymes. It demonstrates that Canna indica could protect membrane integrity thereby restricting the leakage of these enzymes.

VII. CONCLUSION

The Canna indica flowers extract has significant cardioprotective activity when tested in albino rats.

SUMMARY

The aim of this study was to evaluate the cardioprotective properties of Canna indica flowers extract in albino rats.

- The ethanolic extract of Canna indica flowers extract found to contain cardiac glycoside, carbohydrate, proteins, alkaloids, glycosides, saponins, phytosterol, flavonoid, and trepenoids, steroids, tannins, phylobatinins, phenols as chemical constituents
- Treatment with Canna indica flowers extract has protected the heart from isoproterenol and doxorubicin induced cardiotoxicity in albino rats. This was demonstrated by reducing the elevated levels of biomarkers like AST, ALT CPK, LDH. were reduced in ISO and DOX induced cardiotoxicity in rodents by Canna indica flowers extract. Histopathological studies has supported the results.

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