Evaluation of Cardioprotective activity of Ethanolic extract of bark of *Helicteres isora* Linn.

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Abstract

This study evaluates the cardioprotective activity of the ethanolic extract of the bark of *Helicteres isora* Linn. in a laboratory model. *Helicteres isora* Linn., a medicinal plant traditionally used in various cultures for its therapeutic properties, has gained attention for its potential cardiovascular benefits. The ethanolic extract was prepared using a standard protocol and subjected to phytochemical screening, which identified the presence of flavonoids, tannins, and saponins as key phytoconstituents.In vivo experiments were conducted using a rat model subjected to isoproterenol-induced myocardial infarction. The cardioprotective effect of the extract was assessed through measurements of cardiac markers, including serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and aspartate aminotransferase (AST), alongside histopathological analysis of cardiac tissues.Results indicated that administration of the ethanolic extract significantly reduced the elevated levels of cardiac biomarkers compared to the control group, suggesting a protective effect against myocardial injury. Furthermore, histopathological examination revealed a marked reduction in necrosis and inflammatory cell infiltration in the cardiac tissues of the extract-treated group.The findings of this study support the cardioprotective potential of the ethanolic extract of *Helicteres isora* Linn.

INTRODUCTION

Cardiovascular diseases (CVDs) remain a leading cause of morbidity and mortality worldwide, prompting an urgent need for effective preventive and therapeutic strategies. The search for natural compounds with cardioprotective properties has gained increasing attention, as they may offer safer alternatives to synthetic drugs often associated with adverse effects. One such promising source is the bark of *Helicteres isora* Linn., a traditional medicinal plant known for its diverse pharmacological properties.

Helicteres isora, a member of the Malvaceae family, is widely distributed across several tropical and subtropical regions. Traditionally, various parts of this plant have been used in ethnomedicine for the treatment of a range of ailments, including gastrointestinal disorders, liver dysfunction, and respiratory conditions. Recent studies have indicated that *Helicteres isora* possesses notable antioxidant, anti-inflammatory, and antimicrobial activities, suggesting its potential role in combating oxidative stress and inflammation—two critical contributors to the pathophysiology of cardiovascular diseases.

The versatility of phytochemicals found in *Helicteres isora*, such as flavonoids, tannins, and phenolic compounds, has spurred interest in their potential cardioprotective effects. Oxidative stress and inflammation are key factors in the development of atherosclerosis and myocardial infarction; thus, natural antioxidants may play a significant role in mitigating these conditions. Preliminary research suggests that the ethanolic extract of *Helicteres isora* bark could offer protective effects on the cardiovascular system, though comprehensive evaluations of its cardioprotective activity remain limited.

This study aims to evaluate the cardioprotective activity of the ethanolic extract of *Helicteres isora* bark through in vitro and in vivo assessments. We hypothesize that the extract exhibits significant positive effects on cardiac health by enhancing antioxidant capacity, reducing inflammation, and

improving lipid profiles. This investigation could contribute valuable insights into the therapeutic applications of *Helicteres isora* in cardiovascular health and support its traditional use in herbal medicine. Further exploration may pave the way for the development of novel cardioprotective agents derived from this plant, providing a natural adjunct or alternative to conventional therapies.

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The bark of *Helicteres isora* Linn. was collected during December 2023 from Solakkadu, Kollimalai, Namakkal District, Tamilnadu, India, identified and authenticated

EXTRACTION

The dried bark of *Helicteres isora* Linn. was ground into a fine powder with an auto- mix blender. The fine bark powder was suspended in equal amount of ethanol in soxhlet apparatus. Then the fine powder was suspended in an equal amount of ethanol, stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 182.4 g/kg of powdered bark) served as ethanolic extract of *Helicteres isora* Linn. for experimentation. The appearance of resulted extract yield of the extract was dark green color.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The ethanolic extract of bark of *Helicteres isora* Linn. was subjected to preliminary phytochemical screening

PHARMACOLOGICAL STUDIES ACUTE TOXICITY STUDIES

Acute toxicity studies were performed by using OECD guide lines (Organization of Economic Cooperation and Development) 423. It’s a step wise procedure with 3 female animals per step. Depending upon the mortality and/or morbidity status of animals, the average of 2–3 steps are necessary to allow judgment on the acute toxicity of the test substance. It results in the use of minimum number of animals while allowing for acceptable data based scientific conclusion. The procedure uses defined doses (2000mg/kg body weight) and the result allows a test substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause acute toxicity.

EXPERIMENTAL PROCEDURE

Three male Wistar rats weighing 150-220g body weight were used for the study, since the herbal extract are relatively non-toxic, 2000mg/kg bodyweight was selected as the starting dose level of the extract (defined dose by OECD 423 guidelines). 18 hours prior to the administration of test drug, animals were fasted overnight with water *ad libitum*. Body weight of rats before and after administration of test drug

noted and any changes in fur and skin and, eyes and mucous membrane, respiratory system and circulatory system were observed and also sign of convulsion, tremors, diarrheoa, salivation, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted.

Behavioral profile

Alertness, irritability, restlessness and fearfulness.

Neurological profile

Spontaneous activity, reactivity, touches response and pain response.

Autonomic profile

Defecation and urination, lethality or death of animals was observed after 24 hours and 72 hours respectively.

EXPERIMENTAL ANIMALS

Colony inbreed strains of Male Wistar rats of 160-200g body weight were used for pharmacological studies, three different groups with 6 animals in each, were housed at controlled temperature (25 ± 2°C), humidity (60–80% relative humidity) and light dark cycle (12/12 hour); on a standard rodent chow (Hindustan lever pvt ltd., Bangalore) and water *ad libitum*. Animals were handled carefully and acclimatized to laboratory conditions for 14 days prior to experimentation. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethical Committee (), and studies were carried out in accordance with Institutional ethical guidelines.

The animals were fasted and no water, from 12 hours prior to the application of anesthesia, which preceded the sacrifice, blood collection, whose aim was to perform complete blood count and fragments of the left ventricle.

INDUCTION OF MYOCARDIAL INFARCTION:[68]

After the treatment of animals for three weeks with aqueous extract of bark of *Helicteres isora* Linn., isoproterenol (ISO) 85 mg/kg/day (diluted in 2 ml of saline), *s.c* was administered below the skin of the neck in two divided doses at 12 hours intervals to induce myocardial necrosis. Twenty four hour after the first dose of ISO administration, the rats were sacrificed.

METHODOLOGY

The animals were divided into three groups of 6 animals each. Group-1 received normal saline and was considered as normal control animals. Group-2 received normal saline. Group-3 received ethanolic extract of bark of *Helicteres isora* Linn. in a dose of 400 mg/kg/p.o. The treatment was continued for

about 3 weeks. After completion of treatment, all groups of animals except for group 1were fasted overnight and were induced Myocardial Infarction (MI) by giving subcutaneous injection of 85mg/kg of ISO in two divided doses at 12 hours interval. Twenty Four hour after the first dose of ISO administration, ECG of rats taken and were sacrificed.

ANESTHESIA

Twenty-four hours after the last subcutaneous injection, anesthesia was proceeded to perform the sacrifice of animals. The anesthetic technique involved the use of thiopentone Sodium anaesthesia at the dose of 30 mg/kg applied intraperitoneally.

ECG CHANGES

Needle electrodes were placed subcutaneously and changes in Lead II were recorded 12hours after the second dose of ISO on an electrocardiograph. Heart rate and electrocardiograph [ECG] were observed in anesthetized animals (Thiopentone Sodium anaesthesia 30 mg/kg i.p.)for a period of one minute for every 5 minutes. The type of alterations [ST-segment elevation or depression] in normal and experimental animals was considered.

COLLECTION OF SAMPLES

After adequate anesthesia, an incision was performed in an inverted T extending from neck to pubis.

COLLECTION OF BLOOD

We obtained access to the retroperitoneum, the abdominal aorta was dissected in its infrarenal portion. The blood collection was then performed through a puncture of the abdominal aorta with a disposable Jelco number 24 (24 gauge). Using a 5 mL disposable syringe, coupled with Jelco 5 mL of blood were collected. Of this volume, 2 mL were stored in a tube for complete blood count and 3 mL in a tube for biochemical dosages (urea, creatinine, SGOT, SGPT and troponin I). The tubes, properly identified, were then placed in a cooler with ice.

COLLECTION OF PLASMA SERUM

Blood was collected from the retroperitoneum, and serum was separated by centrifuging the blood at 3000 rpm after the blood clots. The supernatant serum was separated and used for estimation of marker enzymes.

COLLECTION OF FRAGMENTS OF THE MYOCARDIUM TO MEASURE THE ACTIVITY OF THE ENZYME LEVELS

After the withdrawal phase of the cardiac apex, we proceeded immediately to the resection of two fragments of the left ventricle muscle. These fragments were weighed on a precision balance, and

placed separately inside cryotubes properly identified and put into container of liquid nitrogen. The cryovials were then stored in a freezer at a temperature of − 70°C until the analysis in a period of up to two weeks.

ESTIMATION OF SERUM LIPID PROFILE

The levels of HDL, LDL and VLDL were estimated in the supernatant serum obtained by using commercially available kits (HIMEDIA, Bangalore).

ESTIMATION OF CARDIAC MARKER ENZYMES

ESTIMATION OF CREATININE-KINASE

Blood serum was isolated and 10% homogenate was prepared by adding 50mM Phosphate buffer, pH 7.4. and homogenate was centrifuged at 7000 rpm for 15 mins. and the supernatant used for the estimation of CK-MB by following the procedure given in the kits.

ESTIMATION OF LACTATE DEHYDROGENASE (LDH) [69]

Blood serum was homogenised in 5ml of 0.1 M Tris-Hydrochloride buffer in ice cold condition. The homogenate was centrifuged at 2500 rpm for 5 mins. The supernatant was used for the estimation of LDH following the procedure given in the kits.

ANTIOXIDANT PARAMETERS

ESTIMATION OF LIPID PEROXIDATION [70]

PROCEDURE:

Lipid peroxidation products of heart homogenate were determined as thiobarbituric acid reactive substances (TBARS). Heart of the Control and treated rats was homogenised in ice-cold 0.9% saline to get 10% homogenate. 0.5 ml of supernatant after differential

centrifugation was allowed to react with 3 ml of 1% Orthophosphoric acid and 1 ml of 0.6% thiobarbituric acid. The tubes was heated in boiling waterbath for 45 mins, cooled and then 4 ml of n-butanol was added to each test tube mixed vigorously and centrifuged for 5 mins at 1000 rpm. The supernatant was used for determination of TBARS at 535 nm against a reagent blank. TBARS were expressed as nmol/L wet weight.

- ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) [71]

PROCEDURE:

0.1 ml of homogenate was taken in a test tube containing 1.2 ml of sodium pyrophosphate buffer (pH 8.3,0.052M). To this mixture 0.1ml of Phenazinemethosulphate (186 µM) and 0.3ml of 300µM Nitrobluetetrazolium will added. The reaction was started by addition of 0.2 ml of NADH (750µM) the reaction mixture was incubated for 90 sec at 30°C. The reaction was stopped by addition of 0.1ml glacial acetic acid. Then the mixture was stirred vigorously with 4.0 ml of n-butanol and allowed to stand for 10mins to separate butanol layer. The butanol layer was separated and used for determination of SOD at 560 nm. The activity of SOD was expressed as units/mg protein.

- **ESTIMATION OF REDUCED GLUTATHIONE (GSH)** [72]

PROCEDURE:

0.5 ml of homogenate was added to each tube containing 0.5 ml Trichloroacetic acid (TCA 10%). The test tube was gently shaken intermittently for 10 mins followed by centrifugation at 3000 rpm for 5mins at room temperature. Accurately 0.1 ml of the resulting clear supernatant was mixed with 1.8ml of the phosphate buffer (0.1M, pH 8) in separate test tubes. Atleast a duplicate was made for each sample. 0.1 ml Ellman's reagent (0.39%) was added to each tube and after 5mins, the optical density was measured at 412 nm against a reagent blank. The data was expressed as mmol/g tissue.

STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's "t" test. (P values < 0.001) was considered statistically significant, it was calculated using graph pad prism Version 6.

RESULTS

PREPARATION OF PLANT EXTRACTS:

In Soxhlet apparatus, the dried 1000 g of *Helicteres isora* Linn. bark powder was taken for extraction for 24 hours. Amount of the extract of *Helicteres isora* Linn. obtained was 182.4 g. Percentage yield was found to be 18.24%.

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE EXTRACT OF Helicteres

isora Linn. BARK:

The results of preliminary phytochemical analysis of the ethanolic extract of bark of *Helicteres isora* Linn. is shown in Table 1.The ethanolic extract of bark of *Helicteres isora* Linn. showed the presence of various phytochemical constituents such as

Table 1
List of Phytochemical Constituents Screened in *Helicteres isora* Linn.

S.No.	Constituents	Ethanolic Extract
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Protein	
4.	Steroids	+
5.	Phenols	+
6.	Tannins	
7.	Flavonoids	+
8.	Gums and Mucilage	
9.	Glycosides	+
10.	Saponins	+
11.	Terpenoids	+
12.	Cardiac Glycosides	
+ Present;– Absent;		

ACUTE ORAL TOXICITY STUDY

The acute oral toxicity study was performed according to OECD 423 guidelines. A single oral administration of a starting dose of 2000 mg/kg body weight, of ethanolic extract of bark of *Helicteres isora* Linn. (EBHI) was administered to 3 male rats and observed. There was no lethality, mortality or any toxic reactions found at any selected dose level until the end of the study period. The results of acute oral toxicity studies are shown in Table 2.

Table 2
Acute Toxicity Study of *Helicteres isora* Linn.

S. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before Test	After test				
1.	EBHI	2g/kg	150	155	No signs of toxicity	Nil	Nil	14 days
2.	EBHI	2g/kg	150	170	No signs of toxicity	Nil	Nil	14 days
3.	EBHI	2g/kg	170	185	No signs of toxicity	Nil	Nil	14 days

ECG CHANGES

Ck-MB LEVELS

Table 3 indicates that the change which occurs due to the pretrearment of ethanolic extract of bark of *Helicteres isora* Linn. in CK-MB enzyme level in the blood serum.

Table 3
Effect of Ethanolic extract of *Helicteres isora* on CK-MB levels in serum

GROUPS	CK-MB (ng/ml)
GROUP 1 (CONTROL)	0.08 ± 0.026
GROUP 2 (NEGATIVE CONTROL)	0.86 ± 0.052****
GROUP 3 (400 mg/kg EBHI)	0.31 ± 0.026**

Values (ng/ml) are expressed as mean ± SEM (n = 6) .Values comparison were made between Group I Vs Group II, III (****$p < 0.0001$, **$p < 0.01$).

LACTATE DEHYDROGENASE LEVELS

Table 4 indicates the changes occur due to the pretreatment of *Helicteres isora* Linn. in the (Lactate Dehydrogenase) LDH enzyme level in the blood serum.

Table 4
Effect of Ethanolic extract of *Helicteres isora* on LDH levels in serum

GROUPS	LDH (IU/L)
GROUP 1 (CONTROL)	93.15 ± 2.73
GROUP 2 (NEGATIVE CONTROL)	201.60 ± 1.18****
GROUP 3 (400 mg/kg EBHI)	135.70 ± 2.71****

Values (IU/l) are expressed as mean ± SEM (n = 6) .Values comparison were made between Group I Vs Group II, III (****p < 0.0001).

REDUCED GLUTATHIONE

Table 9 indicates that the Reduced Glutathione (GSH) levels in the heart tissues, which were measured by making heart homogenate. The study indicated that due to Pretreatment of EBHI, increase in the GSH levels was found.

Table 9
Effect of Ethanolic extract of *Helicteres isora* on GSH levels in heart homogenate

GROUPS	GSH (mmol/gm)
GROUP 1 (CONTROL)	971.5 ± 55.440
GROUP 2 (NEGATIVE CONTROL)	760.8 ± 2.893**
GROUP 3 (400 mg/kg EBHI)	826.4 ± 25.950*

Values are expressed as mean ± SEM (n = 6) .Values comparison were made between Group I Vs Group II, III (**p < 0.01, *p < 0.05).

SUPEROXIDE DISMUTASE

Table 10 indicates that the Superoxide Dismutase (SOD) levels in the heart tissues, which were measured by making heart homogenate. The study indicated that due to Pretreatment of EBHI, increase in the SOD levels was found.

Table 10
Effect of Ethanolic extract of *Helicteres isora* on SOD levels in heart homogenate

GROUPS	SOD (Units/mg protein)
GROUP 1 (CONTROL)	98.65 ± 7.324
GROUP 2 (NEGATIVE CONTROL)	70.01 ± 0.577***
GROUP 3 (400 mg/kg EBHI)	82.79 ± 0.259*

Values are expressed as mean ± SEM (n = 6) .Values comparison were made between Group I Vs Group II, III (***p < 0.001, *p < 0.05).

DISCUSSION

Preliminary phytochemical analysis of the ethanolic extract of bark of *Helicteres isora* Linn. showed the presence of phytochemical such as Alkaloids, Carbohydrate, Steroids, Phenols, Flavanoids, Saponins, Terpenoids, Glycosides.

Acute oral toxicity studies of ethanolic extract of bark of *Helicteres isora* Linn. were performed by using OECD 423 guidelines. Studies did not exhibit any lethality or any profound toxic reactions at a dose of 2000 mg/kg/p.o. According to OECD 423 guidelines for acute oral toxicity study, LD_{50} dose of 2000 mg/kg/p.o of ethanolic extract of bark of *Helicteres isora* Linn. was found to be safe.

The formation of plaques in coronary artery decreases the blood flow to the myocardium and leads to hypoxia ultimately resulting in the development of MI. Isoproterenol, a synthetic catecholamine and β-adrenergic agonist, acts through β-adrenergic receptor which causes severe stress in the Myocardium. [73] ISO is the Catecholamine which produces highly toxic free radicals through auto oxidation mechanism, the free radicals produced by this mechanism is highly toxic than other methods of induction.[74] ISO is proposed as a cardiotoxic agent due to its ability destruct myocardial cells, causing hypoxia, and it disturbs the coronary microcirculation and it elevates the intracellular calcium load. Two consecutive doses of ISO by subcutaneous route leads to myocardial cell death.

The cardioprotective activity of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) is observed by the estimation of the two cardiac marker enzymes CK-MB, LDH in heart. Due to leakage from the heart and into the blood, after induction of MI there is an increase in the cardiac marker enzyme levels in the serum. The protective activity of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) produces significant actions against ISO induced MI. The significant rise observed in the levels of cardiac marker enzyme

in group-II compared to group-I indicates the severity of the necrotic damage to the myocardial membrane. Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue. The release of cellular enzymes creates non specific alterations in the membrane integrity and permeability as a response to β-adrenergic stimulation.[75]

The ECG recordings are done to identify changes occurring in the myocardium during MI. ECG abnormalities are important tools for accurate diagnosis of Myocardial infarction. ST segment elevation was observed in ISO induced MI in rat. The study shows significant alterations of ECG graph which were observed in ISO administered rats as compared to normal control rats. Due to ST segment elevation the intensity of P-wave is reduced along with decrease in QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle and insignificant increase in heart rate. The alterations could be due to the consecutive loss of cell membrane in injured myocardium.[76]

In this study, an elevation of ST-segments was observed in ISO induced rat and pretreatment with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) significantly inhibited ISO induced ST segment elevation suggestive of its cell membrane protecting effects.[77] The appearance of Q-wave and ST segment elevation are some of the indicative signs of ischemia. In the present study no pathological Q wave was observed due to conditions of ischemia. Administration of ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) eliminates the acute fatal complications by protecting the cell membrane damage.

CONCLUSION

From our study it may be concluded that the ethanolic extract of bark of *Helicteres isora* Linn. contains phytochemicals such as Alkaloids, Carbohydrates, Steroids, Phenols, Flavonoids, Glycosides, Saponins, Terpenoids.Acute oral toxicity studies of ethanolic extract of bark of *Helicteres isora* Linn .did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w/p.o, in experimental rats.

In this study, an elevation of ST segments were observed in ISO induced rat and pretreatment with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) significantly inhibited ISO induced ST segment elevation suggestive of its cell membrane protecting effects.The ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg) possess good cardioprotective activity against Isoproterenol induced myocardial necrosis by decreasing the level of CK-MB, LDH in serum and increase in heart. On treating with ethanolic extract of bark of *Helicteres isora* Linn. (400 mg/kg), LDL, Total cholesterol levels are

decreased and HDL levels increased, while compared to negative control group where HDL level was decreased, and LDL and total cholesterol levels were increased.

GSH & SOD levels were also increased in ethanolic extract of bark of *Helicteres isora* Linn. treated group. The ethanolic extract of bark of *Helicteres isora* Linn. was found to be most effective in the functional recovery of the heart.Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Helicteres isora* Linn.

Declarations

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest

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Figures

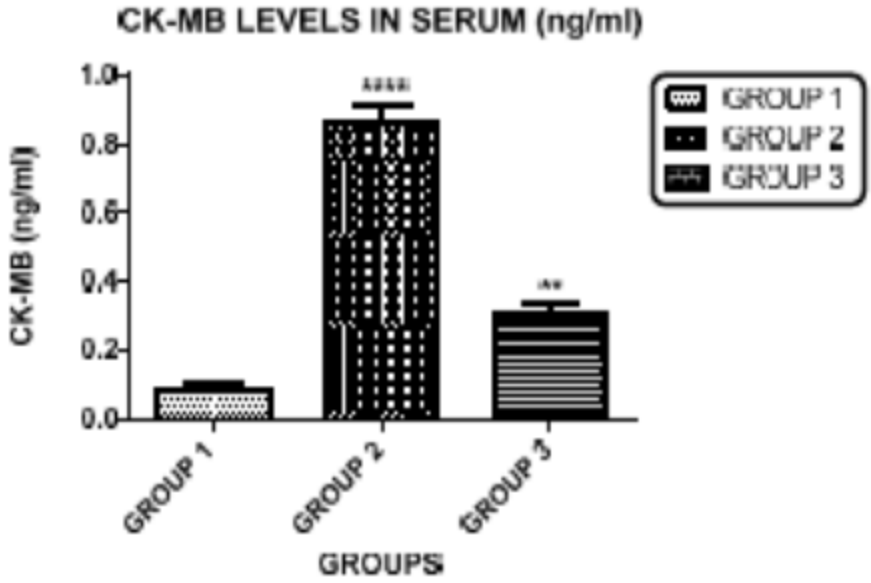

Figure 1

FIG.9: Effect of Ethanolic extract of *Helicteres isora* on CK-MB levels in serum

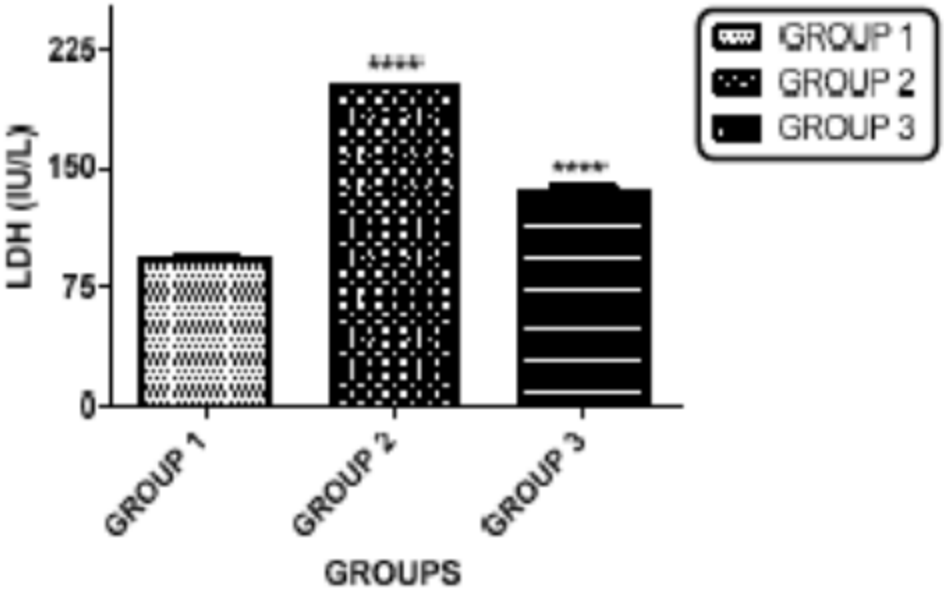

Figure 2

FIG. 10: Effect of Ethanolic extract of *Helicteres isora* on LDH levels in serum

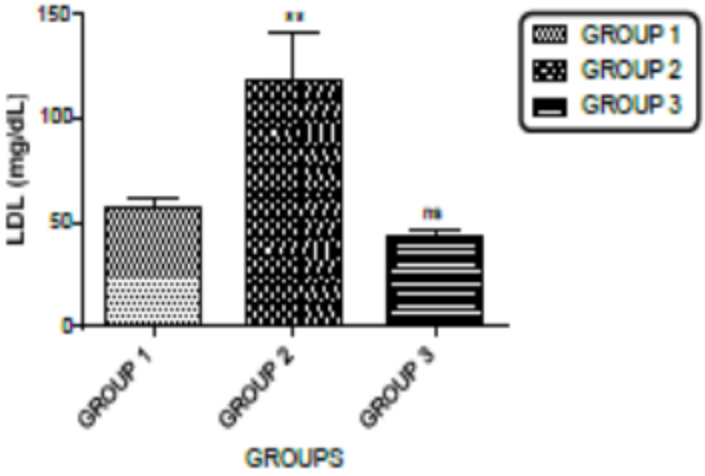

Figure 3

FIG.11: Effect of Ethanolic extract of *Helicteres isora* on LDL levels in serum

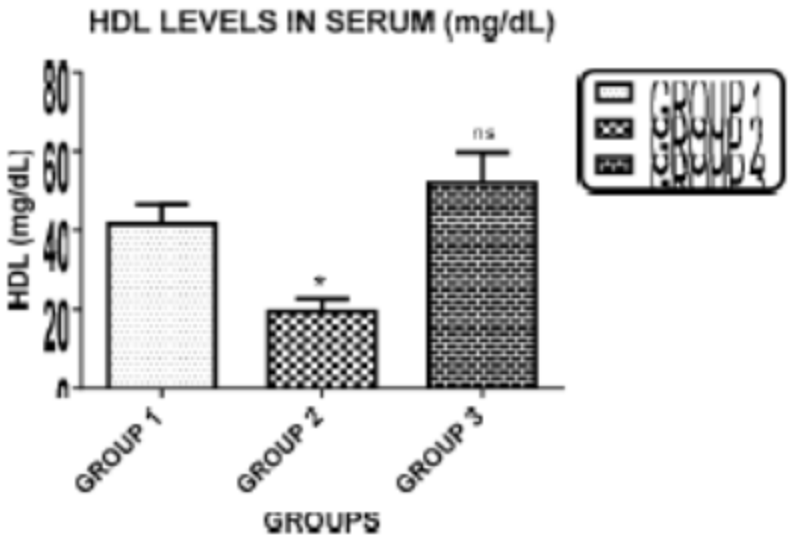

Figure 4

FIG. 12: Effect of Ethanolic extract of *Helicteres isora* on HDL levels in serum

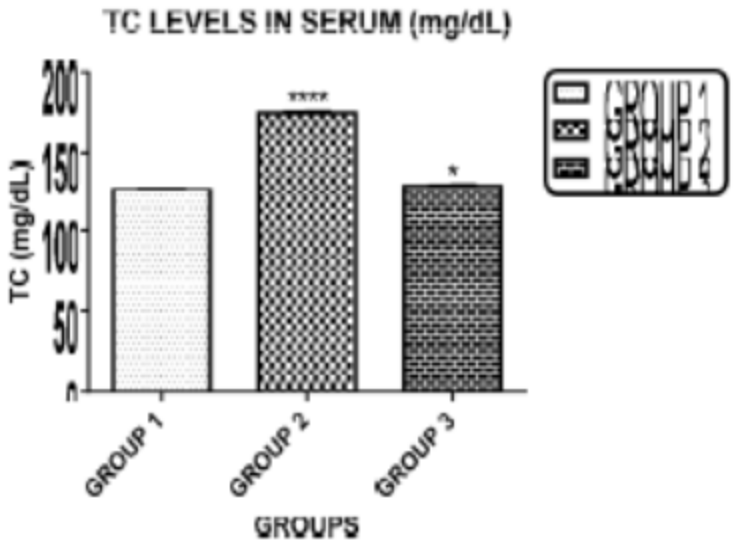

Figure 5

FIG 13: Effect of Ethanolic extract of *Helicteres isora* on Total Cholesterol levels in serum

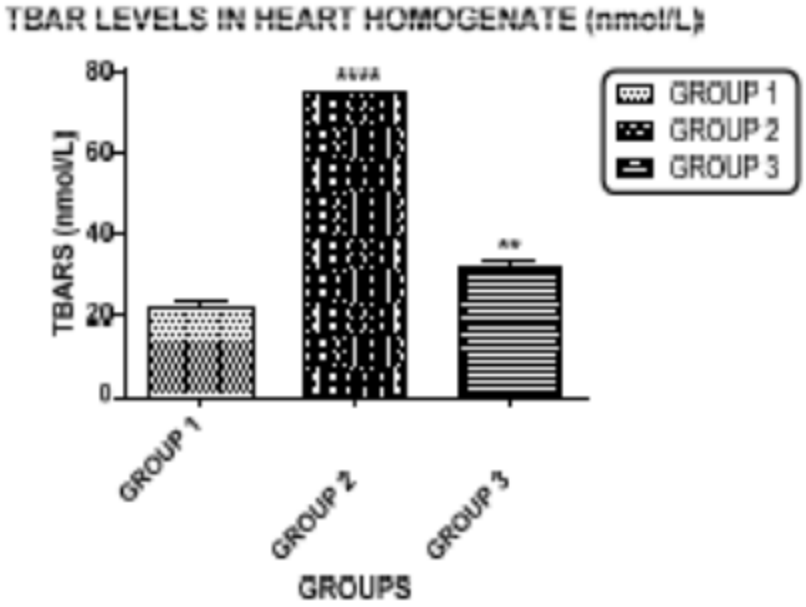

Figure 6

FIG 14: Effect of Ethanolic extract of *Helicteres isora* on TBARS level in heart homogenate

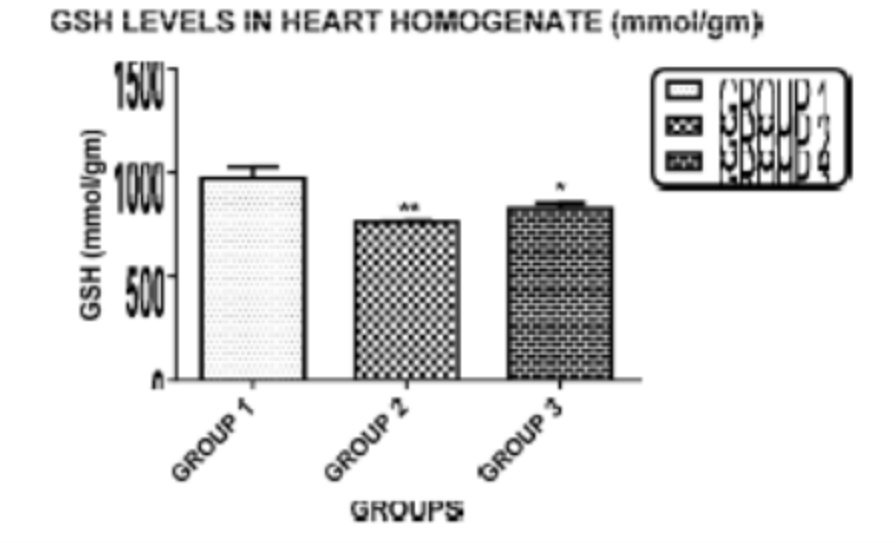

Figure 7

FIG 15: Effect of Ethanolic extract of *Helicteres isora* on GSH levels in heart homogenate

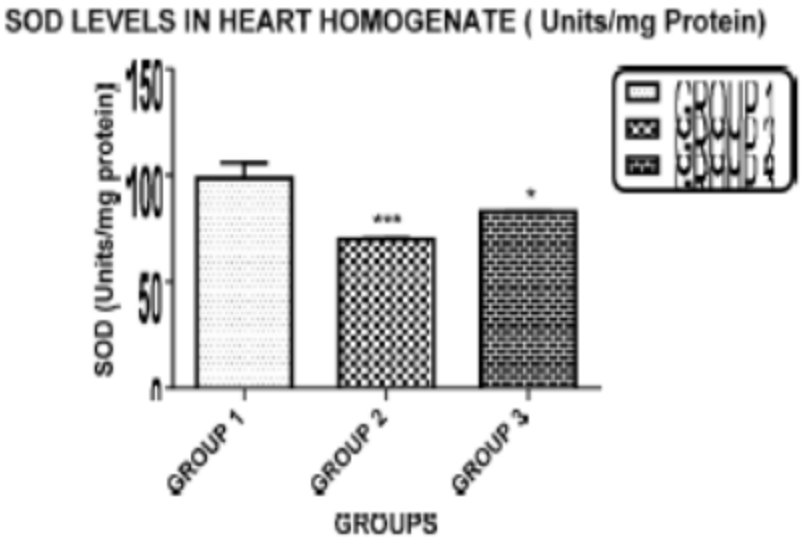

Figure 8

FIG 16: Effect of Ethanolic extract of *Helicteres isora* on SOD levels in heart homogenate

Supplementary Files

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