Prophylactic effects ofHydroethanolic extract of Brassica Cretica Leaves on Myocardial Injury in Rats.

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Abstract:

Background

Cardiovascular diseases (CVDs) are increasingly the leading cause of mortality worldwide. One naturally occurring plant, *Brassica Cretica*, is used to address a variety of illnesses, including heart conditions. There is, however, little information available regarding the plant's effectiveness and safety.

Objective:

To evaluate the prophylactic effects of hydroethanolic extract of *Brassica Cretica* leaves on cyclophosphamide-induced myocardial injury.

Methods:

Rats were randomized into 6 groups of 6 rats each, namely Saline control, positive and negative control, hsydroethanolic extract of Brassica cretica (HEBC) in three dose levels. The prophylactic effects of the hydroethanolic extract was evaluated based on anatomical, biochemical and histopathological methods. The parameters such as body weight, cardiac mass to body mass ratio, troponin-I, alanine transaminase (ALT), aspartate aminotransferase (AST), creatine kinase-myoglobin binding (CK-MB), total cholesterol were observed.

Results

Hydroethanolic extract of Brassica Cretica significantly prevented the deleterious effects of cyclophosphamide on body weight ($P<0.001$), ratio of cardiac mass to body mass ($P<0.01$) Cardiac biomarkers such as, Troponin-I ($P<0.01$), ALT ($P<0.001$), AST ($P<0.01$), CK-MB, ($P<0.001$), Lipid profiles including triglycerides ($P<0.001$) and total cholesterol ($P< 0.01$) were

greatly reduced by hydroethanolic extract of Brassica Cretica. The cyclophosphamide-treated groups were found to have necrosis, edema, and bleeding, while the groups that received 200 mg/kg and 400 mg/kg of the hydroethanolic extract had normal cardiocytes.
Conclusion: Hydroethanolic extractof Brassica Cretica leaves have cardioprotective activities. prophylactic effects againstcardiac injury could be attributed to the protective effects on liver and cardiac biomarkers. However, this requires further in-depth understanding.
Keywords: cardioprotective, cardiac biomarkers, hydroethanolic extract, cyclophosphamide

Introduction

Cardiovascular diseases have risen considerably in occurrence during the last two decades, making them a major global issue.[1-3]CVDs claimed the lives of 17.9 million people which nearly corresponds to 31% of the world's population. More than 75% of CVD deaths occur in developing countries with limited medication availability. Stroke or heart attacks were responsible for four out of every five CVD deaths. [4,5]In Ethiopia, CVDs were responsible for around 10.9 % of all deaths in 2017. [6]Even currently available medications have a lots of side effects and ineffectiveness difficulties. Myocardial injury, which includes myocarditis, ischemia, and degeneration, is the loss of muscular or neuronal function of the heart.[7] Acute myocardial injury can occur as a result of a myocardial oxygen supply-demand mismatch in a variety of cardiac and non-cardiac illnesses.[8,9] Infection, sudden left ventricular failure, and anticancer treatments are all harmful in some way. Myocardial damage develops into myocardial infarction(MI) if ischemia lasts more than 20 minutes. In addition, cardiac injury is occasionally associated with a proinflammatory and prothrombotic condition as a result of platelet aggregation embolization and thrombus from quite vulnerable plaque. Within 10 days of cyclophosphamide therapy, an acute, dose-dependent cardiac damage morphologically characterized by necrosis, bleeding, edema, and consequently, myocyte fibrosis occurs.[10-12] There are a variety of medicines and their combinational therapy available to treat CVDs. However, treatment is expensive in general, with side effects ranging from minor to fatal. Despite its widespread use as a food and traditional medicine for a range of diseases, including heart disease, no pharmacological research on its cardioprotective effects has been completed. This study aimed to fill up some of the gaps by using a cyclophosphamide-induced rat myocardial injury model.

Materials and Methods

Chemicals and Instruments

Analytical-grade chemicals and solvents were used, distilled water, formalin, cyclophosphamide, Enalapril (EN), ethanol.

Collection, Identification, and Preparation of Plant Materials

Brassica Cretica leaves were purchased from a local vendor in Pune, Maharashtra, India. A head (Botanist) of Botanical Survey of India Western regional Centre, 7-Koregaon road Pune-41044,

verified the entire plant. The BSI Herbarium has deposited a voucher specimen number BCRT1 Dated 23 Sep 2021.

Experimental Animals

In this study, male wistar albino rats were employed (weight 200-220g). All of the animals were kept in a polypropylene cage. After one week of acclimatization to the experimental environment, the rats were provided with ad. libitum access to food and water.

Animals and approval from animal ethical committee

The animals were kept in the animal house of Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, under standard housing conditions. The protocol was approved by IAEC (Institutional Animal Ethics Committee) with approval number (MCP/IAEC/07/2021).

Methods

Extraction of Plant Material

The leaves were then air-dried in the shade at room temperature. The coarse powder of Brassica Cretica was macerated for 48 hours at 80°C in 70% ethanol. The filtrate was then dried in ansoven at 40°C. Before being stored in the refrigerator, the heat-dried extract was kept in a desiccator.

Percentage Yield of Hydroethanolic Extract of Brassica Cretica

The Percentage Yield of the Hydroethanolic Extract was caluculated as,

% Yield= (Weight of extracts obtained)/ (Weight of powder used for extraction) ×100

Grouping and Dosing of Animals

The rats were divided in six experimental groups as,

Group-I: Saline control group

Group-II: Negative control group (Cyclophosphamide, 200 mg/kg, i.p.)

Group-III: Positive control group (Enalapril 10 mg/kg diluted in 2% Tween 80 p.o.) for 10 days followed by Cyclophosphamide, 200 mg/kg, i.p. on 11^{th} day.

Group-IV: HEBC 100 mg/kg, p.o. for 10 days followed by Cyclophosphamide, 200 mg/kg, i.p. on 11^{th} day.

Group-V: HEBC 200 mg/kg, p.o. for 10 days followed by Cyclophosphamide, 200 mg/kg, i.p. on 11^{th} day.

Group-VI: HEBC 400 mg/kg, p.o. for 10 days followed by Cyclophosphamide, 200 mg/kg, i.p. on 11^{th} day.

Collection of Blood Samples and Plasma Preparation

On the final day of therapy (day 12), blood was drawn from the animal's retro-orbital plexus into heparinized tubes after administering ketamine 100mg/kg i.p. after 11 days of treatment and

damage infliction. To make plasma, blood samples were centrifuged for 15-minutes at 3500 rpm in a centrifuge. Troponin I, AST, ALT, and lipid profiles were estimated from the clear supernatant using the semi-automatic analyzer.[13,16]

Lipid Profiles

In 2mL of plasma, total cholesterol and triglycerides were measured and compared in each experimental group. [10]

Surgical Removal of the Heart

The rats were sacrificed by cervical dislocation. A trans-abdominal incision was used to cut the diaphragm of rats, exposing the thoracic chamber. After the thorax was split open on both sides after cartilage attachment to the ribs, the heart was revealed and carefully cradled between the fingertips to avoid contusion harm. After excision, the heart was placed in a beaker containing a normal saline 0.9 % solution for washing purposes. The heart was washed and placed on filter paper to absorb moisture before being weighed to estimate the heart's relative weight to body weight. The heart was then placed in a jar containing 10% formalin and kept frozen at 80° for the duration of the experiment.[17]

Measurement of Body Weight and Heart Weight

To determine daily weight gain or loss, the digital weighing balance was used to weigh each rat's body weight and heart weight.[15]

Heart Weight to Body Weight Ratio

The heart weight to body weight ratio was calculated by dividing the rat's heart weight by its body weight. The units used were grams per gram.[18]

Histopathological Studies

The vital organ (Heart) was isolated from sacrificed rats and was fixed in 10% formalin. A specimen of a sample of the heart (cardiac tissue) from positive, negative, normal control groups and treatment groups (100, 200, 400 mg/kg, p.o. of extract) was taken out from animals for histopathological examinations and sent to the pathological lab.

Statistical Analysis

Graphpad prism version 7 was used for data analysis. The data was represented as Mean ± SEM and analyzed by one-way analysis of variance (ANOVA), followed by a Tuke’y post hoc test, with a P-value of 0.05 being regarded statistically significant.

Results

Percentage Yield of the Brassica Cretica Extract

1.2 kg of Brassica Cretica leaves powder was utilized in the plant's Hydroethanolic extraction. The plant's actual yield was 150 g. The plant's percentage yield was 12.5 % (w/w). The color of

the Hydroethanolic extractwas dark brown. After drying in the oven, the fine powder was obtained and homogenized with a mortar and pestle.

Cardioprotective Activity of the Hydroethanolic extract of Brassica Cretica Leaves

Effect of the Hydroethanolic extractsBrassica Cretica on the Rats' Body Weight. As compared to the normal control group, rats treated with cyclophosphamide had a significant (P<0.01) reduction in end body weight. Treatment with test article in two higher doses i.e., 200 mg/kg, p.o. and 400mg/kg, p.o. (P<0.5, p<0.01 respectively) showed the significantimprovement in body weight to normal. Pretreatment of the rats with the usual medication (EN 10mg/kg) resulted in no significant weight loss.

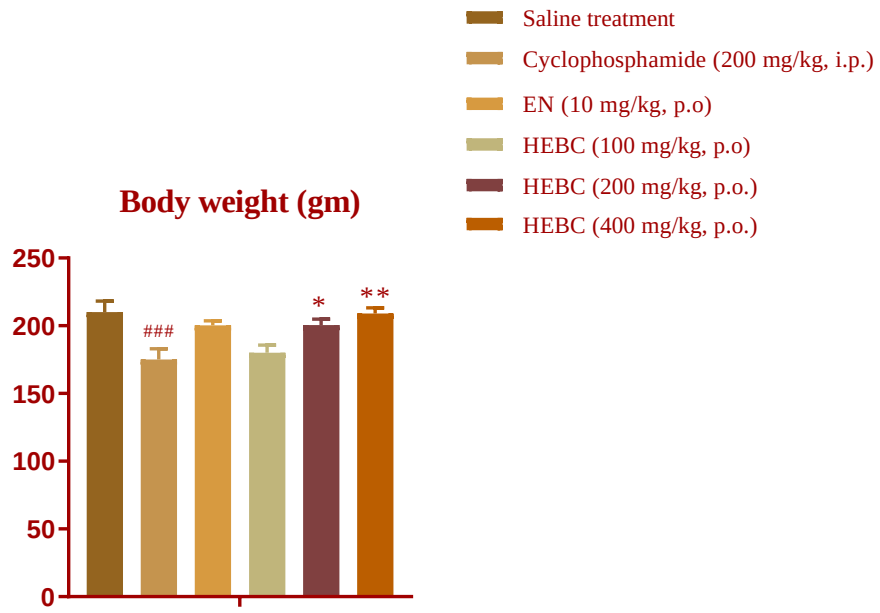

Fig 1: Effect of the Hydroethanolic extract of Brassica Cretica on Body weight, Mean ±SEM (n= 6); analysis was performed using one-way ANOVA followed by Tukey post hoc test; a Compared with normal control: ; ###P<0.001, Compared with normal control: *P<0.05, **P<0.01, ***P<0.001 as compared with cyclophosphamide (200 mg/kg, i.p.) i.e. negative control. (HEBC-Hydroethanolic extract of Brassica Cretica)

Effect of the Hydroethanolicextractof Brassica Cretica on Heart Weight to Body Weight Ratio

Administration of cyclophosphamide (200 mg/kg, i.p.) resulted in a significantincrease in the heart weight to body weight ratio (P<0.001) when compared to the normal control group. In comparison to the cyclophosphamide-treated group, all doses of Hydroethanolic extract of Brassica Cretica leaves and enalapril (EN) treatment demonstrated a significant (P<0.001) decrease in heart weight to bodyweight ratio after pretreatment.

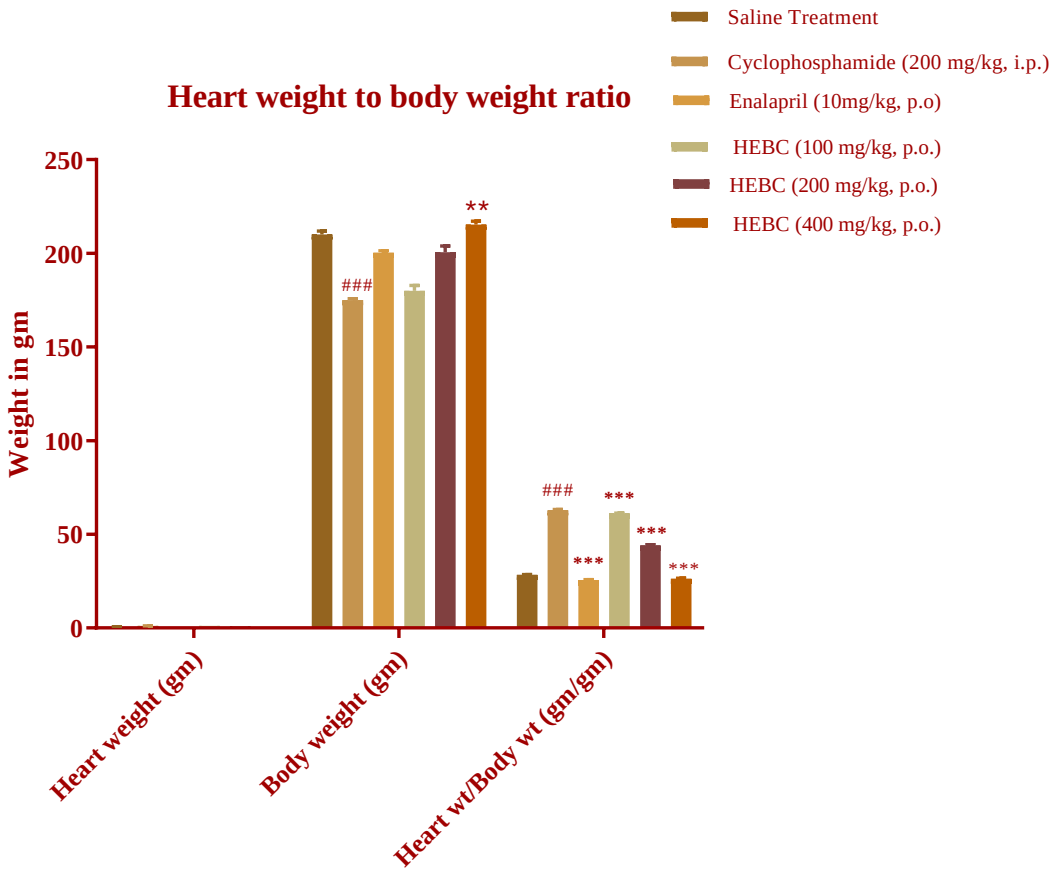

Fig 2: Effect of the Hydroethanolic extract of Brassica Cretica on Heart weight to body weight ratio, Mean ±SEM (n= 6); analysis was performed using one-way ANOVA followed by Tukey post hoc test;$^{###}$P<0.001, Compared with normal control: *P<0.05, **P<0.01, ***P<0.001 as compared with cyclophosphamide (200 mg/kg, i.p.) i.e. negative control. (HEBC-Hydroethanolic extract of Brassica Cretica)

Effect of the Hydroethanolicextractof Brassica Cretica on Cardiac Biomarkers

Troponin-I level was significantly increased among the rats administrated with cyclophosphamide compared to the normal control group (P<0.01) However, pretreatment of the rats with the200mg/kg dose of the Hydroethanolicextractof Brassica Cretica decreased troponin-I level significantly P<0.05 Besides, the EN-treated and 400mg/kg Hydroethanolic extract reduced the troponin I level significantly P<0.01.

An elevation in plasma ALT value was noticed in the cyclophosphamide-treated group compared to the normal control group ($^{##}$P<0.01). All the treatments significantly (**P<0.01) reduced ALT levels as compared to negative control group.

The plasma AST value was elevated in cyclophosphamide-administered group as compared to the normal control group ($^{###}$P<0.001). Treatment with EN as well as all three doses of HEBC (dose dependant manner) significantly (***P<0.001, **P<0.01, **P<0.01, ***P<0.001) reduced the plasma AST levels as compared to negative control group.

CK-MB level was significantly ($^{###}$P<0.001) increased after treatment with cyclophosphamide as compared to the normal control group. However, all the treatments reduced the CK-MB level significantly (**P<0.01) as compared to negative control group.

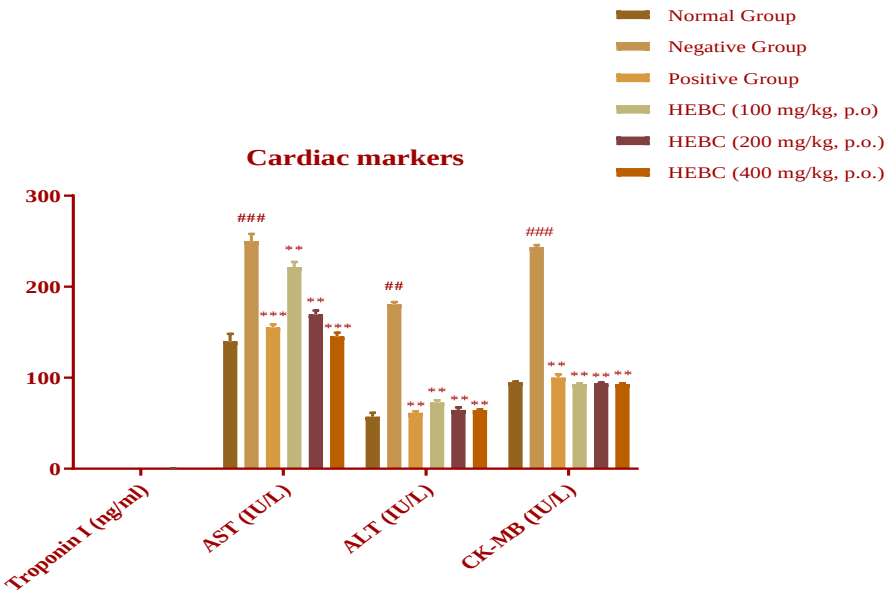

Fig 3: Effect of the Hydroethanolic extract of Brassica Cretica on Cardiac Biomarkers, Mean ±SEM (n= 6); analysis was performed using one-way ANOVA followed by Tukey post hoc test; a Compared with normal control: $^{###}$P<0.001, Compared with normal control: *P<0.05, **P<0.01, ***P<0.001 as compared with cyclophosphamide (200 mg/kg, i.p.) i.e. negative control. (HEBC-Hydroethanolic extract of Brassica Cretica)

Effect of the Hydroethanolic extract of Brassica Cretica on Lipid Profiles

Triglycerides were increased significantly ($^{###}$P<0.001) increasedafter treatment with cyclophosphamide as compared to normal control group. Treatment with EN and all three doses of test article significantly (***P<0.001, **P<0.01, ***P<0.001, ***P<0.001) reduced triglyceride levels as compared to negative control group.

An elevation in plasma total cholesterol value was observed in the cyclophosphamide-treated group as compared with the normal control group ($^{###}$P<0.001). However, treatment with EN and two higher doses ofHydroethanolic extract (200 mg/kg, p.o. and 400 mg/kg, p.o.) reduced plasma total cholesterol levels significantly (***P<0.001, *P<0.5, **P<0.01).

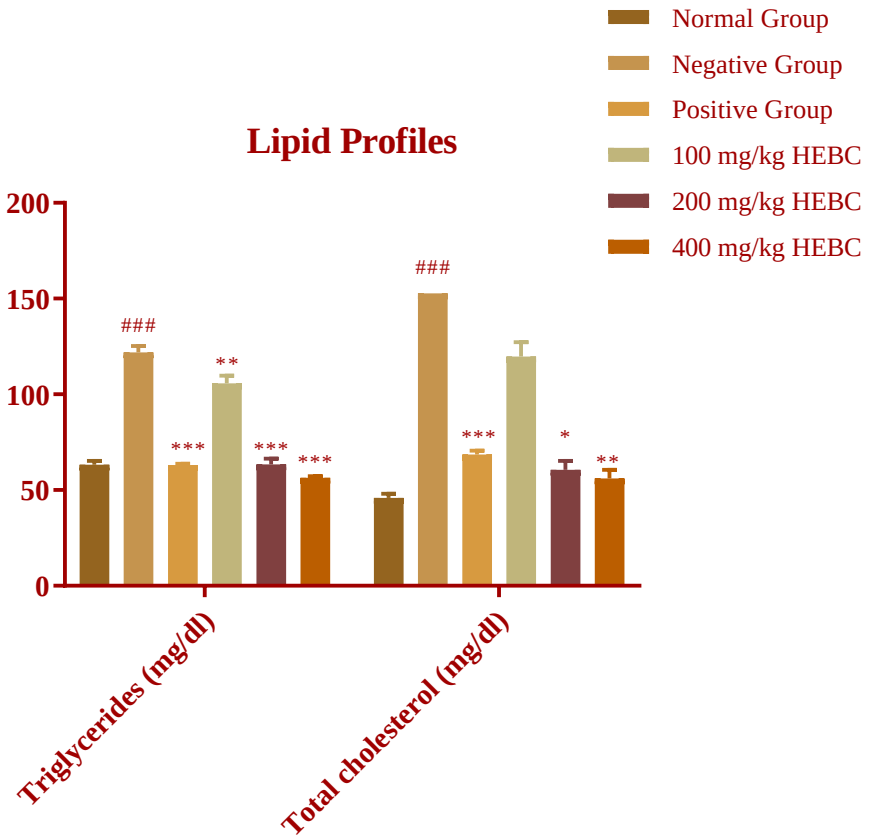

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Fig 4: Effect of the Hydroethanolic extract of Brassica Cretica on Lipid Profile, Mean ±SEM (n= 6); analysis was performed using one-way ANOVA followed by Tukey post hoc test; a Compared with normal control: *P<0.05, **P<0.01, ***P<0.001 as compared with cyclophosphamide (200 mg/kg, i.p.) i.e. negative control. (HEBC-Hydroethanolic extract of Brassica Cretica)

Histopathological Finding

The cardioprotective activity of the hydroethanolic extractof Brassica Cretica leaves were confirmed by the histopathologic examination of the cardiac tissues of control and treated animals. As showed in Figure, the cardiac tissues in the normal control group showed normal

morphological architecture with no cellular necrosis, interstitial space oedema and hemorrhage. However, the cardiac tissues of rats administered with only cyclophosphamide showed necrotic cardiocytes, hemorrhage and oedema. However, the cardiac tissues of the rats treated with EN, 200 and 400mg/kg of Hydroethanolic extract of the plant showed that there were more normal cardiocytes and regeneration of cardiac cells were observed.

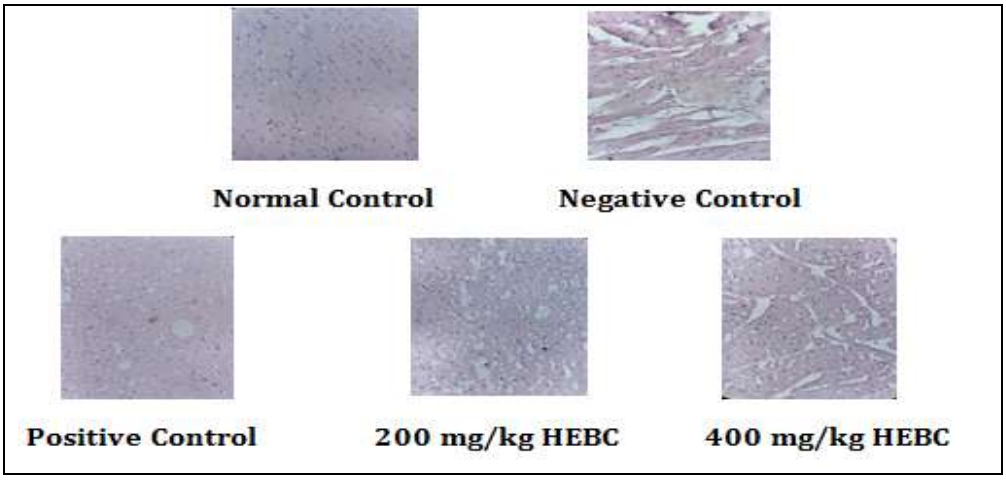

Fig 5: Histopathological changes of cardiac tissue on control and treated groups on experimental rats

Discussion

This research work aimed to investigate the prophylactic effects of Brassica Cretica leaf extracts in an experimental animal model of cyclophosphamide-induced myocardial infarction (MI). The levels of cardiac enzymes (Troponin-I, ALT, AST, and CK-MB), as well as lipid profiles such as triglycerides and total cholesterol, as well as lipid profile such as triglycerides and total cholesterol, were found to be raised in the cyclophosphamide-treated groups, while the Hydroethanolic extract-treated groups rectified the problem. Furthermore, cyclophosphamide therapy resulted in body weight loss and an increase in heart weight, which was reported as an increase in the heart weight to body weight ratio, an issue that was addressed by the plant extract at all dose levels.

Histopathological results confirmed this conclusion, showing that cyclophosphamide has a negative effect on heart tissue and that the condition was improved in the plant extract treated groups. When the cyclophosphamide-treated group was compared to the normal control group, there was a considerable decrease in body weight relative to the initial weight.

The weight loss seen in this study could be due to cyclophosphamide causing anorexia in the experimental rats by harming the hunger center in the hypothalamus or gastrointestinal system. However, when compared to the control groups, all the treatment exhibitedno statistically significant weight loss. This might be possible due to the significant presence of phenolic chemicals in plant, hence challenging cyclophosphamide's appetite-suppressing effects.[15, 19]

When compared to the normal control group, cyclophosphamide administration resulted in a considerable rise in the relative heart weight to body weight ratio. This could be owing to the direct or indirect involvement of cyclophosphamide-metabolites, particularly acrolein, which induces cytoplasmic vacuolization, myocyte disruption, edema, and fibrosis in the cardiac tissue via oxidative stress. [15, 19]Despite this, when compared to the negative control group, the Hydroethanolic extract significantly reduced the heart weight to body weight ratio in all treatment groups.[15,19-20]

One of the most fundamental indicators for diagnosing cardiac injury is plasma troponin I, AST, and ALT levels; however, troponin I is the most sensitive and specific to the heart. During a myocardial infarction, these biomarkers were increased. The explanation for this is that these enzymes were numerous in the heart and were released into the bloodstream as a result of cardiac muscle cell membrane breakdown and rupture. As a result, an increase in cardiac enzymes indicates cellular leakage and the loss of functional integrity of the heart's cell membrane.[11,21-22]AST and ALT are known to be released in the response to liver injury, in addition to cardiac injury. However, AST is more specific to heart injury than ALT is to liver disease. To determine which organ is the more relevant source, the ratio of AST and ALT must be calculated. The AST level is higher than the ALT level in cardiac muscle injury, resulting in an AST/ALT ratio of more than one.[23]

Troponin I, AST, and ALT levels were considerably higher in the cyclophosphamide-treated rats than in the normal control group in the current investigation. Most crucially, the AST/ALT ratio of the cyclophosphamide-treated group was greater than one, implying that the damage to the heart was worse than that to the liver. After treatment with the Hydroethanolic extract of Brassica Cretica, the elevated levels of cardiac enzymes in the plasma were shown to revert to a practically normal profile. By lowering the increased levels of these enzymes, pretreatment of the rats with the EN Hydroethanolic extract stopped the harmful effect of cyclophosphamide on the heart.

As a result, the reduction of increased plasma levels of troponin-I, AST, and ALT by the Hydroethanolic extract of Brassica Cretica to their respective normal values indicate plasma membrane stabilization as well as repair of cyclophosphamide-induced cardiac tissue damage. Polyphenols, flavonoids, and tannins, which are secondary metabolites of this plant, may aid to maintain membrane integrity, and minimizing enzyme leakage. The plant extract's cardioprotective efficacy is related to its ability to stabilize plasma membranes and repair cardiomyocyte damage.[15, 19,24-25]

When compared to controls, the development of MI after Cyclophosphamide injection dramatically raised cardiac marker enzyme activity (CK-MB). Because it is abundant in myocardial tissue and almost absent in most other tissues, CK-MB activity in the serum is a sensitive and significant diagnostic marker of MI.

The increased levels of total cholesterol and triglycerides in the cyclophosphamide-treated group suggest that cyclophosphamide is interfering with lipid production or metabolism.

Cyclophosphamide inhibited cardiac lipoprotein lipase (LPL) production, resulting in an increase in total cholesterol and triglyceride levels in lipid indicators. Lipoprotein lipase is a triglyceride-degrading enzyme that converts triglycerides to fatty acids.[10,22,25-26] Plasma lipid profile levels were reduced in a dose-dependent manner after treatment with Brassica Cretica Hydroethanolic extract. The secondary metabolite of polyphenols that can bind with bile acids to promote their excretion, block hepatic cholesterol production, and induce LPL enzymes may be responsible for this plant's lipid-lowering impact.[19, 26-27]

The histopathologic findings corroborated the activity of the Hydroethanolic extract of Brassica Cretica on biochemical results. The normal control rats' hearts showed normal heart architecture, whereas the cyclophosphamide-treated rats' hearts displayed cardiac necrosis, interstitial edema, and myocardial bleeding. This could be due to cyclophosphamide-metabolites such as acrolein causing the generation of free radicals and oxidative stress. The 100mg/kg Hydroethanolicextract also showed bleeding, edema, and necrosis. These significant pathological alterations, on the other hand, were less severe in rats administered with higher doses of hydroethanolic extract, as well as the conventional medication followed by cyclophosphamide. This suggests that pretreatment of the rats with plant extracts and EN may help to avoid cyclophosphamide-induced cardiotoxicity. As a result, the histopathologic data demonstrated that the Hydroethanolic extract had protective action against cyclophosphamide-induced MI, which is consistent with the findings of previous investigations. [19, 22, 28-30]

Preliminary phytochemical screening of Brassica Cretica Hydroethanolic extract revealed the presence of flavonoids, polyphenols, and tannins in a previous study. As a result, the cardioprotective effect of the Hydroethanolic extract could be attributed to the presence of phytochemicals such as polyphenols, alkaloids, flavonoids, polyphenols, and tannins, all of which have cardioprotective properties either alone or in combination. [31-34]

Conclusion

Brassica Cretica Hydroethanolic extract has strong cardioprotective efficacy in most parameters in a dose-dependent manner, according to the findings of this study. In terms of body weight, heart weight, lipid profiles, and cardiac biomarkers, the Hydroethanolic extract has the most substantial protective impact against the negative effects of cyclophosphamide. The antioxidant polyphenolic components (tannins and flavonoids) of the leaves may have a role in the cardioprotective effect.

Conflict Of Interest

The author declares that there is no conflict of interest.

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