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Research Article

Cnidoscolus Phyllacanthus Hepatoprotective and Antioxidant Properties Against D-Galactosamine-Induced Oxidative Stress in Rats

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Conflict of interest: Nil

Abstract

In this study, the researcher identified *Cnidoscolus phyllacanthus* plant with antioxidant and hepatoprotective potentials. Undoubtedly, the plant world still has many species of plants having therapeutic compounds that have yet to be found. Many plants are continually being examined for their probable pharmacological utility, notably for their hepatoprotective characteristics. Hepatoprotective herbs and preparations are many. Nearly 160 phytoconstituents from 170 plants in 55 families exhibit liver-protective action. 33 patented multi-ingredient plant compositions in India employ more than 87 plants. EECP's antioxidant-boosting and anti-lipid peroxidative properties imply it might help prevent free-radical-mediated tissue damage from hepatotoxicity. Various flavonoids' antioxidant activity has shown that hydroxyl group distribution and amount are important. Ring B hydroxylation gives polyphenols their antioxidant properties. EECP protects rats against D-galactosamine intoxication, especially at 400 mg/kg body weight. This flavonoid improved hepatoprotective and antioxidant activities in rats with D-galactosamine-induced hepatitis.

Keywords: *hepatoprotective, antioxidant, Cnidoscolus phyllacanthu ,d-galactosamine, oxidative stress*

1. Introduction

The curiosity in pharmaceuticals that are derived from higher plants, which are frequently referred to as herbal remedies or herbal medicines (phytomedicines), has essentially developed in all areas of the world [1-3]. The study of phytotherapy for the treatment of chronic diseases may yield an outstanding return in the potential resources of medicinal plants. These plants play major roles in the discovery of new biologically active substances and biotechnological applications in disorder preventive action, and their development and utilisation fit into all existing prevention strategies .The plant known as *Cnidoscolus phyllacanthus* is a member of the Euphorbiaceae family and may be found in its natural habitat in Mexico as well as other areas of Central America. It is well-known for its historical applications in medicine, and researchers have investigated the possibility that it possesses

beneficial features for one's health, such as hepatoprotective and antioxidant qualities[2,3].In this present study, we evaluate the effects of *Cnidoscolus phyllacanthus* on hepatoprotection and antioxidant activity in rats with d-galactosamine-induced oxidative stress. Activity in hepatoprotection, also known as hepatoprotection, is the capacity of a chemical to shield the liver against the effects of a variety of noxious substances and situations. In this investigation, the researchers most likely wanted to determine whether or not *Cnidoscolus phyllacanthus* could protect the liver of the rat from the harm that was caused by d-galactosamine[2-4].

1.A. **Antioxidant** Activity Antioxidants are substances that assist neutralise damaging free radicals in the body, which can cause oxidative stress and damage cells. Antioxidants can be found in fruits, vegetables, and grains. In the study, it is possible that researchers investigated whether or not *Cnidoscolus phyllacanthus* possesses antioxidant activity, with the goal of determining whether or not it may reduce the amount of oxidative stress experienced by the liver[5-8].

1.B. d-Galactosamine: d-Galactosamine is a chemical molecule that is known to promote liver harm in experimental animals. d-Galactosamine is also known as d-galactosamine. It is frequently utilised in the field of research to replicate particular elements of liver disorders and to produce oxidative stress in the liver[5].

1.C. Oxidative Stress: Oxidative stress happens when there is an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to remove them, which results in damage to the body's cells. This can be prevented by maintaining a healthy equilibrium. There is a correlation between it and a number of ailments, including liver disorders.During the course of the investigation, the researchers most likely gave rats either an extract of *Cnidoscolus phyllacanthus* or its active components. The rats were then subjected to d-galactosamine. They would next examine several indicators related to liver health, such as liver enzyme levels, histological alterations, and antioxidant enzyme activity, in order to determine the protective benefits of the plant extract against oxidative stress[3,5].

2. Methodology:

Extraction of solvents (hot percolation method)

Preparation of *Cnidosolus phyllacanthus* extracts in petroleum ether, chloroform, and ethanol.

2.A. Used Equipment

- The apparatus of Soxhlet
- utilised materials
- Chloroform Ethanol Petroleum ether
- Dried in the shade of *Cnidoscolus phyllacanthus*

2.B.Materials and Method

Animals: Albino wistar rats (180-220gm)

CHEMICALS:

D- galactosamine

: Vitamin E

:Ethanolic extract of *Cnidoscolus phyllanthus*

2.C. Selection and Acclimatization of Animals

Albino rats of wistar strains weighing between 180-220gm were produced from animal experimental laboratory, and used throughout the study. They were housed in micronylon boxes in a control environment (temp 25+-2^0c) and 12 hrs dark\ light cycle with standard laboratory diet and water ad libitum[5].

The study was conducted after obtaining Institutional Animal Ethical Committee clearance. As per the standard practice, the rats were segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on a healthy diet and maintained in a hygienic environment in our animal house.

2.D. Treatment protocol

The acclimatized animals were divided into 5 groups of each 6 animals, designated as

• Group 1: Served as normal control and received normal diet and water.

• Group 2: Toxic control received 25mg/kg of D-galactosamine through I.P for 21 days.
• Group 3: Standard control received 25mg/kg of vitamin E orally for 21 days.
• Group 4: The treatment control received 200mg/kg of Ethanol.
extract of *Cnidoscolus phyllanthus* orally for 21 days.
• Group 5: The treatment control received 400mg/kg of Ethanol.
extract of *Cnidoscolus phyllanthus* orally for 21 days.

2.E. Preparation of Drugs

- Ethanolic extract of *Cnidoscolus phyllanthus* was dissolved in 20ml of sterile water and was administered orally at a dose of 200mg/kg and 400mg/kg/rat.
- D-Galactosamine was diluted in sterile water and administered I.P at a dose of 25mg/kg/rat.
- Vitamin E was diluted in sterile water and administered orally at a dose of 25mg/kg

After 24 hours of Galactosamine administration, all animals were mercifully euthanized with Ketamine HCl and 4 ml of blood was extracted by heart puncture and allowed to clot for 30 minutes at room temperature. AST, ALT, ALP, TP, TB, GGPT, and total albumin were assayed from serum separated by cooling centrifuge. The livers were promptly dissected, rinsed with ice-cold saline, and homogenised in 10% phosphate buffer solution (PH 7.4). The liver homogenate was used to measure lipid peroxidation (Lpo), and some fractions were centrifuged at 7000 rpm for 10 min at 40 C in a refrigerated centrifuge to measure SOD, CAT, and GPx. Histopathological examinations used aseptically removed liver samples from each group preserved in 10% formalin [5,8].

2.F. Statistical Analysis : One-way ANOVA and Newmann Keul's multiple range tests performed the statistical analysis. Mean+SEM values. P <0.01 was statistically significant.

2.F. Experimental Work

Table 1: Effect of *Cnidoscolus phyllanthus* and Vitamin E pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine.

Group. No	TREAT-MENT DOSE (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	Total Albmin(g/dl)
I	Normal control 10ml/kg normal saline	44.40±1.52	30.09±1.49	23.68±1.30	5.15±0.08	1.92±0.08	96.90±2.75	3.80±0.16
II	Toxic control 25mg/kg D-galactosa-mine	*a 105.90± 2.40	*a 94.49±1.05	*a 144.10±2.35	*a 3.16±0.22	*a 4.40±0.26	*a 173.42±2.90	*a 2.20±0.07
III	Standard control Vitamin E 25mg/kg	*b 60.10±1.20	*b 40.56±1.06	*b 56.4±1.70	*b 3.90±0.08	*b 2.8±0.15	*b 122.20±1.95	*b 2.90±0.05
IV	Treatment control EECP 200mg/kg	*b 68.65±1.46	*b 54.82±2.72	*b 65.86±2.30	*b 4.60±0.25	*b 3.30±0.20	*b 136.30±3.04	*b 2.54±0.04
V	Treatment control EECP 400mg/kg	*b 62.45±1.15	*b 47.94±0.97	b 58.50±1.95	*b 4.05±0.26	*b 2.95±0.18	*b 130.94±1.23	*b 2.30±0.09

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control(G2) at p< 0.01.

Table 2: Effect of *Cnidoscolus phyllanthus* and Vitamin E pre-treatment on biochemicalliver parameter in D-Galactosamine induced hepatotoxicity.

Group. No.	TREATMENT DOSE(mg/Kg)	SOD(U/mg) Protein	CATA-LASE(U/mg) Protein	GPX(U/mg) Protein	MOA(U/mg) Protein
I	Normal control 10ml/kg Normal saline	132.25±2.40	290.40±2.40	1.10±0.05	3.90±0.17
II	Toxic control 25mg/kg D-galactosa-mine	*a 68.20±1.65	*a 190.75±2.70	*a 0.40± 0.02	*a 7.40±0.12
III	Standard con-trol Vitamin E 25mg/kg	*b 118.05±2.80	*b 260.45±1.92	*b 0.85± 0.02	*b 4.50±0.14
IV	Treatment control 200mg/kg EEPL	*b 96.50±1.60	*b 230.05±1.80	*b 0.55± 0.02	*b 5.60±0.28
V	Treatment control 400mg/kg EEPL	*b 105.65±2.62	*b 240.75±2.65	*b 0.74± 0.02	*b 4.80±0.08

- Values are expressed as Mean ± SEM.
- Values are finding out by using one way ANOVA followed by Newmannkeul's
- multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control(G2) at p< 0.01.

Table 3: Effect of eecp on the levels of non-enzymatic antioxidants in the liver tissue of d-galactosamine –hepatotoxic and control rats

GROUPS	GLUTATHIONE MG/100G TISSUE	VITAMIN-C MG/100G TISSUE	VITAMIN-E MG/100G TISSUE
Normal control 10ml/kg normal saline	132.60±3.45	0.82±0.08	5.92±0.60
Toxic control 25mg/kg D-galactosamine	73.55±1.70*a	0.30±0.02*a	2.40±0.30*a
Standard control Vitamin E 25mg/kg	110.32±2.70*b	0.74±0.07*b	5.60±0.55*b
Treatment control EECP	98.05±2.16*b	0.60±0.04*b	4.92±0.50*b

200mg/kg			
Treatment control EECP 400mg/kg	91.90±1.95*b	0.69±0.06*b	5.02±0.48*b

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.

*b – values are significantly different from Toxic control (G2) at p< 0.01.

3. HISTOPATHOLOGICAL STUDIES OF LIVER TISSUE

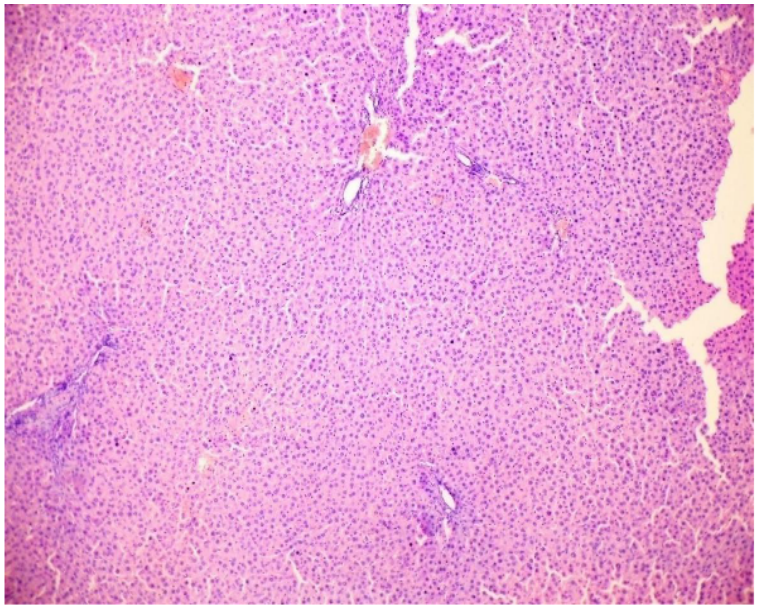

Figure 1: Liver section of GP1 (Normal control)

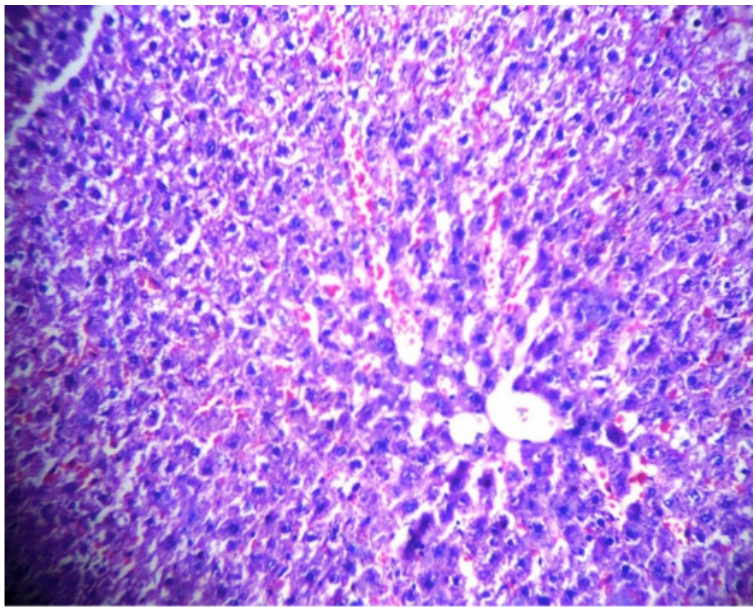

Figure 2: Liver section of GP2 (toxic control)

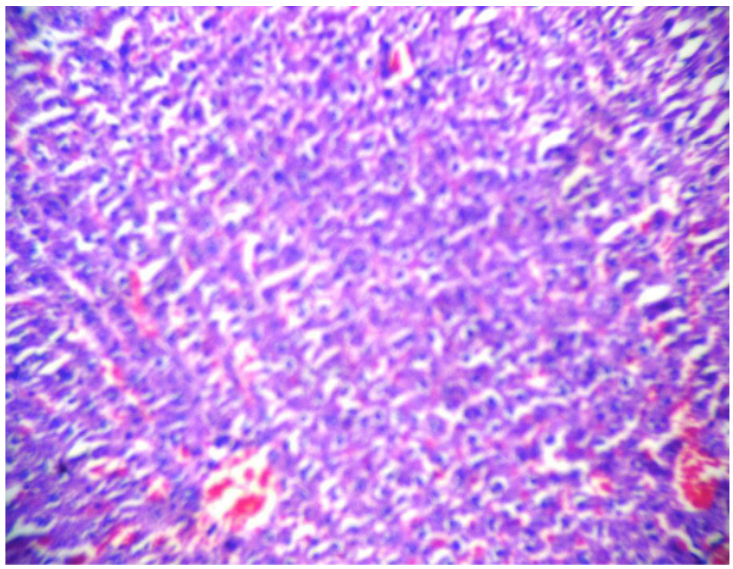

Figure 3: Liver section of GP3 (standard control)

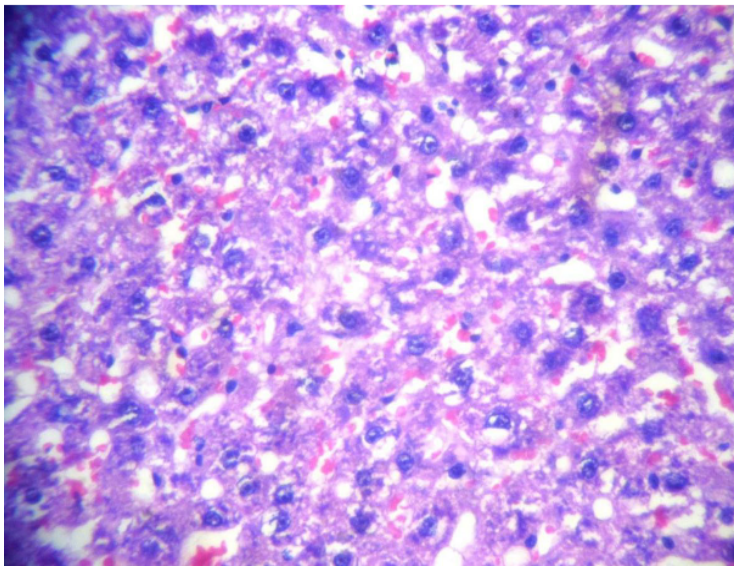

Figure 4 :Liver section of GP4 (*Cnidoscolus phyllanthus*200 mg/kg/rat

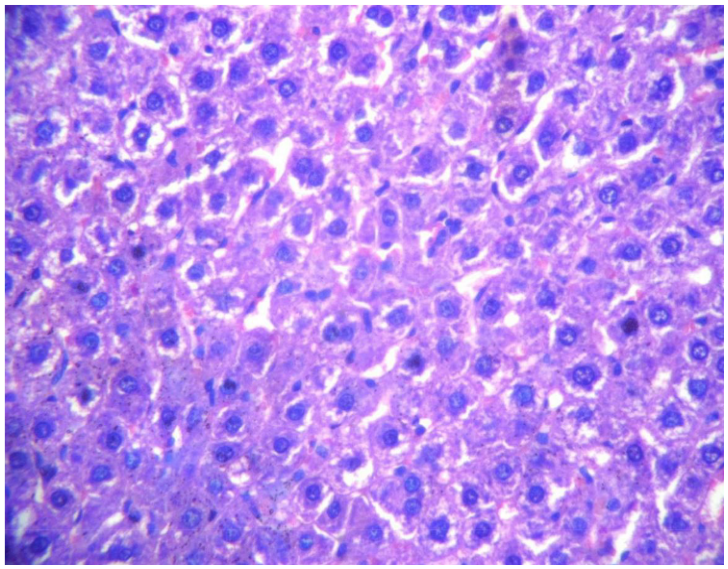

Figure 5: Liver section of GP5 (*Cnidoscolus phyllanthus*400 mg/kg/rat)

4. Result:

4.1.Biochemical Observations

A significant increase in (P< 0.01) Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline phosphatase (ALP), Total bilirubin (TB), and Gamma-glutamyl transpeptidase (GGTP) and a significant decrease in (P< 0.01) Total protein (TP) and Total albumin (TA) levels were observed in animals treated with galactosamine 25mg/kg (Group II) as compared to the normal control group (Group I). Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) at a dose of 200 and 400 mg/kg orally for 21 days decreased the levels of the above indices like AST, ALT, ALP, TB, and GGTP and increased levels of TP and TA significantly (P 0.01) in groups IV and V. Vitamin-E pretreatment produced a significant decrease in (P< 0.01) serum AST, ALT, ALP, TB, and GGTP and a significant increase in TP and TA at (P< 0.01) in group III.

4.2. Biochemical Observation In Liver Homogenate Tissue

In liver homogenate, a significant decrease in SOD, CAT, and GPx levels and an increase in LPO levels were observed in animals treated with galactosamine 25mg/kg (group II) as compared to the normal control group (Group I). Pretreatment with Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) at a dose of 200mg/kg and 400mg/kg orally for 21 days increased the levels of the above indices like SOD, CAT, and GPx levels and decreased levels of LPO significantly (P 0.01) in groups IV and V. In group III, vitamin-E pretreatment caused the levels of liver homogenate enzymes like SOD, CAT, and GPx to go up significantly (P 0.01) and the levels of LPO to go down significantly (P 0.01). Table No. 3 shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C, and Vitamin E in the tissues (liver) of D-galactosamine-hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine are hepatotoxic and significantly decreased in rats. Both doses administered to rats showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats(fig4).

4.3 Histopathological Observations

Normal control animals (Group I) had normal liver architecture, with a central vein that stood out, cytoplasm that was still there, and a nucleus and nucleolus that were easy to see (Fig. 8). The liver sections of galactosamine-treated animals (Group II) showed hepatic cells with serum toxicity characterised by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis, and swelling of vascular endothelial cells (Fig. 9). Vitamin E (Group III) exhibited protection from galactosamine-induced changes in the liver).Ethanolic extract of *Cnidoscolus phyllanthus* (EECP) pretreatment at a dose of 200mg and 400mg/kg (groups IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with preserved cytoplasms (figure 9). EECP pretreatment also caused a marked decrease in inflammatory cells(fig 1-4).

5. Discussion

D-galactosamine is a well-known hepatotoxicant that causes an injury to the liver that is more diffuse and resembles human viral hepatitis than other types of liver damage. Changes in the way liver cells digest their food lead to changes in the way serum enzymes function, which in turn causes the damage that D-galactosamine causes to the liver[1,2]. An increase in serum enzyme levels is suggestive of cellular leakage and a breakdown in the hepatocyte's structural and functional integrity. Damage to the plasma membrane of liver cells causes the release of several enzymes into the bloodstream. These enzymes include aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilurubin, and gamma-glutamyl transpeptidase. Their concentration in the serum can be used as a valuable quantitative measure of the degree of hepatocellular damage as well as the kind of damage. When D-galactosamine made the serum toxic, the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total biluribin, and gamma-glutamyl transpeptidase went up. On the other hand, total protein and total albumin activities were observed to decrease throughout this process. The fact that EECP caused a considerable decrease in the activity of these enzymes

provides evidence that it is able to maintain the structural integrity of the hepatocyte membrane. The effect of the high dose of EECP (400 mg/kg) was superior to that of the low dose of EECP (200 mg/kg). The increased concentration may have led to the formation of additional byproducts, which would have hampered the activity if they had been present in sufficient quantities. After treatment with EECP, the activity of these enzymes dropped a lot. This shows that EECP protects the liver from damage caused by D-galactosamine[5].

Typically, it is believed that the production of the highly reactive hydroxyl radical (OH), which accelerates lipid peroxidation and harms the cell membrane, is what causes the oxidative damage that occurs when D-galactosamine is present. In the kidney, the harmful effects of D-galactosamine were observed to increase lipid peroxidation and decrease antioxidant levels. When rats were given D-galactosamine, researchers found that their livers and kidneys had a lot more thiobarbituric acid reactive compounds, lipid hydroperoxides, and conjugated dienes[8,9]. In the current investigation, we found that the tissues of D-galactosamine-hepatotoxic rats had increased amounts of thiobarbituric acid reactive compounds, lipid hydroperoxides, and conjugated dienes. It has been recognised for a long time that increased lipid peroxidation in different tissues can induce functional deterioration. As a result, the functional degradation of essential tissue that might lead to difficulties may be indirectly caused by increased oxidative stress.

After treatment with EECP and Vitamin E, there was a significant decrease in lipid peroxidation, which may be because both of these chemicals have antioxidant properties, and as a result, there was less lipid peroxidation. EECP is capable of scavenging free radicals and also has antioxidative properties. An imbalance between a cell's or tissue's reactive oxygen species and its antioxidant defence mechanisms can lead to oxidative stress. This imbalance can result in lipid peroxidation, damage to DNA, and the inactivation of a large number of enzymes. Superoxide dismutase, catalase, and glutathione peroxidase are the three enzymes that make up the enzymatic antioxidant defence system[5,11,12]. This system is the natural guardian against lipid peroxidation and contains it. In the tissue of D-galactosamine-hepatotoxic mice, our research revealed that the activity of these enzymes was significantly reduced. The superoxide radical (O2) is what causes damage to the membrane as well as the biological structure of the membrane. Superoxide dismutase defends against this[8,13].

Catalase's primary role is to convert hydrogen peroxide into water at a significantly higher rate than glutathione peroxidase, which is also one of catalase's functions. It is possible that glutathione peroxidase has a significant role to play in the elimination of lipid hydroperoxides. To ensure the effective elimination of oxygen radicals from tissues, maintaining a healthy equilibrium between these enzymes is essential (87). As a result, a decrease in the activity of these enzymes may cause an increase in the production of harmful superoxide radicals and hydrogen peroxide, which can lead to a variety of adverse effects. The injection of EECP resulted in the observation of significant elevations in the activity of these enzymes. The non-enzymic scavengers glutathione, ascorbic acid, and gamma-tocopherol make up the second line of defence. These scavengers eliminate any remaining free radicals that have escaped the breakdown process caused by the antioxidant enzymes. Also, when there are a lot of free radicals, enzyme-based antioxidants stop working, so non-enzymatic antioxidants are probably needed to get rid of these radicals[1,5]. Glutathione, the most abundant non-protein thiol found in living beings, is critically important to the process of coordinating the antioxidant defence system. Glutathione keeps the important thiol group from being oxidised, reacts directly with reactive oxygen species and electrophilic metabolites, and is a substrate for a number of enzymes, including glutathione peroxidase. Because of oxidative stress, rats that have been given D-galactosamine have lower levels of glutathione, which shows that they are using more glutathione. A change in the redox status of glutathione not only weakens the cell's ability to defend itself against hazardous chemicals, but it also leads to an increase in oxidative stress and oxidative damage. In addition to glutathione, gamma-tocopherol and ascorbic

acid are also significant free-radical scavengers that protect the cell membrane from the effects of hazardous substances[10-16]. When it comes to neutralising oxygen-based free radicals, vitamin C and vitamin E work together to produce a synergistic effect. Vitamin C is able to successfully avoid observable oxidative damage in response to all different kinds of oxidative stress by acting as a free-radical scavenger of oxygen radicals and scavenging oxygen radicals. It would appear that ascorbic acid is able to capture the peroxyl radical in the aqueous phase at a rate that is sufficient for lipids, and as a by-product of this reaction, dehydroascorbate is generated. Ascorbate is produced from dehydroascorbate by the thiol cycle. GSSG and GSH work together as a duo in the thiol cycle. As a result, glutathione in the blood maintains the appropriate quantities of the active form of vitamin C within the cells. When there is a decrease in glutathione, the amount of ascorbic acid that is present in the cell also decreases. The drop in btocopherol and ascorbic acid levels in D-galactosamine-hepatotoxic rats may have been caused by an antioxidant defence against increased ROS or a drop in glutathione levels in these rats. Both of these possibilities are possible. In this regard, it was observed that both ascorbic acid and beta-tocopherol levels dropped in liver disorders, particularly in D-galactosamine-hepatotoxic rats. In the rats that were given EECP and Vitamin E, our research found that there was an increase in the levels of these antioxidants[5,17-22].S.M. Lira et al. (2017) conducted research that revealed Phytochemical analysis showed that extract rich in flavonoids, catechins and triterpenoid, which did not show any mortality and did not modify the behavioural patterns of treated mice. Animals given 200 milligrammes per kilogramme of body weight experienced a 29% decrease in glucose levels[1].

Tannins, anthocyanins, and alkaloids are some of the metabolites identified in a study conducted by N.R.L. Morais and colleagues (2016). The antioxidant activity of the leaves was highlighted, with a CI50 of 58.3 ppm being presented by the leaves. This value is comparable to that of vitamin C (43.0 ppm), which was employed as a positive benchmark[2]

A. Aranda-Rickert, L et al., (2011) came to the conclusion that the extract showed to identify a number of different fatty acids. These fatty acids included myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, arachidic acid, and high oleic acid[3].M.A.I. Aloufa etal (2016) obtained According to the results, the essential oil of faveleira does not cause structural mutations at the chromosomal level, does not interfere with the structure and function of the cellular cycle, and does not induce genotoxicity in the metaphase cells of the bone marrow of mice. These findings indicate that the essential oil of faveleira does not cause genotoxicity[4].Since EECP can increase the amount of antioxidants in the body and also has antilipid peroxidative activity, it seems likely that this molecule could help reduce the effects of free radicals, which are what cause hepatotoxicity and tissue damage. Research on the antioxidative effects of different flavonoids has shown that the location and number of hydroxyl groups in the molecule are important[5,17-19]. In a broad sense, the hydroxylation of ring B determines the antioxidative properties of polyphenols. The findings presented here provide further evidence that EECP has a protective effect against D-galactosamine toxicity in rats. This effect is particularly pronounced at the high dose that was used in this study (400 mg/kg body weight). When this flavonoid was given as a supplement to rats with hepatitis caused by D-galactosamine, it improved both its hepatoprotective and antioxidant properties.

6. Conclusion

In summary, the results of the studies we conducted showed that EECP, at both doses, had hepatoprotective and antioxidant activity. A decrease in the blood levels of hepatic marker enzyme activities supported this. From the two dosages that were examined, the higher one—400 mg/kg/body weight—exhibited more promising hepatoprotective and antioxidant effects. This dosage is also comparable to the conventional medicine vitamin-ethno-botanic. There are a number of Indian ethnobotanical traditions that suggest a rich repertory of medicinal plants that are utilized by the population for the treatment of liver disease (such as jaundice, liver gallstones, hepatitis, and so on). On the other

hand, there were not sufficient scientific examinations into the hepatoprotective effects that these plants possessed. Recent advancements in the investigation of such plants have led to the isolation of around 170 distinct phytoconstituents from plants belonging to approximately 55 families that display hepatoprotective action.

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