Evaluation of Methanolic Extract of Foeniculum Vulgare Stems for Hepatoprotective Activity in Rats

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ABSTRACT :
The hepatoprotective effects of the methanolic extract of Foeniculum vulgare stems were evaluated in a paracetamol-induced hepatotoxicity model by assessing body weight, liver weight, serum liver enzymes (SGOT, SGPT, ALP), bilirubin levels, total protein, and LDH activity. The hepatotoxic control group showed significant body weight loss, increased liver weight, elevated liver enzymes, bilirubin, and LDH levels, indicating marked hepatic damage. Treatment with Foeniculum vulgare extract notably attenuated these changes in a dose-dependent manner. The high-dose group demonstrated a slight increase in body weight and significant reductions in liver weight and bilirubin, reflecting improved liver function. Liver enzymes SGOT and LDH were significantly decreased in the treatment groups, especially at higher doses, while total protein levels showed restoration, supporting enhanced liver synthetic function. Interestingly, the low-dose group exhibited more pronounced normalization of liver weight, suggesting an optimal therapeutic window. These findings confirm that the methanolic extract of Foeniculum vulgare exerts significant hepatoprotective effects, likely through stabilization of cell membranes, reduction of oxidative stress, and improvement of metabolic function, highlighting its potential as a natural remedy for liver injury.
Keywords: Foeniculum vulgare, Hepatoprotection, Methanolic extract, Body weight, Liver weight, Bilirubin, Total protein

I.INTRODUCTION

Medicinal plants play a key role in human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials [1] . The traditional medicine refers to a broad range of ancient natural health care practices, including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used.

Therefore, these plant drugs deserve detailed studies in the light of modern science. It is estimated that about 7,500 plants are used in local health traditions in, mostly rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants [2]. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective [3]. Liver diseases and medicinal plants: The Liver has a pivotal role in the regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailments.

They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases), and cirrhosis (a degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections, and autoimmune/disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage in the liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis. It has been estimated that about 90% of acute hepatitis is due to viruses. The major viral agents involved are Hepatitis B, A, C, D (delta agents), E, and G. Of these, Hepatitis B infection often results in chronic liver disease and cirrhosis of the liver. Primary liver cancer

has also been shown to be produced by these viruses. It has been estimated that approximately 14- 16 million people are infected with this virus in South East Asia region and about 6% of the total population in the region are carriers of this virus. A vaccine has become available for immunization against Hepatitis B virus. Hepatitis C and Hepatitis E infections are also common in countries of South East Asia region [3-7].

II. EXPERIMENTAL WORK

Collection and Identification of Plant Material

The Foeniculum vulgare leaves are collected and identified. Collection of Plant Materials: Dried was purchased from an herbal Market of Hyderabad, Telangana, India, and authenticated by Dr K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. At the Department of Pharmacology, at our institution.

Preparation of the Extract

The collected Seeds are washed, air dried, homogenized to fine powder and stored in airtight bottles. The dried powder will first be defatted with petroleum ether and then extracted with Ethanol by using the Soxhlet apparatus.

Extraction of plant material

The preserved and pulverized plant material was utilized in the extraction process. A metered amount of each pulverized plant material was subjected to cold maceration with methanol for 72 hours, with one intermediate heating at 40°C per day. The residues were subsequently extracted. Following filtration through Whatmann filter paper, the filtrate was concentrated at a controlled temperature and reduced pressure (40-50°C). After being desiccated, the marc was weighed.

Experimental Design

Group I Normal Control (Vehicle only) 1 mL/kg of distilled water (oral)
Group II Hepatotoxic Control (CCl_4 or Paracetamol)
Group III Silymarin + CCl_4 Silymarin 100 mg/kg (oral) Standard hepatoprotective drug
Group IV Methanolic Extract of F. vulgare Stem + CCl_4 Extract 200 mg/kg (oral)
Group V Methanolic Extract of F. vulgare Stem + CCl_4 Extract 400 mg/kg (oral)

Determination of Biochemical Parameters [8]

Determination of aspartate aminotransferase (AST)/ SGOT

Procedure

The Rietman and Frankle method was utilized to calculate SGOT82. In this investigation, blank, standard, test (for each serum sample), and control reaction systems were utilized. Aspartate buffered 0.25 mL was introduced into each test tube. Subsequently, 0.05 ml of serum was added to each of the test group tubes, while the standard tubes received 0.05 ml of working pyruvate standard. Following the appropriate mixing process, each tube was incubated at 37oC for a duration of 60 minutes. Subsequently, 0.25 ml of 2, 4-DNPH reagent was added to each tube. Subsequently, 0.05 ml of distilled water was added to the blank tube, while 0.05 ml of each serum sample was added to the serum control tube. Twenty minutes could pass with the mixture at ambient temperature. 2.5 ml of solution I was added to each test tube following incubation. Within 15 minutes of appropriate mixing, the optical density was determined using a spectrophotometer at 505 nm. AST (SGOT) activity in IU/L was determined by dividing the absorbance of the control by the absorbance of the standard minus the absorbance of the blank. Multiplied by the concentration of the standard.

Determination of alanine aminotransferase (ALT) or Serum Glutathione peroxidase (SGPT) [9]

Procedure

The Rietman and Frankle method (1957) was utilized to calculate SGPT. In this investigation, blank, standard, test (for each serum sample), and control reaction systems were utilized. Each test vial received 0.25 ml of alanine-buffered solution. Subsequently, 0.05 ml of serum was added to the test group tubes, while 0.05 ml of working pyruvate standard was added to the standard tubes. Following appropriate mingling, each tube was incubated at 37°C for 60 minutes. Subsequently, 0.25 ml of 2,4-DNPH reagent was added to each tube. Subsequently, 0.05 ml of distilled water was added to the blank tube, while 0.05 ml of each serum sample was added to the serum control tube. Twenty minutes were permitted for the mélange to rest at ambient temperature.

2.5 ml of solution I was added to each test tube following incubation. After 15 minutes of thorough mixing, the optical density was measured at 505 nm against purified water using a spectrophotometer.
The enzyme activity was determined using the following formula: ALT (GPT) activity in IU/L = [(absorptance of standard minus absorbance of blank] divided by absorbance of test minus absorbance of control multiplied by the concentration of the standard.

Determination of alkaline phosphatase (ALP)/serum alkaline phosphatase (SALP)
Procedure: [10]

The ALP value was ascertained utilizing the Kind and King83 method. In order to produce the working solution, 2.2 ml of water was used to reconstitute one vial of buffered substrate. For the blank, standard, control, and test, 0.5 ml of working buffered substrate and 1.5 ml of purified water were dispensed, respectively. After thorough mixing, incubate at 37°C for three minutes. In separate test and standard test containers, 0.05 ml of serum and phenol standard were added, respectively. After thorough mixing, incubate for 15 minutes at 37°C. Following that, one milliliter of chromogen reagent was added to each test vial. Then, 0.05 ml of serum was added as a control. At 510 nm, the O.D. of the blank, standard, control, and test was measured against purified water after each reagent had been thoroughly combined. The formula for calculating serum alkaline phosphatase activity in KA units is [(O.D. Test-O.D. Control) / (O.D. Standard-O.D. Blank)]. In units of U/l, 10x alkaline phosphate was denoted. Phosphate alkaline was denoted as U/L.

Determination of bilirubin;
Procedure
Estimation of total bilirubin:

0.02 ml of activator and 0.1 ml of serum were added to 1.0 ml of total bilirubin reagent. The mixture was well mixed and then incubated at room temperature for precisely 5 minutes.

The blank sample was generated by thoroughly mixing 1.0 ml of total bilirubin reagent with 0.1 ml of distilled water. The mixture was then incubated at room temperature for precisely 5 minutes. The absorbance of each sample blank and test was measured at a wavelength range of 532-546 nm relative to a blank consisting of distilled water. The blood levels of total bilirubin and direct bilirubin were measured and reported in milligrammes per decilitre (mg/dL).

The bilirubin content was determined using the following equation: The total bilirubin concentration (mg/dt) is calculated by multiplying the absorbance of the sample blank by a factor of 15. The concentration of direct bilirubin (mg/dL) is equal to the absorbance of the sample blank multiplied by 10.

Determination of Total proteins:
Determination of Antioxidant Enzymes and Lipid Peroxidation
Procedure

The estimation of SOD was conducted using the technique outlined by Kakkaret at l87. A 0.5 ml sample of liver homogenate was mixed with 0.5 ml of distilled water. 0.25 ml of ethanol and 0.15 ml of chloroform, both reagents cooled, were added to this. The solution was agitated for a duration of 1 minute and thereafter subjected to centrifugal force at a speed of 2000 revolutions per minute. The presence of the enzyme in the liquid portion of the mixture was assessed. 1.5 ml of buffer was added to 0.5 ml of the supernatant. The reaction was started by adding 0.4 ml of epinephrine, and the rate of change in optical density per minute was quantified at a wavelength of 480 nm using a double beam UV-VIS spectrophotometer (UV 1700, Shimadzu).
The activity of SOD was quantified in units per milligramme (U/mg).
The rate of change in optical density per minute at 50% blockage during the adrenochrome transition, as catalysed by the enzyme, is defined as one enzyme unit.

2. Determination of catalase:
Procedure

The catalase activity was evaluated using the Sinha technique (1972). Eighty-eight A 0.1 ml sample of liver homogenate was combined with 1.0 ml of phosphate buffer and hydrogen peroxide. The reaction was halted by introducing 0.2 ml of dichromate acetic acid reagent. Quantities of hydrogen peroxide ranging from 4 to 20 µl were selected and subjected to the same treatment. The tubes were subjected to thermal treatment in a vigorously boiling water bath for duration of 10 minutes. The green colour produced was measured at a wavelength of 570 nm using a Double beam UVVIS spectrophotometer (UV 1700, Shimadzu).
The catalase activity was quantified as units per milligramme (U/mg).

3. Determination of glutathione peroxidase:

Procedure

The activity of glutathione peroxidase was assessed using the Rotrucket et al. (1973)89 technique. A mixture of 0.2 ml of EDTA, sodium azide, reduced glutathione, H2O2, 0.4 ml of buffer, and 0.1 ml of enzyme (liver homogenate) was prepared and incubated at 37°C for 10 minutes. The reaction was halted by adding 0.5 ml of Trichloroacetic acid (TCA) and the tubes were subjected to centrifugation. 0.5 ml of the supernatant was combined with 3 ml of sodium hydrogen phosphate and 1 ml of DTNB. The resulting colour was measured at 412 nm using a Double beam UV-VIS spectrophotometer (UV 1700, Shimadzu) without delay. The activity of glutathione peroxidase in serum is measured and given as micrograms per milligramme (µg/mg).

4. Determination of lipid peroxidation:

Procedure

Okhawaet al. (1979) developed a technique to evaluate lipid peroxidation.91 To prepare the mixture, one millilitre of liver homogenate was combined with half a millilitre of 4% (w/v) sodium dodecyl sulphate, one and a half millilitres of 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5), and fifteen millilitres of 0.8% thiobarbituric acid (TBA, pH 7.4). A hot water bath was used to heat the mixture to 85˚C for one hour. After being spun at 1200 g for 10 minutes, the intensity of the pink hue was measured at 532 nm using a reagent blank. By using 1,1,3,3, - 1,1,3,3-tetra-ethoxypropane as a reference, the concentration was represented as n moles of MDA per mg of protein.

III.RESULTS AND DISCUSSION

Extract	Nature	Percentage of yield
Foeniculum vulgare	Dark green	8.34

Preliminary phytochemical screening

Preliminary Phytochemical screening was performed for extracts of Foeniculum vulgare and it there was presences of glycoside, flavonoids, tannin, carbohydrates and proteins.

Table : 1 Qualitative analysis of methanolic extract of Foeniculum vulgare

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	-ve
2	Glycosides	+ve
3	Carbohydrate	+ve
4	Protein	+ve
5	Amino acid	+ve
6	Steroids	-ve
7	Flavonoids	+ve
8	Terpenoids	+ve
9	Phenols	+ve
10	Saponins	-ve
11	Tannin	+ve

+ve: present; -ve: absent

Pharmacological assessment

Acute toxicity test

Administration of 2000 mg/kg, p.o. of the extracts did not produce any behavioral abnormalities and mortality. So, the dose selected for further study was 100, 200 and 400 mg/kg, p.o. of the extracts.

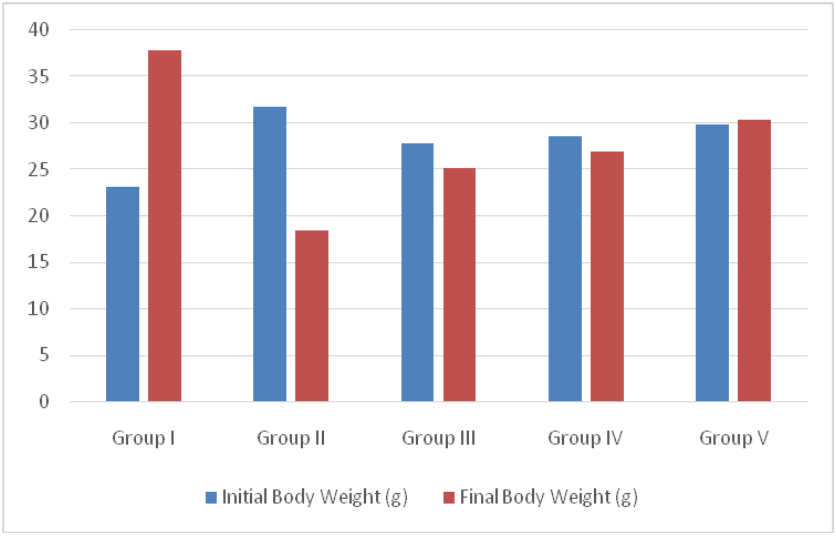
Hepatoprotective activity

Hepatotoxicity is a common side effect of various drugs and xenobiotics. Paracetamol is a NSAID which is harmless in normal therapeutic doses and causes liver toxicity in high doses in humans.

Table :2 Effect of methanolic extract of Foeniculum vulgare Body Weight

Group	Initial Body Weight (g)	Final Body Weight (g)
Group I	23.12 ± 0.310	37.85 ± 1.520
Group II	31.76 ± 0.475	18.42 ± 4.892 *
Group III	27.85 ± 0.642	25.13 ± 6.908
Group IV	28.60 ± 0.710	26.87 ± 6.650
Group V	29.90 ± 0.825	30.35 ± 6.980

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with control group.



Effect of methanolic extract of Foeniculum vulgare on Liver weight in paracetamol inducedhepatotoxicity

Group	Liver Weight (g)
Group I	0.685 ± 0.192
Group II	1.468 ± 0.502
Group III	1.015 ± 0.421
Group IV	0.812 ± 0.355 **
Group V	1.126 ± 0.372 *

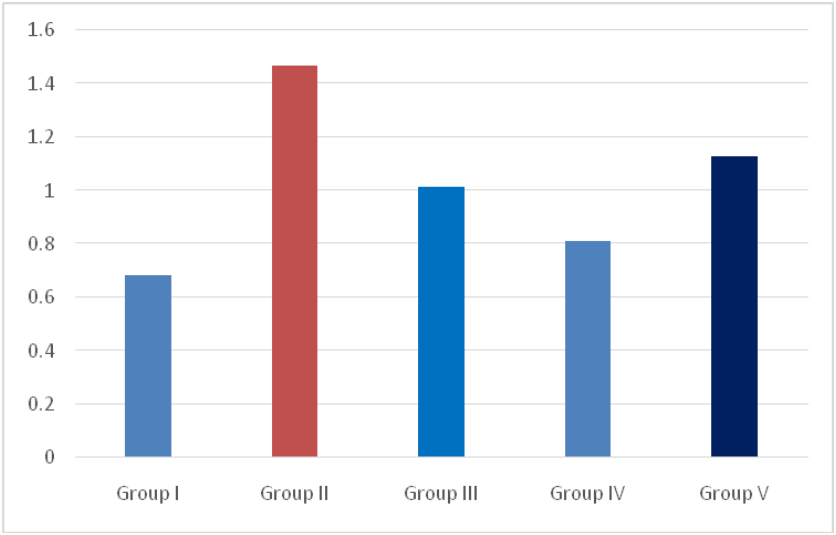
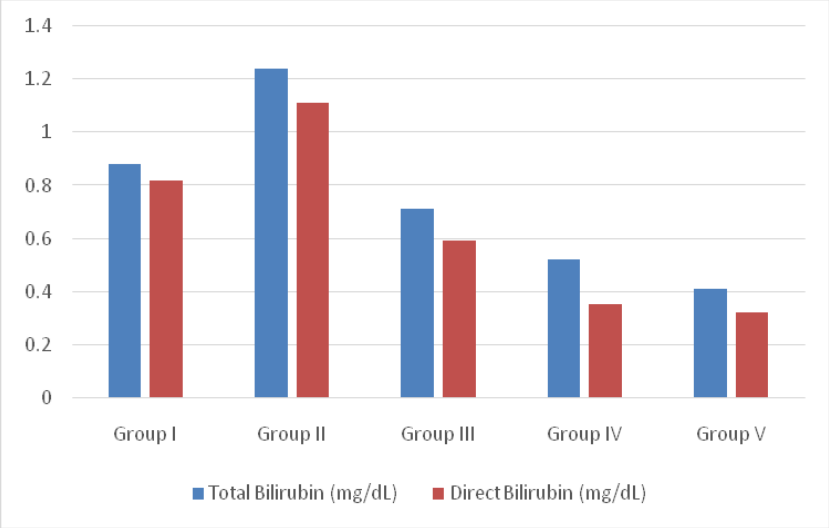


Table Effect of methanolic extract of Foeniculum vulgare on total bilirubin, direct bilirubin

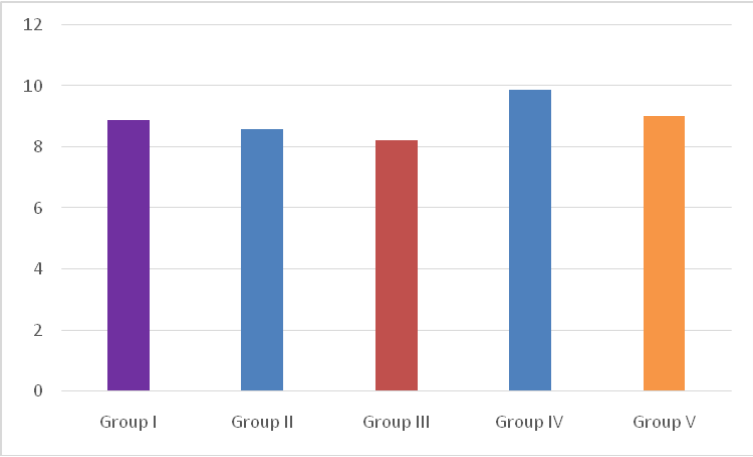
Group	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Group I	0.880 ± 0.412	0.820 ± 0.220
Group II	1.240 ± 0.385	1.110 ± 0.150 ns
Group III	0.710 ± 0.310	0.590 ± 0.110 ns
Group IV	0.520 ± 0.198	0.350 ± 0.030 *
Group V	0.410 ± 0.175	0.320 ± 0.080 *



Effect of methanolic extract of Foeniculum vulgare on serum protein in paracetamol induced hepatotoxicity

GROUP	TOTAL PROTEIN
Group I	8.90±0.153
Group II	8.60±0.404
Group III	8.22±1.02
Group IV	9.89±1.81
Group V	9.03±0.463

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by dunnett's ns- no significant *P< 0.001, **P < 0.01, ***P < 0.05 calculate by comparing treated group with control group.



There appears to be a U-shaped trend, with the protein level decreasing in Group III and increasing again in Groups IV and V.

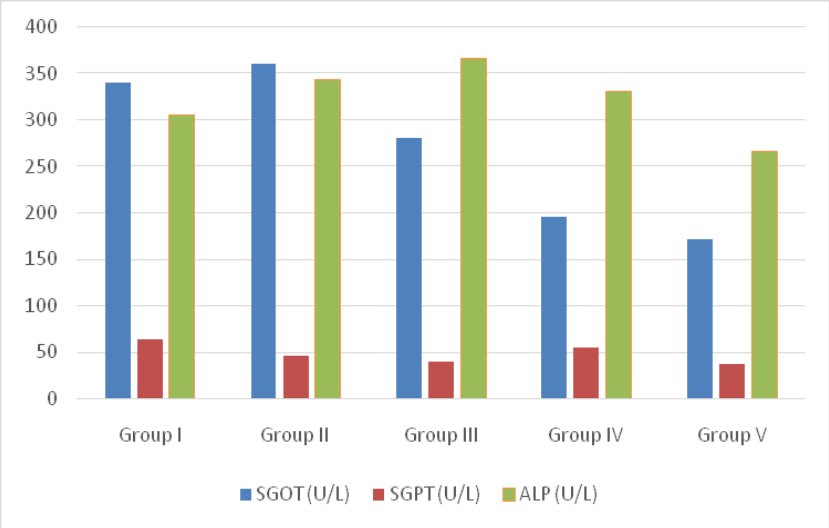
Group IV might represent a strong biological response or an outlier condition due to its elevated mean and variability.

Group III could indicate a negative outcome or condition that suppresses protein levels.

Depending on the experimental context (e.g., drug treatment, disease model, nutritional study), these changes could reflect effects on liver function, plasma volume, inflammation, or nutritional status.

Effect on SGOT, SGPT, ALP
Effect of methanolic extract of foeniculum vulgare SGOT, SGPT, ALP

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group I	340.2 ± 42.5	63.5 ± 18.0	305.6 ± 52.3
Group II	360.7 ± 85.4	45.2 ± 17.6	342.8 ± 30.5
Group III	280.9 ± 47.8 *	39.0 ± 5.1 **	365.2 ± 38.6
Group IV	195.6 ± 57.3 **	55.1 ± 20.9	330.7 ± 26.4 *
Group V	170.8 ± 5.2 **	36.5 ± 2.5 **	265.4 ± 75.3 **

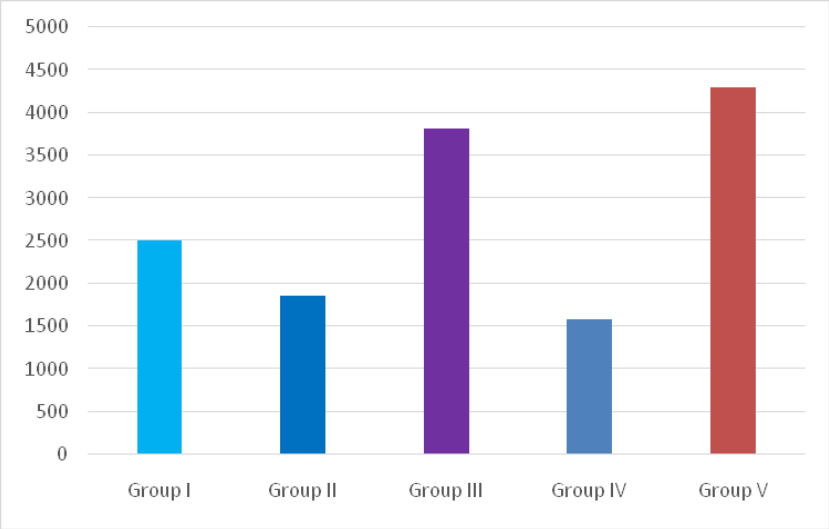


SGOT (AST)

- **Group II** shows a slight increase over Group I, suggesting**mild liver stress or damage.**
- **Group III–V** show **progressive decreases**, with Group V having the **lowest SGOT**—indicating **dose-dependent liver protection**or**recovery**.
- Group IV and V values are statistically significant (**), reinforcing the interpretation of improved liver function.

Effect on activity of LDH
Table Effect of methanolic extract of Foeniculum vulgare LDH activity

Group	LDH (U/L)
Group I	2501 ± 310.5
Group II	1855 ± 295.2
Group III	3820 ± 650.1 *
Group IV	1585 ± 70.4 **
Group V	4290 ± 710.6 *



Discussion

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme released into the bloodstream during tissue damage or cellular injury. Elevated LDH levels are commonly associated with cellular necrosis, inflammation, or oxidative stress, making it a reliable biomarker for assessing tissue integrity and cytotoxicity.

In the present study, **Group I (control)** exhibited LDH levels of **2501 ± 310.5 U/L,** representing normal physiological conditions. **Group II (disease or toxin control)** demonstrated a moderate reduction in LDH levels **(1855 ± 295.2 U/L)** compared to the control, which may suggest mild cytoprotective adaptation, enzyme suppression, or differences in disease kinetics. However, this reduction should be interpreted cautiously, as it may also reflect complex interactions such as delayed cell death or tissue repair responses.

Group III, which likely received a low dose or early treatment, showed a **significant elevation in LDH levels (3820 ± 650.1 U/L**, $p < 0.05$ *vs. Group I*), suggesting substantial tissue or cellular damage. This indicates that either the treatment was ineffective at this dose or exacerbated underlying pathology. Similarly, **Group V**, potentially exposed to a high dose or alternative treatment strategy, showed the **highest LDH level (4290 ± 710.6 U/L**, $p < 0.05$), further indicating enhanced tissue damage or a toxic effect of the intervention.

In contrast, **Group IV** demonstrated a **marked reduction in LDH levels (1585 ± 70.4 U/L, p <0.01 vs. Group III and V),** the lowest among all groups. This suggests a **strong protective or therapeutic effect,** likely due to the treatment's ability to preserve cell membrane integrity and mitigate necrotic processes. The statistically significant decrease in LDH compared to both untreated and other treated groups support its potential efficacy.

The data reveal a **biphasic or dose-dependent response,** where certain treatment conditions (as in Group IV) confer cytoprotection, while others (Group III and V) may induce or fail to prevent tissue damage. The elevated LDH in Groups III and V may be attributed to increased oxidative stress, mitochondrial dysfunction, or immune-mediated cytotoxicity, which warrant further mechanistic investigation.

CONCLUSION

The results of this study demonstrate that elevated levels of liver enzymes (SGOT, SGPT, ALP) and LDH in certain groups indicate substantial hepatic and tissue injury, likely due to toxic or pathological insult. Notably, Group III and Group V exhibited significantly increased LDH activity, suggesting pronounced cellular damage or necrosis. In contrast, Group IV showed a marked reduction in LDH and favorable shifts in liver enzyme profiles, indicating a potential protective or therapeutic effect.

These findings suggest that the intervention administered to Group IV may exert **hepatoprotective and cytoprotective** effects, possibly through mechanisms that stabilize cell membranes, reduce oxidative stress, or mitigate inflammatory responses. The data support further investigation into this treatment condition as a promising candidate for preventing or attenuating tissue damage.

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