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**Original Research Article**

# Pharmacological Assessment of the Hydro-alcoholic Extracts of Leaves of *Eryngium foetidum* Linn. (EFHA)

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## Abstract

The present study is aimed at assessment of the innate pharmacological potential of leaves of *Eryngium foetidum*. The study objective includes the evaluation of the different hydro-alcoholic concentrations of *Eryngium foetidum* extracted and to find correlates with its yield value and the biological effect in animal models of hepatoprotectivity. The plant was collected from a rural garden of Assam. The plant was subsequently authenticated at the Botanical Survey of India, Shibpur, Howrah and a specimen number was accorded a reference number and retained for future reference. The extraction process was carried out by maceration and the hydro-ethanol extract was prepared in different ratios of 30:70, 50:50 and 70: 30 respectively. The yield of the three different extracts was compared and the acute toxicity study was performed as per OECD guidelines 423 after obtaining prior permission from the Institutional animal ethics committee (IAEC). The study, however, showed no mortality or untoward behavioural morbidities in the experimental animals post the oral acute toxicity study at doses up to 2000 mg/ kg body weight. Subsequently the efficacy study of the drug was performed using 18 Swiss albino mice (n=3) as a prospective pilot interventional efficacy study to evaluate the beneficial effect of the drug in hepatotoxicity induced by Carbon tetrachloride ($CCl_4$) in murine models. The experimental groups were: negative control, disease control, standard (silymarin treated) and hydro-alcoholic extract groups. The findings were statistically analyzed using two way-ANOVA at CI of 95% with $p<0.05$ from the efficacy study shows promise in putative role of this indigenous plant in the amelioration of hepatotoxicity and poses no signs of morbidity or mortality upto a dose of 2000 mg/kg body weight with the drug. Follow up studies with larger sample size are however, warranted to corroborate findings to definitely arrive at a meaningful conclusion regarding the standing and the hepatoprotective potential of this indigenous drug.

## Article Info

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## Keywords

*Eryngium foetidum*
Hydro-alcoholic extract
Hepatoprotective activity
Medicinal plants

## Introduction

Medicinal plants are used for the preparation of wide spectrum of derivatives ranging from traditional extracts with high standard contents of active constituents to chemically pure compounds. With the development of analytical technology late in the 19th century, came the efforts to identify and isolate the active chemical

moieties in medicinal plants, so that the more potent, safe and efficacious and high quality drugs could be developed. These discoveries enabled the quality process of conventional medicine to begin, and from these evolved the conventional medicines of today (Wild, 2003). These cover phytochemicals such as alkaloids, terpenes, flavonoids, essential oils, glycosides, etc. Moreover, the bioactive compounds from the medicinal plants can serve as leads for developing new synthetic drugs in modern medicines. However, some of the plant compounds remain to be unique due to their structural complexity. Likewise the present study is based upon evaluative hepatoprotective aspects of *Eringium foetidum* on the topical anti-inflammatory activity of Phytosterols (Garcia et al., 1999), suppressors of inflammatory mediators macrophages (Mekhora et al., 2012).

The plant is rich in calcium, iron, carotene, and riboflavin and its harvested leaves are widely used as a food flavoring and seasoning herb for meat and many other foods. Several modern drugs are used to treat disorders but their prolonged use may cause severe adverse side effects, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Plant drugs are known to play a vital role in management of diseases. Hyperacidity, also known as acid dyspepsia is the condition of excreting more than the normal amount of hydrochloric acid in the stomach. Exhaustive literature survey revealed that the carminative potentialities of *Eringium foetidum* has not been exploited and thus an attempt was made to scientifically evaluate the antacid property. In ethnomedicine the plant is used to treat burns, ear ache, fever, hypertension, constipation, seizures, asthama, stomach ache, worms, infertility complications, snake bites, arthritis, diarrhea and malaria (Shavandi et al., 2012). This comprises bioactive polyphenolic compounds, these polyphenolic compounds have shown a wide range of biological activities such as anti-inflammatory, hepatoprotective, antioxidant, antithrombotic, anticarcinogenic, free radical scavenging, anti mutagenic, anti microbial properties too (Modnicki and Balserek, 2009).

*Eringium foetidum* has long been used to treat fever, vomiting, diarrhea, hypertension, arthritic pain, and convulsions (Saenz et al., 1997). Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab et al., 2003).

The medicinal effects of plants are due to metabolites especially secondary compounds produced by them. Therefore, there has been an interest in finding natural antioxidants from *Eryngium foetidum* in the present study. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones (Aswathy and Oommen, 2014). Various medicinal plants have been used to treat for various diseases in all over the world. Nowadays, Indian medicinal plants are belonging to about 40 families were investigated as liver protective drugs (Handa and Sharma, 1986).

*Eryngium foetidum* is a tropical perennial and annual herb in the family Apiaceae. In India, the herb is mainly found in the north-eastern state of Assam, where it is known by the local name Man Dhonia, in Manipur, it is known by the local name Awa phadigom or Sha maroi.Mizoram, it is known as Bahkhawr, Tripura as Bilati dhonia and in Nagaland, Burma dhania. It is also found in the Andaman & Nicobar Islands, and in a few parts of Tamil Nadu, Kerala and Karnataka. It is unknown in other parts of India. In Trinidad and Tobago,it is known as Bhandhania by the Indian community and Shandon Beni by the other locals.In tropical regions of world (America, South Asia, Pacific Islands), Spiny corriander. In English-speaking Caribbean countries knows it as Celantro. In Surinam, it is known as Sneki wiwiri. In Peru, where these coriander is known as Culantro. In Brazil it is known as Coentro-bravo.

The present study objectives included to assess the pharmacognostical and pharmacological potential of hydro-alcoholic extracts of leaves of the indigenous herb *Eryngium foetidum*. To evaluate the different hydro-alcoholic concentrations of *Eryngium foetidum* extracted and correlate it with its yield value and the biological effect in animal models of hepatoprotectivity. To perform the oral acute toxicity study of *Eryngium foetidum* in toxicity models according to Organisation for economic co-operation and development (OECD) guidelines.

## Materials and methods

### Plant selection

*Eryngium foetidum* the traditional medicinal plant is reported to possess many therapeutic activities. From ancient text and literature its, anticlastrogenic (Promkum et al., 2012), anthelmintic (Bencao, 1999), antioxidant (Nebija et al., 2009), antimalarial, etc. can be deciphered. From the plant for the present study was selected from some rural areas of Tangla, Assam. From the traditional medicinal healers we got know about the plant hepatoprotective potential. To unravel the hepatoprotective activity of this plant the study was designed. Many studies had been carried out on its antioxidant properties and many phytochemical screening was carried out earlier by many researchers and scientists but evaluation of hepatoprotective activity was not yet done. This is how plant was selected for the present study.

### Plant collection

The plant was collected from a rural garden of Tangla, Udalguri B.T.A.D, Assam. No flowers were budded on the plant in the month of July/2014, so the young plants were collected.

### Plant identification

Plant identification is done at Botanical Garden, Kolkata. *Eryngium foetidum* plant was collected along with its leaves and roots at morning time from a rural garden, Tangla, Assam. The herbarium specimen was submitted to Authentication Office of Herbarium, Royal Botanical Garden, Kolkata.

### Extraction

The powdered leaves of *Eryngium foetidum* stored in pharmacological laboratory were further dried under shade for a week before extraction started. Then the powdered materials were weighed in three separate beaker and transferred into 500 ml beaker respectively. Over the 3 beakers menstrum (hydro-alcoholic solvent) was poured in different concentration and ratio a given in Table 1. Then the mixture of solvent and crude powder contained in each beaker was mixed properly with a gentle shaking and stirring with a glass rod. After the mixing is over it was transferred to three separate stoppered conical flask which was rinsed with ethanol and dried earlier to prevent the men strum from evaporating. All the stoppered conical flasks are kept, shaken, processed and preserved in a same manner to avoid batch to batch variation.

### Profile of leaf constituents (Cardozo et al., 2004)

Fresh leaves consist of 86–88% moisture, 3.3% protein, 0.6% fat, 6.5% carbohydrate, 1.7% ash, 0.06% phosphorus and 0.02% iron. Leaves are an excellent source of vitamin A (10,460 I.U./100 g), B2 (60 mg %), B1 (0.8 mg %), and C (150–200 mg %) On dry weight basis, leaves consist of 0.1–0.95% volatile oil, 27.7% crude fiber, 1.23% calcium, and 25 ppm boron.

**Table 1.** Quantity of crude leaves and solvent with its different ratio of concentration.

| Sl. no. | Crude leaves (in g) | Solvent (menstrum) | Ratio |
|---|---|---|---|
| 1 | 25 | 30% hydro-alcoholic solvent | 3:7 |
| 2 | 25 | 50% hydro-alcohol solvent | 5:5 |
| 3 | 25 | 70 % hydro- alcoholic solvent | 7:3 |

Physical properties of the evaporated final product of the extracts were found to be:

Nature - Semisolid in nature
Colour - Dark brown
Odour – Aromatic
Solubility - Soluble in water, ethanol

### Acute toxicity study (Ghosh, 2011)

An Oral acute toxicity study as per OECD guidelines 423 was performed on the extracts to check for any mortality or behavioral changes in Swiss albino mice up to a dose of 2000 mg/ kg body weight (n=5).

### Pharmacological evaluation of hepatoprotective activity:Carbon tetrachloride model of hepatotoxicity in rodent models

Carbon tetrachloride $CCl_4$ is widely used for the experimental induction of liver damage (Parola et al., 1992; Castro et al., 1974; Poli, 1993). The principle causes of $CCl_4$ is induced hepatic damage in lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals. $CCl_4$ impairs hepatocytes directly by altering the permeability of the plasma, lysosomal, and mitochondrial membranes.

Highly reactive free radical metabolites are also formed by the mixed function oxidase system in hepatocytes *via* CYP2E1, causing severe centrilobular necrosis. This model has been used extensively to examine the pathogenesis of cirrhosis. $CCl_4$ in liquid paraffin in ratio of 1:1 ($CCl_4$: liquid paraffin) was prepared for injecting through intra peritoneal route in albino mice with respect to body weight.

**Table 2.** Experimental grouping and study design.

| Group (n=6) | Intervention | Inducing phase | Treatment |
|---|---|---|---|
| I | Positive Control | Ratio $CCl_4$ 1:1 | No treatment |
| II | Standard | Ratio $CCl_4$ 1:1 | Silymarin 70mg/kg |
| III | Test group 1 | Ratio $CCl_4$ 1:1 | EFHA 3:7 |
| IV | Test group 2 | Ratio $CCl_4$ 1:1 | EFHA 1:1 |
| V | Test group 3 | Ratio $CCl_4$ 1:1 | EFHA 7:3 |

## Estimation of biochemical parameter

The biochemical parameters, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum creatinine and serum albumin were analysed using standard procedures (Bergmeyer et al., 1980; King et al., 1980). The results obtained were subjected to two-way ANOVA $p<0.05$ followed by post hoc Tukey's test.

## Results and discussion

## Serum hepatospecific markers - Serum glutamate pyruvate transaminase (SGPT)

The changes in SGPT across different study groups expressed on Day 0 and on Day 15 are given in Table 3 and Fig. 1 and the unit of measurement is IU/L.

**Table 3.** Comparison of SGPT across study groups (IU/L).

| Group | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Day-0 | 47±0.5 | 52±0.6 | 44±1.1 | 55±1.0 | 49.5±0.6 |
| Day-15 | 44.83±0.4 | 97.50±0.9 | 64.76±1.3 | 62.33±1.3 | 55.5±1.3 |

## Serum glutamate oxaloacetate transaminase (SGOT)

The changes in SGOT across different study groups expressed on Day 0 and on Day 15 are given in Table 4 and Fig. 2 and the units of measurement is IU/L.

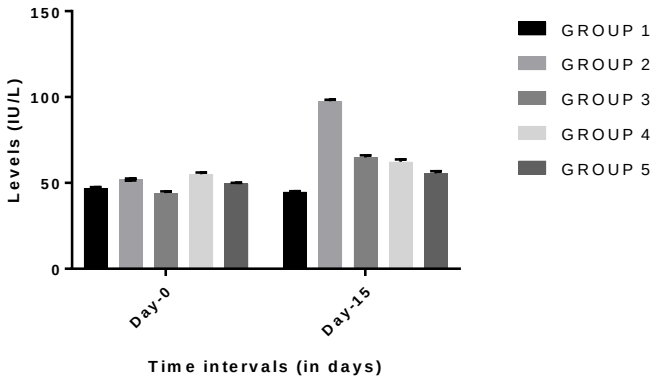


**Fig. 1:** SGPT changes across treatment groups. N.B: Significance was assessed by two-way ANOVA $p<0.05$ followed by post hoc Tukey's test.

**Table 4.** Changes in SGOT levels across study groups on Days 0 and 15 respectively. Values are Mean ± Standard Error of Mean (SEM) (n=6) (IU/L).

| Group | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Day-0 | 41.5±1.3 | 46±1.4 | 51±1.2 | 48.6±1.3 | 44.33±0.9 |
| Day-15 | 39±1.9 | 84.5±1.2 | 63±1.4 | 49.5±1.1 | 47.5±1.0 |

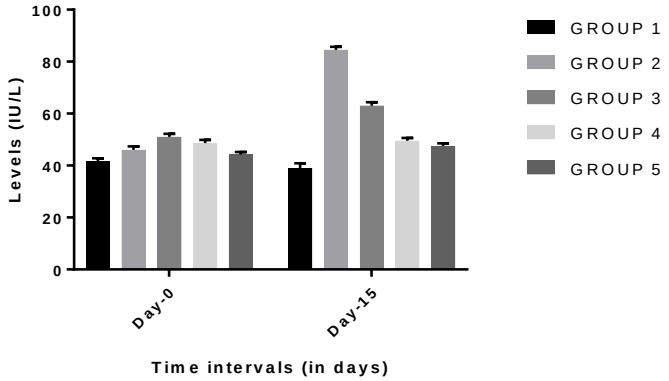


**Fig. 2:** SGOT changes across treatment groups. N.B: Significance was assessed by two-way ANOVA $p<0.05$ followed by post hoc Tukey's test.

## Serum creatinine

The changes in serum creatinine across different study groups as expressed on Day 0 and on Day 15 are given in Table 5 and Fig. 3 and the unit of measurement expressed is mg/100ml.

**Table 5.** Comparison of changes in serum creatinine levels in study groups (n=6). Mean ± Standard Error of Mean (SEM) (n=6) (mg/100ml).

| Group | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Day-0 | 0.32±0.01 | 0.27±0.02 | 0.31±0.02 | 0.37±0.02 | 0.39±0.01 |
| Day-15 | 0.33±0.02 | 0.36±0.01 | 0.37±0.02 | 0.41±0.01 | 0.42±0.01 |

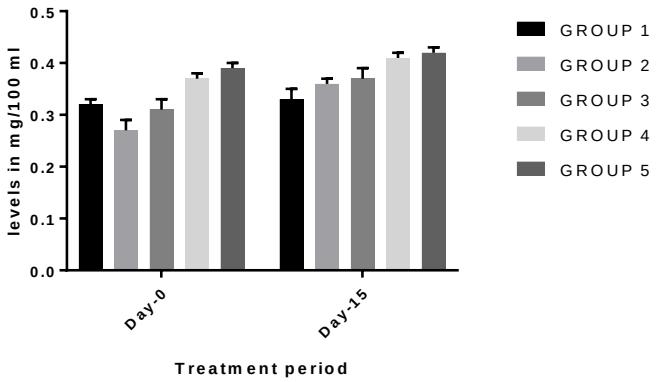


**Fig. 3:** Changes in creatinine levels. N.B: Significance was assessed by two-way ANOVA $p<0.05$ followed by post hoc Tukey's test.

## Serum albumin

The changes in serum albumin across different study groups expressed on Day 0 and on Day 15 are given Table 6 and Fig. 4. The unit of measurement is mg/100ml.

**Table 6.** Comparison of changes in serum albumin levels in study groups. Values are Mean± SEM (n=6) (100mg/ml).

| Group | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Day-0 | 3.30±0.3 | 3.8±0.4 | 3.7±0.6 | 3.91±0.9 | 3.78±0.8 |
| Day-15 | 3.21±0.8 | 5.1±0.4 | 4.69±0.9 | 4.57±1.1 | 4.23±0.9 |

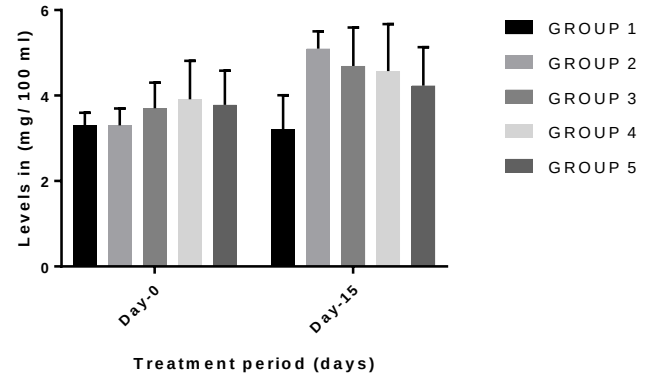


**Fig. 4:** Changes in albumin levels across treatment groups. N.B: Significance was assessed by two-way ANOVA $p<0.05$ followed by post hoc Tukey's test.

The findings revealed that all the extracts under study did not cause any lethality in Swiss albino mice upto a dose of 2000 mg/kg body weight. After maceration for 15 days *Eryngium foetidum* extract of ratio 3: 7 gave 1.1 g yield and the ratio 7: 3 gave 0.97 g and the ratio 5: 5 gave 0.28g the lowest amount of semisolid extract in the container. Therefore according to the result we found that the ratio of 3: 5 (menstrum: crude) powder gave the maximum yield of crude *Eryngium foetidum* extract. The toxicological parameters of the study were explored that confirmed that the Oral Acute Toxicity study did not reveal any untoward toxicity in Swiss albino mice at doses of 2000 mg/ kg body weight. The biological activity result is awaited till the completion of treatment and histological studies of the same. From the pharmacognostical study we can conclude *Eryngium foetidum* and its pharmacognostical parameter, we have found that the yield of the plant was found to be greatest for 3:5 ratio of the menstrum crude extract.

The efficacy study of the drug was performed using 18 swiss albino mice (n=3) as a pilot prospective, interventional efficacy study to evaluate the beneficial effect of the drug in hepatotoxicity caused by Carbon tetrachloride ($CCl_4$) in murine models. The experimental groups were: negative control, disease control, standard (silymarin treated) and hydro-alcoholic extract groups. The findings from the efficacy study shows promise in putative role of this indigenous plant in the amelioration of hepatotoxicity and poses no signs of morbidity or mortality upto a dose of 2000 mg/kg body weight with the drug.

## Conclusion

The extraction process of *Eryngium foetidum* was carried out by maceration and the hydro-alcoholic (ethanol) extract was prepared in the ratio of 30:70, 50:50 and 70:30 respectively. The yield of the three different extracts was compared and the acute toxicity study was performed as per OECD guidelines after obtaining prior permission from IAEC of the institute. No mortality or untoward physiological or psychological morbidities was reported in the experimental animals post the oral acute toxicity study. The revelations from the efficacy study shows a potential putative role of this indigenous plant in the amelioration of hepatotoxicity and poses no signs of morbidity or mortality up to a dose of 2000 mg/kg body weight with the drug. Follow up studies with larger sample size are however, warranted to corroborate findings to definitely arrive at a meaningful conclusion regarding the standing and the hepatoprotective potential of this indigenous drug of the north-east and its possible greater role in the future phyto-therapeutics of liver disorders.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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