ejpmr, 2017,4(01), 432-436

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF HEPATOCYTES REGENERATOR POTENTIALITY OF EE-LEF AGAINST CCL_4 INDUCED RAT HEPATOCYTES

Mahendar Boddupally[*1], Monica Chopra[2], Dr. S. Shoba Rani[3], Shubhankar Saha[4]

[1]Assistant Professor, Department of Pharmacology, Nalanda College of Pharmacy, Cherlapally-508001, Nalgonda, Telangana State, India.
[2]Senior Lecturer, Department of Pharmacy, Meera Bai Institute of Technology, Maharani Bagh, New Delhi-110065.
[3]Assistant Professor, Department of Pharmaceutical analysis and Quality assurance, JNTUH, Kukatpally-500072, Telangana State, India.
[4]Department of Pharmacology, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh-517561.

***Corresponding Author: Mahendar Boddupally**
Assistant Professor, Department of Pharmacology, Nalanda College of Pharmacy, Cherlapally-508001, Nalgonda, Telangana State, India.
DOI: 10.20959/ejpmr20171/2482

Article Received on 10/11/2016 **Article Revised on 01/12/2016** **Article Accepted on 22/12/2016**

ABSTRACT: The liver is the only visceral organ that possesses remarkable capacity to regenerate. The liver can regenerate after either surgical removal or after chemical injury. It is known that as little as 25% of the original liver mass can regenerate back to its full size. The process of regeneration in mammals is mainly compensatory growth because only the mass of the liver is replaced not the shape. Liver regeneration involves replication of the liver cells, mainly hepatocytes, followed by other cells such as biliary epithelial cells and sinusoidal endothelial cells. Once cell proliferation is completed, the newly divided cells undergo restructuring, angiogenesis and reformation of extracellular matrix to complete the regeneration process. The main objective of present research work is to screen the bio molecules present in EE-LEF and evaluate the *in vivo* hepatocytes regenerators' potentiality. etc. The *in vivo* hepatocytes regenerator's potentiality was performed against CCl_4 induced rat hepatocytes. The results obtained from the present experimental data **were** indicated that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin due to CCl_4 intoxication were reduced significantly in rats, after treatment with **EE-LEF**. Treatment with **EE-LEF** at a **500 mg/kg b. w.** significantly decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by **26.1%, 47.16%, 24.34% and 43.58%** respectively. Silymarin used as standard drug showed a reduction of **55.09%, 68.98%, 57.46% and 60.68%** receiving CCl_4 alone. So depending upon the present data it was confirmed that the biochemical parameters of the group treated with **EE-LEF** was significantly lower than the CCl_4-treated group.

KEYWORDS: Regeneration, hepatocytes, angiogenesis, endothelial cells, SGPT etc.

INTRODUCTION[1,2,3,4]

Taxonomy

Botanical Name: Enhydra fluctuans Lour
Family: Asteraceae (family description)
Genus: Enhydra
Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Epithet: fluctuans Lour.
Common Names: Harkuch, Hingcha
Local names: kankong-kalabau (Tag.).
Indian Name: Helencha
Part used: Leaf
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Asterales
Species: E. fluctuans
Bengali Name: Hingcha sag.

Habitat

Grows in swampy ground in Tropical climate. Native to India, Bangladesh, Burma, Sreelankha and several places in south east Asia. Hingcha or Kankong-kalabau is found in Rizal Province in Luzon, being occasional along the banks of small streams in and about Manila. It was certainly introduced, being found also in tropical Africa and Asia to Malaya. In Bengal it is commonly known as Hingha and grows plenty in ponds & lakes.

Description & Uses

Perennial herb of swampy ground in coastal areas, till recently considered as a single species under the first name, but now recognized to be two: E. fluctuans only in the Niger Delta, but widespread in the tropics, and E. radicans from Senegal to Dahomey and Fernando Po. No usage of either species is recorded for the Region. The leaves of E. fluctuans are somewhat bitter and are eaten as a salad or vegetable in several tropical countries. In

Zaïre E. fluctuans has been reported a favourite food of the hippopotamus.

This plant is a prostate, spreading, annual herb. The stems are somewhat fleshy, 30 centimeters or more in length, branched, rooting at the lower nodes, and somewhat hairy. The leaves are stalkless, linear-oblong, 3 to 5 centimeters in length, pointed or blunt at the tip, usually truncate at the base, and somewhat toothed at the margins. The flowering heads are without stalks, are borne singly in the axils of the leaves, and excluding the bracts, are less than 1 centimeter in diameter. The outer pair of the involucral bracts is ovate and 1 to 1.2 centimeters long; the inner pair is somewhat smaller. The flowers are white or greenish-white. The acheness are enclosed by rigid receptacle-scales. The pappus is absent. Flower colour: beige, white.

Fig1: E. Fluctuans flowering plants

Fig 2: E. Fluctuans plants

Edible uses

According to Burkill the young parts are used as a salad in several countries, including Malaya. Sometimes they are steamed before they are eaten. Guerrero reports that in the Philippines the leaves are pressed and applied to the skin as a cure for certain herpetic eruptions. In bengal it is washed, chopped and cooked as Sag fry or boiled with rice and eaten with boiled rice with boiled potato, salt and mastered oil. Burkill reports that the young parts and the leaves of the plant are somewhat bitter and are used by the Malays as a laxative. Caius says that the leaves are useful in diseases of the skin and of the nervous system. The fresh juice of the leaves is prescribed in Calcutta as an adjunct to tonic metallic medicines, and is given in neuralgia and other nervous diseases. The leaves are antibilious. The expressed juice of the leaves is used as a demulcent in cases of gonorrhea; it is taken mixed with the milk of either a cow or a goat. As a cooling agent, the leaves are pounded and made into a paste which is applied cold to the head. Watt quotes Forsyth, who states that the plant is useful in torpidity of the liver. An infusion should be made the previous evening. It is boiled with rice and taken with mustard oil and salt.

Medicinal uses

laxatives,; paralysis, epilepsy, convulsions, spasm; skin, mucosae. They are said to be a laxative, antibilious and demulcent. They are used in India in skin and nervous affections, and in the Philippines are applied to certain herpetic eruptions, anti oxidant, anti inflammatory etc.

MATERIALS AND METHOD

Drugs and chemicals used

Standard drug silymarin was purchased from retail shop and other chemicals used for extraction purpose and phytochemical tests provided by the institutional store were of laboratory grade.

Experimental animals

White male albino Wister rats weighing about 200-250gm was used, they were obtained from the animal house of C. L. Baid metha College of Pharmacy, chennai. They were kept under observation for about 7 days before onset of experiment to exclude any intercurrent infection, had free access to normal diet and water.

Methodology for Soxhlet extraction

First the dried leaves of **Enhydra fluctuans Lour** are triturate to make fine powder and the powered material is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol is heated on a water bath or on a heating mental. As the solvent boil, its vapours rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the

organic substance behind.[6] Afterwards the ethanolic extract of leaves of **Enhydra fluctuans Lour (EE-LEF)** transfer in a clean and dried beaker and are concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Phytochemical screening and characterization biomolecule rodoxanthine.[7-10]

Preliminary phytochemical screening of EE-LEF have shown the presence of diverse bioactive molecules such as: carbohydrates, proteins and aminoacids polyphenols, carotenoids, phytosterols and alkaloids which are confirmed by their specific qualitative cofirmatory chemical tests.

Evaluation of acute oral toxicity[11]

In the present study the acute oral toxicity of the **EE-LEF** was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b. w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of **CPCSEA: IAEC/XXIX/05/2016.**

Experimental protocol for Hepatoprotetive activity[12]

A total of 30 rats were taken and divided into 5 groups of 6 rats each

A. **Group I:** Normal Control Group [**NCG -** (only the vehicle (1 mL/kg/day of 1% CMC; p.o.)].
B. **Group II:** Negative Control Group **[Neg.CG** – (CCl4 1 mL/kg (1:1 of CCl_4 in olive oil) i. p].
C. Group III: Positive Control/Standard Group **[SG -** CCl_4 1 mL/kg (1:1 of CCl_4 in olive oil) i.p.+ Standard Silymarin 100 mg/kg orally (p. o.) for 7 days]
D. **Group IV:** CCl_4 1 mL/kg (1:1 of CCl_4 in olive oil) i.p + EE-LEF (500 mg/ kg b. w., p.o.)]

Collection of blood

On the 8th day, blood was collected by retro orbital puncture, under mild ether anesthesia after 8 hr fasting. Blood samples were centrifuged at 3000 rpm for 20 mins. Serum was separated and stored at – 200^0 C until biochemical estimations.

Biochemical Analysis

The Serum samples were analyzed for
(I) Alanine Aminotransferase (ALT) (SGPT)
(II) Aspartate Aminotransferase (AST) (SGOT)
(III) Alkaline Phosphatase (ALP)
(IV) Serum Bilirubin

Histopathological Analysis

The liver tissue was dissected out and fixed in 10% formalin solution. It was then dehydrated in ethanol (50%-100%), cleared in xylene and embedded in paraffin wax. Afterwards thick sections (5-6 mm) were made and then stained with hematoxylin and eosin dye for photo microscopic observation. The whole biochemical and histopathological analysis was carried out at V.H.S Hospital in Chennai.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of EE-LEF have shown the presence of diverse bioactive molecules such as: carbohydrates, and aminoacids polyphenols, carotenoids, phytosterols, tannins and alkaloids which are confirmed by their specific qualitative cofirmatory chemical tests.

Acute oral toxicity study

(i) Acute oral toxicity studies were performed according to the OECD guideline 423 method.
(ii) This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes.
(iii) The **EE-LEF** was administered initially at a dose of 2000 mg/kg b.w and 1% CMC (p.o) and observed 14 days mortality due to acute toxicity.
(iv) Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded.
(v) Since no sign of toxicity observed at 2000 mg/kg b.w. to the group of animals, the $\mathbf{LD_{50}}$ value of the **EE-LEF** expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD50 < 2500 mg/kg).
(vi) From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there $\mathbf{LD_{50}}$ cut of values > 2000 mg/kg b. w.

Table 1: for the dose selection by Acute toxicity class method (OECD) guide lines 423 of EE-CS

Sl. No.	Treatment group	Dose mg/kg	Sign of toxicity	Onset of toxicity	Duration
2	EE-LEF	500	No	No	14 days

Hepatoprotective activity

Biochemical analysis

The effects of **EE-LEF** on liver marker enzymes and serum bilirubin levelsare displayed in **table 1 and fig: 3 and 4.** The data exhibited that Normal Control Group (NCG) demonstrated a normal range of AST, ALT, and

bilirubin levels while the CCl_4-treated group showed elevated levels of AST, ALT, and bilirubin, thus confirming that CCl_4 causes hepatocellular degeneration at higher doses. The elevation of cytoplasmic AST and ALT is considered an indicator for the release of enzymes from disrupted liver cells. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. The Results displayed in **table 1 and fig 3 and 4: were** indicated that the elevated levels of SGOT, SGPT, ALP and Serum bilirubin due to CCl_4 intoxication were reduced significantly in rats, after treatment with **EE-LEF**. Treatment with **EE-LEF** at a **500 mg/kg b. w.** significantly decreased the SGOT, SGPT, ALP, Serum Bilirubin levels by **26.1%, 47.16%, 24.34% and 43.58%** respectively. Silymarin used as standard drug showed a reduction of **55.09%, 68.98%, 57.46% and 60.68%** receiving CCl_4 alone. So depending upon the data of **table 1** it was confirmed that the biochemical parameters of the group treated with **EE-LEF** was significantly lower than the CCl_4-treated group.

Histopathological Analysis

The results of light microscopy examination of the transverse section of control, CCl_4-treated and treated with **EE-LEF** rat livers were represented **in fig 4.** It was revealed that the liver section of animals treated with CCl_4 showed a high degree of damage characterized by cell vacuolation, pyknotic and degenerated nuclei and wall of bile capillaries. Liver sections of these rats indicated necrosis, ballooning and degeneration in hepatic plates and loss of cellular boundaries.

Table: 1 for the assessment of Biochemical parameters

Groups	AST(SGOT) IU/L	ALT(SGPT) IU/L	ALP(SALP) IU/L	Sr. bilirubin mg/dL
I	53.00	46.60	139.2	0.58
II	202	204.4	399.2	1.17
III	90.80	63.40	169.8	0.46
IV	149.6	108.0	302.0	0.66

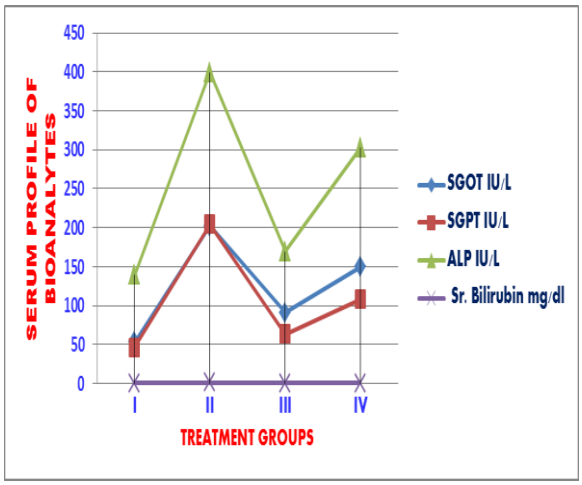

Figure 3: Comparison of serum profile of bioanalytes.

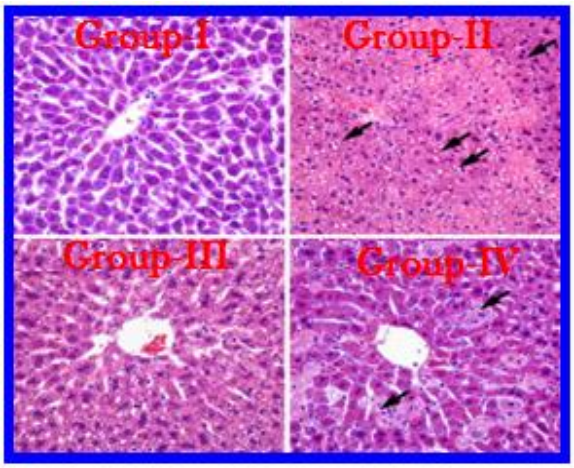

Fig 4: Histopathological analysis of rat liver sections using H&E staining. Group-I: Section from a normal control rat liver. Group-II: The liver section obtained from CCl_4-intoxicated rats shows a variety of cavitations and necrosis in hepatocytes. Group-III: Liver tissue section prepared from the std. drug silymarin-treated group shows centrilobular regeneration with restoration of c.v, ss and hepatocytes with mild necrosis. Group-IV: Liver tissue section prepared from the 500 mg/kg EE-LEF-treated group shows less cavitation and necrosis than C. E: Liver tissue section prepared from the std. drug silymarin-treated group shows centrilobular regeneration with restoration of c.v, ss and hepatocytes with mild necrosis.

CONCLUSION

From the above experimental data, here I concluded that the EE-LEF contained various bioactive molecules which were identified by their specific qualitative tests and executed moderate to good hepatoprotective activity. When compared with standard drug silymarin it displayed that EE-LEF had the ability to restore and regenerate the CCl_4 induced hepatocytes.

REFERENCES

1. http://www.aluka.org/action/showMetadata?doi=10.5555/AL.AP.UPWTA.1_928
2. http://www.fivetastes.com/vegetables/helencha.html
3. http://www.bpi.da.gov.ph/Publications/mp/pdf/k/kankong-kalabau.pdf
4. http://vaniindia.org.whbus12.onlyfordemo.com/herbal/plantdir.asp
5. http://www.virboga.de/Enhydra_fluctuans.htm
6. ARUN BHAL, B. S BHAL. Advanced Organic Chemistry, First Edition, 2010; 13.

7. P. C Dandiya, P. K. Sharma, Bio-chemistry and clinical pathology, second edition, 17-18, 24, 47-48.
8. Dr. G. Devala Rao, A Manual of Practical Biochemistry, 17.
9. Jaswant Kaur, PV Chemistry of Natural Products, edition, 2010; 113-114, 116, 344-346, 381.
10. C.K. KOKATE, A.P. PUROHIT, S.B.GOKHALE, Pharmacognosy, 42 Edition, A.1.
11. "OECD guidelines – 423" for testing of chemicals, 2001; 1-14
12. Garrat DC. The Quantitative Analysis of Drugs, Japan, Chapman and Hall, 1964; 456.