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The Role of Alkaloid as Hepatoprotective and Anti-Inflammatory Agent from Fenugreek Seed

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PRASHANT RAGHAV*, DANISH AHMED, VIKAS KUMAR

DEPARTMENT OF PHARMACEUTICAL SCIENCES
SHUATS NAINI ALLAHABAD

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ABSTRACT

The present study was aimed to extract the Hepatoprotective and anti-inflammatory principle/s in various solvents extract of Fenugreek seeds. The powder of Fenugreek seeds was directed to soak for extraction purposes in various solvent (Alcohol,CCl_4 and Acetone). Then initially phytochemical screening was done with the obtained different solvents extract for identified the various Phytochemical like alkaloid, glycoside, saponins, fatty acids, etc. In concluded screening results the alkaloid was present in the various solvent extract. Therefore the HPTLC has been done with all extract of different solvents to confirmation of alkaloid presence and all the results obtained by HPTLC like Rf value, retention time has matched with the carpaine alkaloid. So that alkaloids may be present in alcoholic, ccl4 and acetone extracts. Because alkaloid has the phagocyte character so this can be used as eliminators for various exogenous and endogenous toxicants. Now the obtained extracts evaluated using the Hepatoprotective activity by using the liver enzyme detection methods (SGOT, SGPT, and ALKP.TB.TP etc) and Anti-Inflammatory activity by using carrageen induced inflammation methods. Pharmacognostical analysis of fenugreek seed is reported to contain alkaloids, phospholipids, sterols, fatty acids, flavonoids, and glycosides etc. This study investigates Pharmacological potential as Hepatoprotective and Anti-Inflammatory agent of alkaloid components of dried *Trigonella foenumgraecum* (L) seeds.

INTRODUCTION

Trigonella foenumgraecum (L), commonly known as fenugreek is an annual herb belonging to the family Papilionaceae and is cultivated throughout the country [1]. Fenugreek is native to Eastern Europe and parts of Asia but now widely cultivated almost all over the world for its leaves and seeds, which are commonly used as leafy vegetables and condiments, respectively [2]. The leaves and seeds of the plant are widely used as spice in food preparations and as ingredient in traditional medicine [3]. Fenugreek has strong spicy and seasoning type sweet flavor. The whole seed or its ground powder is used in pickles vegetable dishes and spice powder and the dried seeds are used as condiments. In traditional Indian medicine system, fenugreek has been used extensively for curing several disorders. India is the major producer of fenugreek and it has been mainly used for culinary and medicinal purposes. In this context, fenugreek is extensively cultivated in most regions of the world for its medicinal value. Fenugreek seeds have been known and valued as medicinal material from very early times. Its seeds are considered as commercial source of a steroid diosgenin, which is importance to the pharmaceutical industry [4]. The biological and pharmacological actions of fenugreek are attributed to the variety of its constituents, namely: steroids, N-compounds, polyphenolic substances, volatile constituents, amino acids, etc [4]. The liver is a vital organ and its strategic location and multidimensional functions support almost every other organ in the body. Liver is also the main organ for metabolism and elimination of drugs [5, 6]. At the same time liver is prone to many diseases like allergy to food and involves immune system as well. Liver damage can also be caused by drugs, particularly anti-tubercular drugs, general anesthetics, paracetamol and some anti-cancer drugs. Alcoholic liver diseases with cirrhosis (formation of fibrous tissue in liver) caused by excessive alcohol consumption is a common occurrence. Liver toxicity not only occurs from direct toxicity of the primary compound but also from reactive metabolite or immunologically-mediated response.

MATERIALS AND METHODS

Materials

Alcohol and PCM were used as toxicant for liver toxicity, and carrageenan, chemical for inflammation in this study. Alcohol, Silymarin were purchased from Sigma Aldrich Mumbai and carrageenan purchased from CDH New Delhi.

Preparation of seed extracts:-

Apparently, healthy plant seeds were collected, washed thoroughly in tap water and dried in room temperature for 20 days**.** The dried 500 g seed was powdered and soaked separately in 100 ml n-Hexane, Acetone, Ethanol, CCl_4, and Water by keeping it in a shaker for 3 days. The extracts were filtered into a Petri plate using Whatman filter paper. It is set for evaporation. The dried sample is evaluated for its pharmacological activity.

Optimization of dose:-

A total of 20 mice were randomly allotted to one control and 3 treatment groups. The drug in each case was suspended in 0.1% CMC. The suspended extract was administered orally in three doses, namely 0.5, 1.0, and 5 g/kg body weight. The toxic symptoms observed were autonomic responses, motor activity and CNS excitation, etc. The animals were observed for 24 h for all signs of toxicity and mortality. Acute treatment with 0.5 g/kg was found to cause significant lowering in blood glucose levels in the treatment groups as compared to the control; hence, this dose was selected as the pharmacologically active dose. After optimization of dose random screening of test compound has been done with their pharmacological potency. On the basis of their potencies three different solvent extracts has been selected for rest of experiment e.g-alcohol, CCl_4 and acetone extracts.

Pharmacological Activity:-

Alcohol Induced Hepatotoxicity:-

Hepatic injury in rats was induced separately by administration of equal dose of alcohol (0.4 ml/kg, i.p.). Liver damage was monitored by raised biochemical marker enzymes (SGOT, SGPT, ALKP, TP, TB and TCHL)[7,8,9,10,11].

Experimental Design:

Group-I-Normal Group (with saline water 10 ml/kg)

Group-II-Control Group (with alcohol 0.4ml/kgi.p)

Group-III-Standard Group (with Silymarin 100mg/kg i.p)

Group-IV-Test Group (EEF 500mg/kg p.o)

Group-V- Test Group (EEF 1000mg/kg p.o)

Group-VI- Test Group (CEF 500mg/kg p.o)

Group-VII- Test Group (CEF 1000mg/kg p.o)

Group-VIII- Test Group (AEF 500mg/kg p.o)

Group-IX- Test Group (AEF 1000mg/kg p.o)

Male Albino Wister rats weighing 150–200 gm were employed for assessing the Hepatoprotective activity. They were fed with a standard pellet diet and water *ad libitium*. Nine groups of 6 rats was kept in a plastic cage and maintained at 25oC to 28oC with 40-70% RH and 12 hr light/dark cycles and were fastened for 12 hours prior to the experiment. The nine groups of 6 rats in each group, the first group served as normal control, which was untreated which received saline. The 2nd group as toxic control received alcohol (0.4 ml/kg b.w, p.o.,1.25% solution of alkyl alcohol in water) on the first day only to produce toxicity in liver and thereafter no treatment of fractions. 3rd group as standard received alcohol on the first day and thereafter received treatment with standard drug silymarin at a dose of 100 mg/kg body weight, p.o for 14 days. The group 4th to 9th received alcohol on the first day and then treated with different extracts of fenugreek seeds as ethanol, ccl_4 and acetone dose of 500 and 1000 mg/kg body weight, p.o for 14 days. All dosing was started at the same time in the morning to avoid the effects of biological rhythm changes.

Method of analysis

On 14th day the blood samples of four rats from each group were withdrawn by puncturing the retro orbital plexus under ether anesthesia. The blood samples were allowed to clot for 30-40 min. at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min. and various biochemical parameters were estimated. Biochemical marker enzymes (SGOT, SGPT, ALKP, TP, TB and TCHL) were carried out by reported methods.

*Paracetamol induced hepatotoxicity***:** The hepatotoxic effect of paracetamol (acetaminophen) is due to formation of hepatotoxic metabolite [12]. Paracetamol is commonly used as analgesic and antipyretic drug but causes liver damage in high doses [13,14]. Administration of paracetamol produces necrosis of centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesions [15]. The paracetamol

is broken down to sulphate and glucuronide conjugates after that it's metabolized to reactive intermediate. It's depolluted by conjugation with glutathione. The covalent binding of N-acetyl- P-benzoquinone imine, an oxidative product of paracetamol to sulphydryl groups of protein, result in lipid peroxidative degradation of glutathione and causes cell necrosis in the liver [13,14]. In various studies, different doses of paracetamol have been used to produce hepatotoxicity. They vary from 1 g/kg to 3 g/kg; p.o. single dose of paracetamol administration on 10th, 5th or 3rd day of experiment [15,16].

Animals: Male *Wistar* rats weighing 200 ± 20g were used for this study. The animals were kept inpolypropylene cages and maintained at 25 ± 5ºC under 12 h light/dark cycle. The animals were allowed free access standard pellet diet and water ad libitum.

Experimental protocol: Animals were divided into 9 groups of six rats each and treated orally as below for 15 days.

Group-I-Normal Group (with saline water 10 ml/kg)

Group-II-Control Group (with PCM 500mg/kgi.p)

Group-III-Standard Group (with Silymarin 100mg/kg i.p)

Group-IV-Test Group (EEF 500mg/kg p.o) + PCM as group II

Group-V- Test Group (EEF 1000mg/kg p.o) + PCM as group II

Group-VI- Test Group (CEF 500mg/kg p.o) + PCM as group II

Group-VII- Test Group (CEF 1000mg/kg p.o) + PCM as group II

Group-VIII- Test Group (AEF 500mg/kg p.o) + PCM as group II

Group-IX- Test Group (AEF 1000mg/kg p.o) + PCM as group II

Method of analysis

On 15th day the blood samples of four rats from each group were withdrawn by puncturing the retro orbital plexus under ether anesthesia. The blood samples were allowed to clot for 30-40 min. at room temperature. Serum was separated by centrifugation at 3000 rpm at 4°C

for 10min and used for measurement of various biochemical markers like (SGOT, SGPT, ALKP, TP, TB and TCHL) were carried out by reported methods.

Anti-inflammatory Activity:-

***Effect of various extracts doses of fenugreek seeds in carrageenan induced rat paw edema*:**-Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay. Edema was induced by sub-plantar injection of 0.1ml of 1% freshly prepared solution of carrageenan in distilled water into the right-hind paws of each rat of all the groups except the normal group-I. Animals of standard/test group-III, IV, V, VI, VII, VIII, IX were treated with the single dose of standard drug (Diclofenac sodium 4mg/kg) and various extracts doses of test compound e.g-500 mg/kg and 1000 mg/kg, respectively 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at "0 hour" and then at 1, 2, 3, 4, 5 and 24th hour after carrageenan injection. Increase in paw thickness was measured as the difference in paw thickness at "0 hour" and paw thickness at respective hours.

- **Experimental Protocol:**
- Group-I-Normal Control Group (with saline water 10 ml/kg)
- Group-II- Control Group(with carrageenan 0.1ml of 1% w/v)
- Group-III-Standard Group (with Diclofenac Sodium 4mg/kg i.p)
- Group-IV-Test Group (EEF 500mg/kg p.o)
- Group-V- Test Group (EEF 1000mg/kg p.o)
- Group-VI- Test Group (CEF 500mg/kg p.o)
- Group-VII- Test Group (CEF 1000mg/kg p.o)
- Group-VIII- Test Group (AEF 500mg/kg p.o)
- Group-IX- Test Group (AEF 1000mg/kg p.o)

STATISTICAL ANALYSIS:- Data was put across as the mean ± SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA)

followed by Dunnett's 't' test, which was used to identify difference between groups. P value <0.05was considered as significant.

RESULTS AND DISCUSSION

Hepatoprotective activity of various extracts of fenugreek seeds against alcohol:- This screening model for Hepatoprotective activity of fenugreek seed extracts was carried out with alcohol reagent. Alcohol was the induction agent for liver toxicity during the experiment. After observation, all the test group of experiment three test groups showed signifies results in comparison with standard drug group. The all selected 3 test groups showed magnificence pharmacological activity which were like-alcoholic extracts>CCl_4 extracts> acetone extracts. In all test group which was treated by fenugreek seeds extracts all biochemical markers which caused the liver toxicity had been reduced in dose dependent manner.

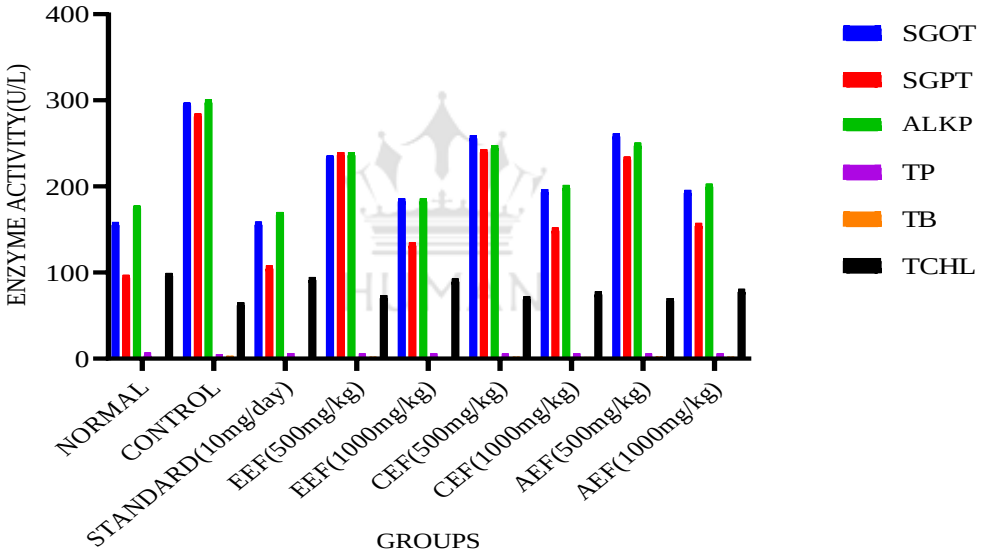


Figure No. 1: Hepatoprotective activity of various extracts of fenugreek seeds against alcohol

Hepatoprotective activity of various extracts of fenugreek seeds against paracetamol:- This screening model for Hepatoprotective activity of fenugreek seed extracts was carried out with paracetamol compound. Generally, PCM is used as an antipyretic drug in humans, so this compound is also responsible for the liver toxicity in many treated people. Here this drug is used as an toxicant for this model. After observation, all the test group of experiment three

test groups showed signifies results in comparison with standard drug group. The all selected 3 test groups showed magnificence pharmacological activity which were like-alcoholic extracts>CCl_4 extracts> acetone extracts. In all test group which was treated by fenugreek seeds extracts all biochemical markers which caused the liver toxicity had been reduced in dose dependent manner.

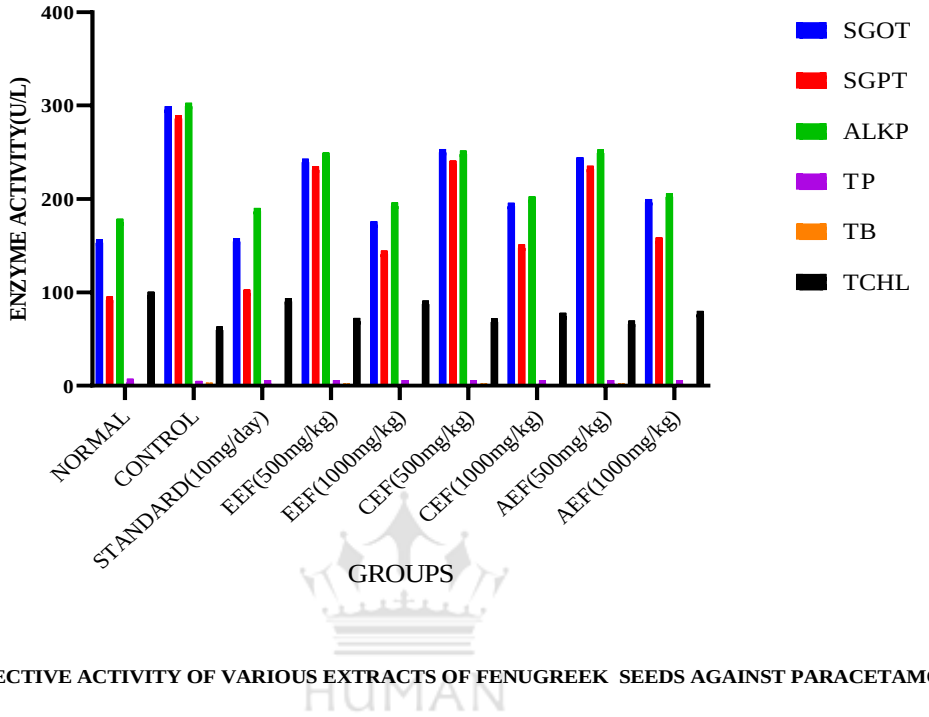
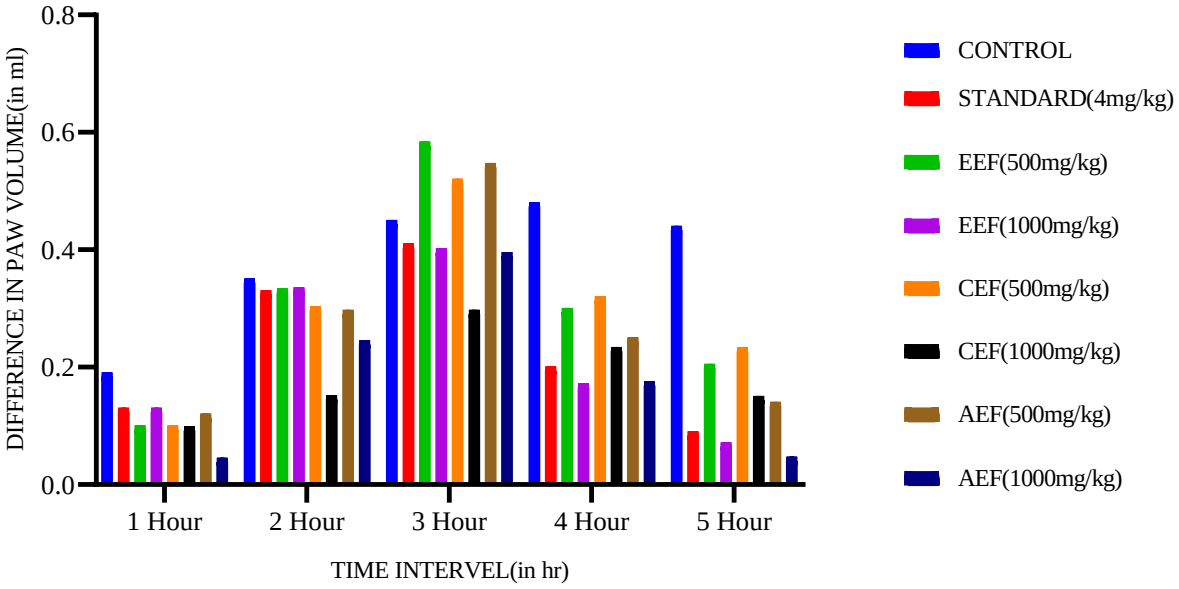


Figure No. 2: Hepatoprotective activity of various extracts of fenugreek seeds against paracetamol

Effect of various extracts doses of fenugreek seeds in carrageenan induced rat paw edema:-Whenever the liver gets toxic the inflammation is already our in liver during this condition. So the inflammation is also a major condition in imbalance functioning of liver. Some time in many people it is showing in form of fatty liver and liver enlargement in size from normal size generally which is called as liver cirrhosis. In this screening model, all three extracts showed better potency in reduction of inflammation mediators. The all selected 3 test groups showed magnificence pharmacological activity which were like-alcoholic extracts>CCl_4 extracts> acetone extracts.



CARAGEENAN INDUCED PAW EDEMA TEST

Figure No. 3: Effect of various extracts doses of fenugreek seeds in carrageenan induced rat paw edema

Present study focuses on nutritional and health benefits of fenugreek. Fenugreek is rich in fiber, protein, and bioactive components giving it promising nutritional and health beneficial properties. Major health beneficial properties of fenugreek like antidiabetic, antioxidant, anticarcinogenic, hypoglycemic activity and hypocholesterolemic activity have been discussed in this research article. Based on these medicinal properties, as reported by scientific findings, fenugreek can be recommended and can be made a part of our daily diet as its liberal use is safe and various health benefit can be drawn from this natural herb. Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by acetaminophen higher doses in experimental animals and humans. For screening of Hepatoprotective agents, alcohol and paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. Administration of paracetamol caused a significant elevation of enzymes level such as AST, ALT, ALP and bilirubin level has been attributed to the damage structural integrity of liver because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity. The co administrations of all examined plants extract have prevented the increased serum marker enzymes AST, ALT, ALP level and bilirubin level. This is in agreement with the

commonly accepted view that serum levels of AST, ALT and ALP return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes the traditional system of medicines viz. Ayurvedic, Unani etc. can provide us with valuable guidelines to the selection, preparation and application of herbal formulation for hepatic dysfunction. A large number of medicinal plants have been used traditionally for immunomodulation and hepatoprotection. In order to validate the effects of these compounds, their efficacy in experimental models of hepatic dysfunction needs to be investigated.

CONCLUSION

There are lots of advances in modern medicine, but unfortunately, there are very few drugs that protect the damaged liver and help in regeneration of hepatic cells. Traditional uses of herbal medicines have been documented since long historical period and they are widely acknowledged to be safe and effective and recognized as a form of alternative medicine in conventional scientific based health care system. In recent decade, complementary and alternative medicine approach using medicinal plants for prevention and treatment of diseases have been gaining importance. Medicinal plants exhibit efficacy in treatment of a number of diseases which are not otherwise cured by synthetic drugs. In this research study basically, the treatment has been done with natural remedy which obtained from fenugreek seed in different solvents extract form. After seeing all the observation and interpretation of obtained data the study got finalized that alcoholic extracts showed magnifies potency and CCl^4 extract give better potency against all toxicant but acetone extract not showing better activity but it also showed some pharmacological potency against toxicant during the whole experiments.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Image *Author -1*	***PRASHANT RAGHAV– Corresponding Author*** *DEPARTMENT OF PHARMACEUTICAL SCIENCES SHUATS NAINI ALLAHABAD* *3C/210 BUDHI VIHAR MAJHOLA MORDABAD-244001*
Image *Author -2*	***Dr. DANISH AHMED*** *DEPARTMENT OF PHARMACEUTICAL SCIENCES SHUATS NAINI ALLAHABAD*
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