EVALUATION OF ANTI-HYPERURICEMIC PROPERTY OF *TRIGONELLA FOENUM GREACUM* SEEDS EXTRACT IN ETHAMBUTOL INDUCED HYPERURICEMIA IN RAT

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ABSTRACT

This study's goal is to determine the role of *Trigonella foenum-graecum* (fenugreek) extract of seeds on hyperuricemia as well as on the kidneys of hyperuricemic rats which were induced combined dose of ethambutol 250 mg/kg and adenine 100 mg/kg and this was treated by the extract of *Trigonella foenum-graecum* with a higher dose of 500 mg/kg along with a lower dose 250 mg/kg and the std. drug allopurinol 10 mg/kg for 21 days one-hour next of induced dose administered intragastrically and the different biochemical parameter was evaluated on 21 th day UA in serum, BUN, and CRE, and on the same day the rats were sacrificed. TFGE administration reduced the concentrations of UA, BUN, as well as CRE in serum meanwhile treatment with TFGE reverse the accumulated uric acid crystal in the kidney which was determined by the histopathological study of kidneys.

TLC and HPLC were the two methods to identify various chemical constituents existing in the *Trigonella foenum-graecum* which is accountable for the hypouricemic property of the *Trigonella foenum-graecum* along with this the plant shows different types of pharmacological action which is already reported in other research.

Two different doses were given to the rats to find out the best dose of *trigonellinea foenum-graecum* but there was no discernible variation between the LD treatment and HD treatment therefore the LD of TFGE can be taken for the treatment of hyperuricemia

In summary, the research showed that TFGE exhibited anti-hyperuricemic properties by increasing the rate of excretion of uric acid from the body

Keywords- *Trigonella foenum-graecum,* hyperuricemia, uric acid, creatinine, blood urea nitrogen, histopathological, biochemical

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1. INTRODUCTION

1.1 HYPERURICEMIA

Uric acid (UA) level in the blood 7.0 mg/dl or above in men and 6.0 mg/dl in women are considered to be hyperuricemia. UA is the metabolized ultimate outcome of endogenous and exogenous purine **Maiuolo et al. [26]**

The exogenous source is dependent upon the diet which is a high-protein diet and Endogenous uric acid production occurs mostly in the liver, colon, and other tissue muscles, as well as the kidney., and vascular endothelium **Choudhary et al. [6]** Between 300 and 400 mg per day, there is a fairly consistent production and elimination of purines about 2/3 rd of the UA load is eliminated by the kidneys, while the other 1/3rd leaves the body through the G.I system.

Renal excretion of UA is one of the major regulators of serum uric acid **Ruiz et al. [33]** Additionally, studies have linked hyperuricemia to vascular conditions that are closely related to each other, such as vascular dementia, Type 2 DM, and kidney failure, as well as to conditions that are risk factors for CVS, such as hypertension, metabolic syndrome, and coronary artery disease**.** **Jin et al. [16]**

1.2 PATHOPHYSIOLOGY

With an M.W of 168 Da and the chemical formula $C_5H_4N_4O_3$), UA is an organic compound with a heterocyclic ring. A number of enzymes synthesize the two purines that is adenine, and guanine, into UA. **Maiuolo et al. [26]**The equilibrium between the two purine metabolism and excretion is responsible for maintaining balanced levels of UA **Yanai et al. [46]** .the rate of catabolism is done by uricase enzyme into urate which is not present in humans. The liver mainly synthesizes uric acid and another source of uric acid is from the breakdown of food sources almost one-third, by the breakdown of endogenous purine compounds

Desai et al. **[10]** adenine and guanine are converted into hypoxanthine, then the enzyme xanthine oxidase converts this hypoxanthine to xanthine then xanthine oxidase again converts xanthine to uric acid which is irreversible. **Teng et al. [39]**

The kidneys are responsible for eliminating uric acid roughly 95% of UA is filtered out easily by the help of glomerulus filtration in the bowman's capsule but subsequently almost fully reabsorbed in the PCT. the presence of transmembrane channels which operated the transport of urate in the kidney are URAT1 and the OAT family regulates the serum uric acid level **Becker et al. [3]** This determines the net urate excretion.

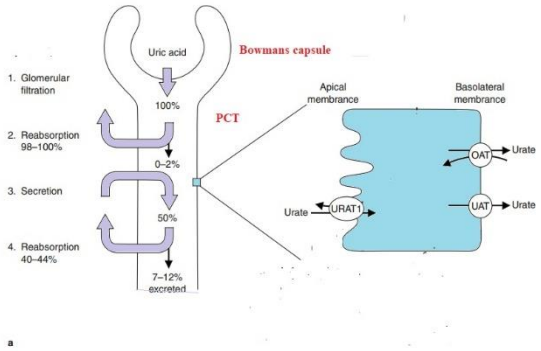


Figure 1.1 Uric acid transport in the nephron

1.3 EPIDEMIOLOGY

Purine-rich. diet overconsumption is the major reason behind the prevalence of hyperuricemia globally **Xu et al. [43]** The incidence of hyperuricemia is rising in countries having high-income and the world developing economically with a Western lifestyle **Li et al. [23]** The majority of epidemiological studies demonstrate that high-income countries typically have higher prevalence rates of hyperuricemia than underdeveloped countries. Information from the nationally representative National Health and Nutrition Examination Survey 2007-2016 revealed that the prevalence of hyperuricemia in the US remained stable between 2015 to16, with pervasiveness rates of 20.2% for men and 20.0% for women**.** In Ireland, from 2006 to 2014, the pervasiveness of hyperuricemia rose in both in males 19.7% to 25.0% and in women 20.5% to 24.1% **Kumar A.U et al. [22**

1.4 Current Treatment and Management

1.4.1 Classification

Classification of the anti-hyperuricemic drug is explained in table no 1.1 according to **Tripathi K.D. [40]**

All guidelines still recommend using inhibitor of Xanthine Oxidase Inhibitors (XOIs) as the first choice of treatment. With the exception of the ACR guidelines, which recommend either allopurinol or febuxostat, allopurinol is the first-line medication among them. When monotherapy is ineffective, the second choice is uricosurics, and all recent guidelines still support the use of combinations of drugs together **Sattui and Gaffo. [36]**

Disease-modifying anti-hyperuricemic drugs.

Acute gouty arthritis		
	NSAID	Naproxen, piroxicam, diclofenac Indomethacin, eterocoxib
	Colchicine	
	Glucocorticoids	Prednisolone, Triamcinolone
Chronic gouty/Hyperuricemia		
	Uricosurics	Probenecid, Sulfinpyrazone
	Synthesis inhibitor	Allopurinol Febuxostat

Table 1.1: Drug for the treatment of hyperuricemia

The first priority in the treatment of hyperuricemia is to prevent the pain of acute episodes which are marked by a cellular inflammatory response brought on by urate crystal deposition **Gliozzi et al. [13]** These events are controlled with the medications, colchicine, glucocorticoids, indomethacin, and celecoxib (NSAIDs) with different modes of action.

1.4.2 Mode of Action of Drug

The action of NSAIDs results from up-regulated COX-2's suppression of prostaglandin synthesis, It is regarded as the key factor in triggering the inflammatory reactions that characterize waves of gouty arthritis. Colchicine reduces inflammation by preventing the production of IL-1beta in monocytes that have been activated by monosodium urate (MSU). Additionally, it inhibits the formation of superoxide anion, chemotaxis, attachment to cellular substrata, and the Tyrosine kinases and phospholipases are downregulated in neutrophils during phagocytosis lysosomal enzymes are released **Cronstein et al. [9]**The well-known anti-inflammatory effects of glucocorticoids is due to their interaction with receptors of glucocorticoids, which are found in target cells, almost all of which are bodily tissues. The glucocorticoids' capacity is to prevent the activation of transcription factors that promote inflammation including NF-B and activating protein-1 and inhibit the synthsesis of IL-1beta**. Gllozzi et al. [13]**

There are two main classes to treat hyperuricemia uricosuric medications: sulphinpyrazone, probenecid, and benzbromarone, which reduce urate reabsorption in the renal tubule and raise urine uric acid excretion and the other one Uricostatic medications, such as allopurinol, reduce uric acid production by competitively inhibiting xanthine oxidase. **Suresh and Das [36]**

1.5 Newer Treatment of Gout

Interleukin-1 (IL-1), IL-8, and TNF-a are among the cytokines that contribute to the pathogenesis of gout, but IL-1b appears to be the main mediator **Charles A. Dinarello. [10]** Thus In order to control inflammation of l hyperuricemia, the effectiveness of IL-1 blocking and TNFα blocking medications is a good development **Busso and So [4]**

- Anakinra
- Rilonacept
- Canakin

1.6 Medicinal Plant in Hyperuricemia

There are several medicinal plants having anti-hyperuricemic properties that can reduce the level of UA in the body either by increasing its outflow or by acting on xanthine oxidase

Numerous compounds that are produced from plants have anti-hyperuricemic properties. By blocking the XO enzyme, chemical components such lignans, triterpenoids, and xanthophyll can reduce inflammation while phenolic compounds, flavonoids, tannins, essential oils, alkaloid and glucosides have the ability of gout treatment. The dual effects of tannins, lignans, and essential oils include a decrease in uric acid production and uricosuric activity. **Ling and Bochu. [10]**

2. MATERIAL AND METHOD

2.1 PLANT RESOURCE

The seed and the plant was collected from the local market of Prayagraj (U.P) India in the month of November, December and it was authenticated from the Botanical Survey of India, Prayagraj by the taxonomist of the institute with voucher accession no 2022-2023/827 collected seed are air dried under shade and moisture was evaporated and then it was powdered.

2.2 DRUG CHEMICAL AND INSTRUMENT

Ethambutol and allopurinol was a got free sample from IPCA Pharmaceuticals Sikkim, adenine was obtained from CDH, .India. HCL, KOH, ferric chloride, iodine, nitric acid, sulphuric acid Fehling's solution A and B, potassium permagnate, n-hexane, formic acid, sodium hydroxide, copper sulfate were obtained from Geetraj corporation and chemical laboratory. Acetic acid, ethyl acetate, picric acid, chloroform, ethanol, and formaldehyde, were obtained from the United Institute of Pharmacy, Naini Prayagraj. Aalpha-naphthol, millons reagent, dragendroff reagent, and biuret reagent obtained from Fizmerk India chemicals.

2.3 PREPARATION OF PLANT EXTRACT

The seeds were physically examined and washed to remove any extraneous substances, including weeds, stones, dirt, and grit. The seeds were pulverized using a laboratory grinder (Philips). all the smallest particle were removed through 0.420 mm mesh. The leftover material was once again crushed and sieved. **Baquer et al [2]**

The 50-gm seed powder of fenugreek was defatted using hexane as the solvent for 4 hours in a Soxhlet system. **Wani et al. [41]** Afterward, 80% ethanol was used to extract the defatted powder by ultrasound-assisted extraction (UAE) **Dsouza et al. [12]** A sealed conical flask containing twenty gram of defatted seed powder of fenugreek was used to conduct an extraction using ethanol 80% as the solvent and kept on the ultrasound bath and Throughout the extraction process, the ultrasonic bath's temperature was maintained at 35 C. **Wani et al. [31]**

Then the Purification of the extract is done by Using a separating funnel, To eliminate the fat content, the dried form of the extract was washed with 50 ml of diethyl ether after diluting with 50 ml of distilled water. The aq. surface was extracted using 50 ml of H_2O and saturated n-butanol 4 times after the ether layer was removed. To extract crude, the butanol mixture was added with distilled water in a small amount to eliminate contaminants. The residual butanol mixture was then transferred to the RBF after this rotating evaporation was done under vacuum at a temperature of 55 degrees Celsius and kept in a tight container in a refrigerator.

2.4 PHYSIOCHEMICAL ANALYSIS

The physiochemical analysis of the drug was done under the guidelines and the procedure of , **Khandelwal. [19] and Khan et al. [18]**

2.4.1 Foreign matter

Drugs ought to be made from the plant's verified portion. They must be completely devoid of any insects, mould, and visible and excreta contaminants like stones. Sand, poisonous and dangerous foreign objects, and chemical remnants **Patwekar et al [32]**

Procedure- The seeds were weighed after being divided into 10 gm, and the presence of foreign matter was calculated after the separation of overseas matter that was found using a lens (6x) .

2.4.2 Moisture content determination (loss on drying)

After precisely weighing the drug, It was kept in an evaporating dish and heated to 105 degrees Celsius in a hot air oven. for five hours and weighed again to determine the loss of drying and determine the percentage of moisture content.

2.4.3 Determination of ash values

Ash values were calculated in order to find low-quality products, used-up drugs, and sand or other earthy materials. Using water-soluble ash and acid-insoluble ash, it can also be used as a method of identifying the chemical components**.**

2.4.4 Total ash value

A tared silica crucible was filled with exactly 3 gm of air-dried powder, which was then burned until carbon-free at a temperature of no higher than 4500C. The mixture was then cooled, weighed, and the calculation were made to determine the total ash present in the crude drug with respect to the drug dried normally in air.

2.4.5 Acid insoluble ash

The ash produced was heated in 25ml of diluted hydrochloric acid for five minutes. The residue was gathered on free ash filter paper, cleaned with dist. water, lighted, and weighed. In order to determine acid-insoluble ash

2.4.6 water-soluble ash

On a filter paper(ash-free), the non-soluble material was gathered, washed with hot distilled water, and burnt at lower temperature to a weight of constant. The weight of the non-soluble substance is divided by the ash weight. The weight disparity represents the water-soluble ash. The amount of water-soluble ash in the medication that had been air-dried was calculated.

3.1 PHYTOCHEMICAL ANALYSIS

after extraction, the phytochemical screening was done to determine the presence of different phytochemicals available in the extracts by following the guidelines and procedures of **Khandelwal.[19] ,** and **Mahmood and Yahya.[25]**

3.1.1 Tests for Carbohydrates and Glycosides

3.5.1.1 Molisch test

Two to three drops of a 1% alcohol-naphthol solutionist tube's walls. When two liquids converge, a brown ring that indicates the presence of carbohydrates appears.

3.5.1.2 Legal's test

After adding a few drops of sodium nitroprusside solution and 1 ml of pyridine, the sample was turned alkaline by adding sodium hydroxide solution. The presence of glycosides is indicated by their pink-to-crimson appearance.

3.5.1.3 Borntrager's test

The formation of pink color when ammonium solution was added in same amounts to the chloroform layer which was formed after treating the sample with chloroform and the formation of the pink color showed that glycosides is present.

3.5.2 Test for alkaloids

3.5.2.1 Dragendorff's test

Separately, the material was mixed with a few drops of diluted hydrochloric acid. and treated with 2-3 drops of dragendroffs reagent formation of Reddish brown ppt indicated a positive result that alkaloid is present

3.5.2.2 Mayer's test

Separately, 2 ml of the sample were mixed with a few drops of the diluted hydrochloric acid and treated with Mayer's reagent formation of Cream color ppt indicated positive result for the presence of alkaloid.

3.5.3 Tests for Steroids and Triterpenoids

3.5.3.1 Libermann-Burchard test

The sample added a few drops of acetic anhydride, which caused it to boil and cool. The upper layer turned green to show the presence of steroids after adding concentrated sulfuric acid from the test tube's side, which caused a brown ring to form at the intersection of the two layers. Triterpenoids also contribute to the development of a rich red hue.

3.5.3.2 Salkowski test

After the sample has been exposed to a few drops of strong sulfuric acid, a lower layer that is yellow in colour forms as proof that triterpenoids are present**.**

3.5.4 Test for proteins and free amino acids

4.5.4.1 Million's Test

Small amounts of the substance were dissolved in a few ml of purified water and incorporated to Million's reagent processing; the red colour indicates the presence of protein and free amino acids.

3.5.4.2 Ninhydrin test

The presence of free amount of amino acid and protein was determined by dissolving the in water in small amount then treated with ninhydrin reagent which gives red color

3.5.5 Test for flavonoids

3.5.5.1Alkaline reagent test

Presence of flavonoid was determined by observing bright yellow color when slowly magnesium hydroxide was added drop wise that turns colorless when acid added drop wise.

3.5.5.2 Shinoda's test

After the sample had been dissolved in alcohol and a piece of magnesium added, concentrated hydrochloric acid was gradually added, followed by heat. The presence of magenta colour indicates the presence of flavonoids.

3.6 THIN LAYER CHROMATOGRAPHY OF EXTRACT

3.6.1 Preparation of sample

the sample is dissolved in ethanol (1 mg mL–1) Shaked for 5 to 10 min and the insoluble particles were removed with the help of filter paper.

3.6.2 Preparation of stationary phase

Mixing of 30 g of Silica Gel-G with 60 ml of distilled H_2O created a slurry. They were applied using a spreader device on a cleaned glass TLC plate 20 × 5 cm and the layered plates were air-dried for 15 minutes and then allowed to activate at 110 degrees delicious**. Bran and Hardman. [4]**

3.6.3 Saturation of chromatographic chamber

The chamber where the plates will be kept is made saturated by adding a suitable amount of mobile phase into the chamber and the area is mounted with filter paper in order to get uniformity.

3.6.4 Application of test sample

Using a micropipette, samples were deposited on plates at a distance of 10 mm - lateral border and 7 mm from the plate's lower edge.

3.6.5 Preparation of mobile phase

The different mobile phase was utilized for the effective partition of a constituent such as n-Heptane/ethyl acetate (7:3, V/V), Methanol/water (4:1, V/V), Chloroform/acetone (7:3, V/V), Butanol/water/acetic acid (4:1:1, V/V/V), Toluene: Ethyl acetate: Formic acid (6:5:1, v/v)

3.6.6 Development of chromatogram

After the plates had dried (15 min later), the separated bands is seen by misting them with a modified anisaldehyde reagent and heating them for 3 min at 105 °C.

3.6.7 Observation of the Chromatogram

The path traveled is observed under the shorter and the longer wavelength of UV light in daylight with the help of a needle the spots were marked retention factor was calculated After completing the TLC, the plate was observed under UV radiation at 254 to 350 nm the figure are estimated by the help of visible light. And the Rf value was calculated

$$\text{RF value} = \text{DSU} / \text{DSV}$$

DSU is the solute's travel distance

DSV is the solvent's travel distance

3.7 HPLC ANALYSIS

About 2 gm of fenugreek ethanolic extract was mixed with ethanol and used for the estimation of HPLC. **Kagus et al. [21]** Ethanolic extracts were analyzed, using an automated injector series pumping system for HPLC, Quantification, and validation Agilent Technologies' RP-Columns (250 mm 4.6 mm, 5.0 m) were used for separation, It involved injecting 10 L of phosphate buffer solution with a pH of 2.6 and isocratically eluting aceto-nitrile and water(5:95)and the flow rate was maintained. By comparing their retention durations to references, phenolic chemicals were identified

3.8 PHARMACOLOGICAL EVALUATION

3.8.1 Experimental Animals

Male Wistar rats were purchased from Chakraborty Enterprises, Kolkata, West Bengal (Reg. No. 1443/PO/BT/s/11/CPCSEA) Laboratory with a weight of the Rats 180–220 gram and were kept at a consistent 24 2 °C, 60 ± 5% relative humidity, and a 12 h day night cycle in a room. Over the length of the study, they received standard chow ad libitum, and prior to the start of the studies, they had a week to get used to the lab environment**. Oh et al. [31]**

3.8.2 Acute oral toxicity assessment

For the toxicity assessment, the Organisation for Economic Co-operation and Development's (OECD) criteria were followed. Three groups of the rats (n = 4) were randomly selected: gp.-A, gp.-B, and gp.- C and B and C received a single dose of 5000 mg/kg body weight, while A-Group received deionized water as a control. Up to the conclusion of the trial, all rats were provided access to free access to sterile water and standard animal feed (rat chow), as well as oral extracts of *Trigonella foenum-graecum* seeds.

The rats were intensively watched for the first 4 hours and then daily for 2 weeks after receiving Trigonella foenum-graecum seed extracts. They are watched for behavioral indicators of toxicity (such as agitation, dullness, and anxiety), respiratory pattern, ocular, skin, and diarrheal symptoms. Throughout the period of observation, the rats' patterns of food and water consumption were noted. For two weeks, digital scaling was used to weigh all of the animals once a week.

3.8.3 Selection of dose *Trigonella foenum-graecum seeds* extract

After giving the highest dose of the extract of the test drug no mortality was found so we have selected the dose of 1/10 th of the maximum dose which is 500 mg/kg as a higher dose and 250 mg/kg as a lower dose for our experiment.

3.8.4 Experimental Induction of Hyperuricemia

Wistar albino rats were given an oral dose of a freshly prepared solution of adenine (100 mg/kg) and ethambutol (250 mg/kg) once daily at 9:00 a.m. for three weeks. **Zhu et al[48]**

3.8.5 Experimental Design and Procedure

Wistar albino rats are divided into 5 groups. Each group contains 06 animals for the research.

Group 1 Normal control group was given water in a volume of 10 mL/kg .

Group 2 Negative control group was administered with combined dose adenine (100 mg/kg) and ethambutol (250 mg/kg) by gavage.

Group 3 Positive control group hyperuricemia induced by ethambutol and adenine treated by standard drug Allopurinol (10 mg/kg)

Group 4 Hyperuricemia induced by ethambutol and adenine treated by 250mg/kg of seed extract of *T. foenum-graceum*

Group 5 Hyperuricemia induced by ethambutol and adenine treated by 500mg/kg of seed extract of *T. foenum-graceum*

3.9 Model for Anti hyperuricemic Property

The male Wistar rats 180–220 g were purchased from Laboratory Animal Center Chakraborty Enterprises, Kolkata, West Bengal (Reg. No. 1443/PO/BT/s/11/CPCSEA) Laboratory Rats and housed in cages Before a week of the experiment began, the animals were kept in a room that was kept at 24 2 °C, 60 5% RH, and at 12 h day and night cycle to help them get used to the lab environment. Throughout the period of the study, they received the normal diet at their discretion. **Meng Z et al 2015**

The rats were separated into five groups, each with six rodents. **Zhou et al. [47]** All the group except the normal group was induced hyperuricemia by giving ethambutol (250 mg per kg) and adenine (100 mg/per kg) orally daily at 9:00 a.m. with the help of oral gavage for 21 days The positive control group was treated with standard drug allopurinol 10 mg /kg **Wu et al. [42]**

3.10 BIOCHEMICAL PARAMETER

3.10.1 URIC ACID, BUN, CREATININE

The blood sample was collected on the 21st day after fasting the experimental animal for twelve hours and sacrificed by the help of ether anesthesia after sacrificing the rats Blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UA) levels were measured in a serum sample for determining the result **Yamamoto et al. [45]**

3.11 HISTOPATHOLOGICAL STUDY

After sacrificing the experimental animal on the 21 days of the model the kidney and the liver detach after dissecting the animal **Y. Chen, et al**. **[7]** and were stored in an airtight container with formalin (10%v/v) at a temperature below 37ºC and incubated in a good hygienic place for evaluation of histopathology Finally, light microscope was used to examine kidney histological lesions. **Oh et al. [31]**

The histopathology was done at United Diagnostic and Research, Prayagraj

3.12 ANALYSIS OF STATICAL DATA

The evaluation of statical data was executed with the help of one-way ANOVA by Newman Keuls method used this analysis was done by “GraphPad\Prism 9\prism.exe" window software

4. RESULT

4.1 ANALYSIS OF PHYSIOCHEMICAL PARAMETER

Trigonella foenum greacum seeds were crushed and sieved through a 40-mesh screen to create a coarse powder. The plant material was ground up and put through the normal process for finding out physicochemical properties.

S.NO	Parameter	%W/W
1	Foreign matter	2.50%
2	Loss on drying	1.60%
3	Ethanolic extractive value	16.45%
4	Aqueous extractive value	10.24%
4	Total ash value	6.66%
5	Acid insoluble ash value	1.54%
6	Water soluble ash value	2.66%

Table 4.1physiochemichal parameter analysis of coarse powder of seeds of *Trigonella foenum greacum*

The different physiochemical parameter was performed on seeds of *Trigonella foenum greacum* first the foreign matter was determined, the loss of drying and determine moisture content in percent Determination of total ash values, Acid insoluble ash, Water-soluble ash and ethanolic extractive value was found to be greater than aqueous extractive value.

4.2 PHYTOCHEMICAL ANALYSIS

After extraction, the phytochemical screening was done to assess the presence of different phytochemicals in the extracts of seeds of *Trigonella foenum greacum*

S.NO	Phytochemical Test	Names	Aqueous Extract	Ethanolic extract
1	Alkaloids	Mayer	+	+
		Dragendorff	+	+
2	Phenolic compounds	Lead acetate	+	+
3	Carbohydrate	Bendict	-	+
		Molish	+	+
4	Glycosides	Killer-Llni	+	+
5	Steroids	Salkowskis	-	+

		Libermann-Burchard	+	+
6	Saponins	Foam test	+	-
		Mercuric chloride	+	+
7	Protein	Biuret	-	-
		Ninhydrin	+	+
8	Flavonoids	Lead acetate	-	-
		Ferric chloride	+	+
9	Terpenoids	Libermann-Burchard	+	+
		Trim-Hil	-	-

Table 4.2 phytochemical parameter analysis of extract of seeds of *Trigonella foenum greacu* screening shows the presence of Steroids, Triterpenoids, alkaloids, flavonoids, proteins, carbohydrates, Phenolic compounds, Carbohydrate, and glycosides

4.3 THIN LAYRER CHROMATOGRAPHY

The quantitate and the qualitative estimation was done by using TLC and HPLC of the extract of seeds of *Trigonella foenum greacum.* A no of phytoconstituent is present in the extract this method help in the determination of the constituent by the help of different solvent system of different ratio that is The different mobile phase was used for the effective separation of constituent such as n-Heptane/ethyl acetate (7:3, V/V), Methanol/water (4:1, V/V), Chloroform/acetone (8:2, V/V), Butanol/water/acetic acid (4:1:1, V/V/V) separated bands were visualized by spraying with a modified anisaldehyde reagent and observed under shorter and the longer wavelength of U.V

S.NO	**Solvent system**	**R_f**
1	n-Heptane/ethyl acetate	0.47
2	Methanol/water	0.85
3	Chloroform/acetone	0.77
4	Butanol/water/acetic acid	0.94
5	Water/chloroform/acetone	

Table 4.3 TLC analysis of an ethanolic (80%) extract of *Trigonella foenum greacum* seed showing different R_f value

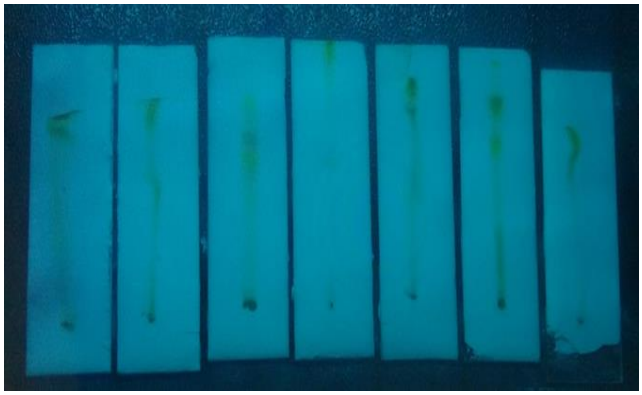
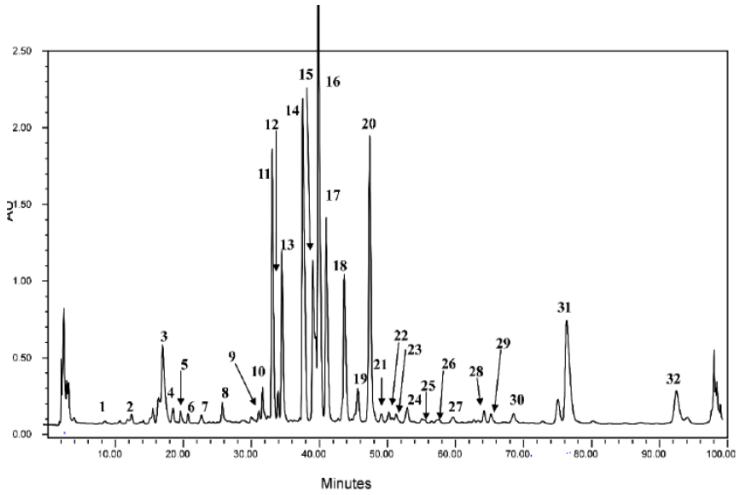


Figure 4.1 shows different spots of chemical constituents of extract of *Trigonella foenum greacum* on the TLC plate



4.4 HPTLC ANALYSIS

Figure 4.2- HPLC of ethanolic extract of fenugreek with different peaks

The ethanolic sample extract has a number of chromatographic peaks, as seen by the HPLC separation profile. the prominent peaks signify the presence of several chemical components.

Peak	**λmax (nm)**	**Compound Identification**
1-8	228-316	Phenolic acid
9, 11, 13, 14, 17	234-338	Non - Acylated flavonoid glycoside
10, 12, 15, 16, 18, 20-30	234-344	Acylated flavonoid glycoside

Table 4.4 HPLC profile of fenugreek showing different chemical constituent

4.5 PHARMACOLOGICAL EVALUATION

4.5.1 Hypouricemic property

The negative group are on a vehicle no drug was given to all six rats in the experimental period, in the positive group hyperuricemia was induced by the combined dose of Ethambutol 250 milligram per kg and Adenine 500 milligram per kg in each group except the negative control.

The standard group was treated with standard dose of Allopurinol 10 mg/kg The other two groups was treated with the *Trigonella foenum greacum.* Seeds extract with low high dose of 500 mg/kg and low dose of 250 mg/kg . all the dose was given for 21 days. The oral administration of TFGE 250 mg/kg 500mg/kg cause a dose-dependent fall in uric acid level recoded as 392.69±13.15 µmol/l - 257.01±16.52µmol/l and 392.69±13.15 µmol/l-254.21±9.11µmol/l respectively the hyperuricemic rat treated allopurinol 10 mg per kg which is a standard drug and the result recorded as 392.69±13.15 µmol/l -191.52±13.53 µmol/l

Table 4.5 Hypouricemic activity effects of Allopurinol and TFGE on uric acid in Ethambutol-adenine induced hyperuricemia

Group	Uric acid level (µmol/l)	BUN levels (µmol/l)	Creatinine levels (µmol/l)
Negative Control	**138.19±2.46**	**30.41±0.67**	**40.25±1.40**
Positive Control	**392.69±13.15***	**44.28±0.78***	**72.48±1.65***
Allopurinol 10 mg/kg	**191.52±13.53****	**33.99±0.58****	**46.98±0.66****
TFGE 250 mg/kg	**257.01±16.52****	**40.14±1.15****	**52.02±0.89****
TFGE 500 mg/kg	**254.21±9.11****	**38.58±1.10****	**51.42±0.65****

The data represent as mean ±SD of six rats in each group p 0.05 *in comparison of positive group with negative group and p 0.01 **show the significant difference as compared to the positive group

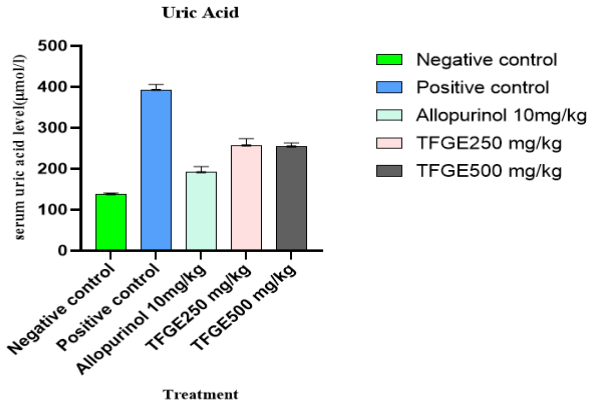


Figure 4.3 Graphics represent the uric acid level of experimental animal

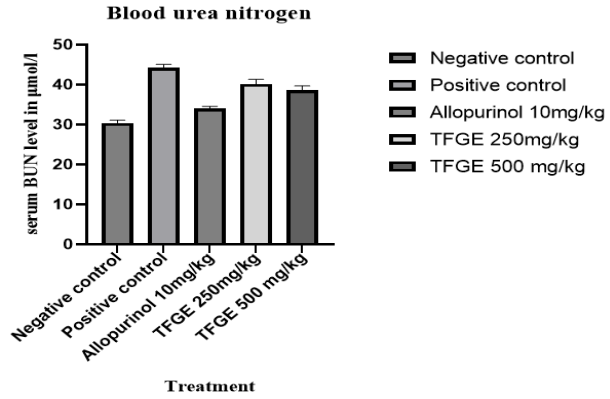


Figure 4.4 Graphics represent the BUN level of experimental animal

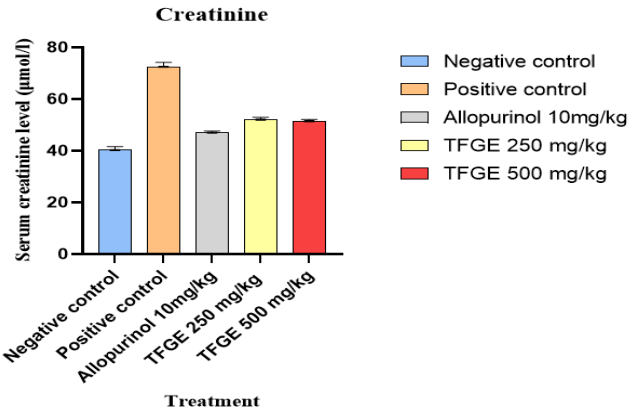
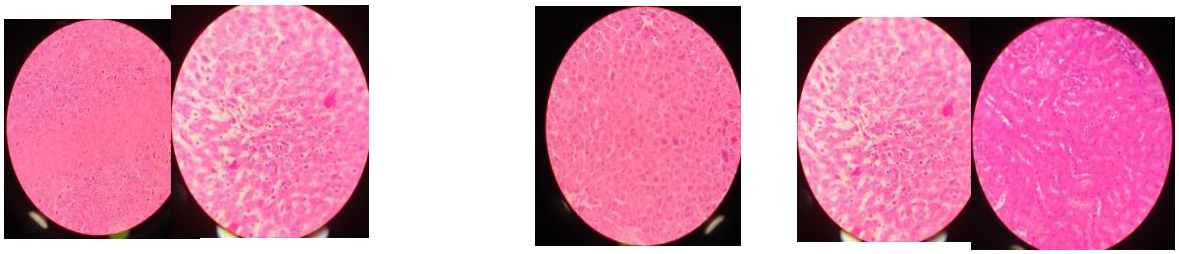


Figure 4.5 Graphics represent the creatinine level of experimental animal

4.5.2 HISTOPATHOLOGY



a)Negative b)Postive c)Standard treated d)TFGE 250 mg/kg e)TFGE 500mg/kg

Comparing the elevated serum UA level to the positive group, renal injury is associated with it. The comparison among the negative group and the different treatment groups is done with the hyperuricemic group and as a clear pathological indicator of hyperuricemia several organized structural alterations were noticed. Using a histological severity score, the tissue damage caused by ethambutol-adenine-induced hyperuricemia was quantified and kidney histological changes of negative control rats and positive induced rats were compared. the kidney tissues under a negative control Rats' morphology was normal and no sign of inflammation seen this group rat, while ethambutol-adenine-treated rats displayed a number of distinct histologic alterations, including edoema, proximal tubule necrosis, and microscopic borders between nearby proximal tubule cells. Tubulointerstitial and glomerular lesions were lessened compared to the positive group with the low-dose, high-dose treatment group (TFGE 250 mg/kg), (TFGE 500 mg/kg), and standard control ones (Allopurinol, 10 mg/kg) treated groups, the proximal tubule cells were controlled, and the cytoplasm was comparatively clear.

DISCUSSION

Over the past few decades, hyperuricemia occurs because to uric acid underexcretion or overproduction, hyperuricemia has been on the rise. UA values are greater in hyperuricemic patients, resulting in the uric acid crystal accumulating in the joints and producing excruciating, swelling, and redness. After the chronic period, kidney injury is brought on by changes in excretion that also lead to a considerable increase in level of CRE and BUN which is the most important biochemical parameter for the detection of kidney nephrotoxicity or kidney damage **Yamamoto et al [47]**. Less than half of the treatments, while being regularly used for individuals with hyperuricemia, were successful in lowering serum UA levels **Y. Chen, et al [7]**. There is no doubt that we need anti-hyperuricemic drugs with greater effectiveness and fewer side effects. *Trigonella foenum greacum.* show a variety of therapeutic property which has been already reported.

Different chemical and physical factors were evaluated in the study. The physiochemical parameter which includes foreign matter, to find out the presence of any organic or inorganic matter, extractive value, for the selection of the best solvent for extraction and the nature of the chemical constituent; percentage of loss on drying, to ascertain how much moisture is present in the crude form of the drug; estimation of ash value to estimate the quality, purity, and identification of the crude form of the drug.

Then, the results of the phytochemical examination revealed the existence of steroids, triterpenoids, proteins, flavonoids, alkaloids, carbohydrates, phenolic compounds, carbohydrates, and glycosides. TLC and HPLC qualitative methods were performed the identify the chemical constituents.

The hyperuricemia was induced by the combined dose of ethambutol and adenine **H. Wu et al.[42]** Ethambutol decreases the level of excretion of uric acid when given a dose of 250 mg/kg and adenine increases the level of purine in the body at a dose of 100 mg/kg and both show a combined effect and cause hyperuricemia, In the current investigation, we discovered that rats had higher-than-normal levels of blood uric acid, creatinine, and BUN as well as UA crystal deposition treated with ethambutol and adenine because of transmembrane channels URAT1 and the OAT which operate the transport of urate in the kidney is responsible for the cause of **Becker, et al [3]** induced hyperuricemia and according to a renal histological investigation, introduce of adenine and ethambutol to rats caused tubular ectasia with UA crystal accumulation. This hyperuricemia was treated by TFGE with two doses the low dose of 250 mg per kg and the high dose of 500mg/kg for 20 days and the result we found a significant decrease in the amount of uric acid in comparison with the positive group although the level of BUN and creatinine were also decreased and shows as a positive result. The standard drug was found to be a more positive result and show greater decreases in uric acid levels, BUN, and creatinine.

In the histological investigation, shows the UA crystal accumulation decreased in the treated group of allopurinol and TFGE.

This result shows that the *Trigonella foenum greacum seed* extract increases rate of excretion of uric acid from the body and shows hypouricemic property.

5. CONCLUSION

Hyperuricemia puts people at risk for developing diabetes mellitus, CVS disease, metabolic syndrome, and chronic renal disease. The rise in hyperuricemia over the past few decades has received a lot of attention. It is already understood that hyperuricemia is directly linked with excess production and low excretion of uric acid (UA) in patients. So for that, a multidisciplinary approach is needed for the management of hyperuricemia.

In conclusion, our research supported that TFGE could decrease the UA level and show hypouricemic properties in ethambutol-adenine hyperuricemia. *Trigonella foenum greacum* seed extract doses of 250 mg/kg and 500 mg/kg decrease elevated serum uric acid and substantial shifts in levels of Scr and BUN.

The histological investigation shows the UA crystal accumulation decreased in the treated group of allopurinol and TFGE both in the low and high doses.

The ethanolic extract of *Trigonella foenum greacum* seed shows the presence of proteins, triterpenoids, alkaloids, glycosides, steroids, carbohydrates, phenolic molecules, carbohydrates, and flavonoids.

There was no significant difference between treatment with a low dose of TFGE 250 milligram per kg and a high dose of 500 mg/kg so the treatment can be done by selecting the low dose of TFGE

Therefore, according to our findings' may be employed as a novel possible drug or supplement in the treatment of hyperuricemia.

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