ejpmr, 2018,5(3), 326-330

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIUROLITHIATIC ACTIVITY OF *GREWIA FLAVESCENS JUSS*. ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS.

G. N. Pramodini[1]*, Dr. Shaik Mohammed Khasim[2] and Dr. Parwez Alam[3]

[1]Associate Professor, Shadan College of Pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.
[2]Director, Shadan College of Pharmacy, Peerancheru, Hyderabad-500091, Telangana, India.
[3]Associate Professor, Shadan College of Pharmacy, Peerancheru, Hyderabad-500091, Telangana, India.

***Corresponding Author: G. N. Pramodini**

Associate Professor, Shadan College of Pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.

Article Received on 25/12/2017 **Article Revised on 15/01/2018** **Article Accepted on 05/02/2018**

ABSTRACT

Objective: To study the antiurolithiatic activity of grewia flavescens (tiliaceae) in ethylene glycol induced urolithiasis in male wistar rats. **Method**: The urolithiasis was induced in rats by oral feeding of ethylene glycolated water (0.75%) for 28 days. Ethanolic extract of EEGF (100mg/kg, 200mg/kg, and 400mg/kg) was administered orally from 1st day to 28th. **Results**: it was observed that the inducing agent ethylene glycol elevated the formation of kidney stones and kidney inflammation which resulted in decrease in urine output. Treatment with ethanolic extract of EEGF from 15th days significantly ($P<0.001$) reduced the kidney stones and resulted in restoring of kidney activity such as glomeruli and renal tube functioning. **Conclusion**: 28 days administration of ethylene glycol showed calcium oxalate crystals in renal tubules in negative control group. Treatment with EEGF decreases the calcium oxalate crystals on dose dependent manner and restored activity of glomeruli and renal tube functioning. Thus the results of our study reveal that EEGF effective in treatment of ethylene glycol induced urolithiasis in rats when compared to the standard drug cystone.

KEYWORDS: Antiurolithiasis, *Grewia flavescens*, Tiliaceae, Ethylene glycol.

1) INTRODUCTION

Urolithiasis is a complex process which is a consequence of an imbalance between promoters and inhibitors in the kidneys.[1] Greek ouron, "urine" and lithes, "stone". is the condition where urinary calculi are formed or located anywhere in the urinary formation system, or the process of stones formation in the kidney, bladder and /or ureter (urinary tract). Kidney stones are a common cause of blood in the urine and pain in the abdomen, flank, or groin. Kidney stones occur in only every 20 people at some time in their lives.[2] Nephrolithiasis (renal stones, urinary stones, urolithiasis, and renal calculi) affects great number of patients world wide[3] urolithiasis has affected humankind since antiquity and can persist with serious consequences throughout a patient's lifetime.[4]

Kidney stones are hard, solid particles that form in the urinary tract, in many cases; the stones are very small and can pass out of the body without any problems. However, if a stone (even a small one) blocks the flow of urine, excruciating pain may result and prompt medical treatment may be needed. Recurrent stone formation is a common part of the medical care of patients with stone diseases, calcium containing stones and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium, phosphate to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%[5] since it is one of the major problem that is disturbing the life style of young population. So, herbal extract formulation had their significance in the targets only one aspect of urolithiasis pathophysiology, most plant based therapy have been shown to be effective at different stages of stone pathophysiology.[6]

Grewia Flavescens popularly known as "donkey berry" is a shrub or small tree, often seen in groups along the edges of roads, river banks and dry rivers, growing in large uniform groups'. The plant parts are being used in India as folk medicine. The leaves were reported to be useful in ulcerated tongue, colic pain, wounds, cholera and dysentery. *Grewia Flavescens* is a multi stemmed shrub / small tree up to 5m height. Its bark is dark grey-brown belongs to Tiliaceae family. The plant is used traditionally as Anthelimentic, CNS depressant[7] nearly 40 species of this genus are found in India, some of which are well known for their medicinal value.[8,9]

Traditionally *Grewia Flavescens* root powder and decoction of roots were used to remove kidney stones and in urinary tract infections.[10] The present study is based on the traditional claim and an effort has been made to expedite /explore the scientific validity of the antiurolithiatic activity of EEGF whole plant extract in invitro model.

2) MATERIALS AND METHODS

Plant collection and identification

The crude *Grewia Flavescens* juss whole plant was collected from Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India. in the month of January, 2016. The plant was authenticated by plant taxonomist Dr. K. Madhava Chetty, Assistant Professor, Dept of Botany, Sri Venkateshwara University, Tirupati, AP, India.

The *Grewia Flavescens juss* whole plants were cut into proper size and washed 3 times with drinking water then dried in shade with proper care. The dried plant material were blended into course powder and passed through sieve 60.

Preparation of extract

The course powder 500gm was subjected to maceration and transferred to stoppered flask and treated with pure ethanol until the powder is fully immersed at room temperature. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours from time to time to ensure better extraction . This process is repeated for 7 days, followed by exhaustive maceration for 5 days by using solvent ethanol. The solvent was decanted and filtered with filter paper and recovered with the help of rotary vaccum evaporator. The extract was dried under desicator and stored in an air tight container [11]. The final extract was then subjected to investigate the antiurolithiatic activity.

Animals

Healthy adult male wister rats weighing between 150-200gm were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (Temperature: 25+/-2^{o}) and maintained on 12-h light; 12-h dark cycle they were provided with regular rat chow and drinking water ad libitum. The animal care and experimental protocols were approved by institutional animal ethical committee (IAEC) Registration number (1412/PO/Re/S/2011/CPCSEA).

3) ACTUE TOXICITY STUDIES

The acute toxicity studies of ethanolic extract of *Grewia flavescens* was determined as per the OECD guidelines no 423 (Acute toxic class method). It was observed that the test extract was safe even at 2000 mg/kg dose. This dose was taken as a therapeutic dose for the determination of antiurolithiatic effect. The LD_{50} of the EEGF was greater than 2000mg/kg. Hence, it is practically a nontoxic crude drug. So 1/10th (200mg/kg) and 1/5th (400mg/kg).[12]

4) PHARMACOLOGICAL SCREENING OF ANTIUROLITHIATIC ACTIVITY

Ethylene glycol induced hyperoxaluria model was used to assess the antilithialic activity in albino rats. Animals were divided into six groups containing six animals in each. group I served as control and received regular food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to group II-V for induction of renal calculi for 28 days. Group III received standard antiurolithiatic drug cystone (750mg/kg body weight) from 15th till 28th day. Group IV and V received EEGF at high dose (400mg/kg) medium dose (200mg/kg) and low dose (100mg/kg) p o from 15th to 28th day.[13]

ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY

a) Effect of EEGF plant extract on the kidney weight

The weights of the kidney of normal, induced, standard drug treated and extract treated group rats were weighed. The group III animals gained the least body weight as compared to the normal. control and extract treated groups. In addition, the wet weight of kidney were taken and compared between the groups.

b) Collection of urine

Urine samples were collected on 28th day for 24 h by keeping the animals in individual propylene metabolic cages. Animals had free access to drinking water during the urine collection period.

c) Histopathology of kidneys

To confirm the incidence of urolithiasis the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. the kidneys were cleaned off from extraneous tissue and transferred to 10^{o} F neutralized formalin solution (P^{H} 7.4) sections of kidney was fixed in paraffin, stained with hemotoxylin and eosin and observed for histopathological studies.

5) STATISTICAL ANALYSIS

Statistical analysis was carried out using graph pad prism (version 5) software. results were expressed as mean +/- SEM. Group of data were compared applying one – way ANOVA followed by Dennett's multiple comparison test. Differences between the data were considered significant at $P<0.05$.

6) RESULTS AND DISCUSSION

Kidney weight and urinary volume

The kidney weight was significantly increased ($P<0.001$) in ethylene glycol induced group II animals when compared to the control group animals. Whereas the standard treated group animals was shown significant ($P<0.001$) reduction in the kidney weight when compared to the EEGF induced group I animals. Similarly a significant ($p<0.001$) decrease in the kidney weights also identified in EEGF treated group (Table-1).

The urinary volume was significantly ($P<0.001$) decreased in ethylene glycol induced group II animals. Whereas the standard treated group animals was shown significant ($P<0.01$) increase in urine output when compared to the ethylene glycol induced group II animals. Similarly a significant increase in the urinary volume is also identified in EEGF treated ($P<0.05$) animals.

Table 1: Effect of Anti-urolithiasis activity of EEGF in wistar rats: Ethylene Glycol induced Urolithiasis Model:

S.No.	Treatment	Kidney wt. in gm.	Urine out put
1	Control	0.8550±0.033	7±.035
2	Disease control	1.380±0.056	11±0.35
3	Standard cystone	0.9575±0.0188***	7.125±0.42***
4	EEGF 100mg/kg	1.373±0.035ns	9±0.20**
5	EEGF 200mg/kg	1.145±0.033**	8.05±0.21***
6	EEGF 400mg/kg	1.045±0.039***	7.325±0.25***

*P<0.05, **P<0.01, ***P<0.001 Compared with Disease control, ns = Not significant Compared with Disease control.

Histopathology of kidneys

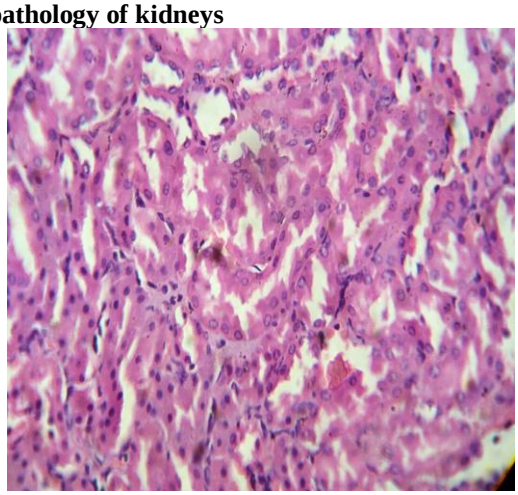


Figure 1: Normal control.

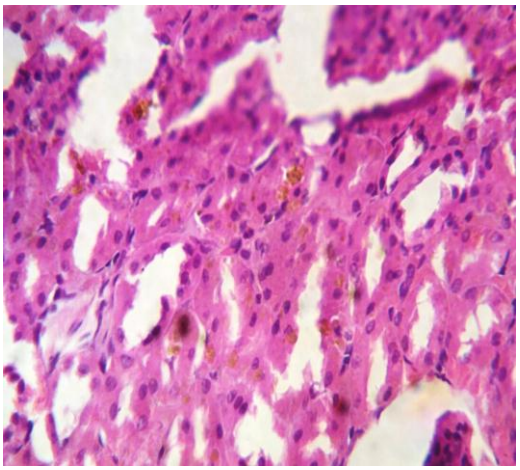
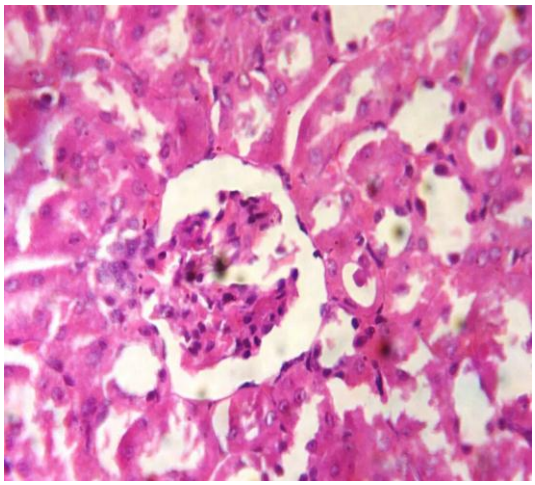
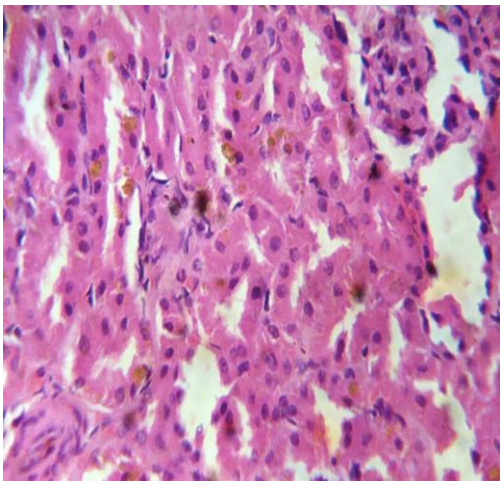


Figure 2: Negative control.



Figrue 3: Standard cystone 750 mg/kg.



Figrue 4: Low dose EEGF100mg/kg.

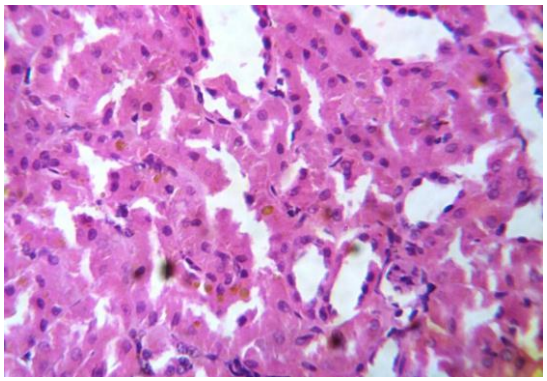


Figure 5: Medium dose EEGF 200mg/kg.

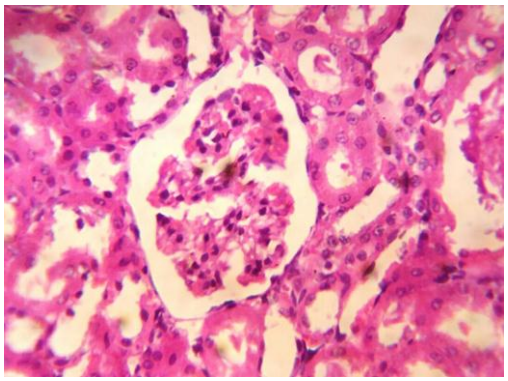


Figure 6: High dose EEGF 400mg/kg.

Histopathology of kidneys

Kidneys of rats stained with hematoxylin and eosin (100 × magnification) - (a) control group showing normal architecture of renal cortex and medulla; (b) urolithiatic group showing golden brown crystals in renal tubules and occasionally in glomerulai; (c) standard group no abnormalities were detected; (d) EEGF group treated with 100mg/kg extract showing mildly multifocal mild degree deposits of golden brown crystals in renal tubules and occasionally in glomerulai; (e) EEGF group treated with 200mg/kg extract showing minimally multifocal mild degree deposits of golden brown crystals in renal tubules and occasionally in glomerulai; (f) EEGF group treated with 400mg/kg extract no abnormalities were detected in renal tubules and glomerulai.

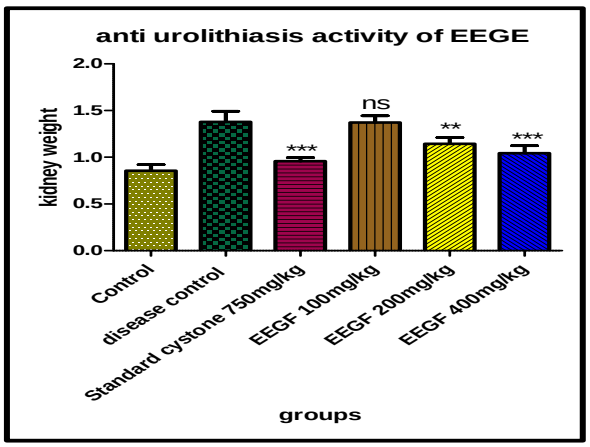


Figure 7: Effect of Anti –Urolithiasis activity of EEGF whole plant in ethylene glycol induced model (Kidney weight).

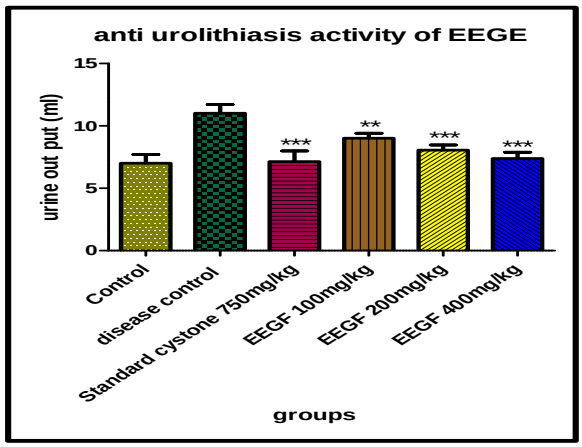


Figure 8: Effect of anti Urolithiasis activity of EEGF whole plant in ethylene glycol induced model (Urine output in ml).

7) CONCLUSION

Present study was conducted to evaluate the antiurolithiatic activity of ethanolic extract of whole plant parts of *Grewia Flavescens* juss. The extract prevented the formation of stones supporting the traditional claim.

28 days administration of ethylene glycol showed calcium oxalate crystals in renal tubule in negative control group. Treatment with (EEGF) ethanolic extracts of *Grewia Flavescens* juss. decreased calcium oxalate crystals on dose dependent manner and restored activity of glomeruli and renal tubule functioning. Histological findings also supported the study. The antiuroliatic activity may be apparently due to flavonoids, saponins and phenolic compoumds which were present in the whole plant. Thus the results of our study reveal that EEGF is effective in treatment of ethylene glycol induced urolithiasis in rats compared to the standard drug cystone. Further work has to be perfoemed to isolate the active chemical constituents responsible for the antiurolithiatic activity.

8) REFERENCES

1. M. Nishanthi, B.Vijaya kumar and M.Vijay Aanandhi. Antiurolithiatic activity of the plant extract of peperomia tetraphylla on ethylene glycol induced urolithiasis on rats. Rasayana journal of chemistry, 2016; 9(2): 294-299.
2. Fayed Ibrahim, Afrah Alasbohy. Parsley: mechanism as antiurolithiasis remedy. International journal of medical and health research., June, 2017; 3(7): 35-40.
3. Khashayar Sakhaee, Naim.M. Maaloof and Bridget Sinnott. Kidney stones 2012: Pathogenisis, Diagnosis and management. The journal of clinical endocrinology and metabolism, 2012; 97(6): 1847-1860.
4. Samrik K. Shah, Kruti M.Patel, Pinal M.Vaviya. Evaluation of Antiurolithiatic activity of Betula Utilis in Rats using ethylene glycol model. Asian Journal of Pharmaceutical Research, 2017; 7(2).
5. Unnati Atodariya, Roshni Barad, Siddhi Upadhyay, Umesh Upadhyay. Antiurolithiatic activity of Dolichos Biflorus Seeds. Journal of pharmacognosy and phytochemistry, 2013; 2(2): 209-213.
6. P.Rajeshwari, G.Rajeshwari, SK.Jabbikulla, I. Vishnu Varshan. Indian Journal of pharmacognostical and pharmaceutical sciences, 2013; 5(suppl 3): 599-601.
7. Rama Mohan Reddy. M, Yanadaiah j.p, Rvindra Reddy.K, Lakshman Kumar.D, Balamuralidharan, Assessment of antidiabetic activity of ethanolic extract of Grewia flavescens. Juss leaves against Alloxan induced diabetis in rats. Journal of Global trends in pharmaceutical sciences. 2013; 4(2): 1086-1090.
8. G.N.Pramodini, Dr.K.Appanna Chowdry, Perwez Alam and Seema Firdouse. Antiulcer activity of ethanolic extract of Grewia flavescens. Juss Whole Plant in Rats. EJPMR, 2017; 4(06): ne436-440.
9. Praveen kumar Goyal, pharmaceutical and pharmacological properties of the genus Grewia: A Review; Indian journal of pharmacy and pharmaceutical sciences, 2012; 4(suppl 4)L: 72-78.
10. Neha Sharma, Bakeet singh Tanwer and Rekha Vijayvergia. Study of medicinal plants in Aravali

regions of Rajasthan for treatment of kidney stones and urinary tract trobles. Indian journal of pharma Tech Research, 2011; 3(1): 110-113.
11. Harbone J B. Phytochemical methods: A Guide to modern Techique of plant analysis.chapmam and hall, London. 1984.
12. G.N.Pramodini and Dr.Appanna chowdary; Study of Acute toxicity and Anti-inflammatroy activity of ethanolic extract of Grewia flavescens. Juss Whole Plant in Rats. EJPMResearch., 2017; 4(4): 595-599.
13. Elias. Edwin Jarald, Pankaj Kushwah, Sheeja Edwin, Suhail Asghar Showkal Ahmad Patni. Effect of Unese on ethylene glycol-induced urolithiasis in rats. Indian Journal of Pharmacology, 2011; 43(4): 466-468.