Antilithiatic Influence of *Butea monosperma* Lam and *Nigella sativa* Linn on Ethylene Glycol Induced Nephrolithiasis in Rats

Sumayya Sikandari* Md Liyaqat Ahmed, Pratima Mathad

Department of P G Studies and Research in Botany, Gulbarga University, Kalaburagi-585106 Karnataka, India
Email: sumayya_gadwal@rediffmail.com
Department of Pharmacology, Luqman college of Pharmacy, Kalaburagi-585106 Karnataka, India

ABSTRACT

The effect of aqueous extracts of dried seeds powder of *Butea Monosperma* plant and *Nigella Sativa* plant against Ethylene glycol induced renal calculi in albino wistar rats has been studied in this research. A renal calculus was induced in rats by ingesting 0.75% Ethylene glycol in drinking water for one month. Ethylene glycol treated rats showed significant high increase in the level of promoters such as calcium, phosphorous, Potassium, BUN and low concentration of inhibitors such as magnesium and citrate contents in the urine samples. Histopathological studies also confirmed the deposition of calcium oxalate crystals in kidneys. Separate oral administration of *Butea Monosperma* aqueous suspension to one group of Ethylene glycol induced urolithic rats and *Nigella Sativa* aqueous suspension to another group of Ethylene glycol induced urolithiatic rats (2g/kg body wt/day upto one month) had reduced the concentration of calcium, oxalate, BUN, Creatinine, Phosphorous, and diminished the crystals deposition in the kidneys. The result of the present study confirmed that Butea Monosperma aqueous extracts and *Nigella Sativa* aqueous extracts can be used as curative agent for urolithiasis.

Keywords: Urolithiasis, *Butea Monosperma, Nigella Sativa,* Unani Medicinal Plants,

INTRODUCTION

Urinary calculi or kidney stone formation is considered as one of the ancient major disorder related to Urinary system. It is estimated that 12% of world population experiences renal stone disease 70% in male and 40% in female. However the treatment present in modern medical system such as Lithotripsy, Kidney Dialysis and Surgical operations are either too costly or not without side effects and does not shows the 100% efficacy & the re-occurance is common. Hence the search for antilithiatic drugs from natural sources such as plants has assumed greater importance nowadays, People since from ancient times are taking benefit from the nature for curing many number of diseases. The Indian plants are constantly being explored for possible antilithiatic effects (Jha U et al 2011). The present study was designed to investigate the antilithiatic activity of such two selected plants they are *Nigella sativa* and *Butea monosperma.* These two plants were selected based on the claim from local practitioners, & Ethonobotanists.

Nigella sativa also known as Black cumin (Krishna jirige) has been used from centuries in Unani medicinal system throughout the Middle East, India and Northern Africa. It is an annual flowering plant with pale blue flowers that belongs to Rannunculaceae family. The fruits of this plant contain angular black seeds, the seeds are considered to be the most valuable part contributing benificial health effects. *Nigella sativa* as a natural remedy has been documented to possess numeric therapeutic values, including antibacterial, antioxidant, antifungal, antiparasitic, and antiasthematic property (Aljabre, et al, 2005. Randhawa et al, 2005). The seeds of this plant are also used to cure diabetes, tumour, hypercholesterolemia, hypertension, inflammation, and gastrointestinal disorders (Tasar, *et al*, 2012; Terzi,et *al*, 2010; Meddah *et al*, 2009; Nagi and Almakki, 2009. Ghannadi *et al.* 2005).

Butea monosperma also known as flame of forest is a wild, medium sized tree, belongs to the family Fabaceae. It is locally called as Palas, found in western and central part of India, Burma and few Asian countries. It is a medium sized tree grows upto 20-40 feet height with crooked branches and large trifoliate leaves, large orange colour flower giving the appearance of "Flame of Forest" its pods are flat and with single seed. In literature, the *Butea monosperma* is known for several properties. The flowers are widely used by tribals and local practitioners in the treatment of Hepatic disorders, Viral Hepatitis, Antihepatotoxic, Antimplantation, Hypoglycemic, Urinary disorders, Diuretics , Kidney stone etc. Every part of the plant has its own medicinal importance (M V Patil,

Shubhangi pawar, and D A Patil, 2006). The seeds are also used in the treatment of bleeding piles, urinary stones, abdominal troubles, intestinal worms,etc (Anil kumar and Krishanu samanta 2012).

MATERIALS AND METHODS

Male albino wistar rats weighing 35-40gms were purchased from Luqman pharmacy college, Gulbarga. They were housed in well-ventilated cages maintained in air conditioned laboratories with 12 hours dark/ 12 hours light cycles. They were fed with standard diet purchased from VRK Nutritions, Pune. They had free access to drink water. The animals were maintained in these conditions for one week before the experimental session starts. This study was approved by the Animal Ethical Committee and was carried out according to there guidelines.

ANTILITHIATIC ACTIVITY

The Experimental animals were divided into five groups of six animals each designated as G-1, G-2, G-3, G-4 and G-5. The animals of G-1 were fed with normal food and water only and the animals of this group served as the control. The G-2 animals received Cystone 4.5mg/100gm body weight orally along with the 0.75% of Ethylene glycol for one month and served as the standard (+vecontrol). The animals of G-3 received only 0.75% Ethylene glycol (stone inducer) in drinking water and served as negative control. The animals of G-4 received the 0.75% of Ethylene glycol in drinking water along with the aqueous extract of Plant-1 i.e. *Nigella Sativa Linn* (200mg kg^{-1} body weight /daily) upto one month. The animals of G-5 received the 0.75% of Ethylene glycol in drinking water along with the aqueous extract of Plant-2 i.e. *Butea Monosperma Lam* (250mg kg^{-1} body weight /daily) upto one month. The below mentioned Table No.1 clearly shows the experimental design.

EXPERIMENTAL PROTOCOL

Table No.1 showing experimental design

Sex of animals	Sl/no	Drug administration
Females	Group-1	No administration of drugs except standard food and water.
Males	Group-2	Standard +ve control (0.75% Ethylene glycol in drinking water+Cystone 4.5mg/100gm)
Males	Group-3	Negative control (0.75% Ethylene glycol in drinking water) serve as stone inducer.
Males	Group-4	Aqueous extract of Plant-1 *Nigella Sativa* (200mg kg^{-1} body weight /24hrs) + 0.75% Ethylene glycol in drinking water.
Males	Group-5	Aqueous extract of Plant-2 *Butea Monosperma*(250mg kg^{-1} body weight /24hrs) + 0.75% Ethylene glycol in drinking water.

Relative weights of the Animals

All the experimental animals were divided into five groups of four animals each, the four animals of each group were named as Head(H), Body(B), Tail(T), and Head body(HB) by marking on there body with marker for conviniency, before giving the doses every day throughout the experimental session the animals of all five groups were weighed and there weights were tabulated in Table No 2.

Relative weights of the kidneys

At the end of 30 days, the animals were mild anaesthetized and sacrificed by cervical decapitation. The Kidneys of all the five Groups were removed and weighed; the mean weights of the kidneys of four animals of every group were tabulated in the Table No.3. Immediately after weighing, kidneys were quickly dissected into ice cold saline. They were trimmed free of connective tissue for further Histopathological studies.

Measure of Urinary pH and total urinary output

During the experimental session of one month the urine samples of each individual group were collected at regular intervals of one week to observe and compare the variations in the pH of urine samples and total urinary output of each group with respect to

the positive and negative standard groups, the observed pH values are tabulated in Table No.4. and measured Urinary output of all the five groups in ml is tabulated in Table No.5.

Biochemical assays

The Urine and Blood samples were collected from all the five groups separately in sterile glass containers and were subjected to analyze Calcium, Magnesium, Phosphorous, Potassium, Uric acid, Creatinine, and Proteins in urine samples. BUN and Proteins in the blood samples. The results were tabulated in below mentioned Table No. 6

Histological assays

The tissue pieces taken from the kidney of the rats fixed by neutral buffered formalin (10%) and subsequently embedded in paraffin. The sections of 5µm thickness trimmed with the help of microtome machine were stained by Haematoxylin and Eosin to study the histopathological changes and calcium oxalate crystal depositions.(Fig : 1)

Total number of Calcium Oxalate Deposits in 10 microscopic fields of kidney slices

The total number of calcium oxalate (Caox) crystals depositions in 10 microscopic fields individually of each group was calculated by observing the kidney sections under stereomicroscope to observe the changes in the size of crystals, along with the microscopic observation of kidney sections, the urine samples of all the five groups collected at the last day of experimental session were also examined to see the presence of any crystals. The results are tabulated in Table No 7.

Results

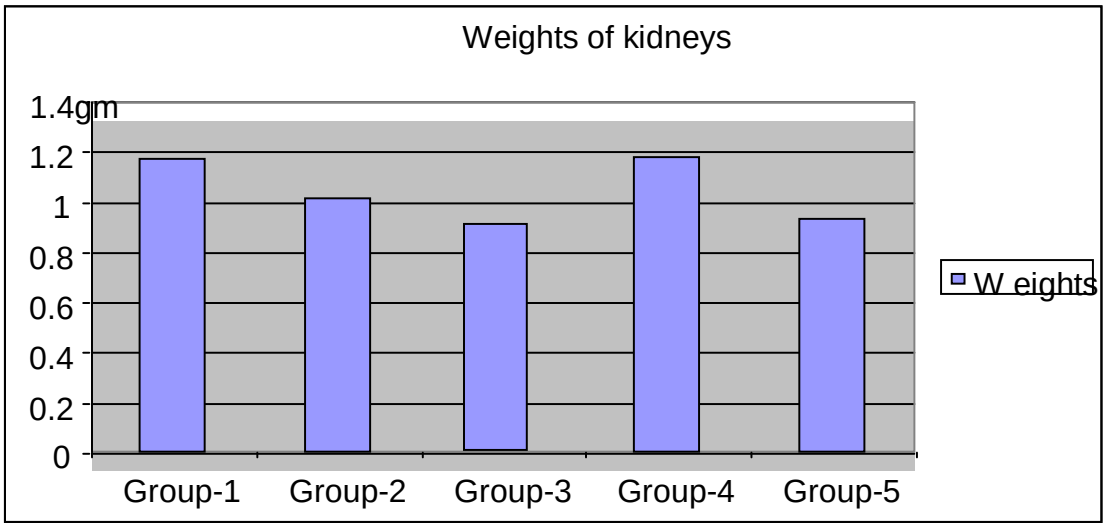
Table no. 2: showing Weights of Animals in Grams from 1st day to 30th day

		1st day	10th day	20th day	30th day
Control	H	50	80	125	140
	B	48	75	128	145
	T	47	79	126	138
	HB	51	80	125	142
Standard	H	50	80	95	130
	B	46	65	105	140
	T	47	70	100	130
	HB	42	60	80	110
-ve control	H	45	67	102	125
	B	46	69	105	130
	T	48	71	75	90
	HB	50	73	60	70
N.S.E	H	55	80	125	125
	B	50	68	95	145
	T	46	80	120	110
	HB	40	55	85	115
B.M.E	H	55	70	130	150
	B	65	71	115	120
	T	45	75	115	150
	HB	48	80	120	95

All the experimental animals were divided into five groups of four animals each, the animals were named as Head(H), Body(B), Tail(T), and Head body(HB) for conviniency, before giving the doses every day throughout the experimental session the animals of all five groups were weighed, It was observed that the body weights of all the five groups was increasing, but at the same time the weights of control group and standard group was increasing at very faster rate, the body weights of 4th group i.e *Nigella sativa* aqueous extract treated group and 5th group i.e *Butea monosperma* aqueous extract treated group were found near to standard group with slight variations. Whereas very less increase in the body weight of Ethylene glycol treated group was observed.

Table No .3 : Comparision of Weights of kidneys of all the five groups on the last day of Experiment after dissection.

	Groups	Weights of kidneys	Mean weights
Normal	Group-1	1.260	1.173±0.06
		1.020	
		1.240	
Standard	Group-2	1.020	1.015±0.03
		1.010	
		1.015	
Induction	Group-3	0.970	0.910±0.01
		0.850	
		0.910	
Plant-1	Group-4	1.150	1.182±0.02
		1.160	
		1.140	
Plant-2	Group-5	1.055	0.936±0.04
		0.860	
		0.895	



At the end of the study, the weight of the kidneys was comparatively lower in Group C i,e negative control compared with Group A control, and Group B i,e positive control .The administration of Aqueous extract of *Nigella sativa* in Group D at equivalent dose of 200 mg/kg (Group D). Increased the weight of kidneys in this Group. The administration of Aqueous extract of *Butea monosperma* in group E at equivalent dose of 250mg/kg (group E). Increased the weight of kidneys. The Table No.3 clearly shows actual weights of kidneys of all the five groups after the completion of experimental session.

Table No. 4: Showing pH of Urine sample

	7^{th} day	14^{th} day	21^{st} day
Group-1	3.4	3.9	4.2
Group-2	3.3	3.7	4.1
Group-3	3.0	2.6	3.7
Group-4	3.2	3.7	4.0
Group-5	3.1	2.9	3.9

The ph of urine samples observation at regular interval of every one week showed that during the first week the pH values of all the five groups were similar to one another and near to the pH three, but the pH value of negative control group was drastically

decreased during the second week as well as third week, the pH values of 4th group i.e *Nigella sativa* aqueous extract treated group and 5th group i.e *Butea monosperma* aqueous extract treated group were found near to standard group with slight variations.

TABLE : 5 DETERMINATION OF URINARY OUTPUT IN ml/24 hrs

Days	Group-1	Group-2	Group-3	Group-4	Group-5
1	9.2	9.1	8.7	9.7	8.6
7	9.7	8.6	9	10	9.4
14	10.4	9.9	9.1	10.4	10.2
21	10.3	11.1	9.3	10.8	11.4
28	12.1	12.5	9.5	11.2	12.1

In urinary output determination control rats (Group-1) did not show any significant variations from 1st day - 30th day, In all the groups considerably there was increase in the urinary output, but less increase in the urinary output of negative control from (8.7ml-9.5ml), moderate increase in the standard group(9.1ml-12.5ml), in Nigella sativa group-4(9.7ml-11.2ml), in Butea monosperma group-5(8.6ml-12.1ml).

Table No. 6: BIOCHEMICAL ANALYSIS

SL/ NO	URINARY EXCREATION LEVEL	GROUP-1	GROUP-2	GROUP-3	GROUP-4	GROUP-5
1	CALCIUM	4.58±0.03	10.89±0.04	0.460±0.03	6.10±0.04	2.95±0.02
2	MAGNESSIUM	1.65±0.01	0.02±0.01	1.22±0.01	0.91±0.01	0.67±0.01
3	POTASSIUM	4.21±0.01	5.36±0.03	0.03±0.01	4.68±0.04	3.82±0.11
4	URIC ACID	13.12±0.02	12.43±0.03	11.09±0.01	13.56±0.01	12.55±0.01
	SERUM ANALYSIS					
5	BUN	1.48±0.04	2.29±0.02	15.26±0.03	1.89±0.05	0.43±0.01
6	PROTEINS	2.20±0.01	4.49±0.01	1.91±0.01	2.94±0.02	3.21±0.01
7	Ph OF Urine sampes	4.2	3.7	4.1	4	3.8

Determination of Urinary Calcium in mg/24hrs

The Biochemical analysis of various elements in the urine and serum of animals at the end of experimental session was carried out. The level of calcium estimated in the 3rd Group i.e negative control Ethylene treated group was fallen to 0.460±0.063, whereas in the 1st control group it was 4.58±0.03, in the Cystone treated positive control 2nd group it was 10.89±0.04 and in the *Nigella sativa* aqueous extract treated 4th group 6.10±0.04 and *Butea monosperma* aqueous extract treated 5th group 2.95±0.02. The values of 4th and 5th groups were found very near to control group and positive control group. The excreation of calcium through urine may reduce the risk of calcium getting deposited in the kidney for the formation of Calcium oxalate stones.

Determination of Urinary Magnessium in mg/24hrs

The level of magnessium estimated in the urine sample of 3rd Group i.e negative control Ethylene treated group was increased up to 1.22±0.01, whereas in the 1st control group it was 1.65±0.01, in the Cystone treated positive control 2nd group it was 0.02±0.01 and in the *Nigella sativa* aqueous extract treated 4th group 0.91±0.01 and *Butea monosperma* aqueous extract treated 5th group 0.67±0.01. The values of 4th and 5th groups were found very near to control group and positive control group. The magnessium is considered as inhibitor for stone formation, increase in its production and its level in the urine sampe is a good sign and indication that the induced drug or extract is effective in the inhibition of stone formation.

Determination of Urinary Potassium in mg/24hrs

The level of Potassium estimated in the urine samples of 3rd Group i.e negative control Ethylene treated group was fallen to 0.03±0.01, whereas in the 1st control group it was 4.58±0.03, in the Cystone treated positive control 2nd group it was 10.89±0.04 and in the *Nigella sativa* aqueous extract treated 4th group 6.10±0.04 and *Butea monosperma* aqueous extract treated 5th group 2.95±0.02. The values of 4th and 5th groups were found very near to first control group and second positive control group. The

excess of potassium if it is continuously getting excreated through urine the risk of potassium getting deposited in the kidney for the formation of stone decreases upto some extent.

Determination of Urinary Uric acid in mg/24hrs

The level of uric acid estimated in the 3rd Group i.e negative control Ethylene treated group was fallen to 11.09±0.01, whereas in the 1st control group it was 13.12±0.02, in the Cystone treated positive control 2nd group it was 12.43±0.03 and in the *Nigella sativa* aqueous extract treated 4th group 13.56±0.01 and *Butea monosperma* aqueous extract treated 5th group 12.55±0.01. The values of 4th and 5th groups were found very near to control group and positive control group. The excreation of uric acid and its presence in urine samples is the indication that both the plant samples are effective in reducing the uric acid concentration in the kidneys and eliminating it through urine.

Determination of BUN (Blood Urea Nitrogen) in mg/24hrs

On 30th day after 2 hours of last dose, animals were anaesthetized. Blood was collected from orbital venous plexus in non-heparinized tubes and centrifuged at 2000 rpm for 20 min to obtain serum, which was used for further analysis. The level of BUN estimated in the 3rd Group i.e negative control Ethylene treated group was increased to 15.26±0.03, whereas in the 1st control group it was 1.48±0.04, in the Cystone treated positive control 2nd group it was 2.29±0.02 and in the *Nigella sativa* aqueous extract treated 4th group 1.89±0.05 and *Butea monosperma* aqueous extract treated 5th group 0.43±0.01. The values of 4th and 5th groups were found very near to first control group and second positive control group. Decrease in its level in both treated groups i.e 4th and 5th group is the indication of decrease in its toxicity in the bood.

Table 7 Total number of caox Deposits in 10 microscopic fields of kidney slices

Groups	No of Deposition
Group-1	NIL
Group-2	7.00±0.58
Group-3	29.67±0.88
Group-4	5.67±0.33
Group-5	3.67±0.33

Microscopic observation of rat bladder urine revealed that urine of the control group rats was more or less devoid of any crystals whereas, in the sections of Ethylene Glycol treated group ‘C’ the number of depositions in 10 microscopic fields were 29.67±0.88 which was significantly higher than group 'B'(Positive control) 7.00±0.58. In group 'D'(Nigella sativa) 5.67±0.33 & in group 'E'(Butea monosperma) 3.67±0.33, the number of deposits were significantly lower than group 'C'. However both the extracts were able to decrease the number of crystal deposits in kidneys. Therefore the benificial action of both the extracts on human kidneys can be suggested without any side effects, toxicity and also an alternative treatment to chemical drugs.

Histopathological analysis

Analysis of Haematoxylin and Eosin stained kidney sections also supported the serum and urine biochemistry results, showing normal structure and architectural intactness without any apparent damages in control group rats and there were no calcium oxalate deposits or other abnormalities in the nephron segments. In positive control group B, deposits were composed of only one or two polygonal crystals depositions. In negative control group C the number of deposits were exceeding from nine to ten, which was significantly higher than control group A. Interestingly, similar to first control group there was no or only one to two crystals depositions were observed in *Nigella sativa* seeds extract treated group-4 and *Butea monosperma* seeds extract treated group-5

Fig 1.a

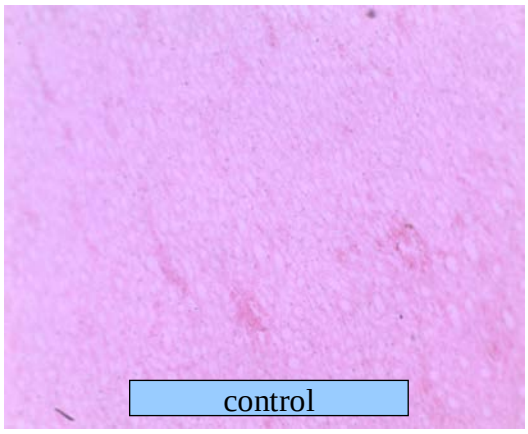


Fig 1.b

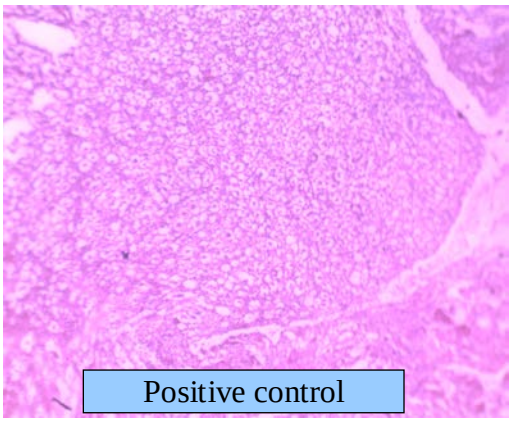


Fig 1.c

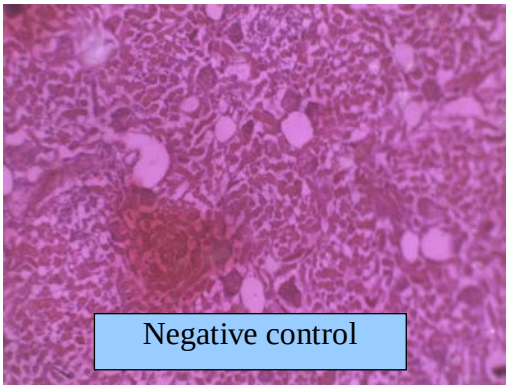


Fig 1.d

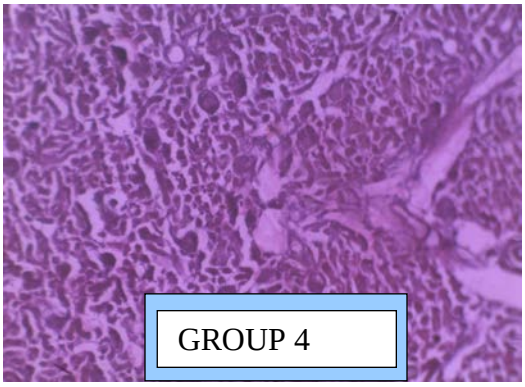


Fig 1.e

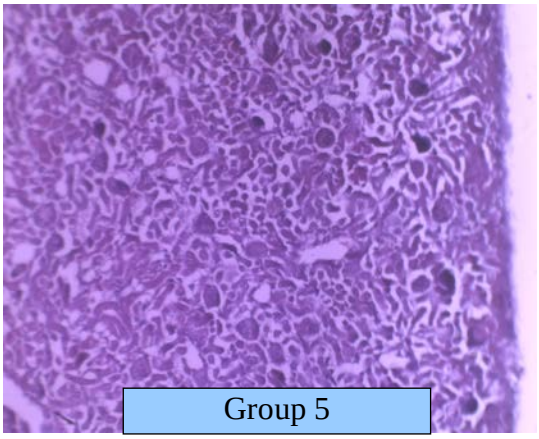


Figure : 1.a Normal structure of kidney
Figure : 1.b Section of kidney showing one calculi deposition
Figure : 1.c Section of kidney showing eight to nine calculi depositions
Figure : 1.d Section of kidney showing two to three calculi depositions
Figure : 1.e Section of kidney showing two to three calculi depositions reduced in size.
.

DISCUSSION

Ethylene glycol is the dihydroxy alcohol derivative of ethane. It is utilised in antifreeze solutions, coolants, glass cleaners, cosmetics, paints etc. The charecteristics of ethylene glycol are clear, colourless, sweet-tasting liquid. When ethylene glycol is metabolised by the body, it produces four toxic metabolites they are glycoaldehyde, glycolate, glycolic acid, and glyoxylate. All of this metabolites may cause destruction of the tissue that primarily form calcium oxalate crystal deposition and metabolic abnormalities, especially a high anion gap metabolic acidosis, lactic acidosis, and hypocalcemia. The Oxalic acid combines with calcium to form calcium oxalate crystals, which deposits in the kidneys. This can result in Hypocalcemia, Hematuria, proteinuria, increased creatinine and renal failure. (A' Liyatur Rosyidah and sri widyarti. 2013).

The results of present study clearly indicates that aqueous extracts of *Nigella sativa* seeds and *Butea monosperma* seeds, showed comparable activity to that of cystone in terms of inhibiting the formation of calcium oxalate precipitate. The reduction of stone forming constituents in urine and there decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, thus aqueous extract of both the plants could be further analysed and characterised for its active compound and mechanism invoved in it, this study could lead to a new drug for the patients with urolithiasis.

Thus, this study provides a basis for utility of aqueous extracts of *Nigella sativa* seeds and *Butea monosperma* seed in the treatment of renal and urinary calculi and it is in accordance with earlier study by Mousa-Al-Reza Hadjzadeh *et al.* 2007 and put forth the possibility of use of *Nigella sativa* seeds as the therapeutic agent to treat urolithiasis. The seeds of *Butea monosperma* in accordance with the review of *Butea monosperma* by Firdaus Rana and Mazumder Avijit *et al.* 2012.

The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. In the present study, observed Hypocalciuria in ethylene glycol induced urolithic rats might be a factor favouring the nucleation and precipitation of calcium oxalate

CONCLUSION

Kidney stone disease has afflicted humankind since ancient times and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. Inspite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of urolilithiasis. Recently, there is with increasing evidence that many healthy natural food and medicinal herbal and supplements have the potentials to solve the problem of eradicating many number of diseases releated to mankind. The Aqueous seeds extract of *Nigella sativ*a and *Butea monosperma* were able to reduce the growth of urinary stones, decreased the number of calcium oxalate depositions and restored the structure of kidneys in rats. Therefore, the beneficial action of these both the plants may be suggested. However, further studies must clarify the mechanism.

In conclusion, The presented data indicated that administration of aqueous extract of *Nigella sativa* and *Butea monosperma* seeds powder to rats prevented urolithiasis induced with Ethylene glycol and reduced the growth of calcium oxalate stones, reduced the number of stone deposits, both the extracts were found effective in reducing the renal tissue injury, decreasing the crystal size, thus can simply be swept by urine and helps in restoring normal kidney architecture and weights of the kidneys. In this comparative study it was found that the Nigella sativa aqueous extract and Butea monosperma aqueous extract results were very near to standard drug i,e cystone, In comparision to both the plant extracts Nigella was very near to standard and Butea was near to Nigella but both were very far from the -ve control results. Further, experimental and clinical studies are required to elucidate the chemical constituents of the extracts and mechanism responsible for the pharmacological activities. This study has supported the folk information, and claim regarding antiurolithiatic activity of both the plants *Butea monosperma* (Ethnomedicines) and *Nigella Sativa.* (Al Tib Al Nabvi)

The number of caox deposits in 10 microscopic fields of kidney slices in group 'C' was 29.67±0.88 which was significantly higher than group 'B'(Positive control) 7.00±0.58. In group 'D'(Nigella sativa) 5.67±0.33 & in group 'E'(Butea monosperma) 3.67±0.33, the number of deposits was significantly lower than group 'C'. However both the extracts were able to decrease the number of crystal deposits in kidney's .Therefore the benificial action of both the extracts on human kidneys can be suggested without any side effects, toxicity and also an alternative treatment to chemical drugs.

REFERENCES:

1. Firdaus rana Mazumdar Avijit. Review on *Butea Monosperma*, 2012; 2(4); 1035-1039.

2. Piyush Gupta and N.S Chauhan. Phytochemical and Pharmacological Review on *Butea Monosperma*, 2012; 3(7); 255-258.

3. Liyatur Rosyidah and sri widyarti. Induction of Nephrolithiasis in Experimental Animal, 2013; The third basic science international conferences; 2013.

4. Abulfazl Khajavi Rad , Mousa-Al-Reza Hajzadeh and Ziba Rajaei. Preventive effect of *Cynodon dactylon* against ethylene giycol-induced nephrolithiasis in male rats, 2011; 1(1); 14-23.

5. Ambersing Rajput and Suboth Chandra pal. Phytochemical Screening Antibacterial Activity and Physicochemical Evaluation of Leaves of *Butea Monosperma*, 2011; 3(3); 189-191.

6. Jha U, Shelke TT and Oswal Rj. Pharmacological Screening of *Musa paradisica* Linn against Ethylene Glycol Induced Renal Calculi, 2011; 2(3); 995-998.

7. Anshu Aggarwal and Surinder K Singla. Preventive and Curative effects of *Achyranthes aspera* Linn. Extract in experimentally induced nephrolithiasis, 2012; 5; 201-208.

8. M V Patil and D A Patil. Ethnobotany of *Butea monosperma* (Lam.) Kuntze in North Maharashtra, India. 2006; 5(4); 323-325.

9. Ravindra Kumar,Tirath Kumar and Harish Chander. Pharmacological evaluation of ethanolic extract of *Kigelia pinnata* fruit against ethylene glycol induced urolithiasis in rats, 2012; 2(1); 63-72.

10. Priya TThambi and Sabu M Chacko. Studies on Diuretic effect of *Lagerstroemia Speciosa* Linn.Leaf extracts in normal rats, 2013; 4(3); 61-69.
11.A.Tamil Selvan and B.Jayalaxmi. Antiurolithiatic activity of Triphala Karpa Chooranam, 2013; 3(3); 267-272.

12.Anand Tiwari Vivek Soni and Vikas Londhe. An overview of Potent Indigeneous Herbs for Urinary Tract Infirmity:Urolithiasis, 2012; 5(1); 7-12.

13.Eman A.Moussa and Jawaher A.A. AL Mulhim. Modulating effect of Nigella Sativa on renal structural changes by Monosodium Glutamate in Female Mice, 2013; 5(2); 33-45.

14.P Soundararajan, R Mahesh and T Ramesh. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats, 2006; vol 44; 981-986.

15.Atef M. Al-Attar. Antiliathic Influence of *Spirulina* on Ethylene Glycol-Induced Nephroletheasis in Male Rats, 2010; 6(1); 25-31.

16. A liyatur Rosiyidah and srihrene third basic International conference.2013

17.Piyush Gupta, 2012.Phytochemical and pharmacological review on *Butea monosperma*, International Journal of Agronomy and plant production Vol.3(7), 255-258, 2012.

18. Aggarval, A. S.K Gandhi, and Tondon. Preventive and curative effect of *Achyranthes aspera* Linn. Extract in experimentally induced nephrolithiasis. Indian Journal of Experimental Biology, vol.50,pp 201-208, march 2012.

19.Salih H.M Aljabre, Omar M. Alakloby, Mohammad A. Randhawa.
Dermatological effects of *Nigella sativa.* Journa of Dermatology and Dermatological surgery.Volume 19 Issue 2 July 2015, Pages 92–98.