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Research Article

Evaluation of Effect of *Rosmarinus officinalis* L. on Ethylene Glycol Induced Kidney Stone in Rats

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Abstract

The present study evaluation of effect of *Rosmarinus officinalis L.* on ethylene glycol induced kidney stone in rats showed that the ethanolic extract of *Rosmarinus officinalis* possess the diuretic activity and effective in the treatment of kidney stone. The activity was evaluated against ethylene glycol (0.75%) induced kidney stone in albino rats. Animals were divided into five groups, containing six animals in each. Group I serve as normal control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was feed to Groups II to V for induction of renal calculi for 28 days. Group III received standard anti-urolithiasis drug, cystone (750mg/kg body weight) from 15th to till 35th day. Group IV and V received ethanolic extract of *Rosmarinus officinalis* at dose of 100mg/kg and 200mg/kg respectively from 15th to till 35th day.

The serum creatinine level, serum urea level, urine calcium level, protein level in urine, serum uric acid was increased and serum calcium level, protein level in serum was decreased in all experimental groups except normal control group after ethylene glycol administration till day 14.

The animals treated on the day 14th with standard drug (Cystone 750mg/kg) and *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), were observed significant decreased serum creatinine level, serum urea level, urine calcium level, protein level in urine and serum uric acid and increased serum calcium level, protein level in serum compared to normal control group on the day 28th and 35th.

This indicates that the ethanolic extract of *Rosmarinus officinalis* (200mg/kg) have protective role against kidney stone.

Keywords: Kidney Stone, Ethanolic extract, *Rosmarinus officinalis,* Ethylene glycol, uric acid, creatinine.

INTRODUCTION

Kidney stone is very common and an increasing disorder of humans. Kidney stone formation results from a succession of physicochemical events like supersaturation, nucleation, growth, aggregation and retention within the kidneys. [1]

Based on chemical composition there are four main types of chemical stones given as follows -Calcium Stones, Struvite or Magnesium Ammonium Phosphate Stones, Uric Acid Stones or Urate and Cystine Stones. The chemical composition of kidney stones depends on the abnormalities in urine composition of various chemicals. Size, shape, and chemical compositions of stones are different. [2]

Calcium Stones - Calcium stones are most common renal stones that comprises about 80% of all urinary calculi. The main constituent of calcium stones is calcium hydrogen phosphate or hydroxyapatite. [3,4,5]

Uric Acid Stone - High concentration of uric acid in urine forms uric acid stones. Uric acid stones are more frequently associated with conditions such as gout.[6]

Struvite Stone - Certain bacteria have the ability to convert urea in urine into ammonium. Ammonium then combines with phosphate and magnesium to form stones. [7,8]

Cystine Stone - Cystine stones are formed due to a genetic abnormality. Amino acids are needed in the human body to make proteins. Due to a defective gene, amino acids are sometimes not absorbed properly by kidneys. The unabsorbed amino acid or cystine builds up to form cystine stones. [9,10]

PLANT PROFILE - *Rosmarinus officinalis* (Family- Lamiaceae)

Geographical source – Rosemary is indigenous to South Europe and South Asia. It is cultivated in Mediterranean basin and India.[11]

Morphological characters – Rosemary is evergreen, usually erect, bushy shrub up to 2m tall and wide. Stem indistinctly quadrangular finely grey pubescent.

Leaves – The linear leaves are about 1 cm (0.4 inch) long and somewhat resemble small curved pine needles. They are dark green and shiny above, with a white underside and curled leaf margins.

Flowers - Its flowers are white, pink, purple or dark blue. [12,13]

Phytochemicals - The main constituents of the rosemary essential oil are camphor, 1,8-cineole, α-pinene, borneol, camphene, β-pinene and limonene. [14]

Regarding the extracts, the phytochemicals mainly present in *R. officinalis* are rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol. [15,16]

Pharmacological Properties - *Rosmarinus officinalis* has following pharmacological activities-

Anti-bacterial, antioxidant, anti-inflammatory. Anti-microbial activity, anti-proliferative activity, hepatoprotectivity, anti-cancer activity, anti-diabetic activity, anti-depressant activity, neuroprotective activity and diuretic activity. [17,18,19]

MATERIALS AND METHODS

Plant sample -

Collection, Authentication and Storage of Herb: The herb has been collected from local market, with the help of field Botanist. The herb has been authenticated by Dr. H. S. Gaur University, Department of Botany, Sagar (M.P). Herbarium number Bot/Her/B1/1364, (Ref. no. Bot/179).

Preparation of Plant Extract -

A) **Petroleum ether extraction:** The dried and powder herb were packed in soxhlet apparatus and extracted with petroleum ether at 60-80^{0}C for 36 hours and completion of extraction were confirmed by pouring a drop of extract from the thimble on a filter paper, which was does not show the presence of any oil spot on that.

B) **Ethanol extraction:** After completely evaporating the petroleum ether, the herb were extracted with semi polar solvent (ethanol) then packed in soxhlet apparatus at 60^{0}C temperature for 36 hours and completion of extraction was confirmed by poured a drop of extract from the thimble on a filter paper, which does not show the presence of any oil spot on that. And, this indicated the complete exhaustive testing of herb. The alcoholic extract was concentrated and dried. Semisolid extract was obtained. [20]

Animal Model for Induction of Kidney Stone

Mature Sprague-Dawley rats (200–225gm) were taken from the animal house of SIPS, Sagar. All experiment was according to CPCSEA (SIPS/EC/2023/58) guidelines.

Ethylene glycol induced Urolithiasis in Rats model

Ethylene glycol-induced hyperoxaluria model was used to assess the anti-lithiatic activity in albino rats. Animals were divided into five groups, containing six animals in each. Group I serve as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was feed to Groups II to V for induction of renal calculi for 28 days. Group III received standard anti-urolithiatic drug, cystone (750mg/kg body weight) from 15th to till 35th day. Group IV and V received - *Rosmarinus officinalis* extract at dose of 100mg/kg and 200mg/kg from 15th to till 35th day.

Groups of Animals (6 Animals in each group)

Group I: Positive Control group (Vehicle treated group)
Group II: Negative Control group (Disease Induced group)
Group III: Standard group (Cystone 750mg/kg)
Group IV: Test group I - Ethanolic Extract– 100mg/Kg
Group V: Test group II -Ethanolic extract– 200mg/kg

OBSERVATIONS AND RESULT

A. PHARMACOGNOSTIC STUDY.

S. No.	Phytochemical Test of Ethanolic extract of *Rosmarinus officinalis*	Observations	Results
1.	Test for alkaloids Dragendroff's Test Mayer's Test Wagner's Test Hager's Test	 Reddish brown ppt. Brown ppt. Reddish brown ppt. Yellow ppt.	 + + + +
2.	Test for Carbohydrates Molisch's Test Fehling's Test Barfoed Test	 Dull violet color Red ppt. Reddish ppt.	 + + +
3.	Test for Glycosides Legal Test Baljet Test Keller killiani's Test	 No change No change No change	 - - -
5.	Test for Flavonoids Alkaline reagent Test Shinoda's Test	 Yellow color turn tocolorless Pink color	 + +
6.	Test for Saponins a. Foam Test	 No change	 -
7.	Test for Phenols and Tannins Ferric Chloride Test Lead acetate Test	 Blue-blank ppt. Yellow ppt.	 + +

(+) Phytoconstituent present and (-) phytoconstituent absent. The ethanolic extract of *Rosmarinus officinalis* gives positive test for alkaloids, carbohydrates, flavanoids, phenols and tannins. It gives negative tests for glycosides and for saponins

B. PHARMACOLOGICAL STUDY

1. Serum Creatinine Estimation (Mg/Dl)

Serum Creatinine (Mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	2.50± 0.007	2.53± 1.006	2.57± 1.032	2.61± 0.098	2.57± 1.012
Negative Control	2.51± 0.018	5.62± 1.012	5.98± 1.320	6.89± 1.320	6.97± 1.310
Standard Group	2.47± 0.032	5.58± 0.092	5.61± 1.026*	4.97±1.303**	4.82± 0.098**
Test Group-I	2.53± 0.038	5.88± 0.098*	5.71± 1.008	5.58± 1.017*	5.26± 1.370*
Test Group-II	2.50± 0.032	5.50± 0.037	5.32± 1.260*	5.02± 1.038*	4.80± 1.360**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone (750mg/kg).

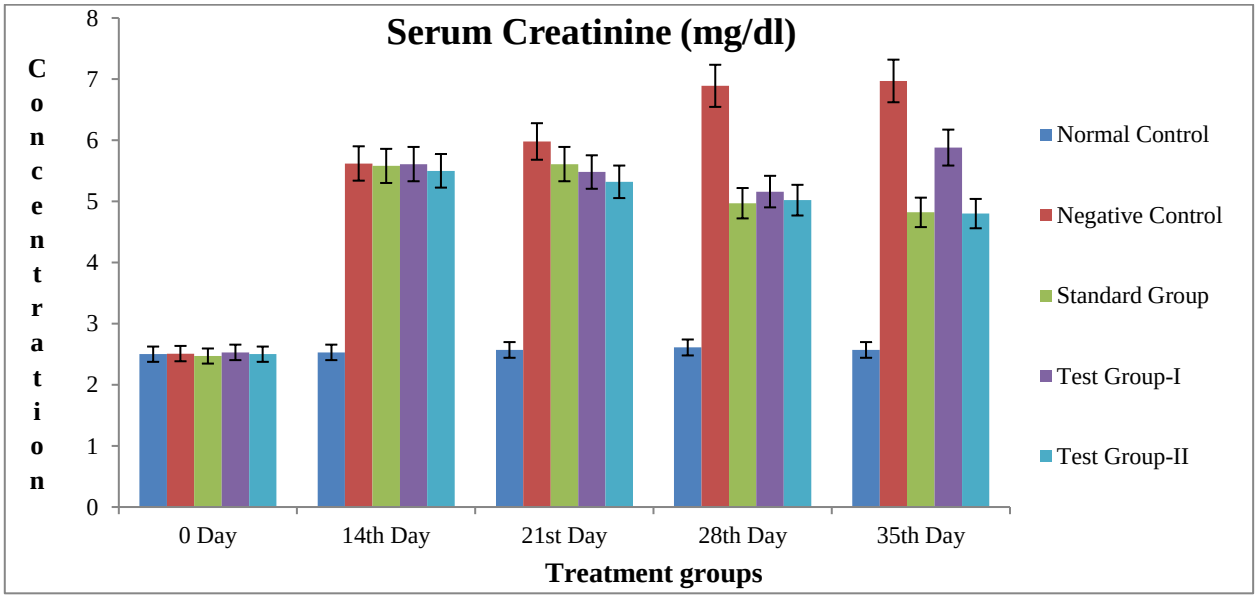


Figure 1: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Serum Creatinine level in Ethylene Glycol Induced Kidney Stone in Rat.

2. Serum Urea Estimation (mg/dl) –

Serum Urea Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	22.27± 2.004	22.23± 1.016	22.31± 2.030	22.37± 1.810	22.23± 2.013
Negative Control	22.23± 1.026	26.16± 1.310	26.93± 2.180	27.18± 1.093	28.18± 1.096
Standard Group	22.98± 1.820	26.13± 1.070	24.97± 0.980**	24.76± 2.010**	24.47± 1.027**
Test Group-I	22.18± 2.030	26.18± 0.720	26.06± 1.030	25.83± 1.097	25.43± 1.210*
Test Group-II	22.26± 2.016	26.16± 1.260	25.01± 1.360*	24.98± 1.380**	24.51± 1.097**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone (750mg/kg).

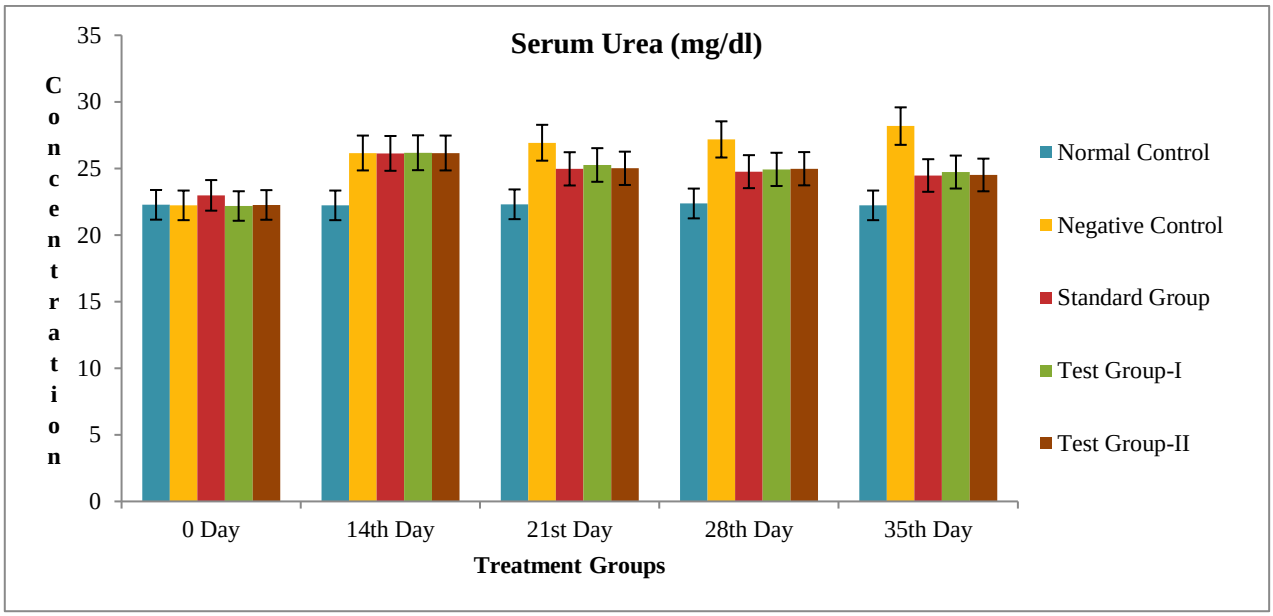


Figure 2: Effect of Ethenolic Extract of *Rosmarinus officinalis* on Serum Urea level in Ethylene Glycol Induced Kidney Stone in Rat.

3. Serum Calcium Estimation (mg/dl)

Serum Calcium Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Positive Control	10.85± 2.013	10.85±2.085	10.82± 2.010	10.83± 1.027	10.85± 2.018
Negative Control	10.83± 2.042	7.58± 1.068	6.36± 1.028	6.01± 2.012	5.83± 2.027
Standard Group	10.85± 1.098	7.53± 2.016	8.36± 2.016**	8.91± 2.016**	9.78± 1.038**
Test Group-I	10.87± 2.045	7.55± 1.061	7.81± 1.086	7.97± 1.031*	8.13± 2.017*
Test Group-II	10.80± 1.087	7.53± 1.002	8.28± 1.036*	8.48± 2.016**	8.52± 1.038**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone (750mg/kg).

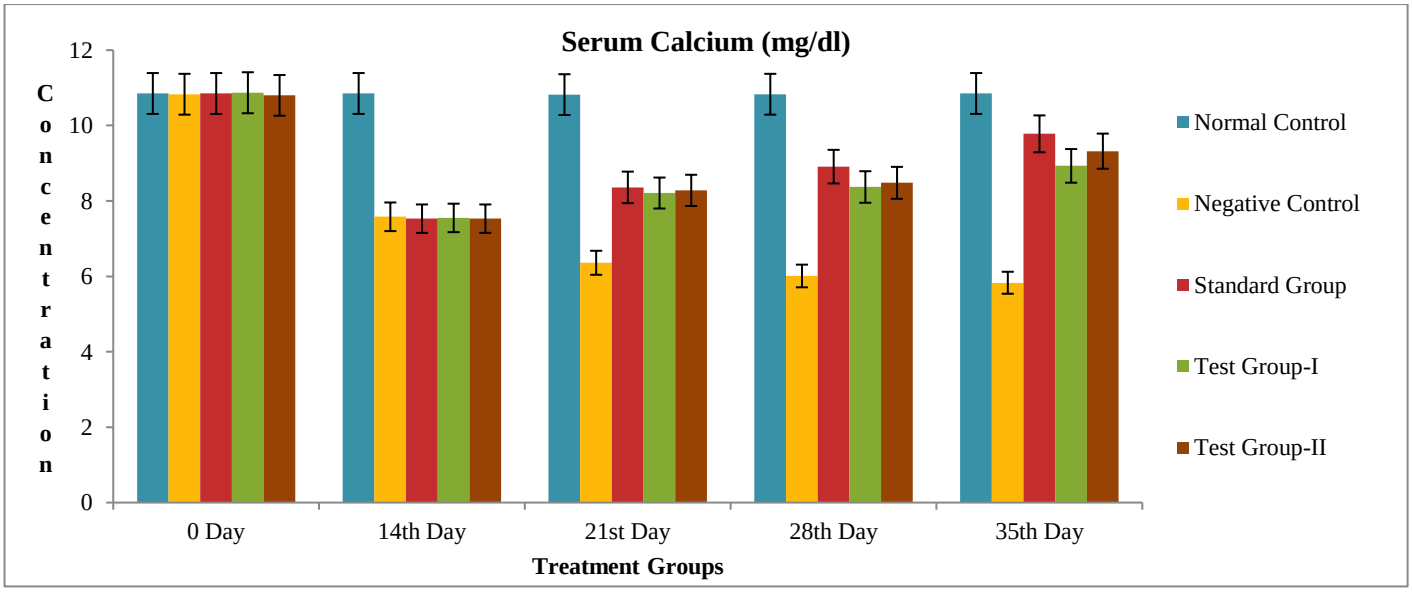


Figure 3: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Serum Calcium level in Ethylene Glycol Induced Kidney Stone in Rat.

4. Urine Calcium Estimation (mg/dl)

Urine Calcium Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	2.64± 0.014	2.68± 0.026	2.67± 0.031	2.65± 0.031	2.68± 0.032
Negative Control	2.81± 0.026	4.93± 0.032	5.01± 0.042	5.69± 0.072	6.28± 0.072
Standard Group	2.83± 0.031	4.81± 0.058	4.76± 0.028*	3.71± 0.065**	3.73± 0.039**
Test Group-I	2.73± 0.023	4.91± 1.012	4.89± 0.021	4.83± 0.061	4.78± 0.037*
Test Group-II	2.80± 0.032	4.87± 1.015	4.80± 0.072	4.03± 0.072**	3.92± 0.026**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone (750mg/kg).

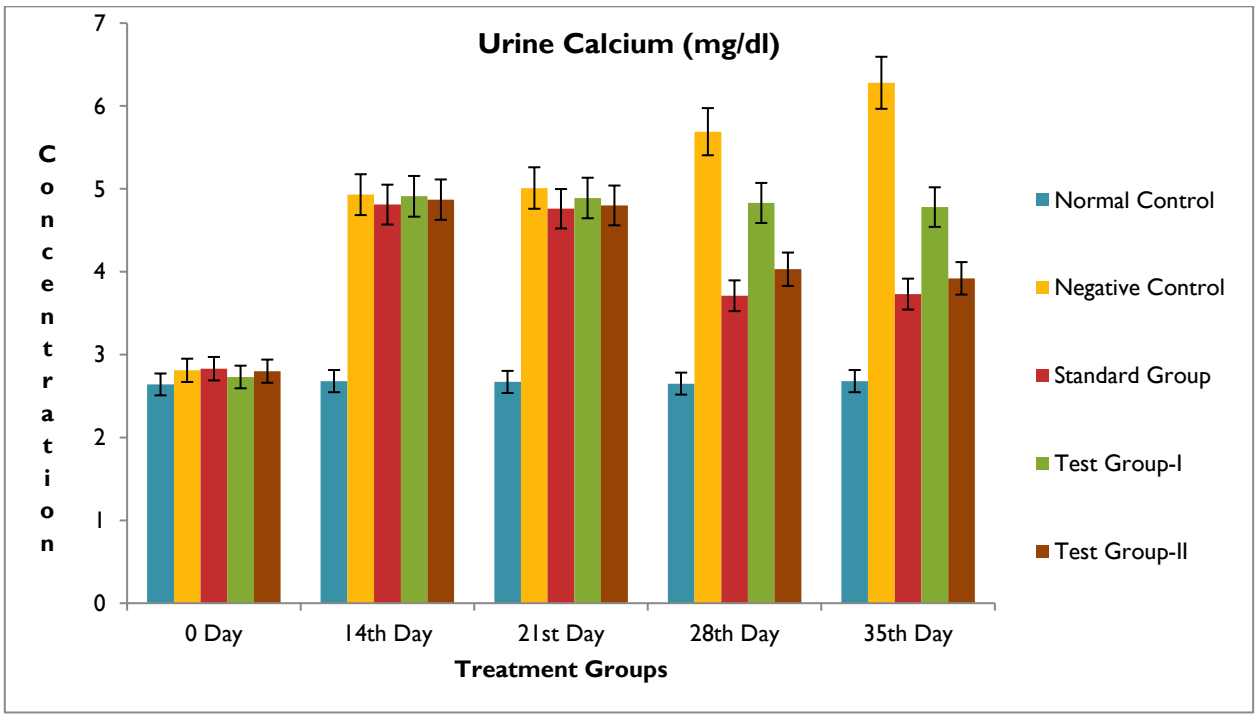


Figure 4: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Urine Calcium level in Ethylene Glycol Induced Kidney Stone in Rat.

5. Serum Protein Estimation (mg/dl)

Serum Protein Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	6.07± 1.026	6.09± 1.620	6.07± 0.980	6.10± 0.970	6.08± 1.020
Negative Control	6.12± 1.080	4.98± 2.160	3.97± 1.016	3.91± 2.010	2.83± 1.037
Standard Group	6.03± 1.160	4.92± 2.030	5.82± 1.980**	6.93± 1.370**	6.02± 2.017**
Test Group-I	6.16± 1.009	4.96± 1.036	5.37± 1.026*	5.62± 2.016*	5.76± 2.360*
Test Group-II	6.08± 1.310	4.93± 1.070	5.72± 2.010*	5.86± 1.580*	5.98± 1.760**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

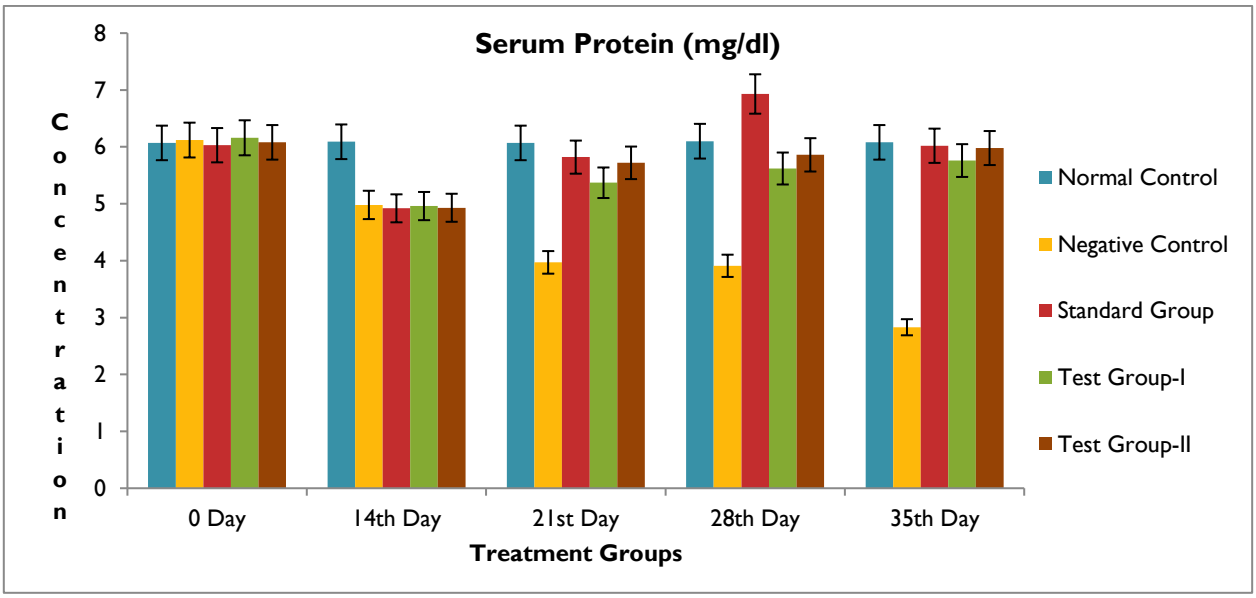


Figure 5: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Serum Protein level in Ethylene Glycol Induced Kidney Stone in Rat.

6. Urine Protein Estimation (mg/dl)

Urine Protein Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	0.86± 0.076	0.84± 0.027	0.98± 0.160	0.84± 1.002	0.88± 0.360
Negative Control	0.86± 0.098	2.76± 1.003	3.23± 1.038	3.77± 1.036	4.03± 1.009
Standard Group	0.84± 0.076	2.78± 1.260	1.46±1.021**	1.32±1.036**	1.27± 1.021**
Test Group-I	0.85± 1.001	2.71± 0.980	1.83± 1.016	1.72± 1.320	1.48± 1.090*
Test Group-II	0.86± 1.003	2.73± 1.031	1.52± 1.036*	1.44± 1.090*	1.30± 2.001**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

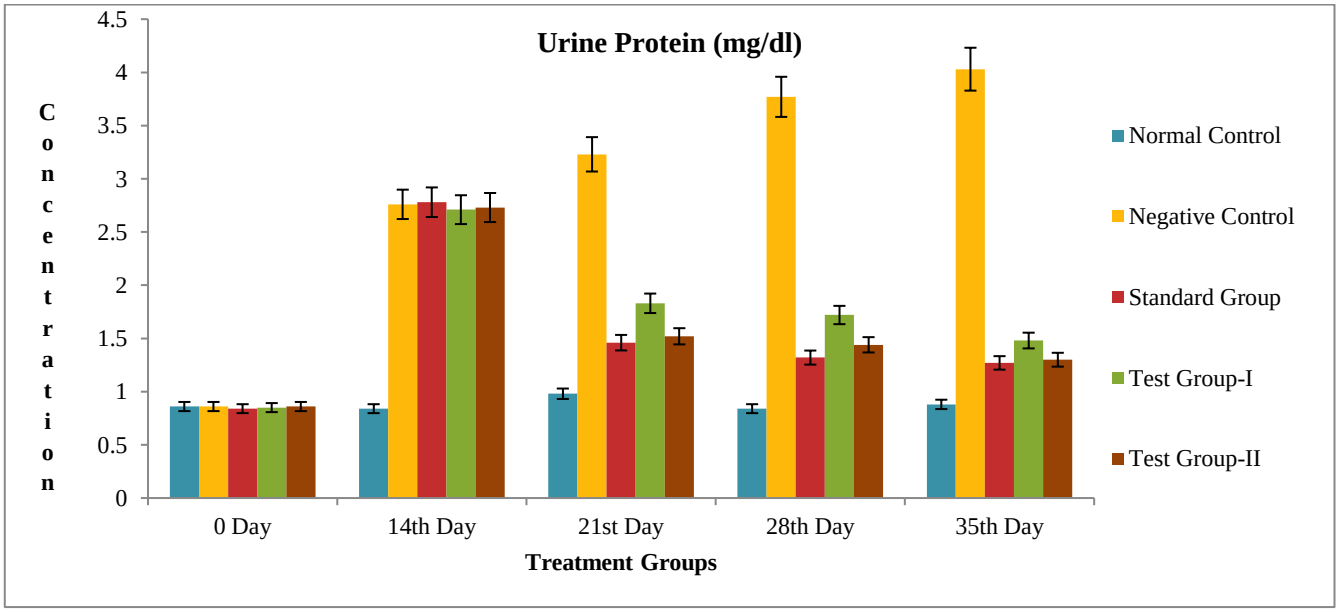


Figure 6: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Urine Protein level in Ethylene Glycol Induced Kidney Stone in Rat.

7. Serum Uric Acid Estimation (mg/dl)

Serum Uric Acid Estimation (mg/dl)					
Groups	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
Normal Control	3.53± 0.720	3.51± 1.016	3.52± 1.020	3.53± 1.026	3.51±1.020
Negative Control	3.58± 1.022	5.68± 0.120	6.12± 0.160	7.26± 0.026	7.83± 0.930
Standard Group	3.56± 1.016	5.51± 1.013	5.32± 0.390**	5.16± 0.091**	4.38± 0.860**
Test Group-I	3.52± 1.023	5.61± 1.038	5.52± 1.036	5.38± 0.920*	5.18± 1.098*
Test Group-II	3.53± 1.036	5.56± 1.028	5.32± 1.061*	5.17± 1.068*	4.46± 1.003**

In the table, 0-day count before induction of stone, day 21st, 28th and 35th counts after starting of treatments. Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Cystone (750mg/kg).

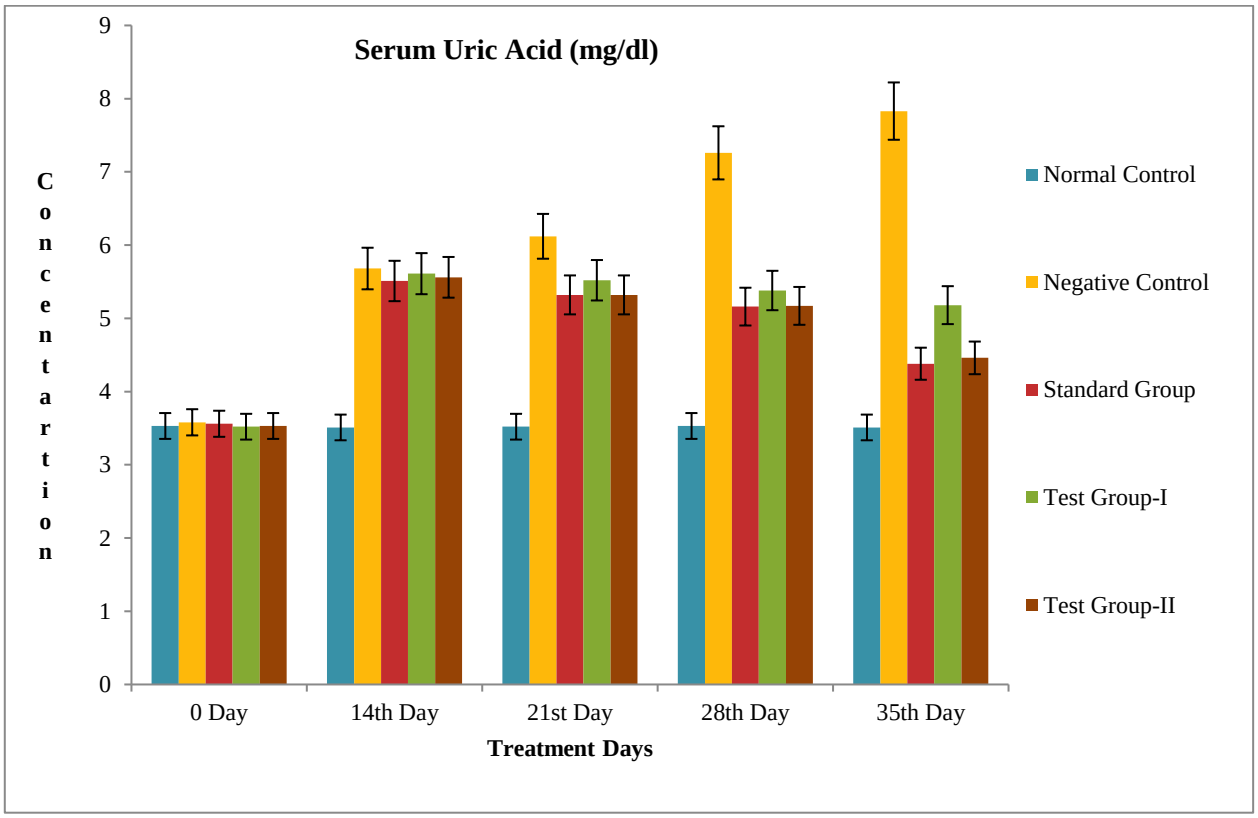
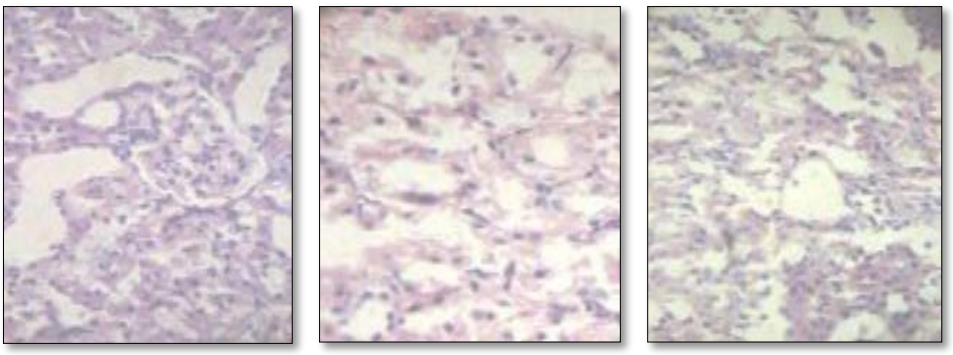


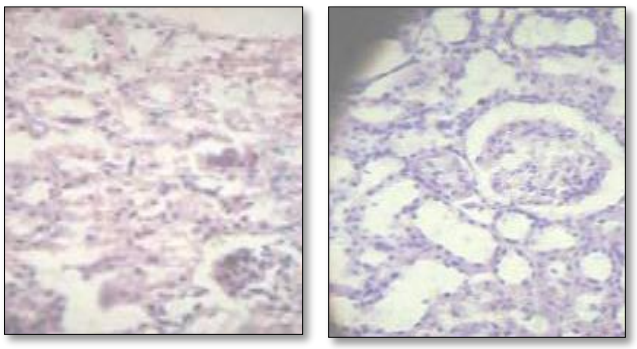
Figure 7: Effect of Ethanolic Extract of *Rosmarinus officinalis* on Serum Uric Acid level in Ethylene Glycol Induced Kidney Stone in Rat.

HISTOPATHOLOGY

To confirm the incidence of urolithiasis (kidney stone), the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. The kidneys were washed, weighed and fixed rapidly with 10% neutralized formalin (pH7.4), and soaked in paraffin, cut at 5µm intervals and the slices were stained with hematoxylin and eosin. Tissue slices were photographed using optical microscopy and observed the pathological changes.



CONTROL GROUP **NEGATIVE CONTROL** **STANDARD GROUP**



TEST GROUP I **TEST GROUP II**

In histopathological observations for gross examination of rat's kidney from control group showed a normal cortical structure of the kidney including glomeruli and distended tubules, sclerotic glomeruli, and increased cellularity between tubules. In ethylene glycol treated group (negative control group) showed cortical structure of the kidney including glomeruli and affected distended tubules, sclerotic glomeruli, and cellularity between the tubules. Group-III, (Cystone 750mg/kg) showed the recovered crypts or small sacs and recovered distended tubules, sclerotic glomeruli, and increased cellularity between tubules.

Group-IV, (100 mg/kg ethanolic extract of *Rosmarinus officinalis*) widespread focal interstitial lymphoplasmacytic infiltration with mild tubulitis, normal glomeruli and arteriolar vessels. There is sign of sclerotic glomeruli and increased cellularity between tubules. Group-V (200 mg/kg ethanolic extract of *Rosmarinus officinalis*) shows minor interstitial lymphoplasmacytic infiltration in glomeruli with occasional penetration of tubular epithelium. In addition, increased cellularity between tubules is clearly visible. From the above results it was noted that the Group-V (200 mg/kg ethanolic extract of *Rosmarinus officinalis*) was most significant.

DISCUSSION

The serum creatinine level of all experimental groups, except normal control group, was increased significantly after the ethylene glycol administration (orally) till day 14. On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant increase in serum creatinine level from normal control animals ($P<0.001$). In the negative control group the serum creatinine level increased to the maximum measurable value of 6.97±1.310mg/dl on day 35th and found to be significant increased ($P<0.001$) compared to the value of day 1st was 2.51±0.018mg/dl. In control animals serum creatinine level remain normal during the entire testing period of 35 days. The animals treated on the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), and extract (100mg/kg and 200mg/kg), only were observed that significant decreased in serum creatinine level ($P<0.001$) compared to normal control group on the day 28th (5.02±1.038) and 35th (4.80±1.360) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The serum urea level of all experimental groups, except normal control group, was increased significantly after the ethylene glycol administration (orally) till day. On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant increase in serum urea level from normal control animals ($P<0.001$). In the negative control group the serum urea level increased to the maximum measurable value of 28.18±1.096mg/dl on day 35th and found to be significant increased ($P<0.001$) compared to the value of day 1 was 22.23±1.026mg/dl. In control animals serum urea level remain normal during the entire testing period of 35 days. The animals treated on the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), only were observed that significant decreased in serum urea level ($P<0.001$) compared to normal control group on the day 28th (24.98±1.380) and 35th (24.51±1.097) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The serum calcium level of all experimental groups, except normal control group, was decreased significantly after the ethylene glycol administration (orally) till day 14. On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant decreased in serum calcium level from normal control animals ($P<0.001$). In the negative control group the serum calcium level decreased to the maximum measurable value of 5.83±2.027mg/dl on day 35th and found to be significant decreased ($P<0.001$) compared to the value of day 1st was 10.83±2.042mg/dl. In control animals serum calcium level remain normal during the entire testing period of 35 days (Table 7.2.3). The animals treated on

the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), only were observed that significant increased in serum calcium level (P<0.001) compared to normal control group on the day 28th (8.48±2.016) and 35th (9.32±1.038) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The urine calcium level of all experimental groups, except normal control group, was increased significantly after the ethylene glycol administration (orally) till day 14 (Table 7.2.4. and Figure 7.2.4). On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant increased in urine calcium level from normal control animals (P<0.001). In the negative control group the urine calcium level increased to the maximum measurable value of 6.28±0.072mg/dl on day 35th and found to be significant increased (P<0.001) compared to the value of day 1st was 2.81±0.026mg/dl. In control animals urine calcium level remain normal during the entire testing period of 35 days (Table 7.2.4). The animals treated on the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), only were observed that significant decreased in urine calcium level (P<0.001) compared to normal control group on the day 28th (4.03±0.072) and 35th (3.92±0.026) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The protein level of all experimental groups, except normal control group, was increased significantly in urine and decreased in serum after the ethylene glycol administration (orally) till day 14 (Table 7.2.5; 7.2.6 and Figure 7.2.5; 7.2.6). On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant increased in urine protein level and significantly decreased serum protein level from normal control animals (P<0.001). In the negative control group the urine protein level increased to the maximum measurable value of4.03±1.009mg/dl and serum protein was decreased to the maximum value of 2.83±1.037 on day 35th and found to be significant data (P<0.001) compared to the value of day 1st was 0.86±0.098mg/dl (in urine) and 6.12±1.080 (in serum). In control animals urine protein and serum protein level remain normal during the entire testing period of 35 days (Table 7.2.5 and 7.2.6). The animals treated on the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), only were observed that significant decreased in urine protein level and increased serum protein level (P<0.001) compared to normal control group on the day 28th (serum protein 5.86±1.580 and urine protein 1.44±1.090) and 35th (serum protein 5.98±1.760 and urine protein 1.30±2.001) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The serum uric acid level of all experimental groups, except normal control group, was increased significantly after the ethylene glycol administration (orally) till day 14 (Table 7.2.7. and Figure 7.2.7). On the day 14th, 21st, 28th and 35th of kidney stone induction, the negative control group observed with significant increased in serum uric acid level from normal control animals (P<0.001). In the negative control group the serum uric acid level increased to the maximum measurable value of 7.83±0.930mg/dl on day 35th and found to be significant increased (P<0.001) compared to the value of day 1st was 3.58±1.022mg/dl. In control animals serum uric acid level remain normal during the entire testing period of 35 days (Table 7.2.7). The animals treated on the day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Rosmarinus officinalis* extract (100mg/kg and 200mg/kg), only were observed that significant decreased in serum uric acid level (P<0.001) compared to normal control group on the day 28th (5.17±1.068) and 35th (4.46±1.003) was observed in ethanolic extract of *Rosmarinus officinalis* (200mg/kg).

The present work has detected the evaluation the effect of *Rosmarinus officinalis* on ethylene glycol induced kidney stone in rats. In kidney stone study, male rats were selected as a model system to induce renal stones because the urinary system of male rats resembles that of humans. Ethylene glycol disturbed oxalate metabolism by way of increasing the substrate available that increase the activity oxalate synthesizing enzymes in rats.

The serum concentration of creatinine, urea, calcium, uric acid and protein were shows significant after administration of *Rosmarinus officinalis* extract. Large numbers of studies have reported that in kidney stones, blood protein level decrease and excretion of protein from the urine increase in kidney stones. In ethylene glycol induced kidney stones in rats showed decreased in serum protein level and increased in urine protein level in disease control group. After the treatment with standard group and with ethanolic extract of *Rosmarinus officinalis*, blood protein level was near of normal level. The negative control group showed the loss of blood protein level may be due to its metabolic and excretion rat from the urine.

Uric acid is known to promote calcium oxalate crystal growth. The predominance of uric acid crystals in calcium stone and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its promoting role in stone formation. In present study, higher concentration of serum uric acid was observed in ethylene glycol induced kidney stone in rats. *Rosmarinus officinalis* extract treatment restored the uric acid at normal level thus reducing the risk of stone formation.

The presence of reduced serum urea in ethanolic extract of *Rosmarinus officinalis* treated animals occurred in a dose dependent manner. Urea is the major nitrogen containing metabolic product of protein degradation. The significant reduction in serum urea concentration as the dose increases may be attributed to liver damage or impairment of urea cycle leading to reduced production of the metabolic product. This reduction in serum urea could also indicate reduction in serum amino-transferases in *Rosmarinus officinalis* extract treated animals.

CONCLUSION

Our present study evaluation of effect of *Rosmarinus officinalis L.* on ethylene glycol induced kidney stone in rats concluded that *Rosmarinus officinalis* extract has diuretic properties. *Rosmarinus officinalis* could be attributed to the presence of flavanoids, alkaloids, Tannins, saponin glycosides and phenolic compounds. The diuretic activity may be due to these constituents whereas drug of *Rosmarinus officinalis*, an established diuretic drug which contains triterpenoid saponins (Achyranthine, Oleanolic acid as glycone) for its activity. The diagnosis parameter mentioned, were helped in performing the experiment, and to know Pharmacological activity of *Rosmarinus officinalis* extract.

In conclusion, the significant effect of ethanolic extract of *Rosmarinus officinalis* in rats was observe, significant effect could be result of synergistic/potentiated action of *Rosmarinus officinalis* extract, since it contains a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of kidney stone.

Rosmarinus officinalis extract showed increased serum calcium and serum protein. It also showed decreased serum creatinine, serum urea, serum uric acid, urine calcium and urine protein.

This indicates its protective role against kidney stone. In summary, *Rosmarinus officinalis* extract treatment reversed the alteration in biochemical, morphological changes in kidney and improved all parameters in kidney stone in rats.

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