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Evaluation of the Effect of *Ocimum basilicum* Leaves on Ethylene Glycol Induced Kidney Stone in Rats

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ABSTRACT

In the 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 increasing the activity oxalate synthesizing enzymes in rats. The serum concentration of creatnine, urea, calcium, uric acid and protein were shown significant after administration of *Ocimum basilicum* 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 a 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 *Ocimum basilicum*, 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. The present work has detected the evaluated the effect of *Ocimum basilicum* on ethylene glycol induced kidney stone in rats.

INTRODUCTION

Herbal medicine refers to medicinal plants in folk medicine to prevent and treat various diseases, the history of herbal plant applications dates back to the ancient time. Herbal phytochemical screening showed numerous bioactive compounds with various biological activities such as alkaloids, polyphenols, essential oils, tannins, quinones, sterols, saponins, etc. The medicinal plants represent good sources of new, safest, biodegradable, and renewable drugs with availability and low costs. Recently, in drug discovery, medicinal plant products were strongly employed and they encompass one-third of traditional products. Patients widely use herbal products to treat joint, skin, gastric, hepatic, respiratory and heart disorders, and also can act as anti-fever, anti-cancer, and anti-proliferation. The kidneys, ureter, urinary bladder, and urethra constitute the urinary system. The kidneys are responsible for maintaining fluid and electrolyte balance, removing waste and extracellular fluid, and assisting in the prevention of hypertension. The kidneys are two bean-shaped organs situated on either side of the vertebral column in the posterior abdomen, just below the level of the diaphragm. Most individuals have two kidneys, each one between the level of the twelfth thoracic and third lumbar vertebrae. The right kidney is slightly lower than the left kidney and is placed under the liver. The kidneys are protected in this location, known as the retroperitoneal space, by surrounding flank and back muscles, fat, and fascia. [1] Each kidney is covered by a thin smooth fibrous membrane called a renal capsule. The renal capsule acts as a protective layer and contains pain receptors. It also serves to prevent kidney swelling. [2] The kidney is divided into two main areas:- • Renal Cortex: A light outer area • Renal Medulla: A darker inner area The medulla has 8 or more renal pyramids, which are cone-shaped parts. Renal columns are the spaces between the pyramids. The renal nerves and arteries enter through the hilum of the kidney. It's also where the ureter and renal vein exit. Lymphatic vessels enter and exit through the hilum as well. The functional unit of the kidney is the nephron. Each kidney contains more than one million nephrons that perform all filtration, secretory, and reabsorption functions. Metabolic end products, unwanted substances or excessive ions such as potassium, sodium, hydrogen or chloride are cleansed from the blood plasma by the nephrons. Healthy nephrons accomplish three major functions:- • Filtration of water-soluble substances. • Reabsorption of filtered water, electrolytes and nutrients. • Secretion of excess substances or waste into the filtrate. [3]

Kidney Stones

Currently, the kidneys and urinary system problems predominate which led to life threatening. Renal diseases are the main reason for kidney failure, cardiovascular diseases, and premature mortality. Nowadays, the world ratio prevalence of kidney problems is elevating day by day. Among the urinary tract diseases, kidney calculi are the most prevalent kidney problem, leading to strong pain closing the flow of urine through the urinary tract, severe hemorrhage, and other risks due to their need to take or break by operation.

A. Calcium Stone

Men are more likely than women to develop calcium stones, which account for 75 to 85 percent of all stones. Calcium can build up to an excess in the human body as a result of a high calcium intake from food and supplements.

B. Uric Acid Stone

Uric acid stones make up 5 to 10% of all stones, and they are more common in men. Uric acid stones are formed when there is a high quantity of uric acid in the urine. When there is problem in purine metabolism, excess of uric acid is formed.

C. Struvite Stone

Struvite stones makeup 10 to 15% of all stones, are mostly encountered in women, and have been connected to urinary tract infections. Struvite stones occur when the kidneys become infected. [7]

D. Cystine Stone

Cystine stones account for 1% of all stones and are seen in people who have cystinuria, a genetic condition. Cystinuria (cystine stones) is an autosomal recessive disorder caused by an inborn mistake in the transfer of the dicarboxylic acids cystine, ornithine, lysine, and arginine (COLA).

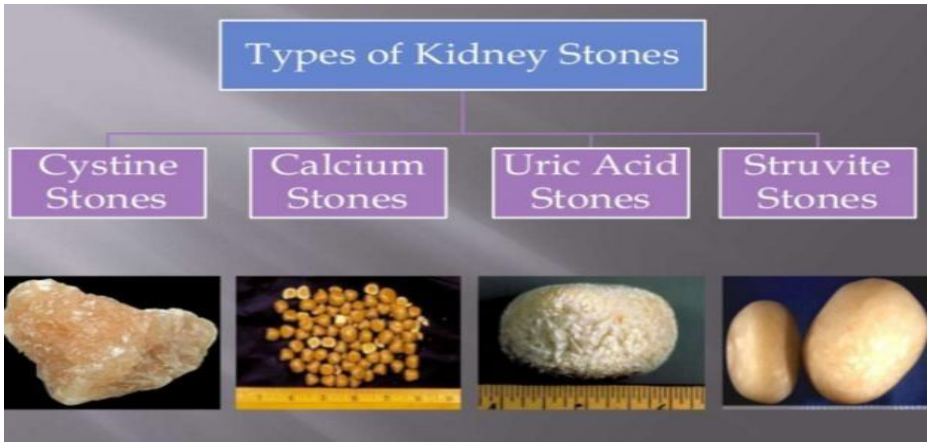

Fig.1.Types of stones

MATERIALS AND METHODS

Collection, Authentication and Storage of Leaves: The leaves have been collected from local market, with the help of field Botanist.

Authentication:

The leaves have been authenticated by Dr. H. S. Gaur University, Department of Botany, Sagar (M.P). Herbarium number Bot/Her/B1/1364, (Ref. no. Bot/169).

Extraction Process

- Petroleum Ether
- Ethanol

Petroleum ether extraction:

The dried and powdered leaves were packed in a soxhlet apparatus and extracted with petroleum ether at 60-80^{0}C for 36 hours. Extraction completion was confirmed by pouring a drop of extract from the thimble on a filter paper, which did not reveal any oil spots.

Ethanol extraction:

After completely evaporating the petroleum ether, the leaves were extracted with semi polar solvent (ethanol) and then packed in a soxhlet apparatus at 60^{o}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 leaves. The alcoholic extract was concentrated and dried. Semisolid extract was obtained. [36-38]

Phytochemical Analysis

Qualitative phytochemical analysis of any plant species is a necessary process as it indicates the presence of constituents and also provides further prospects of the particular plant species for future research investigations.

1) Tests for Alkaloids

0.5 gm of extract was dissolved in 10 ml of dilute Hydrochloric acid (0.1 N) and filter andthis filtrate was used to test the presence of alkaloids.

- Mayer's test
- Wagner's test
- Hager's test

2). Tests for carbohydrates

- **Molisch's test:** Few drops of Molisch's reagent to the filtrate were added, followed by addition H2SO4 by the side of the test tube. The mixture was allowed standing for two minutes and then diluting with 5 ml of distilled water. Formation of a red or dull violet color at the interphase of the two layers was shows positive test. [39-41]

3). Tests for Glycosides:

- Keller killiani's test:

4). Test for Phenols and Tannins

- Ferric Chloride Test:
- Lead acetate Test:

5). Test for Flavonoids

- Shinoda's test for Flavonoids:

6). Tests for Saponins

- Foam Test:

Experimental animals

Apparently healthy Wistar albino rats of either sex of between 150 – 200g were used. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets and water *ad libitum*. Animals were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments[3]. The experimental protocol of study was reviewed and approved by Institutional Animal Ethical Committee (IAEC), Sagar Institute of pharmaceutical sciences, Sagar (M.P.)(Reg. no. SIPS/EC/2018/19.

Acute toxicity (LD50) Test

The mean lethal dose of extracts was determined in albino rats. The LD50 was conductedin a pilot study using nine wistar rats. The rats were randomly divided into 3 groups of 3 rats each and 10,100 and 1000 mg\kg body weight respectively, were administered orally. The animals were monitored for behavioral changes and mortality for 24h. When no death was observed in any of the groups, then animals were given 1250, 1500, 2000, and 5000mg/kg body weight of the extract and monitored for 24 h for change in behavior and mortality. The LD50 is generally calculated as the geographical mean of the least lethal dose that killed a rat and highest dose that did not kill a rat. Usually, the extract dose administered to the animal is either calculated as 1/10th of the observed acute toxicity test or extrapolated from the human dose. In this study, the reference body surface area of rat (0.02m2) was multiplied by the maximum dose (5000 mg/kg b. w) tested during the acute toxicity study that is, $0.02\times5000=100$[49].

Ethylene glycol induced Urolithiasis in Rats model

The 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 served 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 extract at dose of 100mg/kg and 200mg/kg from 15th to till 35th day.[49]

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

RESULTS

S. No.	**Phytochemical Test of Ethanolic extract of *Ocimum basilicum***	**Observations**	**Results**
1.	**Test for alkaloids** a) Dragendroff's Test b) Mayer's Test c) Wagner's Test d) Hager's Test	Reddish brown ppt.Brown ppt. Reddish brown ppt.Yellow ppt.	+ + + +
2.	**Test for Glycosides** a) Legal Test b) Baljet Test c) Keller Killiani's Test	No change No changeNo change	- - -
3.	**Test for Carbohydrates** a) Molisch's Test b) Fehling's Test c) Barfoed Test	Dull violet colorRed ppt. Reddish ppt.	+ + +
4.	**Test for Flavonoids** a) Alkaline reagent Test b) Shinoda's Test	Yellow color turn to colorless Pink color	+ +
5.	**Test for Saponins** a) Foam Test	No change	-
6.	**Test for Phenols and Tannins** a) Ferric Chloride Test b) Lead acetate Test	Blue-blank ppt.Yellow ppt.	+ +

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

Pharmacological Study. Serum Creatinine Estimation (mg/dl)

Serum Creatinine (Mg/dl)							
S.No.	**Bodywt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35thDay**
1	175gm	Positive Control	2.51± 0.007	2.54± 1.006	2.58± 1.032	2.62± 0.098	2.58± 1.012
2	177gm	Negative Control	2.52± 0.018	5.63± 1.012	5.99± 1.320	6.90± 1.320	6.98± 1.310
3	182gm	Standard Group	2.48± 0.032	5.59± 0.092	5.62± 1.026***	4.98± 1.303***	4.83± 0.098***
4	186gm	Test Group-I	2.54± 0.038	5.62± 1.008	5.49± 1.017**a*	5.17± 1.370***a*	5.89± 0.098***a**
5	184gm	Test Group-II	2.51± 0.032	5.51± 0.037	5.33± 1.260***a*	5.03± 1.038***a**	4.81± 1.360***a***

In the table, the 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).

Serum Urea Estimation (mg/dl)

Serum Urea (Mg/dl)							
S.No.	**Body wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	23.27± 2.004	23.23± 1.016	23.31± 2.030	23.37± 1.810	23.23± 2.013
2	177gm	Negative Control	23.23± 1.026	27.16± 1.310	27.93± 2.180	28.18± 1.093	29.18± 1.096
3	182gm	Standard Group	23.98± 1.820	27.13± 1.070	25.97± 0.980**	25.76± 2.010***	25.47± 1.027***
4	186gm	Test Group-I	23.18± 2.030	27.18± 0.720	26.26± 1.030**a*	25.93± 1.097**a*	25.73± 1.210***a*
5	184gm	Test Group-II	23.26± 2.016	27.16± 1.260	26.01± 1.360**a**	25.98± 1.380***a**	25.51± 1.097***a** *

In the table, the 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).

Serum Calcium Estimation (mg/dl)

Serum Creatinine (Mg/dl)							
S.No.	**Body wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	11.85± 2.013	11.85± 2.085	11.82± 2.010	11.83± 1.027	11.85± 2.018
2	177gm	Negative Control	11.83± 2.042	8.58± 1.068	7.36± 1.028	7.01± 2.012	6.83± 2.027
3	182gm	Standard Group	11.85± 1.098	8.53± 2.016	9.36± 2.016***	9.91± 2.016***	10.78± 1.038***
4	186gm	Test Group-I	11.87± 2.045	8.55± 1.061	9.21± 1.086***	9.37± 1.031***a**	9.93± 2.017***
5	184gm	Test Group-II	11.80± 1.087	8.53± 1.002	9.28± 1.036***a*	9.48± 2.016***a**	10.32± 1.038***a***

In the table, the 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 the negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

Urine Calcium Estimation (mg/dl)

Urine Calcium Estimation (mg/dl)							
S.No.	**Body Wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	3.64± 0.014	3.68± 0.026	3.67± 0.031	3.65± 0.031	3.68± 0.032
2	177gm	Negative Control	3.81± 0.026	5.93± 0.032	6.01± 0.042	6.69± 0.072	7.28± 0.072
3	182gm	Standard Group	3.83± 0.031	5.81± 0.058	5.76± 0.028***	4.71± 0.065***	4.73± 0.039***
4	186gm	Test Group-I	3.73± 0.023	5.91± 1.012	5.89± 0.021***a*	5.83± 0.061***a*	5.78± 0.037***a*
5	184gm	Test Group-II	3.80± 0.032	5.87± 1.015	5.80± 0.072**a***	5.03± 0.072***a***	4.92± 0.026***a***

In the table, the 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 the normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to the negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

Serum Protein Estimation (mg/dl)

Serum Protein Estimation (mg/dl)							
S.No.	**Body Wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	7.07± 1.026	7.09± 1.620	7.07± 0.980	7.10± 0.970	7.08± 1.020
2	177gm	Negative Control	7.12± 1.080	5.98± 2.160	4.97± 1.016	4.91± 2.010	3.83± 1.037
3	182gm	Standard Group	7.03± 1.160	5.92± 2.030	6.82± 1.980***	7.93± 1.370***	7.02± 2.017
4	186gm	Test Group-I	7.16± 1.009	5.96± 1.036	5.37± 1.026**a*	6.62± 2.016**a*	6.76±*** 2.360***a*
5	184gm	Test Group-II	7.08± 1.310	5.93± 1.070	6.72± 2.010***a*	6.86± 1.580***a**	6.98± 1.760***a**

In the table, the 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 the normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to the negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

Urine Protein Estimation (mg/dl)

Urine Protein Estimation (mg/dl)							
S.No.	**Body Wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	0.87± 0.076	0.85± 0.027	0.99± 0.160	0.85± 1.002	0.89± 0.360
2	177gm	Negative Control	0.87± 0.098	2.77± 1.003	3.24± 1.038	3.74± 1.036	4.03± 1.009
3	182gm	Standard Group	0.85± 0.076	2.79± 1.260	1.47± 1.021***	1.33± 1.036***	1.28± 1.021***
4	186gm	Test Group-I	0.86± 1.001	2.72± 0.980	1.84± 1.016***a*	1.73± 1.320***a**	1.49± 1.090***a**
5	184gm	Test Group-II	0.87± 1.003	2.74± 1.031	1.53± 1.036***a**	1.45± 1.090***a**	1.31± 2.001***a***

In the table, the 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 the normal control group, b = ns when compared to the normal control group, a*** = P<0.001 when compared to the negative control group, c = ns when compared to standard group. Standard = Cystone(750mg/kg).

Serum Uric Acid Estimation (mg/dl)

Serum Uric Acid Estimation (mg/dl)							
S.No.	**Body Wt.**	**Groups**	**0 Day**	**14th Day**	**21st Day**	**28th Day**	**35th Day**
1	175gm	Positive Control	4.53± 0.720	4.51± 1.016	4.52± 1.020	4.53± 1.026	4.51±1.020
2	177gm	Negative Control	4.58± 1.022	4.68± 0.120	7.12± 0.160	8.26± 0.026	8.83± 0.930
3	182gm	Standard Group	4.56± 1.016	6.51± 1.013	6.32± 0.390***	6.16± 0.091***	5.38± 0.860***
4	186gm	Test Group-I	4.52± 1.023	6.61± 1.038	6.52± 1.036**a*	6.38± 0.920**a*	6.18± 1.098**a*
5	184gm	Test Group-II	4.53± 1.036	6.56± 1.028	6.32± 1.061***a*	6.17± 1.068***a*	6.46± 1.003***a**

In the table, the 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 the 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).

DISCUSSION:

The serum creatinine level of all experimental groups, except the 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 6.98±1.310mg/dl on day 35th and found to be significant increased (P<0.001) compared to the value of day 1st was 2.52±0.018mg/dl. In control animals serum creatinine level remain normal during the entire testing period of 35 days.

The animals treated on day 14th with different groups of drug therapy like standard drugs (Cystone 750mg/kg), *Ocimum basilicum* 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 (9.48±2.016) and 35th (10.32±1.038) was observed in ethanolic extract of *Ocimum basilicum* (200mg/kg).The protein level of all experimental groups, except the 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 increase in urine protein level and significantly decreased serum protein level from normal control animals ($P<0.001$).

CONCLUSION:

In this study the significant effect of ethanolic extract of *Ocimum basilicum* in rats was observe, significant effect could be the result of the synergistic/potentiative action of *Ocimum basilicum* extract, since it contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of kidney stone. *Ocimum basilicum* 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 stones. In summary, *Ocimum basilicum* extract treatment reversed the alteration in biochemical, morphological changes in kidney and improved all parameters in kidney stone in rats.

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REFERENCES:

1. Asplin JR, Favus MJ, Coe FL. Nephro-lithiasis. In: Brenner BM, ed. Brenner and Rector's kidney. 5th ed. Philadelphia: Saunders, 1996: 1893-935.
2. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC; Times trends in reported prevalence of kidney stone in the United state; 1976-1974; Kidney International,2003 May; 63(5);1817-1823.
3. Cavendish, M; "Kidney disorders", Diseases and Disorders;2 (1st ed.). Tarrytown, New York: Marshall Cavendish Corporation. (2008) 490–493.
4. Preminger, GM; "Chapter 148: Stones in the Urinary Tract". In Cutler, RE. The Merck Manual of Medical Information Home Edition (3rd ed.). Whitehouse Station, New Jersey: Merck Sharp and Dohme Corporation; 1299–1349.
5. Cooper JT, Stack GM, Cooper TP. Intensive management of ureteral calculi. Urology 2000;56: 575-8
Dey J, Creighton A, Lindberg JS, Fuselier HA, Kok DJ, Cole FE, et al. Estrogen replacement increased the citrate and calcium excretion rates in postmenopausal women with recurrent urolithiasis. J Urol 2002;167: 169-71.

6. Robbins basic pathology ; 8th edition ; The kidney and its collecting system; 571-573
7. Hoppe, B; Langman, CB (2003). "A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria". Pediatric Nephrology18 (10): 986-981.
8. Curhan GC, Willett WC, Rimm EB, Stampfer MJ. Family history and risk of kidney stones. J Am Soc Nephrol 1997;8: 1568-73.
9. National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Public Health Service, US Department of Health and Human Services. Retrieved 2011-07-27.
10. Barreiro, J. editor, 1989. "Indian Corn of the Americas: Gift to the World." Northeast Indian Quarterly, Spring/Summer 1989. (Gramene Reference ID 9387).
11. Fussell, B., 1992. The Story of Corn. Alfred Knopf, Inc, NY, USA. (Gramene Reference ID 9388).
12. Meng, E. and J. Ekboir, 2000. "Current and Future Trend in Maize Production and Trade." CIMMYT World Maize Facts and Trends. (Gramene Reference ID 9389).
13. Smith, C., et al., 2004. Corn: Origin, History, Technology, and Production. Wiley Series in Crop Sciences. (Gramene Reference ID 9390).
14. Dowswell, C., et al., 1996. Maize in the Third World. Westview Press, USA. (Gramene Reference ID 9391).
15. YasunoriItoh, Takahiroyasui, Atsushi Okada, Keiichi Tozawa, Yutaro Hayashi and KenjiroKohri ; The Journal of Urology ; Vol. 173, 271–275, January 2005.
16. Javed Khan Pathan, Neelam Khan, H.M. Sohel; International Journal of Toxicology and Applied Pharmacology; 1-5; 28 March 2011.
17. Jignesh G.Shah1, Dr. Bharat G.Patel2, Sandip B. Patel1, Ravindra Patel1; Pharmacognosy Communications; Volume 2 | Issue 2 |;34-38; Apr-Jun 2019.
18. Ravindra Kumar, Tirath Kumar, Virender Kamboj, Harish Chander; Asian Journal of Plant Science and Research; 63-72;2019.
19. Solihah, M.A., Wan Rosli, W.I. and Nurhanan, A.R.; International Food Research Journal 19(4): 1533-1538 (2018).
20. Kozan E, Küpeli E, Yesilada E.; J Ethnopharmacol. 2006 Nov 24;108(2):211-6. Epub 2016 May 16.
21. Bhaigyabati Thoudam, T Kirithika, J Ramya, K. Usha; Research Journal of Pharmaceutical, Biological and Chemical Sciences; Vol-2, Issue 4, October-December; 986-992.
22. Guo-Jianyou, Liu-Tongjun, Han-Linna, Liu-Yongmei; Nutrition & Metabolism 2009; Published on 23 Nov 2009; 1-6.
23. Khairunnisa, Hashim Puziah, and Mustafa Shuhaimi reported the “Corn silk in healthcare”; Review article Published in Journal Molecules; 2012;9697-9715.
24. Yaman Husnu, Ikram Hayyom, Abdul karim Shahbozi, Benefits of corn silk (Zea mays) to kidney Shelale Edu. Corporation. 2008; Tajikistan, 4-12.