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Research Article

# ANTILITHIATIC EFFECT OF *HELIANTHUS ANNUUS* LINN. LEAF EXTRACT IN ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED NEPHROLITHIASIS

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**ABSTRACT**

The effect of aqueous and ethanolic extracts of *Helianthus annuus* Linn. (Sunflower) leaves on calcium oxalate nephrolithiasis has been studied in male Albino Wistar rats. Ethylene glycol and ammonium chloride feeding resulted in hyperoxaluria as well as increased renal excretion of calcium and phosphorus. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by treatment using aqueous and ethanolic extracts. From this study, we conclude that both the treatment with aqueous and ethanolic extracts of *Helianthus Annuus* leaves had an inhibitory effect on crystal growth, with improvement of kidney function.

**Keywords**: Ammonium chloride, ethylene glycol, lithiasis, *Helianthus Annuus*.

## INTRODUCTION

Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. Urinary calculi have been found in the tombs of Egyptian mummies dating back to 4000 BC and in the graves of North American Indians from 1500-1000 BC. Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 BC. Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones[1, 2].

Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys. The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Not all standard pharmaceutical drugs used to prevent urolithiasis are effective in all patients, and many have adverse effects that compromise their long-term use. Renal calculi can be broadly classified in two large groups: tissue attached and unattached. Attached calculi are mainly integrated by calcium oxalate monohydrate (COM) renal calculi, with a detectable attachment site to the renal papilla and basically consisting of a core located near to the attachment site (concave zone) and radially striated concentrically laminated peripheral layers. Unattached calculi, with no detectable site of attachment to papilla, are developed in renal cavities of low or reduced urodynamic efficacy and can exhibit diverse composition and structures[3.5]. Several reports have been published since Randall's first description of papillary calcifications and their possible active role in the genesis of COM papillary calculi[4-6]. At present, it seems clear that renal epithelial cell injuries play a decisive role in such a type of renal calculi development, and in fact the lithogenic effect caused by ethylene glycol (EG) must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by EG. Thus, although EG rat model can be questioned as a general model to study renal stone formation, it must be considered as an interesting model to evaluate renal papillary stone development, at least for those stones which genesis is linked to oxidative cell damage[7, 8].

Thus, the first studies on experimental EG renal lithiasis appeared in the 60' decade[9,10], but the importance of the oxidative damage caused by hyperoxaluria was not clearly proposed until the end of the century[11]. From this last period it appeared several prophylaxis proposals on EG induced nephrolithiasis using herbal extracts and antioxidants[12-13]. The effects of these compounds did not seem to be mediated by diuretic or other urinary biochemical changes and positive effects on calcium oxalate lithiasis are most likely due to anti-oxidative effects.

A large number of plant drugs have been used in India since ancient times which claim efficient cure of urinary stones.

***Helianthus Annuus* Linn.** (Asteraceae).

*Helianthus annuus* Linn. is common in Indian gardens, in swampy & malarious districts as its presence purifies the air. Seeds yielded 4.00 p.c. moisture & 46.00 p.c. oil on kernels, and the oils. (Sunflower oil) is used for culinary & table purposes like olive or almond oil, and is also used in scorpion-sting. Its oil-cake is a valuable food for cattle & poultry[14].

An annual plant, markedly pubescent. Stem hispid or hirsute, 1-2m high or higher in cultivation, branched above. Leaves mainly alternate, blades branched ovate, 7-30 cm long or smaller above, usually slightly acuminate at the apex, decidedly toothed, those of the lower leaves cordate at the base, those of the upper cuneate, Ligules of ray flowers 2.5-5 cm long, disk flat, 3-5 cm broad. All parts are often much larger in cultivated forms.

**Distribution: Cultivated in India**

The leaves are emetic, applied in lumbar pain. The leaves have a bitter bad taste, tonic, emmenagogue, aphrodisiac, lessen inflammation, given in insanity, applied in complaints of the chest, liver, lung, used in piles, ophthalmia, ascites, cure diseases of the kidney (Unani)[15].

## MATERIALS AND METHODS

### Preparation of extracts

The fresh leaves of *Helianthus annuus* were collected from local areas of Patan, Maharashtra, India during September-2009 and authenticated at Willingdon College, Sangli, India.

The leaves were dried in shade and were ground to get a coarse powder. The aqueous extract (10%, w/v) of dried leaves was prepared using chloroform water, I.P., by maceration method for 7 days at room temperature and ethanolic extract (10%,w/v) of dried leaves was prepared using 70% (v/v) alcohol by soxhlet method at temperature of 60-70˚C. The extracts were then filtered, concentrated under vacuum and freeze-dried[16].

### Animals

Twenty male Albino Wistar rats weighing approximately 280 g were acclimated for 3 days in cages before experiments commenced. Experiments were conducted in accordance with internationally accepted standard guidelines for the use of animals. Rats had *ad libitum* access to standard chow and tap water, and were kept under a controlled 12 h light/dark cycle at 22 ± 2°C.

**Acute toxicity studies**

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One- tenth of the median lethal dose (LD50) was taken as an effective dose[16].

**Ethylene glycol & ammonium chloride induced urolithiasis model[12]**

The twenty rats were divided into five groups comprising four animals per group. Each group underwent a different treatment protocol for 10 days.

*Group 1:* Normal, *ad libitum* access to regular food and drinking water, and administered 6 µl distilled water per 1 g of body weight by gavage (intra-gastric administration).

Groups 2, 3, 4 and 5 *ad libitum* access to regular food and *ad libitum* access to drinking water containing 0.75% [v/v] ethylene glycol (EG) and 2% [w/v] ammonium chloride (AC) in order to promote hyperoxaluria and CaOx deposition in the kidneys.

*Group 2:* Standard drug (Cystone tab.750 mg);

*Group 3***:** Aqueous extract of *Helianthus Annuus* leaves (500 mg);

*Group 4:* Ethanolic extract of *Helianthus Annuus* leaves (500 mg) and

*Group: 5* Rats were administered 6 µl distilled water/g body weight by gavage (positive control).

All rats were weighed daily.

**Assessment of antiurolithiatic activity**

**Serum analysis**

After the 10-day experimental period, rats were anaesthetized and blood was collected from the retro-orbital region, centrifuged at 10,000 × g for 10 min[17], and the serum collected and analyzed for level of calcium, phosphorus, urea and creatinine using calcium (Biolab Diagnostics), phosphorus (Coral Clinical Systems), urea (Pathozyme Diagnostics), creatinine (Coral Clinical Systems) diagnostic kits.

**Kidney homogenate analysis**

The rats were then sacrificed by cervical dislocation, the abdomen opened and both kidneys removed. The isolated kidneys were cleaned off extraneous tissue and right kidney was preserved in 10% neutral formalin. The left kidney was dried in an oven at 100°C for 24 h, after which the kidney was weighed and then minced in a beaker containing 7 ml 0.5N nitric acid. The mixture was then heated until the liquid became transparent. The calcium content of the mixture was determined using calcium kit (Biolab Diagnostics). The amount of calcium is expressed as µg/g dry kidney. The right kidney was fixed in bouin liquid, soaked in paraffin, cut at 3–4 µm intervals, and the slices stained using hematoxylin and eosin[18,19]. Tissue slices were photographed using optical microscopy under polarized light.

**Statistical analysis**

The result were expressed as Mean ± SEM. Statistical analysis was carried out using one way ANOVA followed by the student-t test. P<0.05 was considered statistically significant. Conventional Windows software was used for statistical computations.

**RESULTS**

**Serum analysis**

Serum analysis showed that urea and creatinine levels were higher in Groups 2, 3, 4 and 5 compared to Group 1 (Fig. 1). These data indicate marked renal damage in the EG/AC-treated rats. The data also showed that urea, creatinine, calcium and phosphorus levels were significantly retained near normal level in rats treated with extracts (Groups 2, 3 and 4) compared to rats treated with EG/AC alone (Group 5, positive control).

**Body weight**

EG/AC-treated rats (Groups 2, 3, 4 and 5) weighed less than the normal rats (Group 1) at the completion of the experiment.

**Calcium levels in the kidneys**

The left kidneys were assessed for calcium levels. EG/AC treatment alone (Group 5) resulted in increased kidney calcium levels compared to the normal rats, while the administration of *Helianthus annuus* extracts reduced this calcium accumulation (Group 2) Fig-3.

**Histological examination**

Histopathological studies clearly revealed that the tissue samples from the control group (Group 1) shows tubules with single epithelial lining along the margin and were of normal size. In Group 5 (positive control), all the tubules showed the presence of crystals, there was marked dilatation of the tubules and total degeneration of the epithelial lining with infiltration of inflammatory cells into the interstitial space. In Group 2, 3, 4 (test group) the specimen showed characters similar to the control group. These morphological findings were consistent with the left kidney calcium level data[20].

**Table 1: Serum biochemical data**

| Groups | Calcium (mg/dl) | Phosphorous (mg/dl) | Creatinine (mg/dl) | Urea (mg/dl) |
|---|---|---|---|---|
| Normal (vehicle) | 6.54±0.07 | 8.00 ±0.03 | 0.37±0.01 | 3.14±0.07 |
| Standard(Cystone) 750 mg | 7.08±0.06* | 7.27 ±0.07* | 0.24±0.02* | 28.98±0.04* |
| Aqueous extract 500 mg | 7.85±0.07* | 8.35 ±0.08* | 0.44±0.01* | 24.37±0.07* |
| Ethanolic extract 500 mg | 7.00 ±0.08* | 8.62 ±0.07* | 0.44±0.01* | 20.97±0.06* |
| Positive control | 9.52 ±0.10* | 8.97 ±0.10* | 0.60±0.03* | 37.64±0.11* |

Values are expressed as Mean ± SEM.,* P<0.05 was considered statistically significant.

**Table 2: Kidney calcium homogenate**

| Groups | Kidney homogenate (Calcium) (µg/gm) |
|---|---|
| Normal (vehicle) | 7.68 ± 0.04 |
| Standard (Cystone) 750 mg | 7.58 ± 0.07* |
| Aqueous extract 500 mg | 4.38 ± 0.09* |
| Ethanolic extract 500 mg | 4.85 ± 0.07* |
| Positive control | 28.67 ± 0.10* |

Values are expressed as Mean ± SEM.* P<0.05 was considered statistically significant.

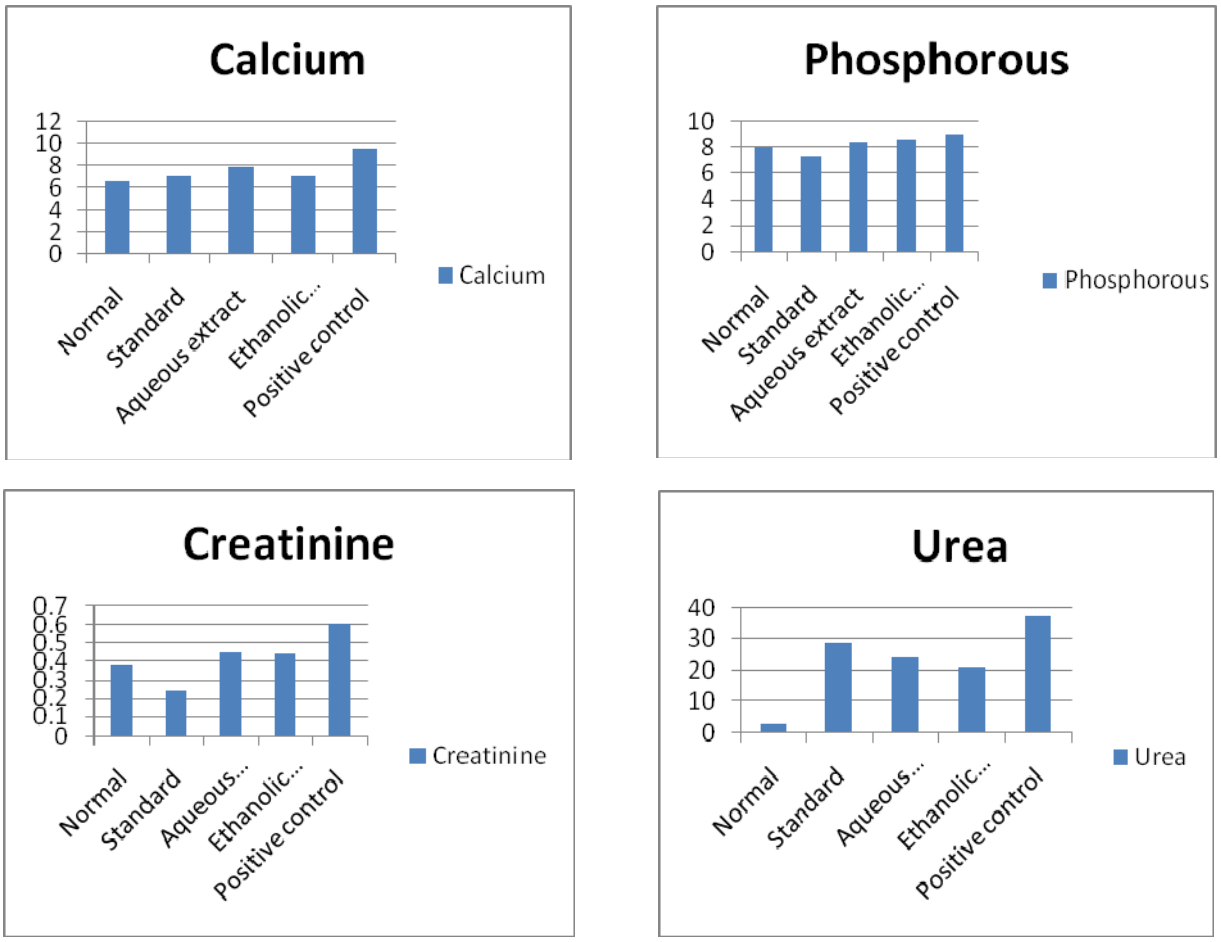



**Fig. 1: Serum calcium, phosphorous, creatinine, urea**

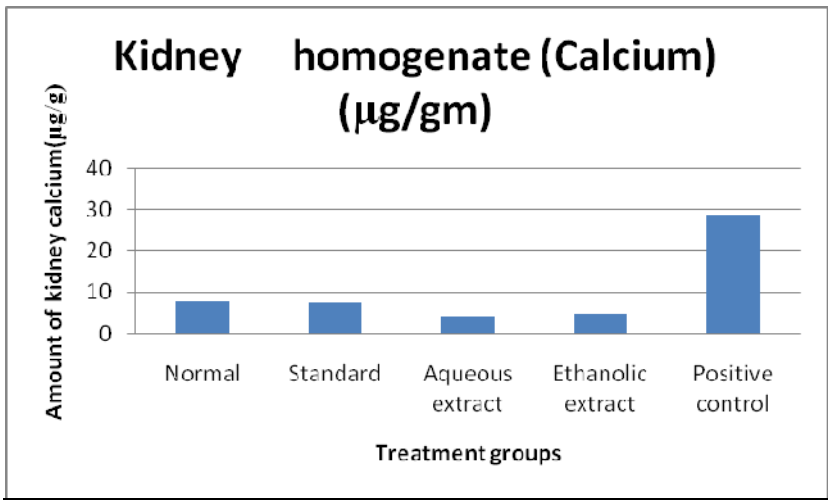
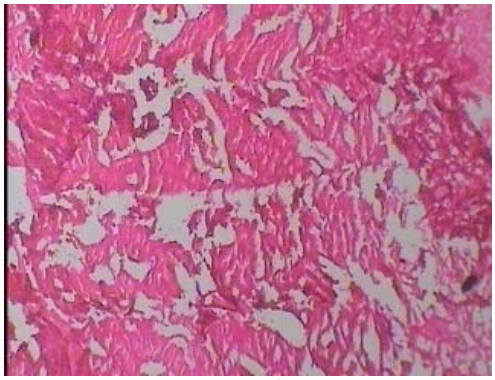
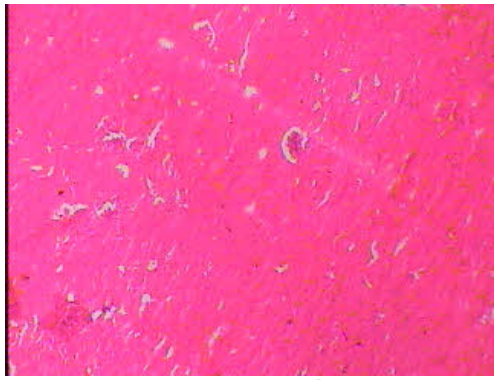


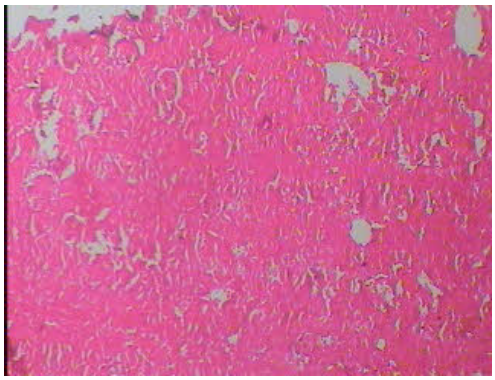

**Fig. 2: Amount of Calcium in left kidney (µg/gm)**



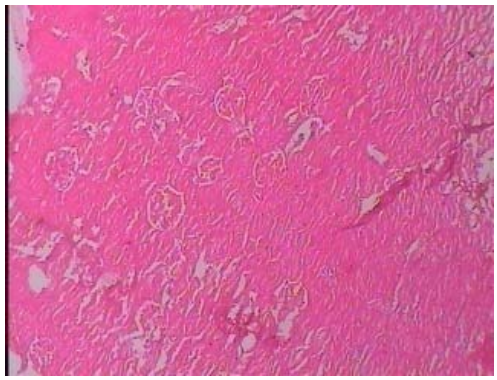
**Normal**



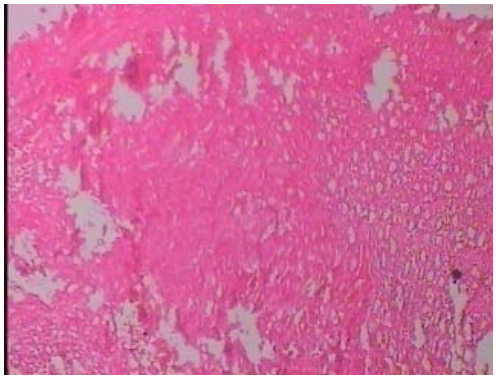
**Positive control**



**Standard (Cystone tablets)**



**Aqueous extract**



**Ethanolic extract**

**Fig. 3: Crystalline formations in the rat kidney cortex, renal parenchyma & renal papilla. Sections were viewed using a BX41 optical microscope and polarized light.**

## DISCUSSION

Urinary lithiasis is generally the result of an imbalance between inhibitors and promoters in the kidneys. Human kidney stones are usually composed of CaOx[1], and several studies have examined the effect of the citrus juices on calcium salt crystallization[21-22]. However, the conclusions from those studies were not consistent. Many in vivo models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease[23-25]. Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans[23]. Rat models of CaOx urolithiasis induced by either EG alone or in combination with other drugs such as AC, are often used to study the pathogenesis of kidney crystal deposition[24]. Using the accelerated model[17], in the present study rats were treated with 0.75% EG and 2% AC for 10 days. All positive control rats (Group 5) developed CaOx depositions during that time.

The present study examined the effect of extracts of *Helianthus Annuus* Linn. on the deposition of CaOx crystals within the rat kidney. The current study analyzed body weight, kidney calcium level, serum concentrations of calcium, phosphorus, urea and creatinine and the histopathology of the kidney. We found that Group 1 rats (normal group) remained active and gained weight, while Group 2, 3, 4 and 5 rats lost weight over the 10 days of treatment. Microscopic examination using polarized light of kidney sections derived from nephrolithiasis rats showed intra-tubular and interstitial crystal deposits. These crystal deposits were observed in

the kidneys of all Group 5 rats. Rats treated with aqueous & ethanolic extracts had far less kidney calcification and lower renal tissue calcium levels than the positive control rats (Group 5) (Table 1 and 2).

## CONCLUSION

The aqueous & ethanolic extracts of *Helianthus annuus* Linn. Significantly reduced the elevated level of calcium oxalate ions which is consider as one of the inhibitor of crystallization. The histopathological findings also show sign of improvement after treatment with extract. All these observation provided the basis for the conclusion that *Helianthus annuus* L. leaves extract inhibit the stone formation induced by ethylene glycol treatment.

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