



**IN VIVO EVALUATION OF COMBINED DOSAGE OF *APIUM GRAVEOLENS DULCE*
AND *BRYOPHYLLUM PINNATUM KURZ* FOR ANTIGOUT ACTIVITY**

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ABSTRACT

The present study aimed at the In vivo evaluation of combined dosage of *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* for antigout activity. The activity was performed for 3 days using ethanolic extract of leaves and stalk of *Apium graveolens dulce* and fresh juice extract of leaves of *Bryophyllum pinnatum kurz* at 500mg/kg and 106.4mg/kg doses. The study conducted against Albino rats (Wistar strain). In this study Allopurinol was used as a standard drug. The results were noted in terms of highest reduction in uric acid formation showing reduction in BUN, creatinine, uric acid and Xanthine oxidase level in serum and uric acid in urine. The combination of ethanolic extract of leaves and stalk of *Apium graveolens dulce* and fresh juice extract of leaves of *Bryophyllum pinnatum kurz* shows significant effect by reducing the formation of uric acid.

KEYWORDS: Allopurinol, *Apium graveolens dulce*, *Bryophyllum pinnatum kurz*, Potassium oxonate.

INTRODUCTION

Gout is usually characterized by repeated attacks of inflammatory arthritis described as red, tender, hot, and swollen joint. Pain usually comes rapidly within twelve hours. In the most of cases the joint at the base of the big toe is affected. It may also result in nodule like formation also known as tophi, urolithiasis, or urate nephropathy. The main reason of causing gout is the combination of diet and genetic factors. It occurs more commonly in those who eat a lot of meat, drink a lot of beer, or are obese. Elevated levels of uric acid in the blood are the important mechanism. Gout occurs at increased levels of uric acid causing the uric acid to crystallize and deposit in joints, tendons and surrounding tissues.^[1]

The most important approach in the treatment of gout is the use of xanthine oxidase (XO) inhibitors, which are effective in reducing blood serum and urinary uric acid levels and as well as the development of tophaceous deposits.^[2] So, food components which consist of xanthine oxidase inhibitor can reduce the formation of uric acid and reduce the inflammation.^[3]

Treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), steroids or colchicine improves symptoms. Once the acute attack reduces, levels of uric acid can be lowered by changing the way of living and in those with frequent attacks, allopurinol or febuxostat provides prevention for a longer time. Incidence of gout in India is not given very clearly.^[1]

The prevalence is 0.12% as per International League of Nations Against Rheumatism, Community Oriented Program for Control of Rheumatic Diseases (ILAR COPCORD) study in Bhigwan village of India.^[5]

ApiumgraveolensDulce (family - Apiaceae) Celery is a marshland plant variety, it has been cultivated as a vegetable. Its stems, leaves, and seeds are used in the preparation of food. Celery seed is used as a spice and its extracts are used for medicinal purpose. Investigation of plant showed the use of all parts of celery plant.^[4]

The seed of the plant can be used as antipasmotic, antirheumatic, antibronchitis, and antiasthma. The root can be used to treat urolithiasis, and the constituent flavonoid present in plant can be used to treat diseases associated to uric acid because of its xanthine oxidase inhibitory activity, it can also be used as a diuretic. The Leaves and stalk are used for arthritis, rheumatism, gout, urinary tract inflammation, and especially for rheumatoid arthritis.^[6]

Bryophyllum pinnatum kurz (family – crassulenscent) commonly known as panfuti, life plant is an herb of about one meter in height, with opposite, glabrous leaves (with 3-5 deeply crenulated, fleshy leaflet). They widely grow in hot and humid areas, around the dwelling places, along road sides and in abandoned farm and fields. It grows widely and used as folk medicine in tropical Africa, India, China, Australia and tropical America Madagascar, Asia and Hawaii.

Incidence of gout in India is not given very clearly. The occurrence is 0.12% as per International League of Nations against Rheumatism, Community Oriented Program for Control of Rheumatic Diseases (ILAR COPCORD) study in Bhigwan village of India.

The cause of Gout is due to elevated levels of uric acid, often related to relatively high levels in the blood. This can occur because diet, genetic predisposition, or under excretion of uric acid. Allopurinol is the most frequently used XO inhibitor prescribed for the treatment of gout. Allopurinol can cause the side effects, such as nephrolithiasis. Thus, the development of new anti-gout agents with greater efficacy and a broader safety profile is greatly needed. Focus on plant research has increased all over the world lately, and a large number of evidence has collected to show immense potential of medicinal plants used in various traditional systems.^[7]

Hence the present study is undertaken to investigate the anti hyperuricemic effect of the plant *Apium graveolens dulce* (celery) and *Bryophyllum pinnatum kurz* (Panfutti) which are traditionally used as antigout agent.

PLANT COLLECTION AND AUTHENTICATION

The plant *Apium graveolens dulce* (celery) was collected in month of October from local region of Sangli, Maharashtra state, India. The plant was identified and authenticated by Dr. S.S Sathe (Asso. Professor Department of Botany) in Rajeramrao College, Jath, Dist: Sangli.

The plant *Bryophyllum pinnatum kurz* (panfutti) was collected in the month of December from local region of Sangli, Maharashtra state, India. The plant was identified and authenticated by Dr. U.S. Yadav (Asso. Professor and HOD of Botany) in Willingdon College of Sangli.

MATERIAL USED

In the present study Allopurinol, Potassium oxonate and xanthine oxidase enzyme were used in antigout activity. All the material was used in laboratory grade.

DOSE SELECTION OF DRUGS

Dose for animal was selected according to LD50.

- 1) *Apium graveolens dulce* – 500mg/kg.
- 2) *Bryophyllum pinnatum kurz* – 106.4mg/kg.

PREPARATION OF PLANT EXTRACT

Preparation of Aqueous extract -Fresh juice was collected from the plant *Bryophyllum pinnatum kurz* by putting the plant leaves in the grinder and extracting the juice with the help of muslin cloth. The extract obtained was used for further use.

Preparation of Alcoholic extract-Fresh leaves and stalks of *Apium graveolens dulce* were washed and air dried for about 6-7 days, and powdered. That powder was extracted with 95% ethanol by soxhlet apparatus for 48 hrs and was used for further oral administration.

IN VIVO PROCEDURE

Albino rats (Wistar strain) [150-280 g] of both sexes obtained from the Pharmacology research laboratory animal house of Appasaheb College of Pharmacy, Sangli.

Male / Female wistar rats weighing 150-280 grams respectively were selected randomly for antigout activity. Animals were kept under standard housing conditions with free access to food and water ad libitum. Rats were kept in polypropylene cages with stainless steel lid. The bedding materials of cages were changed every day. Animal for study were approved by the animal institutional ethical committee under CPSCEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

The anti-gout activity was carried according to standard method (8) in this activity Animals were divided into different groups containing 6 animals in each. Group I was given vehicle P.O, Group II was given special diet {5% fructose+3% uric acid+2% potassium oxonate +0.0001% artificial sweetener (dextrose), + high protein food (soya bean powder)}, Group III was given standard Allopurinol(50mg/kg), and Group IV was given ethanolic extract of *Apium graveolens dulce* (500mg/kg), Group V was given fresh juice extract of *Bryophyllum pinnatum kurz* (106.4mg/kg), and Group VI was given combination of both the extracts. On the third day 24 h urine was collected. Concentrations of uric acid, BUN, creatinine and serum XO activity was determined in blood and uric acid in urine, further the animals were sacrificed by cervical dislocation. The kidney was removed from each animal for Histopathological studies and foot joint of each animal was studied for x-ray determination.

RESULTS AND DISCUSSION

Qualitative phytochemical investigation

Preliminary Phytochemical studies revealed that the ethanolic extract of leaves of *Apium graveolens dulce* contained carbohydrates, flavonoids, alkaloids, steroids, glycosides, phenols, furocoumarins, volatile oils, sesquiterpene, alcohols, fatty acids (Ali Esmail Al-Snafi 2014) and Fresh juice extract of *Bryophyllum pinnatum kurz* contained alkaloids, triterpenes, lipids, flavonoids, glycosides, bufadienolides, phenols and organic acids (Firoz Anwar 2012.) As shown in table no. 1.

Acute oral toxicity study

As per OECD guidelines ethanolic extract of leaves of *Apium graveolens dulce* produced no toxic symptoms or mortality up to a dose level of 5000 mg/kg body weight orally in rat, and hence the drug was considered safe and one tenth of its dose 500 mg/kg was used for our work.

Ali Email Al-Snafi reported LD50 of fresh juice extract of leaves of *Bryophyllum pinnatum kurz* 1064.21 mg/kg produced no toxic symptoms or mortality up to a dose level of body weight orally in rat, and hence the drug

was considered safe and one tenth of its dose 106.4 mg/kg was used for our work.^[9]

Antigout activity of combined dosage of *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* in potassium oxonate induced gout in rats was investigated in the present study.

In the present study there was significant ($p < 0.0001$) increase in creatinine, Uric acid, BUN and serum XO activity and urine uric acid in special diet received rats of gout control group as compared to vehicle group.

As reported by Vaidehi N. Sarvaiya, increased level of uric acid in blood causes gout, which was seen in our research work as well. While in the groups of rat received standard reference compound allopurinol at 50 mg/kg, ethanolic extract of leaves of *Apium graveolens dulce*, 500mg/kg, fresh juice extract of leaves of *Bryophyllum pinnatum kurz* 106.4mg/kg and combination of both these extract showed significant ($p < 0.0001$) reduction in serum creatinine, uric acid, BUN, and urine uric acid in potassium oxonate induced rats as compared to gout control group.

Further, percentage inhibition of serum XO enzyme activity by *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* showed 53% and 41% respectively, when compared to control group. While there was increase in percentage inhibition of serum XO activity in combination of extracts. (54 %).

Vaidehi N. Sarvaiya et.al reported that kidney of PO induced group was seen pale on 4th day as compared to kidney of normal group, which was found similar with our work further there was no change in coloration of treated group.

Vaidehi N. Sarvaiya et.al reported that Histopathological changes in kidneys of PO induced rats showed varying degree of degenerative, as well as necrotic changes. The lesions were mild to severe in nature, and the extent of kidney damage was directly correlated to the degree of urate deposition. Which was found similar with our research work in the gout control group showing changes in atrophy of glomeruli and desquamation of tubular epithelium along with severe congestion, hemorrhage, degeneration, and necrosis of renal tubular epithelium (Figure-2).

Simultaneous treatment of hyperuricemic rats with alcoholic and fresh juice extracts *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* respectively at 500mg/kg and 106.421mg/kg body weight (Figure- 6) and standard treatment allopurinol at 50 mg/kg body weight revealed significant improvements in macroscopical and microscopical renal histomorphological structures (Figure-3).

Radiographic changes were seen in the x-ray of rat foot joint, as shown below by the arrows. Group II showed maximum deposition of uric acid in joint space compared with Group I. The Groups III, IV, V, VI, and VII showed deposition of uric acid much lesser than Group II.

IN VIVO POTASSIUM OXONATE INDUCED MODEL SERUM BIOCHEMICAL ESTIMATION

TABLE NO. 1 Effect of ethanolic and fresh juice extracts of leaves of *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* on serum biochemical estimation.

GROUPS	Normal	Control	Standard	<i>Apium graveolens dulce</i> (ethanolic extract)	<i>Bryophyllum pinnatum kurz</i> (fresh juice extract)	Combination
Uric acid	0.595±0.0329	1.52±0.0093	0.5817±0.0204	0.55±0.0182	0.6033±0.0274	0.515±0.0076
Creatinine	1.125 ±0.0158	1.748±0.02041	0.7417±0.0188	0.77±0.0230	0.7733±0.0162	0.7267±0.0122
BUN	7.278±0.5438	22.34±0.4156	5.138±0.0773	5.967±0.3221	6.867±0.3703	5.633±0.2951
XO activity	1.626 ±0.1477	4.775 ±0.3099	2.467 ± 0.2167	2.027±0.328	0.208±0.2538	1.721±0.3475
XO inhibition %	-	-	64%	53%	42%	54%

Statistical analysis was carried out by one way ANOVA followed by the Dunnett's test at the significance level of $p < 0.0001$, values are expressed as mean ±S.E.M.(n=6)

URINE URIC ACID ESTIMATION

Table No.2 Effect of ethanolic and fresh juice extracts of leaves of *Apium graveolens dulce* and *Bryophyllum pinnatum kurz* on urine analysis.

GROUPS	NORMAL	CONTROL	STANDARD	<i>Apium graveolens dulce</i>	<i>Bryophyllum pinnatum kurz</i>	COMBINATION
Uric acid	263.3±4.302	803±17.42	309.3±18.73	294.5±17.25	292.8±14.93	267±7.65

Statistical analysis was carried out by one way ANOVA followed by the Dunnett's test at the significance level of $p < 0.0001$, values are expressed as mean ±S.E.M.(n=6)

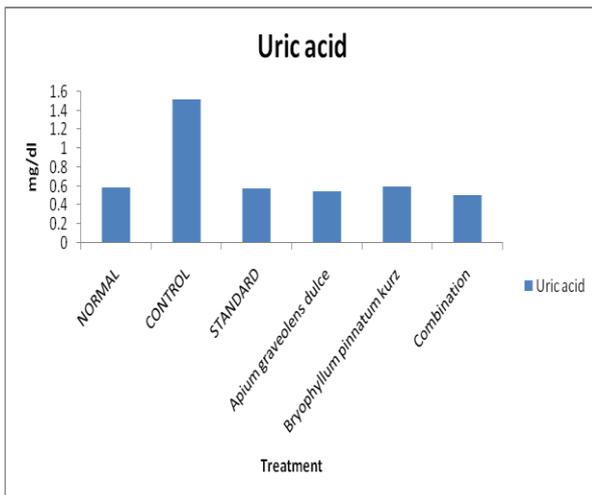


Fig.no: Effect of ethanolic and fresh juice extracts of leaves of *Apiumgraveolensdulce* and *Bryophyllumpinnatumkurzon* Serum uric acid in Potassiumoxonate induced gout.

HISTOPATHOLOGY RESULTS

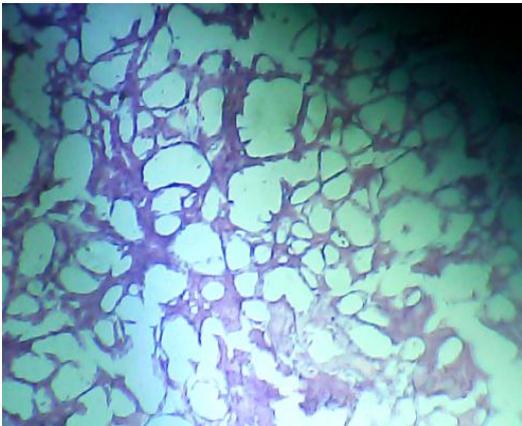


Fig 1: Section of kidney of rat from vehicle control rats (Group I) Did not show any macroscopic and microscopic alterations.

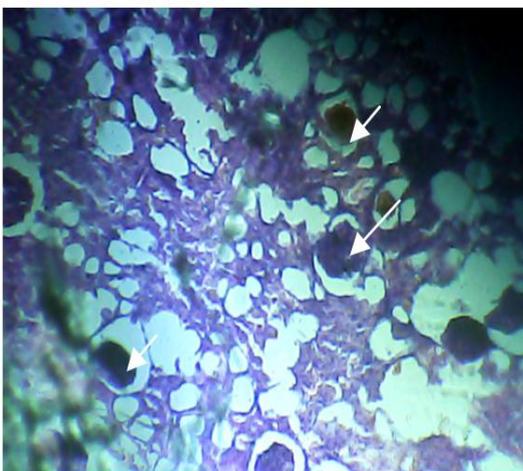


Fig 2: Section of kidney of rat from gout control group (Group II) showing atrophy of glomeruli, severe congestion And hemorrhage with degeneration and necrosis of renal Tubular epithelium.

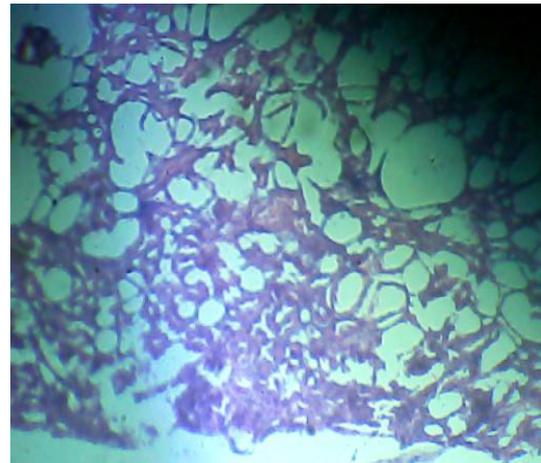


Fig 3: Section of kidney from rat of Group III, Treated with standard allopurinol 50mg/kg revealing, Significant improvements in renal histomorphological structures.

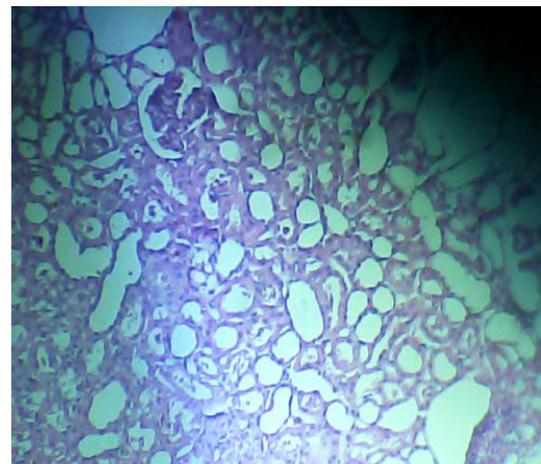


Fig 4: Section of kidney from rat of Group IV, Treated with alcoholic extract of *Apiumgraveolensdulce* of 500mg/kg Revealing significant improvements in renal histomorphological structures.

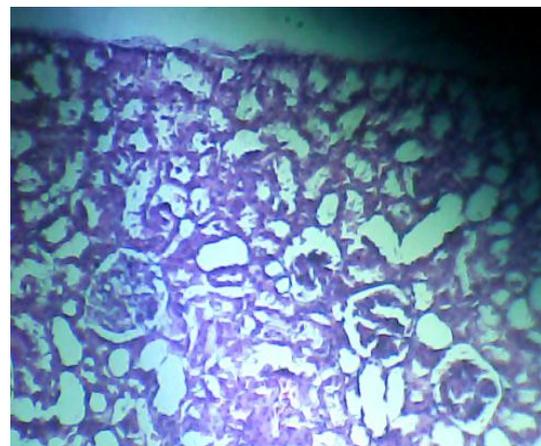


Fig 5: Section of kidney from rat of Group V, Treated with aqueous extract of *Bryophyllumpinnatumkurz* 106.4mg/kg Revealing significant improvements in renal histomorphological structures.

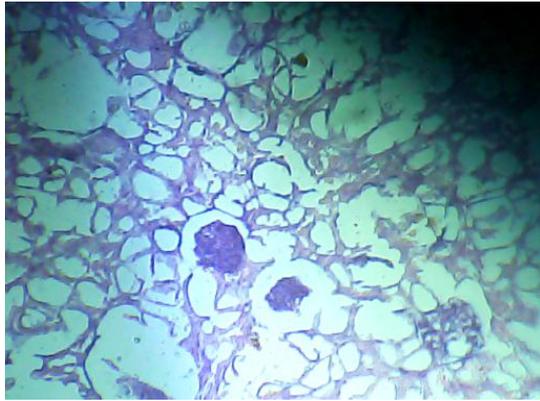
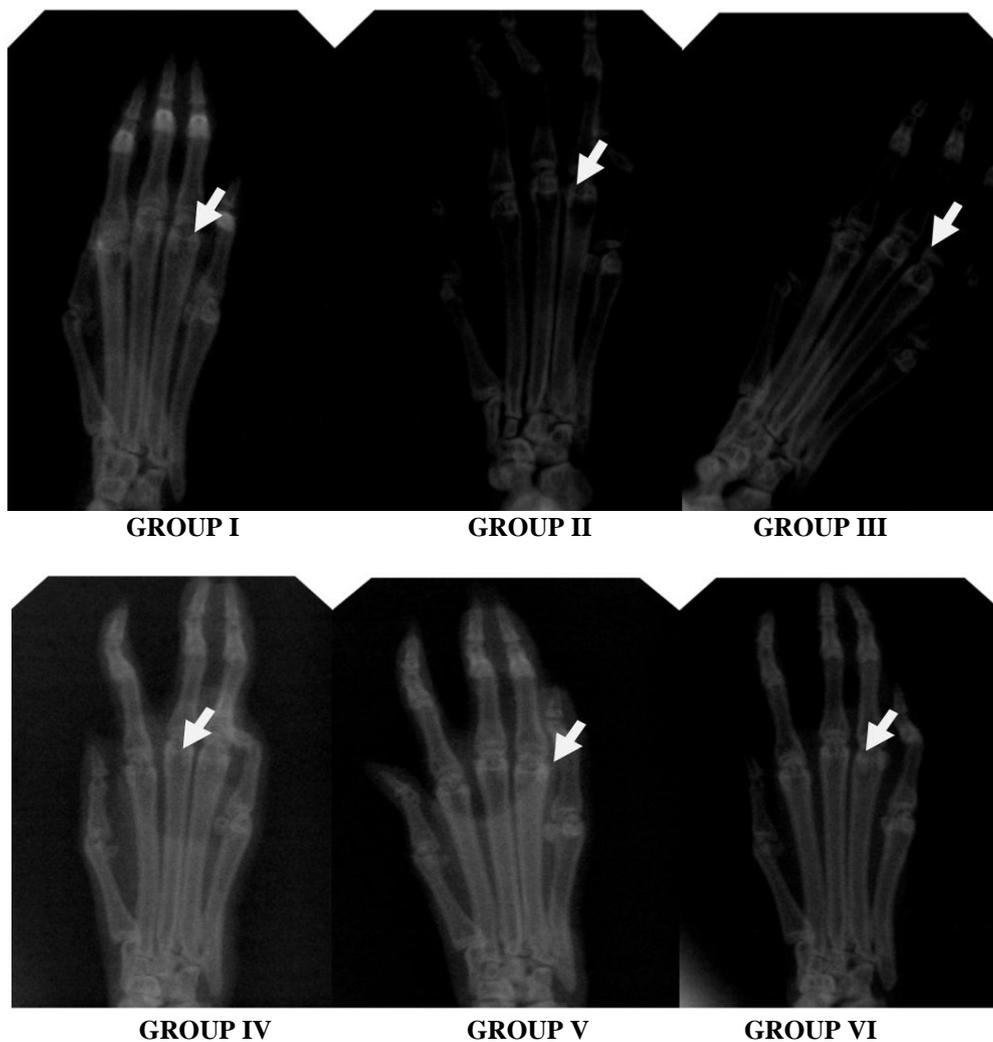


Fig 6: Section of kidney from rat of Group VI, Treated with Combination of ethanolic extract of *Apiumgraveolensdulce* and Aqueous extract of *Bryophyllumpinnatumkurz* 106.4mg/kg revealing significant Improvements in renal histomorphological structures.

RADIOGRAPHY



CONCLUSION

Our study shows that 2 days repeated oral administration of ethanolic extracts and fresh juice extracts of leaves of

Apiumgraveolensdulce and *Bryophyllumpinnatumkurz* at 500mg/kg and 106.4mg/kg body weight produce antinociceptive effect in rats. We suggest that combination effect

of *Apiumgraveolens* dulce and *Bryophyllum pinnatum* kurz have potential for newer therapeutic applications in the future.

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