ORIGINAL ARTICLE

Effect of Apium Graveolens (Celery) Seed Extract on Serum Uric Acid Level of Hyperuricemic Rats and its Comparison with Allopurinol

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Doi: doi.org/10.53685/jshmdc.v2i2.5

ABSTRACT

Background: Plant derived medicines are widely used in traditional culture all over the world.
Objectives: To determine the effect of Celery Seed Extract (CSE) on uric acid levels in hyperuricemic rats and to compare the effect of allopurinol and CSE.
Materials & Methods: It was an animal experimental research study. Group A served as negative control whereas Group B served as positive control. CSE was given orally to three groups of rats (C, D, and E). One hour prior to administration of CSE; potassium oxonate was injected intraperitoneally in all groups except negative control to induce hyperuricemia. Similarly, group F was given allopurinol one hour after injection of potassium oxonate. Blood samples were collected for uric acid estimation.
Results: It was found that administration of both CSE (group C, D, E) and allopurinol (group F) significantly lowered serum uric acid levels ($p<0.001$) as compared to positive control (group B). Serum uric acid lowering effect of both drugs CSE and allopurinol was found to be statistically significant on day 3rd and day 7th and was almost comparable.
Conclusion: Celery seed extract significantly reduces serum uric acid levels in potassium oxonate-induced hyperuricemic rats and its uric acid lowering effect was comparable with that of allopurinol.
Key Words: Celery seed extract, Gout, Uric Acid, Xanthine Oxidase, Allopurinol, Urirc acid level.

How to cite this article: Karim A, Bhatti MSA, Johnson N, Akhter M, Mona S, Safder Z. Effect of Apium Graveolens (Celery) seed extract on serum uric acid level of hyperuricemic rats and its comparison with Allopurinol. JSHMDC. 2021; 2(2): 85-91. doi: 10.53685/jshmdc.v2i2.5

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Received 03.05.2021, Revised 10.06.2021, Accepted 21.12.2021

INTRODUCTION

Gout is a metabolic disorder, due to the deposition of uric salt crystals in the joints and leads to recurrent episodes of arthritis.[1] In people with gout, uric acid levels in the blood exceed normal limits (hyperuricemia) so it is often referred to as uric acid disease.

Hyperuricemia is a metabolic disease which is detrimental to health of humans. It is known as considerable risk factor for gout and is also related to the metabolic syndrome, cardiovascular disease, stroke and chronic kidney disease. [2,3] High uric acid level has also been found to directly inhibit insulin signaling and induces insulin resistance.[4]

Currently, some uricostatic and uricosuric pharmaceutical medicines are used and have proved to be efficient in controlling uric acid levels in serum. But their clinical importance is limited by their harmful effects on liver and kidneys. Hence, attention is being drawn towards natural and herbal products as a management regimen for hyperuricemia. People are accustomed to use medicinal plants as home remedies widely and these are also used as a basic material for pharmaceutical preparations. In the past decade or so, their use has tremendously increased. In the Southeast Asia, celery has been used and prescribed as a folk medicine to manage various diseases like urolithiasis and gout. Celery or apium graveolens extract has been shown to act as an anti-inflammatory, gastro-protective, and hepato-protective agent. It has also been known to be protective against Helicobacter pylori infection.[5] Apium graveolens extract influences mood and cognition in healthy mice.[6] Celery was also found effective in reducing blood glucose levels.[7] Hanaa et al., investigated effects of extracts of the celery, leek, parsley, and molokhiaand found that they have some protective role in treatment of gout.[8] Sedanolide, obtained from celery seed oil, had been consumed as a folk medicine to manage inflammatory-associated diseases such as gout and rheumatism.[9] Hence, it is important to find the serum uric acid lowering effects of CSE and compare them with allopurinol, which is the standard drug for decreasing serum uric acid, so that it may be used for patients of gout and hyperuricemia.

Celery is traditionally used to treat rheumatism and cardiovascular disorders. Hyperuricemia is considered as a predisposing factor for gout and is also associated with coronary artery disease.The objective of present study was to determine the effect of CSE on serum uric acid level and to compare the effect of CSE with allopurinol in animal model of hyperuricemia.

MATERIALS AND METHODS

Celery seeds were collected and ground by using domestic blender into small particle size. 10 g of seed powder was soaked in 100 ml of methanol for 24 hours. The extract was filtered and transferred to the rotary evaporator. While the entire methanol was separated, extract was collected from the collecting flask and stored at 4° C for further tests.

Animals

The present study was conducted on 36 rats that were randomly divided into six groups; each containing six rats. The weight of animals was between 160-205 grams. These animals were studied in a normal and healthy laboratory environment and were given a basal diet as feed and water ad libitum. All the experimental procedures were carried out in the Pharmacology Department of Shalamar Medical and Dental College Lahore. Throughout the experimental period, the animals were seen daily to observe any signs of toxicity and their weight was recorded at regular intervals.

Induction of hyperuricemia in rats

To produce hyperuricemia, 250 mg/kg potassium oxonate was dissolved in 0.9% saline solution and

injected intraperitoneally, except to animals belonging to group A (negative control). Potassium oxonate is a uricase enzyme inhibitor that stops the conversion of uric acid to allantoin and thus artificially increases serum uric acid levels in rats to provide a hyperuricemic animal model.

Methodology

Group A served as negative control group. In other 5 groups hyperuricemia was induced by intraperitoneal injection of potassium oxonate.[10] Group B served as positive control. One hour after the induction of hyperuricemia, group C, D and E were given 100 mg/kg, 200 mg/kg and 400 mg/kg body weight CSE respectively whereas group F was administered 5 mg/kg allopurinol via oral gavage. This regimen was continued for the next seven days.[11,12]

For sample collection, light anesthesia was given to rats and an intracardiac puncture was performed on day zero and also on the last day of the experiment i.e. on day seven. Blood samples were also extracted from tail vein on the first and third day of the experiment after 3 hours of administration of test agent. The samples were kept at room temperature for 1 hour so that they could clot, and were then subjected to a centrifuge for 10 minutes at a speed of 2500 rpm. Serum was separated and stored at -20°C.

Determination of serum uric acid level on day 0, 1, 3 and 7 was done by using enzymatic calorimetric technique, using the commercially available diagnostic kit (Uric acid liquiform monoreagent kit, labtest, France) at Department of Pathology, Shalamar Medical and Dental College Lahore.

Statistical Analysis

Data was analyzed using SPPS version 21. The normality of data was assessed using Kolmogrov-Smirnov test. Standard analysis of variance (one-way ANOVA) was employed to assess difference among groups. Post-hoc tukey's test was used for pair-wise comparison between the study groups. A p value ≤ 0.05 was considered as statistically significant.

RESULTS

In order to induce hyperuricemia, potassium oxonate was injected to all experimental rats through peritoneum except batch A (negative control group). After the occurrence of hyperuricemia, the experimental rats were given as treatment the CSE as well as allopurinol and then the decrease in their uric acid levels was estimated. Estimated levels of serum uric acid in all rats are given in table 1. In group A (negative control), serum uric acid level on day zero was 3.33±0.28 mg/dl (mean±S.D). The average serum uric acid level in all six groups showed insignificant difference on day zero ($p > 0.05$).

In hyperuricemic group B (positive control), serum uric acid level was significantly raised after giving an intraperitoneal injection of potassium oxonate ($p< 0.001$) whereby mean ± S.D level of serum uric was 6.5± 0.22 mg/dl on 3rd day and 9.0 ± 0.45 mg/dl on 7th day of the experiment.

In group C (minimum dose group i.e. CSE 100 mg/kg), mean serum uric acid level was 4.5 ± 0.38 mg/dl on 3rd day and on seventh day it was 4.6± 0.25 mg/dl. These levels were found to be significantly lower than the hyperuricemic group B ($p < 0.001$). Group D (medium dose group i.e. CSE 200 mg/kg) had serum uric acid levels of 3.5 +0.34 on 3rd day and on 7th day it was 3.3 ± 0.43 mg/dl. Likewise, mean serum uric acid level of group E (maximum dose group i.e.

CSE 400 mg/kg) was 3.4±0.3 on 3rd day and on day seven it was 3.0 ± 0.25 mg/dl. Serum uric acid levels of group D and E were significantly lower as compared to hyperuricemic group B (positive control) animals on 3rd and on last day (i.e. day 7) of experiment ($p<0.001$). In group F (5mg/kg allopurinol treated group), a significant decrease (p

< 0.0001) in serum uric acid levels was observed with a mean value of 3.1± 0.42 mg/dl on 3rd day and 2.9± 0.38 mg/dl on 7th day of the experiment (Table 1).

Keeping in view the the results of serum uric acid levels obtained on day 3 and day 7, it can be concluded that administration of both CSE (group C, D, E) and allopurinol (group F) significantly lowered serum uric acid levels ($p<0.001$) as compared to group B (positive control). Serum uric acid lowering effect of both drugs CSE and allopurinol was found to be statistically significant on day 3 and 7; their effect being almost similar. These results are elaborated in figure 1, 2 and 3.

Table 1: Effect of CSE and allopurinol on serum uric acid level in experimental rats

Groups	Zero day mean±SD (mg/dl)	1st day (mean±SD) (mg/dl)	3rd day (mean±SD) (mg/dl)	7th day (mean±SD) (mg/dl)	p value
Group A (-ve Control group)	3.3±0.28	2.9±0.24	3.5±0.34	3.6±0.28	<0.05
Group B (+ve Control group)	3.2 ±0.24	3.4 ±028	6.5 ±0.21	9.0±0.45	<0.0001
Group C (CSE 100 mg/kg)	3.4 ±0.46	3.5 ±0.51	4.5 ±0.38	4.6 ±0.25	<0.0001
Group D (CSE 200 mg/kg)	3.6 ±0.34	3.7 ±0.44	3.5 ±0.34	3.3±0.43	<0.0001
Group E (CSE 400 mg/kg)	3.3 ±0.49	3.5 ±0.38	3.4 ±0.30	3.0 ±0.25	<0.0001
Group F (Allopurinol)	3.0 ±0.34	3.4 ±0.48	3.1±0.42	2.9±0.38	<0.0001

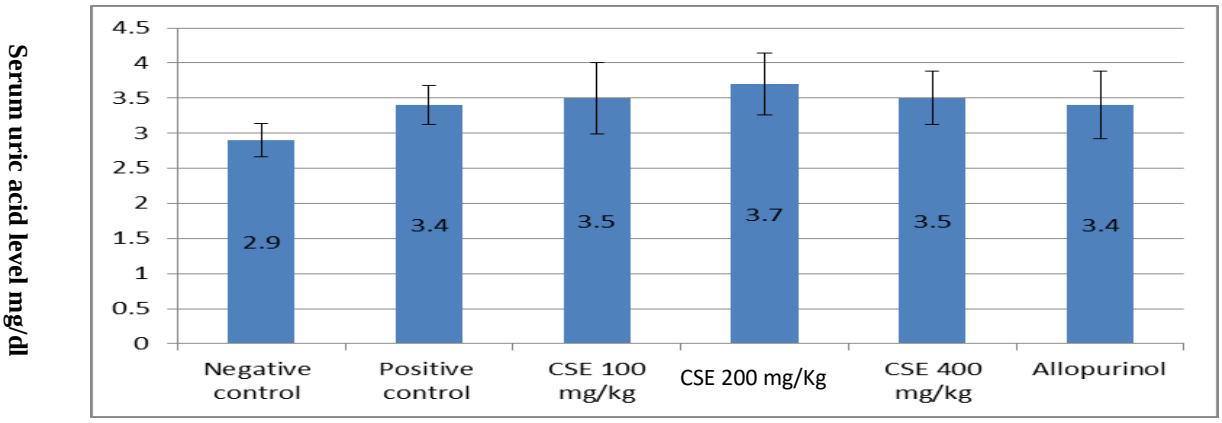

Figure 1: Effect of CSE on serum uric acid levels (mg/ dl) on day 0

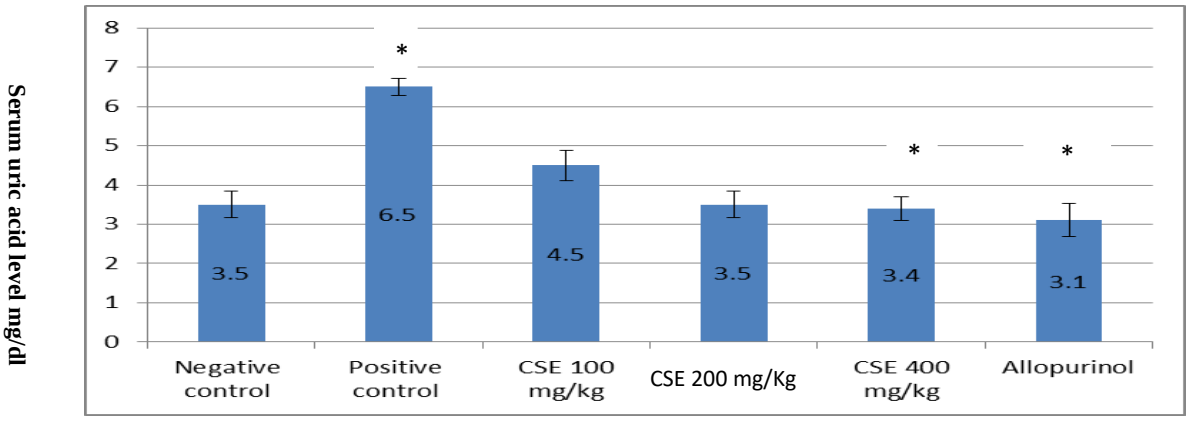

Figure 2: Effect of CSE on serum uric acid levels (mg/ dl) on day 3 (* p ≤ 0.0001)

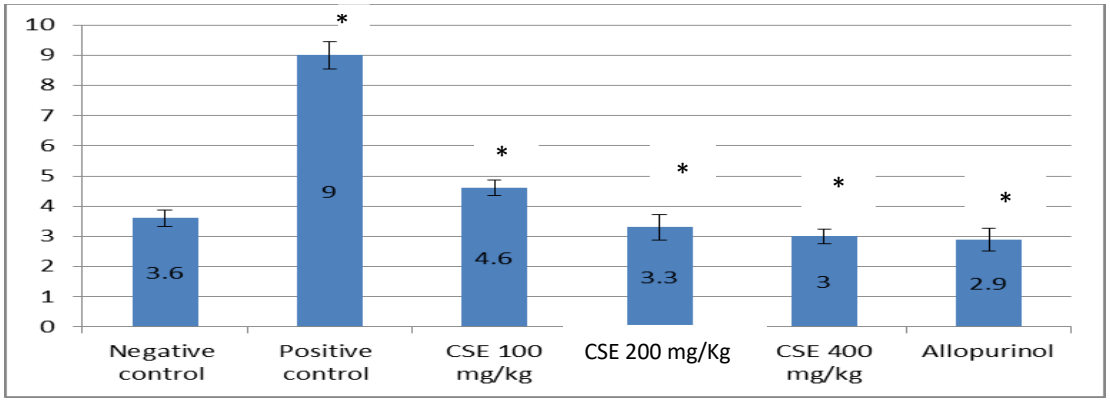

Figure 3: Effect of CSE on serum uric acid levels (mg/ dl) on day 7 (* p ≤ 0.0001)

Uric acid is the end product formed after metabolism of purine. Humans have more levels of uric acid compared with other mammals. Hyperuricemia is defined as high levels of uric acid in the blood that exceeds the normal range. In a normal population, the upper limit of the normal range is 360µmol/L (6mg/dL) for women and 416µmol/L (7.0mg/dL) for men.[13]

Hyperuricemia may be caused by overproduction of uric acid into the body. It may also be induced by certain drugs. Xanthine oxidase inhibitors, such as allopurinol or febuxostat, are commonly used to lower the body's uric acid but they have many side effects.[14] Celery has been found

useful as herbal medicine to treat gout and can be recommended as alternative natural medicine to substitute synthetic drugs as allopurinol.[15] However, scientific evidences for use these natural drugs is still required. The present study demonstrated that CSE lowered the level of uric acid in the Serum significantly, which is in accordance with the study of Dolatti et al; 2018 who found that A graveolens extract can lessen the serum uric acid levels through blocking hepatic xanthine oxidase activity and has shown the practical usage as a beneficial bioactive agent or functional food that can induce hypouricemia.[16] The probable mechanism of action of CSE is inhibition of xanthine oxidase activity.[17] It was observed in a study that oral administration of celery, leek and parsley decreased level of uric acid level and creatinine in gouty rats, which is consistent with this study. Recently Shaopeng et al., 2019 investigated in their study that both aqueous extract and oil extracts of Celery seeds decrease uric acid levels in the sera of mice having hyperuricemia and reduces ankle joint swelling in rats with gouty arthritis.[18]

For recommending these agents as potential therapeutic use, investigations on their toxicology were carried out with an

Alcoholic extract of Celery seeds and found that there were no significant toxic sub-chronic effects on its oral administration in rats.[19]

Recommendations

All these experimental evidences suggest that celery seeds must be further evaluated to make them useful for treatment of hyperuricemia and gout in human beings.

CONCLUSION

The present study demonstrated that methanol extract of CSE possesses hypourecemic activity in Potassium oxonate-induced hyperuricemia in rats. These findings suggest the use of CSE as a dietary supplement for the treatment of hyperuricemia may be beneficial, but the molecular mechanisms underlying these effects require further investigation**.**

Source of funding

Grant for the project was provided by the Shalamar Medical & Dental College, Lahore.

Conflicts of interest

All authors declared no conflicts of interest.

Contributors

AK: contributed to the conception and design of the study.

SB: drafted the work and revised it for intellectual content

NJ: entered, analyzed and interpreted data

MSAB: contributed to writing of literature review

SM: conducted experimental work

ZS: was involved in drug & extract preparation.

All authors approved the final version and also agreed to be accountable for all aspects of the work.

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