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ORIGINAL ARTICLE

Xanthine Oxidase Inhibitory Activity of Ethanolic Extract of *Ficus Carica* Fruit

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ABSTRACT

Hyperuricemia is a purine metabolism disorder characterised by an excess of uric acid in the blood and considered as a risk factor for gout, coronary heart disease, hypertension, diabetes, and a variety of other illnesses. Xanthine oxidase inhibitors have key role in management of hyperuricemia and related disorders but multiple adverse effects associated with these agents have minimized their chronic use. *Ficus carica* fruit has been widely used around world as a therapeutic agent for several disorders. In traditional medicine it has been used to treat gouty arthritis but the effect has not been scientifically established so far.

Objective: This study was conducted to assess the *in vitro* xanthine oxidase inhibition of *Ficus carica* fruit extract.

Method: The ethanolic extract of *Ficus carica* fruit was evaluated *in vitro* at five different doses for its xanthine oxidase inhibition. Xanthine oxidase inhibitory activity was measured spectrophotometrically and inhibitory concentration IC_{50} was calculated. The dose dependant inhibition of *Ficus carica* fruit was compared with inhibition of standard drug allopurinol.

Results: *Ficus carica* fruit extract was found to possess xanthine oxidase inhibition with IC_{50} 27.5 µg/ml in comparison to the allopurinol with IC_{50} 11.0 µg/ml.Thus the agent can be used as alternative to standard drug allopurinol for treatment of hyperuricemia and related disorders.

Conclusion: The study showed that *Ficus carica* fruit has potential to inhibit the xanthine oxidase so can be used as natural source to treat gout and many other xanthine oxidase related medical disorders.

Keywords: *Ficus carica* Extract (FCE), Xanthine Oxidase (XO), Xanthine Oxidase Inhibition (XOI), Hyperuricemeia (HU).

INTRODUCTION

Hyperuricemia is clinicaly depicted by bizarre expanded degrees of uric acid within the blood. Epidemiological information uncovers that the normal serum concentration of uric acid ranges between 6.0 – 7.0 mg/dl. A limit value of 6 mg/dl is favoured because it more correctly identifies a sound populace.[1] Frequency of hyperuricemia is uncommon in premenopausal women due to contrary relationship between female gonadal hormones and serum uric acid levels.[2]

The worldwide predominance of hyperuricemia is going to be hightened due to numerous reasons counting iatrogenic ones. Purines are considered as primary generators yielding uric acid within human body by utilizing XO enzyme. This enzyme catalyzes the hydroxylation of hypoxanthine to xanthine first and xanthine to uric acid at the end.[3] The kidneys are chief organs responsible for elimination of uric acid. Either increased production or reduced elimination of uric acid leads to hyperuricemia.[4]

Xanthine oxidase being a principal enzyme involved in the synthesis of uric acid plays a prevailing part in hyperuricemia and its related clinical conditions.[5] It is considered as the most important contributory factor for development of gouty arthritis characterized by accumulation of uric acid crystals in joint synovium, although associated with other chronic clinical conditions like hypertension, diabetes and renal failure collectively known as metabolic syndrome.[6]

The pharmacotherapy of hyperuricemia is aimed at modulating the activity of enzymes involved in synthesis and of transporters renposible for excretion of uric acid. There are two classes of drugs uricostatic and uricosuric. The first one diminish the uric acid production via inhibiting XO enzyme including Allopurinol and Febuxostat [7] while later limit the reabsorption of uric acid at renal proximal tubules and include probenecid and benbromazone[8]. Drugs included in both groups carry multiple side effects like allopurinol is associated with dose dependant gastrointestinal intolerance and cutaneous rashes.[9] The commonly reported adverse effects with Febuxostat are deranged liver enzymes and headache.[10] Uricosuric drugs are alternative drugs for those intolerant to allopurinol and febuxostat . Probenecid is contraidicated in patients with renal failure and benzbromazone is associated with hepatic toxicity.[11]

Keeping in view multiple adverse effects associated with uric acid lowering drugs, conventional therapeutic plants have drawn attention to be utilized as an alternative in this regard.

Flavonoids , diverse constituents present in various plants have been studied extensively to show their therapeutic benefits in various clinincal conditions. These are found to be effective compounds in inhibiting xanthine oxidase.[12] Xanthine oxidase inhibitory activity has been reportred from traditional plants like, *Phyllanthus niruri, Tradescentia albiflora Cinnamomum cassia, Chrysanthemum indicum, Lycopus europaeus (Lamiatae)* and *Polygonum cuspidatum.*[13]

Ficus carica commonly known as 'fig' is a temperate species. From ancient times it has been cultivated due to its high nutrition value. This plant welcomes consideration of the researchers worldwide for its biological activities. The therapeutic application of *Ficus carica* has been shown within the various traditional systems of medication such as Ayurveda, Unani, and Siddha. It has been utilized to treat diabetes, asthma, and disorder of gastrointestinal tract like diarrhea. Phytochemical analysis reveals that it is a a rich source of flavonoids.[14] *Ficus carica* leaves have been studied for hypouricemic effect and *in vitro* xanthine oxidase enzyme inhibition [15] but fruit has not been studied to evaluate this effect.

This stuy aimed to see the protective effect of this fruit against hyperuricemia via XO inhibibtion test *in vitro* .

MATERIALS AND METHODS

Materials: *Ficus carica* dry fruit 100 grams, Tab. Allopurinol 100mg (GSK), Xanthine powder (Sigma Aldrich), Xanthine oxidase (Sigma Aldrich), Phosphate buffer saline tablets (Linear chemicals) Absolute ethanol (Scharib S.L), Micropipettes (0.5-10ul) Micropippettes (100-1000ul), Yellow and Blue tips for micropippettes, Distilled water, Gloves, Glass test tubes, Spectrophotometre 29 nm (Pictus B Diatron, japan).

Preparation of Extract: One hundred gm of dried Fig fruit was obtained from local market of Lahore and sent to the Botony department of Government College University Lahore for identification. After rinsing with fresh water it was kept at room temperature to parch for three days. Weight of dessicated fruit was found to be constant by the end of this period.

Ethanolic Fig extract was prepared by drenching dried fuit in a ratio of 1:10. The glass bottle was subject to occasional shaking for three days. By using filter paper , supernatant solvent was sieved and evaporated to get chocolate colored paste. After weighing, this crude extract was stored at temperature of 5C°. This process yield 30% ethanolic *Ficus carica* extract.[16]

***In vitro* Xanthine oxidase inhibitory assay:** Spetrophotometer with wavelength of 295 nm was used to measure the xanthine oxidase inhibition in the presence of test agents and xanthine as substrate. A well known xanthine oxidase inhibitor allopurinol was taken as control to compare the inhibitory effect of *Ficus carica*. Both test samples were used in five different concentrations starting from 100 µg/ml in distilled water. Later serial dilutions were made up to 5 µg/ml .

Initially assay mixture contained 1.9 ml of 50 millimoles potasium phosphate buffer having pH 7.5, 0.1 ml of test agents FCE and allopurinol and 0.1 ml of freshly prepared enzyme solution that contained 0.2 units of xanthine oxidase (XO) in one ml of Phosphate buffer. This mixture was incubated at 37°C for fifteen minutes. Later added 1.0 ml of 0.15 mM xanthine solution to start the reaction. This was again followed by reincubation at 37°C for thirty minutes.

To stop the reaction 1N HCL was added to this mixture and absorbance was measured with the help of spectrophotometer. Likewise, blank and control mixtures were prepared. In blank mixture enzyme solution was replaced with phosphate buffer while in control 0.1 ml of distilled water was added instead of test agents to yield optimum uric acid.

All of the determinations were performed in triplicate. Percent xanthine oxidase inhibition was calculated using formula.[17]

$$Percent\ XO\ inhibition = \frac{(Absorbance\ control - Absorbance\ sample)}{(Absorbance\ Control)}\ x\ 100$$

In above formula, absorbance sample was calculated by abstracting absorbance blank from absorbance sample. Half maximal inhibitory concentration (IC_{50}) was determined with help of Microsoft Office Excel 2010 using dose response data.

RESULTS

Both test agents exhibited concentration dependant xanthine oxidase inhibition. At greatest concentration (100 µg/ml) FCE showed 80.1% inhibition in comparison to allopurinol that showed 94% inhibition. Calculated half maximal inhibitory concentration of FCE was 27.5 µg/ml in comparison to allopurinol that was found to be 11.0 µg/ml.

Table 1: Individual absorbance values of *in vitro* Xanthine Oxidase inhibitory Assay (Allopurinol)

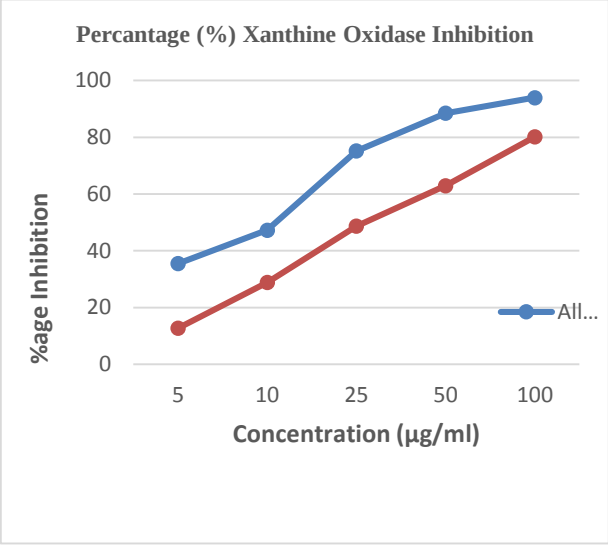
Conc.µg/ml (sample)	Absorbance Sample at given concentration	Absorbance Blank at given concentration
5	Mean= 0.257	Mean= 0.010
10	Mean= 0.223	Mean= 0.021
25	Mean= 0.114	Mean= 0.019
50	Mean= 0.055	Mean= 0.011
100	Mean= 0.035	Mean= 0.012
Absorbance control (Mean) = 0.383		

Table 2: Individual absorbance values of *in vitro* Xanthine Oxidase inhibitory Assay (*Ficus carica* Fruit FCF)

Conc. µg/ml (sample)	Absorbance Sample at given concentration	Absorbance Blank at given concentration
5	Mean= 0.362	Mean= 0.032
10	Mean= 0.309	Mean= 0.040
25	Mean= 0.221	Mean= 0.027
50	Mean= 0.170	Mean= 0.030
100	Mean= 0.103	Mean= 0.028
Absorbance control (Mean) = 0.378		

Table 3: Percent Xanthine oxidase inhibition of FCE compared with allopurinol with IC_{50} values

Percent xanthine oxidase inhibition	Concentration					IC_{50} (µg/ml)
	5 µg/ ml	10 µg/ ml	25 µg/ ml	50 µg/ ml	100 µg/ ml	
Allopurinol	35.5 %	47.2 %	75.2%	88.5%	94.0%	11.0
FCE	12.7%	28.8%	48.6%	62.9%	80.1%	27.5



DISCUSSION

Xanthine oxidase (XO), a versatile enzyme is extensively distributed among different species ranging from microbes to human and even to the multiple mammalian tissues. It is an important group member of enzymes collectively known as xanthine oxidoreductase. In human beings its main function is to catalyse the breakdown of hypoxanthine to xanthine first and then to uric acid as a last step of this reaction involved in the generation of purine bases[5]. The buildup uric acid in the body can initiate several pathlogical processes like diabetes mellitus, hypertension, stroke and hyperlipidemia all of which are important components of metabolic syndrome .[18]

Allopurinol and febuxostat are two available xanthine oxidase inhibiting pharmacological agents used to lower uric acid levels in hummans. Still these agents are considered as superior to uricosuric and anti inflammatory agents in this regard. Besides their effectiveness in this domain, both agents possess multiple adverse effects like stevens jhonson's syndrome and hepatotoxicity which can limit their use.[19] Therefore, it was essential to explore compounds with xanthine oxidase inhibitory activity showing good saftey profile in term of adverse effects as compared to allopurinol and febuxostat.

Flavonoids have been testified to exibit xanthine oxidase inhibitory activity. Moreover, flavonoids also have also been studied for their anti inflammatory properties .[12]

In recent past, the botanical resources have gained popularity for their utilization as a potential source of new drugs and have been extensively searched for new remedies to target multiple disorders. Natural products offer a large pool of XO inhibitors that can be established into clinical products. Thus we took initiative to search *in vitro* xanthine oxidase inhibitory activity of *Ficus carica* dried fruit extract.

Ficus carica dried fruit was selected due to its antioxidant properties and possession of high content of flavonoids.[20] Moreover, due to high content of flavonoids and the ability to inhibit

ROS producing enzymes, *Ficus carica* leaves have been studied their for xanthine oxidase inhibition assay.[15]

In vitro study results showed that similar to allopurinol, FCE exhibited dose dependant xanthine oxidase inhibition. At maximum concentration (100 µg/ml) FCE possessed 80.15% XO inhibition in comparison to allopurinol that is 93.99% at the same concentration. The half maximal inhibitory concentration (IC_{50}) for FCE was found 27.5 µg/ml in comparison to allopurinol that was 11 µg/ml showing that allopurinol is more potent than FCE.

This *in vitro* XO inhibition assay of FCE extract revealed promising results which are compareable to the results of similar studies performed on other food extracts like grape fruit juice (IC_{50} 12.4 µg/ml), cran berry juice (IC_{50} 13.5 µg/ ml), vinegar (IC_{50}15.8 µg/ml) and onion (IC_{50} 33.3 µg/ ml)[21]. In another study, fig leaves were tested for its XO inhibition and found to have percent xanthine oxidase inhibition 34.8 µg/ml [15].

Our results are also compareable to the percent XO inhibition of methanolic extract of the twig of *Cinnamomum cassia* (Lauraceae) (IC_{50} 18 µg/ml), followed by the flower of *Chrysanthemum indicum* (Asteraceae) (IC_{50} 22 µg/ml), the leaves of *Lycopus europaeus* (Lamiatae) (IC_{50} 26 µg/ml) and water extract of the rhizome of *Polygonum cuspidatum* (Polygonaceae) (IC_{50}, 38 µg/ml).[13]

In our previous study we concluded that FCE reduced serum uric acid levels in oxonate induced hyperuricemic rats in dose dependant manner.[22] The results of current study validates the conclusion of previously conducted research and demonstrates the underlying mechanism responsible for antihyperuricemic effect of *Ficus carica* fruit.

For decades, plant derivatives have been explored and found to possess broad spectrum biological activities. Among these derivaties flavonoids are indispensable compounds and have been shown potent inhibiton of various enzymes like cyclooxygenase (COX), lipooxygenase, and xanthine oxidase as well.[23] *Ficus carica* fruit is known as an ironic source of flavonoids so its xanthine oxidase inhibiton potential could be the result of any of the flavonoids present in it.

CONCLUSION

Based on study results, it is concluded that FCE has potential to inhibit the xanthine oxidase (XO) enzyme.

Importance of Study: *Ficus carica* fruit can be used as a natural remedy for the treatment of hyperuricemic related disorders.

Future Recommendations: Further studies can be carried out to discover the active constituent of this fruit accountable for xanthine oxidase inhibition.

Conflict of interest: None.

REFERENCES

1. Das M, Borah NC, Ghose M, Choudhury N. Reference ranges for serum uric acid among healthy Assamese people. Biochemistry research international. 2019 Jan 9;2019.
2. Wan H, Zhang K, Wang Y, Chen Y, Zhang W, Xia F, Zhang Y, Wang N, Lu Y. The associations between gonadal hormones and serum uric acid levels in men and postmenopausal women with diabetes. Frontiers in endocrinology. 2020
3. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. International journal of cardiology. 2016 Jun 15;213:8-14.
4. Arakawa H, Amezawa N, Kawakatsu Y, Tamai I. Renal reabsorptive transport of uric acid precursor xanthine by URAT1 and GLUT9. Biological and Pharmaceutical Bulletin. 2020 Nov 1;43(11):1792-8.
5. Bortolotti M, Polito L, Battelli MG, Bolognesi A. Xanthine oxidoreductase: One enzyme for multiple physiological tasks. Redox Biology. 2021 May 1;41:101882.
6. Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, Lanaspa MA, Nakagawa T, Johnson RJ. Uric acid in metabolic syndrome: from an innocent bystander to a central player. European journal of internal medicine. 2016 Apr 1;29:3-8.
7. Day RO, Kamel B, Kannangara DR, Williams KM, Graham GG. Xanthine oxidoreductase and its inhibitors: relevance for gout. Clinical Science. 2016 Dec 1;130(23):2167-80Li YJ, Chen LR, Yang ZL, Wang P, Jiang FF, Guo Y, Qian K, Yang M, Yin SJ, He GH.
8. Comparative efficacy and safety of uricosuric agents in the treatment of gout or hyperuricemia: a systematic review and network meta-analysis. Clinical Rheumatology. 2022 Aug 29:1-0.
9. Ryu HJ, Song R, Kim HW, Kim JH, Lee EY, Lee YJ, Song YW, Lee EB. Clinical risk factors for adverse events in allopurinol users. The Journal of Clinical Pharmacology. 2019 Feb;53(2):211-6.
10. Jordan A, Gresser U. Side effects and interactions of the xanthine oxidase inhibitor febuxostat. Pharmaceuticals. 2018 May 25;11(2):51.
11. Jansen TL, Tanja G, Matthijs J. A historical journey of searching for uricosuric drugs. Clinical Rheumatology. 2021 Sep 28:1-9.
12. Yuan M, Liu Y, Xiao A, Leng J, Liao L, Ma L, Liu L. The interaction of dietary flavonoids with xanthine oxidase in vitro: Molecular property-binding affinity relationship aspects. RSC advances. 2019;9(19):10781-8.
13. Sakti AS, Widyastano H, Maulidina A, Mistasari D, Eriani DS, Munim AB. Xanthine oxidase inhibitory activity of methanol extract fractions of various Indonesian ethnopharmacological plants. International Journal of Applied Pharmaceutics. 2020 Mar 23:43-6.
14. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry and pharmacology of *Ficus carica*: A review. Pharmaceutical biology. 2018 Nov. 1;52(11):1487-503.
15. Priyoherianto A. Antihyperuricemic Activity of *Ficus Carica* Leave Extract in Mice. International journal of pharmaceutical sciences review and research. 2018;1(1):32-47.
16. Gilani AH, Mehmood MH, Janbaz KH, Khan AU, Saeed SA. Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*. Journal of ethnopharmacology. 2018 Sep 2;119(1):1-5.
17. Kostic DA, Dimitrijevic DS, Stojanovic GS, Palic IR, Đorđevic AS, Ickovski JD. Xanthine oxidase: isolation, assays of activity, and inhibition. Journal of Chemistry. 2020 Oct;2015.
18. King C, Lanaspa MA, Jensen T, Tolan DR, Sánchez-Lozada LG, Johnson RJ. Uric acid as a cause of the metabolic syndrome. Uric Acid in Chronic Kidney Disease. 2018;192:88-102.
19. Li L, Zhang Y, Zeng C. Update on the epidemiology, genetics, and therapeutic options of hyperuricemia. American Journal of Translational Research. 2020;12(7):3167.
20. Benmaghnia S, MeddAahb, Tir-Touil A, Hernandez JA. Phytochemical analysis, antioxidant and antimicrobial activities of three samples of dried figs (*Ficus carica* L.) from the region of mascara. Journal of Microbiology, Biotechnology and food sciences. 2021 Jan 6;2021:208-15.
21. Hwang JY, Pyo YH. Comparison of organic acid contents and xanthine oxidase inhibitory activities of commercial fruit juices and vinegars. Journal of the Korean Society of Food Science and Nutrition. 2020;45(11):1685-90.
22. Irum Z, Khan FA, Parveen F, Bhatti FY, SiddiqueAH, Zaheer Z. *Ficus Carica* Reduces Serum Uric Acid Level in Hyperuricemic Rats. Pakistan Journal of Medical and Health Sciences 2022; 16(1) :243-246.
23. Nile SH, Keum YS, Nile AS, Jalde SS, Patel RV. Antioxidant, anti-inflammatory, and enzyme inhibitory activity of natural plant flavonoids and their synthesized derivatives. Journal of biochemical and molecular toxicology. 2018 Jan;32(1):e22002