DOI: https://doi.org/10.53350/pjmhs22161243

ORIGINAL ARTICLE

Ficus Carica Reduces Serum Uric Acid Level in Hyperuricemic Rats

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ABSTRACT

Background: *Ficus carica* has been broadly used as traditional medicine around the world. Less toxicity of this plant represent its possible uses as therapeutic remedy for several disorders. In folk medicine, *Ficus carica* fruit has been used to treat hyperuricemia and associated conditions like gout and renal stones. However, no scientific work has been done so far to observe these effects.

Aim and Objective: To assess that *Ficus carica* (Fig fruit) extract can lessen the elevated blood levels of uric acid in hyperuricemia induced by potassium oxonate in rats.

Place and Duration of Study: The study was carried out at the Pharmacology Department of Post Graduate Medical Institute, Lahore and completed in six months.

Methods: Thirty-six rats were divided randomly into six groups. Group A was the negative control. Group B was the positive control and rats in this group were given potassium oxonate intraperitoneally (250 mg/kg) to induce hyperuricemia. Group C received potassium oxonate and allopurinol (5mg/kg) also. Group D, E, & F received potassium oxonate and *Ficus carica* in three different doses low (250mg/kg), medium (500 mg/kg), and high (750 mg/kg) respectively. In all groups, potassium oxonate was administered on days one, three and seven while test agents were administered for seven consecutive days. Sampling was done on day zero, one, three and seven.

Results: In experimental animals, *Ficus carica* fruit extract decreased serum levels of uric acid in dose dependent manner. It reduced uric acid levels significantly (p value < 0.001) in medium and high dose extract groups as compared to hyperuricemic control group. Serum uric acid levels in medium dose group E (2.8 $\pm$ 0.54) and low dose group F (2.5 $\pm$ 0.31) were comparable to that of allopurinol group C (2.2 $\pm$ 0.27) though this effect was observed earlier in allopurinol group C indicating quicker onset of allopurinol action.

Conclusion: *Fiscus carica* features antihyperuricemic effects. It resulted in significant decrease in levels of uric acid in serum of the extract treated animals in doses of 500mg/kg and 750mg/kg.

Keywords: *Ficus carica,* Hyperuricemia, Potassium oxonate, Serum uric acid (SUA), Xanthine Oxidase.

INTRODUCTION

Immoderate high levels of serum uric acid results in clinical condition called hyperuricemia. Epidemiological studies reveal that upper normal limit of serum uric acid in females is 6mg/dl while in males it is 7mg/dl. Nonetheless biochemical researchers commend that normal serum uric acid concentration is 6.8-7.0 mg/dl, recommended over statistical definitions, since it becomes insoluble above this level. For exact demarcation of people enjoying good health, preferential normal lowest serum value is 6mg/dl[1]. High levels of estrogen have a protective effect against hyperuricemia which makes hyperuricemia less common in premenopausal females and not uncommon in males[2].

The worldwide escalation of hyperuricemia is about to happen due to multiple causes including the drug induced hyperuricemia. Uric acid is an organic acid which is very less soluble in plasma and water and it is transported via albumin primarily. Degradation of purines in our body yields uric acid. Xanthine oxidase converts hypoxanthine into xanthine which is further converted into uric acid[3]. Abnormal activities of xanthine oxidase result in various pathological patterns of hyperuricemia.

Regulation of serum uric acid levels from blood is done primarily by kidneys via by modifying the rate of the tubular reabsorption and tubular secretion[4]. Some excretion also occurs via BCRP or ABCG2 which is an efflux transporter in intestine[5].

Excessive production or diminished removal of uric acid from body results in hyperuricemia. Sea food, sweet beverages and alcoholism are observed to be the predisposing factors to hyperuricemia. Hyperuricemia is also found to be common in person with gene polymorphism of SLC22A12 and SLC22A12 (GLUT-9)[6].

It is due to raised serum levels of uric acid over period of time that sodium urate crystals are deposited in joint cavities, clinically termed as gouty arthritis. Biochemical reactions produce urate and oxalate from proteins obtained from animal sources salt of calcium oxalate is nucleated by uric acid. It predisposes to formation of kidney stones[7].

Kidney related disorders due to uric acid may include morphological and subsequent functional abnormalities of glomerulus, deposition of connective tissue in renal tubal interstitium that is a definite factor of an abnormal physiological condition called metabolic syndrome[8]. Allopurinol and Febuxostat are two main drugs used as therapeutic remedy. Additionally, increasing urinary excretion of uric acid is also a methodology where drugs like probenecid and bezbromazone are used. While obtaining therapeutic effects, respondents also face adverse effects. Allopurinol, affects bowel movements and induces diarrhea along with remarkable epidermal effects while febuxostat, alters liver enzyme's functionality and cause joint aches[9].

Limited variety of agents available for treatment of increased serum uric acid levels is forcing us to look back to traditional mode of searching for plants with medicinal properties. Many substances like flavonoids are present in extracts of raw plants which are Morin, Quercetin, kaempferol, and rutin. These bioactive substances boost the interest of examining the plant which already seem very important in relation to xanthine oxidase inhibitory behavior[10].

Ficus carica is a plant which possesses a pivotal therapeutic role and is known to have different names in different languages i.e., "fig" in English and "injir" in Urdu . The extraction, screening and identification of the medicinally active substances in *Ficus. carica* reveals that it contains coumarins, flavonoids and glycosides. Systematic investigation on figs discloses their numerous actions on biological system including their antispasmodic, antiplatelet, antidiarrheal, anticancer, anti-inflammatory hypoglycemic, and antioxidant role. A detailed solitary survey on different flavonoids including morin, coumarin, rutin, tannins reveals their strong xanthine oxidase blocking activity

and it is phytochemically approved that *Ficus carica* is very rich with flavonoids. In folk medicine, *Ficus carica* is one of the fruit to be used to treat gout and kidney stones. Due to this reason, this study was planned to observe serum uric acid lowering effect of *Ficus carica* in hyperuricemic rats[11].

MATERIALS & METHODS

The study was performed at Post Graduate Medical Institute (PGMI, Lahore. after approval from Ethical Review Committee for basic Sciences of PGMI. Male Sprague Dawley rats were chosen as experimental animals. Total 36 rats were taken and further divided randomly into 6 groups. Each group contained 6 animals. Rats were kept inside cages in the animal house of PGMI in hygienic environment. The animals were provided natural day and night cycle and adapted to the average laboratory temperature i.e. 25 ± 2C. They were fed on regular rat chow and water *ad libitum.* Veterinary and humane care was delivered according to the guidelines given in "Directions for the use and care of laboratory animals" Animals were acclimatized for one week before the start of the study.

Preparation of *Ficus carica* fruit extract: For preparation of extract, dried fig was soaked in eighty percent ethanol in a ratio of 1 : 10 w/v for 3 days and subjected to the occasional shaking. Whatman filter paper No. 1 was used to filter the supernatant material. Dark brown colored thick paste was obtained after evaporation of solvent[12].

Six study groups were given drugs as described in the table 1. Potassium oxonate, a uricase inhibitor, was administered intraperitoneally to induce hyperuricemia one hour before giving test agents orally on days one[1], three[3], and seven[7]. While allopurinol and three different concentrations of *Ficus carica* extract were administered orally for seven consecutive days[13].

Table 1: Experimental Protocol

Animal group	Drug by intraperitoneal route (Day 1, 3 & 7)	Drugs by oral route (From Day 1 to 7)
Group A	Distilled water	Distilled water
Negative Control	250mg/kg	5ml/kg
Group B	Potassium oxonate	Distilled water
Hyper uricemic Control	250 mg / kg	5ml/kg
Group C	Potassium oxonate	Allopurinol
Drug Allopurinol	250 mg/kg	5mg/5ml/kg
Group D	Potassium oxonate	*Ficus carica*
Low Dose Extract	250mg/kg	250mg/5ml/kg
Group E	Potassium oxonate	*Ficus carica*
Medium Dose Extract	250mg/kg	*500mg/5ml/kg*
Group F	Potassium oxonate	*Ficus carica*
High Dose Extract	250mg/kg	750mg/5ml/kg

Sampling: Three hours after administration of test agents, a drop of blood was obtained by puncturing tail vein and subjected to measure the blood uric acid on days zero (0), one (1) and Three (3) by using uric acid meter (Multisure). Cardiac puncture was done after anesthetizing animals with chloroform on last day (7) of experiment. Blood sample was later analyzed for uric acid by enzymatic end method using commercially available kit made by Linear Chemicals Spain. Uric acid concentration was measured against blank with spectrophotometer at 546nm wavelength.

Statistical analysis: For serum uric acid values obtained on day zero, one and three, statistical tests were not applied because numerical values could not be obtained in negative control group for all three days and for other groups on day zero due to low sensitivity of uric acid meter. Hence descriptive analysis was made on these days. For uric acid levels obtained on day seven, data was transcribed into SPSS20 and found to be normally distributed by Shapiro Wilk test, analysis of variance (ANOVA). Difference between group means was significant with a *p value* <0.001 (ANOVA). To analyze the difference between the group means of serum uric acid *post hoc* Tukey's test was applied.

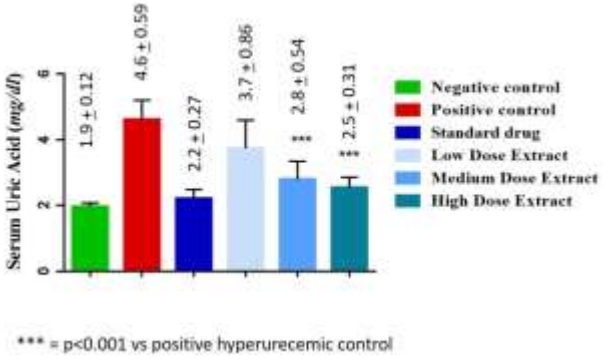
RESULTS

Table. 2. Represents the numerical data of Serum Uric Acid values for the days zero, one, and three (0,1 and 3). The sensitivity of the uric acid meter was to measure values up to 3mg/dl and above. Therefore, uric acid levels below this value could not be measured by the meter and for all the values < 3mg/dl, a sign of "Lo" appeared on the meter. Descriptive analysis on these days revealed that SUA level was similar (Lo= < 3mg/dl) in all groups on day 0. It remained Lo (<3mg/dl) on day 1 and 3 in negative control group A also. Among experimental groups, SUA on day 3 was lowest (Lo =< 3mg/dl) followed by 3.5 mg/dl in high dose extract group F While SUA values were almost similar in medium dose extract group E , low dose extract group D and positive control group B.

Table. 2: SUA of all study groups on uric acid meter.
Lo. refers to the SUA < 3mg/dl

Serum Uric Acid/ SUA (mg/dl)			
Groups	Day zero	Day one	Day Three
Group A Negative control	Lo	Lo	Lo
Group B Positive Control	Lo	4.4	4.5
Group C Drug Allopurinol	Lo	3.1	Lo
Group D Low Dose Extract	Lo	4.5	4.2
Group E Medium Dose Extract	Lo	4.2	4.1
Group F High Dose Extract	Lo	4.0	3.5

On day 7, lowest SUA level was observed in allopurinol group C. In extract treated groups, SUA level was decreased in dose dependent manner. Highest reduction was observed in high dose extract group F. Fig. 1 shows graphical presentation of mean ± SD of all groups.



*** = p<0.001 vs positive hyperurecemic control

Figure 1: Effect of three doses (250mg/kg, 500mg/kg, 750mg/kg) of *Ficus carica* and standard drug allopurinol on Serum Uric Acid (Mean ± SD) of potassium oxonate treated rats. (n = 6)

The mean difference of SUA levels (day 7) among all groups was assessed by *post hoc* Tukey's test. In comparison to the negative control groups, significant increase in serum uric acid level (*p*-value < 0.001) was there in Positive Control and low dose extract groups. While SUA levels in medium dose extract, high dose extract and allopurinol drug groups were very near to negative control and the difference was non-significant.

Three experimental groups i.e Medium Dose Extract, High Dose Extract and Standard drug Allopurinol groups showed significantly low SUA as compared to the Positive Control group (p-value <0.001) .While in contrast to the same experimental groups, SUA in Low Dose Extract treated animal group was significantly higher.

Table 3: Post *hoc* Tukey's multiple comparison test

Group		Mean Difference	Significance
Negative control	Positive Control	-2.650***	< .001
	Allopurinol	-0.250	0.957
	Low Dose Extract	-1 .766***	< 0.001
	Medium Dose Extract	-0.833	0.083
	High Dose Extract	-0.583	0.383
Positive Control	Allopurinol	2.400***	< 0.001
	Low Dose Extract	0.883	0.057
	Medium Dose Extract	1.816***	< 0.001
	High Dose Extract	2.066***	< 0.001
Drug Allopurinol	Low Dose Extract	-1.516***	< 0.001
	Medium Dose Extract	-0.583p	0.383
	High Dose Extract	-0.333	0.867
Low Dose Extract	Medium Dose Extract	0.933*	0.039
	High Dose Extract	1.183**	0.005
Medium Dose Extract	High Dose Extract	0.250	0.957

DISCUSSION

Approximately 18 percent of the general population is affected by hyperuricemia and males are affected predominantly as compared to females[14]. Whenever blood uric acid level exceeds normal value, crystals of monosodium urate tend to precipitate in the tissues and joint cavities resulting in complications like gout, nephrolithiasis and chronic nephropathy. Other medical conditions like high blood pressure, cardiac ischemia, metabolic syndrome renal damage, insulin resistance and diabetes mellitus are directly associated with this[15].

Purine nucleotide catabolism resulting either endogenously or from exogenous sources yields the uric acid product. Inadequate excretion or augmented production of uric acid leads to hyperuricemia.[3] In treatment of hyperuricemia, allopurinol which is a potent xanthine oxidase inhibitor (XOI) is commonly used but its accompanying side effects like diarrhea and aplastic anemia has decreased its use. Other drugs which are helpful in hyperuricemia like probenecid and sulfinpyrazone are nephrotoxic while benzbromarone can result into fulminate hepatic damage[9]. Hence search for a better hypouricemic drug is need of the time. Multiple phytochemicals extracted from natural products have been studied for their anti hyperuricemaic properties especially the plant flavonoids, tannins as well as coumarins were investigated for hypouricemic properties. Procyanidin extracted from grape seeds[16] and flavonoids extracted from *Lippa nodiflora*[17] has shown the ability to manage hyperuricemia by antagonizing the effect of xanthine oxidase enzyme and partly because of their uricosuric properties. Quercetin, another flavonoid present in plants have shown the potential to inhibit uric acid production via xanthine oxidase catalysis.

We selected *Ficus carica* because both fresh and dried fruits are admirable source of bioactive compounds like flavonoids and proven as excellent antioxidant[18]. In traditional medicine, it has been used to treat hyperuricemia induced arthritis and nephrolithiasis[19].

In this study we demonstrated that *Ficus carica* fruit extract decreased serum uric acid in rats treated with uricase inhibitor potassium oxonate. Test agent decreased serum uric acid in dose dependent fashion (medium and low dose) which is comparable to the hypouricemic effect of standard drug allopurinol. Reduction in serum uric acid was more obvious when test agent is administered for more days as compared to the allopurinol so the antihyperuricemic effect was also time dependent. It reflects that allopurinol is quicker in onset of action as compared to the test agent.

Previously there was no study available showing antihyperuricemic effect of *Ficus carica* fruit. So, we made comparison of our experimental results with those obtained via studies performed on herbs, fruits, vegetables and other natural products. The results of our study is comparable to another study in which the onion juice lowered the level of serum uric acid in potassium oxonate induced hyperuricemic rats significantly (*p*-value < 0.001)[13]. Similar results were obtained from studies done on flavonoids isolated from *Zingiber officinale*[20] and essential oils extracted from leaves of *Cinnamomum Osmopholieum*[21] that significantly reduced (*p*-value < 0.001) uric acid level in hyperuricemic rats.

Similar comparisons can be made with other studies like Hypouricemic effect of ethanolic extract of *Aster glehni* leaves ($p < 0.05$)[22], soursop leaves *Siegesbeckia orientalis L* ($p< 0..05$)[23] *Chondrot T* (<0.05)[24], *crudrania tricuspidata* leaves ($p <0.05$)[25], *Davillia formosana* (<0.001)[26] and *Citrus unshiu fruit extract* (<0.01)[27]. All these agents including our test agent *Ficus carica* showed dose dependant reduction in serum uric acid levels.

In said experimental study, three doses 250mg/kg, 500mg/kg, and 750mg/kg of *Ficus carica* were chosen to establish its hypouricemic effect. The study results revealed that *Ficus carica* fruit extract in medium dose (500mg/kg) and high dose (750mg/kg) significantly decreased serum uric acid level. This reduction in serum uric acid was comparable with antihyperuricemic effect of standard drug allopurinol.

Strengths and limitations of study: Our study supports the application of this fruit as an indigenous remedy for treating hyperuricemia and associated conditions. The study is deficient in identifying active constituents responsible and mechanism of lowering serum uric acid which can be investigated further**.**

CONCLUSION

Ficus carica extract significantly reduced serum uric acid in dose of 500mg/kg and 750 mg/kg in potassium oxonate induced hyperuricemic rats. This effect is equivalent to that of 5mg/kg of allopurinol.

Acknowledgement: Contributing authors are grateful to staff of Animal house and Pathology laboratory of Postgraduate Medical institute, Lahore for their cooperation.

Conflict of Interest: No conflict of interest.

Source of Funding: Post Graduate Medical Institute, Lahore.

Disclaimer: Data is part of M. Phil thesis.

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