Journal of Comprehensive Pharmacy

Research Article

Available Online at: www.jcponline.in

ISSN NO: 2349-5669

Evaluation of anti-gout activity of fresh fruit juice of *psidium guajava* against diuretic Induced Gout in Wistar rats

Divya S Madirala,* Rajaram Cuddapah, Nelson K Sadhu.

Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa-516003, Andhra Pradesh, India.

ARTICLE INFO

Article history:

Received 10 March 2014

Accepted 1 April 2014

Available online 19 April 2014

*Corresponding author:

Divya Sree M

E-mail:

divya.sree003@gmail.com

Tel.: +91-9963884338

Abstract

Aim The present study was designed to investigate the anti-gout activity of fresh fruit juice of *Psidium guajava* (FFPG).

Method Five groups of animals containing six in each group were used for the study, group 1 served as normal which were fed normal diet and water; group 2 served as control which were treated with the combination of Hydrochlorothiazide (10mg/kg) p.o. and Spironolactone (20mg/kg) p.o.; group 3 served as standard and treated with Spironolactone (20mg/kg) p.o. and the combination of Hydrochlorothiazide (10mg/kg) and Allopurinol (10mg/kg) p.o.; group 4 served as treatment group and treated orally with low dose of *Psidium guajava* fruit juice and the combination of Hydrochlorothiazide (10mg/kg) and Spironolactone (20mg/kg) p.o. and group 5 served as treatment group and treated orally with high dose *Psidium guajava* fruit juice and the combination of Hydrochlorothiazide (10mg/kg) and Spironolactone (20mg/kg) p.o. all groups were treated for a period of 7 days and finally the effects observed were compared with known anti-gout agent, allopurinol.

Result Fresh fruit juice of *Psidium guajava* (FFPG) significantly reduced the elevated levels of urea, uric acid, and BUN levels in rats. Amazingly high dose (400mg/kg) shows good significance than lower dose (200 mg/kg).

Conclusion These results indicate that FFPG has a significant protective effect against diuretic induced gout in experimental rats.

Keywords Anti-Gout activity, Spironolactone, Hydrochlorothiazide, *Psidium guajava*, Allopurinol.

INTRODUCTION

Gout is one of the most common diseases characterized by an abnormal metabolism of uric acid, resulting in an excess of uric acid in the tissues and blood. Deposition of monosodium urate crystals in joints or kidneys resulting in gouty arthritis and nephrolithiasis [1,2]. Xanthine oxidase (XO) is an important enzyme catalyzing the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid which is excreted by kidneys. Excessive production and/or inadequate excretion of uric acid results in hyperuricemia [3]. XO also serves as an important biological source of oxygen derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as

inflammation, atherosclerosis, cancer and aging. In vitro bioassays are used to examine test material for XO inhibition, as inhibitors of XO may be potentially useful for the treatment of gout or other XO induced diseases [4]. Several authors reported on the XO inhibitory potential of traditionally used medicinal plants [5,6].

Psidium guajava Linn (Family: Myrtaceae) is a semi deciduous tropical tree commonly known as guava or 'Amrood' in north India and is widely grown throughout India for its fruits. *P. guajava* reported to possess many phytoconstituents like flavonoids, terpenoids and glycosides. In addition high percentage of Vitamin C, Carotene, Vit B1, B2, and B6, free sugars (glucose, fructose and sucrose) has been reported to be present in

these fruits [7]. Many studies revealed that *P. guajava* possess anti-bacterial, antispasmodic, antidiabetic, anticough, antioxidant and a narcotic like activities [8].

The present study was undertaken in order to investigate the claim that the plant has anti-gout property. However anti-gout property of *P. guajava* has not been scientifically investigated. Therefore, in the present study anti-gout property of fresh fruit juice of *P. guajava* has been evaluated against diuretic induced gout in the Wistar albino rats.

MATERIALS AND METHODS

Drugs and Chemicals

Diuretics (Hydrochlorothiazide + Spiranolactone) were obtained as a gift sample from Cadila Pharma Ltd India. Allopurinol was obtained from sigma-Aldrich, Bangalore, India. Standard kit of urea, uric acid and BUN were obtained from ERBA diagnostic, India. All other reagents used were of analytical grade.

Collection of fruits

The fresh fruits of *P. guajava* were collected from local market, Kadapa, Andhra Pradesh.

Preparation of the fresh fruit juice

The fresh fruits of *P. guajava* were collected, cleaned, grinded, and filtered by a filter paper. The residue was discarded and fresh juice collected.

Experimental animals

Experimentation animals of either sex weighing 150-200g were obtained from raghavendra enterprises, Bangalore. The animals were housed in stainless steel cages at a controlled room temperature of 24⁰ c, under 12h light and 12h dark cycle. After one week of acclimatization, the experiment animals were divided randomly into 5 groups (n=6). The experimental protocol was approved by the Institutional Experimental Animal Committee of PRRM College of Pharmacy, Utukuru, Kadapa. (Ref: 1423/po/a/11/CPCSEA).

Assessment of anti-gout activity

Five groups of animals containing six each were used for the study

Group 1: Normal: which were fed with normal diet and water.

Group 2: Control: Treated with the combination of Hydrochlorothiazide (10mg/kg) p.o. and Spiranolactone (20mg/kg) p.o. for a period of 7 days.

Group 3: Standard: Treated with Spiranolactone (20mg/kg) p.o. and the combination of Hydrochlorothiazide (10mg/kg) and Allopurinol (10mg/kg) p.o. for a period of 7 days.

Group 4: Treatment 1: Treated orally with low dose of *Psidium guajava* fruit juice and the Combination of

Hydrochlorothiazide (10mg/kg) and Spiranolactone (20mg/kg) p.o. for a period of 7 days.

Group 5: Treatment 2: Treated orally with high dose *Psidium guajava* fruit juice and the Combination of Hydrochlorothiazide (10mg/kg) and Spiranolactone (20mg/kg) p.o. for a period of 7 days.

Collection of blood samples:

The blood samples were collected from the retroorbital venous plexus of rats without any coagulant for the separation of serum, at the regular intervals of the treatment. After collecting the blood in micro centrifuge tubes they were kept for 1 h at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters.

Assessment of serum marker enzymes

Urea, uric acid and BUN were estimated by using standard diagnostic kits.

Statistical analysis

In the present study, all the data was expressed as mean $\pm$ S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

RESULTS

Selection of dose

The doses were selected according to the acute toxicity studies conducted earlier [9].

Anti-gout activity

The plant used in the present study has been chosen from literature to possess anti-inflammatory activity to be tested in a gout model in rat. Rats treated with Diuretics showed significantly increased in levels of Uric acid, Urea and BUN in serum of control group, when compared with normal groups. Oral dose of FFPG at 200 mg/kg & 400 mg/kg FFPG reduces the Urea, Uric acid and Blood Urine Nitrogen levels significantly and dose dependent manner when compared with control group as shown in Figures 1, 2 & 3. The results were tabulated in Table 1.

DISCUSSION

The present study was carried out to evaluate the anti-gout activity of FFPG against diuretic induced gout in Wistar rats. Spiranolactone and Hydrochlorothiazide induced gout is the widely used animal model for the determining the anti-gout property of new therapeutic agents. Rats treated with FFPG shows significant decrease in uric acid, urea and BUN levels. Reduction of uric acid levels in serum on administration of FFPG may

Table 1: Effect of FJPG on diuretic induced gout in Wistar rats

Sl.no.	Treatment groups	Blood urea levels	Blood uric acid levels	Blood urea nitrogen (BUN) levels
1.	Normal	16.0±0.96	2.98±0.12	6.60±0.11
2.	Control (Spiranolactone + Hydrochlorothiazide)	55.17±2.35 ^{###}	11.17±0.83 ^{###}	41.18±3.27 ^{###}
3.	Standard (Spiranolactone + Hydrochlorothiazide + Allopurinol)	23.0±0.96 ^{***}	4.83±0.03 ^{***}	17.15±0.44 ^{***}
4.	Test-1 (Spiranolactone + Hydrochlorothiazide + 200 mg FJPG)	30.33±0.88 ^{***}	4.26±0.05 ^{***}	14.27±0.52 ^{***}
5.	Test-2 (Spiranolactone + Hydrochlorothiazide +400 mg FJPG)	36.50±0.76 ^{***}	5.85±0.04 ^{***}	23.17±0.95 ^{***}

All values are shown as mean ± SEM and n=6.

indicate $p < 0.05$, ## indicate $p < 0.01$, ### indicate $p < 0.001$ when compared to normal group.

* indicate $p < 0.05$, ** indicate $p < 0.01$, *** indicate $p < 0.001$ when compared to CCl₄ group (One-way ANOVA followed by Tukey's test)

Fig 1: Effect of FFPG on Uric acid level. **Fig 2:** Effect of FFPG on Urea level **Fig 3:** Effect of FFPG on Blood Urea Nitrate

reflect xanthine oxidase inhibition and/or inhibition of renal urate reabsorption [10]. Some natural compounds, such as vitamin C, have been reported to produce reduction in plasma uric acid with simultaneous increase in urinary uric acid [11].

Acknowledgement

We thank PRRM College of Pharmacy, Kadapa. Andhra Pradesh, India, for providing experimental facilities.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. B-Rao C, Kulkarni-Almeida A, Katkar KV, Khanna S, Ghosh U, Keche A et al. Identification of novel isocytosine derivatives as xanthine oxidase inhibitors from a set of virtual screening hits. *Bioorg Med Chem*. 2012;**20**(9):2930-2939
2. Flemmig J, Kuchta K, Arnhold J, Rauwald HW. *Olea europaea* leaf (Ph.Eur.) extract as well as several of its isolated phenolics inhibit the gout-related enzyme xanthine oxidase. *Phytomedicine*. 2011;**18**(7):561-566.
3. Lespade L, Bercion S. Theoretical study of the mechanism of inhibition of xanthine oxidase by flavonoids and gallic acid derivatives. *J Phys Chem B* 2010;**114**(2):921-928
4. Sweeney AP¹, Wyllie SG, Shalliker RA, Markham JL. Xanthine oxidase inhibitory activity of selected Australian native plants. *J Ethnopharmacol* 2001;**75**(2-3):273-277
5. Azmi SMN, Jamal P, Amid A. Xanthine oxidase inhibitory activity from potential Malaysian medicinal plant as remedies for gout. *International Food Research Journal* 2012;**19**:159-165
6. Havlik J, Gonzalez de la Huebra R, Hejtmankova K, Fernandez J, Simonova J, Melich M et al. Xanthine oxidase inhibitory properties of Czech medicinal plants. *J Ethnopharmacol* 2010;**132**(2):461-465
7. Mishra K, Seshadri TR. Chemical components of the fruits of *Psidium guajava*. *Phytochemistry* 1967;**7**:641-645
8. Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML et al. Anticough and antimicrobial activities of *Psidium guajava* Linn. leaf extract. *J Ethnopharmacol* 1999;**67**(2):203-212
9. Rai PK, Jaiswal D, Mehta S, Watal G. Anti-hyperglycaemic potential of *Psidium guajava* raw fruit peel. *Indian J Med Res* 2009;**129**(5):561-565
10. Doha A.M, Sahar Y, Al-Okbi. Evaluation of anti-gout activity of some plant food extracts. *Pol. J. Food Nutr. Sci* 2008;**58**(3):389-395
11. Stein HB, Hasan A, Fox IH. Ascorbic acid-induced uricosuria. A consequence of megavitamin therapy. *Ann Intern Med* 1976;**84**(4):385-388

Cite this Article as: Madirala DS,* Cuddapah R, Sadhu NK. Evaluation of Anti-Gout activity of fresh fruit juice of *psidium guavaja* against diuretic Induced Gout in Wistar rats. *J Compr Phar* 2014;1(1):26-29