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

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**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 7days 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 uric, 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 24°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/CPCEA).

**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 ± 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 FJPG 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 FJP on diuretic induced gout in Wistar rats

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

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 FFJP on Uric acid level.

Fig 2: Effect of FFJP on Urea level

Fig 3: Effect of FFJP on Blood Urea Nitrate
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Conflict of interest
No potential conflict of interest relevant to this article was reported.

REFERENCES


