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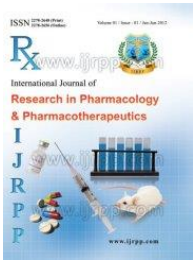

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# Protective effect of *Canthium parviflorum* against potassium oxonate induced hyperuricemia in rats

**Asokan Balakrishnan Ramajayam[1], Sengottuvelu Singaravel[2], Jaikumar Sankarapillai[3*]**

[1]*Department of Pharmacology, Aarupadai Veedu Medical College, Puducherry,*
[2]*Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamilnadu.*
[3]*Department of Pharmacology, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry.*

***Corresponding author: Dr. S. Jaikumar**
**Email: jaipharma2007@gmail.com**

## ABSTRACT

*Canthium parviflorum*, belonging to the Rubiaceae was a valuable source of new secondary metabolites, jused in ayurvedic system of medicine, for various disorders. Traditionally, various parts of *Canthium parviflorum* were used to treat oedema, dysentery, spasm, scabies, diabetes etc. It also used to eliminate uric acid as well as increase the urinary output. Current study was aim to evaluate the antigout property of ethanolic leaf extract of *Canthium parviflorum.* The study was conducted in both *in-vitro* and *in-vivo* models. Xantihine Oxidase inhibition method was used in *in-vitro* model and Potassium Oxonate induced hyperuricemia was adopted for *in-vivo* model. The Xanthine Oxidase inhibitor, Allopurinol was used as reference compound in both the models. Two dose levels (200 and 400mg/kg) of ethanolic leaf extract of *Canthium parviflorum* was evaluated against Potassium Oxonate induced hyperuricemia in SD rats. All the test drugs were administered once daily for 28 days and the protective effect was determined by measuring the plasma uric acid. In *in-vitro* model, the *Canthium parviflorum* showed Xanthine Oxidase inhibition activity and comparable as that of Allopurinol. In *in-vivo* model, both the doses of ethanolic leaf extract of *Canthium parviflorum* exhibited significant reduction in the plasma uric acid levels as compare to Potassium oxonate induced hyperuricemia in rats. From the results it was concluded that, ethanolic leaf extract of *Canthium parviflorum* produced antigout activity against Potassium oxonate induced gout in rats.

**Keywords:** *Canthium parviflorum*, Potassium Oxonate, Xanthine Oxidase, Antigout Activity.

## INTRODUCTION

Gout is a systemic disease that results from the deposition of monosodium urate crystals in tissues. Increased serum uric acid above a specific threshold is a requirement for the formation of uric acid crystals. Genetic predisposition is thought one of the major factors that share in the incidence of gout [1]. Urate is the ionized form of uric acid present in the body. Uric acid is a weak acid with pH of 5.8. Urate crystals deposition in tissues starts to occur when serum uric acid level rises above the normal threshold. Pathological threshold of hyperuricemia is

defined as 6.8 mg/dL [2]. Monosodium urate crystals can be deposited in all tissues mainly in and around the joints forming tophi. The general prevalence of gout is 1–4% of the general population. In western countries, it occurs in 3–6% in men and 1–2% in women. In some countries, prevalence may increase up to 10%. Prevalence rises up to 10% in men and 6% in women more than 80 years old. Annual incidence of gout is 2.68 per 1000 persons. It occurs in men 2–6 folds more than women. Worldwide incidence of gout increases gradually due to poor dietary habits such as fast foods, lack of exercises, increased incidence of obesity and metabolic syndrome [3]. Some factors may affect the solubility of uric acid in the joint. These include synovial fluid pH, water concentration, electrolytes level, and other synovial components such as proteoglycans and collagen. Serum uric acid level in the body is determined by the balance between its production either from purine intake in diet or endogenous production by cellular turnover and its excretion by the kidneys and GIT. Increased production of uric acid is responsible for only 10% of cases of gout while the remaining 90% are caused by its renal under-excretion [4]. Early presentation of gout is an acute joint inflammation that is quickly relieved by NSAIDs or colchicine. Renal stones and tophi are late presentations. Lowering serum uric acid levels below deposition threshold either by dietary modification and using serum uric acid lowering drugs is the main goal in management of gout. This results in dissolution of Monosodium urate crystals preventing further attacks [5].

Allopurinol is the most common clinically used XO inhibitor prescribed for the treatment of gout [6]. Allopurinol can cause the side effects, such as nephrolithiasis, allergic reaction and increase the toxicity of 6-merkaptopurin [7]. Thus, the development of novel hypouricemic agents with greater efficacy and a broader safety profile is greatly needed.

In recent times, focus on plant research has increased all over the world, and a large number of evidence has collected to show immense potential of medicinal plants used in various traditional systems. *Canthium parviflorum* is a shrubby and woody plant found throughout the Western Ghats. *Canthium parviflorum* is a shrubby and woody plant belonging to the family Rubiaceae found throughout the Western Ghats. *Canthium parviflorum* is a thorny sub scandent shrub grows up to 3meters height with spreading branches distributed throughout India in shrub forests and dry plains. Leaves are simple, opposite, small, and acute with axillary spines. Flowers are white, small in axillary cymes. Fruits oblong two-chambered drupe, become yellow when ripe. All the plant parts such as roots, leaves, fruits and stems are pharmacologically useful.

In Ayurvedic system of medicine, *Canthium parviflorum* used as laxative and also to cure gout. Traditionally the roots and leaves are used to cure vitiated conditions of kapha in fever and constipation [8]. *Canthium parviflorum* leaf is used as an astringent and posses wound healing property [9]. Leaf paste is applied externally to treat scabies and ringworm infection [10]. The *Canthium parviflorum* as herbal medicine is used for the treatment of diabetes among major tribal groups in South Tamilnadu [11]. The roots of this plant are traditionally used by the tribes of Orissa in the treatment of swelling of neck. The roots are astringent, sweet, thermogenic, diuretic, febrifuge, constipating, anthelmintic, and tonic. They are used in vitiated conditions of kapha, diarrhoea, strangury, fever, leucorrhoea, intestinal worms, and general debility [12]. Tribes of Orissa state in India use fruits of this plant to treat a headache. It is traditionally used for snake bites [13]. The root and leaf paste of *Canthium parviflorum* are very useful for diuretic [14]. This plant contains high quantities of total carotenoids and beta carotenoids which are very essential for vitamin A activity. The *Canthium parviflorum* is the richest source of β-carotene and 100 g of edible fruit position contains 9.51 mg carotenoids and 6.10 mg of β-carotene [15]. Few of its traditional claims like anticancer [16], antidiabetic and hypocholestromic [17], antimicrobial [18], anti-inflammatory [19], antioxidant activity [20] were scientifically proved. Present study was undertaken to establish the antigout activity of ethanolic leaf extract of *Canthium parviflorum* against Potassium Oxonate induced gout in rats.

## MATERIALS AND METHODS

### Plant Material

The leaves of *Canthium parviflorum* were collected from the region of Wayanad, it was identified as *Canthium parviflorum* and authenticated by the Scientist D, Botanical Survey of India,

Southern Regional Center, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/16/97/2017-18/Sci/01055) had been deposited in the herbarium for future reference.

## Preparation of Extract

The leaves were washed in running tap water to remove the adhering soil matters, and shade dried. After complete drying, the leaves were grinded in to coarse powder using mechanical blender. The coarse powdered leaves were extracted with 90% ethanol by cold maceration process. Its then filtered by clean muslin cloth and concentrated using rotary evaporator. The dried ethanolic leaf extractof *Canthium parviflorum* was stored in desiccators for further studies.

## Animals and Ethical Considerations

Healthy male Sprague – Dawley (SD) rats weighing between 180 - 220 gm were used for this study. The animals were obtained from animal house, College of Veterinary and Animal Sciences, Mannuthy. After the arrival of animals, they were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2$^{o}$C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

## Xanthine Oxidase inhibition *in vitro* assay

The Xanthine Oxidase inhibitory activity was assayed spectrophotometrically based on the previous report [21]. The assay mixture, consisting of 50 µl of test solution, 35 µl of 50 mM phosphate buffer (pH 7.5), and 30 µl of Xanthine Oxidase solution (0.1 U/ml in 50 mM phosphate buffer, pH 7.5), was prepared immediately before use. After preincubation at room temperature for 15 min, the reaction was initiated by the addition of 60 µl of substrate solution (150 µM xanthine in the same buffer). The assay mixture was incubated at room temperature for 30 min. afterwards, 25 µl of 1 N HCl was added, and the absorbance values were measured at 290 nm. Allopurinol, a known inhibitor of Xanthine Oxidase, was used as a positive control. The increased ultraviolet absorption at 290 nm indicated the formation of uric acid, and all determinations were performed in triplicate. The Xanthine Oxidase inhibition was calculated as a percentage (%) = (1 − B/A) × 100, where A is the change in absorbance per min without the test sample and B is the change in absorbance per min with the test material.

## Experimental design

Thirty male Sprague-Dawley rats were randomly divided into five groups of six each. Group I served as normal control (without induction of gout) administered with 0.1% Carboxy Methyl Cellulose solution (CMC). Group II to V animals were administered with 250mg/kg of Potassium Oxonate once daily for 28 days through intraperitoneally to induce hyperuricemia. Gorup II animals were served as gout control, received only Potassium Oxonate. Group III animals served as reference control received Allopurinol (5mg/kg) through oral route. Group IV and V received, 200 and 400mg/kg of ethanolic leaf extract of *Canthium parviflorum* respectively. All the test drugs were administered orally once daily for 28 days, 30 minutes before Potassium Oxonate administration.

On 29$^{th}$ day, all the animals were anesthetized with penotobarbitol sodium (45mg/kg. i.p), blood was withdrawn from sinus puncture, centrifuged and the plasma uric acid was determined [22].

## Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's '*t*' test. P values < 0.05 were considered as significant.

## RESULT & DISCUSSION

**Table 1. Inhibitory effect of ethanolic leaf extract of *Canthium parviflorum* against Xanthine Oxidase.**

| S.No | Test Drug | % Inhibition of Xanthine Oxidase |
|---|---|---|
| 1 | Allopurinol (100µg/ml) | 96.34±2.33 |
| 2 | *Canthium parviflorum* (200µg/ml) | 85.63±1.37 |
| 3 | *Canthium parviflorum* (400µg/ml) | 92.54±1.98 |

The Xanthine Oxidase inhibitory effect of ethanolic leaf extract of *Canthium parviflorum* was studied, and the effect was compared with the reference control Allopurinol (Table 1). The % inhibition of Xanthine Oxidase of clinically used drug Allopurinol was 96.34%. The % inhibition of Xanthine Oxidase of the *Canthium parviflorum* leaves at 200 and 400mg/kg was 85.63 and 92.54% respectively. Both the doses of *Canthium parviflorum* showed comparative Xanthine Oxidase inhibitory effect compared to Allopurinol. Higher dose 400mg/kg of *Canthium parviflorum* showed similar effect as that of the reference control Allopurinol.

**Table 2. Effect of ethanolic leaf extract of *Canthium parviflorum* on Potassium Oxonate induced gout in rats**

| S.No | Drug Treatment | Uric Acid (µmol/L) |
|---|---|---|
| 1 | Group – I Normal Control (0.1% CMC) | 65.36±3.86 |
| 2 | Group – II Gout Control Potassium Oxonate (250mg/kg) | 176.44±8.53 |
| 3 | Group - III Allopurinol (5mg/kg) | 76.05±3.50*** |
| 4 | Group - IV *Canthium parviflorum* (200 mg/kg) | 87.75±3.90*** |
| 5 | Group - V *Canthium parviflorum* (400 mg/kg) | 79.82±2.32*** |

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Gout Control

The antigout effect of ethanolic leaf extract of *Canthium parviflorum* was studied against Potassium Oxonate induced hyperuricemia in ratsand the results were given in table 2. The results showed that, 28 days administration of Potassium Oxonate enhanced the plasma uric acid compared to normal control. Simultaneous administration of Allopurinol and *Canthium parviflorum* significantly (P<0.001) decreased the plasma uric acid compared to induced control. The plasma uric acid levels of Allopurinol was 76.05 µmol/L. Both the doses of *Canthium parviflorum* significantly decreased the elevated levels of plasma uric acid against Potassium Oxonate induced hyperuricemia in rats.

## CONCLUSION

Based on ethnobotonical information, *Canthium parviflorum* was studied for its protective role against Potassium oxonate induced hyperuricemia in rats. From the result, it was concluded that the ethanolic leaf extract of *Canthium parviflorum* exhibited protective role by preventing the elevation of plasma uric acid levels against Potassium oxonate induced hyperuricemia in rats. The leaves of *Canthium parviflorum* are rich in various phytoconstituents like alkaloids, flavonoids, tannins, steroids, saponins, terpenoids etc. Further study may be focused to

isolate theactive principle responsible for its antigout activity.

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