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SCREENING OF HYPOURECEMIC ACTIVITY OF *MURRAYA KOENIGII* IN PYRAZINAMIDE INDUCED HYPERURECEMIA

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ABSTRACT

Background: *Rheumatism is any painful disorder affecting the loco-motor system including joints,* muscles*,* connective tissues*,* soft tissues *around the joints, and bones. Large number of herbal species has been used traditionally or as folk medicine against inflammatory ailments. Many of them have been studied scientifically and proved to be beneficial against inflammatory agents.* ***Objectives:*** *To evaluate the Hypourecemic activity of murraya koenigii leaves in pyrazinamide induced hyperurecemic rats.* ***Methodology:*** *Male Wistar rats, weighing 150-200 gm were used for acute model. Pyrazinimde metabolite pyrazinoate serve as the exchanging anion from inside cells, thereby stimulating anion exchange and urate reabsorption. The blood samples were collected from each rat through tail vein puncture, allowed centrifugation for 15 min; serum was separated and estimated its initial serum uric acid levels using Uric acid kit.* ***Results:*** *The Initial serum uric acid levels of rats were found to be in the range of 3.5-4.5 mg/dl. After one week, daily administration of pyrazinamide at higher dose (250mg/kg) was significantly increases the blood uric acid level upto 10.5 -11.0 mg/ dl. The alcoholic and aqueous extracts of M.koenigii leaves at 250 mg/ kg have comparable hypourecemic activity with that of standard drug Allopurinol.* ***Conclusion:*** *Elevated serum uric acid levels are able to cause acute flares in Pyrazinamide administered rats. Ethanolic extract of curry leaves produced maximum hypourecemic activity whereas the minimum effect was produced by Pet ether extract of curry leaves. M.koenigii leaves having hypourecemic activity may be due to presence of Carbazole alkaloids and Phenolics.*

KEY WORDS

Rheumatism, Hypourecemic activity, Pyrazinimde, Allopurinol, Ethanolic extract.

INTRODUCTION:

Rheumatism is any painful disorder affecting the loco-motor system including joints, muscles, connective tissues, soft tissues around the joints, and bones.46 million people in the United States are living with rheumatic diseases, which are the most common causes of reduced mobility. Plants have played a remarkable role in health care system since ancient times. Traditional based medicines still exert greater deal of importance to people living in developed countries and also lead a discovery of new drug candidates.

Large number of herbal species has been used traditionally or as folk medicine against inflammatory ailments. Many of them have been studied scientifically and proved to be beneficial against inflammatory agents. The success has been attained to isolate various single chemical entities responsible for anti-inflammatory activity.

The apparent rise in the prevalence and incidence of gout over the past several decades may be caused by growing populations with risk factors for this disease, such as advanced age, high intake of purine-rich animal protein, metabolic syndrome, diuretic use, organ transplant, and end-stage renal disease. Men have a greater risk of developing gout than women in all age groups, although the sex ratio tends to equalize with advancing age.

The risk of developing gout is directly related to the degree of hyperuricemia. In a prospective study, the annual incidence of gout (i.e., the proportion of new gout cases diagnosed per year in an at-risk population) was 0.1% in men whose SU levels were <7mg/dL, 0.5% for levels between 7.0 and 8.9mg/dL, and 4.9% for levels >9.0 mg/dL.

One of the most common complaints during pyrazinamide therapy is arthralgia which is often associated with a raised serum uric acid level. Attacks of gout may also occur in a proportion of them and death due to pyrazinamide-induced hyperuricemia has been re-ported by Kass (1965). In the present study Pyrazinimde selected as an inducer of hyperurecemia. Pyrazinamde an anti TB drug, its chronic use or at high doses causes hyperurecemia. Its metabolite pyrazinoate serve as the exchanging anion from inside cells, thereby stimulating anion exchange and urate reabsorption, this study is designed to check the anti hyperurecemic activity of curry leaves in pyrazinamide induced hyperurecemic rats.

The ethno medicinal plant *Murrya koenigii* (Curry-leaf tree) which is native to India exhibits diverse biological activities. *Murrya koenigii* has been used for centuries in the Ayurvedic system of medicine.

The leaves of *Murrya koenigii* contain proteins, carbohydrate, fiber, minerals, carotene, nicotinic acid, Vitamin C, Vitamin A, calcium and oxalic acid. It also contains crystalline glycosides, carbazole alkaloids, koenigin, girinimbin, iso-mahanimbin, koenine, koenidine and koenimbine. Triterpenoid alkaloids cyclomahanimbine, tetrahydromahanimbine are also present in the leaves

Figure1: Leaves of *Murrya koenigii*

METHODOLOGY:

The plant leaves were collected from the local area and identified the same for physical characteristics on morphology of *Murraya koenigii*. The collected plant leaves were washed thoroughly 2-3 times with running water and with distilled water. The leaves were air-dried under shade. The leaves were crushed to make possible fine powder.

Preparation of different extracts by cold maceration method

350 gm of dried leaf powder passed through sieve No 40 was macerated with 250 ml of solvent (Pet ether, chloroform, ethanol and water) in a tightly covered round bottom flask up to seven days period with intermittent shaking. After seven days, the extracts were filtered by using filter paper (Whatman No A-1), marc was pressed. The filtrate obtained from organic solvents like pet ether, chloroform and ethanol was allowed to air dry. The aqueous extract was concentrated in vacuum at 37 ◦ C using a rotary vacuum evaporator.

All dried extract were stored in a desiccators. The colour, appearance of physical nature of the extract were examined and recorded in Table 1.

Table 1:- The Percentage Yield of Petroleum Ether, Chloroform, Ethanol and Aqueous

S,No	Solvent	Nature of Extract	Color	%Yield
1	Pet.Ether (40-60˚C)	Semisolid	Greenish black	3.9
2	Chloroform	Semisolid	Dark green	3.1
3	Ethanol	Semisolid	Green	6.3
4	Aqueous	Semisolid	Brown	7.7

Induction of Hyperurecemia in Male Wistar rats

In the present study Pyrazinimde selected as an inducer of hyperurecemia. Pyrazinamde an anti TB drug, its chronic use or at high doses causes hyperurecemia. Its metabolite pyrazinoate serve as the exchanging anion from inside cells, thereby stimulating anion exchange and urate reabsorption.

Dose titration of Pyrazinamide in rats

Pyrazinamide at higher doses or upon its chronic use elevates serum uric acid. Its metabolite pyrazinoate serve as the exchanging anion from inside cells, thereby stimulating anion exchange and urate reabsorption.

***Animals*:**

Male Wistar rats weighing, 125-150 gm were selected and divided into three groups. Each group having 3 animals. Rats were kept in poly propylene cages and fed on standard laboratory diet. The animals were exposed to 12 hours of darkness and light each.

Estimation of Initial Serum uric acid levels

The blood samples were collected from each rat through tail vein puncture, allowed centrifugation for 15 min; serum was separated and estimated its initial serum uric acid levels using Uric acid kit.

Preparation of different doses of pyrazinamide

Pyrazinamide is soluble in cold water; its water solubility is 15mg/ml. In this work dose titration of Pyrazinamide was done at different doses i.e.150 mg/kg, 200mg/ kg and 250 mg/kg body weight. According to animal body weight dose of pyrazinamide was calculated and prepared according to its solubility.

Experimental design

Group I-treated with Pyrazinamide at dose of 150 mg/kg b.wt

Group II –treated with Pyrazinamide at dose of 200 mg /kg b.wt

Group III- treated with Pyrazinamide at dose of 250 mg/kg b.wt

Administration of Pyrazinamide to rats

The prepared pyrazinamide solution, at different doses was administered by oral route to each rat using oral feeding tube. Like this manner the dose administration was done upto seven days to ten days. Pyrazinamide at its high doses or at chronic use inhibit or reduce the uric acid excretion and elevates the blood uric acid level Excess uric acid in the blood is going to accumulate at the space between the joints and causes inflammation.

Estimation of serum uric acid after one week

After one week of daily administration of pyrazinamide, the serum uric acid levels were estimated by collecting blood from tail vein of each rat.

RESULTS:

Acute toxicity testing of plant extract

The prepared plant extracts from different solvents were evaluated. The acute toxicity studies for the prepared extracts were done in the dose range of 500.1000,2500,5000 mg/kg body weight.

Acute toxicity study was carried out according to OECD guidelines. The extracts were given to rats by oral route at a dose level of 500, 1000 and 2500 mg/kg body weight, to groups of 4 animals. No death occurred within 24 h of dose of 500, 1500 mg/kg but at a dose of 2500 mg/kg 50% mortality was observed. As dose was increased further up to 5000 mg/kg, at that dose all the animals were died. Hence 2500 mg/kg dose was considered as LD50.

The Initial serum uric acid levels of rats were found to be in the range of 3.5-4.5 mg/dl. After one week daily administration of Pyrazinamide at higher dose (250mg/kg) was significantly increases the blood uric acid level up to 10.5 -11.0 mg/ dl. At tenth day we have seen acute flares at the joints of fore limbs, especially in the morning time. This stage indicates the development of acute phase of gout. The significantly elevated serum uric acid levels were maintained up to 30 days of drug discontinuation.

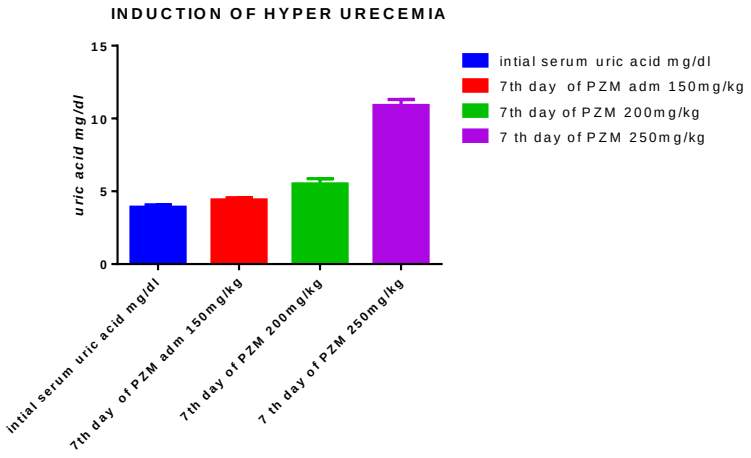

Figure 2: Induction of Hyperurecemia with different doses of Pyrazinamide

Screening of hypourecemic activity of Murraya koenigii leaves

The serum uric acid levels of experimental groups after treatment of different extracts of curry leaves and standard drug was shown in the following Tables.

MKPE: *Murraya koenigii* pet ether extract
MKCE: *Murraya koenigii* chloroform extract
MKEE: *Murraya koenigii* ethanolic extract
MKAE: *Murraya koenigii* aqueous extract

Table 2: Serum Uric acid Levels after treatment with Standard Drug (Allopurinol)

Animal No	Initial serum uric acid in mg /dl	Serum uric acid level in hyper urecemic rats in mg/dl	Serum uric acid level in mg/dl after treatment of Allopurinol
1	4.0	10.5	3.8
2	4.2	10.8	4.0
3	4.0	10.4	4.0
4	4.0	10.2	4.2
5	3.7	9.8	4.2
6	3.9	10.0	4.0

Table 3: Serum Uric acid Levels after treatment with *Murraya koenigii* pet ether extract (MKPE)

S. No	Initial Serum uric acid levels in mg//dl	Serum uric acid level in hyper urecemic rats in mg/dl	Serum uric acid level in mg/dl after treatment of MKPE 250 mg /kg
1	4.0	10.0	9.5
2	4.0	10.2	9.4
3	4.4	10.5	9.5
4	4.2	10.6	9.5
5	4.4	10.8	9.6
6	4.0	10.3	9.3

Table 4: Serum Uric acid Levels after treatment with *Murraya koenigii* chloroform extract (MKCE)

S. No	Initial Serum uric acid levels in mg//dl	Serum uric acid level in hyper urecemic rats in mg/dl	Serum uric acid level in mg/dl after treatment of MKCE 250mg/kg
1	4.0	10.5	9.4
2	4.2	10.6	9.2
3	4.0	10.3	9.3
4	3.6	10.0	9..0
5	3.8	10.0	8.9
6	4.0	10.4	9.2

Table 5: Serum Uric acid Levels after treatment with *Murraya koenigii* ethonolic extract (MKEE)

S. No	Initial Serum uric acid levels in mg//dl	Serum uric acid level in hyper urecemic rats in mg/dl	Serum uric acid level in mg/dl after treatment of MKEE 250 mg/kg
1	3.8	10.0	4.2
2	4.0	10.2	4.5
3	4.0	10.1	4.6
4	4.0	10.4	4.7
5	4.2	10.6	4.8
6	4.4	11.0	5.0

Table 6: Serum Uric acid Levels after treatment with *Murraya koenigii* aqueous extract(MKAE)

S. No	Initial Serum uric acid levels in mg//dl	Serum uric acid level in hyper urecemic rats in mg/dl	Serum uric acid level in mg/dl after treatment of MKAE 250 mg/kg
1	4.0	10.2	6.8
2	4.0	10.4	7.0
3	4.2	10.8	7.2
4	3.7	10.0	6.5
5	3.5	9.8	6.5
6	3.9	10.2	6.9

The alcoholic and aqueous extracts of *M.koenigii* leaves at 250 mg/ kg have comparable hypourecemic activity with that of standard drug Allopurinol. The ethanolic extract of *M.koenigii* leaves was shown maximum hypourecemic activity and Pet ether extract of *M.koenigii* leaves has shown minimum hypo urecemic activity.

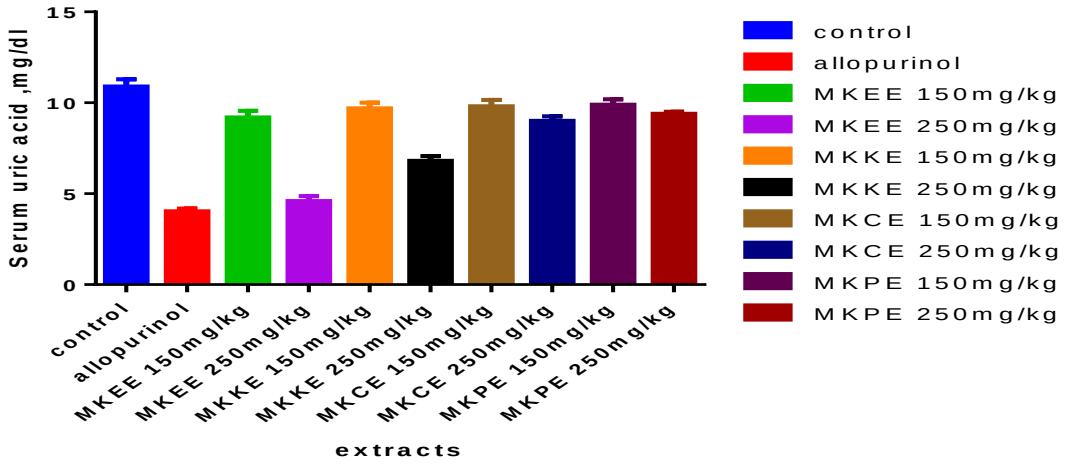

Figure 3: The effect of different extracts of *M.koenigii* leaves on serum uric acid levels at 150 and 250 mg /kg body wt.

CONCLUSION:

This study is revealing that Pyrazinamide at 250 mg/kg b.wt is able to produce significant elevation of serum uric acid levels in one week. Curry leaves are rich in alkaloids, phenolic compounds, steroids and glycosides. Alcoholic and aqueous extracts are only having hypourecemic activity at 250 mg/kg, Ethanolic extract of curry leaves produced maximum hypourecemic activity whereas the minimum effect was produced by Pet ether extract of curry leaves. *Murraya koenigii* leaves having hypourecemic activity is due to presence of Carbazole alkaloids and Phenolics.

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