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PHARMACOLOGICAL EFFECT OF *FICUS BENGHLENSIS* LEAF EXTRACT ON SERUM URATE LEVELS AND VARIUS PARAMETER IN OXONATE INDUCED HYPERURICEMIC ANIMALS.

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ABSTRACT

Background: *Ficus Benghalensis (Moraceae)* Plant Species are widely available in Asian regions and used extensively in the field of Variety of disease Conditions. It includes Hypoglycaemia Activity, Anthelmintic, Anti-Inflammatory, Anti-Diabetic and mainly Anti-Oxidants Activity due to its important chemical Constituents in its leaf like Flavonoids. **Materials and methods:** Leaves of *Ficus Benghalensis* was extracted by Soxhlate Extraction Procedure and tested for its Anti-hyperuricemic activity on Wister Rats of either Sex. **Results:** Thirty six either sex albino rats were divided randomly into six groups. There were three group doses variations of *Ficus Benghalensis* extract that were 200 mg/kg bw, 300 mg/kg bw and 500 mg/kg bw. Intraperitoneal administration of Potassium oxonate (280 mg/kg bw) one hour before final test drug administration of test drugs in animals on 7th day uric acid level in plasma and urine also Total Protein content, Glutathione, Creatinine, Malonaldehyde , ESR various parameter was measured in rats after hours. The results showed that all extracts could reduce uric acid level of rats. The decreasing potency of uric acid level and various parameter in blood was observed at 300 mg/kg and 500 mg/kg dose. This results indicated that *Ficus Benghalensis l*eaves extract may be effective for the prevention and the treatment of hyperuricemia.

KEYWORDS: *Ficus Benghalensis,* Potassium oxonate, uric acid level, hyperuricemia.

INTRODUCTION

Gout is one of the most common inflammatory arthritis types, characterized by elevation in serum uric acid levels and deposition of monosodium urate crystals in and around the joints. The serum uric acid level in the body is a function of balance between the breakdowns of Purine and the rate of urate excretion. The normal serum uric acid level is 2-7.0 mg/dl For adult male and 2-6.0 mg/dl in women.[1] Above this concentration of uric acid in a body is referred as Hyperuricemia condition. The more amount of uric acid is supersaturated in Body fluids and is prone to crystallization and subsequent tissue deposition. The uric acid values however differ for women and children who have lower normal serum uric acid Level.

Hyperuricemia seems to be more prevalent worldwide, probably due to improvements in standard of living, increasing longevity and the usage of certain drugs such as salicylate and pyrazinamide.[2, 3] This has resulted in significant morbidity and increase in costs of the health care system. It is often associated with a number of human diseases.

Clinically reported, the key factor uric acid is related not only to an increased risk of gout, but also to an increased risk of cardiovascular disorder, nephrolithiasis and diabetes.[4]

Uric acid is the insoluble end product of purine metabolism. Approximately two-thirds of body uric acid comes from the breakdown of endogenous purines, with the remainder from dietary purines. It is predominantly excreted through the kidney, and a substantial amount is excreted through the gut. Elevated serum uric acid is one of the major risk factors for gout.[5] Hyperuricemia is defined as serum uric acid levels above 7 mg/dL, which is the solubility limit of urate in body fluids.[6] The level of serum uric acid also appears to be an important risk factor for development of gout. Hyperuricemia leads to the deposition of urate crystals in joints, and shedding of crystals into the synovial fluid triggers a local inflammatory response. Phagocytosis of monosodium urate crystals generally initiates the inflammatory pathway. Cytokines (IL 1s, IL6, TNF-α), neutrophil activation, and formation of inflammation by macrophages and monocytes are the hallmarks of local inflammation.[7-12] Deposition of the crystals can lead to chronic gout and eventually the formation of tophi (deposition of urate crystals in soft tissues). Periarticular urate deposition results in the development of structural joint damage in gout.[13]

The therapeutic approach to treat gout is to use either uricosuric agents or xanthine oxidase inhibitors (XOI). XOIs block the synthesis of uric acid from purines and they are much useful

when compared to other drugs, since they possess lesser side effects. Allopurinol remains to be the dominant clinically used xanthine oxidase inhibitor, however, adverse effects limits its therapy.[14] Thus, there is a need to develop compounds with XOI activity which is devoid of the undesirable side effects of allopurinol. A potential source of such compounds can be obtained from medicinal plants.[15, 16, and 17] Many Indian medicinal plants have been used for the prevention and treatment of gout and related inflammatory disorders, but they lack sufficient scientific evidence.[18]

The Plants have been used in conventional medicine for several thousand years. Awareness Of medicinal plants has been accumulated in the course of many centuries based on different Medicinal systems such as Ayurveda, Unani and Siddha. WHO also recognized the role of traditional system of medicine which depends largely upon the medicinal plants to achieve its goal "Health for all by 2020".[19]

Ficus Benghalensis Plant Species from the Family of *Moraceae*. It is one of the Plant widely Available in Asian regions and used extensively in the field of Variety of disease Conditions, it includes Hypoglycaemia Activity, Anthelmintic Anti-Inflammatory, Anti-Diabetic and Mainly anti-Oxidants Activity due to its chemical Constituents. Also from anciently the Tribal people of Jhabua District in Madhypradesh used these Plants for Joint Diseases and Anti-Hyperuricemia Activity.[20]

MATERIALS AND METHODS

Plant material

The plant material consists of dried powdered leaves of *Ficus benghalensis* belonging to the family Moraceae.

Plant collection

The leaves of plant *Ficus benghalensis* (Family-Moraceae) were collected from the Botanical garden of Bombay Veterinary College, Maharashtra State in the month of September to October 2013. The leaves were authenticated at the G.N. Khalsa College, Mumbai. The fresh leaves were washed and dried at room temperature. When the leaves were sufficiently dry and easily crushable with hands, they were subjected to a grinder to obtain a powdered form of the leaves. This powder was stored in an air tight container for the further extraction process.

Extraction

The air-dried leaves powder of *Ficus benghalensis* was defatted with (60-80 %) petroleum ether (750ml). It was then extracted with ethanol and distilled water (70:30) in Soxhlate apparatus for 16 hrs. The extract was filtered and concentrated in a rotary flash evaporator at 60°C. The concentrated ethanolic extract was poured into excess of distilled water with stirring and filtered. The filtrate that comprises water-soluble portion of extract was extracted in liquid –liquid extractor with hexen. It separated into two layers one is Aqueous and other is oily portion. Hexen extract was concentrated to a small volume and was kept in a refrigerator for 48 hours, which yielded, browny crystals. These crystals were dissolved in water and were tested for the presence of Flavonoids.[21]

Phytochemical screening of *ficus benghalensis* extracts.

Phytochemical analysis for qualitative detection of alkaloids, flavonoid, tannins, and saponins, steroid, glycosides and reduced sugars was performed on the extract as described by C.K. Kokate.[22]

NOTE: Conduction of all the procedures involving animals in the entire study was in compliance with the guidelines issued by IAEC (Institutional Animals Ethics Committee). The number of the approved Protocol for this study, given by the IAEC (Institutional Animals Ethics Committee) was: **IAEC/PCOL-23/2013**.

Acute toxicity studies

Albino Wister Rats weighing between 180-220 g maintained under standard laboratory conditions was used. Animals were divided into six groups consisting of 6 each; the animals received a single oral dose (2000 mg/kg, body weight) of each extract. Animals were kept overnight fasting prior to drug administration. After the administration of the extract, food was withheld for further 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention during the first 4 hours) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and CNS (ptosis, drowsiness, gait, tremors and convulsions) changes.[23]

Selection of dose of the extract.

LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The LD50 of the extract as per OECD guidelines falls under category 4 values with no signs of acute toxicity at doses of 2000 mg/kg. The biological evaluations of the extract were carried out at a dose of 200 mg/kg, 300 mg/kg & 500 mg/kg body weight.

A. In-vivo Studies

I. Potassium Oxonate induced Hyperuricemia

Wistar rats of body weight 180-220 gm were divided into six groups. Consisting of 6 animals such as Control, Disease control, Standard group (Allopurinol 10mg/kg as Standard), Test 1, Test 2 and Test 3 (200mg/kg, 300mg/kg, 500mg/kg of FBLE). Group I received normal saline and served as the control. Group II received potassium oxonate (280 mg/kg *b.w., i.p*) and served as hyperuricemic control 58. Groups III, IV and V received the hydro-alcoholic extract of *Ficus benghalensis* at a dose of 200 mg/kg, 300mg/kg and 500mg/kg orally respectively. Group VI received allopurinol (10 mg/kg, orally) and served as the positive control. The extract of dose administered orally for 7 days. On the 7 th day potassium oxonate (280 mg/kg *b.w.*) was injected intraperitoneally one hour before the final test drug administration to induce hyperuricemia after one hour of the final drug administration, blood was collected by retro-orbital method. The effect of extract on ESR, MDA as an indicator for lipid peroxidation, Glutathione and Creatinine for detection of Renal functioning, Total protein and uric acid content in blood and urine was determined.[23-29]

In-vitro Studies

I. **Assay of XDH/XO activities in Rat liver**

On the 7th day after blood collection, animals were sacrificed and the liver was excised, washed in cold 0.15 M KCl separately and homogenized (10% w/v) using 0.05M potassium dihydrogen phosphate buffer pH (7.5) in 0.5 mM EDTA. The homogenate was then centrifuged at 5000g for 10 min, the lipid layer was carefully removed and the resulting supernatant was further centrifuged at 5000g for 10min.The Supernatant was used for the assay of xanthine oxidase (XO) and xanthine dehydrogenase (XDH) enzymes activities. After preincubation for 15 min at 37ºC, the reaction was initiated by the addition of 1ml of 250µM xanthine (dissolved in phosphate buffer, pH 7.5). After 10 min, the reaction was stopped by the addition 0.5 ml of 0.58 M HCL and the solution was centrifuged at 5000 g for 5 min. The

absorbance of the supernatant was measured at 290 nm against the blank. XO/XDH activities were expressed as nM uric acid formed/ min/ mg protein.[30]

$$\% \text{ Inhibition} = \frac{A \text{ (control)} - A \text{ (Test)} \times 100}{A \text{ (control)}}$$

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dennett's test. Results are expressed as mean ±SEM from six rat in each group. P values < 0.05 were considered significant. Compared with (#) Standard Group & (*) Compared with Disease Control group (one-way ANOVA followed by Dennett's Multiple Comparisons test). $^{###}p<0.001$ shows significant results compared with standard.

RESULTS AND DISCUSSION

Acute toxicity studies

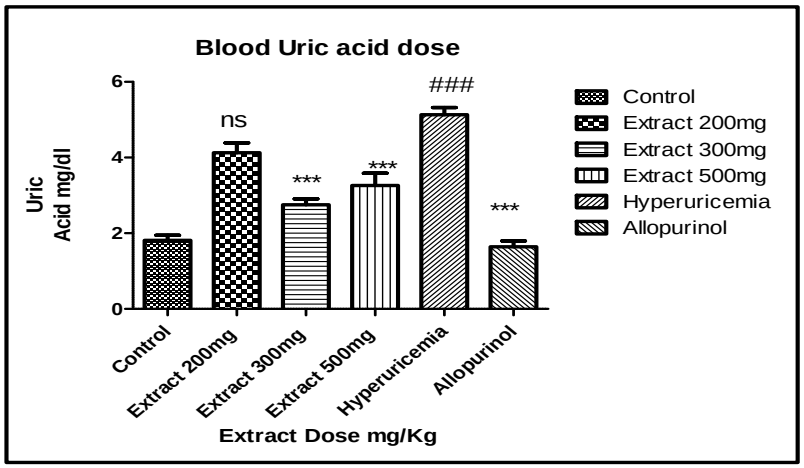
In LD50 studies, it was found that the animals were safe unto a maximum dose of 2000 mg/kg body weight. There were no changes and normal behavior pattern and no signs and symptoms of toxicity and mortality were observed.

Effect of *ficus benghalensis* leaf extract on serum urate level in rat.

Administration of the uricase inhibitor, potassium oxonate resulted in significant (P<0.01) hyperuricaemic in rat, as indicated by an increase in the serum uric acid levels when compared to the control group. Pre-treatment with the *ficus benghalensis* leaf extract (200 mg/kg, 300 mg/kg & 500 mg/kg) of for seven days significantly (P<0.01) reduced the serum urate levels, when compared with the hyperuricaemic control group. The reduction in urate levels produced by 300 mg/kg extract was more potent than that of the other doses of extracts. Administration of 200 mg/kg of *ficus benghalensis* did not produce any significant (P>0.05) reduction in serum urate levels, when compared to the oxonate-treated group. The standard drug allopurinol at a dose of 10 mg/kg elicited significant (P<0.01) reduction of serum urate level compared to hyperuricaemic rats (Table 1).

Table 1: Effect of *ficus benghalensis* leaf extract on serum urate level.

Sr. No.	Treatment	Blood Uric Acid level
1	Control	11.12 ±0.136***
1	Disease Control	30.79±0.263 ***###
2	Standard (Allopurinol 10mg/kg)	9.91±0.158 ***
3	Test 1 (FBHLE 200 mg / kg)	24.64±0.312 **#ns
4	Test 2 (FBHLE 300 mg / kg)	16.5 ± 0.188 ***#ns
5	Test 3 (FBHLE 500 mg / kg)	19.52 ± 0.153 ***



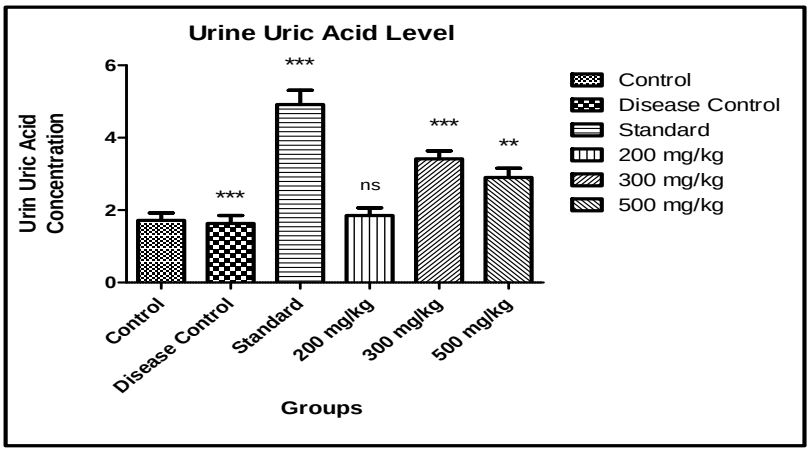
Allopurinol was used as the standard drug in the experiment, while different doses of the extract accounted for the test drug. In this model, the disease control animals showed the highest uric acid level after injection of uricase inhibitors that is Potassium Oxonate. Owing to the Anti-hyperuricemic property, the standard drug (Allopurinol) showed maximum inhibition.

Test drug Showed significant hypouricemic activity at the dose of 300mg/kg and 500mg/kg, but due to taking consideration of solubility in oral dose, the 300mg/kg dose showed maximum activity and less significant inhibition at the lowest dose at 200mg/kg. Xanthine oxidase (XOD) catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid and is a key enzyme in the pathogenesis of hyperuricemia. The ability of extracts of *Ficus benghalensis* to inhibit uricase was investigated in this study.

Administration of the uricase inhibitor potassium oxonate significantly affected various bio-chemical parameters (Urine Uric acid, ESR, Creatinine, MDA, glutathione, total protein content) on Wistar rat's blood. A disease control Animal decreases Uric acid Excretion is decreased when Potassium Oxonate Injection is injected but after standard drug (Allopurinol) administration the uric Acid Excretion in Urine gradually increases. Also, the urine uric acid concentration at dose of 300 mg/kg, and 500 mg/kg show significant result. Due to the oral Administration of the test dose of 300mg/kg and 500 mg/kg increases the uric acid elimination via the urine and decreases the blood uric acid level in the body.

Table 2: Effect of ficus benghalensis leaf extract on Urine uric acid level.

Groups	Normal Urine Uric acid level (mmole)	After Potassium Oxonate Injection Urine Uric acid level (mmole)	After Extract Administration Urine Uric acid level (mmole)
Control	2.78 ± 0.08	2.31 ± 0.43	2.23 ±0.53
Positive Control	1.63 ± 0.024	1.50 ± 0.16 ##ns	1.63 ±0.053 ##ns
Test1 (200 mg/kg)	2.31 ±0.23 ***#ns	1.51 ± 0.56 **#ns	1.81 ±0.074 **###ns
Test 2 (300mg/kg	2.23 ± 0.063 ***##	1.5 ± 0.09 ***###	3.41 ±0.072 ***##
Test 3 (500 mg/g)	2.48 ± 0.25 ***##	1.63 ±0.45***##	2.9 ± 0.13 ***###
Standard	2.76 ± 0.53 ***	1.4 ± 0.31***	4.91 ±0.9***



Effect of *ficus benghalensis* leaf extract on various Parameter in Blood.

An increased serum urate levels compared to normal control group was observed after potassium oxonate injection. Allopurinol (10 mg/kg), significantly (p<O.01) reduce serum urate levels of hyperuricemic rats to values than that found in normal animals. A Seven day treatment with hydroalcholic extracts of leaves at the dose of 300 mg/kg and 500 mg/kg respectively reduce significantly (p<0.01) serum uric acid levels compared to hyperuricemic control group. The present study also investigated the efficacy of orally administered *Ficus benghalensis* extracts and allopurinol on serum non-invasive biomarkers of oxidative stress (malondialdehyde concentration) and lipid-peroxidation (reduced glutathione) levels in rat's blood. As shown in Table significant decrease (p<0.01) in serum MDA levels was observed in rats treated with FBHL extracts (300mg/kg & 500mg/kg) than compared to positive control group, whereas a drastic decrease in glutathione levels is also observed. A similar effect was obtained in all tested

parameters in hyperuricemic animals after extracts administration compared to hyperuricemic control group. In the present study gouty rats showed a significant increase in Erythrocyte Sedimentation Rate (ESR) and creatinine level. The oral administration of Hydro-alcoholic leaves extracts 300mg/kg and 500mg/kg of *Ficus benghalensis* produced the significant reduction in ESR and Creatinine levels 1hours after i.p administration of oxonate. The Hydro-alcoholic extract 300mg/kg and 500mg/kg showed significant ($p<0.01$) decrease in ESR level compared to positive gouty control rats and values are comparable to the standard (allopurinol) whereas in case of reduction of Creatinine levels, leaves extract (300mg/kg)showed more significant ($p<0.01$)reduction than dose of 200mg/kg ($p<0.05$). As shown in Table, the amount of protein content did not vary neither with administration of oxonate nor with the oral dose of extracts. There were insignificant ($p>0.01$) results shown by both Hydro-alcoholic leaves extracts 300mg/kg, and 500mg/kg compared to positive control in the total protein content in the liver tissues of rats treated with the uricase inhibitor, potassium oxonate. It was thus concluded that as per the results, there is no effect of potassium oxonate on protein content in liver tissues.

Table 3: Effect of ficus benghalensis leaf extract on various Parameter in Blood.

Parameter	Control	Disease Control	Standard	200 mg/kg	300 mg/kg	500 mg/kg
ESR (mmh^{-1})	$2.6 \pm 0.04^{***}$	$4.83 \pm 0.39^{###}$	$1.26 \pm 0.15^{***}$	$3.5 \pm 0.19^{***}$	$1.8 \pm 0.02^{**##}$	$2.98 \pm 0.09^{***##}$
Creatinine (mg/dL)	$0.74 \pm 0.02^{***}$	$1.34 \pm 0.06^{###}$	$0.76 \pm 0.07^{***}$	$1.31 \pm 0.24^{**#ns}$	$1.05 \pm 0.06^{**##}$	$1.09 \pm 0.02^{**##}$
Malonaldehyde (µ/mL)	$1.88 \pm 0.02^{***}$	$4.57 \pm 0.39^{###}$	$1.97 \pm 0.12^{***}$	$3.5 \pm 0.08^{***#ns}$	$2.1 \pm 0.04^{***##}$	$2.7 \pm 0.29^{***##}$
Glutathione (µ/mL)	$1.04 \pm 0.11^{***}$	$3.59 \pm 0.15^{###}$	$2.29 \pm 0.10^{***}$	$2.78 \pm 0.03^{***#ns}$	$2.34 \pm 0.12^{***#}$	$2.61 \pm 0.03^{**##}$
Total protein Content (g/dL)	$3.66 \pm 0.03^{***}$	$3.23 \pm 0.29^{###}$	$3.54 \pm 0.01^{***}$	$3.33 \pm 0.11^{***##}$	$3.82 \pm 0.05^{**###}$	$3.38 \pm 0.10^{**##}$

Effect of *ficus benghalensis* leaf extract on XO/XDH activities in rat Liver

Animals treated with potassium oxonate produced a significant ($P<0.01$) increase in XO/XDH enzyme activities in rate liver compared to control group. Pre-treatment of rats with *ficus benghalensis* leaves extract at a dose of 200 mg/kg, 300 mg/kg *b.w and* 500 mg/kg *b.w.* for seven days produced significant ($P<0.01$) inhibition towards XO (19.46%, 33.89%, 29.068% and 59.52%) and XDH (24.6%, 36.436%, 32.12% and 59.82 %) respectively, when compared with the hyperuricemic control. The inhibition of the XO/XDH activities at

the dose of 300 mg/kg and 500mg/kg of leave extract was found to be the highest among the extract tested. On the other hand, the action of the leaves extract (200 mg/kg) on the inhibition of XO (19.46%) and XDH (24.6%) was non-significant ($P < 0.05$). Allopurinol inhibited both XO (59.52%) and XDH (59.82%) activities at a dose of 10 mg/kg, Exhibiting more potent activity than the all test Dose.

Table 4: Effect of *ficus benghalensis* leaf extract on XO/XDH.

Treatment	Dose	XO	XDH	% Inhibition	% Inhibition
		nM Uric acid formed/ min/mg protein		XO	XDH
Control	Saline	1.25 ± 0.07 $^{**\#}$	2.1054 ± 0.04 $^{**\#}$	-	-
Disease Control	280 mg/kg	3.3443 ± 0.11$^{\#\#\#}$	3.4216 ± 0.12$^{\#\#\#}$	-	-
Test 1	200mg/kg	2.632 ± 0.10 $^{**\#ns}$	2.5206 ± 0.22$^{**\#\#ns}$	19.465	24.6
Test 2	300mg/kg	2.09836 ± 0.04 $^{***\#\#\#}$	2.1983 ± 0.07 $^{***\#\#\#}$	33.89	36.436
Test 3	500mg/kg	2.2513 ± 0.14$^{***\#\#}$	2.27062 ± 0.34$^{***\#\#}$	29.068	32.12
Standard(Allopurinol)	10mg/kg	1.3209 ± 0.08 ***	1.3295 ± 0.03 ***	59.52	59.826

CONCLUSION

In conclusion, the data reported in the present study indicates that the extracts of *ficus benghalensis* have significantly reduced the serum urate levels in hyperuricaemic animals. This may be due to the inhibition of XO/XDH activities and the presence of phytochemical constituents. Further investigation to explore the effect of other components of *ficus benghalensis* and define their clinical efficacy would be highly desirable.

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