



**EVALUATION OF ANTIGOUT ACTIVITY OF *BRYOPHYLLUM CALYGINUM* LEAVES
EXTRACTS ON POTASSIUM OXONATE-INDUCED GOUT RAT MODEL**

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ABSTRACT

Objective: The present study has been conducted to evaluate antigout activity of aqueous and alcoholic extracts of *Bryophyllum calycinum* leaves on potassium oxonate-induced gout rat model. **Method:** The study was conducted on Sprague-Dawley rats dividing them in eight groups having six rats in each group. Rats were fasted for 16 hr before potassium oxonate injection. After 1 hr. of Oxonate injection, blood samples were collected for parameter evaluation for control group. Allopurinol was given to the standard group and different test extract, each extract with three different doses i.e. 100, 150, 200 mg/kg were given to treated groups T₁, T₂, T₃, T₄, T₅ for consecutive 5 days. Blood samples were taken on 5th day for evaluation of anti gout activity of test extracts of *Bryophyllum calycinum*. Results for parameter evaluated for treated groups were compared with standard group. **Results:** At the end of study, rats of gout control group showed increase in platelets counts, serum creatinine, uric acid, blood urea nitrogen (BUN), and xanthine oxidase (XO) enzyme level along with alterations in kidney tissues as compared to vehicle control group. Gouty rats treated with aqueous and alcoholic extracts of *Bryophyllum calycinum* at 200 and 400 mg/kg body weight and standard treatment Allopurinol at 5 mg/kg body weight showed reduction in platelets counts, serum creatinine, and uric acid, BUN, and XO enzyme level. **Conclusion:** Oral administration of aqueous and alcoholic extracts of *Bryophyllum calycinum leaves* has shown protection against gout in dose-dependent manner in rats.

KEYWORDS: *Bryophyllum calycinum*, Anti gout activity, Hyperuricemia, Allopurinol.

INTRODUCTION

Gout is a heterogenous group of diseases resulting from the deposition of urate (as monosodium urate monohydrate) crystals in supersaturated extracellular fluids. These crystals cause an acute inflammation response and can cause a permanent tissue damage which is characterized by the appearance of ulceration of the joint cartilage, marginal osteophytosis, erosive lesions and chronic inflammation of synovial membrane.^[1] The underlying metabolic disorder in gout is an excessive concentration of uric acid in the blood. Urate lowering therapy is the main approach in the treatment of gout. The target level of serum uric acid is <6.8 mg/dL to dissolve the urate crystals and inhibit gout attack.^[2,3] The most important approach in the treatment of gout is the development of xanthine oxidase (XO) inhibitors, which are effective in elevating plasma and urinary urate levels and reverses the development of tophaceous deposits.^[4] So, food components which inhibit XO activity can reduce the formation of uric acid and alleviate inflammation. It is because of XO enzyme which has an important role in hyperuricemia, catalyzing

the oxidation of hypoxanthine to xanthine and then to uric acid.^[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-mercaptapurin.^[7] Thus, the development of novel hypouricemic herbal 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.

The plant has been reported to have various phytoconstituents which are used clinically. Phytoconstituents like flavonoids, steroids, bufadenolides, glycosides, organic acids, proteins, amino acids, tannins, saponins, triterpenoids and lipids are used in evaluating for various pharmacological activities. Leaves of this plant are mainly rich in bufadenolides

which are actively used practically for treating various cardiovascular disorders. Bufadenolides like bryotoxin A, B, C similarly act like cardenolide glycosides, digoxin, and digitoxin.^[8] Reported pharmacological activities of this plant are like Herbal tonic, Hepatoprotective, Nephroprotective, Neuropharmacological, Anti mutagenic, Anti ulcer, Anti bacterial, Anti diabetic, Immunosuppressant, Anti hypertensive, Analgesic, anti inflammatory, wound healing, Uterine contractility, Insecticidal, fungi toxic, phytotoxic and Anti cancer activity.^[9]

As per our knowledge, there was lack of reported references regarding antigout activity of aqueous and alcoholic extracts of *B. calycinum* on gout rat model so the present study was planned to explore antigout effect of *B. calycinum* leaves extracts following its oral administration at various doses along with hemato-biochemical evaluation.

MATERIALS AND METHODS

Drugs: Potassium Oxonate and allopurinol was purchased by TCI Chemicals.

Chemicals and Reagents: pet. Ether, acetone, chloroform, methanol was purchased from RANKEM chemicals. Sulfanilamide, o-phosphoric acid, naphthyl ethylene diaminedihydrochloride, was purchased from CDH chemicals. TBA, TCA, Tris hydrochloride, was purchased from LOBA CHEM chemicals.

Biochemical kits: Uric acid kit, creatinine kit, BUN kit was purchased from ERBA MANNHEIM.

Collection and authentication of the plant *Bryophyllum calycinum*

The plant sample was collected from college, authenticated as *Bryophyllum calycinum*, as No. Dis/482/2015/Syst.Bot./Rev.Gen./4-5 by Systemic Botany Discipline, Botany Division, Forest Research Institute, P.O. New Forest, Dehradun-248006.

Method for preparation of various extracts of *Bryophyllum calycinum*.

The sample of plant *Bryophyllum calycinum* (5 kg) was dried and grounded to moderately coarse powder. The coarse powder (2 kg) was packed in a clean dry Soxhlet apparatus. The packed material was extracted using different solvents with increasing polarity. The extract was collected in beaker and evaporated to dryness on a water bath and stored in air tight container.

Preliminary photochemical investigation of the different plant extracts

The solvent extracts of leaves of *Bryophyllum calycinum* was subjected to qualitative chemical test for the identification of various plant constituents.

Experimental Animals

Sprague Dawley rats weighing between 250-280 g was used for Anti-gout activity. Rats of body weight 250-280 g of either sex were bred in the Institutional animal house and used for Anti-gout activity. The animals were housed in standard polypropylene cages and were maintained under controlled room temperature (22±20°C) and humidity (55±5%) along with 12:12 hour light and dark cycle. All the animals were provided commercially available rat normal pellet diet and water ad libitum. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were followed and prior permission was granted from the Institutional Animal Ethics Committee (Reg. No. IAEC/273/CPCSEA/SBS/02/2015) for conducting the animal experimental studies.

Potassium oxonate induced gout in rats.

Gout is directly related with increased serum uric acid level, which results from the over production or under excretion of uric acid in the body, and is highly influenced by a high dietary intake of nucleic acids.^[10] Hyperuricemia, a root cause factor for the development of gout and it is also involved in many diseases such as renal dysfunction, cardiovascular diseases, hypertension, hyperlipidemia, diabetes and metabolic syndrome.^[11,12] Administration of potassium oxonate, a well-known inhibitor of urate oxidase, is widely used to create an animal model of hyperuricemia and gout. In the present study, potassium oxonate administration successfully induced hyperuricemia in rats, with a peak effect 3 hours following administration. The uricase inhibitor potassium oxonate was used to induce hyperuricemia in the rats.^[13] To increase serum urate levels, the rats were given potassium oxonate (320 mg/kg) orally.

In the present study Sprague Dawley rats were divided into 8 groups (n=6).

Normal vehicle: only vehicle (DMSO) was given orally.

Positive control: Potassium Oxonate was given orally (320 mg/kg of body weight) for inducing gout in rats.

Standard group: Potassium Oxonate induced gout was treated with allopurinol (10 mg/kg of body weight) orally for five days.

Treated group 1 (T₁) (Petroleum ether extract): Potassium Oxonate induced gout was treated with pet ether extract with three different doses (100 [1(a)], 150[1(b)], 200 [1(c)] mg/kg) orally for five days.

Treated group 2(T₂) (chloroform extract): Potassium Oxonate induced gout was treated with chloroform extract with three different doses orally for five days.

Treated group 3(T₃) (acetone extract): Potassium Oxonate induced gout was treated with acetone extract with three different doses orally for five days.

Treated group 4(T₄) (methanolic extract): Potassium Oxonate induced gout was treated with methanolic extract with three different doses orally for five days.

Design of experiment

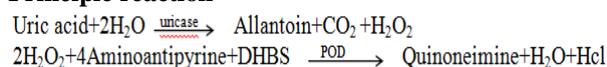
Sprague Dawley rats were divided into 8 groups
↓
Rats were fasted for 16 hr. before potassium oxonate injection.
↓
After 1 hr. of Oxonate injection, blood samples were collected for parameter evaluation for control group. Allopurinol was given to the standard group and different test extract, each extract with three different doses i.e. 100, 150, 200 mg/kg were given to treated groups T₁, T₂, T₃, T₄, T₅ for consecutive 5 days.
↓
Blood samples were taken on 5th day for evaluation of anti gout activity of test extracts of *Bryophyllum calycinum*.
↓
Results for parameter evaluated for treated groups were compared with standard group.

Evaluation Parameters

Determination of serum uric acid level

Uric acid is converted by uricase into allantoin and hydrogen peroxide which in presence of peroxidase (POD) oxidizes the chromogen to a red coloured compound which is read at 500 nm. The final colour of the reaction is stable for 15 min.

Principle reaction



Procedure: Procedure was followed as mentioned in user guide of uric acid kit.

Calculations: Determine absorbance for the standard and unknown samples by using the formula,

$$\frac{\text{uric acid (mg/dL)}}{\text{Abs. of standard}} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{concentration of standard (mg/dL)}$$

Determination of inflammation by plethysmometer

The paw was marked with ink at the level of lateral malleolus and immersed in the water column of a plethysmometer for measuring the paw volume. The paw volume was measured immediately after the Oxonate injection and then at 30min, 1, 2, 3, and 4 hrs. The peak effect of Oxonate usually occurs at three hrs after the injection. The increase in paw volume at six hrs was calculated as percentage and compared with that the volume measured immediately after the injection of oxonate for each animal. The difference of average volume between treated animals and standard groups was calculated.

Calculations

$$\% \text{Inflammation} = \frac{\text{avg. of final paw vol.} - \text{avg. of initial paw vol.}}{\text{Avg. of initial paw vol.}} \times 100$$

Treated group 5(T₅) (aqueous extract): Potassium Oxonate induced gout was treated with aqueous extract with three different doses orally for five days.

Determination of serum creatinine level: Creatinine in alkaline solution reacts with picrate to form a red orange compound under the specific condition of assay; the rate of development of the colour is proportional to the concentration in the sample when measured at 500 nm.

Procedure: procedure was followed as mentioned in user guide of creatinine kit.

Calculations

Determine absorbance change ($\Delta A = A_1 - A_2$) for the standard and unknown samples by using the formula,

$$\frac{\text{Creatinine (Mg/dL)}}{\Delta A \text{ of standard}} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{concentration of standard (mg/dL)}$$

Determination of Malondialdehyde level

The method estimate malondialdehyde (MDA), a product of lipid per oxidation process. One molecule of MDA reacts with two molecules of thiobarbituric acid under mildly acidic conditions to form a pink chromogen, whose intensity is measured colorimetrically at 535 nm.

Procedure: About 2ml of the rat blood sample was added to 2ml of freshly prepared 20% w/v TCA and the mixture was allowed to stand in ice bath for 15 min. After 15 min. samples were centrifuged at 3000 rpm. About 2ml of clear supernatant solution was mixed with 2ml freshly prepared TBA. The solution was mixed and allowed to heat for 10 min. in a boiling water bath. Samples were taken immediately cooled in ice bath for 5 min. and absorbance was measured at 532nm against blank reagent.

Calculations

$$Y = 0.0033x - 0.0107$$

Where,

Y= absorbance of test sample

X= concentration of test sample, Units: nmol/L

Determination of NO level

The method determines nitric oxide concentrations based on enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colourimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acidified NO_2 produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylene diamine to form the chromophoric azo derivative which absorbs light at 540-570 nm.

Procedure: To the 750 μl of blood and standard sodium nitrite, 750 μl of griess reagent was added. Mixture was incubated for 15 min. at room temperature in dark. Blank was prepared with 750 μl of griess reagent. Absorbance was measured spectrophotometrically at 540nm against blank. Each sample was estimated in triplicate.

Calculations

Equation obtained from standard curve of nitrate/nitrite

$$Y = 0.005x - 0.012$$

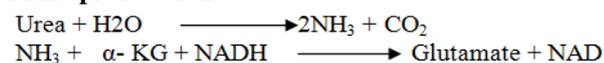
Y= absorbance of test sample

X= concentration of nitrate/nitrite.

Units: $\mu\text{mol/L}$

Determination of Blood Urea Nitrogen: Elevated serum urea concentrations are observed in impaired kidney functions, liver diseases, congestive cardiac failure, diabetes, infections and diseases which impaired kidney infection.

Principle reaction



KG- ketoglutarate

GLDH- glutamate dehydrogenase

The rate of decrease in absorbance is monitored at 340nm and is directly proportional to urea concentration in the sample.

Calculations

Determine absorbance change ($A = A_1 - \Delta A_2$) for the standard and unknown samples by using the formula,

$$\text{Urea (mg/dL)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{concentration of standard (mg/dL)}$$

Determination of erythrocyte sedimentation rate (ESR)

Erythrocyte sedimentation rate is used to diagnose the infection in body. In this evaluation parameter, erythrocytes sedimentation rate is determined in mm/hr. A wester green tube graduated with 0-200mm is like a pipette; it is filled with heparinised blood sample and kept in ESR stand for an hour. After an hour, read the fall in blood level in tube after sedimentation and evaluate the erythrocyte sedimentation rate of the blood sample.

RESULTS AND DISCUSSION

Effect on serum uric acid level: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in serum uric acid level which indicates overproduction of uric acid and under excretion of uric acid through kidneys. Among all the treated groups, methanolic group (T_4) results statistically significant decrease in serum uric acid level. Furthermore, out of the three doses level of methanolic extract of *Bryophyllum calycinum*, 200 mg/kg of body weight results statistically significant decrease in serum uric acid level. (Table. 1 and fig.1).

Effect on serum blood urea nitrogen: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in serum blood urea nitrogen level indicating kidney dysfunction via under secretion of accumulated uric acid through urine. Among all the treated groups, methanolic group (T_4) and aqueous extract (T_5) results statistically significant decrease in serum BUN level. Furthermore, out of the three doses level of methanolic and aqueous extracts of *Bryophyllum calycinum*, 200mg/kg of body weight of both the extracts resulted statistically decrease in serum BUN level. (Table.1 and fig.2).

Effect on serum creatinine level: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in serum creatinine level indicating kidney dysfunction via under secretion of accumulated uric acid through urine. Among all the treated groups, aqueous group (T_5) resulted statistically decrease in serum creatinine level. Furthermore, out of the three doses level of aqueous extracts of *Bryophyllum calycinum*, 200 mg/kg of body weight resulted statistically decrease in serum creatinine level. (Table. 1 and fig. 3).

Effect on malondialdehyde level: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in MDA level indicating oxidative stress. Among all the treated groups, methanolic group (T_4) resulted statistically decrease in MDA level. Furthermore, out of the three doses levels of all the extracts of *Bryophyllum calycinum*, 200 mg/kg of body weight resulted statistically decrease in MDA level. (Table. 1 and fig.4).

Effect on NO level: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in NO level indicating oxidative stress. Among all treated groups, aqueous extract (T_5) resulted statistically decrease in NO level. Furthermore, out of the three doses of aqueous extracts of *Bryophyllum calycinum*, 200mg/kg of body weight resulted statistically decrease in NO level (Table.1 and fig. 5).

Effect on Erythrocyte Sedimentation Rate (ESR): In control group, administration of potassium oxonate (320mg/kg) orally resulted increase in ESR level

indicating increasing chance of infection. Among all the treated groups, aqueous extract (T₅) resulted statistically decrease in NO level. Furthermore, out of the three doses of aqueous extracts of *Bryophyllum calycinum*, 200mg/kg of body resulted statistically decrease in ESR level. (Table.1 and fig. 6).

Effect on inflammation in paw by Plethysmometer: In control group, administration of potassium oxonate (320mg/kg) orally resulted increase paw volume indicating increasing chance of inflammation (figure 7.7.1). Among all the treated groups, methanolic extract (T₄) resulted statistically decrease paw volume. Furthermore, out of the three doses of methanolic extracts of *Bryophyllum calycinum*, 200mg/kg of body weight, resulted statistically decrease in paw volume. (Table. 1, fig. 7 and fig. 8).

Table 1: Different parameters to evaluate anti gout potential.

Experimental	Dose (mg/kg)	Serum uric acid level (mg/kg)	Serum blood urea nitrogen Level (mg/kg)	Serum creatinine level (mg/kg)	Malondialdehyde	Nitric oxide	Erythrocyte sedimentation rate (mm/hr.)	Inflammation by plethysmometer (ml)
Groups					Level (mg/kg)	Level (mg/kg)		
Normal vehicle	DMSO	1.254 ±0.44****	19.56 ±2.85****	0.005 ±0.01****	64.33 ±10.39****	66.72 ±13.05****	0.333 ±0.33****	0.866 ±0.01****
Positive control	Potassium oxonate (320 mg/kg)	5.750 ±0.34	65.143 ±2.51	5.143 ±2.24	88.53 ±35.33	90.03 ±35.35	0.11 ±0.79	2.999 ±1.66
Standard	Allopurinol (10 mg/kg)	1.592 ±0.35****	23.053 ±0.78****	0.063 ±0.03****	77.36 ±2.68****	68.08 ±15.35****	1.766 ±0.26****	0.966 ±1.18****
T ₁ (petroleum ether extract)	a)100 mg/kg	5.477±0.25	66.796 ±3.53	10.323 ±0.76**	92.07 ±0.36****	202.29 ±23.24**	10.166 ±2.72	2.013 ±2.38
	b)150 mg/kg	5.003±0.08	59.351 ±2.70	8.093 ±0.47****	91.58 ±0.48****	202.57 ±32.37**	7.895 ±0.68	1.403 ±2.36****
	c)200 mg/kg	5.223±0.43	57.446 ±2.89	7.575 ±0.89****	91.09 ±2.04****	196.31 ±3.18****	7.165 ±0.87	1.350 ±0.35****
T ₂ (chloroform extract)	a)100 mg/kg	5.073±0.25	55.183 ±3.42	6.468 ±1.152****	91.51 ±0.68****	179.25 ±8.54****	6.443 ±0.27*	1.160 ±4.55****
	b)150 mg/kg	4.510±0.30*	55.183 ±3.96	5.403 ±0.21****	90.41 ±0.12****	176.67 ±10.26****	6.533 ±0.14	1.200 ±0.08****
	c)200 mg/kg	4.333±0.29*	43.376 ±5.46****	5.576 ±0.63****	92.65 ±0.70****	177.23 ±2.58****	7.589 ±0.81	1.119 ±0.37****
T ₃ (acetone extract)	a)100 mg/kg	4.716±0.36	53.833 ±2.37	5.223 ±0.35****	92.28 ±0.85****	174.47 ±4.07****	7.066 ±0.69	1.067 ±0.77****
	b)150 mg/kg	4.423±0.32	46.763 ±1.17**	4.826 ±0.28****	87.99 ±5.61****	163.11 ±2.83****	7.245 ±0.64	0.836 ±0.99****
	c)200 mg/kg	4.382±0.13	41.831 ±0.84****	4.636 ±0.30****	86.60 ±2.10****	160.93 ±3.64****	6.866 ±1.03	0.803 ±1.18****
T ₄ (methanolic extract)	a)100 mg/kg	0.873 ±0.20****	25.777 ±2.30****	2.536 ±1.16****	83.37 ±6.11****	150.07 ±4.64****	6.033 ±0.2*	0.656 ±3.66****
	b)150 mg/kg	0.520 ±0.10****	22.216 ±1.88****	1.986 ±0.79****	75.31 ±2.62****	129.52 ±4.81****	5.440 ±0.41**	0.700 ±0.33****
	c)200 mg/kg	0.282 ±0.21****	20.840 ±0.44****	0.600 ±0.39****	71.85 ±3.82****	118.06 ±2.64****	4.933 ±0.13**	0.226 ±0.87****
T ₅ (aqueous extract)	a)100 mg/kg	1.289 ±0.25****	25.353 ±3.25****	1.880 ±0.88****	76.55 ±2.17****	116.37 ±6.58****	4.066 ±0.13****	0.393 ±0.24****
	b)150 mg/kg	0.818 ±0.24****	23.337 ±6.08****	0.988 ±0.68****	70.25 ±4.03****	110.64 ±5.82****	3.733 ±0.53****	0.276 ±0.35****
	c)200 mg/kg	0.456 ±0.24****	21.956 ±1.05****	0.376 ±0.37****	71.11 ±3.72****	89.18 ±1.68****	2.533 ±0.40****	0.281 ±0.55****

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett's test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

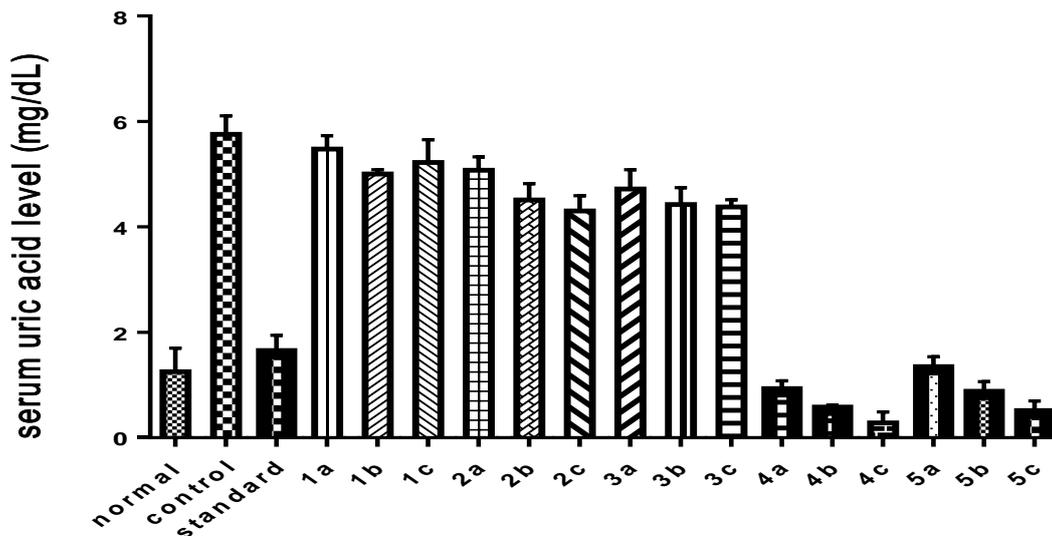


Fig. 1: Graph showing the effect on serum uric acid level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean \pm SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

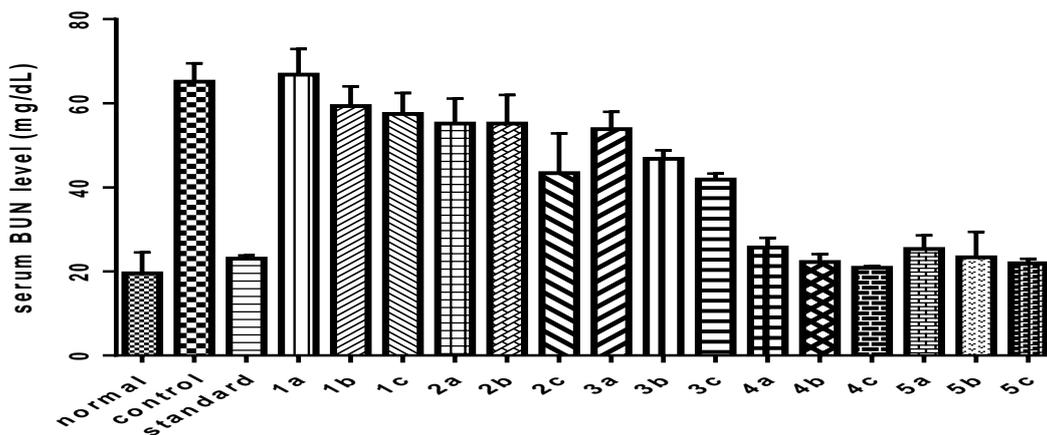


Fig. 2: Graph showing the effect on serum blood urea nitrogen level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean \pm SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

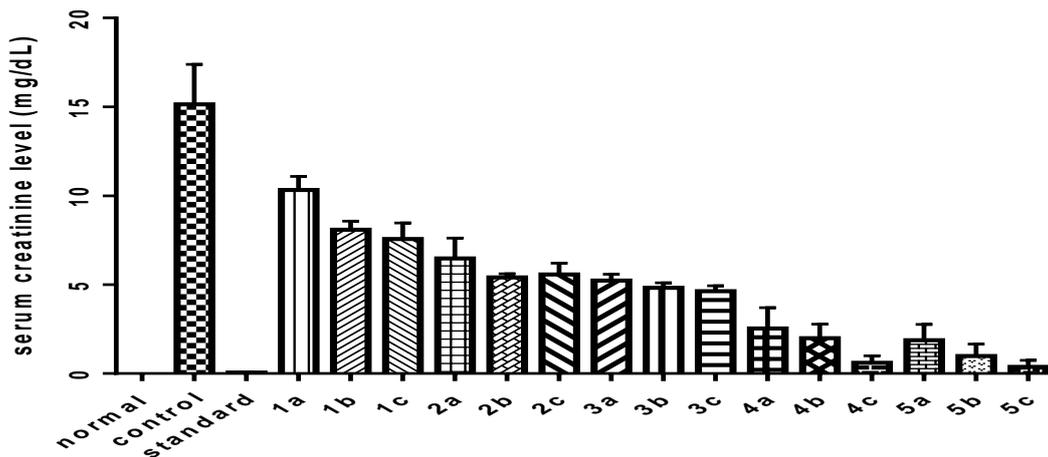


Fig. 3: Graph showing effect on serum creatinine level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

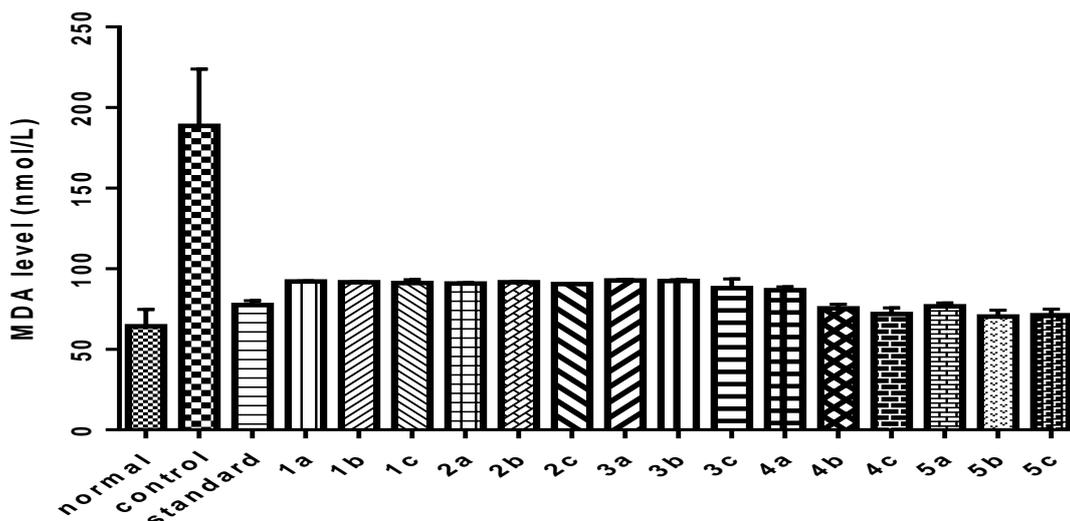


Fig. 4: Graph showing effect on MDA level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

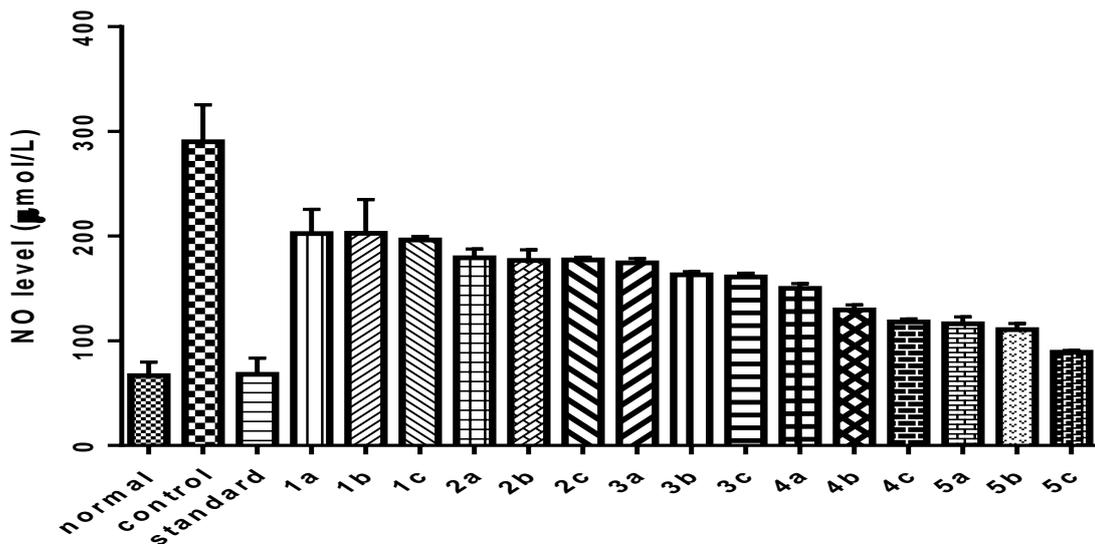


Fig. 5: Graph showing effect on NO level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

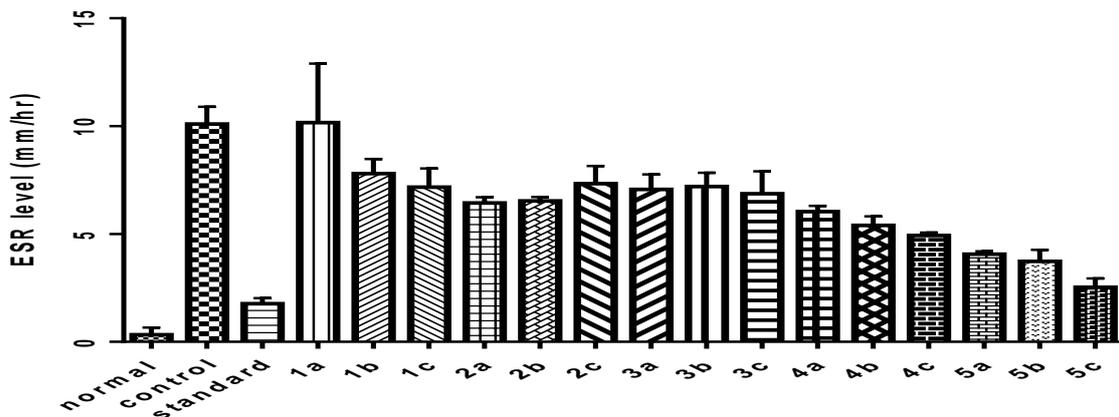


Fig. 6: Graph showing effect on ESR level.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.

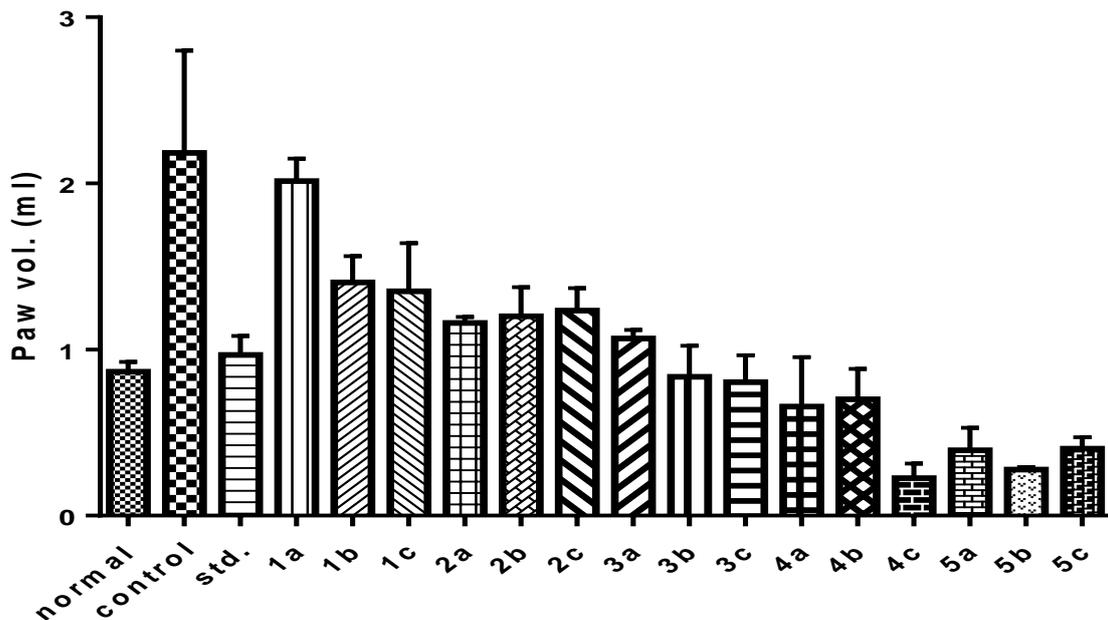


Fig. 7: Graph showing effect on paw volume level in plethysmometer.

Note- The statistical significance of difference between means was calculated by ANNOVA followed by Dunett’s test (post hoc test). Values are expressed as Mean ± SEM, ****P< 0.0001, ***P<0.001, **P<0.05, *P<0.01; n=6 in each group.



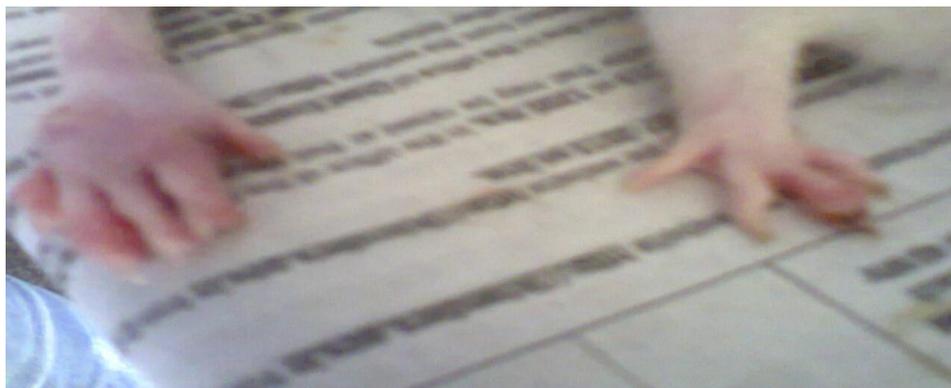


Fig. 8: Image showing rat's paw having inflammatory gout in Metatarsal phalangeal joint.

Gout is a painful inflammatory arthritis which can lead to decrease in quality of life. The disease occurs when body fluids are saturated due to high level of uric acid that eventually settles in the joints.^[14] *Bryophyllum calycinum* leaves are frequently used as an herbal remedy for human disorders including hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint pains, headaches, antifungal and antibacterial and body pains. The leaves are also used for lymphadenitis and ear disease. In the form of poultice and powder they are applied to sloughing ulcer, it is commonly taken as a folk medicine in India to treat renal calculi, which is the result of deposition of urate crystals in kidney, same is the case in gout i.e. deposition of urate crystals in joints. Hence, it was used to check anti gout potential. The phytochemical tests were performed on the petroleum ether extract, chloroform extract, acetone extract, methanolic extract and aqueous extracts and it was found that pet. Ether, chloroform, and acetone extracts contains fatty acids, steroids, organic acids, alkaloids, carbohydrates and methanolic and aqueous extracts contains alkaloids, flavonoids, tannins, sugars and glycosides. The plant *Bryophyllum calycinum*, being a rich source of phytoconstituents, may normalize the serum uric acid level and improve the symptoms of gout.

Xanthine oxidoreductase (XOR) is a widely distributed enzyme which has been extensively studied for more than 100 y because of its abundance in bovine milk which is available on a large scale. Bovine milk XOR is the first studied enzyme.^[15]

The present study was aimed at extraction and evaluation of anti gout potentials of *Bryophyllum calycinum* leaves extract in potassium oxonate induced gout in rats. Actually, potassium oxonate directly interferes in the elimination of uric acid. It is uricase inhibitor; uricase is the enzyme which converts uric acid into more water soluble compound Allantoin. Hence, potassium oxonate is used in Hyperuricemia and anti gout models as it inhibits the activity of uricase enzyme thus retards the conversion of uric acid into Allantoin and favors the accumulation of uric acid in 16 hr. pre fasted animals. First of all acute gout symptoms are seen in metatarsophalangeal joint. Potassium oxonate shows its

peak effect within 2 hr. of injection, so to evaluate the parameters for control group blood samples were withdrawn through retro orbital method after 2 hr. of potassium Oxonate injection.

After statistically evaluating the results for the control group, it was found that serum uric acid level, serum BUN level, serum creatinine level, MDA level, NO level, ESR level and paw volume level was increased beyond the normal ranges hence indicating that Hyperuricemia and gouty condition was successfully achieved in control groups.

In standard group, allopurinol (10mg/kg) as a standard drug was given for 5 days. After evaluating the results, it was found that the serum uric acid level, serum BUN level, serum creatinine level, MDA level, NO level, ESR level and inflammation was statistically significantly reduced as compared to the vehicle control group.

Gouty animals were then given different test extracts, each with different doses that is 100, 150, 200 mg/kg of body weight respectively to evaluate hypouricemic and anti gout activity. Five different test extracts i.e. pet. Ether extracts, chloroform extracts, acetone extracts, methanolic extracts and aqueous extracts were given orally to the gouty animals for 5 days. On the 7th day, blood samples were withdrawn from the animals to evaluate the Hyperuricemia and anti gout potentials in test extracts. It was found that methanolic group (T₄) and aqueous group (T₅) showed significant reduced serum uric acid level, serum BUN level, serum creatinine level, MDA level, NO level, ESR level and inflammation among all the extracts at dose 200 mg/kg of body weight. Moreover, aqueous extract of *Bryophyllum calycinum* shown statistically significant reduction in serum uric acid level, serum BUN level, serum creatinine level, MDA level, NO level, ESR level and inflammation at 200 mg/kg of body weight as compared to methanolic extract.

CONCLUSION

Gout is an inflammatory disease which can be fatal if left untreated, although the body itself undergoes the resolution phase after an initial acute attack but if the recurrent gouty attack persists, post exposure prophylaxis

is necessary. So, to treat this painful inflammatory disease various anti gout drugs are used, among them allopurinol is the first choice drug. But, allopurinol and other drugs used to treat gout like colchicines, indomethacine, etc. Have high toxicity profile along with several adverse effect and incompatibility with other drugs. Hence, moving towards the safe zone in context of health issues, herbal treatment is the best option now-a-days.

After literature review and survey of herbal potentials, it was found that the plant *Bryophyllum calycinum* commonly known as Dardmaar, as the name itself reflects the analgesic action was already proved to have anti inflammatory, analgesic, anti urolithic, anti hypertensive, antidiarrheal, anti ulcer, anti bacterial, anti microbial, anti tumor, anti oxidant, anti diabetic activities and toxicity profile. Considering the pathophysiology of gout, it was found that various inflammatory processes were used in development of gout and uric acid level was found to be elevated in blood and uric acid level was found to be reduced in urine, this implies kidney dysfunction condition and urolithic condition also. Hence, this plant was selected for the anti gout activity as it was previously proved to have anti inflammatory and anti urolithic activity.

After completed the phytochemical screening tests on extracts it was found that pet. Ether, chloroform, and acetone extracts contains fatty acids, steroids, organic acids, alkaloids, carbohydrates and methanolic and aqueous extracts contains alkaloids, flavonoids, tannins, sugars and glycosides. Considering the results after anti gout activity it was found that methanolic extract and aqueous extract showed statistically significant improvement in gouty rats. Hence, it can be concluded that alkaloids and flavonoids present in methanolic and aqueous extracts are responsible for the anti gout activity. Firstly, herbal extracts containing alkaloids show anti inflammatory activity, moreover, colchicines used in gout treatment is also an alkaloid drug. Secondly, flavonoids consist of benzopyran ring in its structure, which may attribute to its xanthine oxidase inhibitory activity. There were several research paper in literature review that studied on the molecular docking studies of allopurinol with xanthine oxidase enzyme exhibited binding interactions and warrants further studies needed for the development of potent xanthine oxidase inhibitors for the treatment of gout. These results clearly indicate that flavonoids especially, Silbinin, Galangin, Apigenin, Baicalin and Chrysin have excellent binding interactions with xanthine oxidase. Hence, it is finally concluded that alkaloids and flavonoids present in methanolic and aqueous extracts are responsible for the anti gout activity.

Further investigations on the isolation and identification of active compounds in the leaves of *Bryophyllum calycinum* can be done to identify the potential chemical entity and possible mechanism of action can be explored

for clinical use in the prevention and treatment of gout and related inflammatory disorders.

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