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RESEARCH ARTICLE

Antihyperuricemic Effects of *Operculina turpethum* Linn (Indian Jalap) Root Extract in Rodent Models

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Abstract

Background: Well-known traditional Indian medication *Operculina turpethum* is used to treat fever, bronchitis, ulcers, haemorrhoids, tumours, obesity, and jaundice. An excessively elevated level of uric acid in the blood is known as hyperuricaemia. The widespread presence of hyperuricemia has been steadily rising globally nowadays. Clinical treatments for hyperuricemia are benzbromarone and probenecid as uricosuric agents and Allopurinol (ALP) and febuxostat as Xanthine Oxidase (XOD) inhibitors. However, only a small number of medications are available due to the poor tolerance of these therapies and their potential for adverse effects, including hepatotoxicity, kidney disorders, gastrointestinal symptoms, and hypersensitivity syndrome. **Aim:** Utilizing both *in vivo* and *in vitro* studies, we examined the Antihyperuricemic potential of *Operculina turpethum* Root Ethanol Extract (OTRE) as well as its underlying mechanisms of action. **Methods:** We investigated the amounts of uric acid in serum as well as the inhibition of xanthine oxidase (XOD) in the liver tissue of a hyperuricemic rat model created by potassium oxonate (PO). In this *in vitro* investigation OTR show strong XOD inhibitory activity when compared with ALP. **Results:** In comparing the IC_{50} values of OTRE-200 was (94.32) and ALP was (1.52). The dose of OTRE-200mg/kg and Allopurinol-Treated (ALT) 10mg/kg groups had significantly lower levels of uric acid (UA) in their urine and blood than the PC group ($p < 0.05$) in PO-induced hyperuricemic rats. Urine UA levels were lower than serum levels in all OTE groups. In rats with PO-induced hyperuricemia, OTRE-200mg/kg reduced serum UA levels and elevated excretion of UA in the urine. **Conclusion:** This study showed that in rats with PO-induced hyperuricemia, OTRE increased UA excretion and decreased serum UA levels. OTRE prevents hyperuricemia by inhibiting the liver's and serum's XO activity *in vivo* and by having antioxidant activity *in vitro*.

Major Findings: This study clarifies that OTRE shows positive benefits and could be beneficial in hyperuricemia and gout.

Keywords: Antihyperuricemic, Ethanolic Extract, *Operculina turpethum* Linn, Xanthine Oxidase

1. Introduction

Hyperuricemia is defined as there is increase in blood UA levels typically greater than 6 mg/dL in women and 7 mg/dL in men[1]. Cardiovascular disease, chronic renal disease, type 2 diabetes mellitus and gout are all markedly elevated in individuals with this condition[2]. The result of purine metabolism is serum UA (SUA)[3]. The other one-third of SUA is generated by high-purine diets, with the remaining two-thirds coming from internal metabolic processes[4]. The kidneys remove between 60 % and 70 % of the UA that is produced by the body; the remainder is expelled via the gut and biliary secretions. After that, gut bacteria continue to degrade it through uricolysis[5]. Among the main causes of hyperuricemia are reduced renal excretion and abnormal UA metabolism[6].

Given that 5 % to 30 % of the general population has a diagnosis of hyperuricemia, the prevalence of the condition appears to be rising globally[7, 8]. Additionally, men are more likely than women to live in industrialized nations[9]. Men and women in India had prevalence rates of hyperuricemia of 14.13 % and 10.53 %, respectively. Alcohol consumption, foods high in purines, and the prevalence of overweight and obesity are all rising

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globally, and they may all be factors in the steady rise in hyperuricemia. Sugar-sweetened beverages are also becoming more popular[10].

Controlling SUA levels requires treating and preventing hyperuricemia because it is brought on by either increased UA production, decreased excretion, or both[11]. The rate-limiting enzyme xanthine oxidase (XOD) oxidizes hypoxanthine to xanthine, which is then transformed into UA[12]. Thus, XOD enzymatic activity can influence SUA production and concentration[13]. As a result, proteins involved in the synthesis and transport of UA by the kidney could be used as effective therapeutic objectives for the control of hyperuricemia.

Benzbromarone and probenecid are uricosuric drugs, and Allopurinol (ALP) and febuxostat are XOD inhibitors that are now utilized in the clinical treatment of hyperuricemia[14]. Nevertheless, these medications have adverse effects that include hepatotoxicity, renal problems, gastrointestinal issues, and hypersensitivity syndrome, and they are poorly tolerated[15]. Consequently, there is a need for safer, more potent treatment drugs for hyperuricemia. Herbal remedies were proposed as novel therapeutic approaches in earlier research to get around the drawbacks of pharmaceuticals for hyperuricemia[16].

Operculina turpethum L. (OT) belongs to the family *Convolvulaceae* and is well known as an indian jalap. While OT was naturalized in the West Indies, but originally from Asia, Africa, and Australia. It can be found in large quantities in India, China, Australia, Sri Lanka, and the Pacific Islands. In earlier research, root and stem extracts of OT have shown antidiabetic, antiulcer, antimicrobial, anticancer and Anti-hemolytic properties[17-21].

Operculina turpethum root was used in this research to evaluate the Xanthin Oxidase (XOD)-inhibitory and antioxidant activities under various solvent extraction conditions (Pet ether, ethanol, and water). The ethanolic extract has shown the maximum % yield, so, ethanolic extract is used for further study. The mechanisms behind the Antihyperuricemic effects of OTR extracts were also extensively studied using a rat model of hyperuricemia induced by Potassium Oxonate (PO).

2. Materials and Methods

2.1 Drugs and Chemicals

We bought potassium dihydrogen phosphate, xanthine, XO, dimethyl sulfoxide (DMSO), and potassium oxonate from Sigma Chemicals Co. Ifars Pharmaceutical Laboratories provided the allopurinol. Sclavo Diagnostic was the source of the UA kit.

2.2 Plant Specimen

Operculina turpethum was collected in August in Malshej Ghat, Junnar, Maharashtra. The taxonomist from the BSI in Pune identified and verified the plant, using a specimen (BSI/WRC/Tech/22) that was placed at BSI Pune.

2.3 Preparation of Extracts

2.4 Continuous Hot Percolation Method

Employing the hot percolation method for 48 hours with a Soxhlet apparatus, the root material of *Operculina turpethum* was extracted with petroleum ether and Ethanol[22]. Under lower pressure, all the solvents were extracted from the collected extract using distillation. The concentrated extract was placed in a vacuum desiccator for later use after being dried in a water bath.

2.5 Maceration

Operculina turpethum root material that had been coarsely pulverized and shade dried was put into an aspirator bottle and extracted using water over the course of seven days, employing the cold maceration method. Following decantation and filtering, distillation over a boiling water bath was used to extract over 80% of the solvent, with the remaining portion being removed under reduced pressure. The extracted material was utilized for additional research after being further dried in a vacuum desiccator.

2.6 Animals

Sixty Sprague-Dawley rats weighing between 150 and 180 g were bought from Lakshmi Bio Farms in Alephata (Junnar, Pune) and assigned at random to several trial cohorts. The animals were placed in a polypropylene cage, with a regulated temperature of 24°C± 1°C and 44-54 percent relative humidity. Animals placed in a room with follow a 12-hour daily light and dark cycle. Unless otherwise indicated, animals were given commercial food pellets and water on a free-choice basis. Before conducting experiments on them, they were at least one week adjusted to the laboratory environment. The Ashvin Rural Ayurvedic College and Hospital (Sangamner, Maharashtra) IAEC authorized

the design of the experiment (Approval No. ACOP/AH/2022/1), and the standards set by the committee were followed in the execution of the study.

2.7 Pharmacological Evaluation

2.7.1 Antioxidative Assays

2.7.1.1 DPPH Radical Scavenging Assay

The DPPH assay was carried out as per a previously published protocol[23]. Ascorbic acid (standard) and OTR extract were dissolved in water to get final concentrations of 10, 100, 200, 400, 800 and 1000mg/ml. When each solution (20 mL) was added, 180 mL of 0.1 mM DPPH dissolved in methanol was present in the 96-well microplate. Absorbance at 517 nm was measured using a microplate reader (Infinited VR M200, Tecan, Switzerland) after 30 min of incubation in the dark at room temperature.

Equation (1) was used to determine the percentage of DPPH activity:

$$DPPH\ scavenging\ activity\ (\ \%) = \frac{(Absorbance\ control\ -\ Absorbance\ sample)}{(Absorbance\ control)} \times 100$$

DPPH radical scavenging activity (%) was plotted against the concentration of ascorbic acid (mg/mL) or plant extract to find the concentration required to reduce DPPH radical scavenging by 50 %. This is known as IC_{50}.

2.7.1.2 ABTS Assay

Modified from a previously published method, the process was followed for the ABTS assay. Preparing the working solution involved mixing equal amounts of the two original solutions and letting them sit at room temperature for 12 to 16 hours in the dark. A stock solution containing 7 mM ABTS and 2.45 mM K_2SO_4 (potassium sulfate) was produced. Using an infinite VR M200 microplate reader (Tecan, Switzerland), the mixture was thinned out by combining 24 mL of methanol and 1 mL of ABTS solution. After that, take the absorbance at 734nm (1.100 ± 0.020 units). With every test, a new ABTS solution was made. 200 milliliters of ABTS radical action solution were combined with 10 milliliters of the sample in 96-well plates. An InfinitedVR M200 microplate reader (Tecan, Switzerland) was employed to calculate absorbance at 734 nm. Three duplicates of each determination were made. The industry standard was Trolox. The Trolox equivalent antioxidant capacity (TEAC)/gram extract is used to express the results.

2.7.2 In Vitro Xo Inhibitory Activity

According to previous study with some modification spectrophotometric assessment of the OTR extract Xanthine Oxidase (XO) inhibitory action was conducted at 290 nm[24]. After that take 0.9 mililiters at 25 °C and a pH of 7.5 in 0.05 M sodium phosphate buffer, 0.1 milliliters of XO enzyme solution and 1 milliliter of OTR extract (100 µg/ml in DMSO) were prepared in cold buffer. 2000 µL of a recently made substrate mixture (xanthine solution 0.15 mM) was added to start a reaction at 25°C after a 15-minute pre-incubation time. Later, mixture underwent a 30-minute incubation stage at 25°C. After that, in the test mixture, add 1 ml of 1N hydrochloric acid to stop the reaction and with the help of UV/Vis spectrophotometer the absorbance at 290 nm was measured. The enzyme solution was replaced with phosphate buffer in the blank, which was prepared in the same way as the absorbance and tested against it. To produce the positive control solution, DMSO was used to dissolve 100µg/ml of allopurinol.

$$XO\ inhibition\ (\ \%) = \{1-\alpha/\beta\} \times 100$$

Where β represents XO›s activity in the presence of OTR extract and α represents XO's activity in the absence of the tested substance (OTR extract). The inhibitory effects of XO were evaluated using OT and allopurinol at various concentrations (1, 2, 3, 4, 5, 10, 25, 50, and 100 µg/ml). The median maximal inhibitory concentration (IC_{50}) was then determined using the dose-response logarithmic curve.

2.7.3 Enzyme Kinetic Analysis of OTR Extract on XOD Inhibition

The mode of XOD inhibition by OTR extract was identified using Lineweaver-Burk plot analysis[25]. Using xanthine as a substrate at varying concentrations (15, 30, 60, and 120 mm), 3 conc. of OTR extracts (125, 250, and 500 mg/ml) were evaluated. The procedure outlined above was followed to carry out the enzyme reaction. Analyzing observed data using non-least squares regression, the plant extract's inhibitory constant (Ki)

for XOD inhibition was discovered. Utilizing Microsoft Excel 2010 and the Solver Add-in, the equation is as follows:

$$V = \frac{V\max.S}{Km + S(1 + I / Ki)}$$

where Km is the Michaelis constant expressed in millimeters (mm), S are the substrate and I is inhibitor concentrations expressed in milliliters per milliliter. v is initial and Vmax are the maximum velocities of the uric acid formation, expressed in milliliters per minute. A non-linear optimization was done using the Generalized Reduced Gradient (GRG) algorithm from the Solver add-in in Microsoft Excel 2013.

2.7.4 In Vivo Antihyperuricemic Activity

Potassium oxonate was administered as a uricase inhibitor to create a hyperuricemic rat model. With a small adjustment to the potassium oxonate dosage, the experiment was carried out in accordance with Liu's method[26]. Rats were randomly assigned to four groups in that 1st group is normal control, 2nd is hyperuricemic control, 3rd is standard (allopurinol), and 4th is test sample (n=6). Using 0.3 % CMC-Na, suspension dosage forms of allopurinol and OTR extract were prepared. Rats were given oral doses of 100 and 200 mg of OTR extract per kg of body weight (bw). The same protocol was followed to administer 10 mg/kg bw of allopurinol as a standard. The suspension's volume was given according to the rat's body weight. One day prior to their use in the experiment, the rats were fasted. The animals were denied water for one hour before the medication was given.

At least one hour prior to the potassium oxonate-induced hyperuricemia experiment, the animals were brought into the lab. Prior to the administration of potassium oxonate, blood was drawn at 0 h to measure the serum UA level. In short, rats were given intraperitoneal injections of PO (250 mg/kg) one hour prior to the drug administration in order to elevate the serum UA level, with the exception of the normal control group. To measure the UA level on the first, second and third hours following drug administration, blood samples from rats were drawn by tail vein (0.1-1ml) bleeding during the period of one, two and three hours[27]. Allow the blood to coagulate for 30 minutes at normal temperature, the serum was extracted by centrifuging the mixture for seven minutes at 10,000 × g. Before the UA level was measured, the serum was kept in storage at -20°C. Using a standard diagnostic kit and an enzymatic-colorimetric approach, the serum UA level was determined in compliance with the manufacturer's recommendations.

2.7.5 Effects of Otr Extract on Xod in Liver Homogenates

The amount of residual XOD activity in the removed livers is determined by measuring the concentration of UA generated from xanthine. To summarize, the livers were homogenized in five volumes of cold 80 mM sodium pyrophosphate buffer, pH 7 point 4, and then centrifuged at 3000 g for 10 min at 4°C. The leftover material was centrifuged once more at 10,000 g for 60 minutes at 40 °C after the lipid layer was removed. The obtained supernatants were then used to measure the residual XOD activity. A 100 ml portion of each liver homogenate was mixed with 800 ml of 80 mm sodium pyrophosphate buffer (pH 7.4), and the mixture was left to incubate at 25°C for 10 minutes. After that, 500 mL of the 120 mm xanthine solution was added, shaken, and left to incubate for 30 minutes. 100 mL of 1N HCL was added to stop the reaction after 0 and 30 minutes. HPLC was used to measure the uric acid concentrations in the samples that were collected after a 10-minute centrifugation at 3000 g. The total protein content of the liver homogenate was determined using the Bradford Protein Assay Reagent (Wako Pure Chemicals, Osaka, Japan). The quantified residual XOD activity was defined as the amount of uric acid produced per minute per milligram of protein.

2.8 Statistical Analysis

The outcomes are displayed as Mean ± Standard error mean.

One-way analysis of variance (ANOVA) and Dunnett's test were used to analyze the extract, standard and control. P values less than 0.05 were deemed statistically significant.

3. Results

3.1 Etraction Yield

Comparing the yield of OTREs by solvent, the ethanol extracts yielded 19:20 percent, which was higher than the yields of water extract (15:60 percent) and pet

ether extract (2:00 percent). Only the ethanol extract is pharmacologically evaluated because the ethanol yield of the OTR extracts is higher Table 1.

3.2 Antioxidant Activity of OTRE

Anthocyanins, polyphenols, ellagitannins, and carotenoids are examples of phytochemicals that have antioxidant activity. These phytochemicals are also useful in preventing long-term illnesses. These phytochemicals have demonstrated their antioxidant capacity in human studies conducted both *in vitro* and *in vivo*. Because OT extract contains secondary metabolites such as phenols and flavonoids, it is being studied for antioxidative effects.

3.2.1 DPPH Scavenging Activity

Table 1. Solvent extractive values percent (W/W) of *Operculina turpethum* root

Name of extract	Method of extraction	Color	Consistency	Percentage yield
Pet ether extract	Soxhlet	Light yellow brown	Slight sticky	2.00w/w
Ethanol extract		Brown	Sticky	19.20w/w
Water extract	Maceration	Greenish brown	Sticky	15.60w/w

Table 2. Effect of *O. turpethum* extract on DPPH free radical Scavenging activity

Conc (µg/ml)	Ethanol extract % inhibition	Ascorbic acid % inhibition
10	28.12±0.54	45.12±0.52
50	46.58±0.45	55.65±0.71
100	59.12±0.89	68.36±0.65
200	65.42±0.68	76.18±0.55
300	72.78±0.38	84.89±0.32
500	81.20±0.51	90.26±0.26
IC_{50} (µg/ml)	50.42±0.42	20.93±0.48

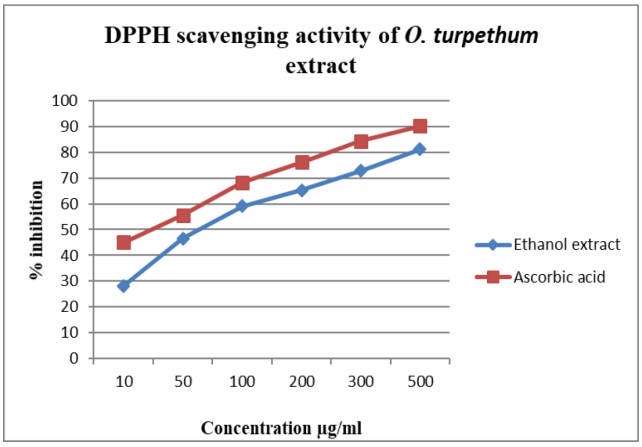


Figure 1. Effect of *O. turpethum* extract on DPPH free radical scavenging activity.

3.2.2 Nitric Oxide (NOS) Scavenging Activity

Table 3. NOS Scavenging activity of *O. turpethum* extract

Conc (µg/ml)	Ethanol extract % inhibition	Ascorbic acid % inhibition
10	20.04±0.62	35.78±0.84
50	29.36±0.45	43.18±0.37
100	42.39±0.36	52.64±0.57
200	50.04±0.48	60.22±0.75
300	61.28±0.72	71.32±0.69
500	67.12±0.49	79.22±0.48
IC_{50} (µg/ml)	155.10±0.53	102.90±0.59

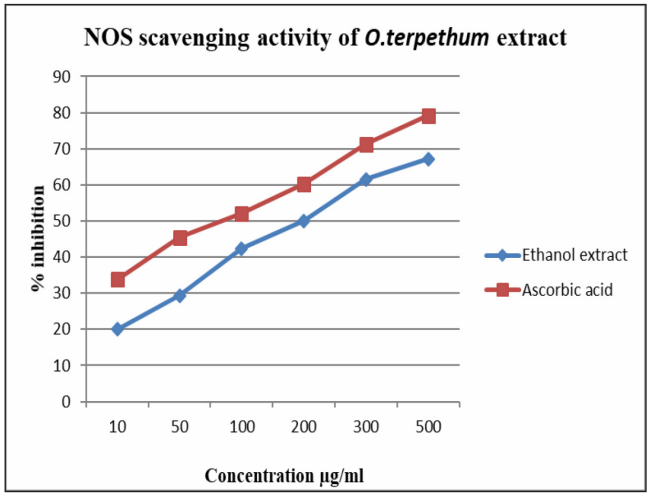


Figure 2. NOS scavenging activity of *O. turpethum* extract.

For both assays, the percentage of inhibition in the turpethum extract was demonstrated in relation to ascorbic acid and was observed to progressively increase with concentration Figures 1 and 2. Ascorbic acid IC_{50} values in the DPPH and NOS assay was discovered to be 20.93 µg/ml and 102.90 µg/ml each. The OTR extract significantly inhibited the DPPH radical in the DPPH assay (IC_{50} Value: 50.42±0.42 µg/ml) Table 2, and in the NOS assay (IC_{50} Value: 155.10+ 0.53 µg/ml)

Table 3, the extract demonstrated the highest radical scavenging activity. This suggests that for assessing OTR antioxidant potential, DPPH is a more effective assay than NOS.

3.3 Effect of *O. turpethum* Extracts on *In Vitro* (XOD) Activity

Allopurinol and OTR extract inhibit xanthine oxidase at different doses as shown in Table 4. For every 100 µg/ml, there was a greater than 50 % inhibition of XO. OTR suppressed XO by 68 % at a dosage of 100 µg/ml, while allopurinol, suppressed it by 92 %. Additionally, the XO inhibitory activity of allopurinol and OTR extract was expressed as an IC_{50}, or the amount of the standard drug or sample that was tested that was required to inhibit xanthine oxidase activity by 50 % in the same experimental conditions.

By using GraphPad Prism to calculate the IC_{50} values based on the logarithmic dose-response curve, the value corresponded to 94.32 µg/ml for OTR extract and 1.52 µg/ml for allopurinol Figure 3.

3.4. XOD Inhibition *In Vitro* of *O. turpethum* Extract by Lineweaver–Burk Analysis

Analysis of the *in vitro* enzyme kinetic data using the Lineweaver-Burk method revealed the existence of OT decreased the Vmax of xanthine oxidase relative to the control with normal alteration in the Km of xanthine Fig.4. This implies a standard example of noncompetitive, reversible inhibition of an enzyme reaction. Ki is measured at 57.69µg/ml for the OT extract.

Table 4. At different concentrations *O. turpethum* and allopurinol, Xanthine oxidase inhibition

Concentration (µg/ml)	XO inhibitory activity (%)	
	Allopurinol	*O. turpethum* extract
1	42±1.4	8±1.2
2	52±1.2	22±0.8
3	58±0.4	31±1.1
4	61±0.6	38±0.2
5	68±1.8	43±0.3
10	75±2.2	49±1.4
25	81±1.2	52±1.8
50	86±0.4	56±2.2
100	92±1.6	68±2.2

3.5 *In Vivo* Antihyperuricemic Activity

Using potassium oxonate, the Antihyperuricemic effects of *O. turpethum* extract were demonstrated in hyperuricemic rats Table 5. A uricase inhibitor that can cause hyperuricemia in rats is potassium oxonate. The potassium oxonate-induced rat is a valuable animal model for hyperuricemia that may be used to assess samples that impact UA levels and to assess potential treatment options for specific illnesses linked to aberrant UA levels. Rats treated with potassium oxonate develop hyperuricemia, as seen by the sharp rise in UA levels when compared to normal rats.

Prior to potassium oxonate therapy, at 0 h, the UA levels in all treatment groups were identical to those rats of the normal control group. Every rat given the vehicle-only to the normal control group (CMC-Na) displayed the same serum UA level. In hyperuricemia rats, the allopurinol 10mg/kg given by orally as the standard resulted in a significant drop in the UA level between the first- and third-hours following therapy ($p<0.05$). Three hours after oral treatment, the UA level of the allopurinol group did not differ from that of the usual control group.

According to these findings, allopurinol treatment resulted in a full recovery and was able to bring the serum UA level down to normal. The hyperuricemic group's profile UA level increased even after the sample was administered, but the extract group's serum UA level differed substantially from the hyperuricemic group at the first and second hours ($p<0.05$), and

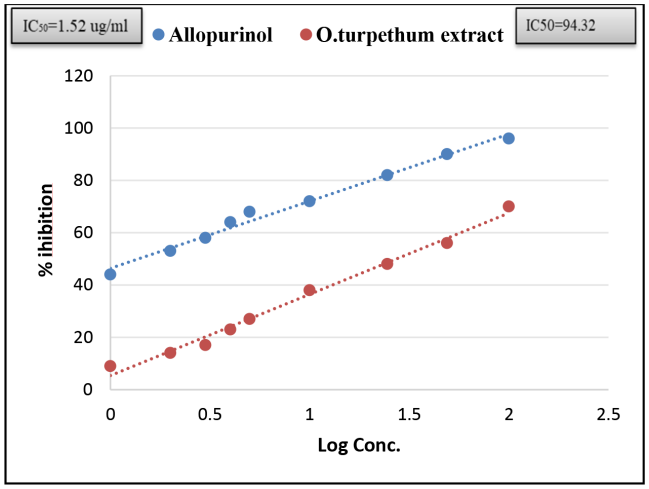


Figure 3. IC_{50} values and xanthine oxidase inhibition of allopurinol and *O. turpethum* extract.

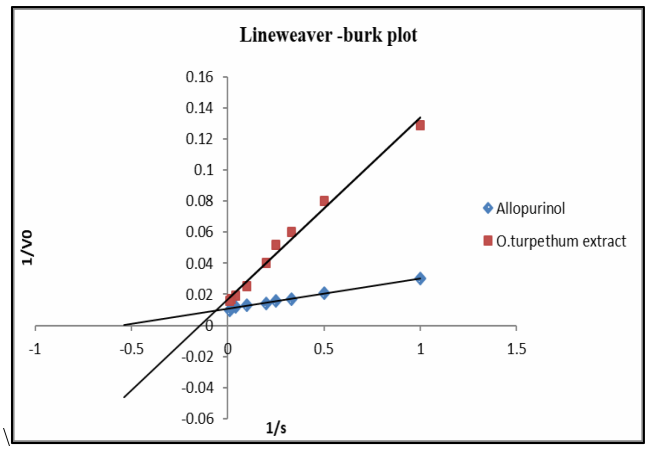


Figure 4. Xanthine oxidase inhibition by *O. turpethum* extract.

Comparing the allopurinol group ($p<0.1$, 0.05, 0.01) to the hyperuricemic control (p, q, r) and the hyperuricemic control ($p<0.1$, 0.05, 0.01) to the normal control (a, b, c).

3.6. Effects of OTR Extract on XOD in Liver Homogenates

Allopurinol at the dose of 10 mg/kg and OTR extract 200 mg/kg significantly suppress XOD activity, reducing the production of UA in liver homogenates by 93.10 % and 71.26 %, respectively Table 6. Results display the significant differences ($p < 0.01$ and $p < 0.05$) compared with the untreated hyperuricemic group. OTR extract at 100 mg/kg decreased the

Table 5. Effect of *O. turpethum* extract on sUA level

Group	Dose (mg/kg)	Serum uric acid level (mg/dl)			
		0h	1h	2h	3h
Normal	-	2.42±0.33	2.22±0.35ry	2.15±0.25r	2.27±0.39
Hyperuricemic control	-	2.34±0.22	3.66±0.26cz	3.90±0.38cz	3.15±0.25cz
Allopurinol	10	2.26±0.34	1.62±0.40br	1.75±0.18r	2.01±0.53r
O. turpethum extract	100	2.50±0.36	2.59±0.19rz	3.10±0.28crz	3.05±0.28a
O. turpethum extract	200	2.32±0.20	2.43±0.16rz	3.11±0.23crz	3.01±0.65

The values for six rats are given as Mean±SEM.

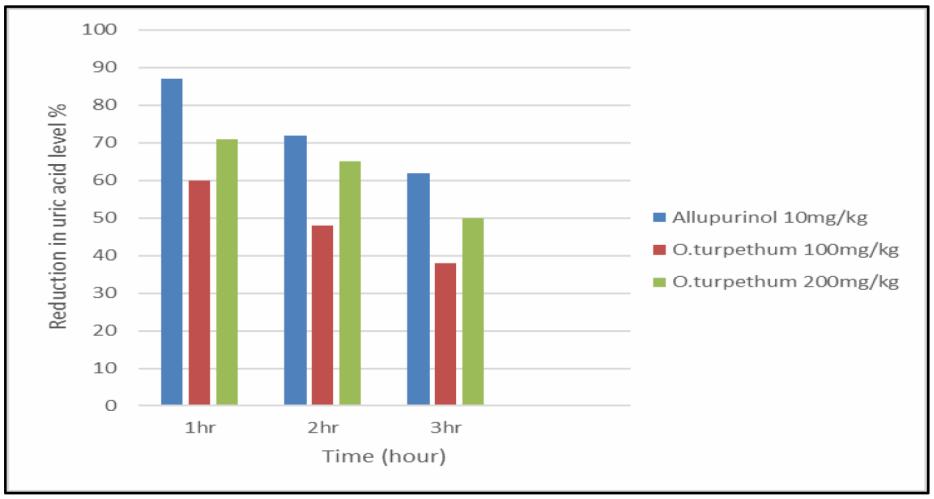


Figure 5. Effect of *O. turpethum* extracts on serum uric acid level.

only the first hour indicated no difference from the normal control. Compared to allopurinol (86 %), the *O. turpethum* extract 200 mg/kg showed potential Antihyperuricemic activity (70 %), although it took longer for the condition to recover to normal Figure 5.

activity of xanthine oxidase in liver homogenates, resulting in a 48.27 % decrease in uric acid synthesis. Nevertheless, there was no discernible difference between these outcomes and the hyperuricemic group's untreated results.

Table 6. Rat liver treated with *O. turpethum* extract in residual Xanthine Oxidase Activity (XOD)

Groups	Dose (Mg/kg)	XOD activity (nanomole/min/mg protein)	(%) Inhibition
Normal	-	0.94±0.07	-
Hyperuricemic control	-	1.74±0.15	-
Allopurinol	10	0.12±0.001**	93.10
O. turpethum extract	100	1.05±0.20	48.27
O. turpethum extract	200	0.50±0.12*	71.26

Six rat per group provided the mean ± SEM for the data. *p < 0.05 when compared to the hyperuricemic group that did not receive treatment. *p < 0.01 in comparison to the group of people with untreated hyperuricemia.

4. Discussion

Hyperuricemia is a condition marked by an excess of urate in the bloodstream consequently of an unbalance in the production and excretion of urate. Globally, the prevalence of hyperuricemia has been steadily rising recently[28]. The treatment of hyperuricemia is currently confined to a few medications, many of which have undesirable side effects. Since 2003, benzbromarone has not been used as a uricosuric agent due to significant hepatotoxicity, which has been observed in most European nations[29]. Despite being primarily used to treat hyperuricemia, ALP, an XO inhibitor, has been linked to serious cutaneous adverse effects[30]. As a result, numerous earlier research projects have been carried out to create safe, side-effect-free therapeutic medicines for hyperuricemia utilizing natural ingredients[31].

This study demonstrated OTRE's Antihyperuricemic, XOD-inhibitory, and antioxidant properties. A higher level of XOD expression and activity results in a higher production of Reactive Oxygen Species (ROS). XOD activity rapidly reacts with nitric oxide to generate superoxide anion, which can form cytotoxic oxidants connected to a variety of diseases, including endothelial dysfunction and cardiovascular disease[32]. Increased localized ROS levels lead to significant cellular damage and the production of additional, more reactive radicals[33]. When oxidative stress is present, XO activity outpaces XDH activity in terms of ROS production, leading to further ROS production. NOX keeps XO levels in check in endothelial cell cultures, whereas XO is the cause of elevated ROS generation[34].

When compared to NOS, OTRE had the greatest XOD-inhibitory activity by DPPH in this investigation. OT extract had the most radical scavenging action in both the NOS and DPPH assays, with an IC50 value of 174.12 + 0.53 g/ml and 20.42 + 0.58 g/ml, respectively. These results imply that OTRE prevents hyperuricemia by blocking ROS production via its XOD-inhibitory action.

In vitro, the XOD (xanthine oxidase) inhibitor activity of the OTE extract was 68 % of that of allopurinol (95 %) and the IC_{50} value of the extract was 94.32 **µg**/ml compared to allopurinol is 1.52 **µg**/ml.

A possible mechanism is that product may reduce blood uric acid levels by inhibiting the action of xanthine oxide enzyme, which inhibits uric acid synthesis. The OTR extract inhibited xanthine oxidase activity *in vitro* significantly. Many bioactive elements, such as polyphenols, have been found in OTR extract[35]. Previous research has shown that these compounds have a high inhibitory action against xanthine oxidase. OTR extract has contains various chemical constituents like Stigmasterol and lupeol. The stigmasterol fundamental mechanism is that they can reduce IL-17, TNF-α and IL-1 levels in hyperuricemic rats by reducing liver XOD activity. Lupeol is primarily responsible for the extract's urate-lowering action[36].

We investigated the potential Antihyperuricemic efficacy of OTR extract in potassium oxonate induced hyperuricemia in rats. OTRE at 200mg/kg dramatically reduced sUA levels when compared to the positive control group ($p < 0.05$). According to these results, OTRE-200 may reduce hyperuricemia by increasing urinary sUA excretion and decreasing blood sUA levels. Uric acid formation in liver homogenate of OTRE-200 was significantly reduced by residual XOD activity, which suppressed XOD activity by 76.0% as compared to allopurinol 90.3%.

5. Conclusion

This study showed that in rats with PO-induced hyperuricemia, OTRE increased UA excretion

and decreased serum UA levels. OTRE prevents hyperuricemia by inhibiting the liver's and serum's XO activity *in vivo* and by having antioxidant activity *in vitro*. This study clarifies that OTRE shows positive benefits and could be beneficial in the hyperuricemia and gout management.

6. Author Contribution

The author, Dhananjay Babanrao Deshmukh, conducted the experiments, performed statistical analysis, and produced the report. Dr. Snigdha Das Mandal provided critical feedback and edited the manuscript. Both writers contributed to the interpretation of the data. All authors have read and approved the published version of the manuscript.

7. References

1. Kim SK. Interrelationship of uric acid, gout, and metabolic syndrome: focus on hypertension, cardiovascular disease, and insulin resistance. J Rheum Dis. 2018; 25(1):19-27. https://doi.org/10.4078/jrd.2018.25.1.19
2. Ni Q, Lu X, Chen C, Du H, Zhang R. Risk factors for the development of hyperuricemia: a STROBE-compliant cross-sectional and longitudinal study. Medicine. 2019; 98(42):e17597. https://doi.org/10.1097/MD.0000000000017597
3. Glantzounis G, Tsimoyiannis E, Kappas A, Galaris D. Uric acid and oxidative stress. Curr Pharm Des. 20051390: 25-32. https://doi.org/10.2174/138161205774913255
4. Perez ES, Medina MAG, Lomeli ML, *et al.* Association between serum uric acid and metabolic syndrome components in prepubertal obese children (Tanner stage I) from Nuevo León, Mexico—a preliminary study. BMC Obes. 2017; 4:25. https://doi.org/10.1186/s40608-017-0160-6
5. Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis. 2012; 19(6):358-71. https://doi.org/10.1053/j.ackd.2012.07.009
6. Stewart DJ, Langlois V, Noone D. Hyperuricemia and hypertension: links and risks. Integr Blood Press Control. 2019; 12:43-62. https://doi.org/10.2147/IBPC.S184685
7. Cicero AFG, Rosticci M, Bove M, *et al.* Serum uric acid change and modification of blood pressure and fasting plasma glucose in an overall healthy population sample: data from the Brisighella heart study. Ann Med. 2017; 49(4):275-82. https://doi.org/10.1080/07853890.2016.1222451
8. Katova T, Simova I, Yotov Y, Peeva K, Georgieva N. Hyperuricemia and cardiovascular risk in the Bulgarian population. Cureus. 2020; 12(1):e101.
9. Song P, Wang H, Xia W, Chang X, Wang M, An L. Prevalence and correlates of hyperuricemia in the middle-aged and older adults in China. Sci Rep. 2018; 8:4314. https://doi.org/10.1038/s41598-018-22570-9
10. Desideri G, Puig JG, Richette P. The management of hyperuricemia with urate deposition. Curr Med Res Opin. 2015; 31(Suppl 2): 27-32. https://doi.org/10.1185/03007995.2015.1087982
11. Lee YS, Son E, Kim SH, Lee YM, Kim OS, Kim DS. Synergistic uric acid-lowering effects of the combination of *Chrysanthemum indicum* Linné flower and *Cinnamomum cassia* (L.) J. Persl bark extracts. Evid Based Complement Alternat Med. 2017; 9764843. https://doi.org/10.1155/2017/9764843
12. Zhang Y, Jin L, Liu J, *et al.* Effect and mechanism of dioscin from *Dioscorea spongiosa* on uric acid excretion in animal model of hyperuricemia. J Ethnopharmacol. 2018; 214:29-36. https://doi.org/10.1016/j.jep.2017.12.004
13. Kang LL, Zhang DM, Ma CH, *et al.* Cinnamaldehyde and allopurinol reduce fructose-induced cardiac inflammation and fibrosis by attenuating CD36-mediated TLR4/6-IRAK4/1 signaling to suppress NLRP3 inflammasome activation. Sci Rep. 2016; 6:27460. https://doi.org/10.1038/srep27460
14. Oh DR, Kim JR, Choi CY, *et al.* Effects of ChondroT on potassium oxonate-induced hyperuricemic mice: downregulation of xanthine oxidase and urate transporter 1. BMC Complement Altern Med. 2019; 19(1):10. https://doi.org/10.1186/s12906-018-2415-2
15. Chen RJ, Chen MH, Chen YL, *et al.* Evaluating the urate-lowering effects of different microbial fermented extracts in hyperuricemic models accompanied with a safety study. J Food Drug Anal. 2017; 25(3):597-606. https://doi.org/10.1016/j.jfda.2016.07.003
16. Bao R, Liu M, Wang D, *et al.* Effect of *Eurycoma longifolia* stem extract on uric acid excretion in hyperuricemia mice. Front Pharmacol. 2019; 10:1464. https://doi.org/10.3389/fphar.2019.01464
17. Pulipaka S, Challa SR, Pingili RB. Comparative antidiabetic activity of methanolic extract of *Operculina turpethum* stem and root against healthy and streptozotocin-induced diabetic rats. Int Curr Pharm J. 2012; 1(9):272-8. https://doi.org/10.3329/icpj.v1i9.11618
18. Ignatius V, Narayanan M, Subramanian V, *et al.* Antiulcer activity of indigenous plant *Operculina turpethum* Linn. Evid Based Complement Alternat Med. 2013; 2013:272134. https://doi.org/10.1155/2013/272134
19. Kiran B, P N, D J B C. In-vitro evaluation antibacterial potentiality of aqueous extract of *Operculina turpethum* Silva (root). Eur J Pharm Med Res. 2017; 4(1):261-3.

20. Pourfraidon Z, Sharma C. Biological activity of prominent anti-cancer plants using brine shrimp lethality test. J Microb World. 2009; 2(3):201-4.
21. Sharma V, Singh M. *In vitro* antiarthritic and hemolysis preventive: membrane stabilizing efficacy of ethanolic root extract of *Operculina turpethum*. World J Pharm Pharm Sci. 2013; 2(4):302-12.
22. Harborne JB. Phytochemical methods—a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; 1973; 49-188.
23. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compos Anal. 2006; 19:669-75. https://doi.org/10.1016/j.jfca.2006.01.003
24. Yumita A, Suganda AG, Sukandar EY. Xanthine oxidase inhibitory activity of some Indonesian medicinal plants and active fraction of selected plants. Int J Pharm Pharm Sci. 2013; 5:293-6.
25. Sato VH, Sungthong B, Rinthong PO, Nuamnaichati N, Mangmool S, Chewchida S, Sato H. Pharmacological effects of Chatuphalatika in hyperuricemia of gout. Pharm Biol. 2018; 56(1):76-85. https://doi.org/10.1080/13880209.2017.1421235
26. Liu X, Chen R, Shang Y, Jiao B, Huang C. Lithospermic acid as a novel xanthine oxidase inhibitor has anti-inflammatory and hypouricemic effects in rats. Chem Biol Interact. 2008; 176(2-3):137-42. https://doi.org/10.1016/j.cbi.2008.07.003
27. Sunarni T, Leviana F, Fidrianny I, Wirasutisna KR. Antihyperuricemic and xanthine oxidase inhibitory activities of fractions from ethanolic leaves extract of *Stelechocarpus burahol*. Asian J Pharm Clin Res. 2016; 9(6):255-8. https://doi.org/10.22159/ajpcr.2016.v9i6.14314
28. Zitt E, Fischer A, Lhotta K, Concin H, Nagel G. Sex- and age-specific variations, temporal trends and metabolic determinants of serum uric acid concentrations in a large population-based Austrian cohort. Sci Rep. 2020; 10(1):7578. https://doi.org/10.1038/s41598-020-64587-z
29. Jansen TL, Reinders MK, Van Roon EN, Brouwers JR. Benzbromarone withdrawn from the European market: another case of "absence of evidence is evidence of absence"? Clin Exp Rheumatol. 2004; 22(5):651.
30. Ramasamy SN, Korb-Wells CS, Kannangara DR, *et al*. Allopurinol hypersensitivity: a systematic review of all published cases 1950-2012. Drug Saf. 2013; 36(10):953-80. https://doi.org/10.1007/s40264-013-0084-0
31. Arimboor R, Rangan M, Aravind SG, Arumughan C. Tetrahydroamentoflavone (THA) from *Semecarpus anacardium* as a potent inhibitor of xanthine oxidase. J Ethnopharmacol. 2011; 133(3):1117-20. https://doi.org/10.1016/j.jep.2010.10.027
32. Gliozzi M, Malara N, Muscoli S, Mollace V. The treatment of hyperuricemia. Int J Cardiol. 2016; 213:23-7. https://doi.org/10.1016/j.ijcard.2015.08.087
33. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. J Physiol. 2004; 555(Pt 3):589-606. https://doi.org/10.1113/jphysiol.2003.055913
34. Liu N, Xu H, Sun Q, *et al*. The role of oxidative stress in hyperuricemia and xanthine oxidoreductase (XOR) inhibitors. Oxid Med Cell Longev. 2021; 2021:1470380. https://doi.org/10.1155/2021/1470380
35. Ahmad T, Husain MK. A review on *Operculina turpethum*: a potent herb of Unani system of medicine. J Pharmacogn Phytochem. 2017; 6(1):23-6.
36. Oliviero F, Scanu A, Luisetto R, Ramonda R, Spinella P, Sfriso P, Galozzi P. Anti-inflammatory and hypouricemic effect of bioactive compounds: molecular evidence and potential application in the management of gout. Curr Issues Mol Biol. 2022; 44:5173-90. https://doi.org/10.3390/cimb44110352