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Preclinical evaluation of herbal plant in potassium oxonate-induced hyperuricemia

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Objective: The present study was undertaken to investigate the anti-gouty arthritic activity of *Adhatoda vasica* Nees aqueous leaves extract (AVLE) using potassium oxonate-induced hyperuricemia in mice.

Material and methods: Potassium oxonate (PO) induced hyperuricemia in mice were determined by in vivo experiments. PO causes hyperuricemia in one hour after i.p. administration. Thus mice were administered PO followed by AVLE and standard drug treatment of allopurinol study conducted for 7 days finally measuring the serum levels of uric acid, xanthine oxidase, and xanthine dehydrogenase etc.

Results: The evaluation of anti-gouty arthritic potential by oral administration of AVLE (100–400 mg/kg) evoked a significantly decrease in the serum levels of uric acid, xanthine oxidase (XOD), xanthine dehydrogenase (XDH) in treated mice. Finally, the study concluded that in AVLE the flavonoid constituents impart enzyme inhibitory activity thus reducing levels of xanthine oxidase, xanthine dehydrogenase, and serum uric acid.

Conclusions: The results obtained in this study indicate that AVLE possesses potential anti-gouty arthritic activity.

Keywords: XOD-Xanthine oxidase, XDH-Xanthine dehydrogenase, PO-Potassium oxonate.

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INTRODUCTION

Adhatoda vasica Nees (family Acanthaceae) is a shrub 1–2.5 m high with opposite ascending branches. The leaves are simple, opposite, 7–19 cm long and 4–7 cm wide. The flowers are white, pink or purple. The plant grows throughout the Indian peninsula up to an altitude of 1300m. The names *Adhatoda zeylanica* Medic and *Justicia adhatoda* Linn are used synonymously. It is commonly known as Basak (Bengali); Aradusi, adusa (Gujrati); Arusa, baansa, adulsa (Hindi); Bansa, basuti, bhekkar (Punjabi), and shwetavasa, vasa, vasaka (Sanskrit) and Malabar nut (English) in different languages and regions of India (Anjaria and Bhatt, Glossary 1995). The plant has been used in the indigenous system of medicine in India for over 2000 years. It is a well-known drug in Ayurvedic and Unani medicine for the treatment of various ailments of respiratory tract in both children and adults (Manjunath, 1948). The leaves were used for stomach catarrh with constipation, gout, urinary stone (Madaus, 1938) and warmed leaves used externally for rheumatic pains and

dislocation of joint (Rao and Jamir, 1982). All the parts of the plant have been used for their therapeutic beneficiary effect from ancient times (Atal, 1980). Antioxidant and radical scavenging activity and anti-inflammatory effect (Srinivasarao et al., 2006). The plant is used as an ingredient of numerous popular formulations including cough syrups used in combination with ginger and *tulsi* where it exerts its action as an expectorant and antispasmodic (Atal, 1980). Bisolvon, a branded drug containing *Vasaka* as an ingredient is used to clear the airways by decreasing the mucus secretions and opening the air passages. There are various herbal formulations, viz. Kada, Fermiforte, Spirote available for the treatment of various kinds of respiratory disorders (Iyengar et al., 1994; Shete, 1993).

Beneficial effect of food in treatment of disease is due to the presence of biologically-active ingredients with new components of beneficial effects towards diseases. Gout is characterized by hyperuricaemia and recurring attacks of arthritis, associated with metabolic disorder

of purine metabolism and in later causes chronic arthritis, formation and renal failure (Golding, 1989).

It is a chronic metabolic disorder characterized by the deposition of monosodium urate crystals in joints and other tissues. These crystals may lead to an acute inflammatory response and can make an everlasting tissue damage which is characterized by the arrival of ulceration of the joint cartilage, marginal osteophytosis, geodic and erosive lesions and prolonged inflammation of synovial membrane (Dalbeth & Haskard, 2005; Corrado et al., 2006). Raised oxidative stress has been reported in patients suffering from gout (Urano et al., 2002). Prevention of purine-rich foods is significant for gout controlling (Beneke, 2003). The utmost important approach in the treatment of hyperuricemia is the inhibition of xanthine oxidase, which are effective in decreasing urinary and plasma urate levels and reverses the development of tophaceous deposits (Nuki & Simkin, 2006). So food components which inhibit xanthine oxidase activity may decrease the formation of uric acid and alleviate inflammation. This is because of xanthine oxidase is a crucial enzyme playing an important role in hyperuricemia, catalyzing the oxidation of hypoxanthine to xanthine and then to uric acid (Unno et al., 2004). Also inhibiting the renal urate reabsorption and oxidative stress has an important effect in gout controlling.

However, its anti-gouty arthritic potential has not been scientifically explored. Disturbances in this metabolic system are associated with several disease conditions. And treatment of hyperuricemia and gout is based on the experience of traditional medicine systems (Theoduloz et al., 1988; Chiang et al., 1994; Guerrero and Guzman, 1998; Owen and Johns, 1999), their uses in modern medicine suffer from the lack of scientific evidence. Attention has been focused on identifying their phytochemicals, which possess the ability to inhibit XDH/XO activities and thereby reduce the urate levels. Flavonoids have been shown to be inhibitors of the activity of XO in vitro study (Nagao et al., 1999).

Thus, there are several preclinical studies that can be employed for the evaluation of the anti-gouty arthritic potential of a compound. Therefore, in the present study, an attempt has been made to evaluate *Adhatoda vasica* aqueous leaves extract (AVLE) for its Anti-gouty arthritic activity.

MATERIALS AND METHODS

Drug material

Potassium oxonate was procured from Sigma-Aldrich, USA.

Chemicals and drugs

Allopurinol tablets procured from Zyloric® GlaxoSmithKline Pharmaceuticals, Batch no-N385. *Adhatoda vasica* aqueous leaves extract (AVLE) was obtained from Saiba Industries Gujrat, India.

Animals

The study was approved by Institute's animal ethical committee and confirmed to national guidelines on the care and use of laboratory animals (CPCSEA/IAEC/PC-10/07-2K8). Swiss albino mice 25-30 g were obtained from Yash farms, Pune used for the study. The animals were maintained at 25 ± 2 °C in the Institute's animal house with food (Nutrivet, Pune, India) and water ad libitum.

Selection of dose

AVLE has weighed accurately and prepared appropriate stock solution (100 mg/kg, 200mg/kg, and 400 mg/kg) using distilled water as a vehicle. The drug solutions were prepared fresh daily.

Anti-gouty arthritic activity

Potassium oxonate (PO) induced Gout in mice

Animals: Swiss male albino mice, 25-30 g, will be required.

Drug: Aqueous leaves extract of *Adhatoda vasica*

Inducing agent: Potassium oxonate (PO).

Groups	Dose and Route
Control	0.9%saline solution (1 ml/kg)
PO control	Potassium oxonate (250 mg/kg, i.p.)
Standard	Potassium oxonate (250 mg/kg, i.p.)+ Allopurinol (10 mg/kg orally) on 1 st ,3 rd and 7 th day
AV 100	Potassium oxonate (250 mg/kg, i.p.)+ Aqueous extract of <i>Adhatoda vasica</i> (100mg/kg) on 1 st ,3 rd and 7 th day
AV 200	Potassium oxonate (250 mg/kg, i.p.)+ Aqueous extract of <i>Adhatoda vasica</i> (200mg/kg) on 1 st ,3 rd and 7 th day
AV 400	Potassium oxonate (250 mg/kg, i.p.) + Aqueous extract of <i>Adhatoda vasica</i> (400mg/kg) on 1 st ,3 rd and 7 th day

Procedure

Procedure: Divide mice into six groups (n=6). Withdraw food, but not water from all the animals on 1.5 h before the final drug administration on the 7th day of study, inject intraperitoneally (i.p.) with potassium oxonate (250 mg/kg) to increase the serum urate level. Group I control which will receive saline, Group II hyperuricemic control will orally receive 0.9% saline (1ml/kg) solution for 1, 3 and 7 days, respectively. Group III will serve as Standard will receive orally Allopurinol (10mg/kg) for 1st, 3rd and 7th days respectively. AV 100, AV 200 and AV 400and will receive the extract of *Adhatoda vasica* at 100, 200 and 400 mg/kg respectively for 1, 3 and 7 days, respectively. Collect whole blood samples 1hr after final drug administration. Blood was allowed to clot, Centrifuge to obtain the serum. Measuring serum parameters such as uric acid, xanthine oxidase (XOD), xanthine dehydrogenase (XDH) levels (Rasool and Varalakshmi, 2006).

Statistical analysis

The values were calculated as mean \pm SEM. The significance of the difference of the mean value with respect to control group was analyzed by one-way ANOVA followed by Dunnett t-test using software GraphPad Prism 6.0. $P < 0.01$ or above was considered to be significant.

RESULTS

Evaluation of uric acid

The results obtained for serum uric acid (Group I) were 5.74 ± 0.1523 mg/dl, 2.76 ± 0.123 n moles of uric acid/mg of protein, 2.141 ± 0.126 n moles of uric acid/mg of protein. For negative control (Group II) we got 10.3 ± 0.425 mg/dl, 6.923 ± 0.352 n moles of uric acid/mg of protein, 6.961 ± 0.3636 nmoles of uric acid/mg of protein. For rats, treated with AVLE100 (test Group III) we got 8.716 ± 0.679 mg/dl, 6.366 ± 0.2348 nmoles of uric acid/mg of protein, 6.328 ± 0.4575 nmoles of uric acid/mg of protein. Rats treated with AVLE 200 (test Group IV) we got 7.883 ± 0.3655 mg/dl, 5.683 ± 0.2926 nmoles of uric acid/mg of protein, 5.7 ± 0.2394 nmoles of uric acid/mg of protein. AVLE 400 (test Group V) we got 7.016 ± 0.2651 mg/dl, 4.733 ± 0.2404 nmoles of uric acid/mg of protein, 4.416 ± 0.1797 nmoles of uric acid/mg of protein. Rats treated with allopurinol (standard Group VI) shows values of 6.073 ± 0.03528 mg/dl, 2.605 ± 0.144 nmoles of uric acid/mg of protein, and 3.5058 ± 0.1358 nmoles of uric acid/mg of protein respectively. P value of all groups was compared against control group I. Control rats (Group I) showed normal concentrations of serum uric acid lower than all other groups. Negative control rats (group II) showed the highest values for serum uric acid which were significantly higher than the corresponding values obtained for control animals with $P < 0.001$. Rats treated with AVLE 100 (group III), AVLE 200 (group IV), AVLE 400 (Group V) showed significant decrease in serum uric acid with $P < 0.001$ than negative control.

Evaluation of XOD

Control rats (Group I) showed normal concentrations of XOD activity lower than all other groups. Negative control rats (group II) showed the highest values for XOD activity which were significantly higher than the corresponding values obtained for control animals with $P < 0.001$. Rats treated with AVLE 100 (group III), AVLE 200 (group IV), AVLE 400 (Group V) showed significant decrease XOD activity with $P < 0.001$ and $P < 0.01$ for XOD and $P < 0.001$ and $P < 0.001$ for XDH for groups III, IV and V respectively. However, it can be noted that in comparison to the negative control all the test groups show notable inhibition in XOD activity. In mice treated with standard allopurinol (Group VI), it can be observed that allopurinol almost completely inhibits the rise in XOD, with values similar to the control. It can be

observed that AVLE 200, AVLE 400 show a slightly better XOD inhibition as compared to than allopurinol standard.

Evaluation of XDH

Control rats (Group I) showed normal concentrations of XDH activity lower than all other groups. Negative control rats (group II) showed the highest values for XDH activity which were significantly higher than the corresponding values obtained for control animals with $P < 0.001$. Rats treated with AVLE 100 (group III), AVLE 200 (group IV), AVLE 400 (Group V) showed significant decrease in XDH activity with $P < 0.001$ for XDH for groups III, IV and V respectively. However, it can be noted that in comparison to the negative control all the test groups show notable inhibition in XDH activity. In mice treated with standard allopurinol (Group VI), it can be observed that allopurinol almost completely inhibits the rise with a slightly significant XDH activity with $P < 0.01$.

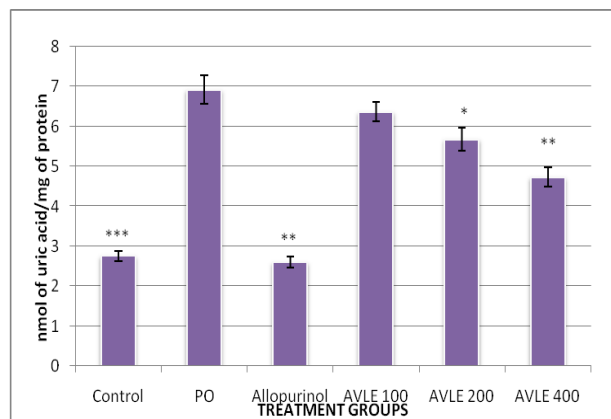


Figure 1. Effect of vehicle, PO, AVLE 100, AVLE 200, AVLE 400 and allopurinolon XOD levels.

Table 1. Effect of AVLE on serum uric acid, XOD, XDH levels.

TREATMENT GROUPS	SERUM URIC ACID	XOD	XDH
Normal control	5.74 ± 0.152	2.76 ± 0.123	2.141 ± 0.1266
PO control	10.3 ± 0.4258	6.923 ± 0.352	6.961 ± 0.3636
Allopurinol	$6.073 \pm 0.03528^{**}$	$2.605 \pm 0.144^{**}$	$3.5058 \pm 0.1358^{**}$
AVLE 100	8.716 ± 0.679	6.366 ± 0.234	6.328 ± 0.4575
AVLE 200	$7.883 \pm 0.3655^*$	$5.683 \pm 0.2926^*$	5.7 ± 0.2394
AVLE 400	$7.016 \pm 0.2651^{**}$	$4.733 \pm 0.2404^{**}$	$4.416 \pm 0.1797^*$

Values are expressed as mean \pm S.E.M. n = 6. Significant values were compared with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ ANOVA followed by Dunnett's test, all groups compared to PO control group.

DISCUSSION

Gout is a metabolic disorder related to an excess of circulating uric acid resultant in the deposition of monosodium urate crystals (MSU) in tissues. This hyperuricemia can occur through less uric acid excretion or overproduction and can be determined in utmost patients. A number of reversible factors contribute to increased urate production, with a high purine-containing diet, obesity and consistent alcohol intake (Bieber and Terkeltaub, 2004; Choi et al., 2004). After formation, the MSU crystals may be deposited in joints, usually in the big toe or ankle, triggering neutrophil infiltration, swelling and severe pain (Desaulniers et al., 2001). Estimations from the Third National Health and Nutrition Examination Survey (NHANES III) indicate that 0.5% of the total population suffering from a gout attack. Gout is currently considered as to be the most common form of inflammatory arthritis in men over 40 years old. Exceeding rheumatoid arthritis (Lawrence et al., 1998). The choices for the treatment of chronic gout are allopurinol, which is an inhibitor of the xanthine oxidase enzyme, probenecid, which is a uricosuric drug that promotes the renal excretion of uric acid and non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, that inhibit COX enzyme activity (Cronstein and Terkeltaub, 2006; Terkeltaub, 2010). An alternative drug that has been used to treat gout attacks is colchicine, which is an alkaloid derived from the autumn crocus *Colchicum autumnale* (Roberge et al., 1993). However, around 50% of patients are noncompliant with the prescribed medication, especially if they are having recurring gout flares (Gaffo and Saag, 2010). Moreover, each of these agents is allied with dangers, possibly severe adverse effects and drug-drug interactions. Thus, many gout patients end up opting for treatments based on folk medicine (Terkeltaub, 2010). The disease has a very long course of relapses and remissions and thus causes gross deformity. Treatment for Arthritis is mostly a lifetime process and hence above-mentioned drawbacks need to be addressed. Some side effects are bone marrow suppression, cardiovascular complications, hepatotoxicity, renal impairment, etc.

Though a large number of new drugs and therapies have been developed over the past few decades, even today, no ideal drug treatment is available to completely cure or check the progress of this disease. Hence, many of arthritic patients commonly prefer complementary and alternative medicines which emphasize the need of a cost effective drug with minimal side effects.

The objective of the present study was to evaluate the anti-gouty arthritic activity of the aqueous leaves extract of *Adhatoda vasica* Nees. *Adhatoda vasica* Nees (Acanthaceae) is commonly known as "Malabar nut". The decoction of *Adhatoda vasica* Nees is used in rheumatism. Earlier studies also reports the presence of phytosterols like alkaloids, carbohydrates, proteins and

trace elements are present in the leaves *Adhatoda vasica* Nees.

In present study *Adhatoda vasica*, aqueous leaves extract (AVLE) was used. In acute oral toxicity study, the extract was found to be safe up to the dose of 2000 mg/kg. From this data, three doses of 100, 200 and 400 mg/kg were selected for testing dose-dependent anti-gouty arthritic potential of *Adhatoda vasica*. The preliminary phytochemical analysis results showed the prominent presence of alkaloids, triterpenoids, saponins, tannins, glycosides and flavonoids in AVLE. The anti-arthritic effect of AVLE was confirmed by measuring the serum levels of uric acid, XOD and XDH etc clinical endpoints for evaluating the efficacy of any anti-gouty arthritic agent. AVLE showed a significant anti-gouty arthritic effect in a dose-dependent manner. It was found that 200, 400 mg/kg dose of AVLE was effective.

The present study results indicate that AVLE possesses significant anti-hyperuricemic activities. The effect might be due to potential phytochemicals found to be present in preliminary phytochemical analysis such as phytosterols, alkaloids, triterpenoids, saponins, tannins, and flavonoids.

The ideal requirement of an Anti-gouty arthritic agent includes anti-inflammatory and anti-hyperuricemic properties. The current research work indicates that *Adhatoda vasica* aqueous leaves extract demonstrated significant anti-hyperuricemic and anti-inflammatory activity. This suggests that *Adhatoda vasica* aqueous leaves extract could be a valuable addition to the current anti-arthritic therapies. Moreover, these studies strongly validate the claims of the tribal use of this plant as an anti-arthritic agent. Also, it paves way for further investigation of the chemical constituents responsible for the activity.

CONCLUSION

All the characteristic features of gouty arthritis such as lowering the raised enzymes levels of uric acid, XOD, XDH were restored by AVLE treatment in the study protocol and hence can be used for the treatment protocol. From the present work, it can be concluded that *Adhatoda vasica* leaves extract (AVLE) might act as an anti-gouty arthritic agent, with anti-hyperuricemic activity. The phytochemicals like alkaloids, flavonoids, triterpenoids, tannins, proteins, glycosides, vitamin C etc. Present findings support the tribal use of *Adhatoda vasica* as an anti-arthritic agent.

CONFLICT OF INTEREST

None declared.

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