PRECLINICAL EVALUATION OF HERBAL PLANT IN POTASSIUM OXONATE INDUCED HYPERURICEMIA IN MICE

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XOD-Xanthine oxidase, XDH-Xanthine dehydrogenase, AVLE-Aqueous leaves extract of Adhatoda vasaka, PO-Pottasium Oxonate.

A B S T R A C T

Objective: The objective of the study to perform Preclinical Evaluation Of Herbal Plant In Potassium Oxonate Induced Hyperuricemia In Mice.

Method: The present study was undertaken to investigate anti-gouty arthritic activity of aqueous leaves extract of Adhatoda vasaka using Potassium Oxonate induced hyperuricemia in mice. Potassium oxonate (PO) induced hyperuricemia in mice were determined by in vivo experiments. PO causes hyperuricemia in 1h after ip 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, xanthine dehydrogenase, etc.

Results: The evaluation of anti-gouty arthritic potential by oral administration of AVLE (100-400 mg/kg) evoked a significantly decreased the serum levels of uric acid, xanthine oxidase (XOD), xanthine dehydrogenase (XDH) in treated mice.

Conclusions: The results obtained in this study indicate that AVLE possesses potential anti-gouty arthritic activity. Finally, the study concluded that in AVLE the flavonoidal constituents impart enzyme inhibitory activity thus reducing levels of xanthine oxidase, xanthine dehydrogenase and serum uric acid.

INTRODUCTION

Adhatoda vasica (L.) 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 L. are used synonymously. It is also known under the common name Malabar nut tree and the Sanskrit name Vasaka. 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 (Manjunath, 1948). The leaves was 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). Anti-oxidant and radical scavenging activity (Srinivasarao et al., 2006) Anti-inflammatory effect (K.alam et al., 2011)

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; Kong et al., 2000), their uses in modern medicine suffer from the lack of scientific evidences. Attention has been focused on identifying their phytochemicals, which possess ability to inhibit XDH/XO activities andthereby reduce the urate levels. Flavonoids have been shown to be inhibitors of the activity of XO in 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 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

Pottasium oxonate was procured from Sigma-aldrich, USA.
Chemicals and drugs

1. Allopurinol Tablets: Zyloric® GlaxosmithKline Pharmaceuticals, Batch no-N385
2. Adhatoda vasica aqueous leaves extract (AVLE) was obtained from Saiba Industries Gujarat, 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 gms 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 was weighed accurately and prepared appropriate stock solution (100 mg/kg, 200 mg/kg, 400 mg/kg) using distilled water as a vehicle. The drug solutions were prepared fresh daily.

Anti-Gout 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)

Study of the effect of Adhatoda vasica in potassium oxonate induced hyperuricemia in mice:

The results obtained in table 1 below for serum uric acid, XOD, XDH and of liver homogenate Control rats (group I) showed normal concentrations of serum uric acid, XOD and XDH activity lower than all other groups. Negative control rats (group II) showed the highest values for serum uric acid, XOD and 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 serum uric acid with P<0.001 than negative control and significant XOD and XDH 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 and XDH activity. In mice treated with standard allopurinol (group VI) it can be observed that allopurinol almost completely inhibits the rise in uric concentration and XOD, with values similar to the control, with a slightly significant XDH activity with P <0.01. It can be observed that AVLE 400 show a slightly better XOD inhibition as compared to than allopurinol standard.

### Table 1: Effect of vehicle, AVLE 100, AVLE 200, AVLE 400 and allopurinol on uric acid levels

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

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 rats will orally receive 0.9% saline (1 ml/kg) solution for 1, 3 and 7 days, respectively. Group III will serve as Standard which will receive orally Allopurinol (10 mg/kg) for 1, 3 and 7 days, respectively. AV 100, AV 200 and AV 400 and will receive extract of Adhatoda vasica at 100, 200 and 400 mg/kg respectively for 1, 3 and 7 days, respectively. Collect whole blood samples 1h 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.1,2

RESULTS

The values were calculated as mean±SEM. The significance of the difference of the mean value with respect to control group was analyzed by one way ANOVA followed by Dunnet’s t-test using software Graphpad Prism 6.0. P<0.01 or above was considered to be significant.3,6
of these agents is recurring gout with the prescribed Colchicum which is an alkaloid (Cronstein and Terkeltaub, 2006; Terkeltaub, 2010). Another acid, an uricosuric drug that stimulates the renal excretion of uric acid is probenecid, which is an inhibitor of the xanthine oxidase enzyme, probenecid, which for the treatment of chronic gout are allopurinol, which is an allopurinol and can be readily determined in most patients. A number of reversible factors contribute to increased urate production, including a high purine diet, obesity and regular alcohol consumption (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, causing neutrophil infiltration, swelling and excruciating 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 has suffered from a gout attack. In addition, gout is currently considered to be the most common form of inflammatory arthritis in men over 40 years old. exceeding rheumatoid arthritis in men over 40 years old. The disease has a very long course of relapses and remissions and thus causes gross deformity (Winstanley et al., 1996). 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.

Although 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 (Shankaranarayanan et al., 2009) 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 report the presence of phytosterols like alkaloids, carbohydrates, proteins, and trace elements are present in the leaves Adhatoda vasica Nees.

In present study A. 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 dependant anti-gouty arthritic potential of AVLE. 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, XDH etc clinical endpoints of arthritic effect of AVLE was confirmed by measuring the, serum levels of uric acid, XOD, XDH etc clinical endpoints of AVLE. AVLE showed significant anti-gouty arthritic effect in a dose dependant 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 where restored by AVLE treatment in the study protocol and hence can be used for treatment protocol. From the present work, it can be concluded that *Adhatoda vasica* leaves extract...
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(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.

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References

4. Yun-Wei Shi a, Cai-Ping et al., 2012Uricosuric and nephroprotective properties of Ramulus Moriethanol extract in hyperuricemic mice Journal of Ethnopharmacology
6. Pamela Gasse, Nicolas Riteau et al., 2010 Uric Acid Is a Danger Signal Activating NALP3 Inflammasome in Lung Injury Inflammation and Fibrosis.

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