

## EFFECT OF ARISTOLOCHIA INDICA ON DIURETICS INDUCED GOUT

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### Summary

In the present study, the aqueous and alcoholic extracts of '*Aristolochia Indica*' belongs to family 'Aristolochiaceae' was analysed for its influence on the drug induced 'Gout' and its possible mechanism of action in inhibiting Hyperuricemia to support the folklore claim. Diuretic drugs such as Thiazides, Amiloride causes Hyperuricemia. 10mg/kg thiazide and 2mg/kg amiloride were used in combination orally to induce Hyperuricemia in animals (Rats). The animals were divided into four groups, Group 1 served as control in which the combination of 10mg/kg Thiazide and 2mg/kg Amiloride were administered orally for a period of 15 days. Group 2 served as standard in which the animals were orally administered with the combination of 10mg/kg Thiazide and 2mg/kg Amiloride and were further treated with 50mg/kg probenecid. Group 3 served as Test 1 in which, combination of 10mg/kg Thiazide and 2mg/kg Amiloride were administered orally and were further treated with 400mg/kg alcoholic leaf extract of *Aristolochia indica*. Group 4 served as Test 2 in which, combination of 10mg/kg Thiazide and 2mg/kg Amiloride were administered orally and were further treated with 400mg/kg of aqueous leaf extract of *Aristolochia Indica*. The investigations were carried out for a period of 15days, at the end of 3, 6, 9, 12 & 15<sup>th</sup> day, urine samples were collected from each animal and the uric acid level was estimated using commercial uric acid kit. It was observed that the standard drug probenecid, alcoholic and aqueous leaf extracts of *Aristolochia indica* were found to maintain normal uric level when compared to control. It was concluded that *Aristolochia indica* extracts were found to inhibit the drug induced hyperuricemia. Further research on this may give some beneficial information for gout patients who are undergoing diuretics therapy.

**Keywords-** *Aristolochia Indica*, Uricase POD-method, Gout, Diuretics.

### Introduction

The Aristolochiales are a group of paleoherbs, a basal group flowering plants<sup>1</sup>. The plant is commonly known by the names birthwort & snakeroot, which refers to its use in traditional medicine for postpartum infections and snakebite respectively. *Aristolochia Indica* is the plant belonging to family Aristolochiaceae, different parts of the plant bears many medicinal properties<sup>2</sup>.

The plant has been used in skin diseases where there is morbidity of vata, pitta & kapha. It is used as an appetizer, aphrodisiac & anthelmentic<sup>3</sup>. The fresh juice of the leaves is a popular antidote to snake poison. The leaves & barks are used in bowel complaints of children, diarrhoea & in intermittent fevers. As a blood purifier the dried roots & rhizomes are used as a gastric stimulant & bitter tonic. The root is used in skin diseases. It heals wounds & destroys the toxic effect of all poisons. In olden days, it was used against snakebites. In traditional medicine the underground parts of the plant are rubbed with honey & given to treat leprosy & macerated with black pepper. The roots & stems of the plant are used in ethno veterinary aches and pains, rheumatism<sup>4</sup>, anthrax, madness, antibacterial effect<sup>5</sup>, antineoplastic effect, antiarthritic effect<sup>4</sup> & snakebite.

The major chemical constituents present in the aristolochia extracts are Phenanthrene derivatives<sup>6</sup> like Aristolochic acid<sup>7</sup> aristolochic acid-D, aristolochic acid-D methyl ether lactum, aristo lactum  $\beta$ -D glucoside/aristolochic acid, aristolochic acid, methyl ester, methyl aristolochate, aristolamide, aristolonic acid, aristolonitrite. Quinones like Aristolindiquinone, lactones like Aristololide, Alkaloid like Aristolochine, Terpenes like mono & sesquiterpenes including linalool,  $\beta$ -caryo phyllene,  $\alpha$ -humulene, ishwarone, caryophyllene oxide, ishwarol, ishwarane & aristolochene &  $\alpha$ -terpinolene<sup>8</sup>.

The extracts are found have various medicinal activities like, Antiestrogenic activity<sup>9</sup>, Abortifacient activity, Interceptive activity<sup>10</sup>, Antitumour activity<sup>11</sup>, antifertility<sup>12</sup>, Immunomodulatory activity and anti-inflammatory activity.

Among that the leaves were predicted to have antihyperuricemic effect according to folklore claim<sup>13</sup>. Hyperuricemia is increased uric acid concentration in blood having drug induction as one of the reasons. *Aristolochia indica* leaf extracts were said to have influence on this drug induced gout.

Drug induced gout or hyperuricemia is a common problem nowadays. Reports suggest that diuretic drugs like amiloride, ethacrinic acid, frusemide and thiazides induce hyperuricemia<sup>14</sup> on high doses and on prolonged use. Hence there is a search for antihyperuricemic drugs that can effectively control the diuretics induced Hyperuricemia<sup>16</sup>. It was also found that various drugs act by controlling the hyperuricemia, such as Allopurinol that was found to act by inhibiting the synthesis of uric acid<sup>15</sup> and probenecid like uricosuric drugs<sup>17</sup> that act by increasing the rate of excretion of uric acid level, NSAIDS are found to reduce the pain and inflammation associated with gout. In this work we concentrate on the qualitative activity of anti hyperuricemic effect of *Aristolochia indica* whole plant extracts by comparing with standard uricosuric agent like probenecid.

The objective of the present study includes extraction of active principle from *Aristolochia indica* by soxhlet extraction method and to analyse the influence of aqueous and alcoholic leaf extracts on the diuretics induced Gout<sup>18,19</sup> and the possible mechanism of action and to compare with the standard drug probenecid on animals such as rats.

### Materials and Methods

#### Collection of Plant Material:

*Aristolochia indica* whole plants were collected & they were authenticated by Regional forest research centre, Rajahmundry & department of Pharmacognosy, GIET School of Pharmacy, Rajahmundry. The plants were air dried under shade & coarse powdered and used for extraction.

#### Extraction of the active principle:

The powdered whole plant of *Aristolochia indica* was extracted by Soxhlet apparatus with ethanol as solvent<sup>20</sup>. The extract was concentrated under reduced pressure in freeze drier & this extract was used for the Pharmacological investigation.

The aqueous extract was prepared by taking the powder of *Aristolochia indica* whole plant and it was macerated in distilled water for several days. The extract was filtered through muslin cloth & then with Whatman No:40 filter paper, the extract was evaporated to dryness by slow heating and continuous stirring in a water bath. The residue was collected & it was used for Pharmacological investigation.

#### Estimation of uric acid in urine by using commercial uric acid kit (Uricase- POD method)<sup>21,22</sup>

Young wistar Albino rats of either sex weighing between 150-200g obtained from Animal house department, under standard Laboratory conditions were kept in quarantine for 24 hours. Albino rats of either sex were distributed into '4' groups, each consists of six rats.

Group 1 served as control in which animals were treated orally with a combination of 10mg/kg Thiazide and 2mg/kg Amiloride for a period of 15 days.

Group 2 served as standard in which animals were treated orally with a combination of 10mg/kg Thiazide and 2mg/kg Amiloride and were further treated with 50mg/kg probenecid orally.

Group 3 served as Test 1 in which 10mg/kg Thiazide and 2mg/kg Amiloride were administered orally in combination and were further treated with 400mg/kg alcoholic leaf extract of *Aristolochia indica*.

Group 4 served as Test 2 in which 10mg/kg Thiazide and 2mg/kg Amiloride were administered orally in combination and were further treated with 400mg/kg aqueous leaf extract of *Aristolochia indica*.

Investigation was carried out for a period of 15 days and at the end of 3,6,9,12 and 15<sup>th</sup> day the urine samples were collected from each animal<sup>23</sup> and the uric acid level was estimated using commercial liquid gold uric acid kit manufactured by Span diagnostics limited, India. The uric acid level was also measured on day 1 of the study and also compared with the readings of last day (15<sup>th</sup> day).

The experiment was conducted in accordance with the guidelines given by “*Institutional Animal Ethics Committee*” (IAEC) approval number- GSP/PY/05/2008.

### Results and Discussion

It was observed that the standard drug probenecid, alcoholic and the aqueous leaf extracts of *Aristolochia indica* were found to maintain normal uric acid level on drug induced hyperuricemia when compared to control group of animals till the end of the study, where as the control animals maintained high levels of uric acid till the end of the study. The values are tabulated in Table-I.

**Table No I-** Average uric acid levels of *Aristolochia Indica* extracts and probenecid treated on drug induced Hyperuricemic rats. \*P<0.05 \*\*P<0.01 \*\*\*P<0.001

Group	Average Uric acid level in the urine gms/day				
	Day3	Day6	Day9	Day12	Day15
<i>Control</i>	0.361±0.012	0.432±0.043	0.562±0.022	0.985±0.032	1.556±0.044
<i>Aqueous extract</i>	0.463±0.022	0.569±0.032	0.655±0.013	0.774±0.0298*	0.798±0.032**
<i>Alcoholic extract</i>	0.447±0.019	0.574±0.006	0.673±0.042	0.738±0.026***	0.739±0.032**
<i>Standard</i>	0.381±0.033	0.478±0.036	0.558±0.029	0.607±0.016***	0.655±0.023***

### Conclusion

Since the aqueous and alcoholic extracts of *Aristolochia indica* reduces the elevated uric acid level similar to standard drug probenecid, we can assume the extracts of *Aristolochia indica* to follow the same mechanism of probenecid in reducing the uric acid level i.e. by causing decrease in distal tubular secretion of uric acid and in high doses increases its excretion rate by blocking tubular reabsorption. Hence it can be concluded that the extracts of *Aristolochia indica* may produce uricosuric effect similar to

probenecid. Further research on this may give some beneficial information about the role of extracts on uric acid synthesis and also it may help gout patients who are undergoing diuretics therapy.

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