ORIGINAL ARTICLE

# Sub-chronic safety evaluation of aqueous extract of *Alangium salvifolium* (*L.f.*) *Wangerin* leaves in rats

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## ABSTRACT

Conventionally, the juice and extract of *Alangium salvifolium* leaves have been used for the treatment of diabetes, wound healing, dog bite, and as a poultice in rheumatism. To carry out the sub-chronic toxicity and thereafter safety evaluation of *A. salvifolium* leaves. The aqueous extract of *A. salvifolium* leaves was administered orally at the doses of 200, 400, and 800 mg/kg/day for 90 days. All the animals were observed daily for general behavior, changes in body weight, food, and water consumption. At the end of the treatment period, biochemical and hematological parameters were analyzed; and the animals were sacrificed for histopathological examination of heart, lungs, liver, and kidney. The general behavior and water intake were normal in all the rats. The increase in body weight was observed in female rats of all the groups while body weight was decreased in high dose group animals of both sexes. Hematological parameters were not disturbed by the continuous use of extract. A significant decrease in glucose level was observed in intermediate- and high-dose group animals while urea and creatinine level were significantly high in animals of high-dose group. Although histopathological examination of most of the organs exhibited no structural changes, some tubal damage in kidneys was observed in high-dose group animals. The high dose of extract has shown mild signs of toxicity on kidney function test, but no toxic response was observed on hematological and liver biochemical parameters. The extract also exhibited hypoglycemic potential.

**Key words:** *Alangium salvifolium*, phytoconstituents, sub-chronic toxicity

## INTRODUCTION

The use of medicinal plants, either as an extract pure compound or as a derivative, is being used for various therapeutic purposes. The World Health Organisation in 2008 reported that 80% of Asian population uses herbal medicinal products for their primary health care and the data are same for developed countries also.[1] As a result of inadequate knowledge regarding mode of action, potential side effects, interactions with other pharmaceuticals; the indiscriminate use of herbal products can result is serious toxic effects.[2,3] Some extremely toxic phytoconstituents such as digoxin and strychnine have been extracted for various therapeutic purposes, hence careful investigation is required before use of such medicinal plants.[4] Since safety continues to be a major issue with the use of herbal medicines, it is important that the relevant regulatory measures are to be taken to protect public health by ensuring that all herbal medicines are safe and of suitable quality; and have passed the standard quality and toxicity studies.

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*Alangium salvifolium* (Syn. *Alangium lamarckii;* Family: Alangiaceae) is a deciduous, thorny, armed or unarmed plant which is cultivated extensively in India due to its various therapeutic uses.[5-8] This plant is commonly known as Ankol or Ankola in Hindi and Ankolam, Aranji in Tamil and Malayalam The widespread uses of this plant are due to various phytoconstituents such as flavanoids, terpenoids, phenolic glycosides, tannins, steroids, β-carboline, and quinolizidine alkaloids such as alangine, alangicin, marckindine, tubulosine deoxytubulosine, emetine, ankorine, and alangimarckine.[9-11] Leaf juice is applied externally and taken internally in case of dog bite. Leaves are also used as a poultice in rheumatic pains, wound healing, diabetes, and hemorrhages.[12]

Other reported therapeutic uses of the plant include antiulcer,[13] antidiarrheal,[14] hepatoprotective,[15] anticancer,[16] analgesic and anti-inflammatory.[17] Despite potential therapeutic uses of the plant, no data is available on safety or toxicity; hence, the present sub-chronic toxicity study was designed to evaluate the safety profile of aqueous extract of leaves.

## MATERIALS AND METHODS

### Plant material, extraction, and preliminary phytochemical screening

*A. salvifolium* leaves were procured from the local market of Lucknow and was identified by Dr. S. Rajan, Department of Botany, Government College of Ooty, Tamil Nadu (specimen voucher no. LSPS/04/H/001/2013). Leaves were shade dried, crushed, and extracted by continuous hot extraction using water as solvent. After filtration, the extract was concentrated by rotary evaporator and stored in a desiccator. Qualitative analysis of the extract was carried out to identify the various phytoconstituents such as alkaloids, phenols, flavonoids, tannins, saponins, and glycosides.

### Animals

The present study was carried out after the approval from Institutional Animal Ethical Committee and Sprague-Dawley rats were procured from National Institute of Pharmaceutical Education and Research, Mohali. The protocol approval number of the study was 954/PO/ac/06/CPCSEA/Nov-2012/I. The study was performed as per guidelines of Organization for Economic Co-operation and Development (408) for oral sub-chronic toxicity studies in rodents. The animals were acclimatized to environmental conditions for 7 days before the start of the study.

### Sub-chronic toxicity study

The rats were randomly divided into four groups: control group, low-, medium-, and high-dose group (Group I–IV respectively); and each group contained 16 animals (8 males and 8 females). Rats were administered with aqueous extract by oral gavage once daily for 90 consecutive days. The control group received 2 ml/100 g of distilled water while Group II, III, and IV were administered with dose of 200, 400, and 800 mg/kg/day, respectively. During the treatment period, all animals were observed once daily for the changes in eyes, skin, fur, occurrence of secretions, food intake, water intake, and mortality. At the end of the study, the blood was collected from the anesthetized animals by retro-orbital puncture for biochemical and hematological analysis. The animals were euthanized by cervical dislocation, and histopathological examination of kidneys, lungs, heart, and liver was carried out.

### Hematological and biochemical analysis

Complete blood count and biochemical analysis were performed after the completion of the study. Lipid profile, liver function test, and kidney function test were carried out to evaluate the effect of the extract on important organ systems.

### Histopathology

At the end of the study, the animals were sacrificed by cervical dislocation and subjected to gross and histopathological alterations. In the gross examination, changes in organ size, shape, and any visible lesions were recorded.

### Statistical analysis

Results are shown as mean ± standard deviation. The statistical significance of the differences among the treatments and control was verified using one-way analysis of variance. $P < 0.05$ was considered statistically significant.

## RESULTS

During the 90-day observation period, all the groups (control as well as treatment) did not show any sign of morbidity or mortality. No unusual behavior or physical changes were observed in animals.

### Animals body weight changes and food consumption

A marked reduction in the body weight of male animals was observed in intermediate and high dose groups from 9$^{th}$ week onward, but the data were not statistically significant. In contrast, significant increase in the body weight was observed in female rats in all dose groups with respect to control group. Changes in body weight also supported the less food consumption in male animals only in intermediate and high dose group from 10$^{th}$ week onward [Figures 1-4].

### Hematological analysis

Treatment group animals showed no significant changes in the hemoglobin and red blood cells level in both male and female animals in comparison to control group animals [Table 1].

## Biochemical analysis

Blood glucose levels were significantly decreased in both male and female rats of intermediate-dose group ($P < 0.05$) and high-dose group ($P < 0.01$). Significant reduction in the cholesterol and triglyceride levels were reported at the dose of 400 mg/kg and 800 mg/kg (at $P < 0.01$) [Table 2]. Serum glutamic oxaloacetic transaminase (SGOT) level was significantly reduced at the dose of 200 mg/kg ($P < 0.05$) and 800 mg/kg ($P < 0.05$) in male rats. No significant change was observed in the serum glutamate pyruvate transaminase (SGPT) level of both control and treated rats. Total serum protein test measures the total amount of protein including globulin and albumin in the blood. Total serum protein levels were significantly increased at the dose of 400 mg/kg ($P < 0.05$) and 800 mg/kg ($P < 0.01$) in female rats but no significant change was observed in male rats. All the treatment groups exhibited similar level of alkaline phosphatase as compared to control group [Table 3]. Serum urea and creatinine level were significantly increased in both male and female rats of high-dose group ($P < 0.05$) in comparison to control group animals. No significant change was observed in the uric acid level in all groups [Table 4].

## Histopathological examination

All the animals were subjected to necropsy after the completion of 13-week study period. For histopathological examination, the heart, kidneys, liver, and lungs were isolated and examined [Figures 5-8].

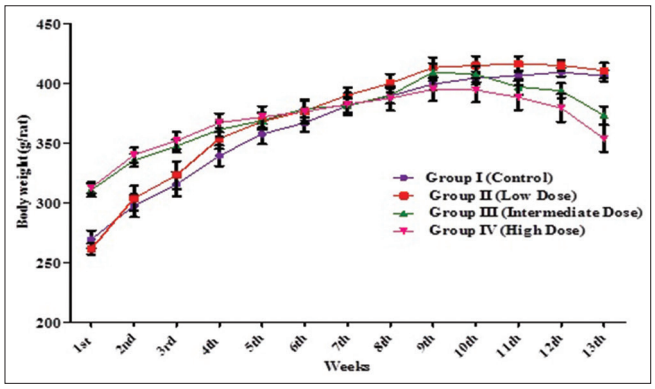


**Figure 1:** Effect of aqueous extract of *Alangium salvifolium* leaves on body weight of male rats (g/rat)

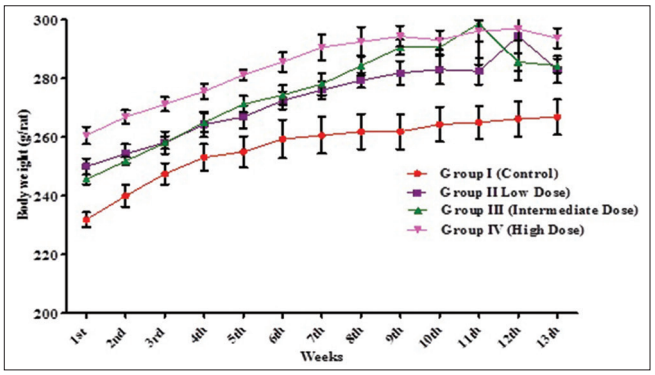


**Figure 2:** Effect of aqueous extract of *Alangium salvifolium* leaves on body weight of female rats (g/rat)

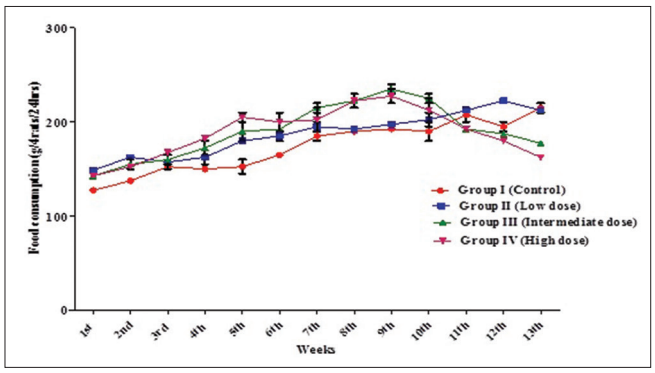


**Figure 3:** Effect of aqueous extract of *Alangium salvifolium* leaves on food consumption of male rats

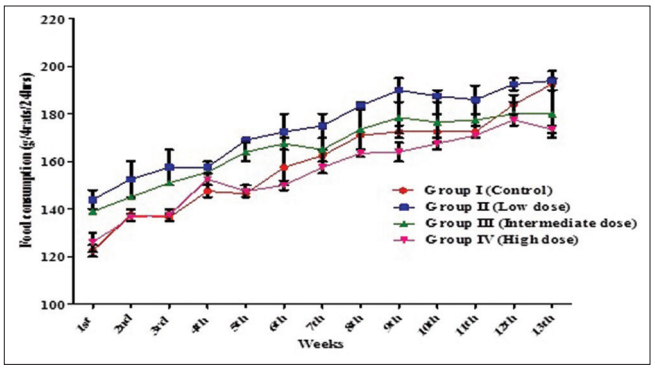


**Figure 4:** Effect of aqueous extract of *Alangium salvifolium* leaves on food consumption of female rats

**Table 1: Effect of aqueous extract of *Alangium salvifolium* leaves on hematological parameters**

| Parameters | Control | | Dosage (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 200 | | 400 | | 800 | |
| | Male | Female | Male | Female | Male | Female | Male | Female |
| HGB (g/dL) | 12.48±0.24 | 12.05±0.24 | 12.54±0.32 | 12.00±0.12 | 12.74±0.62 | 12.09±0.31 | 12.32±0.27 | 11.57±0.44 |
| RBC (cells/µl) | 7.30±0.157 | 07.30±0.15 | 08.06±0.21 | 07.39±0.10 | 08.15±0.44 | 07.32±0.22 | 07.99±0.21 | 07.16±0.27 |
| WBC (cells/µl) | 8.07±0.63 | 5.14±0.54 | 9.77±1.21 | 5.09±0.27 | 11.07±0.66 | 5.83±0.53 | 6.95±0.25 | 04.56±0.45 |
| PLT (cells/µl) | 373.5±0.55 | 403.13±0.43 | 398±0.73 | 390.63±0.75 | 450.57±0.34 | 353.7±0.67 | 430.87±0.99 | 540.8±0.24 |

Control versus treatment groups, values are mean±SEM ($n$=8). RBC: Red blood cell, WBC: White blood cell, PLT: Platelet, HGB: Hemoglobin

**Table 2: Effect of aqueous extract of *Alangium salvifolium* on glucose and lipid profile**

| Gender | Treatment groups | Parameters (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | Glucose | TG's | Cholesterol | HDL | LDL |
| Male | Control | 116.84±1.80 | 67.25±1.48 | 66.63±1.70 | 37.38±1.49 | 15.80±2.15 |
| | 200 mg/kg/day | 111.84±3.17 | 60.38±1.66 | 58.00±2.17 | 36.5±1.99 | 12.83±2.45 |
| | 400 mg/kg/day | 103.09±3.04* | 53.00±1.87** | 50.75±3.46** | 35.75±1.44 | 08.55±1.93 |
| | 800 mg/kg | 90.60±3.20** | 46.50±2.38** | 44.94±2.17** | 31.20±1.13 | 06.57±1.98 |
| Female | Control | 107.29±1.75 | 63.63±1.62 | 65.88±2.43 | 34.88±2.43 | 18.28±3.83 |
| | 200 mg/kg/day | 102.29±1.49 | 56.88±2.28 | 62.99±1.45 | 33.65±2.10 | 17.18±1.97 |
| | 400 mg/kg/day | 93.54±3.20* | 50.25±2.47** | 51.50±1.04** | 33.25±0.53 | 10.20±1.58 |
| | 800 mg/kg | 85.41±2.67** | 46.88±3.48** | 45.25±1.40** | 30.00±1.60 | 07.38±1.24 |

*$P<0.05$, **$P<0.01$, control versus treatment groups. Values are mean±SEM ($n=8$). SEM: Standard error of mean, TG: Triacylglycerol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

**Table 3: Effect of aqueous extract of *Alangium salvifolium* on liver function test**

| Gender | Treatment groups | Parameters | | | | |
|---|---|---|---|---|---|---|
| | | SGOT (U/L) | SGPT (U/L) | Total protein (mg/dl) | Total bilirubin (mg/dl) | Alkaline phoshatase (U/L) |
| Male | Control | 160.31±1.64 | 67.25±1.48 | 05.95±0.19 | 0.28±0.01 | 104.11±3.56 |
| | 200 mg/kg/day | 151.00±2.86* | 60.38±1.66 | 05.69±0.15 | 0.28±0.07 | 104.73±3.40 |
| | 400 mg/kg/day | 157.32±2.11 | 53.00±1.87 | 06.20±0.21 | 0.29±0.04 | 96.13±1.98 |
| | 800 mg/kg | 142.26±2.33** | 46.50±2.38 | 05.76±0.17 | 0.31±0.06 | 94.13±1.66 |
| Female | Control | 115.06±3.19 | 20.85±0.62 | 05.23±0.12 | 0.198±0.01 | 91.83±1.92 |
| | 200 mg/kg/day | 112.56±1.83 | 21.63±1.28 | 05.64±0.37 | 0.215±0.02 | 89.21±2.10 |
| | 400 mg/kg/day | 107.56±3.10 | 19.60±1.07 | 06.35±0.28* | 0.234±0.02 | 87.75±3.21 |
| | 800 mg/kg | 106.31±3.32 | 22.76±1.28 | 06.60.±0.27** | 0.238±0.04 | 86.37±3.38 |

*$P<0.05$, **$P<0.01$, control versus treatment groups. Values are mean±SEM ($n=8$). SEM: Standard error of mean, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate pyruvate transaminase

**Table 4: Effect of aqueous extract of *Alangium salvifolium* on kidney function test**

| Gender | Treatment groups | Parameters | | |
|---|---|---|---|---|
| | | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine (mg/dl) |
| Male | Control | 39.87±1.38 | 3.13±0.12 | 0.67±0.02 |
| | 200 mg/kg/day | 39.25±1.53 | 3.07±0.18 | 0.69±0.02 |
| | 400 mg/kg/day | 44.87±1.69 | 3.27±0.17 | 0.73±0.01 |
| | 800 mg/kg | 47.50±1.51 | 3.56±0.11 | 0.99±0.03 |
| Female | Control | 37.50±1.65 | 3.12±0.23 | 0.49±0.17 |
| | 200 mg/kg/day | 38.62±1.83 | 2.27±0.12 | 0.52±0.05 |
| | 400 mg/kg/day | 40.37±1.69 | 3.18±0.29 | 0.59±0.01 |
| | 800 mg/kg | 45.75±1.51* | 3.26±0.32 | 0.68±0.01* |

*$P<0.05$, control versus treatment groups. Values are mean±SEM ($n=8$). SEM: Standard error of mean

## DISCUSSION

Plants and plant-derived products have been used for prevention and control of diseases from ancient times.[18] Medicines, functional foods, and nutritional supplements are increasingly being used worldwide. These medicines are perceived as to be safe or free from toxic effects, but a number of adverse effects are reported from these agents and scientific validation of herbal products is essential.[19]

In present investigation, the aqueous extract of *A. salvifolium* leaves was evaluated for sub-chronic toxicity study after oral administration. Conventionally, this plant is used for various therapeutic purposes due to a number of bioactive phytoconstituents such as alkaloids, flavonoids, steroids, and glycosides. The reported therapeutic actions of the plant include antidiabetic,[6,12] anticancer,[14] antioxidant, larvicidal, pesticidal, and skin disorders.[20,21]

After oral administration of aqueous extract for 13 weeks, none of the treatment group had shown any toxic effect. The first symptom observed is the change in body weight of animals; as it is a sensitive indicator for toxic responses to drugs, to hormonal variations, liver disorders, and decreased absorption of essential nutrients,[22,23] In contrast

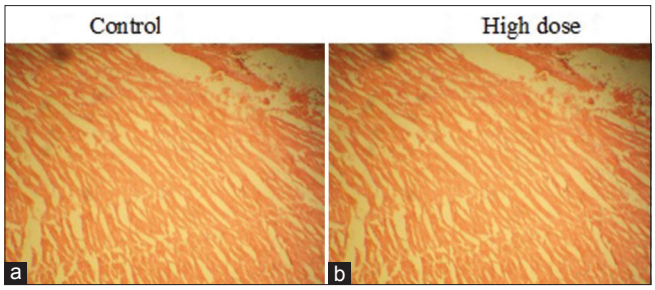


**Figure 5:** (a-b) Effect of aqueous extract of *Alangium salvifolium* leaves on microscopy of heart

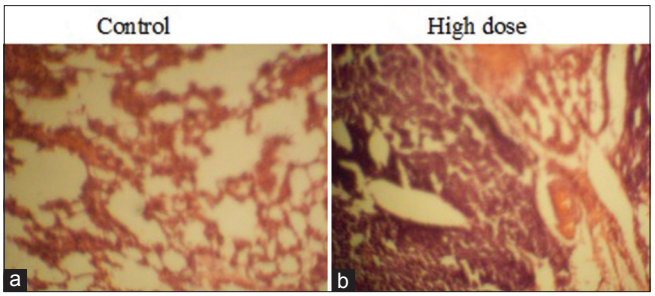


**Figure 6:** (a-b) Effect of aqueous extract of *Alangium salvifolium* leaves on microscopy of lungs

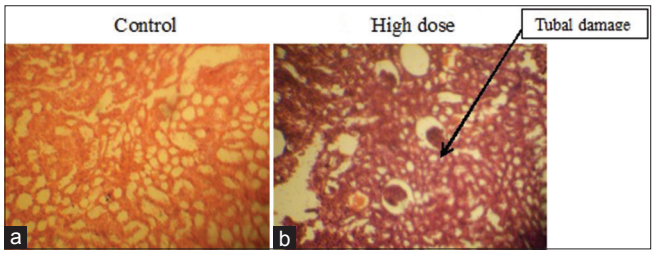


**Figure 7:** (a-b) Effect of aqueous extract of *Alangium salvifolium* leaves on microscopy of kidneys

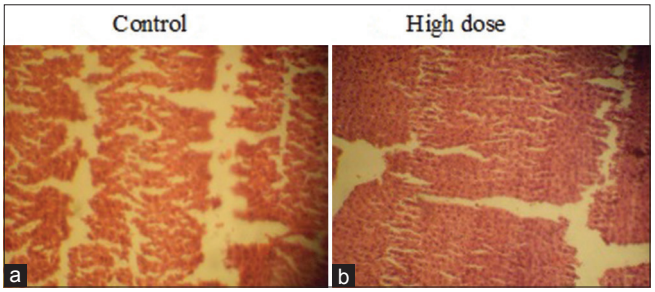


**Figure 8:** (a-b) Effect of aqueous extract of *Alangium salvifolium* leaves on microscopy of liver

to male animals, significant increase in body weight of female animals was seen during the study [Figures 1 and 2].

Hematopoietic system is a sensitive target for toxic compounds and is an important index of physiological and pathological conditions in man and animals.[24] No significant change suggested the nontoxic nature of extract to the hematopoietic system.

High dose group animals showed a significant decrease in blood glucose level in comparison to control and other treatment groups. It may be attributed to the hypoglycemic activity of the extract.[5] This reduction in blood glucose level can be correlated with the decreased level of triglyceride in intermediate and high dose groups.[25]

Significant reduction in the blood cholesterol and triglyceride levels at the dose of 400 mg/kg and 800 mg/kg suggested the hypolipidemic activity of the extract which indicates potential benefits of extract against cardiovascular diseases. Serum cholesterol and proteins are mainly regulated by liver and any change in serum concentrations of these parameters suggest some alterations in liver functions.[26] SGOT level was significantly reduced at the dose of 200 mg/kg (<0.05) and 800 mg/kg (<0.01) in male rats. Both control and treated rats exhibited comparable SGPT level. Total serum protein levels (albumin and globulin) were significantly increased at the dose of 400 mg/kg (<0.05) and 800 mg/kg (<0.01) in female rats, but no significant change was observed in male rats. The elevated total serum protein level indicates dehydration, high protein and high-calorie diet, multiple myeloma, chronic liver disease, and chronic infections.[27] To see the effect of extract on cellular damage, alkaline phosphatase level was measured and showed no significant change at all dose levels in both male and female animals as compared to control.

Kidney function test was performed to see the effect of the extract on urea, uric acid, and creatinine levels as these are considered as important markers for kidney dysfunction.[28] Serum urea and creatinine levels were increased significantly in both male and female animals of high dose group in comparison to control group. Serum uric levels were not altered in any of the treatment group animals.

No remarkable structural changes were observed on histopathological examination of heart, lungs, and liver [Figures 5, 6 and 8]. Minimal tubal damage in kidneys was observed in high dose group of both sexes [Figure 7] which can be also correlated with significant changes in kidney function tests. It can be concluded that the plant should be taken cautiously in case of patients with kidney dysfunction.

## CONCLUSION

It is concluded that the aqueous extract of *A. salvifolium* did not induce any toxicological effects on the majority of the parameters. The extract showed hypoglycemic activity and, therefore, can be of therapeutic value in the management of diabetes. The hypoglycemic effect of the plant can be correlated to the reduced triglyceride and cholesterol levels as they may decrease insulin resistance. Significant alterations in kidney function test parameters were observed

which was further supported by histopathological analysis. All these findings suggested antidiabetic and hypolipidemic potential of the plant up to a dose of 400 mg/kg, but it has to be further supported by chronic toxicity study.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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