INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

# PRECLINICAL TOXICOLOGICAL INVESTIGATION OF SIDDHA FORMULATION VISHA SANJEEVI BY ACUTE AND 28 DAYS REPEATED ORAL TOXICITY STUDIES IN WISTAR RATS

T. Subathra [1]*, P. Shanmugapriya [2], M. Murugesan [3]
[1] Lecturer, Department of Noi Anuga Vidhi including Research Methodology, Sri Sai Ram Siddha Medical College and Research Centre, Sai Leo Nagar, Poonthandalam, West Tambaram, Chennai, Tamil Nadu, India
[2] Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, Chennai, Tamil Nadu, India
[3] Former Dean and Head, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, Chennai, Tamil Nadu, India
*Corresponding Author Email: drsuba.bsms@gmail.com

Article Received on: 05/08/19 Approved for publication: 02/12/19

**DOI: 10.7897/2230-8407.1012325**

**ABSTRACT**

Snakebite is one of the important public health problems of tropical countries including India. Siddha system of medicine has numerous preparations as indicated for treating snake bite one such novel preparation is vishasanjeevi (VS) comprises of single herbal component that is leaf of *Nicotiana tabacum*. The main aim of the present study is to carry out the toxicity profile of the formulation vishasanjeevi in both male and female wistar rats. In the acute study, a single dose of 5, 50, 300 and 2000 mg/kg was orally administered once and monitored for 14 days. In the sub-acute study, repeated doses (73.8, 184.5 and 369 mg/200g body weight/day) of the test drug vishasanjeevi were administered for a period of 28 days. Results of the acute toxicity study indicate that there was no mortality up to a maximum dose of 2000 mg/kg of vishasanjeevi administered rats. No significant changes in body weight and other clinical signs including gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuromuscular coordination of the vishasanjeevi treated rats. In sub-acute study there was a marked decrease in serum SGOT and SGPT level in vishasanjeevi treated rats when compare to that of the control group. Histological observation depicts mild degeneration and inflammatory changes in Low, Mid and high dose treated group. In conclusion the formulation vishasanjeevi was good safety index but at the same time, shown to have interaction with vital organs such as liver and kidney with fluctuation in biochemical index, there by long term exposure of drug requires extra precautionary measures.

**Keywords:** Siddha, *Nicotiana tabacum*, Vishasanjeevi, Safety profile, OECD, Acute, Sub-acute toxicity, Histopathological parameters

## INTRODUCTION

Medicinal plants play a vital role on the surmounting the dreadful diseases that affects the human life consistently since centuries back. Indian system of medicine is provoked by having the bioactive phototherapeutics as its innumerable part in several formulations. Numerous single herb Siddha formulations also strongly advocate the potential role of medicinal herb in treating snake poison. Research on the therapeutic potential of plants has surged over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases.[1] However, while voluminous pharmacological studies have been conducted to ascertain the subjective traditional uses of various medicinal plants, very few plants have been thoroughly evaluated for their detrimental effect. Reports of efficacy are, by far, more numerous than those on toxicity.[2,3] There is, therefore, a need to further the investigation of herbal remedies and phytochemicals to incorporate the observations of short and long-term toxicity manifestations and to ensure effectual open communication of such findings.

*Nicotiana tabacum* (Tobacco) belongs to a family of Solanaceae. It is native to tropical and subtropical America but today it is cultivated throughout the world. In India, the leaves of tobacco plant have been used as a sedative, antispasmodic, vermifuge, antiseptic, emetic and narcotic. The decoction of leaves also applied for muscle relaxation and relieving pain.[4] Discarded tobacco leaves are valuable because of the presence of bioactive compound. However, tobacco leaf is rich in polyphenols which possess various bioactive that affect the quality of tobacco leaf.[5,6] Nicotine, which is isolated from leaves of tobacco in associate with zinc has shown antibacterial activity against ten different strains of gram positive and gram-negative bacterial strain. The anti-nociceptive activities of methanolic leaf extract of tobacco using by tail immersion, hot plate and acetic acid has revealed the abdominal constrictions in albino Wistar mice.[7] Tobacco has also known for its antifungal activity.[8] As a traditional medicine, for the treatment of tuberculosis and coughs were also screened for activity against *Mycobacterium tuberculosis*.[9]

Still now there is no proper documentation with respect to the safety nature of this novel medicinal herb, hence present study aimed at toxicity profile of extrapolating the toxicity profile of the formulation vishasanjeevi using acute (OECD 423) and sub-acute (OECD 407) repeated oral toxicity studies in both male and female wistar rats in accordance with regulatory guidelines.

## MATERIALS AND METHODS

### Animal

Healthy adult Wistar albino rats were used for the study. The animals were housed in polypropylene cages and were kept in well ventilated with 100% fresh air. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ± 2˚C and relative humidity 50–65%. They were provided with standard pelleted feed and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of the National institute of siddha, Chennai, Tamil Nadu, India with the IAEC approval number: NIS/IAEC/I/2013/11 dated 10.1.2013

### Acute toxicity Study

The animals were fasted overnight (08- 12 hrs) with free access to water. Study was conducted with single oral administration of study drug Vishasanjeevi (VS) at the dose of 5, 50, 300, 2000 mg/kg (p.o) to different group of experimental rats. The animals were observed continuously for the first 24 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S, C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention.[10] Body weight was recorded periodically. At the end of the experiment all animals were subjected to gross necropsy and observed for pathological changes.

### Sub-Acute Toxicity Study

Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the start of treatment. The female rats used for the study were nulliparous and non-pregnant. The animals were randomly divided into control group and drug treated groups of 40 wistar albino rats (20 males and 20 females) were selected and divided into four groups. Each group consists of 10 animals (05 Males and 05 Females). First group served as a control and the other three groups were treated with test drug vishasanjeevi (73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight) for 28 days.

The rats were weighed periodically and observed for signs of toxicity pertain to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of the 28th day, the animals were fasted overnight with free access to water. On the 29$^{th}$ day, the animals were sacrificed with excess dose of anesthesia as listed in the CPCSEA annexure. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetraacetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.[11]

### Hematological analysis

Blood samples were analyzed using established procedures using automated mind ray hematology analyzer 2800. Parameters evaluated includes Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean Cell Hemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophils, Basophils, Lymphocytes and Monocytes.

### Biochemical analysis

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.[12]

### Histopathological evaluation

Vital organs were harvested, and the histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic analysis. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.[13]

### Statistical analysis

The statistical analysis will be carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.[14]

## RESULTS

### Assessment of clinical signs in rats treated with Vishasanjeevi on Acute toxicity study

The dose of Vishasanjeevi used for acute toxicity study is 5, 50, 300, 2000 mg/kg. No mortality observed at this dose level, further no significant change with respect to clinical signs of acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

**Table 1: Clinical signs in rats on acute toxicity study**

| Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **5** | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| **50** | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| **300** | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| **2000** | + | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - |

1. Alertness; 2. Aggressiveness; 3. Pile erection; 4. Grooming; 5. Gripping; 6. Touch Response; 7. Decreased Motor Activity; 8. Tremors; 9. Convulsions; 10. Muscle Spasm; 11. Catatonia; 12. Muscle relaxant; 13. Hypnosis; 14. Analgesia; 15. Lacrimation; 16. Exophthalmos; 17. Diarrhoea; 18. Writhing; 19. Respiration; 20. Mortality

**Table 2: Body weight of Male rats in Sub-Acute toxicity study**

| Groups | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| **Control** | 173.3 ± 3.51 | 177.33 ± 2.51 | 179.66 ± 2.51 | 184.66 ± 2.51 | 187.33 ± 2.08 |
| **Low dose** | 174.2 ± 4.54 | 177.4 ± 4.92 | 180.4 ± 4.39 | 184 ± 3.67 | 187.6 ± 3.36 |
| **Mid dose** | 175 ± 5.24 | 178.2 ± 4.60 | 181 ± 4.18 | 184 ± 3.64 | 188.2 ± 3.34 |
| **High dose** | 173.4 ± 4.50 | 176.8 ± 4.76 | 179.8 ± 4.14 | 183.6 ± 3.57 | 187.6 ± 3.78 |

Values are mean ± S.D (n = 5 per group of per sex). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 3: Body weight of Female rats in Sub-Acute toxicity study**

| Groups | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| **Control** | 140 ± 4.47 | 143.8 ± 5.26 | 147.6 ± 5.54 | 152 ± 5.24 | 156.8 ± 5.26 |
| **Low dose** | 140.8 ± 4.32 | 144.2 ± 4.14 | 148.4 ± 3.97 | 152.4 ± 4.03 | 156.4 ± 4.03 |
| **Mid dose** | 140.6 ± 6.02 | 144.3 ± 5.50 | 149.3 ± 6.50 | 153.3 ± 5.03 | 156.6 ± 5.50 |
| **High dose** | 141.6 ± 5.45 | 144.2 ± 4.81 | 150.4 ± 4.50 | 153.6 ± 4.33 | 157.2 ± 5.16 |

Values are mean ± S.D (n = 5 per group of per sex). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 4: Food (g/day) intake of rats in Sub-acute toxicity study**

| Groups | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| **Control** | 51.6 ± 5.54 | 51.8 ± 5.39 | 52.1 ± 5.12 | 51.9 ± 5.56 | 52.2 ± 5.09 |
| **Low dose** | 52 ± 5.75 | 51.9 ± 5.21 | 52.3 ± 5.31 | 52 ± 4.47 | 52.2 ± 5.43 |
| **Mid dose** | 52.2 ± 5.11 | 52.3 ± 3.67 | 52.6 ± 5.73 | 51.9 ± 5.63 | 52.3 ± 5.18 |
| **High dose** | 52.1 ± 5.40 | 52.2 ± 4.44 | 52.5 ± 5.07 | 52.3 ± 4.33 | 52.6 ± 4.88 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 5: Water (ml/day) intake of rats in Sub-acute toxicity study**

| Groups | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| **Control** | 32.6 ± 2.91 | 32.9 ± 3.16 | 33 ± 3.33 | 33.1 ± 2.42 | 33.5 ± 2.92 |
| **Low dose** | 32.8 ± 4.08 | 32.9 ± 3.87 | 32.9 ± 3.65 | 33.2 ± 3.13 | 33.5 ± 2.91 |
| **Mid dose** | 32.8 ± 3.77 | 33 ± 2.36 | 33.1 ± 2.59 | 33.3 ± 2.71 | 33.3 ± 2.54 |
| **High dose** | 32.9 ± 4.32 | 33.1 ± 3.23 | 33.2 ± 2.41 | 33.3 ± 2.62 | 33.4 ± 2.33 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 6: Hematological parameters of rats treated with Vishasanjeevi in Sub-acute oral toxicity study**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **PCV (%)** | 37.66 ± 4.61 | 43.33 ± 3.26 | 45.4 ± 6.12 | 44.33 ± 7.06 |
| **RBC ($mm^3$)** | 4.12 ± 0.51 | 4.71 ± 0.36 | 4.95 ± 0.68 | 4.75 ± 0.79 |
| **Hb (g/dl)** | 12.06 ± 1.58 | 13.9 ± 1.10 | 14.6 ± 2.0 | 14.3 ± 2.24 |
| **MCV (fL)** | 91.4 ± 0.36 | 91.93 ± 0.41 | 91.91 ± 0.67 | 91.16 ± 0.85 |
| **MCH (pg)** | 29.23 ± 0.15 | 29.45 ± 0.21 | 29.51 ± 0.19 | 29.71 ± 0.31 |
| **MCHC (%)** | 32 ± 0.3 | 32.01 ± 0.27 | 32.11 ± 0.43 | 32.58 ± 0.20 |
| **TC (cells/µL)** | 8600 ± 608.27 | 8633 ± 436.65 | 8483 ± 783.36 | 8733 ± 900.37 |
| **DC – N (%)** | 29 ± 5.56 | 29.6 ± 6.84 | 33.83 ± 6.21 | 29.16 ± 4.87 |
| **DC – L (%)** | 70 ± 5.56 | 68.4 ± 6.91 | 64 ± 6.60 | 66.83 ± 7.16 |
| **DC – E (%)** | 1 ± 0 | 1.83 ± 0.98 | 2.16 ± 1.94 | 2.33 ± 1.36 |
| **DC – M (%)** | 0 | 0 | 0 | 0 |
| **PLT (Lakhs/µl)** | 2.06 ± 0.10 | 2.85 ± 0.85 | 2.35 ± 0.27 | 2.4 ± 0.33 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 7: Effect of Vishasanjeevi on Renal Parameters after 28 days Treatment**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Pl. urea (mg/dl)** | 31.33 ± 4.93 | 28.5 ± 6.3 | 30.66 ± 3.14 | 29.16 ± 5.07 |
| **Sr. creatinine (mg/dl)** | 0.97 ± 0.20 | 0.95 ± 0.14 | 0.99 ± 0.16 | 0.95 ± 0.09 |
| **Sr. uric acid (mg/dl)** | 5.46 ± 1.43 | 4.34 ± 0.85 | 4.7 ± 1.24 | 4.3 ± 0.93 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 8: Effect of Vishasanjeevi on Hepatic Parameters after 28 days Treatment**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Sr. bilirubin – Total (mg/dl)** | 0.66 ± 0.05 | 0.72 ± 0.08 | 0.75 ± 0.13 | 0.73 ± 0.16 |
| **Sr. bilirubin – Direct (mg/dl)** | 0.3 ± 0.1 | 0.34 ± 0.05 | 0.33 ± 0.05 | 0.33 ± 0.08 |
| **Sr. bilirubin- Indirect (mg/dl)** | 0.36 ± 0.05 | 0.38 ± 0.10 | 0.41 ± 0.11 | 0.4 ± 0.16 |

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 9: Effect of Vishasanjeevi on Hepatic protein Parameters after 28 days Treatment**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Sr. proteins – Total (g/dl)** | 5.93 ± 0.25 | 5.78 ± 0.31 | 5.75 ± 0.20 | 5.73 ± 0.35 |
| **Albumin (g/dl)** | 3 ± 0.35 | 3.18 ± 0.10 | 3.36 ± 0.27 | 3.13 ± 0.37 |
| **Globulin (g/dl)** | 2.9 ± 0.26 | 2.6 ± 0.27 | 2.55 ± 0.36 | 2.6 ± 0.51 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 10: Effect of Vishasanjeevi on Hepatic enzyme markers (Liver function) after 28 days Treatment**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **SGOT (IU/L)** | 175.66 ± 65.75 | 147 ± 21.50 | 142.5 ± 15.64 | 106.66 ± 34.33 |
| **SGPT (IU/L)** | 200 ± 53.50 | 152.2 ± 24.28 | 155.33 ± 17.9 | 122.33 ± 32.63 |
| **Sr. ALP (IU/L)** | 574.66 ± 175.44 | 418.8 ± 162.93 | 526.83 ± 99.18 | 470.16 ± 193.3 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 11: Effect of Vishasanjeevi on Serum Lipid profile of rats after 28 days Treatment**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Total Chol. (mg/dl)** | 75.33 ± 12.74 | 83.4 ± 8.44 | 85.16 ± 34.75 | 84.66 ± 5.81 |
| **TGL(mg/dl)** | 83.66 ± 28.88 | 84.2 ± 22.99 | 87.83 ± 29.6 | 88.66 ± 27.94 |
| **HDL(mg/dl)** | 24 ± 3.60 | 22.8 ± 4.14 | 24.66 ± 3.32 | 23.5 ± 4.03 |
| **LDL(mg/dl)** | 34.66 ± 10.69 | 41 ± 10.90 | 42.33 ± 49.33 | 40.83 ± 12.05 |
| **VLDL(mg/dl)** | 16.66 ± 5.50 | 16.6 ± 4.39 | 19.16 ± 13.81 | 18.66 ± 5.42 |
| **Sugar (R) (mg/dl)** | 90 ± 20.88 | 87.3 ± 7.57 | 86.66 ± 27.54 | 88.33 ± 30.34 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Table 12: Quantitative data on absolute Organ weight (gm) of rats in sub-acute toxicity study**

| Organs | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Heart** | 0.53 ± 0.10 | 0.58 ± 0.17 | 0.62 ± 0.10 | 0.68 ± 0.13 |
| **Lungs** | 1.13 ± 0.21 | 1.3 ± 0.34 | 1.75 ± 0.73 | 1.75 ± 0.84 |
| **Liver** | 7.24 ± 1.77 | 7.14 ± 2.29 | 7.64 ± 1.68 | 7.43 ± 1.13 |
| **Rt. Kidney** | 0.73 ± 0.08 | 0.78 ± 0.21 | 0.77 ± 0.20 | 0.74 ± 0.13 |
| **Lt. Kidney** | 0.79 ± 0.09 | 0.78 ± 0.21 | 0.75 ± 0.19 | 0.73 ± 0.15 |
| **Spleen** | 0.47 ± 0.21 | 0.44 ± 0.09 | 0.53 ± 0.13 | 0.48 ± 0.10 |
| **Brain** | 1.41 ± 0.12 | 1.55 ± 0.08 | 1.62 ± 0.09 | 1.58 ± 0.11 |
| **Stomach** | 1.26 ± 0.16 | 1.41 ± 0.09 | 1.38 ± 0.18 | 1.37 ± 0.21 |

Values are mean ± S.D (n = 10 per group of which 5 males and 5 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test

**Effect of Vishasanjeevi on Body weight of Rats in Sub-acute toxicity study**

No significant change was observed in body weight of both male and female rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 2 and 3.

**Quantitative data on the food and water intake of rats treated with Vishasanjeevi for 28 days in Sub-acute toxicity study**

No statistically significant differences were recorded in food and water intake observation of rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 4 and 5.

**Effect of Vishasanjeevi on Hematological parameters of rats in Sub-acute oral toxicity study**

No statistically significant differences were recorded in hematological parameters of rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 6.

**Effect of Vishasanjeevi on Renal biochemistry profile of rats in sub-acute toxicity study**

No statistically significant differences were recorded in serum renal biochemistry parameters of rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 7.

**Serum Hepatic Bio-chemistry profile of rats in Sub-acute oral toxicity study**

No statistically significant differences were recorded in serum hepatic biochemistry parameters except the level of SGOT and SGPT of rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 8 - 10.

**Effect of Vishasanjeevi on Serum Lipid profile of rats in sub-acute toxicity study**

No statistically significant differences were recorded in serum lipid profile parameters except the rats treated with Vishasanjeevi at 73.8 mg/200 g, 184.5 mg/200 g and 369 mg/200 g body weight. The results were tabulated in Table 11.

**Quantitative data on absolute Organ weight of male rats belongs to control and drug treated group in sub-acute toxicity study**

No statistically significant differences were recorded in organ weight of male rats treated with Vishasanjeevi at low, mid and high dose. The results were tabulated in Table 12.

**Effect of Vishasanjeevi on Histopathological changes of Male rat in Sub-acute oral toxicity study**

Microscopic observation of vital organs belongs to male rats presenting the following architecture as shown in Figure 1.

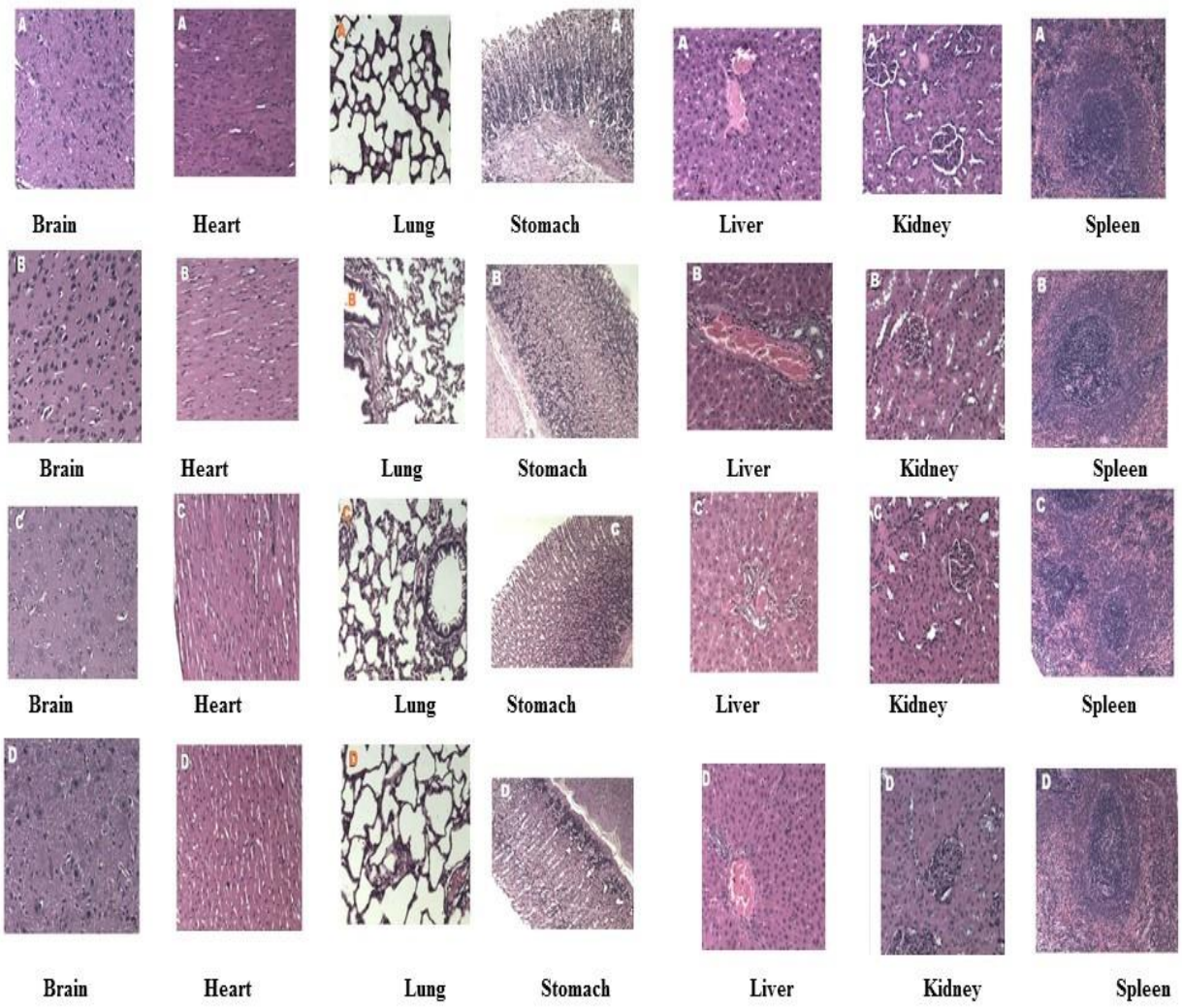


**Figure 1: Histopathology of rats belongs to control, Low, Mid and high dose treated group**

## DISCUSSION

Medicinal plants and plant based natural products have been reported to possess anti venomous properties assayed in laboratories and correlating them with ethno pharmacological studies.[15] Natural inhibitors of snake venoms have been reported by researcher in recent times.[16] Certain compounds such as β-sitosterol, stigmasterol[17], other poly phenols isolated from plants were found to be effective against snake venom.[18] Some plants used for snake venom neutralization traditionally have been tested pharmacologically for their anti-snake venom efficacy. Folk plants against snakebites are in traditional practice since ancient time in Southern part of Tamil Nadu, India.[19] Anti-snake venom botanicals from ethnomedicine and their pharmacological and clinical studies have been reported.[20]

As per the regulatory and safety standard requirements it become mandate for the researcher and investigators to ascertain the dose optimization and validation procedure for investigational products before extrapolating actual therapeutic trials.[21,22] This core practice enhance the potential of evaluating of safety and benefits on those who volunteering the trials pertains to herbal preparation. These standardized operational procedures provoke exemplary way on careful monitoring of drug induced adverse events and also to formulate the risk- benefit ratio determination in clinical trial programme.[23]

In acute toxicity study, there was no mortality up to a maximum dose of 2000 mg/kg body weight of Vishasanjeevi after per oral administration. The changes in body weight and other Clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuromuscular coordination have been used as an indicator of adverse effect. Since no remarkable changes were observed in animal behavior, body weight and organ weight at dose in treated rats as compared to control group, it can be inferred that siddha formulation Vishasanjeevi is nontoxic at the administered dose up to 2000 mg/kg.

Results of the present investigation showed that there was no sign of toxicity and no mortality after single and repeated administration of the test drug Vishasanjeevi at varying doses (73.8 mg, 184.5 mg and 369 mg/200 g body weight) in tested rats. There was no significant difference in mean body weight, food/water intake, behavioral, C.N.S, C.V.S, A.N.S vitals in control and test group rats. Further no changes in the gross observation of all the vital organs in both male and female rats. Single and repeated oral administration of the siddha drug Vishasanjeevi may be safe and considered as relatively non-toxic at the tested dose levels.

## CONCLUSION

The results of the present study have strongly suggested that the siddha drug vishasanjeevi is safe and well tolerated at the tested oral doses in both acute and sub- acute toxicity studies, since no deleterious changes were observed in animal clinical signs, behavior, hematology, serum biochemistry and histopathological parameters. Further the drug has a wide margin of safety and shall be advised for therapeutic benefits at clinical level with proper preclinical validation. Hence from the results, it was concluded that the drug vishasanjeevi were safe and no significant toxicity related events will be encountered during the study.

## ACKNOWLEDGEMENT

I wish to thank the Director of NIS and the faculties of Department of Nanju Maruthuvam, NIS for their support in carrying out the study. I wish to acknowledge my thanks to The Noble research solutions, Chennai, Tamil Nadu, India for their technical assistance in publishing this research work.

## REFERENCES

1. Benzie IF, Wachtel-Galor S. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. CRC Press-Taylor and Francis; Boca Raton, FL, USA; 2011. Herbal Medicine.
2. Ekor M. The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. Frontiers in Pharmacology 2014; 4: 1-14.
3. Chalut DS. Toxicological risks of herbal remedies. Paediatr. Child Health 1999; 4: 536–538.
4. Zaidi MI, Wattoo FH, Wattoo MHS and Tirmizi SA: Anti-bacterial activities of nicotine and its Zinc complex. African Journal of Microbiology Research 2012; 6: 5134- 5137.
5. Ruiz JM, Bretones G, Baghour M, Ragala L, Belakbir A and Romero L: Relationship between boron and phenolic metabolism in tobacco leaves. Phytochemistry 1998; 48: 269-272.
6. Bazinet L, De Grandpre Y, Porter A. Electromigration of tobacco polyphenols. Separation and Purification Technology 2005; 41: 101-107.
7. Trease WGE, Vans CE. Pharmacology. Bailliere Tindall, London 1996; 113: 89- 122, 313-544.
8. Ponstein AS, Vloemans SAB, Buurlage MBS, Elzen PJM, Melchers LS, Cornelissen BJC. A Novel Pathogen and Wound Inducible Tobacco (*Nicotiana tabacum*) Protein with Antifungal Activity. Plant Physiology 1994; 104: 109-118.
9. Adeleye A, Conubogu V and Ayolabi CI: Screening of crude extracts of twelve medicinal plants and "wonder cure" concoction used in Nigeria unorthodox medicine for activity against *Mycobacterium tuberculosis* from tuberculosis patient's sputum. African Journal of Biotechnology 2008; 7: 3182-3187.
10. OECD guideline for testing of chemicals. Guideline 423; 2001.
11. OECD Guide lines 407 for testing of chemicals. Repeated dose 28-Day Oral Toxicity Study in Rodents; 2008. p. 2- 8.
12. Jain N, Sharma P, Sharma N, Joshi S C. Haemato-biochemical profile following sub acute toxicity of malathion in male albino rats. Pharmacology online 2009; 2: 500–506.
13. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 7th ed, Churchill Livingstone, London; 2013.
14. Visweswara Rao. Biostatistics, A manual of statistic methods for use in Health, Nutrition and Anthropology, Rajkamal Electrical press, Delhi; 2007. p. 226-312.
15. Soares AM, Ticli FK, Marcussi S, Lourenço MV, Januário AH, Sampaio SV, Giglio JR, Lomonte B, Pereira PS. Medicinal plants with inhibitory properties against snake venoms. Current Medicinal Chemistry 2005; 12: 2625–2641.
16. Sanchez EE, Rodriguez-Acosta A. Inhibitors of snake venoms and development of new therapeutics. Immunopharmacology and Immuno toxicology 2008; 30: 647–678.
17. Gomes A, Das R, Sarkhel S, Mishra R, Mukherjee S, Bhattacharya S, Gomes A. Herbs and herbal constituents active against snakebite. Indian Journal of Experimental Biology 2010; 48: 865–878.
18. Pithayanukul P, Laovachirasuwan S, Bavovada R, Pakmanee N, Suttisri R. Anti-venom potential of butanolic extract

of *Eclipta prostrata* against Malayan pit viper venom. Journal of Ethno pharmacology 2004; 90: 347–352.
19. Samy RP, Thwin MM, Gopalakrishnakone P, Ignacimuthu S. Ethnobotanical survey of folk plants for the treatment of snakebites in Southern part of Tamil Nadu, Indian Journal of Ethno pharmacology 2008; 115(2): 302–312
20. Selvanayagam ZE, Gnanavendhan SG, Balakrishna K, Rao RB. Anti snake venom botanicals from ethnomedicine. Journal of Herbs, Spices and Medicinal Plants 1995; 2: 45–100.
21. Watanabe S, Imanishi J, Satoh M, Ozasa K. Unique place of Kampo (Japanese traditional medicine) in complementary and alternative medicine: A survey of doctors belonging to the regional medical association in Japan. The Tohoku Journal of Experimental Medicine 2001; 194: 55–63.
22. Yakubo S, Ito M, Ueda Y, Okamoto H, Kimura Y, Amano Y, Togo T, Adachi H, Mitsuma T, Watanabe K. Pattern classification in kampo medicine. Evidence-Based Complementary and Alternative Medicine 2014; 14: 1-5.
23. Mogami S, Hattori T. Beneficial effects of rikkunshito, a Japanese kampo medicine, on gastrointestinal dysfunction and anorexia in combination with Western drug: A systematic review. Evidence-Based Complementary and Alternative Medicine 2014; 14: 1-8.

**Cite this article as:**

T. Subathra *et al*. Preclinical Toxicological Investigation of Siddha formulation Visha Sanjeevi by Acute and 28 Days Repeated Oral Toxicity Studies in Wistar Rats. Int. Res. J. Pharm. 2019;10(12):30-36 http://dx.doi.org/10.7897/2230-8407.1012325

Source of support: Nil, Conflict of interest: None Declared