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# RESEARCH ARTICLE

# ACUTE ORAL TOXICITY STUDY OF *Satyrium nepalense* D. DON. TUBERS EXTRACT ON ALBINO WISTAR RATS

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## ABSTRACT

*Satyrium nepalense* D. Don. belonging to family Orchidaceae is a highly valuable ethnomedicinal herb. Its toxicity study was not conducted earlier. So, acute oral toxicity of tuber extract of *Satyrium nepalense* D. Don. was assessed in albino Wistar rats with a single dose of the extract at 300mg/ Kg body weight and 2000 mg/Kg body weight. Mortality/viability and clinical signs were recorded on test day 0 (prior to administration), after 24 hours of drug administration, on test days 7 and 14. Rats showed no clinical signs of toxicity and no gross pathological changes. All animals appeared normal throughout the experimental procedure. The seed extract was found non-toxic to rats and helped in weight gain with LD> 2000 mg/Kg body weight. The conclusion drawn from this study specify that oral administration of *Satyrium nepalense* is not connected with any toxicologically significant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts.

***Key words:*** *Satyrium nepalense tubers, Acute Toxicity, OECD Guideline 423, Lethality (LD50), Salam-mishri.*



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## INTRODUCTION

*Satyrium nepalense* D. Don. is a highly valuable ethano-medicinal plant belonging to the family Orchidaceae and usually found at higher altitudes (1500-3000 m) of the Indian Himalayan Region.[1] It is a terrestrial herb, about 30-60 cm long, with pink colour spike inflorescence, 2-3 leaves and two tubers with ariel roots underneath the ground (Gaur, 1999). Its tubers are known as *Salam-mishri* and their decoction is commonly used by local inhabitants of hilly areas of Uttarakhand (India) as an energizing tonic and also to cure diseases like fever, diarrhoea, dysentery, and malaria etc (Babbar and Singh, 2016). In some parts of western Himalayan region, it is also known as *Hathjadi* and its tubers are consumed by the native people as food, tonic, and aphrodisiac (Joshi, 2019). In Sikkim, tubers are used for reducing cold, cough, fever, and also for proper child development and growth (Panda, 2013). Phytochemical screening of its tubers showed the presence of glycosides, alkaloids, saponins, flavonoids, unsaturated sterols, and triterpenes (Mishra, 2012). This plant is also reported to exhibit various pharmacological activities like antibacterial, antifungal and antioxidant (SINGH DK, 2019). The pre-clinical safety studies on this medicinal plant are not reported till date. So, this study was designed to evaluate acute oral toxicity of *Satyrium nepalense* D. Don tubers extract on albino Wistar rats.

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## MATERIALS AND METHODS

***Plant Material:*** The tubers of *Satyrium nepalense* D. Don were collected from sloppy grass fields of Surkunda hills, Tehri Garhwal (Uttarakhand). The plant was identified from BSI with reference no.: BSI/NRC/Tech./Herb. (Ident.)/2019-20/776. The plant material was stored in ambient conditions for further study.

***Preparation of Extract:*** The powdered tubers of *Satyrium nepalense* D. Don were subjected to extraction using Ethanol: Water (50:50) by cold maceration for 72 hours. The extract was evaporated to dryness in a rotary evaporator and then stored in an airtight container.

***Experimental Animals:*** Female Wistar rats (9-11 weeks, weighing between 130-200 g) were used for the experiment as they are more sensitive for toxicity studies. All animals were maintained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (22 ± 2 °C) with access to drinking water and pellet diet (Lipton India Ltd.) ad libitum. This study was performed in a CCSEA approved laboratory under registration number 2005/PO/RcBt/S/18/CPCSEA with IAEC approval no. BMRL/AD/CPCSEA/IAEC/2020/11/2 dated 14/11/2020 following all ethical practices as laid down in the guidelines for animal care.

***Chemicals:*** The solvents and chemicals required for the study were purchase from Sigma-Aldrich, India.

# METHODOLOGY

The study procedure described in this study meet the requirements of OECD guidelines for testing of chemicals (no. 423) on conduct of Acute Oral Toxicity (Adopted: 17th December 2001).[8, 9] Female Wistar rats were randomly divided into three groups (n=3) after acclimatization for 15 days. As there was no toxicity study reported earlier for this extract, the starting dose level was selected as 300mg/kg body weight. Group I (normal control) received normal laboratory diet and water. The animals in Group II& Group III received a single dose of the extract by oral administration at 300 & 2000 mg/Kg body weight, after being fasted for approximately 18 hours but with free access to water. The food was provided again approximately 3 h after providing the dose. The extract was formulated in distilled water at a concentration of 200 mg/ml and the dose volume of 10 ml/Kg was administered.

# RESULTS AND DISCUSSION

The various observations including changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somato-motor activity and behaviour pattern in Group I, II and III are tabulated below in Table 2. Hematological findings for gross pathology are tabulated in Table no.3 and mean body weight at day 0 & Day 14 of all the groups are tabulated in Table no. 4. No clinical signs of toxicity and mortality were seen in any animals in the study. The rats in both groups were found to be clinically healthy and had a linear bodyweight increment till fourteen days. There was no gross pathological change being observed. The test drug (HAESN) was found non-toxic up to 2000 mg/kg body weight in albino Wistar rats. So, it is safe for consumption up to 2000 mg/kg in albino Wistar rats.

**Table 1. Groups with sample size & dose**

| Groups | Sample Size | Dose (Oral) |
|---|---|---|
| Group I (Control) | n=3 | 0.5 % CMC |
| Group II | n=3 (repeated twice) | 300 mg/Kg HAESN |
| Group III | n=3 (repeated twice) | 2000 mg/Kg HAESN |

**Table 2. Observations in skin, eyes, mucous membranes etc.**

| Group I, Group II & Group III | | | | | | |
|---|---|---|---|---|---|---|
| Observations | After 30 min. | After 4hr. | After 24hr. | After 48hr. | After 1 week | After 2 week |
| Skin & fur | N | N | N | N | N | N |
| Eyes | N | N | N | N | N | N |
| Mucous membrane | N | N | N | N | N | N |
| Salivation | Ab | Ab | Ab | Ab | Ab | Ab |
| Lethargy | Ab | Ab | Ab | Ab | Ab | Ab |
| Sleep | N | N | N | N | N | N |
| Convulsions | Ab | Ab | Ab | Ab | Ab | Ab |
| Diarrhoea | Ab | Ab | Ab | Ab | Ab | Ab |
| Morbidity | Ab | Ab | Ab | Ab | Ab | Ab |
| Mortality | Ab | Ab | Ab | Ab | Ab | Ab |

**Table 3. Haematology findings**

| S.No. | Parameters | Group I | Group II | Group III |
|---|---|---|---|---|
| 1. | Hb (g/dL) | 13.3 ± 0.4 | 12.6 ± 0.5 | 12.2 ± 0.4 |
| 2. | TLC (cells/cu.mm) | 5721.5.5 ± 106.2 | 5770.3 ± 100.8 | 5716.6 ± 102.6 |
| 3. | Neutrophil (%) | 47.83 ± 1.7 | 48.5 ± 1.5 | 49.5 ± 1.8 |
| 4. | Lymphocyte (%) | 36.66 ± 1.66 | 36.67 ± 1.6 | 39.83 ± 1.6 |
| 5. | Monocyte (%) | 3 ± 0.3 | 2.8 ± 0.3 | 3 ± 0.3 |
| 6. | Eosinophil (%) | 2.5 ± 0.2 | 2.33 ± 0.3 | 2.16 ± 0.3 |
| 7. | Basophil (%) | 1.0 ± 0.3 | 1.0 ± 0.3 | 1.0 ± 0.3 |
| 8. | RBC (millions/cu.mm) | 4.3 ± 0.3 | 4.5 ± 0.3 | 4.3 ± 0.3 |
| 9. | Total Bilirubin (mg/dL) | 0.68 ± 0.04 | 0.7 ± 0.02 | 0.6 ± 0.05 |
| 10. | SGOT (IU/L) | 83.0 ± 2.1 | 82.0 ± 2.1 | 83.1 ± 2.1 |
| 11. | SGPT (IU/L) | 39.5 ± 1.7 | 40.3 ± 1.6 | 39.8 ± 1.7 |

**Table 4. Mean Body weight**

| Body Weight (gm) | Group I | Group II | Group III |
|---|---|---|---|
| Day 0 | 39.8 ± 1.7 | 148.16 ± 2.5 | 143 ± 2.9 |
| Day 14 | 163.5 ± 1.8 | 159.3 ± 1.3 | 156.17 ± 2.3 |

The animals were observed daily during the acclimatization period, mortality/viability and clinical signs were recorded. All animals were observed for clinical signs during first 30 min and at approx. 1, 2, 3 and 4 h after administration on test day 0 and once daily during test days 1-14. Mortality/viability was recorded during first 30 minutes and at approx. 1, 2, 3 and 4 h after administration on test day 0 and twice daily during days 1-14 (at least once on day of sacrifice). Body weights were recorded on test day 0 (prior to administration), test days 7, 14 and at death. All the animals were sacrificed at the end of the observation period under Halothane anaesthesia in euthanasia chamber and discarded after gross/ macroscopic pathological changes were observed and recorded.

***Statistics:*** Body weight & haematology data was evaluated by One-way ANOVA. Values were expressed as mean ± SEM. P value (p)< 0.05 or < 0.001 was considered to be statistically significant as compared to control.

# CONCLUSION

Hydroalcoholic extract of tubers of *Satyrium nepalense* D. Don. was assessed for its oral toxicity in the rats. It was found that the high dosage (2000mg/Kg body weight) of the extract was non-toxic to rats and helped in weight gain. To summarize, the results of this study collectively specify that oral administration of tubers of *Satyrium nepalense* D. Don. is not connected with any toxicologically significant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts.

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