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Original Article

An Oral Acute Toxicity Study of Extracts from *Salvia Splendens* (Scarlet Sage) As Per OECD Guidelines 423

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ABSTRACT

Toxicology may be defined as the study of harmful, poisonous and adverse effects of drugs and other chemicals constituents found in plants, which may increase the chances of mortality or weakness in the general health, physically as well as mentally. The purpose of the study was to test the acute oral toxicity of the extracts of the plant. Acute toxicity of petroleum ether, ethyl acetate and methanol fractionate of *Salvia Splendens* (Scarlet Sage) *was* evaluated in Swiss mice. The acute toxicity studies were carried out based on OECD guidelines 423 and fixed dosage studies was adopted where the limit dose is 2000 mg/kg body weight of test animal. The animals were orally administered a single dose of 5, 50, 300, 2000 mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000 mg/kg body weight) did not produce mortality or changes in general behavior of the test animals. These results indicate the safety of the oral administration of petroleum ether, ethyl acetate and methanol fractionate of *Salvia Splendens* (Scarlet Sage)

Key Words: *Salvia Splendens* (Scarlet Sage), Acute Oral Toxicity, OECD Guidelines 423, Lethality (LD_{50}).

INTRODUCTION

Salvia Splendens of family Lamiaceae/Labiatae (Mint family) is commonly known as Scarlet sage. It is perennial native to Brazil, growing at high altitude with high humidity. It reaches 1.3 m tall . *Salvia Splendens* possesses phyto-chemicals which are of Medicinal importance.[1-5] Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used. The main aim of our study was to evaluate the extracts for their toxic effects before it can be used for applications that are of importance to the public. Hence the of petroleum ether, ethyl acetate and methanolic fractionate extract of *Salvia Spendens* were analysed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000 mg/kg body weight was used following OECD guidelines 9 10. Depending on the duration of drug exposure to animals toxicological studies may be three types such acute, sub-acute and chronic toxicological studies.

In **acute toxicity studies**, single dose of drug given in large quantity to determine immediate toxic effect. Acute toxicity studies are commonly used to determine LD_{50} of drug or chemicals and natural products. In **sub-acute toxicity studies**, repeated doses of drug are given in sub-lethal quantity for a period of 15 to 20 days. Sub acute toxicity studies are used to determine effect of drug on biochemical parameters of tissues. In **chronic toxicity studies**, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic Potential of drug [5]. **Delayed death** means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period. **Dose** is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

The present study has been undertaken to estimate the toxic effects of petroleum ether, ethyl acetate and methanolic extract from *Salvia Splendens* in swiss Albino mice (female) at the dosage of 5, 50, 300 and 2000 mg/kg body weight of an animal for a period of 14 days using OECD 423, results and observation were recorded accordingly and are texted here to present publicly.

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MATERIAL AND METHODS

Plant material, Authentication and extraction procedures: *Salvia Splendens* plant were collected from Bhopal (Madhya Pradesh) and Hazaribag, (Jharkhand) and was authenticated by Dr. V.P. Prasad, Scientist-C, Botanical Survey of India, Government of India, Howrah, (West Bengal). The specimen no. PY/JVD 1026/2011 had been submitted to Faculty of Pharmaceutical sciences, Jyoti Vidhyapeeth Women's University, Jaipur (Rajasthan). The air dried leaves were made into coarse powder and extracted with methanol, ethyl acetate and petroleum ether and percentage yield were calculated. The dried Plant were extracted with Hot continuous soxhlet apparatus for 72 hours with three different solvents i.e., methanol, ethyl acetate and petroleum ether and concentrated to dryness under reduced temperature.

Experimental Animals: Animals were selected as per the OECD guidelines. Healthy young and nulliporous, non pregnant Swiss female mice weighing from 20-30 g of 2 – 4 weeks old were selected, because literature survey of LD_{50} Test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive [6]. To acclimatize with the laboratory conditions, randomly selected animals marked to permit individual identification were kept in clean polypropylene cages for 5 days prior to start an experiment.

Table 1: Following laboratory conditions were maintained As Per OECD 423

S. No.	Condition	Requirement
1	Room Temperature	220C (±30C)
2	Humidity	50 to 60%
3	Light and Dark Period	12/12 Hours
4	Bedding	Clean Sterilized Husk
5	Oral Feed	Conventional Laboratory Diets, Like Standard Pellet Chow.
6	Distilled Drinking Water	Unlimited Supply

Mode of administration: The test substance was administered in a single dose by gavage using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).[7]

Administration of doses: Paragraph 16 of OECD guideline 423 suggests that The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. [7] Paragraph 16 of OECD guideline 423 suggests that Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the period. [7]

Test substance administration volume: The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

Number of animals and dose levels: Paragraph 18 of OECD guideline 423 suggests that three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses. [7] Paragraph 19 of OECD guideline 423 suggests that when available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight. [7] Paragraph 20 of OECD guideline 423 suggests that the time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals. [7] Paragraph 21 of OECD guideline 423 suggests that exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered (see Annex 3). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a

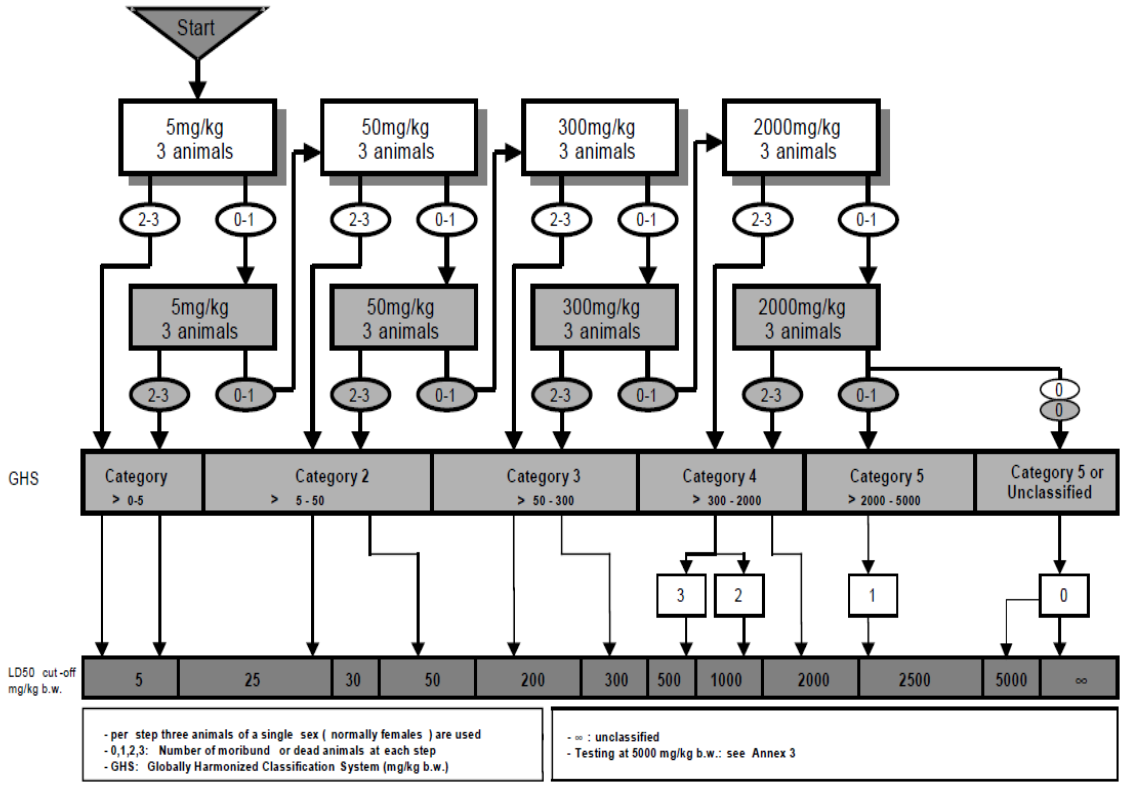
test have a direct relevance for protecting human or animal health or the environment.[7]

Methodology: Paragraph 22 of OECD Guideline 423 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed.[7] Paragraph 23 of OECD Guidelines suggests a limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals (see Annex 3). If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.[7]

Observation period: Animals were observed individually after at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes. Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation.[7]

Test Procedure followed: Prior to the dosing, the animals were fasted overnight for 24 hours. Following the period of fasting, the fasted body weight of each animal was determined as stated in paragraph 26 of OECD Guidelines 423 and the dose was calculated according to the body weight as per the Annex 2a of OECD Guidelines 423 and as stated in Paragraph 23 of OECD Guidelines 423. Annex 2a as under: [7].

ANNEX 2a: TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT



As per above Annex 2a the starting dose of 5 mg/kg body weight of an animal was used. After the different extracts of petroleum ether, ethyl acetete and methanol extracts of different parts of *Salvia Splendens* was orally administered, animals were observed keenly for about 48 hours, within the first 30 minutes time period no morbidity of animals was observed and in the next 4 hours there is also no death of the animals were found in the methanolic extracts of *Salvia Splendens*, all three animals were survived. But there is morbidity in the extracts of petroleum ether and ethyl extract of the plant.

As there is no mortality was indexed in the methanolic extracts of *Salvia Splendens* at the dosage of 5 mg/kg body weight of an animal, the same procedure was again followed with the next three animals, and the results were found same. Since then the dose was brought up to the higher limit and a dose of 2000 mg/kg body weight of an animal was introduced into the second selected group under same laboratory conditions and were fasted for overnight and the dose was given.

Signs recorded during acute toxicity studies: Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then these were maintained for a further 14 days with a daily observation. [7]

Statistical Analysis: Data are presented as a mean ± SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way ANalysis Of VAriance (ANOVA). All data were analysed using student's t-test. P values < 0.05 were considered as the level statistical significance.

RESULTS

The present study conducted as per the OECD guidelines 423 revealed that the extracts did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD_{50} was determine being in excess of 2000 mg/kg body weight. So, testing the extracts at a higher dose may not be necessary.

Table 2 and 3 indicates the parameters observed before and after the administration of the test substance for the three extracts of *Salvia Splendens*. All the parameter were observed normal in the methanolic extract of leaves , roots and stem of the *Salvia Splendens*. The animals were orally administered a single dose of 5, 50, 300, 2000 mg/kg body weight. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000 mg/kg body weight) did not produce mortality or changes in general behavior of the test animals. (Table No. 4). The medium lethal dose (LD_{50}) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. From the statistical analysis of the dosage administered to the animals, it was found that the values are significant at 5%.

DISCUSSION

From the experiment performed as per the OECD Guidelines 423, the results reveal that the methanolic extract of *Salvia Splendens* have been found non-toxic at 2000 mg/kg body weight of experimental animals as it is observed during the experiments. At lower limit dose of 5 mg/kg body weight, No significant changes were observed in body weight and wellness parameters used for evaluation of toxicity. Behavioral pattern, salivation, sleep of the treated as well as the control animals were found to be normal. Tremors, lethargy, diarrhea and coma did not occur in any of the animal. The methanolic extract is the safer extract other than petroleum ether extract and ethyl acetate extract of *Salvia Splendens*.

LD50 Value: As per observations and calculations from Acute Oral Toxicity (OECD Guidelines 423), the LD_{50} value of methanolic extract of leaves , roots and stem of *Salvia splendens* was found to be more than 5 mg/kg body weight but less than 2000 mg/kg body weight.

CONCLUSION

Methanolic extract of *Salvia Splendens* exhibit some toxic effects when given orally at concentration of more than 2000 mg/kg body weight. However the normal and insignificant changes in wellness parameters and the safe use of methanolic extract at a dose of 2000 mg/kg body weight.

Table no. 2: Effects of SSME on acute oral toxicty test in mice.

		Dose mg/kg							
S.No.	**Response**	**5**		**50**		**300**		**2000**	
		Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Aggressiveness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	Grooming	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
4	Gripping	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Touch Response	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6	Decreased Motor activity	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7	Tremors	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
8	Convulsions	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
9	Analgesia	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
10	Muscle spasm	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
11	Catatonia	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
12	Muscle Relaxant	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Hypnosis	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
14	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Exophthalmos	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
16	Diarrhoea	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
17	Writhing	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
18	Respiration	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Mortality	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

SSME – *Salvia Splendens* Methanolic Extract.

Table no. 3 : Effects of SSPEE and SSEAE on acute oral toxicty test in mice.

S.No.	Response	Dose mg/kg							
		5		50		300		2000	
		Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Aggressiveness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
3	Grooming	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
4	Gripping	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Touch Response	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6	Decreased Motor activity	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
7	Tremors	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Positive
8	Convulsions	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
9	Analgesia	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
10	Muscle spasm	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
11	Catatonia	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
12	Muscle Relaxant	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Hypnosis	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
14	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Exophthalmos	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
16	Diarrhoea	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
17	Writhing	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
18	Respiration	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Mortality	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

SSPEE – *Salvia Splendens* Petroleum Ether Extract.
SSEAE – *Salvia Splendens* Ethyl acetate Extract.

Table no. 4 : Acute oral toxicity according to OECD-423

S.No.	Dose (mg/kg)	Leathality		
		S 1 – Methanolic Extract (leaves)	**S 2 – Methanolic Extract (Roots)**	**S 3 – Methanolic Extract (stem)**
1	5 mg/kg	0/3	0/3	0/3
2	5 mg/kg	0/3	0/3	0/3
3	50 mg/kg	0/3	0/3	0/3
4	50 mg/kg	0/3	0/3	0/3
5	300 mg/kg	0/3	0/3	0/3
6	300 mg/kg	0/3	0/3	0/3
7	2000 mg/kg	0/3	0/3	0/3
8	2000 mg/kg	0/3	0/3	0/3

S 1—Methanolic Extract of the leaves of *Salvia Splendens*.
S 2- Methanolic Extract of the Roots of *Salvia Splendens*.
S 3 – Methanolic Extract of the Stem of *Salvia Splendens*.

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