

TOXICITY AND ANTICOAGULANT ACTIVITY OF SALVIA SPLENDENS

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ABSTRACT

Toxicological sulfides carried out on aqueous extract of *Salvia splendens* commonly known as Red Sage reveals that the drug is toxic only in higher doses and causes haemorrhages. LD₅₀ of *S. splendens* is 1287.3 mg/Kg. *Salvia splendens* possesses anticoagulant property. The aqueous root extra, of *Salvia splendens* increases the clotting time of dog's plasma by increasing the normal prothrombin time from 10-15 seconds to 35 seconds. Furthermore, the anticoagulant activity depends upon the pan of the plant i.e. flowers, aerial parts and roots.

Introduction

The remarkable diverse use of the genus *Salvia* belonging to the family "Labiateae" holds a good reputation in medicinal folk lore by earning the name of "All Round Healer" (Shihkin, 1976).

Salvia splendens is commonly known, as "Red Sage" or "Scarlet salvia". *Salvia splendens* Ker.-Gawl (Nasir, 1972) is an ornamental plans. While not much data regarding its biological application and chemical evaluation is available in Eastern and Western literature, an ample data regarding other members of this genus is available which are widely used for curing skin lesions/diseases, diarrhoea, cold, fever, kidney trouble and in epilepsy etc. The major use of this genus is in beverage and culniary industry, as a perfuming and flavouring agent (Shihkin, 1976). Literature citation also reveals the presence of diterpenses, triterpenes and coumarios (Savona, 1978, 1979; Albulesco, 1978). Coumarin is an oral anticoagulant. The major pharmacological effect of oral anticoagulants is inhibition of blood clotting by interference with hepatic synthesis of vitamin K dependent clotting factors i.e. II, VII, IX & X. Literature citation also reveals that the various oral anticoagulants which are available for clinical use differ only quantitatively (Dipalma, 1980).

Keeping in mind the saying of (Goldstein, 1974). All things are poison, for there is nothing without poisonous qualities, it is only a dose which makes thing poison. It was decided to assess its toxicity and dose ratio for safe therapeutic application as well as for its anticoagulant activity.

Material and Methods

Seeds of *Salvia splendens* were cultivated under observation. It was noticed that the plant took six months to grow and to attain maturity. Furthermore, it can be cultivated twice a year. After six months, fully grown mature plants were removed from their beds and washed and dried in air for 24 hours, then chopped into small pieces. Chopped plant was soaked in commercial alcohol for 72 hours and then extracted. Three repeated extractions were taken. Then the extract was partitioned between water and petroleum ether. Aqueous layer was separated and evaporated under reduced pressure at room temperature into a semi-solid mass which was used for toxicological study and was named as aqueous part. This had a pH of 6.6 Moisture content of aerial parts of *Salvia splendens* was found to be 49.78%, Roots 53.37% and Flowers 37.65% respectively.

Toxicity Studies

Male adult healthy albino rats and mice reared at P.C.S.I.R. Laboratories at room temperature weighing 250gm and 30gm respectively were used for oral toxicity test and for Acute Intravenous Toxicity Test.

Animals used for testing were housed in polyethylene cages with sliding perforated stainless steel covers. The cages were 12.0x8.5 in at the top, 10.5x8.0 in. at the bottom and 6.5 in. high, normal feed was given to animals. Water was supplied freely by means of inverted bottles which were placed on the top of stainless steel cover. To facilitate the movement of the rats and mice in cages, saw dust was spread on the floor of cages. The saw dust was changed on the next day. Each cage contained eight animals. Cages were marked with their respective dose. Each dose was repeated thrice to confirm the results.

Oral Toxicity

The oral toxicity of *Salvia splendens* was also carried out on rats by feeding the drug for 7 days in a dose of 500, 1000, 1500, 2000 mg/kg body weight by means of Canula and Syringe. Care was taken not to injure the animal while feeding

Intravenous Toxicity

I.V. toxicity was done on healthy mice weighing 30gm. Drug in different doses as given in Table-I was injected in the tail vein. Observations were made immediately after injecting the drug and for a period of 7 days.

Prothrombin Time

Prothrombin time was calculated by CaCl_2 method as described by (Fred, 1961).

Table-1: Acute Toxicity Test in Mice

Total No. of animals	Dose in mg/30gm	Dose in mg/kg body wt.	Mortality		Percentage of Mortality
			Death	Survived	
8	80	2666.66	8	-	100%
8	70	2333.33	8	-	100%
8	60	2000.00	8	-	100%
8	50	1666.66	8	-	100%
8	40	333.33	8	-	100%
8	30	1000.00	8	-	0%

Results and Discussion

Oral Toxicity

No mortality was seen in any cage at given doses. The animals were kept under observation for 15 days and were found to be normal and healthy.

Intravenous Toxicity

Acute toxicity test was conducted to evaluate any biological effect on the body. As there is a rapid systemic distribution of the test material or drug through out the animal body in a short time. Test material reaches all organs of the body in a period of time limited only by the time required for the blood to circulate and the time necessary for translocation of the test material from capillaries to extracellular fluid.

For determining acute toxicity, first an initial rough dose of 80mg/30gm i.e. 2666.66 mg/Kg body weight was given. The dose was found to be highly lethal. The dose range was then gradually narrowed by reducing 10mg each time ultimately a dose of 30mg/30gm i.e. 1000 mg/kg body weight was achieved which showed no mortality as shown in Table-I.

MLD50 (Minimum lethal dose which kills 50% of animal) was calculated by further increasing the dose from 1000mg/kg. MLD50 of *Salvia splendens* was found to be 1287.33mg/kg (or 37.5mg/30gm). While a dose of 1166.66mg/kg was found to be safe and effective as no mortality was observed at this concentration. Dose of 1200mg/kg exhibited 10%. 1233.33mg - 30%, 1266.66mg-60% and 1287. 33mg-50% mortality respectively as shown in Table-II. The thresh-hold of the drug starts at a dose of 1200mg/kg.

Table-II: Minimum lethal Dose and Safe Effective Dose

Dose in mg/kg	No. of animals	Death	Survival	%Mortality	LD50	F.D.
1000	10	-	10	0%	-	-
1166.66	10	-	10	0%	-	E.D.
1200	10	1	9	10%	-	Thresh-hold
1233.33	10	3	7	30 %	-	-
1266.66	10	6	4		-	-
1287.33		3	5	50 %	LD50	-

All animals exhibited following signs and symptoms when the drug was used intravenously. In higher doses all animals exhibited decreased locomotor activity and sensitivity to sound. Loss of consciousness, Tremor, Rapid shallow respiration. Right hind limb was drawn inside while left hind limb was extended or stretched outward. Body stretched and lower abdominal portions of the body seemed to be paralysed because during walking animal dragged the lower portion on the ground. In higher doses animal took no time to die but as the concentration or the strength of the drug was lowered the animal took 1-3 minutes to die. Autopsy revealed presence of fluid and blood in abdominal cavity. On gross examination there were haemorrhagic spots on heart, liver and lung while G.I. tract was normal. There was retention of urine and bladder was swollen like a balloon. From the observations made, it can be concluded that the aqueous extract of *Salvia splendens* might act by decreasing the tone of the fundus of the bladder or by increasing the tone of the vesical sphincter which results in the retention of urine.

Aqueous extract of *Salvia splendens* was tested for its anticoagulant activity. The plant was found to increase the clotting time. Normal prothrombin time of dog was found to be increased from 35 seconds to 3.15 minutes (30mg dose). The increase in clotting time depends upon the concentration of drug and the part of the plant used. Roots of *Salvia splendens* were found to have more activity as compared to aerial parts and flowers. Presence of coumarin has been reported in Labiateae family and also in *Salvia splendens* (Albulesco, 1978). As the drug was found to increase the prothrombin time which is a main character of coumarin compound. Prolongation of prothrombin time depends upon the suppression of the formation of factors II, VII, IX and X by the liver commonly (Hull, 1978) known as Prothrombin, Hageman factor, Christmas factor and Stuart factor.

Autopsy finding revealed the presence of fluid and blood in the abdominal cavity of the animal which indicated that this may be due to direct effect of over dosage which resulted in marked hypoprothrombinemia and haemorrhages. Haemorrhage is a main major character which ultimately resulted in death.

Conclusion

From the above it can be concluded that *Salvia splendens* can be used as an anticoagulant in a dose of 30mg. It is only toxic in higher doses while not in lower doses.

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