



Preparation of Non- toxic dose of aqueous extract of Strychnine from the stem pieces of *Strychnos-nux-vomica*.

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BACKGROUND:

Strychnine, an alkaloid derived from seeds, stem and roots of the tree *Strychnos – nux- vomica*. Interestingly, Strychnine possesses two diverse properties. It acts as a stimulating agent and on the other hand, it is also an agent causing convulsions or violent hysterical spasms and the poison acts straight away. People discovered the fatal properties of strychnine in ancient times and made use of the seeds to poison their arrow heads. Later, Europeans imported the fatal strychnine seeds from India in the 15th century to eliminate the increasing rodent populace. In Germany it was used as a rodenticide in the early 16th century. More recently, in the 19th century many physicians added very small portions of strychnine to tonics as they supposed that the substance possessed invigorating properties and was able to influence the central nervous system.

By the early 1900s, strychnine became widely available in cathartic pills and caused a significant number of fatalities from suicidal and unintentional ingestions in the United States (US). In the early 1900s, strychnine toxicity was a major cause of toxicological death in children. The number of strychnine exposures in the United States has fallen significantly since its elimination from nonprescription preparations in 1962.

Currently, physicians administer restricted measures of strychnine to enhance the activities of the muscles as well as a remedy for people suffering from alcohol poisoning or to get rid of the toxicity caused by other depressant medicines. In addition, strychnine has been found to be effective in treating certain neurological disorders. Hence, physicians use mild doses of the substance to kindle particular centers in the nervous system. Today, physicians also use controlled doses of strychnine to treat acute constipation as the substance has been found to stimulate the intestinal movements. However, this is done very selectively and only in specific cases. Recent researches have revealed strychnine can be used as a hypoglycemic, antioxidant, hypocholesteremic agents etc..

In most of the studies and the literature, many of the researchers have used the seed extracts of *Strychnos- nux- vomica* tree to get strychnine alkaloid for the observation of medicinal benefits and the toxicological effects and very few researchers had used stem part for the plant for their research. And more over, the strychnine alkaloid was extracted from the seeds by applying some physical and analytical techniques which are time consumed, laborious and costly affairs. Again it is a time consuming process to

separate the safety dosage of the strychnine from the isolated seed extract.

It is easy to isolate moderate concentrations of strychnine and other components of the stem and can be used for the study of hypoglycemic and hypo cholesterolomic effects of the diabetes induced rabbits and also for the investigations of various other activities. The aqueous extract of *Strychnos-nux- vomica* can be used for hypo glyceemic and hypocholesterelomic studies which contain the strychnine alkaloid below the toxic dosage levels. The principle behind the preparation of aqueous extract of strychnine from the stem pieces of *Strychnos- nux- vomica* is the partial solubility of the strychnine alkaloid in water.

Doubtless that the strychnine alkaloid is a toxic substance when it is consumed in higher concentrations by any animal. The LD50/ LDLo data of strychnine is given below for human and some other animals.

Human data

The maximum exposure limit is approximately 0.15 mg/m³. There is one report of one case on ingestion of 3.75 g that was not fatal (Poisindex, 1989b).

Adults

The lethal dose for adults varies. The minimal oral human lethal dose ranges from 30 to 120 mg. When given intravenously or subcutaneously, the lethal dose is significantly lower.

Children

The lethal dose in children may be as low as 15 mg (Goodman & Gilman, 1985).

Fatal doses of strychnine are reported to be as low as 5 to 10 mg, but, more significantly, survival can follow ingestion of very high doses (over 3500 mg). Ingestion of 75 one-grain tablets (4.8 g) was fatal.

Toxic strychnine blood concentration: 0.2 mg/dL; Lethal strychnine blood concentration: 0.9-1.2 mg/dL

A child died of strychnine poisoning after ingesting an unknown number of cathartic pills, each containing 0.8 mg of strychnine nitrate, an amount only twice the maximal "therapeutic" dose for a child of about the same age.

Reported Fatal Dose:

Lethal dose to man: 30-60 mg/kg. (Lewis et al , 1996)

The human lethal oral dose has been estimated to vary between 100 and 120 mg. (Zenz.C et al 1994)

The lethal dose of strychnine is reported to be approximately 15 mg for children and 30 to 100 mg for adults. Toxic strychnine blood concentration: 0.2 mg/dL; Lethal strychnine blood concentration: 0.9-1.2 mg/dL (Gossel et al 1994)

Acceptable daily intake (ADI)

The maximum permissible atmospheric concentration of strychnine is 150 μg per m^3 (Reynolds, 1982).

Animal data:

Animal	Root of Administration	LD ₅₀ / LDLo value
Rat	Oral-rat:	16 mg/kg
	Intraperitoneal	2500 $\mu\text{g}/\text{kg}$
	Subcutaneous	1200 $\mu\text{g}/\text{kg}$
Mouse	Intravenous	960 $\mu\text{g}/\text{kg}$
	Oral-mouse	2 mg/kg
	Intraperitoneal	980 $\mu\text{g}/\text{kg}$
Dog	Subcutaneous	474 $\mu\text{g}/\text{kg}$
	Intravenous	410 $\mu\text{g}/\text{kg}$
	Oral	1100 $\mu\text{g}/\text{kg}$
Cat	Subcutaneous	350 $\mu\text{g}/\text{kg}$
	Intravenous	250 $\mu\text{g}/\text{kg}$
	Oral	750 $\mu\text{g}/\text{kg}$
Rabbit	Subcutaneous	750 $\mu\text{g}/\text{kg}$
	Intravenous	330 $\mu\text{g}/\text{kg}$
	Oral	600 $\mu\text{g}/\text{kg}$
Pigeon	Subcutaneous	700 $\mu\text{g}/\text{kg}$
	Oral	350 $\mu\text{g}/\text{kg}$
Chicken	Subcutaneous	21 mg/kg
	Oral	1 mg/kg
Duck	Subcutaneous	3 mg/kg
	Oral	1 mg/kg
Frog	Subcutaneous	35 $\mu\text{g}/\text{kg}$

(NIOSH, 1983-84 Supplement)

Solubility:

1 g of strychnine dissolves in:

6400 mL water

3100 mL boiling water

150 mL alcohol

35 mL chloroform

180 mL benzene

200 mL toluene

260 mL methanol

320 mL glycerol

220 mL amyl alcohol

Very slightly soluble in ether.

Solubility: Very insoluble in water (0.0115 g/100 ml) but fairly soluble (20%) in chloroform.

Solubility of strychnine:

160 mg/l at 25⁰ C water

6.7 g/l ethanol

One gram dissolves in 182 ml ethanol, 6.5 ml chloroform,

150 ml benzene, 250 ml methanol, 83 ml pyridine.

Very slightly soluble in ether.

Slightly soluble in ethanol and acetone; insoluble in ethyl ether.

Soluble in chloroform, slightly soluble in alcohol and benzene, slightly soluble in ether.

In water, 160 mg/l @ 25 deg C.

Biological half-life by route of exposure:

Half-life=10 hours.

Strychnine concentrations were measured in a patient who attempted suicide. The half-life of 10 hours suggested that normal hepatic function can efficiently degrade strychnine even when the quantity ingested is high enough to cause severe poisoning. The elimination constant ($K_{el}=0.07 \text{ h}^{-1}$) indicates that 7% of the strychnine in the serum at any one moment would be eliminated in 1 hour (Edmunds et al.,1986).

pH: Saturated solution has pH 9.5

Strychnine is an alkaloid found in seeds of *Strychnos-nux-vomica* Loganiaceae, beans of *Strychnos ignatii*(1), *Strychnos toxifera*(4), and other species of *Strychnos*, Loganiaceae(2,3). The most important *Strychnos* species are *S. nux vomica* L. and *S. ignatii* Berg. Seeds from these species contain 2-3% of total alkaloids, of which approximately one-half of the first and two-thirds of the second is strychnine(3,4).

The dried ripe seeds of *Strychnos nux vomica* (Loganiaceae), containing not less than 1.2% of strychnine.

The seeds of *Strychnos nux vomica* contain 1.1 to 1.4 percent of strychnine and brucine, another alkaloid.

The aqueous extract of strychnine from the stem pieces of *Strychnos-nux-vomica* is differ from the extract of seeds in many aspects. In this plant seeds, brucine concentration is greater than strychnine. But in stem, strychnine is greater in concentration than brucine. The isolation of the alkaloid from seeds needed a laborious procedure and it is generally followed methanol extract way and more over the concentration of the strychnine would be more than the lethal dose. But the aqueous extract of strychnine from stem is simple and the concentrations would be with in the safety dosage when it is used 15 grams stem in 150 ml of water for 12 hours per day.

MATERIAL AND METHODS

Plant material

Strychnous nux vomica stem pieces were collected from Srisailam, one of the great shrines of Lord Shiva, located in Nallamala forest, Andhra Pradesh state, India. Botanical identification was done by Prof. D. Durgaiyah, Director, Plant Anatomy Research Centre, Medicinal plant research unit, Hyderabad, Andhra Pradesh.

Aqueous Extraction procedure

The stem pieces (no- 5) weighed about 10- 50grams was soaked in different vessels containing 150 ml of normal drinking water each at room temperature overnight (12 hours). The stem pieces were then taken out and the aqueous extracts were ready for quantitative analysis of the alkaloid, Strychnine.

Quantitative analysis:

Calorimetric method:

By using the calorimetric methods, it was found that 150 ml of the aqueous extract of *strychnos- nux- vomica* stem contains 0.720 mg strychnine. Though the strychnine alkaloid is insoluble in water, it has partial soluble nature in water. The extracts were prepared freshly whenever required and the stem pieces were reused repetitively for 4 times.

The concentration of the strychnine in aqueous extracts of *Strychnos- nux- vomica* stem is constant at room temperature.

Table-1 Concentration of the strychnine in aqueous extract of 10 grams of stem of *Strychnos- nux- vomica* is soaked in various volumes of drinking water. Stem weight- 10 gms.

Volume of water(in ml)	Day-1(mg)	Day-7(mg)	Day-14(mg)
50	0.160	0.120	0.043
100	0.325	0.247	0.102
150	0.480	0.360	0.157
200	0.620	0.457	0.203
250	0.740	0.549	0.245

Change of concentration of strychnine with dilution

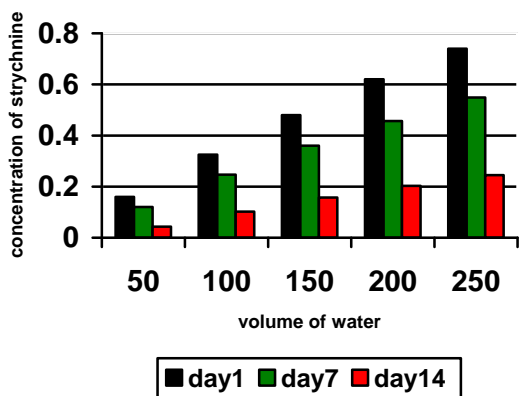
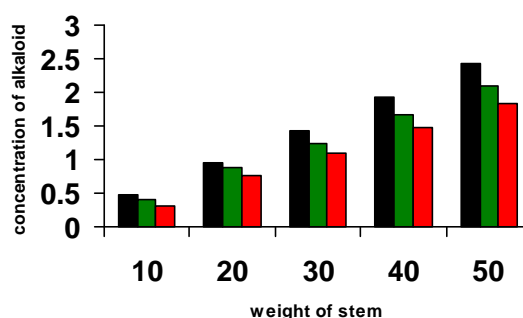


Table-2 Concentrations of strychnine in aqueous extract of various weights of stem of *Strychnos- nux- vomica* soaked in 150 ml of water.

Weight of the stem(in mg)	Day-1(mg)	Day-7(mg)	Day-14(mg)
10	0.480	0.373	0.294
20	0.946	0.748	0.584
30	1.435	1.126	0.885
40	1.935	1.487	1.176
50	2.426	1.889	1.478

Change of concentration of strychnine with weight



In general 100 gm of *Strychnos- nux- vomica* stem contains 500- 800 mg of strychnine alkaloid. The stem piece taken for the investigation had 675.245 mg of strychnine determined by methanol extract method. And other same stem piece which had same weight was used for determination of concentration of strychnine in aqueous extract. On continuous soaking of the stem piece for 12 hours daily for 49 days in 150 ml of water, gradually the strychnine concentration decreases in the water due to gradual decreased rate of diffusion of the alkaloid from the stem into water and very minute quantities were seen after 6 weeks and became zero concentration on 49th day in the water. The total strychnine concentration found in aqueous extract for the total 48 days was 116.930 mg and the remaining strychnine 558.315mg was remaining in stem and did not diffuse from the stem. That can be obtaining only by powdered form through methanol extract procedure.

Table: 3 The reducing concentration (mg) values of strychnine of the aqueous extract of *Strychnos- nux- vomica* stem piece having 100 gm weight soaked for 49 days

1 week (Day 1-7)	2 week (Day8-14)	3 week (Day 15-21)	4 week (Day22-28)	5 week (Day29-35)	6 week (Day36-42)	7 week (43-49)
4.820	3.582	2.876	2.429	2.161	2.060	0.901
4.628	3.447	2.793	2.380	2.136	2.048	0.742
4.427	3.317	2.724	2.334	2.117	2.031	0.579
4.246	3.207	2.657	2.294	2.098	1.846	0.421
4.067	3.109	2.597	2.256	2.088	1.579	0.326
3.896	3.024	2.542	2.221	2.080	1.252	0.120
3.732	2.944	2.481	2.188	2.075	1.052	0.000

RESULTS

The property of partial solubility of strychnine is helped in preparation of aqueous extract of strychnine of *Strychnos-nux-vomica* stem. The ratio of the dissolution of the alkaloid from the stem pieces was constant. The rate of diffusion of alkaloid from the stem pieces of *Strychnos-nux-vomica* is same in all cases and in all conditions. When 10 gm of stem pieces were soaked in 10, 20, 30, 40 and 50 ml water, the concentration of alkaloid diffused out was directly proportional to the volume of water (Table- 1). In this context, the weight of the stem is constant (10 gm) and volume of water differs.

In an another observation, when different weights of stem pieces were soaked in 150 ml of different water samples, the diffusion rate of strychnine was found to be rationale. It was also observed, the concentration of alkaloid diffused into 150 ml of water from the various stem piece weights was also constant. When the weights are gradually increasing, the alkaloid concentration was also increased. The concentration of alkaloid diffused was directly proportional to weights of stem pieces (Table-2). Here the volume of water is same and weight of stem is changed.

100 gm of *Strychnos-nux-vomica* stem contains total 675.245 mg of strychnine alkaloid which is lower than the seed's alkaloid concentration. The total amount of alkaloid which is diffused from the 100 gm of stem pieces soaked in 150 ml of water for 12 hours/ day for 49 days was found 116.930 mg and remaining 558.315 mg was there still in the stem piece which could not be extracted through diffusion in water (Table-3). And that is not extracted out through water.

DISCUSSION

The safety dosage of strychnine alkaloid can be obtained through the aqueous extract method rather than other methods like methanol extract. In methanol extraction method, the stem pieces are dried and powdered and soaked in methanol and total alkaloid is extracted, but this bulk concentration of alkaloid is not safe to use for treatment. It has to be weighed and take in required ranges and dissolved in water for then ready for treatment. 15 gm of stem soaked in 150 ml of water for 12 hours/day for 10-15 days can provide effective concentrations of strychnine alkaloid.

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