

# Preparation of non-toxic dosage of aqueous extract of Strychnine alkaloid from the stem pieces of *Strychnos- nux- vomica* plant for the controlling of blood sugar level in Diabetes induced rabbits.

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## Abstract:

Diabetes, a metabolic disorder, at its simplest explanation, is a condition in which high blood glucose levels known as hyperglycaemia is observed due to the failure of the body in metabolizing the blood glucose properly. The ancient treatment for controlling blood sugar level was started with herbals. Though various ways are available today to control the blood sugar level, the most following and dependable safe way is Ayurvedic treatment at present. Among those herbal treatments, one of the traditional methods is strychnine alkaloid treatment. In some of the states of India especially Chhattisgarh, Jharkhand, Uttaranchal, Kerala and Andhra Pradesh, still the tribes and some other people have been following the use of aqueous extract of strychnine alkaloid for reducing the over blood sugar level.

There are several reports available regarding the harmful effect of over dosage of strychnine consumption. The consumption of over dosage of strychnine is very dangerous which causes immediate hypoglycaemic and neurological disorders and leads to death also observed in Human beings. In view of this, a safe dosage of strychnine alkaloid extract method is explained with its hypoglycaemic effect on Alloxan induced diabetes rabbits in this article.

The aqueous extract of strychnine alkaloid from stem pieces of the plant *strychnos- nux- vomica* is very simple, cheap and the concentrations would be within the safety dosage when certain quantity of stem piece and specific amount water is used. Depending upon the principle, the partial solubility of the strychnine alkaloid in normal water, the aqueous extract is prepared. When it is used a 15 grams of piece of stem, soaked in 150 ml of drinking water for only 12 hours per night and reused for 15 days at room temperature, The alkaloid concentration in aqueous extract is found in the range between 0.720 mg to 0.328 mg from day 1 to 15 respectively which is a non toxic dosage and effectively reduced blood glucose levels in diabetes induced rabbits. The concentration of the alkaloid in the aqueous extract is measured calorimetrically.

Almost all the vegetative and reproductory parts of the plant body are distributed with strychnine alkaloid in different concentrations. The preparation of aqueous extract of the alkaloid strychnine from the stem pieces of *Strychnos- nux- vomica* is differ from the seeds in many aspects. In this plant seeds, the alkaloid Brucine (2,3- dimethoxy strychnine) 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. The aqueous extract is done in different conditions. Different weights of the stem pieces were taken in constant volume of water and found the concentration of diffused alkaloid for unit of time was directly proportional to the weight of the stem. In another case, specific weight of stem pieces were taken in different volumes of water and the concentration of diffused alkaloid for unit of time was found directly proportional to the volume of water.

The aqueous extract of strychnine from stem is simple and the concentrations would be within the safety dosage when it is used 15 grams stem piece in 150 ml of water for 12 hours per day. This aqueous extract contains 0.720 mg of alkaloid which is a safety dosage for the rabbit and effectively reduced the blood sugar level from 343 mg/dl to 220mg/dl in alloxan induced diabetes rabbits.

**Key Words:** Hyperglycaemia, Diabetes mellitus, Cathartic pills, Hypocholesteremic effect, Sinocular puncture

## INTRODUCTION:

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease, so India became 'Diabetes capital' of the world. In 2000, India (31.7 million) topped the world with the highest number of patients with diabetes mellitus followed by China (20.8million) with the United States (17.7 million) second and third places respectively. According to Wild et al., The prevalence of Diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. Due to high prevalence of diabetes worldwide, extensive research is still being

performed to develop new anti diabetic agents and determine their mechanism of action. There are different methods have been following by different physicians and different people all over the world by using allopathic, homeopathic medicines, and different surgical methods, diet control, yoga and physical exercises for controlling blood sugar level.

Strychnine is an alkaloid found in stem, roots and 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).[(1) Budavari S; The Merck Index. 12th ed. Whitehouse Station,NJ: Merck and Co., Inc. p. 1512 (1996) (2) Franz DN; p. 359 in The Pharmacological Basis of Therapeutics 5th ed; Gilman LS, Goodman A eds NY,NY: MacMillan (1975) (3) Cordell GA; Kirk-Othmer Encycl Chem Tech NY: Wiley 1: 918 (1978) (4) Kametni T et al; Ullmann's Encycl Indust Chem. Deerfield Beach,FL: VCH Publ VA1: 590 (1985)]

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.

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.

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. It is easy to isolate moderate concentrations of strychnine alkaloid and other components of the stem and can be used for the study of hypoglycaemic 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

hypoglycaemic 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/ LD Lo data of strychnine is given below for human and some other animals.

#### Human data

The maximum exposure limit is approximately 0.15 mg/m<sup>3</sup>. 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.[Ellenhorn, M.J., S. Schonwald, G. Ordog, J. Wasserberger. *Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning*. 2nd ed. Baltimore, MD: Williams and Wilkins, 1997., p. 1660]

Toxic strychnine blood concentration: 0.2 mg/dL; Lethal strychnine blood concentration: 0.9-1.2 mg/dL [Gossel, T.A., J.D. Bricker. *Principles of Clinical Toxicology*. 3rd ed. New York, NY: Raven Press, Ltd., 1994., p. 421]

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.[Hayes, W.J., Jr., E.R. Laws, Jr., (eds.). *Handbook of Pesticide Toxicology*. Volume 2. Classes of Pesticides. New York, NY: Academic Press, Inc., 1991., p. 618]

#### Reported Fatal Dose:

Lethal dose to man: 30-60 mg/kg.[Lewis, R.J. *Sax's Dangerous Properties of Industrial Materials*. 9th ed. Volumes 1-3. New York, NY: Van Nostrand Reinhold, 1996., p. 3025] T

he human lethal oral dose has been estimated to vary between 100 and 120 mg. [Zenz, C., O.B. Dickerson, E.P. Horvath. *Occupational Medicine*. 3rd ed. St. Louis, MO., 1994, p. 640]

The lethal dose of strychnine is reported to be approximately 15 mg for children and 30 to 100 mg for adults.

[Gossel, T.A., J.D. Bricker. *Principles of Clinical Toxicology*. 3rd ed. New York, NY: Raven Press, Ltd., 1994., p. 351]

**Acceptable daily intake (ADI)**

The maximum permissible atmospheric concentration of strychnine is 150  $\mu\text{g per m}^3$  (Reynolds, 1982).

**Animal data:**

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

(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 and Very slightly soluble in ether. (Sax, 1988; Windholz, 1983)  
Solubility: Very insoluble in water (0.0115 g/100 ml) but fairly soluble (20%) in chloroform.

**Solubility of strychnine:**

160 mg/L at 25<sup>0</sup> C water [USEPA-NIH; Oil and Hazardous Materials Technical Assistance Data Systems (OHM-TADS) (1986)]

6.7 g/L ethanol [Worthing, C.R., S.B. Walker (eds.). The Pesticide Manual - A World Compendium. 7th ed. Lavenham, Suffolk, Great Britain: The Lavenham Press Limited, 1983., p. 496]

1 gm dissolves in 182 ml ethanol, 6.5 ml chloroform, 150 ml benzene, 250 ml methanol, 83 ml pyridine. [Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 1512]

Very slightly soluble in ether. [Budavari, S. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., 1996., p. 1512]

Slightly soluble in ethanol and acetone; insoluble in ethyl ether. [Lide, D.R. (ed.). CRC Handbook of

Chemistry and Physics. 79th ed. Boca Raton, FL: CRC Press Inc., 1998-1999., p. 3-316]

Soluble in chloroform, slightly soluble in alcohol and benzene, slightly soluble in ether. [Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 12th ed. New York, NY: Van Nostrand Reinhold Co., 1993, p. 1097]

In water, 160 mg/l @ 25 deg C. [Seidell A; Solubilities of Organic Compounds. NY, NY: d. Van Norstrand Co., Inc (1941)]

**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 [The Merck Index. 10th ed. Rahway, New Jersey: Merck Co., Inc., 1983., p. 1268]

**MATERIAL AND METHODS****Plant material**

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

**Experimental work done at:** Chemical Analysis was done in R.V Labs, Guntur, Andhra Pradesh state, India. Research work on rabbits was done in Ranchi Veterinary College, Rabbit Farm Unit, Kanke, Jharkhand, India. The experimental protocol has been approved by the institutional animal ethics committee, proposal number being IAEC.049/2011.

**Procedure for the aqueous extraction of alkaloid strychnine:**

Though the strychnine alkaloid is insoluble in water, it has partial soluble nature in water. Based on this principle, the stem pieces are soaked in different vessels containing normal drinking water over night/12 hours at room temperature. The stem pieces were then taken out and the aqueous extracts were ready for quantitative analysis of the alkaloid, Strychnine.

The extracts were prepared freshly when ever required and the stem pieces were reused repetitively in each context.

**Quantitative analysis:****Calorimetric method:**

The concentrations of the strychnine alkaloid are measured by using the calorimetric method.

**Case-1 Constant weight of the stem and different volumes of water.**

The rate of diffusion of the alkaloid and the concentration of the strychnine in aqueous extracts of a specific quantity of *Strychnous- nux- vomica* stem is constant for various volumes of water at room temperature:

When 5 pieces of each 10 grams weight stems are taken and soaked in five different volumes of water 50, 100, 150, 200, 250 ml water respectively for 12 hours/ night, (only over nights, day time it must be removed from water) for 14 days, and the concentrations measured calorimetrically for every 7 days gap i.e on day 1,7 and 14, the concentration of the alkaloid is found to be gradually decreased rationally. Since the same piece of stem has been reusing, the rate of diffusion is decreasing from day 1 to 14. ( table-1)

When 10 gm piece of stem is soaked in 50 ml water, day-1 concentration is 0.160 mg, day -7 is 0.120mg and the day 14 concentration is 0.043 mg. The concentration of alkaloid has been decreasing gradually and the observation is same in remaining volumes of water also.

**Day-1:**The alkaloid concentration difference in 50, 100, 150, 200 and 250 ml of water for 10 gm piece of stem soaked on day 1 is almost all constant, and the difference is around 160 mg for every 50 ml volume of water increased serially.

**Day-7:**The alkaloid concentration difference in 50, 100, 150, 200 and 250 ml of water for 10 gm piece of stem on day 1 is almost all constant, and the difference is around 120 mg for every 50 ml volume of water increased serially

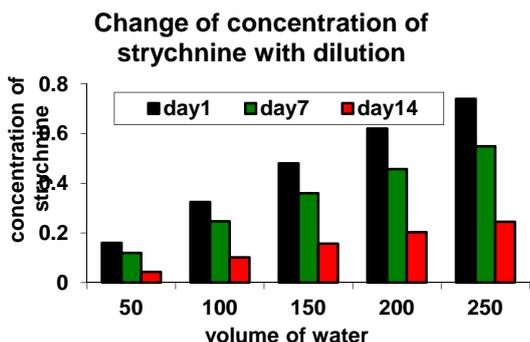
**Day-14:**The alkaloid concentration difference in 50, 100, 150, 200 and 250 ml of water for 10 gm piece of stem on day 1 is almost all constant, and the difference is around 55 mg for every 50 ml volume of water increased serially.

From this, it is observed that the rate of diffusion of the alkaloid for a specific weight of the piece of the stem is directly proportional to the volume of the water.

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

Stem weight- 10 gm. (Stem weight is constant, volume of water is different)

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



**Case-2 Constant volumes of water and different weights of the stem pieces.**

The rate of diffusion of the alkaloid and the concentration of the strychnine in aqueous extracts of a specific volume of water for various weights of the stem of *Strychnous- nux- vomica* is constant at room temperature:

When 10, 20, 30, 40 and 50 grams weights of the stem pieces are taken and soaked in 150 ml water, 12hours/night daily (removed from water in day time) and reused for 14 days, the concentration of the alkaloid diffused is constant on each day.

**Day-1**  
The alkaloid concentration difference in 10, 20, 30, 40 and 50 grams of piece of stem soaked in 150 ml water on day 1 is almost all constant and the difference is around 480 mg for every 10 gm weight of stem increased serially.

**Day-7**  
The alkaloid concentration difference in 10, 20, 30, 40 and 50 grams of piece of stem soaked in 150 ml water on day 7 is almost all constant. and the difference is around 375 mg for every 10 gm weight of stem increased serially.

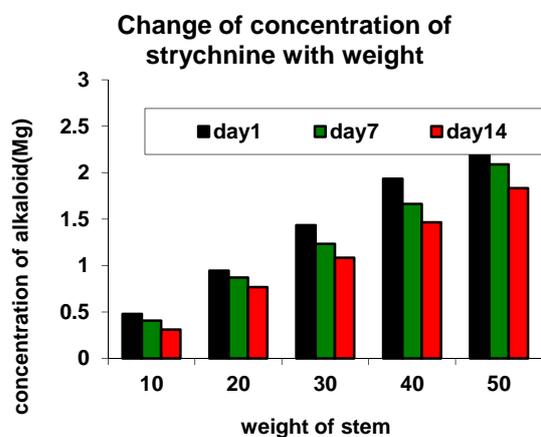
**Day-14**  
The alkaloid concentration difference in 10, 20, 30, 40 and 50 grams of piece of stem soaked in 150 ml water on day 14 is almost all constant. and the difference is around 290 mg for every 10 gm weight of stem increased serially

From this it is observed that the rate of diffusion of the alkaloid for a specific volume of water is directly proportional to the weight of the piece of the stem soaked.(Table-2)

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

Volume of water-150 ml (Volume of water is constant, weight of stem piece is different)

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



**Table: 3 The concentration (in mg) 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 (Day 8-14)	3 week (Day 15-21)	4 week (Day 22-28)	5 week (Day 29-35)	6 week (Day36-42)	7 week ( Day 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

**Case – 3 The concentration variation of 100 grams of stem piece soaked in 150 ml of water continuously for 49 days.****The rate of diffusion of the alkaloid decreases on continuous soaking of the stem piece:**

When 100 grams of the piece of stem taken and soaked in 150 ml of water daily for 12 hours/night (removed from water in day time) continuously for 49 days, the concentration gradually decreased from 4.820 mg to zero milligrams on 49<sup>th</sup> day (Table-3).

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/night, continuously 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 49<sup>th</sup> 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.

**Large scale separation of alkaloids from stem:**

Large scale separation of Strychnine from *strychnos-nux-vomica* L. can be done by p<sup>H</sup> zone refining counter current chromatography. The experiment was performed with a two phase solvent system composed of n-hexane-ethyl acetate-1-propanol- water (1 : 3: 1.5 : 4.5 v/v) where tri methyl amine(10 Mm) was added to the upper stationary phase as a retainer and HCl (5Mm) was added to the mobile phase as an eluter.

From 1.5 gm of crude extract, 176 mg of strychnine was obtained in a single run. The purity of the strychnine was 95.1% as determined by HPLC. The structure of isolated compound confirmed by Electron Ionization – Mass Spectrometer (EI- MS).[ Committee of national pharmacopoeia. Pharmacopoeia of P.R. China, Chemical industry press. Beijing 2005, 45-48; Ito y; Weisz, A. P<sup>H</sup> zone - refining counter current chromatography, US 1994; 1-7]

The aqueous extract of strychnine alkaloid from the stem pieces of *strychnos-nux-vomica* by this natural and simple

method has safe concentration of alkaloid rather than the chemical extraction method by using methanol and it can be used for decreasing the higher blood glucose level in alloxan induced diabetic rabbits.

**Hypoglycaemic effect of aqueous extract:**

15 grams of piece of *Strychnos-nux-vomica* stem is taken and soaked in 150 ml of normal drinking water for overnight for 12 hours and concentration of alkaloid is found 0.720mg. This is orally given to alloxan induced diabetic rabbits and hypoglycaemic effects of alkaloid are observed.

Newzealand white male and female rabbits weighing 1 to 1.5 Kg were used in this study. The rabbits were maintained under standard laboratory conditions at 25±2°C, relative humidity 50±15% and normal photo period (12 hr dark and 12hr light) were used for the experiment. The young animals were placed in a cage with no food for 24 hours before experiment. They were then divided into two groups, one being taken for controls. Only the most definitely healthy animals were taken. Blood-sugar was determined by MacLean's method, heart-blood being generally taken.

Diabetic mellitus in rabbits were induced by intra peritoneal injection of Alloxan 120 mg/kg dissolved in 1% citrate buffer (pH 4.5) in either sexes of New Zealand rabbits, fasted for 24 hours. Animals are considered to be diabetic if they had plasma glucose level of greater than 260 mg/dl in addition to polyuria, hyperphagia and decrease in body weight. After 96 hours of Alloxan injection, 1 ml of blood sample was withdrawn from animals by sinocular puncture under anaesthetized condition in tubes containing potassium oxalate and sodium fluoride as anticoagulant and plasma glucose level was determined using commercially available GOD POD Kit using auto analyzer.

Animals were grouped according to the approximately same plasma glucose level and body weight. The New Zealand rabbits were divided into four groups:

**Group I:** Normal control rabbits received distilled water

**Group II:** Diabetic control rabbits received Alloxan (120mg/kg)

**Group III:** Diabetic rabbits orally received aqueous extract of *Strychnos nux vomica stem* (150 ml/kg) for four weeks from the third day of Alloxan treatment.

**Group IV:** Diabetic rabbits orally received standard drug Glipizide (0.5mg/kg) for four weeks from the third day of Alloxan treatment.

**Table-4 Effect of aqueous extract of Strychnous-nux- vomica stem on the blood glucose level (BGL-mg/dl) in Alloxan induced diabetic rabbits**

S. No	Groups	0 week	1 week	2 week	3 week	4 week	Difference between 4 <sup>th</sup> and 0 week in blood sugar
1	Normal control	180 ± 5	225 ± 15	235 ± 10	238 ± 5	235 ± 5	↑035
2	Diabetic control	280 ± 4	295 ± 10	310 ± 15	340 ± 15	390 ± 10	↑110
3	Standard	350 ± 8	325 ± 5	280 ± 8	240 ± 4	198 ± 5	↓132
4	Aqueous extract	343 ± 5	300 ± 7	270 ± 3	240 ± 8	220 ± 6	↓123

Normal blood sugar level of rabbit is from 190mg/dl to 260 mg/dl.

Each value is represented as mean±SEM, No. of animals (n) = 12, \*\*p<0.01 Vs Normal control, ££ p<0.01 Vs Diabetic control, one way ANOVA followed by Dunnett's Test

At the end of experiment rabbits were fasted overnight and scarified by cervical decapitation. Blood is collected; plasma and serum were obtained and used for determination of various biochemical parameters like Blood glucose level, total protein, Total cholesterol, Serum creatinine & Blood urea nitrogen. The liver was carefully removed, homogenized and the homogenate was used for the estimation of glycogen level.

It is found that the strychnine aqueous extract received group of rabbits and Glipizide received standard group of rabbits show all most all similar reduced blood sugar levels.

#### RESULTS

The property of partial solubility of strychnine in water 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 at room temperature. In case- 1 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 case- 2, when different weights of stem pieces were soaked in 150 ml of water samples, the diffusion rate of strychnine was found to be 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 constant and weight of stem is changed.

In case-3, 100 gm of stem piece gradually lost its diffusible alkaloid from day 1 to day 49 and became zero at the end.

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 by water soaking method.

After the non toxic dosage of aqueous extract of strychnine alkaloid is obtained from the weight of 15 gm stem soaked overnight for 12 hours in 150 ml of water which contain 0.720 mg, orally given to the test group of rabbits from the day 3 of alloxan treatment continuously for 4 weeks and

blood sugar levels were checked on 0, 1, 2, 3,4 weeks. The blood sugar levels reduced from 343 mg/dl to 220mg/dl. Total sugar level is reduced by 123 mg/dl by the end of 28 days. From this the hypoglycaemic effect of strychnine is observed (Table-4). The animal group have not faced any ill effect during the experiment, so the safety, nontoxic dosage concentration of aqueous extract of strychnine for rabbit is found 0.720 mg/kg body weight.

#### 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. The alkaloid concentration was measured calorimetrically and found the range 0.720 mg to 0.328 mg from day 1 to 15 respectively which is a safety dosage for rabbits for controlling the blood sugar level.

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