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Research Article

A COMPARATIVE TOXICOLOGICAL EVALUATION OF STRYCHNINE AND ASHODHITA KUPILU IN WISTAR ALBINO RATS

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ABSTRACT

Strychnine is a natural alkaloid obtained from the dried seeds of *Strychnos nux vomica*. It occurs as colourless, odourless, rhombic prisms, having an intensely bitter taste. The seeds contains about 1.1 -1.4% strychnine. It is a spinal poison & the chief chemical constituent that accounts for the toxic manifestations and is 10 to 20 times more poisonous than brucine. Tonic – clonic convulsions are the characteristic clinical manifestation seen in strychnine poisoning. Kupilu is one among the upavishas that comes under the category of sthava visha. References regarding the drug kupilu are mentioned in the classical texts according to the time period. There is no reference of kupilu in vedakala and samhitakala and was not reported in the 'BrihatTrayee' texts of Ayurveda. In samgrahakala, the reference of visamusti as one of the ingredient of the formulation Agnitundirasa can be seen in Sharnagadhara samhita. Toxicity is the degree at which a substance can damage a tissue or organ. It can be acute, sub-acute and chronic. In the present study 5 groups were taken; group1 – control, group2 – ashodhita kupilu (1/5th LD₅₀), group3- ashodhita kupilu (1/10th LD₅₀), group4- strychnine (1/5th LD₅₀) and group5- strychnine (1/10th LD₅₀). Significant results were observed in the 1/5th LD₅₀ strychnine received group when compared to ashodhita kupilu (1/5th LD₅₀) treated group.

Keywords: Strychnine; Ashodhita kupilu; Toxicity;

INTRODUCTION

Strychnine is one of the oldest poisons known to man. The source of this potent toxin is *Strychnos nux vomica* Linn. plant. It is principally found in the seeds but can be isolated from bark, leaves and root also. It is white, odourless, bitter crystalline powder¹. The fatal dose of strychnine in adult is 60 to 100mg². Clinical features of toxicity vary according to the routes of administration. Once the strychnine is consumed it produces powerful and uncontrollable muscle contractions³.

Kupilu (*Strychnos nux vomica* Linn.) is one of the Sthava vishas that comes under the category of upavishas. The poisonous part is the seeds of ripened fruit. The seeds are flat, circular discs having a diameter of 2½ cm and a thickness of 6mm⁴. Strychnine is the most important active principle present in seeds. Of all constituents strychnine is the most important and the seeds contain about 1.1 to 1.4 percent⁵.

Toxicity is the degree at which a substance can damage a tissue or organ. It can be acute, sub-acute and chronic. Kupilu contains many phytochemical constituents amongst them strychnine is the chief component that accounts for its toxicological manifestations. Hence the present study is intended to compare the toxicity of strychnine alone and ashodhita kupilu as a whole drug.

MATERIALS & METHODS

The ripened fruits of kupilu (*Strychnos nuxvomica* Linn.) will be collected from natural habitat from nearby places in Udupi district and they will be authenticated. Strychnine will be procured from the certified agency.

Animals: In bread wistar strain albino rats of either sex of body wt. ranging from 150-200 g. were obtained from central animal house of the SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. They were maintained at standard housing conditions and fed with standard animal pellet and provided with tap water ad libitum during the experiment. The institutional animal ethical committee (SDMCAU APPROVAL NO.9/IAEC/12-3-2015) permitted the study.

Method

Acute toxicity study will be conducted as per OECD 425 guidelines and sub-acute toxicity study will be conducted as per AYUSH 170 guidelines.

Phage 1 – AOT study

A total of 7 healthy wistar strain albino rats of either sex weighing 150-200g were selected and test drug ashodhita kupilubeeja churna was administered at a fixed dose of 175mg/kg, 550mg/kg and 2000mg/kg as per the AOT software.

Maximum tolerated dose will be calculated by employing OECD 425 guide lines with AOT Software.

Table 1: Identification of Animals, its desired dose, body weight & calculated dose (AOT Study)

Sl. No.	Identification the animal	Desired dose	Body weight (grams)	Calculated dose (ml)
1	Head	175	220	0.76
2	Neck	550	247	2.70
3	Body	2000	251	3.35
4	Tail	550	268	1.47
5	Abdomen	2000	250	3.33
6	Forelimb	550	247	2.70
7	Hind limb	2000	251	3.35

Out of the 7 rat's, death occurred in dosage of 2000mg/kg.

The LD 50 value was found to be 1098mg/kg with a confidence limit of 550 to 2000mg/kg.

Phase 2 – SUB ACUTE TOXICITY STUDY

Wistar strain albino rats of either sex weighing 150-200g in groups of 5 for each ashodhita kupilu and strychnine in 1/5th and 1/10th LD₅₀ dose were administered orally for duration of 28 days⁹

Dose for rats:

AshodhitaKupilu – From AOT study

LD₅₀ = 1098mg/kg

1/5th of LD₅₀ = 219.6 mg/kg

1/10th of LD₅₀ = 109.8 mg/kg

Strychnine

LD₅₀ = 2.5 mg/kg (2500 µg/kg)

1/5th of LD₅₀ = 500µg/kg

1/10th of LD₅₀ = 250µg/kg

Ashodhita kupilu beeja churna was given as solution in 50mg & 100mg gum acacia for groups 2 and 3 respectively. Toxicity was assessed in terms of body weight, behaviour, food conversion ratio, histological study of vital organs and tissues (Brain, Spinal cord, Heart, Lungs, Spleen, Uterus, Testis, Kidney, Liver), bio- chemical assessment (S.G.O.T., S.G.P.T., Serum Alkaline Phosphate, Serum Creatinine, Triglyceride, Cholesterol, Blood Urea & Blood Glucose) and haematological changes (Haemoglobin, W.B.C., R.B.C., P.C.V., M.C.V., M.C.H., R.D.W.C.V., R.D.W.S.D., M.C.H.C. & Platelet count).

RESULTS

Table 2: Effect of Ashodhita kupilu and strychnine on Food conversion ratio in Wister strain albino rats during sub-acute toxicity study

Groups	7 th day	14 th day	21 st day	28 th day
Control	5.89 ± 0.65	5.35 ± 0.47	5.13 ± 0.68	4.22 ± 0.04
1/5 th LD ₅₀ Ashodhita kupilu	5.72 ± 0.57↓	5.69 ± 0.29↑	6.11 ± 0.68↑	5.68 ± 0.60↑
1/10 th LD ₅₀ Ashodhita kupilu	5.71 ± 0.43↓	5.56 ± 0.66↑	5.87 ± 0.95↑	6.52 ± 0.50 ↑
1/5 th LD ₅₀ Strychnine	7.95 ± 0.88↑	4.81 ± 0.83↓	11.30 ± 6.12↑	5.14 ± 0.39↑
1/10 th LD ₅₀ Strychnine	7.11 ± 7.17↑	5.29 ± 0.45↓	5.75 ± 0.41↑	4.98 ± 0.73↑

Data: MEAN ± SEM

There was a significant increase in food conversion ratio in 1/10th LD₅₀ashodhitakupilu group and non-significant increase & decrease in 1/5th LD₅₀ashodhitakupilu group and strychnine (1/5th LD₅₀, 1/10th LD₅₀) when compared to the normal control group.

Table 3: Effect of Ashodhita kupilu and strychnine on % changes in body weight of Wister strain albino rats during sub-acute toxicity study

Groups	7 th day	14 th day	21 st day	28 th day
Control	8.96 ± 3.50	14.72 ± 4.39	13.88 ± 4.50	19.70 ± 6.51
1/5 th LD ₅₀ Ashodhita kupilu	10.84 ± 2.60↑	18.37 ± 5.22↑	24.84 ± 6.25↑	28.42 ± 7.23↑
1/10 th LD ₅₀ Ashodhita kupilu	4.95 ± 2.21↓	8.51 ± 3.20↓	9.17 ± 3.44↓	12.80 ± 4.44↓
1/5 th LD ₅₀ Strychnine	5.32 ± 2.31↓	8.39 ± 4.44↓	18.15 ± 5.24↑	18.08 ± 5.22↓
1/10 th LD ₅₀ Strychnine	12.70 ± 3.17↑	20.44 ± 4.15↑	23.05 ± 4.28↑	30.73 ± 6.4↑

Data: MEAN ± SEM

Gradual increase in the body weight in relation to food and water has been observed in both the ashodhitakupilu and strychnine administered groups which were found to be statistically non-significant.

Table 4: Effect of Ashodhita kupilu and strychnine on organ weight of Wister strain albino rats

Organs	Control	1/5 th LD ₅₀ Ashodhita kupilu	1/10 th LD ₅₀ Ashodhita kupilu	1/5 th LD ₅₀ Strychnine	1/10 th LD ₅₀ Strychnine
Brain	1.69 ± 0.07	1.52 ± 0.06	1.62 ± 0.05	1.46 ± 0.03	1.53 ± 1.15
Cervical spinal cord	2.68 ± 0.08	2.59 ± 0.06	2.39 ± 0.21	2.42 ± 0.12	2.53 ± 0.15
Thoracic spinal cord	4.73 ± 0.20	3.82 ± 0.16	3.14 ± 0.30	4.29 ± 0.12	5.79 ± 0.19
Lumbar spinal cord	5.28 ± 0.30	2.80 ± 0.10	2.51 ± 0.21	4.95 ± 0.14	5.82 ± 0.21
Heart	0.74 ± 0.03	0.72 ± 0.02	0.72 ± 0.07	0.68 ± 0.01	0.79 ± 0.02
Lungs	1.99 ± 0.10	1.21 ± 0.12	1.57 ± 0.10	1.75 ± 0.09	1.6 ± 0.05
Liver	7.69 ± 0.54	8.70 ± 0.89	7.69 ± 0.87	7.28 ± 0.51	9.14 ± 0.65
Spleen	0.85 ± 0.09	1.13 ± 0.18	0.96 ± 0.11	0.89 ± 0.12	1.00 ± 0.09
Kidney	1.67 ± 0.12	1.39 ± 0.14	1.38 ± 0.18	1.72 ± 0.09	1.91 ± 0.13
Testis	2.81 ± 0.09	2.95 ± 0.09	2.90 ± 0.01	2.83 ± 0.03	3 ± 0.05
Uterus	1.04 ± 0.02	1.03 ± 0.07	0.94 ± 0.07	1.15 ± 0.11	1.20 ± 0.12

Data: MEAN ± SEM

Thoracic part of spinal cord shows significant decrease in Ashodhitakupilu group (both 1/5th and 1/10th LD₅₀) and significant increase in 1/10th LD₅₀ strychnine group; Lumbar part of spinal cord shows significant decrease in the Ashodhitakupilu groups and lungs show significant decrease in the 1/10th LD₅₀ of Ashodhitakupilu group.

Table 5: Effect of Ashodhita kupilu and strychnine on haematological parameters of Wister strain albino rats

Parameter studied	Control	1/5 th LD ₅₀ ashodhita kupilu	1/10 th LD ₅₀ ashodhita kupilu	1/5 th LD ₅₀ strychnine	1/10 th LD ₅₀ strychnine
Hb	15.36 ± 0.25	15.75 ± 0.26	14.87 ± 0.48	15.4 ± 0.41	14.85 ± 0.25
Total WBC	7212.5 ± 745.33	8871.42 ± 1232.1	9614.28 ± 1351.3	8871.42 ± 1232.1	9966.66 ± 1913.9
Total RBC	7.55 ± 0.16	7.67 ± 0.12	7.38 ± 0.23	7.92 ± 0.23	7.67 ± 0.14
PCV	42.73 ± 0.62	43.68 ± 0.75	41.6 ± 1.56	44.47 ± 1.33	41.86 ± 0.80
MCV	56.73 ± 0.74	57.03 ± 0.71	56.41 ± 0.71	56.18 ± 0.65	54.6 ± 0.29
MCH	20.3 ± 0.26	20.48 ± 0.25	20.11 ± 0.24	19.41 ± 0.17	19.3 ± 0.19
MCHC	35.88 ± 0.15	36 ± 0.05	35.72 ± 0.23	34.58 ± 0.21	35.43 ± 0.19
RCDW	13.55 ± 0.48	13.4 ± 0.69	14.18 ± 0.86	13.64 ± 0.16	14.13 ± 0.43
RCDWS	26.51 ± 0.84	26.23 ± 1.32	27.62 ± 1.71	27.17 ± 0.39	26.7 ± 0.60
PLATELETS	5.89 ± 0.27	6.55 ± 0.42	6.66 ± 0.21	6.08 ± 0.38	6.09 ± 0.11

Data: MEAN ± SEM

In the strychnine administered group there was significant decrease in the MCH and MCHC as compared to normal control.

Table 6: Effect of Ashodhita kupilu and strychnine on biochemical parameters of Wister strain albino rats

Parameter studied	Control	1/5 th LD ₅₀ ashodhita kupilu	1/10 th LD ₅₀ ashodhita kupilu	1/5 th LD ₅₀ strychnine	1/10 th LD ₅₀ strychnine
SGOT	180.25 ± 5.30	136.62 ± 6.12	114.14 ± 3.83	112.85 ± 4.85	165.33 ± 8.52
SGPT	136 ± 7.21	85.25 ± 8.51	58 ± 1.97	63 ± 11.48	97.83 ± 5.92
ALP	340.62 ± 55.30	436.25 ± 59.07	285.28 ± 51.22	392.71 ± 43.58	360.83 ± 21.5
S.UREA	36.25 ± 1.68	38.62 ± 1.65	36.85 ± 1.28	27.85 ± 4.17	35.16 ± 3.14
S.CREATININE	0.82 ± 0.03	0.4 ± 0.03	0.57 ± 0.04	0.52 ± 0.04	0.72 ± 0.13
CHOLESTEROL	54.12 ± 2.24	70.87 ± 3.69	47.57 ± 1.64	77.71 ± 1.98	51.33 ± 2.86
TRIGLYCERIDE	116.75 ± 27.50	85.5 ± 15.71	80.71 ± 9.23	72.28 ± 9.33	104.83 ± 9.14
SUGAR	120.25 ± 5.35	124.62 ± 4.77	120 ± 4.81	127 ± 3.85	119.16 ± 5.05

Data: MEAN ± SEM

The serum biochemical parameters such as SGOT and SGPT were significantly reduced in Ashodhitakupilu as well as Strychnine group as compared to normal control group. There is significant decrease in creatinine in Ashodhitakupilu(1/5th LD₅₀) and strychnine(1/10th LD₅₀) groups and also significant increase

in Ashodhitakupilu(1/10th LD₅₀). In the lipid profile, there was significant increase in cholesterol level in both 1/5th LD₅₀ groups of Ashodhitakupilu and strychnine. There is a significant increase in sugar in strychnine (1/5th LD₅₀) group.

HISTOPATHOLOGICAL PLATES OF VITAL ORGANS

Figure 1

Figure 2

LUNGS: Moderate fluid cell exudation were observed in both figure

Figure 3

Figure 4

SPLEEN: Moderate pigment deposition was observed in both the figures

DISCUSSION

The present study was undertaken with the objective of assessing the ashodhita kupilu and pure strychnine on different parameters. In the haematological group of parameters there were no remarkable differences between the two sets. The significant changes observed i.e. MCH and MCHC were not substantiated by changes in the related parameters. In biochemical group of parameters- almost similar changes were observed in majority of the cases. However, elevation of serum sugar was the major difference. In Ashodhita kupilu group elevation was observed in serum creatinine but was not attendant with changes in serum urea level- hence considered as self limiting. It may be indicative of higher muscular activity. Serum cholesterol was elevated in both the sets- indicating lipid mobilization potential in both raw and pure products. Histopathological examination did not reveal any major change – indicating whatever changes that occur due to administration of these two sets does not involve organ damage. The differences are mainly functional in nature. Ponderal parameters showed major difference in the weight of spinal cord. Decrease was observed with ashodhita kupilu while increase in weight was observed in strychnine group. The exact reason is not known. However, it does not degenerative changes for the decrease or oedema or hypertrophy as the cause of the increase. Another major difference observed is with respect to fecal weight and fecal water content. This may be due to the presence of additional factors in ashodhita kupilu which might have increased water absorption from the surrounding cells besides adding to the fecal weight. However, this difference may not be of much importance from toxicological point of view.

CONCLUSION

Gradual increase in body weight in relation to food and water intake was noted in both 1/5th LD₅₀ and 1/10th LD₅₀ of ashodhita kupilu & strychnine groups. Food conversion ratio exhibited

marginal increase in 1/10th LD₅₀ ashodhita kupilu group. Haematological investigations suggest significant decrease of MCH & MCHC in 1/5th LD₅₀ strychnine received groups. Biochemical investigations suggest significant decrease of SGOT & SGPT in both 1/5th and 1/10th LD₅₀ of ashodhita kupilu and strychnine treated groups. Significant decrease of serum creatinine in 1/5th LD₅₀ of ashodhita kupilu and strychnine; significant increase in 1/10th LD₅₀ of ashodhita kupilu. Cholesterol level increased significantly in 1/5th LD₅₀ of both groups. Significant increase in sugar level of 1/5th LD₅₀ strychnine treated group. Histopathological examinations suggest fluid exudation in lung of 1 animal of 1/5th LD₅₀ ashodhita kupilu and 1/10th LD₅₀ strychnine group; mild to moderate cell and fluid exudation in 1 animal of 1/10th LD₅₀ strychnine received group. Mild to moderate intensity pigment deposition in spleen of 1 animal of 1/10th LD₅₀ ashodhita kupilu; moderate to significant pigment deposition in 3 animals of 1/5th LD₅₀ strychnine received group; moderate deposition in 1 animal of 1/10th LD₅₀ of strychnine treated group. The assessment of haematological, biochemical and histopathological tests suggests that 1/5th LD₅₀ strychnine received group produced more toxic effects compared to 1/5th LD₅₀ ashodhita kupilu group.

List of abbreviations

W.B.C. - White blood corpuscles
R.B.C. - Red blood corpuscles
P.C.V. - Packed cell volume
M.C.V. - Mean corpuscular volume
M.C.H. - Mean corpuscular haemoglobin
R.D.W.C.V. - Red cell distribution width coefficient variation
R.D.W.S.D. - Red cell distribution width standard deviation
M.C.H.C. - Mean Corpuscular Haemoglobin Concentration
S.G.O.T. - Serum glutamic oxaloacetic pyruvic transaminase
S.G.P.T. - Serum glutamic pyruvic transaminase
A.L.P. - Alkaline Phosphatase

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