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CENTRAL NERVOUS SYSTEM STIMULANT ACTIVITY OF 4-HYDROXY-3-METHOXY STRYCHNINE, AN ALKALOID FROM THE ROOTS OF STRYCHNOS NUXVOMICA LINN

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ABSTRACT:

The objective of the study is to isolate alkaloids having similar activity to that of strychnine and brucine from *Strychnos nuxvomica* and to evaluate them. Methanolic extract from the roots of *S. nuxvomica* after chromatography afforded stigmasterol, β-amyrin and an alkaloid 4-hydroxy-3-methoxy strychnine. The alkaloid exhibited potent central nervous system stimulant activity at lower doses (0.2 mg kg^{-1}, i.p) but displayed depressant, convulsant and toxic effects at higher doses (1 mg kg^{-1}, i.p). This activity is much similar to that of strychnine and indicate that the *S. nuxvomica* possess several such alkaloids which may be responsible for toxic activity of the drug synergistically.

Key words: *Strychnos nuxvomica*, strychnine, 4-hydroxy-3-methoxy strychnine, alkaloid, locomotor activity, and toxicity.

INTRODUCTION:

Strychnos nuxvomica Linn. Is a small tree grown extensively in India and Southern Asia[1, 2, 3]. It is called as "Vishamusti" in the local language. *Strychnos* is the largest genus of small trees and climbing shrubs of the family Loganiaceae with around 190 species. Only seven species among these were found to consists of strychnine, a toxic constituent (LD_{50} 0.2 mg kg^{-1}, parenteral) that has been intensively used both as a rat poison and as a tonic[3]. Literature survey revealed that *S. nuxvomica* has been found to have cytoprotective, antitussive[4], antitumour, antiarthritic[5], toxic[6], convulsant[7] properties. In addition it has been used in digestive disorders, epilepsy, debility[8], in platelet aggregation[9], in throat irritation[10] and improving blood circulation[11]. Chemically the plant is reported to contain indole alkaloids[12-15] iridoids[1] and glucomannans[16].

MATERIALS AND METHOD:

Isolation of 4-hydroxy-3-methoxy strychnine:

The roots of *S. nuxvomica* (1.5 Kg) were collected from Manipal, Karnataka, India during June 2005 and authenticated by Dr.M.Venkaiah, Taxonomist, Department of Botany, Andhra University, Visakhapatnam. A voucher specimen (SG-SNR-28/6/05) was deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry. The roots were air dried, powdered and extracted with petroleum ether (3000 mL) to remove the fatty material and further extracted with methanol (3000 mL) using soxhlet apparatus. The extracts were concentrated under vaccum to get the corresponding residues (12 g and 20 g) respectively. The methanolic residue was acidified to pH 2 with dilute sulphuric acid. The acidic extract was dissolved in water and made alkaline with sodium hydroxide solution, which gave a colourless precipitate. The precipitate was column chromatographed over silica gel, upon elution with petroleum ether, benzene, chloroform and methanol in the order of increasing polarity, afforded SNR-01 (stigmasterol), SNR-02 (β-amyrin) and SNR-03 (4-hydroxy-3-methoxy strychnine).

Central Nervous System (CNS) stimulant and depressant activity:

The alkaloid (4-hydroxy-3-methoxy strychnine) was tested for CNS stimulant activity, the method suggested by Dews[17] *et al* (1953) and Kulkarni[18] *et al* (1975) was followed. Male albino mice (Purchased from National Institute of Nutrition, Hyderabad, India) weighing 20-25 g were selected. Animals were maintained under standard laboratory conditions at 25 ± 2^{o}C, relative humidity 50 ± 15% and normal photoperiod (12 h dark/ 12 h light) were used for the experiment, commercial pellet diet and tap water *ad libitum.* Approval from the institutional animal ethical committee was taken before the study. They were divided into 5 groups (A, B, C, D and E) each consisting of 5 animals. The groups were fasted for 18 h before the test. Stock solution (0.5 mg ml^{-1}) of the test drug was prepared using N/10 HCl and the test drug was administered intraperitonially to all groups, a dose of 0.2 mg kg^{-1} to group A, 0.5 mg kg^{-1} for group B, 1 mg kg^{-1} for group C, 10 mg kg^{-1} for group D and 0.2 ml of N/10 HCl (vehicle) as control for group E were given.

After administration of test drug for the groups they were observed for locomotor activity in photoactometer (Techno Model) at regular intervals for a period of 120 minutes (10, 20, 30, 40, 50, 60, 90 and 120). Each mouse was placed individually in the activity cage and the activity score of all the groups were recorded before and after treatment. The percentage increase or decrease in locomotor activity was calculated and it is as given in the table no:1.

Statistical analysis

Data obtained from the study were expressed as mean ± SEM, statistical analysis was performed using student's *t*-test, p-values less than 0.05 were considered statistically significant.

Table no.1
Activity score of locomotor activity before and after treatment

Group	Locomotor activity								
	Before treatment	After treatment							
		10 min	20 min	30 min	40 min	50 min	60 min	90 min	120 min
A	118.6 ±7.51	144* ± 7.89 (+21.41)	170*** ± 6.14 (+43.33)	154.6**± 6.55 (+30.35)	142.2* ± 1.96 (+19.89)	134.6 ± 3.12 (+13.49)	125.4 ± 8.15 (+5.73)	122.4 ± 5.74 (+3.2)	119.4 ± 6.11 (+0.67)
B	128.6 ±1.17	93.4*± 6.88 (-27.37)	91* ± 10.33 (-29.23)	94.2 ± 11.69 (-26.74)	114 ± 12.47 (-11.35)	130 ± 11.92 (+1.08)	140 ± 11.92 (+8.86)	141.2 ± 11.42 (+9.79)	142.4 ± 9.76 (+10.73)
C	130.6 ±0.26	10.8***± 0.73 (-91.73)	5.6*** ± 0.51 (-95.71)	7.2*** ± 2.03 (-94.48)	8*** ± 1.38 (-93.87)	11.6*** ± 3.06 (-91.11)	21.2*** ±5.36 (-83.76)	31.6***± 7.15 (-75.80)	36.6***±7.60 (-71.97)
D	124.6 ±5.51	-	-	-	-	-	-	-	-
E (Control)	116.2±11.1	119.2 ± 10.25 (+2.58)	119.8 ± 15.90 (+3.09)	117.8 ± 13.00 (+1.37)	121.8 ±12.85 (+4.81)	123 ± 11.73 (+5.85)	115.8 ±11.00 (-0.34)	121.6 ± 12.39 (+4.64)	115 ±12.67 (-1.03)

Student's *t*-test, *p<0.05, **p<0.01, ***p<0.001

Note: Values expressed in parenthesis are percentage change in activity (-) indicates decrease in activity (+) increase in activity

RESULTS AND DISCUSSION:

In group A the activity was increased after the drug treatment and percentage increase in locomotor activity was observed. In group B, the activity scores were decreased after drug treatment and the animals were survived which indicated depressant activity. In group C, the activity score was decreased and delayed toxicity was observed in which death occurred due to convulsions after 36 hr. In group D, treated with 10 mg kg^{-1} dose showed convulsions within two minutes of treatment and led to death. There was no change in the locomotor activity of control group (Group E) whereas the test drug displayed central nervous system stimulant activity at low doses and depression, toxicity at higher doses. The toxicity produced resembled with the toxicity produced by the strychnine. It is known that strychnine type of alkaloids in small doses increase the activity of certain functions in the central nervous system by stimulating respiratory and vasomotor centres, at higher doses they cause tonic convulsions and finally death through respiratory or spinal paralysis or by cardiac arrest.

CONCLUSION:

In the present study it was found that the test drug showed strychnine like activity and produced stimulation at low doses and depression, death at higher doses. This indicates that the tested compound (4-hydroxy-3-methoxy strychnine) exhibited similar actions like that of strychnine. It is observed that there are a number of alkaloids which where isolated and being isolated from S*trychnos* species and all these molecules have indole ring system and differ in few groups, but however possess similar kind of activity with a slight difference in potency. The toxic nature of the drug may be due to synergistic activity of the alkaloids. However further studies are required to establish the role of individual components in a mixture of compounds.

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