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# Evaluation of the sleep modulating effects of methanolic extracts of *Strychnos spinosa* and *Strychnos innocua* fruits in mice

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**Abstract**

This study aimed to evaluate the sleep modulating properties of *Strychnos spinosa* and *Strychnos innocua* fruits in Swiss albino mice. The depressant effect of the extracts on the central nervous system was studied using a diazepam-induced sleep model in mice. The mice were divided into four groups of six mice each. The test groups received *Strychnos spinosa* and *Strychnos innocua* fruits extracts at the doses of 250, 500, and 1000 mg/kg body weight intraperitoneally. In contrast, the control group received distilled water at 10 ml/kg. Diazepam (Ranbaxy®, India) was used as a standard drug and administered 30 minutes after the initial treatment. The lethal doses ($LD_{50}$) of the two extracts were estimated to be >5000 mg/kg. The results obtained from the onset of sleep of rats administered methanol extracts of *Strychnos spinosa,* and *Strychnos innocua* fruits showed that the extracts at 250, 500, and 1000 mg/kg, respectively, did not significantly ($p > 0.05$) alter the onset of sleep when compared with the control. The onset of sleep in the groups treated with *Strychnos spinosa* fruit extracts indicated a dose-dependent decrease pattern. The study also revealed a prolonged duration of sleep in all the experimental groups treated with the two extracts compared to the control group. The treated groups of 250, 500 and 1000 mg/kg significantly ($p < 0.05$) prolonged the duration of diazepam-induced sleep in a dose-dependent manner when compared to the control group. The group treated with 1000 mg/kg of *Strychnos innocua* fruit extract was significant ($p < 0.05$) compared to the control group. In conclusion, the high $LD_{50}$ observed in this study suggests that the two extracts are relatively safe and contain promising bioactive ingredients that cause sleep modulation in mice.

*Keywords*: Extract, Mice, Sleep modulation, *Strychnos innocua*, *Strychnos spinosa*

**Introduction**

*Strychnos spinosa* and *S. innocua* are flowering plants that belong to the family *Loganiaceae*. The genus *Strychnos* is widely distributed around tropical regions in the world. *Strychnos* possess indole alkaloids in their leaves, stems and roots, which have been reported to be poisonous. Strychnine and curare are the commonest alkaloids associated with the plant (Orwa *et al*., 2009). *Strychnos* is an important genus due to various reported therapeutic effect it possesses in Africa and Asia, such as therapy against fever, wound, snakebites, poisoning, tonic and emetic properties against gastrointestinal diseases, treatment against parasitic and skin diseases including leprosy. *S. innocua* is used in different regions of Africa to treat chronic malaria (Neuwinger, 1996). Scientists over the years have discovered alkaloids from plant-based sources are beneficial in the field of surgery. They are used as local and general anaesthetics to suppress brain activity, nerve transmission, muscle relaxation and control of pain (Brown *et al*., 2018). Various parts of *Strychnos* plant used for medicinal purposes include: decoction of the root to treat gonorrhea and chlamydia infection, fresh roots are used to treat snakebite and root infusions in Uganda to treat theileriosis in cattle (Tabuti *et al.,* 2003; Orwa *et al*., 2009; Chinsembu, 2016). The bark and twigs are used to dilate the cervix during childbirth (Orwa *et al*., 2009), the leaves are used to treat malaria and also serve as antibacterial agent (Koné *et al.*, 2004; Asase *et al.,* 2005). The seeds have emetic properties and the fruit pulp is used as a remedy for dysentery and as eardrops (Orwa *et al*., 2009).

Toxicity of *Strychnos* species differ according to the part of the plant and this is associated with strychnine. The root is where strychnine undergo biosynthesis and it is rich in alkaloids and have a greater pharmacological activity than other plant parts. In India, pastoralist allow their cows to graze on the leaves of *Strychnos nux vomica* (Bisset, 1974). There is paucity of information regarding the modulatory effect of *Strychnos spp.* on sleep. Thus, this study was carried out to evaluate the sedative properties of *Strychnos spinosa* and *Strychnos innocua* fruits methanol extracts in mice.

**Materials and Methods**

*Ethical clearance*

The approval for the conduct of this study was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2018/077.

*Experimental animal and management*

A total of 72 Albino mice consisting of both sexes each weighing between 17 and 30 g were used for this study. They were fed with commercial poultry feed (Vitafeed®), while water was provided *ad libitum* throughout the study period in the Animal House, Department of Pharmaceutical Sciences, Ahmadu Bello University Zaria.

*Experimental design*

Plant collection and identification: The matured fruits of *Strychnos spinosa* and *Strychnos innocua* were randomly collected from different locations in Zaria, Kaduna State, Nigeria. The fruits were authenticated at the Herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria, Nigeria and the plants were given a Voucher identification number of 901 and 902 for *Strychnos spinosa* and *Strychnos innocua* respectively. The fruits were stored at the Herbarium for reference purpose.

Methanol extraction of *Strychnos spinosa* and *Strychnos innocua* fruits: Methanol extraction was carried out at the Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria, Nigeria. The method used was as described by Donatus & Ephraim (2009). The two fruits were dried in the shade and were pounded together using mortar and pestle to a coarse powder. The powder was packed into white cotton bags and put into Soxhlet apparatus (Sigma) and extracted exclusively using 3.5 L of 70% methanol (Sigma- Aldrich®) at $70^0$C.The methanolic extract was concentrated by evaporation at room temperature on warm waterbath (HH-S 21.6, double row six holes, HINOTEK China) to separate the solvent from the extract (oily liquid) at a temperature of $93.3^0$C.

Acute toxicity studies of *Strychnos spinosa* and *Strychnos innocua* fruit extracts: Twenty-four (24) mice were used for the intraperitoneal median lethal doses of the two extracts (12 each) using the method described by Lorke (1983).

Diazepam-induced sleep in mice: The method described by Rakotonirina *et al*. (2001) was used for the diazepam-induced sleep model for each of the extracts (consisting of 24 mice per extract). Each extract has 4 groups I-IV consist of 6 mice each and were treated with distilled water (10 mL/kg), 250, 500 and 1,000 mg/kg of the fruit extract (*S. spinosa/S. innocua*) respectively, intraperiotoneally. Thirty minutes later, diazepam (Roche®) at 2mg/kg intraperitoneally was administered to all the groups

respectively. All the animals for the two fruit extracts were observed continuously for the induction time, time of onset of action and recovery time, until the mice were fully recovered.

*Data analysis*

Values obtained were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used followed by Tukey's *post-hoc* test for multiple comparisons of groups. GraphPad Prism version 8.0.0 for windows (GraphPad software San Diego, California, USA) was used for the analysis. Values of $p < 0.05$ were considered significant.

**Results**

The median lethal dose of the two extracts was estimated to be greater than 5000 mg/kg suggesting that they are relatively safe intraperitoneally. No significant ($p > 0.05$) difference in the onset of sleep was observed in all the groups treated with *Strychnos spinosa* fruit when compared to the control. (Table 1). There was a significant ($p < 0.05$) increase in the duration of sleep in *S. spinos*a extracts (80.10 ± 0.24; 115.40 ± 0.51 and 138.80 ± 0.37) respectively in all the treated groups in a dose-depended manner when compared to the control (17.60 ± 4.2) (Table 2). No significant ($p > 0.05$) difference in the onset of sleep was observed in all the groups treated with *Strychnos innocua* fruit when compared to the control. (Table 3). The sleep modulatory effect of *Strychnos innocua* fruit extract on diazepam-induced sleep is shown in Table 4. The group treated with the highest dose (1000 mg/kg) showed significant ($p < 0.05$) increase (79.40 ±1.76) in the duration of sleep when compared to the control (17.00 ± 4.23) (Table 4).

**Discussion**

The lethal intraperitoneal doses ($LD_{50}$) of the two extracts were estimated to be greater than 5000 mg/kg, hence suggesting that the extracts are safe (Matsumura, 1985; Loomis & Hayes, 1996) or practically nontoxic (Lorke, 1983). The safety of these extracts observed in this study agrees with the earlier cytotoxicity study on *S. spinosa* by Adamu *et al.* (2014), who reported an $LC_{50}$ of 361.48 µg/ml on monkey kidney Vero cells *In vitro* using the MTT (3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyl-2H-tetrazolium hydrobromide) assay.

The high value of $LD_{50}$ established in this study has revealed the safe nature of these two extracts and may explain the reasons behind animals and humans eating the fruits without visible detrimental effects. The results obtained on the onset of sleep of animals treated with methanol

**Table 1:** Effect of methanol extract of *Strychnos spinosa* fruit on diazepam-induced sleep-in mice (onset of sleep)

| Treatment (mg/kg) | Onset of sleep (min) |
|---|---|
| Control (10 ml/kg) distilled water + Diazepam (2 mg/kg) | 4.60 ± 0.40 |
| *Strychnos spinosa* (250 mg/kg) + Diazepam (2 mg/kg) | 4.20 ± 0.14 |
| *Strychnos spinosa* (500 mg/kg) + Diazepam (2 mg/kg) | 4.00 ± 0.31 |
| *Strychnos spinosa* (1000 mg/kg) +Diazepam (2 mg/kg) | 3.70 ± 0.27 |

Values are expressed as mean ± SEM (n=6)

**Table 2:** Effect of methanol extract of *Strychnos spinosa* fruit on diazepam-induced sleep-in mice (duration of sleep)

| Treatment (mg/kg) | Duration of sleep (min) |
|---|---|
| Control (10 ml/kg) distilled water + Diazepam (2 mg/kg) | 17.60 ± 4.20 |
| *Strychnos spinosa* (250 mg/kg) + Diazepam (2 mg/kg) | 80.10 ± 0.24* |
| *Strychnos spinosa* (500 mg/kg) + Diazepam (2 mg/kg) | 115.40 ± 0.51** |
| *Strychnos spinosa* (1000 mg/kg) + Diazepam (2 mg/kg) | 138. 80 ± 0.37*** |

Values are expressed as mean ± SEM (n=6), *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$ compared to control

**Table 3:** Effect of methanol extract of *Strychnos innocua* fruit on diazepam-induced sleep-in mice (onset of sleep)

| Treatment (mg/kg) | Onset of sleep (min) |
|---|---|
| Control (10 ml/kg) distilled water + Diazepam (2 mg/kg) | 4.60 ± 0.40 |
| *Strychnos innocua* (250 mg/kg) + Diazepam (2 mg/kg) | 4.4 ± 0.24 |
| *Strychnos innocua* (500 mg/kg) + Diazepam (2 mg/kg) | 4.40 ± 0.51 |
| *Strychnos innocua* (1000 mg/kg) + Diazepam (2 mg/kg) | 3.8 ± 0.37 |

Values are expressed as mean ± SEM (n=6)

**Table 4:** Effect of methanol extract of *Strychnos innocua* fruit on diazepam-induced sleep-in mice (duration of sleep)

| Treatment (mg/kg) | Duration of sleep (min) |
|---|---|
| Control (10 ml/kg) distilled water + Diazepam (2 mg/kg) | 17.00 ± 4.23 |
| *Strychnos innocua* (250 mg/kg) + Diazepam (2 mg/kg) | 53.80 ± 1.72* |
| *Strychnos innocua* (500 mg/kg) + Diazepam (2 mg/kg) | 51.80 ± 1.44* |
| *Strychnos innocua* (1000 mg/kg) + Diazepam (2 mg/kg) | 79.40 ± 1.76** |

Values are expressed as mean ± SEM (n=6), *p < 0.05, **p < 0.001 compared to control

extract of S*trychnos spinosa* and *Strychnos innocua* fruits indicated that the extract at the doses of 250-1000 mg/kg did not significantly alter the onset of sleep compared with the control. The onset of sleep in the groups treated with *Strychnos spinosa* fruit extract showed a dose dependent decrease pattern. The study also revealed a prolonged duration of sleep in all the experimental groups treated with the two extracts compared with the control group. The prolonged duration of sleep was also observed to be statistically significant only in the group treated with 1000 mg/kg compared to the control group. The faster onsets and the significantly prolonged duration of diazepam-induced sleep time observed in this study indicate that the two methanol extracts potentiate diazepam induced sleep in mice and thus possess a sleep-inducing property (Rakotonorina *et al.*, 2001). The sedative properties of these two fruits extracts may be due to the presence of bio-active ingredients such as alkaloids, saponins, and flavonoids. Several researchers have reported that alkaloids, saponins, and flavonoids are responsible for sedation (Won *et al.,* 1980; Wagner *et al.,* 1983; Dubios *et al.,* 1986; Amos *et al.,* 2001; Musa *et al.,* 2006; Viswanatha-Swamy *et al.,* 2006; Musa *et al.,* 2008). The results from this study demonstrate a relationship between the CNS depressant effect of the two extracts and diazepam, which could suggest that the extract of *S. pinosa and S. inocua* mimics the action of benzodiazepines.

Several works conducted on phytochemical investigation suggest that flavonoids and neuroactive steroids are ligands for $GABA_A$ receptors in the CNS, indicating that they can act as benzodiazepine-like agents (Protapaditya *et al.,* 2011). It has also been reported that some flavonoids exhibit high-affinity binding to the benzodiazepine site of $GABA_A$ receptors. Saponins and Triterpenoids have also been agonistic activities at the $GABA_A$ receptor complex (Uma *et al.,* 2011; Kumaresan *et al.,* 2011; Khatoon *et al.,* 2014), suggesting that they act as benzodiazepines-like molecules. Therefore, the CNS depressant activity exhibited by these extracts may be due to the phytoconstituents present in them. Even though there is no evidence on the specific constituent responsible for the CNS depressant effects.

We, therefore, predict that the action of these extracts may be due to the direct activation of GABA receptors (Kavita *et al*., 2013). It may also be due to enhanced affinity for GABA or an increase in the duration of the GABA-gated channel opening (Shans-Ud-Doha *et al.,* 2013) or by potentiating GABAergic inhibition in the CNS via membrane hyperpolarisation leading to a reduction in the firing rate of critical neurons in the brain (Kavita *et al*., 2013)

Conclusively, the relatively low toxicity and the Central Nervous Depression effects of these extracts, as demonstrated in this study, suggest that the two extracts are relatively safe and contain bioactive ingredients that cause sleep modulation in mice.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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