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Behavioural Effects of Hydro-methanolic Crude Extract of Aerial Part of *Indigofera pulchra* in Mice

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Abstract: Indigofera pulchra is used traditionally as prophylactic against snake-bite, treatment of infected wounds and as anti-inflammatory. In this study, the behavioural effects of hydromethanolic of the aerial part extracts of *Indigofera pulchra* were investigated in mice. The results revealed that the extract significantly ($p< 0.05$) prolonged the onset and reduced the duration of sleep at the dose tested (200mg/kg). The extract significantly ($p<0.05$) increased exploratory activity at the dose tested (400mg/kg). It produced no significantly motor coordination deficits in mice at the doses tested (100,200 and 400mg/kg). The intraperitoneal median lethal dose in mice was 2,154 mg/kg while the preliminary phytochemical screening revealed the presence of tannins, saponins, steroids and flavonoids. This result suggests that hydromethanolic extract of *Indigofera pulchra* might contains biologically active principles that are stimulative in nature and lend pharmacological credence to the ethnomedical use of the plant.

Key words: *Indigofera pulchra*, diazepam, behavioral and motor coordination

INTRODUCTION

One important area in which herbal medicines enjoy high patronage world- wide is in the management of pain and inflammations. The herbs are used in combination with other ritual for treatment of Snake-bites. A good number of natural product scientists believe that the initial selection of plants with diverse application in traditional medicine might be encouraged by their easily noticeable CNS effects. (Amos *et al*., 2001).

Indigofera pulchra (willd) is an annual non– climbing herb or shrub that can grow up to 1m tall (Herper, 1976). It is a dicotyledonous plant and belongs to the family leguminosae – papillionaideae (synonym: fabaceae), a family of largely herbs, shrubs and trees with a great variety of habit which are distributed in the temperate and tropical areas (Dallmitz and Watson, 2000). Previous pharmacological studies on the methanol extract of the aerial part of this plant showed that it exhibited venom detoxifying activities (Abubakar *et al.,* 2006). Recent studies have shown that it also possesses antidiabetic activity (Tanko *et al*., 2008).

To the best of our knowledge there is no report on the effect of this plant on central nervous system. The present study was undertaken to provide scientific basis for a possible behavioral and consequently central nervous system, effect of this plant that has various uses in ethnomedicine.

MATEIALS AND METHOD

Plant material: *Indigofera pulchra* aerial parts was collected from Samaru- Zaria in the month of October 2007 and was authenticated by A.U.Gallah of the Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria where a voucher specimen (No.6558) was deposited..

Extraction: The aerial parts were air-dried and made into powder using pestle and mortar The air- dried powdered plant material about 2kg grams was extracted with 30% aqueous and 70% methanol using soxhlets apparatus, the solvent was removed in-vacuo to yield a residue (100gms) referred to as the hydro-methanolic crude extract.

Experimental animals: Seventy five (75) Swiss albino mice of either sex were obtained from the animal house facilities of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria. The mice, maintained on Excel feeds, Ilorin and water *ad libitum,* were housed in polypropylene cages at room temperature throughout the study. All experimental protocols were approved by the University Animal Ethics Committee. The experiments were conducted in a quiet laboratory between the hours of 900h and 1600h.

Acute toxicity study: The lethal dose (LD_{50}) of the plant extract was determined by the method of Lorke (1983) using 12 mice. In the first phase, mice were divided into 3 groups of 3 mice each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight intraperitoneally. They were observed for 24 hours for signs of toxicity. In the second phase, 4 groups containing one mouse each were injected with four more specific doses of the extract (i.p). The median lethal dose (LD_{50}) was calculated using the second phase.

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Phytochemical screening: The preliminary phytochemical screening of *Indigofera pulchra* crude extract was carried out in order to ascertain the presence or absence of various constituents utilizing standard conventional protocols (Trease and Evans, 1983; Harbone and Baxter, 1993).

Drugs used: All chemicals and drugs were obtained commercially and were of analytical grade.

(a) Test for exploratory activity in mice (Hole Board Test): Animal were randomly divided into five groups of 5 mice each. The first group was injected with normal saline which served as a negative control. Mice in the second group received diazepam 1.5mg/kg which served as a positive control, while third and fourth and fifth groups received the extract at the doses of 100, 200 and 400mg/kg respectively.

The exploratory activities of the extract in mice following intraperitoneal administration was determined using the hole board test (File and Wardil, 1975). The apparatus used consists of a white wooden board (40 x 40cm) with four equidistant holes (1cm diameter x 2cm depth).

Each mouse was placed singly at one corner of the board. It was allowed to move about and dip its head into the holes. Poking the nose into a hole is a typical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in five minutes (enough time to exhibit curiosity otherwise) was recorded. The test was carried out 30 minutes after intraperitoneal treatment with the extract at the doses 100, 200 and 400 mg/kg Normal saline 1ml/kg and Diazepam 1.5 mg/kg. Vogel and Vogel (1997) reported that Benzodiazepines tend to suppress nose poking at relatively low doses which was used as control.

(b)Mouse beam walking assay : This method offers improved sensitivity over the mouse Rota rod in determining motor coordination deficits induced by psychotropic agents (Stanley *et al.,* 2005). Mice were allowed to walk from a start platform along a ruler (80cm long and 83cm wide) elevated 30cm above the bench by metal supports to a goal box (enclosed Hamster house). Several trials were performed for each mouse and designed such that the mice tested are aware that there was a goal box that could be reached. A ruler was used because the mouse found this easy to cross and at the same time, it induced minimum anxiety (Stanley *et al.,* 2005).

Animals were randomly divided into five groups of 5 mice each. The first group was injected with normal saline which served as a negative control. Mice in the second received diazepam 1.5mg/kg which served as a positive control, while third and fourth and fifth groups received the extract at doses of 100, 200 and 400 mg/kg.

Once the animals had been tested on the ruler, they were moved immediately to the beam test. The beam was made of wood, 8mm in diameter, 60cm long and elevated 30cm above the bench by a metal support. The animals were placed at one end of the beam and allowed to walk to the goal box thirty minutes after treatment with the extract. Mice that fell were returned to the position they fell from with a maximum time of 60 seconds allowed on the beam. The number of foot slips (one or both hind limbs slipping from the beam were recorded with the aid of tally counter. The number of foot slips is a measure of motor coordination deficit. (Stanley *et al.,* 2005).

(c) Diazepam-induced sleep in mice: The method described by Beretz *et al.,* (1978) and modified by Rakotonirina *et al.,* (2001) was adopted in this study. Adult mice of either sex were divided into 4 groups of five mice in each group. The first group was administered normal saline (1ml/ kg),second third and fourth groups were administered the extract of *Indigofera pulchra* at the doses 100, 200 and 400mg/kg intraperitoneally. Thirty minutes later, diazepam ($1.5 mg\ kg^{-1}$) was administered to all the mice via similar route previously mentioned. Each mouse was then observed for the onset and duration of sleep. The criterion for sleep is the loss of rightening reflex, in which the mice cannot roll back when turned over (Miya *et al.,* 1973). The interval between loss and recovery of rightening reflex was used as the index of hypnotic effect (Fujimori, 1965; Soulimani *et al.,* 2001).

Statistical analysis: The various values were expressed as Mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of $p<0.05$ were considered as significant (Duncan *et al.,* 1977). The treated groups and control groups were analyzed separately for statistical significance.

RESULTS

Phytochemical analysis: Freshly prepared extract was subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of tannins, saponins steroids and flavonoids.

Acute toxicity study (LD_{50}): The sign of toxicity were first noticed after 8–10 hours of extract administration. There was decreased locomotor activity, decreased feed intake, and prostration after 8 hours of extract administration. The median lethal dose (LD_{50}) in rats was calculated to be 2.154 mg/kg body weight intraperitoneally.

Table 1: The effect of hydromethanolic extract of *Indigofera pulchra* on sleep-induced by Diazepam. The dose of 200mg/kg of the crude extract significantly increased the onset of sleep and also decreased the duration of sleep when compared to the control group treated with diazepam only. The dose of 400mg/kg of the crude extract only significantly increased the decrease the duration of sleep when compared with of diazepam induced sleep group.

Table 1: Effect of aerial part of *Indigofera pulchra* extract on Diazepam induced sleep in mice

Treatment	Sheeping Time	
	Onset	Duration
group 1 Diazepam 1.5mg/kg	3.00±0.32	73.4±6.87
Group 2 100mg/kg	4.20±0.80^{ns}	80.4±1.80^{ns}
Group 3 200 mg/kg	7.00±1.09^{a}	30.6±4.80^{a}
Group 3 400mg/kg	4.40±0.69^{ns}	55.4±7.8^{a}

Table 2: Effect of aerial part of *Indigofera pulchra* extract on Motor coordination in mice

Treatment	Time Spent on The beam (minutes)	Number of Foot Slips
Control (Normal saline)	3.80±0.50	0.00±0.00
Group 2 Diazepam 1.5mg/kg	10.6±0.80 ^{a}	6.6 ±1.07 a
Group 3 100mg/kg	3.12±0.44 ^{ns}	0.20±0.20^{ns}
Group 4 200mg/kg	4.22±0.29 ^{ns}	0.60±0.40^{ns}
Group 5 400mg/kg	6.10±0.65 ^{ns}	1.20±0.58^{ns}

Values are given as mean ± SEM, n=5; experimental groups are compared with control. Values are statistically significant at a=P<0.05; ns=not significant.

Motor Coordination (Foot slips and time spent on the beam): Table 2:The extract had no significant effect on the number of foot slips and time spent obtained as the animals walked across the beam balance at all the three doses tested, thirty minutes post administration. The standard drug, diazepam significantly increased the number of foot slips and time spent on the beam when compared to the control normal saline.

Exploratory activity: Table 3: The effect of hydromethanolic crude extract of *Indigofera pulchra* on exploratory activity in mice. The extract at the doses tested (100,200 and 400, mg/kg) there was a significant increase in the number of head dips with the dose of 400mg/kg in the hole-board experiment when compared with the control normal saline.

Table 3: Effect of aerial part of *Indigofera pulchra* extract on exploratory behaviour in mice

Treatment	Exploratory Activity Number of Head Dips
group 1 Control (Nomal saline)	5.20±0.86
Group 2 Diazepam 1.5mg/kg	3.60±0.50^{ns}
Group 3 100mg/kg	6.20±0.80^{ns}
Group 4 200mg/kg	9.40±2.6^{ns}
Group 4 400mg/kg	11.4±0.87

Values are given as mean ± SEM, n=5; experimental groups are compared with control. Values are statistically significant at a=P<0.05; ns=not significant.

DISCUSSION

The extract *Indigofera pulchra* aerial part significantly and dose-independently reduced the onset and prolonged the duration of sleep induced by diazepam. By potentiating the diazepam-induced sleep, the extract seems to possess sleep inducing properties (Guillemain and Tetau, 1980; Rakotonirina *et al.,* 2001). Sedative-hypnotic agents act to increase gamma amino butyric acid (GABA) mediated synaptic inhibition either by directly activating GABA receptors or, more usually, by enhancing the action of GABA on $GABA_A$ receptors. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at $GABA_A$ receptors (Johnston, 2005).The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission.

The extract at the dose of 400 $mgkg^{-1}$produced significant increase in the exploratory behaviour pattern as shown in the head dip result. According to File and Wardill (1975), the hole-board experiment is a measure of exploratory behaviour in animals. A decrease in this parameter reveals a sedative behaviour while an increase indicates stimulatory activity (File and Pellow, 1985) and it has been accepted as a parameter for the evaluation of anxiety conditions in animals (Crawley, 1985). Decrease in exploratory activity by reduction in head dip is a measure of CNS depressant activity (Adzu *et al.,* 2002). While an increase is a measure of CNS stimulatory activity.

The extract had significant decrease on the time spent on the beam balance. It also cause a significant decrease in the number of foot slips. The duration of time spent on the beam balance has been found to be a sensitive measure at determining Benzodiazepine induced motor coordination deficits and is a good predicator of doses producing clinical sedation (Stanley *et al.,* 2005).

It has been reported that saponins show a potent sedative activity when tested in similar models (Dubois *et al.,* 1986). The flavonoid, hispidulin has been reported to act as a positive allosteric modulator across a range of $GABA_A$ receptor subtypes (Kavvadias *et al.,* 2004). Alkaloids are the most important secondary metabolites in many plants that are held responsible for their sedative and anxiolytic action (Elizabetsky and Costa-Campos, 2006). Results of the phytochemical screening however show that the extract does not contain alkaloids. It is therefore plausible to suggest that the sedative activity of extract may be due to the presence of saponins and flavonoids among other phytochemical constituents which have been shown to be present in the extract.

Standard drugs, Diazepam act selectively on $GABA_A$ receptor which mediates fast inhibitory synaptic transmission throughout the CNS. Benzodiazepines bind to the gama-sub-unit of the $GABA_A$ receptor, that causes an allosteric (structural) modification of the receptor results with an increase in $GABA_A$ receptor activity.

Benzodiazepines do not substitute for GABA, which bind at the alpha sub-unit but increase the frequency of channel opening events which leads to an increase in chloride ion conductance and inhibition of the action potential. These drugs also exert a marked taming effect, allowing animals to be handled more easily. (Argyropoulos *et al.*, 2000).

The extract significantly produced an increase in the exploratory behavior pattern as shown in the head dip results the activity resides at he dose of 400 mg/kg when compared to the normal saline control group. Hole board model indicated that head-dipping behavior was sensitive to changes in emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in head-dipping behavior. The extract high dose (400mg/kg) shows increased in number of head-dipping in the hole-board test. It could be argued that the increased head dipping in mice at high dose is merely an artifact of the hyperactivity induced by the extracts. But the effect of diazepam, low and high doses were found to be insignificant when compared with control. Increase in exploratory activity in mice as demonstrated by increase in head-dip is a measure of CNS stimulant activity. This increase in exploratory behavior shows neuroactive property of the extract and its possible application in anxiety conditions. The time taken to reach goal/box is correlated with neuroactivity. The dose of diazepam 1.5mg/kg shows a significant ($p<0.05$) increase in time taken to reach goal box when compared with control. While the tested doses of the crude extract did not show any significant change in time taken to reach goal box when compared with control. This suggested that the extract did not exhibit pronounced sedative effect. It is therefore plausible to suggest that the non sedative activity of the extract may be due to the absence of alkaloid among other phytochemical constituents which have been shown to be absence in the extract.

The three doses of the extract did not show significant changes in the number of foot slips when compared with untreated control group. However, the dose of diazepam showed a significant ($p<0.05$) increase in the number of foot slips made has been found to be a sensitive measure at determining benzodiazepine induced motor coordination deficits and is a good predicator of drug producing clinical sedation (Stanley *et al.*, 2005). Since the three doses of the extract did not show significant increase in the number of foot slips, it indicates that the extract seems not to posses a sedative property. It also exhibit anxiolytic activity by increase in exploratory behaviour which further support the neuroactive property of the extract. Increase in head dip is a measure of CNS stimulant activity.

The results obtained from these experimental models clearly confirmed the anxiolytic activity of the extract of *Indigofera pulchra*. Pytoconsttituents like flavonoids and saponins were reported for their anxiolytic effect. So these active principles might be responsible for anxiolytic activity.

CONCLUSION

This study; therefore suggest that the hydromethanolic crude extract of *Indigofera pulchra* does not possesses central sedative property rather possesses neuroactive property. In onset and duration of sleep, the extract exhibits excitation of the CNS, which confirms its stimulant activity.

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