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The Effects of Ethanolic Extract of *Vitex Doniana* Stem Bark on Peripheral and Central Nervous System of Laboratory Animals

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ABSTRACT

This study evaluated the depressant effects *V. doniana* stem bark ethanolic extract. The stem bark of *V. doniana* (2kg) was macerated for five days with 95% ethanol, filtered and evaporated in vacuo and the extracts concentrate yields was calculated to be 14.8%w/w. The intraperitoneal LD₅₀ in rats estimated with 95% confidence limit was 2154.06mg/kg while no mortality was recorded with the oral route even on administration of 5000mg/kg extract. Thus the extract can be administered with a certain degree of safety. Pre-liminary phytochemical analysis was conducted to ascertain the primary and secondary metabolites present in the extract using standard procedures. The results revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, steroids and terpenes. Alkaloids and anthracenosides were absent in the extract. The administration of doses of 50 mg/mL and 100 mg/mL of the extract produced 70% and 80% local anesthesia respectively on rabbits. Xylocaine exerted local anesthetic effect of 35% and 50% at a concentration of 0.3 and 1.0 mg/ml respectively. The result shows that the extract has significant ($P < 0.01$) local anesthetic effect when compared to xylocaine. Antinociceptive activity of the ethanolic extract was evaluated using acetic acid induced pain and heat. The extract demonstrated significant antinociceptive activities dose dependently when compared to control. The activity being more pronounced at higher dose of 600mg/kg which gave the high percentage protection of the abdominal constriction induced by acetic acid. About 80 and 100% of the treated rats with 400mg/kg and 600mg/kg of the extracts slid down the board. Effect of the extract of pentobarbitone sleep time in Albino Wister rats was also evaluated. The ethanolic stem bark extract of *V. doniana* increase the sleeping time together with the pentobarbitone from 72.3 ± 3.07 at a doses (100mg/kg of extract and 35 mg/kg of the pentobarbitone) to 181 ± 0.35 at a dose of 400mg/kg and 35 mg/kg respectively.

Keywords: Vitex, Phytochemical, Local Anesthetic, Analgesia, muscle relaxant, pentobarbitone sleep

INTRODUCTION

The use of plants for medicinal purpose has been there for thousands of years. Folk medicine both ancient and modern has been a source of useful chemotherapy. Nearly all cultures of the world, both ancient and the recent have heavily depended on plants as a therapeutic agents used in various forms.

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Plants play a major role in the treatment of diseases and still remain the foremost alternative for a large majority of people (Adiaratou *et al.*, 2005). Plants are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Gilani and Rahman, 2005). All civilizations have always had traditions of using herbs to promote healing. Plants still remain the basis for development of modern drugs and medicinal plants have been used for years in daily life to treat diseases all over the world (Ates and Erzdogrul, 2003). According to Ayitey-Smith (1989), traditional medicine evolved from environmental resources, which the people of a community adapted in desperation for survival from disease. On the African continent, traditional medical practices date as far back as 4000 years. It was the sole medical system for health care before the advent of orthodox or modern medicine. The demand for herbal medicines is increasing rapidly due to their lack of side effects. Further as health care costs continue to escalate, the attraction for low-cost remedies has stimulated consumers to re-evaluate the potential of alternatives (Bouldin *et al.*, 1999). Literature has shown that some plants have found application in medicine. In Africa, particularly in Nigeria, herbal medicine has become a part of peoples culture though this form of medicine is not well organized as in China and India (Gbile, 1980). According to Gbile and Adesina (1986), the Nigerian flora has made and would continue to make great contributions to health care of Nigerians. *Vitex doniana* Sweet, a plant commonly known black plum, in English, Prunier noir in French, dinya in Hausa, ucha koro in Igbo, oori-nla in Yoruba and ngarmi in Kanuri is a medium-sized deciduous tree, 8-18m high, with a heavy rounded crown and a clear bole up to 5m. *V. doniana* is from *Verbenaceae* family and abundantly occurring in savannah regions. It can be found throughout tropical Africa. (Han-Jurgen, 1990; Huchinson and Dalziel, 1964; and Kokwaro, 1976). Studies have established central and peripheral effects of the root bark extract whereas reports on the stem bark is scanty if not unavailable.

MATERIALS AND METHODS

Sample collection and identification

The stem bark of *Vitex doniana* leaves were collected in Kawuri village of Konduga (11°39'6"N 13°25'10"E) Local Government Area of Borno state of Nigeria. The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S.S. Sanusi of the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The herbarium specimen with a voucher number 555C was deposited at the Post Graduate Research Laboratory, Department of Chemistry. The stem bark of the *Vitex doniana* was cleaned and air-dried in the laboratory. Five kilogram (5kg) of the stem bark of *Vitex doniana* was pulverised into a coarse powder using mortar and pestle.

Extraction of stem bark of *V. doniana* and Phytochemical analysis

The weighed powdered air-dried sample (2kg) was macerated with 95% ethanol for five days filtered and evaporated

in vacuo at 40°C using a rotary evaporator. The extract concentrate was labeled and the percentage yield was calculated in w/w. The ethanolic extract was subjected to qualitative chemical screening for identification of the primary and secondary metabolites such as flavonoids, alkaloids, sterols, triterpenes, saponins, anthracenosides, tannins, polyuronides, emodol, etc as described by (Ioan, 1982; Sofowora, 1993a&b and Trease and Evans 2002).

Pharmacological Investigations

Animals

Sixty (60) Albino wister rats of both sexes weighing 100-150g, fifty (50) mice of both sexes weighing 20-30g, and two (2) male rabbits were used for the experiments. They were obtained from a colony of rats maintained at the animal house of the Institute of Trypanosomiasis Research Vom, Nigeria. They were housed in clean cages and had access to feeds (ECWA FEEDS) and water *ad libitum*. They were allowed to acclimatize for two (2) weeks in the Veterinary Physiology, Pharmacology and Biochemistry Laboratory before the commencement of the study. All the animals were handled according to the guiding principles of biomedical research involving animals (CIOMS, 1985) as certified by the ethics committee of Faculty of Veterinary Medicine, University of Maiduguri.

Acute Toxicity Evaluation (LD₅₀)

The acute toxicity (LD₅₀) value of the crude stem bark ethanolic extract of *Vitex doniana* was determined using standard procedures described by Lorke (1983). In this study two different routes of administration were considered, that is the oral and intraperitoneal. In phase I rats were divided into 3 groups of three rats for each route and then treated with crude ethanolic extract at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal and orally and observed for 24 hours for mortality. In the phase II, the animals in group for each route will be divided into 4 groups (one animal in group 1, two in group 2, three in group 3 and five in group 4) and doses of 1600mg/kg, 2900mg/kg and 5000mg/kg of ethanolic extract were administered orally and intraperitoneally. The LD₅₀ value was calculated as the geometric mean of the surviving group and the death group in the second phase for which 0/1 and 1/1 was observed.

Local Anesthetic Evaluation

The method described by Shetty and Anika (1982) was used and as adopted by Abdurahaman, (2004). Four identical and symmetrical and circular regions were shaved on the dorsum of the Male rabbits with the 2-shaved circles on the thoracic regions and 2 others on the lumbar regions 24 hours before the commencement of the experiment. Two concentrations (1.0 and 0.3 mg/ml) of xylocaine each were injected intradermally in the left thoracic and left lumbar shaved regions respectively to form wheals, which were encircled with a marker. The encircled regions were each pricked with needle 10 times at 5 minutes interval for 30 minutes starting with zero i.e before the injection of the drug or extract. The number of responses to pain by the rabbits when pricked with needle was recorded as positive.

Effect of Extract on Acetic Acid-Induced Writhing in Mice

Adult mice weighing 20-30g was used for the experiment. The abdominal constriction resulting from i.p. injection of acetic acid (0.6% v/v) consisting of a contraction of abdominal muscle, together with a stretching of hind limbs, was carried out according to the procedure described by Santos *et al.*, (1994); Correa *et al.*, (1996); Nwafor, (1998); Abdulrahman, (2004). The animals were divided into 4 groups of 6 mice per cage.

Group I served as control while groups 2-4 was pretreated with graded doses (mg/kg) of *V. doniana* extract (i.p.) 30 minutes later, acetic acid was administered. The number of writhing movements was counted for 30 minutes. Antinociception was expressed as the reduction of the number of abdominal constriction between control rats (water treated rats) and rats pretreated with the extract.

Effect of Extract on Thermally-Induced Pain in Mice

The effect of extract on hot plate-induced paw was investigated in mice (20- 30g). In these experiments, the electric hot plate was maintained at $45 \pm 1^\circ\text{C}$ (as it will be connected to the thermostatically controlled water bath).

Animals was placed into a glass beaker of 50cm³ diameter on the heated surface and the time(s) between placement and the shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30s cut-off is used to prevent tissue damage. The animals were divided into 4 groups of 5 mice per cage. Group I served as control and received only distilled water. Groups 2-4 was pretreated with graded doses (g/kg) (i.p.) of *V. doniana* stem bark. 10 minutes prior to their being placed on the hot plate. The number of jumps per minute was used as a criterion of discomfort.

Muscle relaxant Activity by Inclined Board Method

Twenty rats of both sexes were divided into four (4) equal groups. The method of Kitano *et al.*, (1983), used by Abdulrahman, (2004) was adopted. The rats was placed one after the other on the smooth surface of a board inclined at 35°C in the horizontal before and after 30 minutes after treatment with varying doses of the extract i.p. The rats were allowed a minimum of 10 seconds to remain on the board. The rats that slip down the board before 10 Seconds was counted as positive for muscle relaxation

Effect of ethanolic stem bark extract of *Vitex doniana* on pentobarbitone sleeping time

Twenty four rats of both sexes were randomly divided into five groups of six rats each. Group 1 (control) was treated with pentobarbitone (35mg/kg) only, while groups 2-4 were treated respectively with 100, 200 and 400mg/kg of the ethanolic extract plus pentobarbitone (35mg/kg). All treatments were done intraperitoneally (I.P) and pentobarbitone was given 30 minutes before extract administration. The rats were given free access to food and water *ad libitum*. The time of pentobarbitone administration, the onset of sleep and the time of awakening were recorded.

Statistical Analysis

Data expressed as mean \pm SD and mean \pm SEM. Test of Significance between control and treatment means were carried out by Analysis of Variance (ANOVA) using Graph Pad Software (2000).

RESULTS AND DISCUSSIONS

The extracts concentrate yields of ethanol is 14.8% w/w. Preliminary phytochemical analysis of the ethanolic stem bark extract of *V. doniana* revealed the presence of tannins, phlobatannins, saponins, carbohydrates, cardioactive glycoside, flavonoids, steroids and terpenes. Alkaloids and anthracenosides are absent in the extract. The phytochemicals found are implicated to have much medicinal importance.

The intraperitoneal LD₅₀ in rats was found to be 2154.06mg/kg. On administration of 5000mg/kg dose of the extract via oral route, there was no dead which makes it impossible to estimate LD₅₀ via oral route using the Lorke method (1983). According the classification of Clarke and Clarke (1977), substances that have an intraperitoneal LD₅₀ between 50 and 500mg/kg are considered toxic and Onyeyilli *et al* (2000) categorized an intraperitoneal LD₅₀ of 1400mg/kg under low toxicity. The fact that LD₅₀ was obtained is an indication that the extract could be administered with some degree of safety both on the oral and intraperitoneal route. The toxicity observed may be due to various chemical present in the stem bark of *V. doniana*. The administration of doses of 50 mg/mL and 100 mg/mL of the extract produced 70% and 80% local anesthesia respectively on rabbits. Xylocaine exerted local anesthetic effect of 35% and 50% at a concentration of 0.3 and 1.0 mg/ml respectively (figure 1 and appendix 1). The result shows that the extract has significant ($P < 0.01$) local anesthetic effect when compared to xylocaine. The effect of the extract at 100mg/mL appeared to be superior than that of xylocaine (1mg/mL). This report appeared to be similar and support the findings of (Abdurahaman *et al.*, 2007, Onyeyili *et al.*, 1998; Aji *et al.*, 2001 and Sandabe *et al.*, 2002) who used this method to evaluate the local anesthetic properties of other plants.

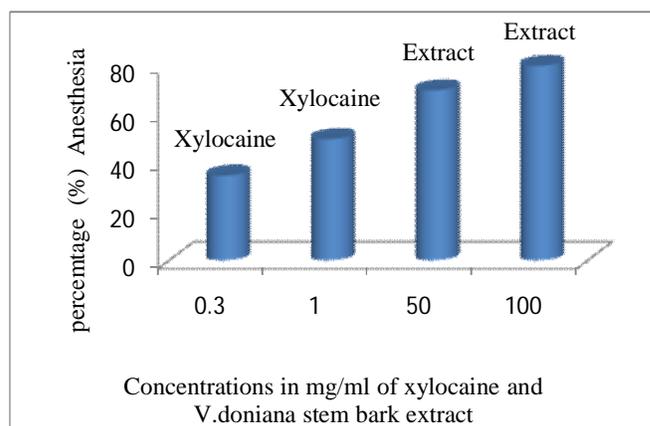


Fig. 1: Percentage(%) Anaesthesia and concentrations in evaluation of Local Anesthetic activity of stem bark ethanolic extract of *V. doniana*.

The ethanolic stem bark extract demonstrated significant antinociceptive activities ($P < 0.05$; $P < 0.001$) dose dependant as 39.0 ± 0.71 , 45.8 ± 0.89 and 55.8 ± 0.37 (Mean number of writhings) respectively for various doses (600, 400 and 200mg/kg *i.p.*) as compared to the control (66.0 ± 0.64) as shown in figure 2 and appendix 2. The activity is more pronounced at a high dose of 600mg/kg which give the highness percentage of inhibition (40.9%) of the abdominal constriction induced by acetic acid. This was found to be significantly lower than pentozocin (20mg/kg) in the extent to which the writhing or stretching induced by acetic acid was reduced. The abdominal constriction method used in evaluating the activity is a very sensitive one and can detect antinociceptive activity of substance at a dose that cannot be detected by other methods such as tail-flick test (Koster *et al.*, 1959; and Collier *et al.* 1968).

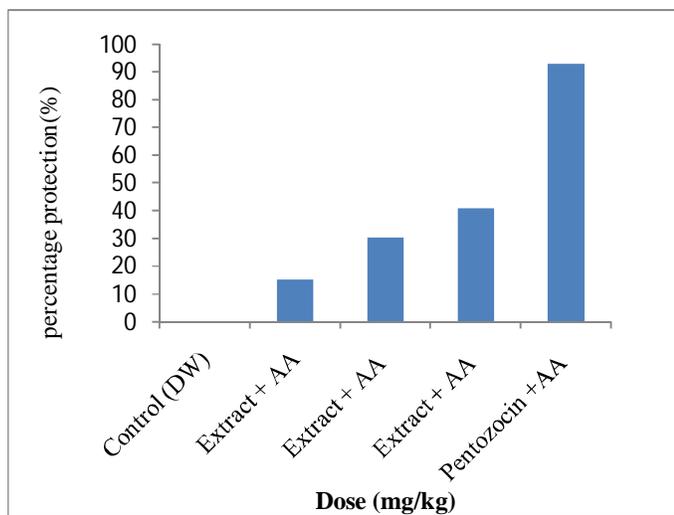


Fig. 2: Percentage protections and various doses of stem bark *V. doniana* ethanolic extracts in evaluating the effects of extract on acetic acid (0.6 %) induced wriths in mice.

The analgesic activity may be due to presence of bioactive compounds such as flavonoid and tannins that are present in the extract. Flavonoids and tannins possessed analgesic and /or anti-inflammatory activities (Ahmadiani *et al.*, 1998 ; Ahmadiani *et al.*, 2000). Also extract appeared to induce an increase in pain threshold to Eddy's hot plate when compared to control (figure 3 and appendix 3). The extract doses (200-600mg/kg) have significantly increased the time of pad licking. However pentozocin significantly increased the time of pad licking with effect superior when compared to the extract. The analgesic superiority of the pentozocin is expected, since pentozocin is a narcotic analgesic used to alleviate deep-seated pain (Turner, 1965; Besra *et al.* 1996).

Fifty percent of the rats treated with 100mg/kg of the ethanolic extracts and 100% of the treated rats with 600mg/kg of the extracts slid down the board. As can be seen from the table, there was a close dose dependant activity on the inclined board test. (Figure 4 and Appendix 4). This shows that the stem bark of *V. doniana* possesses muscle relaxant activity as demonstrated by its effect of inclined board test that can evaluate the muscle

relaxant activity (Kashara and Hikino, 1987; Abdurahaman *et al.*, 2000 and Abdurahaman *et al.*, 2007). This is also in agreement with earlier reports with other plant species that have been established to have muscle relaxant activities (Onyeyili *et al.*, 1998; Effraim, 1999; Sandabe , 2002).

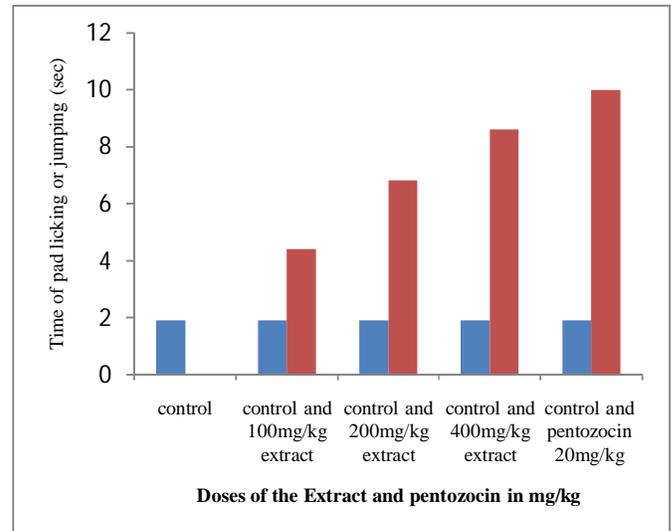


Fig. 3: Effect of ethanolic stem bark extract of *Vitex doniana* on thermal nociception in mice.

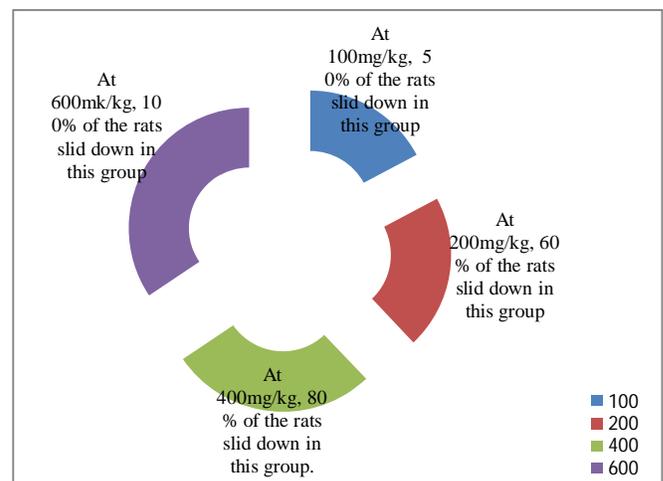


Fig. 4: Percentage (%) of rats that slid down against various doses of the *V. doniana* extract using inclined board method.

The ethanolic extract of *V. doniana* significantly ($P < 0.05$) increased *in* sleeping time of pentobarbitone dose dependently (table 6) at a dose of 100mg/kg, the duration of sleep was 72.3 ± 3.07 when compared with control which was 85 ± 0.23 minutes (table 1.) However with increased in extract dose (200mg/kg and 400mg/kg), there was significant potentiation of the sleeping time. Barbiturates like pentobarbitone have depressant activities on the central nervous system producing effects similar to those of inhalation anesthetic (Rang *et al.*, 1999). The extract and pentobarbitone prolongation of sleeping time appeared to have corroborate the observation of Fujimori (1965) and Abdurahaman *et al.*, (2007)

Appendix. 1: Local Anesthetic effects of stem bark ethanolic extract of *V. doniana*.

Dose/extract	conc. (mgmL ⁻¹)	No. of positive response* over time (min)							Total out of 36	Anesthesia (%)
		0	5	10	15	20	25	30		
Xylocaine	0.30	0	2	3	3	3	4	4	16	35
	1.00	0	2	4	5	5	5	6	27	50
Extract	50	0	3	5	5	5	6	5	30	70
	100	0	4	5	5	6	6	6	32	80

*Positive response indicate failure to twitch, 6= maximum anesthesia, 0= No anesthesia,

Appendix. 2: Effects of stem bark ethanolic extract of *V. doniana* of acetic acid (0.6 %) induced writhes in mice.

Treatment	Dose (mg/kg)	Mean ±SEM	No. of Writhes	Percentage Protection (%)
Control (DW)		66.0 ± 0.64		0.00
Extract + AA	200	55.8 ± 0.37*		15.0
Extract + AA	400	45.8 ± 0.89*		30.1
Extract + AA	600	39.0 ± 0.71*		40.9
Pentozocin +AA	60	4.60 ± 0.40**		93.0

AA= Acetic acid, Dw = Distilled water, *P < 0.05 significantly different from the control, ** P < 0.001 significantly different from the control

Appendix. 3: Effect of stem bark extract of *Vitex doniana* on thermal nociception in mice.

Treatment Groups	Dose(mg/ kg)	Time of pad Licking or jumping (Sec.)	No. of rats used
Control (DW)		1.90 ± 0.09	5
Extract	100	4.40 ± 0.55*	5
Extract	200	6.80 ± 0.44*	5
Extract	400	8.60 ± 0.55**	5
Pentozocin	20	10.0 ± 0.54**	5

Dw = Distilled water, * P < 0.05 is significantly different from the controlethanolic, ** P < 0.001 is significantly different the control.

Appendix. 4: effect of ethanolic stem bark extract of *V. doniana* on muscle relaxation by inclined board

Extract dose (mg/kg)	Control	100	200	400	600
No. of rats that slid down board	0	50	60	80	100

In conclusion, the ethanolic stem- bark extract of *V. doniana* produces substantial depressant effect on both the central and peripheral nervous system. The peripheral effect is seen from the local anaesthetic activity of the extract which was superior to that of xylocaine at 100mg/kg and 1mg/ml respectively for the extract and standard drug. The central nervous activities were shown by its ability to potentiate pentobarbitone sleep, analgesic activities with acetic acid and heat as well as muscle relexant effects. These activities and the neuropharmacological effects were observed with the aqueous root-bark extract of this plant (Abdurahaman, 2004, Abdurahaman *et al.*, 2007). Thus this plant could be a good source of psychotherapeutic agent

Table. 1: Effect of methanolic leaf extract of *vitex doniana* leaf on pentobarbitone sleep time.

S/N	Treatment	Onset of sleep ± SEM (min)	Sleep time ± SEM (min)
1.	Control (35 mg/kg) Pentobarbitone	4.48 ± 0.17	85 ± 0.23
2.	Extract (100mg /kg)+ pentobarbitone (35mg/kg)	3.21 ± 0.40	72.3 ± 3.07
3.	Extract (200mg/kg) +	2.92 ± 1.21	143 ± 3.25*

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