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Evaluation of the Effects of the Aqueous Extract of *Vitex doniana* Root-Bark on the Peripheral and Central Nervous System of Laboratory Animals

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Abstract: Aim of this study to investigate the effects of aqueous extract of *Vitex doniana* on the peripheral and central nervous systems and possibility to use it as folk medicine. The aqueous extract of *Vitex doniana* was soxhlet extracted with distilled water and concentrated *in vacuo* to give a yield of 8.5% w/w. The LD₅₀ following intraperitoneal administration was estimated to be 980 mg kg⁻¹. The aqueous extract of *Vitex doniana* from the study produced substantial depressant effects on both the peripheral and central nervous system. The aqueous extract induced sleep on its own at dose of 400 mg kg⁻¹ and potentiated sodium thiopental sleeping time in a dose dependant manner. It also showed significant (p<0.05) muscle relaxant activities and produced analgesia and weal anesthetic effect. The extract was able to confer 80% protection to rats treated with convulsive dose of PTZ, while it conferred 100% protection to rats treated with convulsion dose of strychnine.

Key words: *Vitex doniana*, sleeping time, analgesic, anticonvulsant, local anaesthetic, muscle relaxant

INTRODUCTION

For thousands of years nature provided has medicine for human illness and most of these remedies were obtained from higher plants. All plants in reaction to stress, infection, danger or environmental changes produce diverse chemicals which have therapeutic potential for a number of ailments and are useful as medicines (Williamson *et al.*, 1996; Winks, 1999). These chemicals which are secondary metabolites have also employed in traditional medicine. In deed the primary health care of 70-80% of the world population is based on the use of medicinal plants derived from traditional system of medicine and local processes (LMPTK, 2006).

Today developing and under developed countries as well as western countries are changing to alternative medicine due to the more holistic approach and substantially fewer side effects as apposed to the a depleted immune system upon prolonged use of allopathic medicine (LMPTK, 2006).

Several natural products used in folklore medicine have been verified using modern scientific methods with the World Health Organization recommending more efforts in this direction (Abdulrahman and Onyeyili, 2001; Zhang, 1997).

Medicinal plants exudates, gums, resins etc are used for the treatment of different diseases, which may

manifest themselves by symptoms such as pain, neuralgia, dyspepsia and anorexia (Choprai *et al.*, 1958).

Many reports are also available on the importance of *Vitex doniana* in African traditional medicine (Bolza and Keating, 1972; Hallam, 1979; Van Maydell, 1990)

A number of single and compound drug formulations of plant origin are indicated for the treatment of psychiatric disorders (Abdulrahman, 1992). These natural plant products used in different forms appear to have encouraging results. However, pharmacological studies on *Vitex doniana* according to literature survey are limited. There is therefore a need to investigate the effects of aqueous extract of *Vitex doniana* on the peripheral and central nervous systems and thus see if there is any scientific basis for its use in folk medicine.

MATERIALS AND METHODS

Plant collection, identification and extraction: The fresh root-bark of *Vitex doniana* were collected in the month of April 2003 from Bulabulin Ngarannam village in the outskirts of Maiduguri Metropolis and properly identified by Dr. S. S. Sanusi of Department of Biological Sciences, University of Maiduguri and voucher specimen (735B) were deposited in the Department of Chemistry Research Laboratory. The

root-bark of *Vitex doniana* was washed with distilled water to remove sand particles and air-dried in the laboratory.

The air-dried root-bark (500 g) was pulverized and soxhlet extracted with distilled water for 6 h. The extract was evaporated *in vacuo* and stored in a capped conical flask inside a refrigerator at 4°C until it was used. Fresh solution was prepared from the stock on the day of the experiment.

Animals and treatments: Sprague Dawley rats weighing 150-200 g were purchased from National Veterinary Research Institute, Vom and used for this study. They were housed in standard rat cages in the Department of Veterinary Physiology and Pharmacology Laboratory, University of Maiduguri, Maiduguri, Borno state Nigeria where they were allowed two weeks to acclimatize before the start of the experiment. They were allowed free access to food (ECWA Feeds Nigerian Ltd., Jos, Nigeria) and water *ad libitum*. Cross bred (New Zealand White x Local breed) rabbits (2.4-2.5 kg) obtained from Maiduguri Monday market were also used. The rabbits were housed in cages in the above named Department and were fed groundnut hay and commercial feed (ECWA Nig. LTD, Jos, Nigeria) and water was provided *ad libitum*.

Five groups of five rats each were used for the acute toxicity study. They were dosed intraperitoneally (i.p.) with varying doses (400, 600, 1000, 1200 and 1600 mg kg⁻¹) of aqueous extract of *Vitex doniana*. The rats were allowed access to food and water *ad libitum* and observed over a period of 24 h for clinical signs and death. The LD₅₀ with 95% confidence interval was determined using the arithmetic methods of Aliyu and Nwude (1982).

Effects of water extract of vitex doniana on sodium thiopental sleeping time in rats: Thirty rats of both sexes were randomly divided into 6 groups of five rats each and treated using standard procedures (Turner, 1965).

All the rats were given food and water *ad libitum* during the experiment. The time of sodium thiopental administration, the time of onset of sleep and the time of awakening were recorded. The results obtained were subjected to one-way ANOVA.

Local anaesthetic effects of aqueous root-bark extract of *Vitex doniana* was evaluated using the method described by Shetty and Anika (1982).

Anticonvulsant evaluation: Six groups (A, B, C, D, E, F) of five rats each were housed in clean cages and were given food and water *ad libitum*. The procedures adopted for the study were those described by

Takagi *et al.* (1960) and Maeda *et al.*, 1981). The results obtained were analyzed by one-way ANOVA.

The onset of convulsion, number of convulsions per minute and the duration of convulsions were recorded.

Analgesic effects: Five groups (A, B, C, D and E) of five rats each were used for acetic acid induced writhing. The method described by Corea *et al.* (1996) was employed.

The number of writhing movements that occurred was counted for 10 min starting 5 min after acetic acid injection for each group of animals. Using the number of writhes/stretches, the percentage analgesia was calculated using the formula of Hernandez-Perez *et al.* (1995).

$$\text{Protection \%} = \frac{\text{Mean writhing of control} - \text{Mean writhing of treatment group}}{\text{Mean writhing of control}} \times 100$$

For study on thermal nociception the rats were divided into five groups of five rats each. The rats were kept on Eddy's hot plate with a constant temperature of 45±1°C and time was recorded for either pad licking or jumping to occur as reported by Turner,(1965).

Muscle relaxation effects: The effect of aqueous extract on hindlimp grip reflex was determined using the method described by Bolon and St. Omar (1989). Rats unable to grasp the wire with hind paws within 15 sec were recorded as positive for loss of hindlimb grip reflex.

Twenty-five rats of both sexes were divided into five equal groups were employed for muscle relaxation activity by the inclined board. The method of Kitano *et al* (1983) was adopted. The rats were placed one after the other on a smooth surface of a board inclined at 35° to the horizontal before and 30 min after treatment with varying doses.

RESULTS

The dose of water root-bark extract of *Vitex doniana* that produced mortality was 1000 mg kg⁻¹ while the dose that causes 100% death was 1600 mg kg⁻¹. The symptoms of toxicity observed with extract administration were dose dependent. Ten to fifteen minutes after administration of extract all the rats in the various groups grouped together and were very weak. Those that received 600 mg kg⁻¹ dose and above were deeply sedated and slept. Signs observed before death included loss of appetite, lethargy, paralysis of hind limb, which progressed to fore limbs, difficulties in respiration and coma. Mortality was recorded 5 h after 1600 mg kg⁻¹ extract of *Vitex doniana* treatment.

The LD₅₀ with 95% confidence limit of the extract was estimated to be 980 mg kg⁻¹.

The aqueous extract of *Vitex doniana* significantly (p<0.01) increased the sleeping time of sodium thiopental dose-dependently in rats (Table 1). At a dose of 50 mg kg⁻¹ the duration of sleep was 31.2±0.75 min compared to the control, which was sodium thiopental only (28.8±0.57 min). The increase in the dose of the extract to 100 mg kg⁻¹ resulted in a duration of sleep of 38.0±0.63 min, while at doses of 200 and 400 mg kg⁻¹ of the extract the duration of sleep rose to 44.8±0.77 and 49.8±0.80 min, respectively. The aqueous extract only at the dose of 400 mg kg⁻¹ produced a duration of sleep of 35.8±0.58 min.

The aqueous extract of *Vitex doniana* produced 55.6 and 80.6% local anaesthesia at 25 and 100 mg mL⁻¹ concentration, respectively on rabbits. On the other hand xylocaine exerted local anaesthetic effect of 66.6 and 72.2% at concentrations of 0.3 and 1.0 mg mL⁻¹, respectively. The result shows that the extract has significant (p<0.01) local anaesthetic effect when compared to xylocaine (Table 2).

The aqueous extract of *Vitex doniana* at 300 and 600 mg kg⁻¹ provided 60% and 80% protection, respectively to rats against PTZ induced convulsion. However, in rats that were treated with convulsive dose of strychnine, the extract conferred 60% protection at 300 mg kg⁻¹ while the 600 mg kg⁻¹ dose conferred 100% protection (Table 3).

The mean onset of spasm was increased by the extract in PTZ treated rats by 15 and 48.68%, respectively in animals treated with 300 and 600 mg kg⁻¹ of the extract while in strychnine treated rats the mean onset of spasm was reduced to 16%. The mean number of spasms per minute were increased by 85% following treatment with extract at 600 mg kg⁻¹ in PTZ poisoned rats, while the mean number of spasms in strychnine

treated rats were reduced by 23% in the group given 300 mg kg⁻¹ of the extract and 66% in the animals treated with 600 mg kg⁻¹ of the water extract.

The time lapse between convulsions and death was initially reduced by 3% and then increased by 28% by the *Vitex doniana* extract in the rats that received PTZ. While in the rats that were given strychnine there was reduction in the time lapse between convulsion and death by 31% (Table 3).

The aqueous extract significantly (p<0.01) reduced the number of writhes induced by injection of acetic acid to rats in a dose-dependent manner (Table 4).

Administration of aqueous extract of *Vitex doniana* root-bark i.p. to rats appears to induce an increase in pain threshold when compared to the control.

The effect was dose-dependent with the time for pad licking significantly (p<0.01) increased with increased dose of extract.

The administration of 200 mg kg⁻¹ of the extract resulted in the time of pad licking of 2.0±0.01 sec compared to control (1.30±0.36 sec), while the administration of 600 mg kg⁻¹ of the extract resulted in 5.0±0.00 sec time of pad licking. Pethidine on the other hand produced the highest reaction time of 8.30±0.54 sec compared to the extract doses used (Table 5).

Sixty percent of the rats that received extract dose of 50 mg kg⁻¹ were unable to grasp wire with hind limb, while

Table 1: Effects of Aqueous Extract of *Vitex doniana* on sodium thiopentone sleeping time

Groups	Extract treatment (mg kg ⁻¹)	Onset of sleep ±SEM	Sleeping time (min) ±SEM
A	Thiopentone (20 mg kg ⁻¹)	8.6±0.01	28.8±0.51
B	50.0	5.6±0.65	312.0±0.75
C	100.0	3.8±0.07	38.0±0.63
D	200.0	4.8±1.37	44.8±0.77
E	400.0	7.6±0.46	49.8±0.80
F	400.0*	6.0±1.16	35.8±0.58

*Extract alone

Table 2: Local anaesthetic effect of aqueous extract of *Vitex doniana* root-bark on rabbits

Drug/Extract	Conc. (mg mL ⁻¹)	No. of positive response* over time (min)							Total out of 36	Anaesthesia (%)
		0	5	10	15	20	25	30		
Xylocaine	0.30	0	3	3	5	4	4	5	24	66.67
	1.00	0	3	3	4	5	5	6	26	72.20
Vitex extract	25.00	0	2	3	3	4	4	4	20	55.57
	100.00	0	4	4	5	5	5	6	29	80.56

*Positive Response indicate failure to twitch; 6 = Maximum Anaesthesia; 0 = No Anaesthesia

Table 3: The effect of aqueous extract of *Vitex doniana* root-bark on Pentylentetrazole (PTZ) and strychnine induced convulsion

Extract pretreatment (mg kg ⁻¹)	Convulsant treatment	Mean No. spasms per min (±SEM)	Mean onset of convulsion (min)	Mean onset of death (±SEM) (min)	Quantal death	Survival (%)
Control	PTZ (100 mg kg ⁻¹ , S.C.)	8.20±0.42	7.6±1.01	19.0±2.60	3/5	40.0
300		13.25±1.6	11.3±5.6	16.5±1.22	2/5	60.0
600		15.25±1.1	17.7±0.47	27.0±0.00	1/5	80.0
Control	Strychnine (2 mg kg ⁻¹ , i.p.)	17.60±2.4	7.2±0.92	16.7±1.88	3/5	40.0
300		13.50±1.67	6.0±0.50	4.5±1.88	2/5	60.0
600		6.0*	6.0*	0.00	0/5	100.0

* Only one rat in this group convulsed

Table 4: Effect of aqueous extract of *Vitex doniana* root-bark on acetic acid (0.6%), induced writhing in rats

Treatments	Dose of extract (mg kg ⁻¹)	No. of writhes per 10 min	Protection (%)
Control	-	65±0.57	0.0
Extract + AA	200	57±1.0*	12.3
Extract +AA	400	44±1.43**	32.2
Extract + AA	600	35±1.16***	46.2
Pethidine +AA	20	4±0.01	93.9

A.A. = Acetic acid

Percentage Analgesia was calculated using the formular of Hernandez-Perez *et al.* (1995)

$$\text{Protection \%} = \frac{\text{Mean writhing of control} - \text{mean writhing of treatment group}}{\text{Mean writhing of control group}} \times 100$$

* p<0.05, ** p<0.01, *** p<0.005, Significantly different from controls (student's t-test)

Table 5: Effect of aqueous extract of *Vitex doniana* root-bark on thermal nociception in rats

Treatment group	Dose (mg kg ⁻¹)	Time for padlicking or jumping (sec)	No. of rats used
Control (DW)	-	1.30±0.36	5
Extract	200	2.00±0.01	5
Extract	400	3.00±0.037	5
Extract	600	5.00±0.00	5
<i>Pethidine</i>	20	8.00±0.54	5

DW = Distilled H₂O

Table 6: Effects of aqueous extract of *Vitex doniana* root-bark on hindlimb grip reflex in rats

Dose of extract (mg kg ⁻¹)	No. of rats used	Rats unable to grasp wire with forepaws (%)
50	5	60
100	5	80
200	5	80
400	5	100
Control	5	00

Table 7: Effects of aqueous extract of *Vitex doniana* root-bark on muscle relaxation (inclined board method)

Groups	Dose of extract (mg kg ⁻¹)	No. of rats that slid down board (%)	No. rats used
A	50	40	5
B	100	60	5
C	200	80	5
D	400	100	5
E	Control	00	5

80% of the rats treated with 100 and 200 mg kg⁻¹ of extract, respectively and all the animals that were treated with 400 mg kg⁻¹ of the extract were unable to grasp wire with fore paws (Table 6).

Forty percent of the rats treated with 50 mg kg⁻¹ of the extract and 60% of the rats treated with 100 mg kg⁻¹ of the extract slid down the board, while 80 and 100% of the rats treated respectively with 200 and 400 mg kg⁻¹ of the extract slid down the board (Table 7).

DISCUSSION

The aqueous root-bark extract of *Vitex doniana* from the present study produced substantial depressant effects on both the peripheral and the central nervous system. The central action of the root-bark extract was shown by its ability to induce sleep, its effects on thiopentobarbitone and pentobarbitone sleeping time, strychnine and pentylenetetrazole induced convulsions, analgesic and muscle relaxant activities. The extract induced sleep on its own and appeared to potentiate barbiturate-sleeping time in a dose dependant manner indicating a pharmacological action.

The extract of *Vitex doniana* potentiated the anaesthetic effect of thiopentobarbitone and pentobarbitone dose dependently. The increase in dose of extract from 50 to 400 mg kg⁻¹ in thiopental treated rats resulted in increased duration of sleep from 31.2±0.75 to 49.8±0.80 min, while the dose increase from 100 to 400 mg kg⁻¹ of the extract in pentobarbitone treated animals resulted in increased duration of sleep from 59.33±2.65 to 161.33±1.67 min. Thiopental and pentobarbitone are ultra-short and short acting barbiturates, which are capable of inducing sleep for a period of 25 min (Range 20-30 min) and 65 min (Range 55-85 min), respectively.

The extract alone was observed induce sleep in rats for a period of 35.8±0.58 min and may have acted synergistically with the barbiturates to increase the duration of sleep dose dependently. Barbiturates are known CNS depressants. Extract from other plants have also been observed to potentiate the sleeping time of barbiturates and other CNS depressants (Asuzu and Abubakar, 1995; Onyeyili *et al.*, 1998; Chopra *et al.*, 1958; Aji *et al.*, 2001; Sandabe, 2002). The extract (400 mg kg⁻¹) on its own was observed to induce sleep within 10-15 min of administration. This is an indication of sedative and depressant action on the central nervous system and agrees with similar experiments in mice and rats using other plant extracts (Asuzu and Ugwuere, 1990; Asuzu and Abubakar, 1995).

The depressant activity of the extract may be attributed in part to an action on the cerebral mechanism involved in the regulation of sleep (Kaul and Kulkarni, 1978; N'gouemo *et al.*, 1994).

The CSN effect of water extract of *Vitex doniana* demonstrated by its ability to abolish the stimulant effect of strychnine at the dose of 600 mg kg⁻¹, however at a lower dose of 300 mg kg⁻¹ it conferred 60% protection to rats treated with convulsive dose of strychnine. The extract also reduced the mean onset of spasm by 16%.

Strychnine is known to act on the spinal cord by blocking the action of glycine, which is the main inhibiting transmitter acting on the motor neurons (Travell and Gold, 1935) and also facilitate multisynaptic reflexes (Franz, 1975). Since barbiturates are normally used to treat animals poisoned with strychnine (Onyeyili *et al.*, 2000), it is suggested that the root-bark extract of *Vitex doniana* could have similar mode of action with the barbiturates.

The extract was also observed to confer 60 and 80% protection on rats pretreated with 300 and 600 mg kg⁻¹ doses, respectively and subsequently treated with convulsive dose of pentylenetetrazole, indicating anticonvulsant effect. This agrees with similar studies carried out earlier in rats using different plants extracts (Asuzu and Abubakar, 1995; Effraim, 1999; Chopra *et al.*, 1958; Sandabe, 2002). This result appears to disagree with the assertion that most drugs with anticonvulsant activity do not completely abolish PTZ seizures but only decrease the seizure (Loscher *et al.*, 1991). Pentylenetetrazole acts on the brain stem by stimulating the medulla (Franz, 1975). The result also appears to be in agreement with the work of Asuzu and Abubakar (1995) in which almost all plants screened for anticonvulsant effects acted by increasing the time of onset of convulsions. The protection of rats given convulsive doses of strychnine and PTZ could be an indication that the root bark of *Vitex doniana* produced depressant activity both on the brain stem and the spinal cord. The ability of the extract to protect animals from induced seizures shows that it contains chemical components capable of antagonizing chemically induced seizures.

The root-bark extract of *Vitex doniana* possessed muscle relaxant activity as was shown by its effects on the inclined board test that can evaluate muscle relaxant activity (Kashara and Hikimo, 1987; Abdurahman *et al.*, 2000). In addition, the extract abolished the hind limb grip reflex, which measures motor coordination requiring synergistic limb activity as well as muscle strength (Attman and Sundarsan, 1975; Laguna *et al.*, 1993). The finding appear to be in agreement with earlier reports from other plant species that have been established to have muscle relaxant activities (Asuzu and Abubakar, 1995; Onyeyili *et al.*, 1998; Effraim, 1999; Sandabe, 2002).

The result of this study also shows that the root-bark extract of *Vitex doniana* induced some analgesic activity in rats. The 600 mg kg⁻¹ dose of the extract conferred 46.2% protection from writhes or stretches induced by acetic acid on the rats. This was found to be significantly lower than the effect of pethidine (20 mg kg⁻¹) in the extent to which the writhing or stretching induced by acetic acid was reduced. Acetic acid is believed to trigger the production of noxious substances within the peritoneum, which causes the writhing response

(Bartoli *et al.*, 1987). The extract appears to reduce the production of noxious substances (irritants) and thereby reduced the number of writhes in the rats. The result of the present study supports the finding of Effraim *et al.* (1999) and Sandabe (2002) who used this method to study extracts of other plants having analgesic activity. The plant also produced analgesia when pain was induced with heat. The extract doses (200, 400, 600 mg kg⁻¹) increased the time taken for pad licking significantly. Pethidine (20 mg kg⁻¹) significantly increased the time of pad licking when compared with the extract. The analgesic superiority of pethidine over the extract is expected, since pethidine is a narcotic analgesic used to alleviate deep-seated pain (Turner, 1965; Besra *et al.*, 1996).

Vitex doniana root-bark was observed to produce local anaesthesia in rabbits, which agrees with earlier reports on other plant species (Onyeyili *et al.*, 1998; Aji *et al.*, 2001; Sandabe, 2002). The local anaesthetic effect commenced 5 min after extract was injected intradermally (just as observed for xylocaine) and the effect lasted for over 30 min even with the lower concentrations. The effect of the 100 mg mL⁻¹ extract appears to be superior to the effect of xyloacaine (1 mg mL⁻¹).

In conclusion the root bark of *Vitex doniana* induced significant depressant effects on the central and peripheral nervous system. It potentiated barbiturate sleeping time and induced local anaesthesia, analgesia, anticonvulsant and muscle relaxant activities; hence the plant could be a potent source of psychotherapeutic agent(s).

REFERENCES

- Abdurahman, F.I., 1992. Studies in natural products chemistry: The *moraceae* in african traditional medicine and management of psychiatry in borno state. M.Sc. Thesis, University of Maiduguri, Maiduguri.
- Abdurahman, F.I., P.A. Onyeyili and Z.I. Samaila, 2000. The depressant effect of aqueous extract of *Ficus sycamoros* Linn. Nig. J. Trop. Agric., 3: 43-49.
- Abdurahman, F.I. and P.A. Onyeyili, 2001. Photochemical screening and Pharmacological activities of the stem-bark of *Terminala avicennoides*. Bull. Anim. Health Prod. Afr., 49: 236-242.
- Aji, B.M., P.A. Onyeyili and U.A. Osunkwo, 2001. The central nervous effects of *Mitragyna africanus* (Wild) stem-bark extracts in rats. J. Ethnopharmacol., 77: 143-149.

- Aliyu, Y.O. and N. Nwude, 1982. Veterinary Pharmacology and Toxicology Experiments. 1st Edn., Baraka Press and Publishers. Zaria, pp: 43-45.
- Asuzu, I.U. and E.E. Ugwueze, 1990. Screening of *Icacina trichatha* extract for pharmacological activity. *J. Ethnopharmacol.*, 28: 151-158.
- Asuzu, I.U. and I. Abu bakar, 1995. The effect of *Icacina trichatha* tuber on the nervous system. *Phytother. Res.*, 9: 21-25.
- Attman, J. and K. Sudarshan, 1975. Postnatal development of locomotion in laboratory rat. *Anim. Behav.*, 23: 896-899.
- Bartoli, A., A. Galli, C. Ghelardi, A. Giotti, M. Malcongio, P. Malmber-Aiclio and P.L. Zucchini, 1987. Antinociception induced by systemic administration of local anaesthetic depends on a central cholinergic mechanism. *Br. J. Pharmacol.*, 92: 711-721.
- Besra, S.E., R.M. Sharma and A. Gomes, 1996. Anti-inflammatory effect of petroleum ether extract of leaves of *Litchie chinensi Gaerm (Sapindaceae)*. *J. Ethnopharmacol.*, 54: 1-6.
- Bolza, E. and W.G. Keating, 1972. Africa Timber. The properties, uses and characteristic of 700 species. Melbourne Common in Scientific and Industrial Research Organization.
- Bolon, B. and V.E.V. St. Omar, 1989. Behavioural and development effects in sucking mice following material exposure to the mycotoxin secaconic acid. *D. Pharmacol. Biochem. Behav.*, 34: 229-231.
- Chopra, R.N., K.L. Nanda and D. Kapoor, 1958. Indigenous drugs of India. 2nd Edn., Dhara and sons. Calcutta, pp: 1-20.
- Corea, C.R., D.J. Kyle, V. Chakravorty and J.R. Calixto, 1996. Antinociceptive profile of pseudopeptide B2 bradykinin receptor antagonist NPC18688 in mice. *Br. J. Pharmacol.*, 117: 552-558.
- Effraim, K.D., 1999. Studies on some central nervous system activities of extracts of *Zizyphus spina-christi* and *Ocimum gratissimum* in Rodents. Ph.D Thesis, Usmanu Danfodio University Sokoto, Nigeria
- Effraim, K.D., H.A. Salami and P. Nwafor, 1999. The effect of aqueous seed of *Trigonelli foenum graecom (Fenugreek)* on hematological parameters in albino rats. *Afr. J. Biomedical. Res.*, 2: 47-51.
- Franz, D.N., 1975. Central Nervous System Stimulants. In: The Pharmacological Basis of Therapeutics. Goodman, L.S. and A. Gilman (Eds.), Macmillan Publishing Co., New York, pp: 359-366.
- Hallam, G.M., 1979. Medicinal uses of flowering plants in Gambia. 1st Edn., Vol. I. Department of Forestry, Yundum, pp: 208.
- Hernandez-Perez, M., R.M. Rabanal, M. Carman dela Tore and B. Rodriguez, 1995. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an O-naphthoquinone diterpenoid from *Saliva aethiopsis* roots and two hemisynthetic derivatives. *Plant Medica.*, 61: 505-509.
- Kashara, Y. and H. Hikimo, 1987. Central action of *Ganoderma lucidum*. *Phytother. Res.*, 1:17-20.
- Kaul, P.N. and S.K. Kulkani, 1978. New drug metabolism inhibitor of marine origin. *J. Pharm. Sci.*, 67: 1293-1296.
- Kitano, T., K. Horikoni and J. Kamiya, 1983. Studies on hypnotic and anti-convulsant actions on 2-methyl-3-(co-toly) quanzolone-4. *Nippon Yakurijoka Zasshi.* 56: 1421-1428.
- Laboratory for medicinal plants and traditional knowledge, LMPTK. [www.friht.org.in/htm/about%20laboratory visited 25/07/06](http://www.friht.org.in/htm/about%20laboratory%20visited%2025/07/06).
- Laguna, M.R., R. Villar, I. Cadavid and J.M. Calleja, 1993. Effects of *Chlorella stigmatophora* extract on the central nervous system. *Planta Medica.*, 59: 125-130.
- Loscher, W., C.P. Fassbender and B. Nothing, 1991. The role of technical, biological and pharmacological factors in laboratory evaluation of anti-convulsant drugs III. Pentylenetetrazole seizure models. *Epilepsy Res.*, 8: 171-189.
- Maeda, S., K. Sudu and M. Aburada, 1981. Pharmacological studies on *Shizandra* fruits. 1. Genera, pharmacological effects of gonisin A and shizandrin. *Yakugaku Zassi*, 101: 1030-1034.
- N'gouemo, P., H. Pamou-Tchivounda, M. Bakdy-Mounfinier, B. Koudogbo and C. Nguemby-Binda, 1994. Some pharmacological effects of Ethanolic extract of *Trema guinensis* on central nervous system. *Planta Medica.*, 60: 305-307.
- Onyeyili, P.A., J.A. Akinniyi and T.U. Yakubu, 1998. Sedative, convulsant and muscle relaxant effects of *Ficus platyphylla* stem-bark in rats. *West Afr. J. Biol. Sci.*, 7: 161-168.
- Sandabe, K.U., 2002. Pharmacological and Toxicological studies of aqueous extract of *Ficus sycomorus* L. stem-bark in laboratory. Ph.D Thesis. University of Maiduguri, Maiduguri, Nigeria.
- Shetty, S.N. and S.M. Anika, 1982. Laboratory Manual of Pharmacology and Toxicology. 1st Edn., Billing and Sons, Londo, pp: 45.
- Takagi, H., T. Ban and T. Takushima, 1960. Studies on hypnotic and anticovulsant action of 2-methyl-1, 3-(O-toly)-quanzole-4. *Nippon Yakurigaku Zasshi.* 56: 1421-1426.
- Travell, J. and H. Gold, 1935. Mechanisms of action of strychnine on respiration. *J. Pharmacol. Exp. Ther.*, 53: 168-178.

- Turner, R.A., 1965. Screening Methods in Pharmacology. Vol. 1, Academy Press, New York, pp: 106.
- Von Maydell, H., 1990. Trees and shrubs of the Sahel: Their characteristics and uses. Verlag Josef Margaret Scientific Books, Muhestrabe 9, D-6992-Weikershein, pp: 397.
- Williamson, E.M., D.T. Okpako and F.J. Evans, 1996. Pharmacological Methods in Phytotherapy Reseach. Vol.1. John Wiley and Sons. England.
- Winks, M., 1999. Function of Plant Sms and Their Exploitation in Biotechnology. Sheffield Academic Press, Sheffield.
- Zhang, X., 1997. Traditional medicine an alternative in the world. An overview. In: The international symposium on Herbal Medicine. King Fahad Hospital, Jeddah, Saudi Arabic. March 24-27, pp: 20.