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HISTOMORPHOLOGICAL EFFECT OF *RAUWOLFIA VOMITORIA AFZEL* ON THE PONS OF SLEEP-DEPRIVED WISTAR RATS

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ABSTRACT
Insomnia is a condition where sleep latency is greater than thirty minutes, sleep efficiency (time asleep/time in bed) is less than 85% or sleep disturbance more than three (3) times a week. The aim of this study was to ascertain the histomorphological effect of Rauwolfia vomitoria Afzel on the pons of sleep-deprived wistar rats. The aqueous leaf extract was obtained by the rotary extraction method. Thirty (30) animals were used in the experimental protocol; they were acclimatized and randomly distributed into six groups of five animals each. Group 1 served as the control, group 2 (non-insomnia induced treated with 20mg/kg of the extract), group 3 (insomnia-induced only), group 4 (insomnia-induced that was treated with 1 ml Diazepam), group5 (insomnia-induced that was treated with 10mg/kg of R.vomitoria) and group 6 (insomnia-induced that was treated with 40mg/kg of R.vomitoria). Sleep disturbance was established using a sleep tracker. Drug administration was for seven days. Anxiogenic behavioral pattern was observed using the elevated plus maze. The animals were anaesthetized, the pons extracted and processed histologically for paraffin wax embedding, sectioned, stained with Hematoxylin and Eosin (H and E) and viewed with a light microscope after which photomicrographs were obtained. Photomicrographs served as results and provided the basis for comparative studies. Histological sections of the insomnia-induced group revealed highly degenerating pontine cell bodies and in some cases disappearance of nucleus and degenerating pontine neurons. Sections of the insomnia-induced animals that were treated with a low dose (10mg/kg) of R.vomitoria extract revealed scattered reappearing nucleus with more vacuolations as compared to the insomnia-induced animals treated with Diazepam and insomnia induced animals treated with a high (40mg/kg) dose of R.vomitoria extract. The study indicates that the aqueous leaf extract of Rauwolfia vomitoria in high doses are of more therapeutic effect in ameliorating insomnia.

KEYWORDS: *Rauwolfia vomitoria Afzel, histomorphological, insomnia-induced, pons, wistar rats.*

INTRODUCTION
It is characterized by difficulty in falling or (and) staying asleep. Insomnia may be termed primary if condition is not affiliated to other health conditions and secondary if otherwise. It may be termed transient, acute or chronic depending on how long it lasts. Factors associated with primary insomnia includes- age factor and female gender[2], physiologic factors like daytime nap, poor sleep hygiene, medications like those used to treat cold, environmental factors like extreme heat[3] etc. Factors associated with secondary insomnia includes- specific health conditions such as allergies, circulatory diseases, nocturia- eg diabetes mellitus or insipidus[4,5], substance abuse[6] etc. Insomnia has pervasive influence upon daily life function compromising social and occupational performance hence should interrupted positively.[7]

Rauwolfia vomitoria (common names- Poison devil's pepper, African serpent wood, African snake root or Swizzle stick) has been reported to be varying therapeutic effects. When administered intramuscularly in dose 2-4mg, it has been used to treat psychosis and schizophrenia. The leaves has a traditional reputation as a fever reducing agent and an antidote for snake bites.[8] The leaves has been postulated to contain the important hypotensive and sedative alkaloids – reserpine and rescinnamine. The histological result of a research carried out on the effect of aqueous extract of *Rauwolfia vomitoria* root bark on the cytoarchitecture of the cerebellum and neurobehavior of adult male wistar rats where oral doses of 600mg/kg and 500mg/kg body weights of the extract was administered to rats in group A and B while the control received distilled water for seven days revealed distortions of the cerebellar cells and layers of the experimental groups compared to the control. The neurobehavioral test revealed locomotion and exploratory activities in the experimental groups compared to the control.[9]

MATERIALS AND METHODS

Collection, identification and preparation of plant material

The leaves of *R.vomitoria Afzel* were obtained from a forest in Onu-Akpaka, Amoji village in Ndiagu, Akpugo, Nkanu West Local Government Area of Enugu State, Nigeria. It was identified and authenticated in a Herbarium, Plant Science Department, University of Port Harcourt, Rivers State.

Experimental animals

Thirty (30) adult female wistar rats of weights between 100 – 240 grams were randomly selected and used for this study. The rats were bought from animal house of Physiology, Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt and acclimatized for two weeks. The animals were kept in wooden cages and sawdust served as animal bedding, they were fed with grower mash manufactured by Grand Cereals Nigeria Limited and tap water ad libitum.

The animals were randomly distributed into six (6) groups of five (5) animals each. Group 1 served as the control group, group 2 served as the non-insomnia-induced group that was treated with *R.vomitoria* (20g/kg) extract. Group 3 served as the insomnia-induced group only, group 4 served as the insomnia-induced group that was treated with 1 milligram Diazepam, group 5 served as the insomnia-induced group that was treated with a low dose (10g/kg) of *R.vomitoria* extract, group 6 served as the insomnia-induced group that was treated with high dose (40g/kg) of *R.vomitoria* extract. Weight differences were accounted for at different stages of the experiment.

Insomnia induction

Animals in group 3, 4, 5 and 6 were induced with insomnia by exposing animals to continuous lightening for 72 hours (3 days) using an electric bulb of 100 watts connected to a power output. An alternative source of light - rechargeable lamp (Multifunctional LED Emergency Lamp, KM-7650) designed such that it automatically turns on immediately there is a power failure was available. A sleep tracker that gives a graphical representation of the wake time, light and deep sleep times was coupled around the trunk of the wistar rats with the tracker sensor facing the ventral surface of the animal to track sleep modalities within the hours of 6.00 PM to 6.00 AM and 8.000PM to 8.00 AM after the exposure of experimental animals to uninterrupted light while the control was tracked before the induction.

Administration of extract

Drug administration was based on body weight. Insomnia-induced animals were administered oral doses of extract and diazepam (depending on group) after insomnia induction.

Histological tissue processing

Animals were anaesthetized by an intraperitoneal (I.p) injection 0.2ml Pentobarbitone Sodium and perfused intracardially with 50ml of 0.9% normal saline and then 100ml of 4% paraformaldehyde in 0.15M Phosphate Buffer Solution. The pons was harvested and post fixed in 4% formaldehyde for about 4 to 5 hours. Tissue was further subjected to routine histological tissue processing using the hematoxylin and Eosin (H and E) stain.

RESULTS

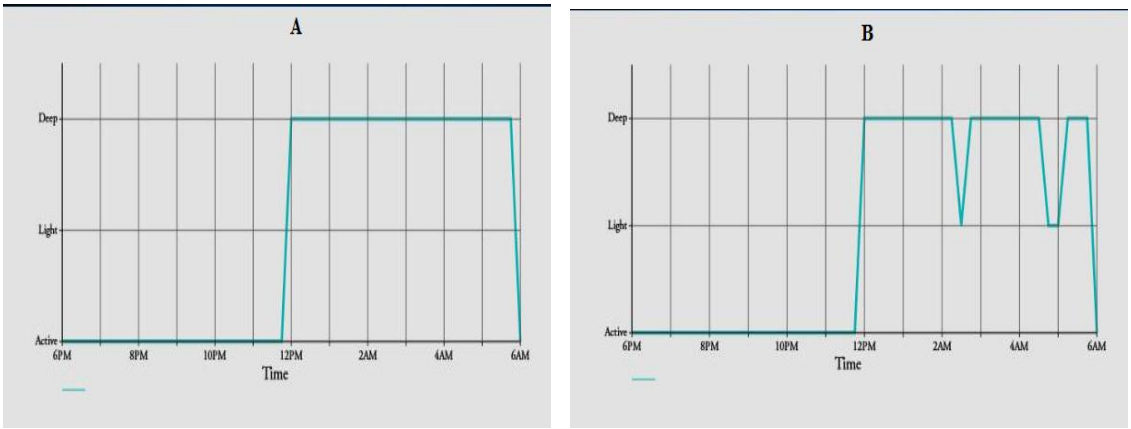

Figure 1

A: Graph of sleep modalities in the control (before exposure to light) from 6 pm – 6 am (Active = 6 hours; Deep sleep = 6 hours; Light sleep = 0 hour).

B: Graph of sleep modalities in the insomnia induced animal (after exposure to light) from 6 pm – 6 am (Active = 6 hours; Deep sleep = 5 hours 15 mins; Light sleep = 45 mins).

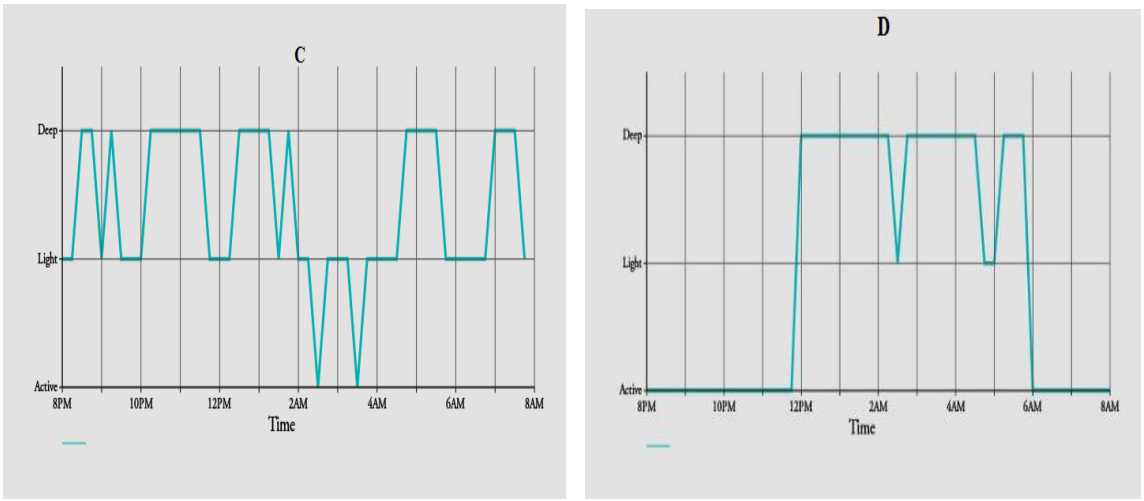

Figure 2

C: Graph of sleep modalities in the control animal from 8 pm – 8 am Active = 30 mins; Deep sleep = 5 hours 15 mins; Light sleep = 6 hours 15 mins).

D: Graph of sleep modalities in the insomnia induced animal from 8 pm – 8 am Active = 6 hours; Deep sleep = 5 hours 15 mins; Light sleep = 45 mins).

Photomicrographs

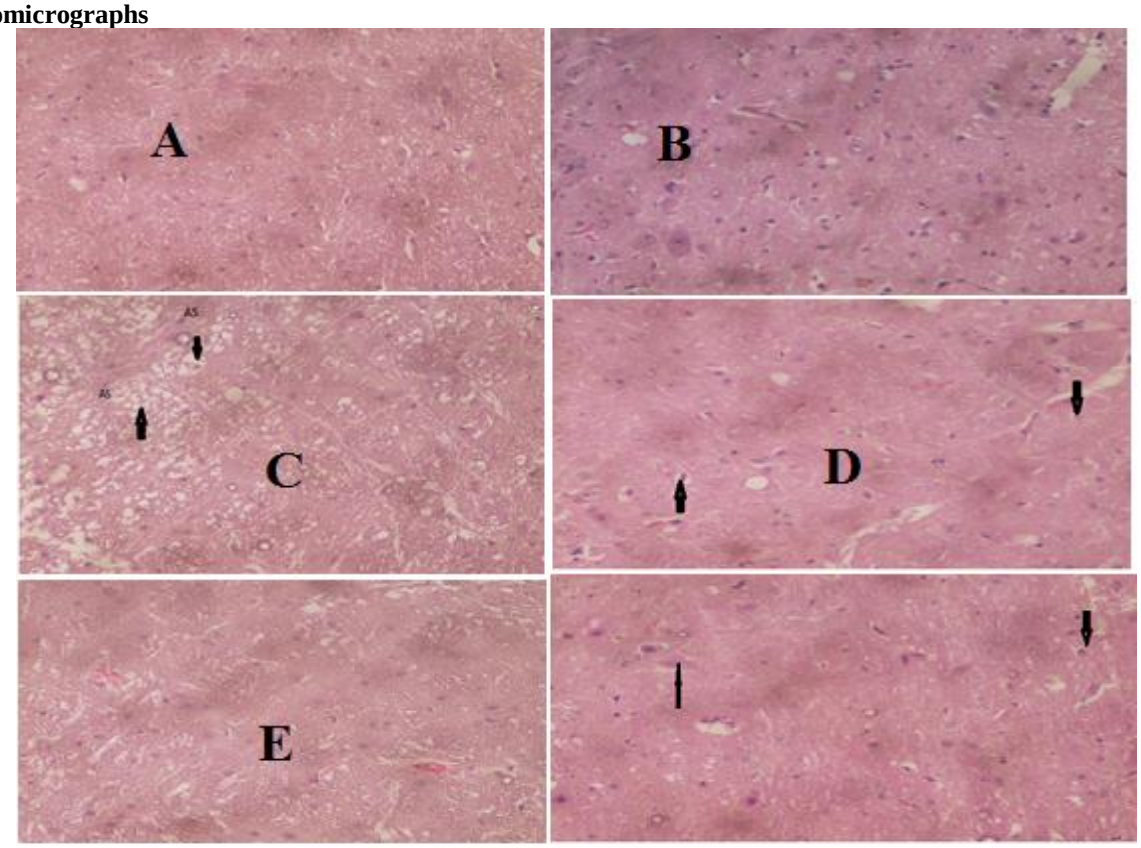

Figure: Photomicrographs of experimental groups.

A: Normal control sections of the pons of wistar rat. B: The pons of wistar rat that were administered 20g/kg *Rauwolfia vomitoria* extract showing well delineated nucleus with no pathology. C: The pons of insomnia-induced wistar rat showing highly vacuolated pontine cell bodies and infiltration of astrocytic cells (AS). D: The pons of insomnia-induced wistar rat that was treated with 1 milligrams Diazepam showing scattered reappearing nucleus with fewer vacuolation. E: The pons of insomnia-induced wistar rat that was treated with 10g/kg of *R. vomitoria* extract showing reappearing pontine nucleus with reduced vacuolations. F: The pons of insomnia-induced wistar rat that was treated with 40g/kg of *R. vomitoria* showing obscured vacuolation.

DISCUSSIONS

Phytochemical analysis revealed relative high abundance of alkaloids[10], flavonoids and saponin as postulated.[11] Carbohydrates, cardenolide, saponin and fixed oils were abundant. Tannin was absent.

The median lethal dose of the leaf extract was reported to be above 5g/kg[11] but the result (50g/kg) of this study exceeds the reported dose. There was no death or adverse effects recorded hence the median lethal of the aqueous leaf extract of *R.vomitoria* is relatively high and the extract considered safe.

Physiological changes like decreased body weights and motility and general state of tiredness was observed after the induction of insomnia in the experimental animals connoting the symptoms of insomnia.[1]

It has been reported that continuous exposure of rats to light induces a marked and transient decline in the Random Eye Movement sleep (REM) and Non – Random Eye Movement sleep.[12,13,14] Also aversive and anxiogenic conditions can be induced in a rat through laboratory room light.[15] Based on calculations with the use of anxiogenic formular (Anxiety = time spent in the open arm / time spent in open and closed arm).

The ascending order of anxiety across the experimental groups includes: *Group 2< Group 6< Group 4< Group 1< Group 5< Group 3.*

A general weight gain were observed in the animals treated with Diazepam and *R.vomitoria* at the end of the experiment. Increased appetite has been reported to be an adverse effect of *Rauwolfia vomitoria* Afzel[16] hence this may be the reason for the increase in weight since increased appetite may have resulted to increased food consumption and invariably an increase in weight of experimental animals. Since the same effect was observed in the animals administered Diazepam, this may suggest that both drugs may be targeted with similar therapeutic objectives.

Results from histological micrographs however revealed that the low dose of *R.vomitoria* administered yielded an insignificant degree of obscuration in vacuolations compared to the animals treated with a high dose of *Rauwolfia vomitoria* extract and Diazepam.

CONCLUSION

Insomnia results in the vacuolations of cellular bodies with disappearing nucleus and degenerating neurons in the pons. Vacuolations in cells is generally an adaptive physiological response presumably for damage limitations. However the aqueous leaf extract in high doses is of similar and more therapeutic effect than Diazepam than the low dose extract in reversing this effect. Thus the leaf extract of *Rauwolfia vomitoria Afzel* has the potential of antagonizing the effect of insomnia induction on the histomorphology of the pons and should be further researched.

CONFLICTS OF INTEREST

There is no conflict of interest.

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