Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 10 (2)

Neurobehavioural Analysis of Insomnia-Induced Wistar Rat administered orally with Aqueous Extract of *Rauwolfia vomitoria* Afzel.

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ABSTRACT

Person with insomnia have symptoms such as difficulty falling asleep, waking up often during the night and having trouble going back to sleep, waking up too early in the morning, feeling tired upon waking. The aim of this research was to investigate wistar rats' neurobehavioural activities that have been deprived of sleep and given aqueous leaf extract of *Rauwolfia vomitoria Afzel.* Thirty (30) adult female wistar rats of weights between 100 - 240g were randomly selected and used for this study. 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. The groups were labeled Group 1, 2, 3, 4, 5 and 6 respectively. The animals' sleep pattern was distorted by exposure to bright light at night for three (3) consecutive days to induce insomnia. Neurobehavioural test was conducted using Elevated Plus Maze (EPM). Scoring was made possible by the use of an Automated tracking and scoring software (Noldus Ethovision video tracking, Hamilton-Kinder infrared photo beam tracking) and this was done in a noise and human traffic free area/zone. Data from state of anxiety using grooming time and rearing time were taken and analysed. The result indicated that the group induced with insomnia only, experienced the highest state of anxiety. The other groups that were given the extract (at high dose and low dose) after light exposure had a reduced state of anxiety as indicated by the bar chart from EPM scores. The extract shows greater potential in the reversal of sleep distortion in albino wistar rat.

Key Words: Neurobehaviour, *Rauwolfia vomitoria,* Elevated Plus Maze,

INTRODUCTION

In ancient time, sleep was thought to be a function of the eye but modern medical practice has revealed that there is a conjoint effort of several parts of the brain whose neurons ceaselessly transmit signals. A thorough review of the Pons may thus give clues on this plaque – insomnia, as it contains some sleep centers. Insomnia also known as trouble sleeping is coined from the Latin word 'in' (no) and 'somnus' (sleep). Insomnia has been defined by the Diagnostic Classification Steering Committee of the American Sleep Disorders Association, as a sleep latency (time taken to fall asleep) that is greater than thirty (30) minutes, sleep efficiency (time asleep/time in bed) less than 85% or sleep disturbance more than three (3) times a week. It is sleep disorder characterized by difficulty in falling or (and) staying asleep. Inadequate sleep occurs when an individual reports one or more deficit in initiating or maintaining sleep. People with insomnia have one or more of the following symptoms: Difficulty falling asleep, waking up often during the night and having trouble going back to sleep, waking up too early in the morning, feeling tired upon waking[1].

There are basically two types of insomnia; Primary and Secondary. Primary insomnia means that a person is having sleep problems that are not directly associated with any other health condition or problem. Secondary insomnia means that a person is having sleep problems due to some underlying factors such as health conditions (like asthma, arthritis, cancer e t c). More so, insomnia can be termed transient, acute or chronic. This classification is based on how long it lasts and how often it occurs. It can be transient, short term (acute insomnia) or can last a long time (chronic insomnia). Transient insomnia occurs when symptoms last from a few days to some weeks, acute insomnia has its symptoms persisting for several weeks. Insomnia is termed chronic when it lasts a least months and sometimes years and majority of its cases being secondary.

It is of public health importance to understand which factors are associated with an individual experiencing insomnia. The major causes of primary insomnia are: Socio-demographic factors like significant life stress (job loss/change, shift in work, relationships, financial stress, death of a loved one, divorce e t c), increasing

age and female gender, lower income[2], environmental factors like noise, light, extreme temperatures etc, medications (like those used to treat cold, allergies, e t c) lifestyle behavioral factors which includes; smoking, alcohol and caffeine consumption, being overweight, physiologic factors like poor sleep hygiene (daytime naps, stimulation prior bed time), sleep environment [3]. Interference in normal sleep schedule (jet lag) may also interfere with sleep.

General symptoms of insomnia includes; sleepiness during the day, general tiredness, irritability, problem with concentration or memory. Insomnia can sap not only your energy level and mood but also your health, work performance and quality of life. It has a negative impact on both physical and mental health[1].

Herbal remedies as an alternative management of insomnia has taken a new verge. Herbal medicine also called botanical medicine or phytomedicine, refers to herbs, herbal materials, herbal preparations and finished herbal products that contain part of plants or other plants materials as active ingredients[4]. Herbal effect has to do with the synergistic action of nature's formula. Drugs and herbs are used differently but both can be extremely beneficial when used appropriately[5]. The use of herbs for the treatment of diseases is popular in developing countries for historical and cultural reasons[6,8] and is more economical because of the rising cost of orthodox drugs in the maintenance of personal health and well-being[7]. The increasing widespread use of herbal medicine has prompted the WHO to promote the integration of traditional medicine and complementary and alternative medicine into the national health care systems of some countries[9]. Herbal medicine has also allowed for research into the pharmacological activities of plants and their metabolites that influence biological processes and reverse diseased states[10].

The aim of this research is to investigate the neurobehavioural analysis of the aqueous leaf extract of *Rauwolfia vomitoria Afzel* on the Pons of insomnia - induced wistar rat.

MATERIALS AND METHODS

Experimental Animal: Thirty (30) adult female wistar rats of weights between 100 - 240g 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 (2) 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 sawdust was changed constantly at intervals to provide a clean and suited environment. The environment was well ventilated. The animals were randomly distributed into six (6) groups of five (5) animals each. The groups were labeled Group 1, 2, 3, 4, 5 and 6 respectively. Group 1 served as the control group, group 2 served as the non-insomnia-induced group that was treated with *R.vomitoria (200mg/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 (200mg/kg) of *R.vomitoria* extract, group 6 served as the insomnia-induced group that was treated with high dose (600mg/kg) of *R.vomitoria* extract.

The rats were weighed at inception, after acclimatization, subsequently during the experiment and before sacrificing. The dose of extract administered was dependent on the weight of the animal. The route of administration of the extract and drug was oral using oral-gastric tube. The animals were exposed to light and darkness within72 hours (L:D 24:00).

Administration of Extract and Drug: The aqueous leaf extract of *Rauwolfia vomitoria Afzel* were administered to the experimental animals in groups 3, 4 and 6 by oral gavage having determined the median lethal dose (LD_{50}). Diazepam was administered to experimental group 5 by oral gavage too while the control group received normal saline and distilled water.

Group 1 (N_1=5) represented the control, non-insomnia induced animals which were administered normal saline and distilled water.

Group 2 (N_2=5) represented non-insomnia induced animals which was administered a moderate dose (200mg/kg) aqueous leaf extract of *R.vomitoria Afzel*.

Group 3 (N_3=5) represented animals induced with insomnia only.

(N_4=5) represented insomnia-induced animal which was treated with 1 milligram of diazepam.

Group 5 (N_5=5) represented the insomnia-induced animals that was treated with a low dose (200mg/kg) of the aqueous leaf extract of *R.vomitoria* extract.

Group 6 (N_6=5) represented insomnia-induced animal which was administered a high dose (600mg/kg) of the aqueous leaf extract of *R.vomitoria* extract.

N_x = number of animals

Behavioural Analysis: Behavioural pattern such as anxiety test was conducted on the animals in group one having induced insomnia and the animals in the other groups after the administration of the plant extract using the elevated plus maze. Results were recorded appropriately.

The Elevated Plus Maze (EPM): The elevated plus maze (EPM) is a rodent model of anxiety that is used as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research. The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40

– 70 cm from the floor. The model is based on rodent's aversion of open spaces. This aversion leads to the behaviour termed thigmotaxis, which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space. In elevated plus maze, this translates into a restriction of movement to the enclosed arms. Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries in to open arms/total entries into open or closed arms). The total number of open arm entries and number of closed-arm entries are usually employed as measures of general activity.

The apparatus consists of two sets of opposing arms approximately 30×5 cm extending from a central (5×5 cm) region. Two arms are enclosed with 15 cm high walls. The remaining two arms are open. Wood was used as the construction material. A slightly raised lip (0.25cm) was provided on three sides of the open arms to minimize falls. Walls of the enclosed arms were opaque and dark. Variability in external factors (e.g light level differences) was also minimized. Similar behavioral measures were scored for this version of the test during the 5–minutes session. Scoring from a videotaped session minimized environmental variables introduced by the presence of the investigator that may impact anxiety-related behaviors. Automated tracking and scoring software (Noldus Ethovision video tracking, Hamilton-Kinder infrared photo beam tracking) was used.

RESULTS

Table 1: Summary Of Behavioral Parameters Across The Experimental Groups

Parameters	Control	Extract only	Insomia induced only	Insomia + Diazepam	Insomia +Low dose of Extract	Isomia + High Dose of Extract
Open Arm Entries	2.00	5.00	I.00	2.00	1.00	4.00
Closed Arm Entries	3.00	9.00	3.00	4.00	4.00	6.00
Time Spent in Open Arm (seconds)	10.00	18.00	5.00	11.00	11.00	12.00
Time Spent in Closed Arm (Seconds)	260.00	280.00	293.00	268.00	284.00	270.00
Grooming (seconds)	60.00	80.00	181.00	65.00	120.00	82.00
Rearing	4	3	3	7.00	7.00	8.00

Measure of Anxiety

(a) Anxiety = time spent in the open arm / time spent in open and closed arm

Group 1 (Control) = 10 / 270 = 0.037
Group 2 (Extract only) = 18 / 298 = 0.06
Group 3 (Insomnia only) = 5 / 298 = 0.016
Group 4 (Insomnia+Diazepam) = 11 / 279 = 0.039
Group 5 (Insomnia+Low dose) = 11 / 295 = 0.037
Group 6 (Insomnia+High dose) = 12 / 282 = 0.042

(b) Anxiety = open arm entry / sum of closed and open arm entries

Group 1 (Control) = 2 / 5 = 0.40
Group 2 (Extract only) = 5 / 14 = 0.35
Group 3 (Insomnia only) = 1 / 4 = 0.25
Group 4 (Insomnia+Diazepam) = 2 / 6 = 0.33
Group 5 (Insomnia+Low dose) = 1 / 5 = 0.20
Group 6 (Insomnia+High dose) = 4 / 10 = 0.40

Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries in to open arms/total entries into open or closed arms).

Ascending Order of Anxiety

Group 2 < Group 6 < Group 4 < Group 1 < Group 5 < Group 3

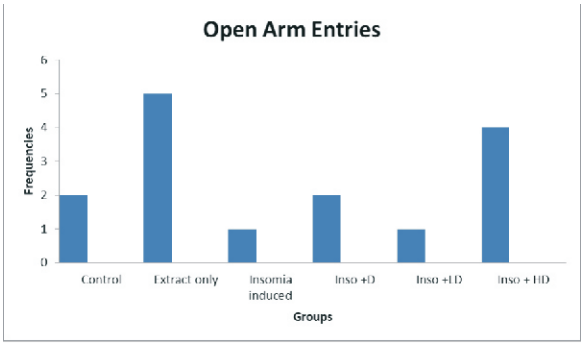

Chart 1: Bar Chart Showing the Mean of Open Arm Entries across the Experimental Groups

The result shows that the group that was treated with the extract only and the group that was insomnia-induced that was treated with a high dose (600mg/kg) of *Rauwolfia vomitoria* extract had a high frequency values in open arm entries indicative of exploratory tendencies (reduced anxiety) whereas, the group that was induced with insomnia only and the group that was insomnia-induced that was treated with a low dose (200mg/kg) of *Rauwolfia vomitoria* extract had a low frequency indicative of anxiety.
The control group and the group that was treated with Diazepam showed reduced anxiety.

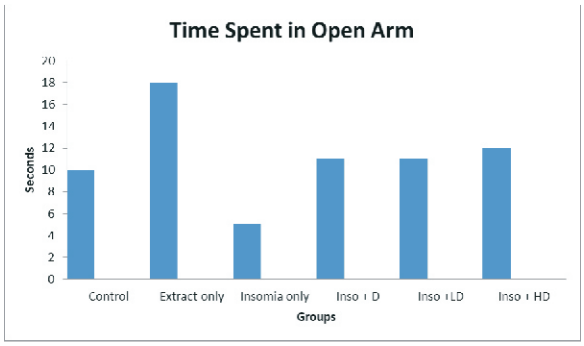

Chart 3: Bar Chart Showing the Mean of Time Spent in the Open Arm across the Experimental Groups

The group that was induced with insomnia spent the shortest time in the open arm when compared to the other experimental groups and this is an indication of anxiety. The group that received the 'extract only' spent more time in the open arm, which is indicative of reduced anxiety and increased exploration.

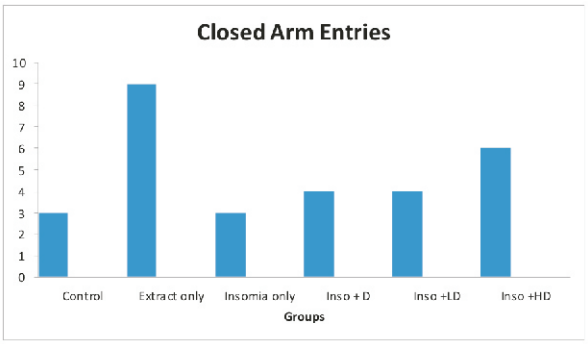

Chart 2: Bar Chart Showing the Mean of Close Arm Entries across the Experimental Groups

It was observed that the experimental group that was treated with the extract only and the group that was insomnia-induced that was administered a high dose (600mg/kg) of *Rauwolfia vomitoria* showed increased locomotive activity in the closed arm which is an indication of increased anxiety.

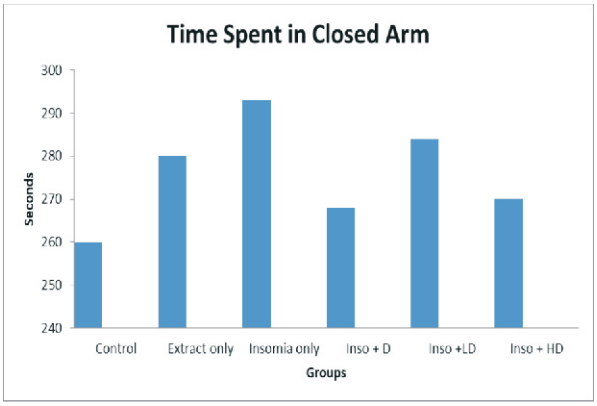

Chart 4: Bar Chart Showing the Mean of Time Spent in the Closed Arm across the Experimental Groups

The result showed that the animal that was induced with insomnia spent more time in the closed arm when compared with those in the other experimental groups, indicating a state of anxiety. The animals that was deprived of sleep and administered a low dose of *R.vomitoria* extract showed a significant level of anxiety when compared with the group sleep-deprived and treated with high dose of aqueous extract of *R.vomitoria* afzel . This indicates that the group treated with the low dose was more anxious than the group treated with high dose. The increase in the time spent in the closed arm is a general indication for anxiety among rodents as they tend to remain confined to the closed arm.

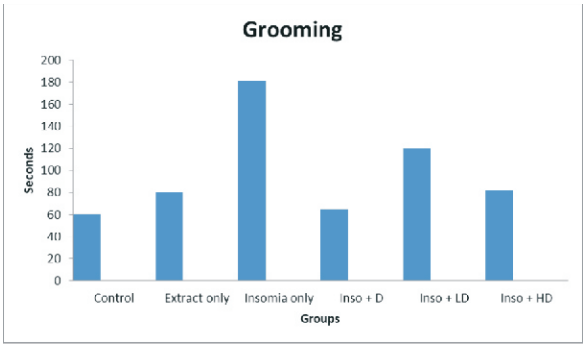

Chart 5: Bar Chart of the Mean of Grooming across the Experimental Groups

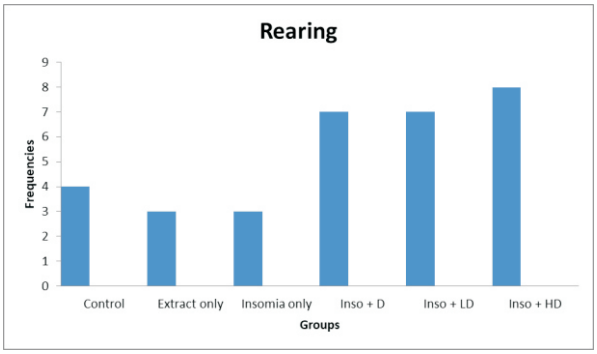

Chart 6: Bar Chart Showing the Mean of Rearing across the Experimental Groups

Grooming (the time the rat spent in scratching itself with its claws or licking itself) was relatively high in the group that was insomnia-induced, this indicated a state of anxiety. Less time of groomng was observed in the control, the group that was insomnia-induced and treated with low dose of *R.vomitoria* and the group that received the extract only. This indicates reduced state of anxiety. The time of grooming reduced more in the insomnia-induced animal treated with high dose of extract (600mg).

Rearing period was lower in the group administered with the extract only and the group that was deprived of sleep. This result showed decreased rearing frequencies which is an indication for emotional state.

DISCUSSION

The neurobehavioral test using the elevated plus maze compared parameters like number of open arm entries, number of closed arm entries, time spent in the opened arm, time spent in the closed arm, grooming and rearing amongst all the experimental groups. The group that received the extract only showed the highest degree of exploratory activities I e was least anxiety. Calculations on the measure of anxiety (time spent in the open arm / total time spent in open and closed arm) was done across the experimental groups. The group administered the aqueous leaf extract of *Rauwolfia vomitoria* extract only had the highest values. As suggested by Eluwa *et al.,* [11] and Odo *et al.,* [12], anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries in to open arms/total entries into open or closed arms). Hence anxiety was least in the animals that were administered *Rauwolfia vomitoria* extract only. Based on this calculation, the ascending order of anxiety across the experimental groups includes: Group administered *Rauwolfia vomitoria* extract only < Insomnia-induced group that was treated with high dose of *Rauwolfia vomitoria* extract < Insomnia-induced group treated with Diazepam < Control group < Insomnia-induced group treated with low dose of *Rauwolfia vomitoria* extract < Insomnia-induced group only.

The experimental group that was induced with insomnia exhibited the highest degree of anxiety when compared to the other experimental groups. This group remained almost confined to the closed arm throughout and spent the least time in the opened arm when compared to the other groups. The experimental group that was insomnia-induced and treated with Diazepam also exhibited some degree of exploratory activities (with some degrees of confinement in the closed-arm of the EPM which is almost similar to the group that was insomnia-induced and was treated with a low dose of *R.vomitoria* extract. The group that was insomnia induced and treated with a high dose of *R.vomitoria* showed lesser degree of exploratory activity (with lesser time of confinement in the closed-arm of the EPM) when compared with the groups that were insomnia-induced and treated with Diazepam and low dose of *R.vomitoria*.

CONCLUSION

Sleeep disturbance is highly observed in albino wistar in the presence of room light, which greatly affect the behaviour of the animals under such exposure (increased anxiety). However, when the extract was administered, there was considerable reduction of anxiety. This suggests anxiolytic property of *Rauwolfia vomitoria afzel* extract.

ACKNOWLEDGEMENT

Many thanks to the Departments of Anatomy and Pharmacology for their assistance.

CONFLICT OF INTEREST

Nil

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