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Evaluation of the sedative and anti-oxidative activity of *Rauwolfia vomitoria* methanol leaves extract using mature Swiss albino rats

Daniel Ikechukwu Oraekei *, Chukwuka Benjamin Uzodinma, Cynthia Onyinyechi Ezeugwu and Marcellus Ejike Nnamani

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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Abstract

There is growing recognition that many adolescents obtain insufficient and/or poor quality sleep, which is regarded both as an epidemic of sleep deprivation amongst adolescents and an important public health problem. Adolescents are thought to optimally require approximately nine hours of sleep per night. However, a meta-analysis found that 53% obtain less than eight hours of sleep on school nights, and 36% report difficulty in falling asleep. This study therefore evaluated the effects of *Rauwolfia vomitoria* in the treatment of insomnia as well as its anti-oxidative potentials. *Rauwolfia vomitoria* 1,600 g leaf powder was extracted using cold maceration in methanol for 72 hours with intermittent shaking. Phytochemical analysis, total phenolic content, acute toxicity study, invitro anti-oxidative studies and sedative activity of the extract were done using standard methods. The phytochemical analysis showed the presence of alkaloids, saponins, tannins, flavonoids, steroids and terpenoids. Mean total phenolic content was estimated as 120.95 ± 1.31 mgGAE/g. No mortality was observed in both phases of the acute toxicity test. The LD_{50} was greater than 5,000 mg/kg body weight. In the anti-oxidant assays, at doses of 0.8 and 1.6 mg/ml, the methanol extract was at the same range of percentage inhibition of free radicals with the standard drug. The extract also showed reduced mean sleep onset similar to that of diazepam but more potent at higher doses. In conclusion, this study indicated rapid, long-lasting and significant anti-oxidant and sedative activities of *Rawoulfia vomitoria* methanol leaves extract which was however dose dependent.

Keywords: Anti-oxidative activity; Insomnia; Phenobarbital sleeping time; *Rauwolfia vomitoria;* Sedative activity

1. Introduction

There is growing recognition that many adolescents obtain insufficient and/or poor quality sleep, which is regarded both as an epidemic of sleep deprivation amongst adolescents and an important public health problem [1]. Adolescents are thought to optimally require approximately nine hours of sleep per night [2]. However, a meta-analysis found that 53% obtain less than eight hours of sleep on school nights, and 36% report difficulty in falling asleep [3]. It has also been noted that insomnia is the most prevalent sleep disorder among adolescents [4]. Insomnia in this sense was defined as chronic dissatisfaction with sleep quantity and/or quality. Approximately 8-11% of young people meet diagnostic criteria for insomnia at any one time [5]. A number of factors may combine to increase vulnerability to insomnia in adolescence. These include firstly, a progressive reduction in the accumulation of homeostatic sleep pressure during wakefulness, which leads to a reduction in sleep drive [6]. The homeostatic sleep-wake system controls the need to sleep; with pressure increasing the longer an individual stays awake and decreasing as sleep occurs. Accumulated sleep drive before going to bed helps to control the quantity and quality of sleep. Secondly, adolescence is associated with a delay in the timing of sleep, which is related to a lengthening of the intrinsic period of the endogenous circadian oscillator [7]. The endogenous circadian oscillator is an internal clock system that regulates the daily, 24-hour rhythmic cycle, in concert with environmental time signals (e.g., the dark–light cycle). The secretion of melatonin is

* Corresponding author: Daniel Ikechukwu Oraekei; Email:di.oraekei@unizik.edu.ng

consistently associated with the human circadian system and melatonin is released later in the evening amongst adolescents than children, which delays the onset of evening sleepiness [8]. In other words, sleep/wake rhythms are delayed in adolescents compared to children, denoting the natural inclination of adolescents to go to bed later and sleep later. Combined with individual Chrono types such as preference for morningness or eveningness), the circadian system helps regulate optimum sleep schedules and sleep window; the timing of sleep is therefore delayed even further in adolescents with evening Chrono types and circadian rhythm disruptions [9]. Thirdly, adolescents develop responsibilities and social interests such as homework, employment, friendships) that encourage remaining awake later into the evening [10]. Fourthly, parental control over bedtime is lessened during adolescence [11]. Fifthly, electronic devices have a deleterious impact on sleep in adolescence, including delaying sleep onset and reducing sleep duration [12]. Sixthly, caffeine intake increases in adolescence, contributing to sleep initiation and maintenance problems [13]. Furthermore, adolescents are subject to the same physiological susceptibilities and psychological and social vulnerabilities that cause insomnia in adults such as predisposition to cognitive-emotional hyper-arousal [14]. These physiological maturational processes and psychological/social factors may interact in adolescence so that reduced sleep propensity in the late evening becomes permissive of continued waking activities and delayed bedtimes [15]. This can have two potential sleep related consequences including prolonged sleep onset latency (SOL) and poor sleep efficiency (SE), and sleep restriction, because school starts early in the morning. Adolescent insomnia severely impacts future health and functioning, and is thought to precipitate and maintain many emotional and behavioral problems, particularly anxiety and depression [16]. Sleep, arousal, and affect represent overlapping regulatory systems, with dysregulation in one system impacting on the others, so sleep disruption during key periods of maturational development may provide a pathway toward later affective dysregulation, and vice versa [17]. Adolescence is therefore a critical developmental window for understanding the mechanisms underlying the association between insomnia and internalizing symptoms to inform early intervention and prevention. There has been an increasing rise in cases of insomnia in recent times and most cases is caused by stress and anxiety. Oxidative stress and reactive oxygen species have been implicated in insomnia. In a certain study, reactive oxygen species (ROS) accumulate in neurons during the waking state, and sleep has a defensive role against oxidative damage and dissipates ROS in the brain. In contrast, insomnia is the source of inequality between ROS generation and removal by an endogenous antioxidant defense system. The relationship between insomnia, depression, and anxiety disorders damages the cardiovascular systems' immune mechanisms and functions [18]. Pharmaceutical anti-insomnia drugs are scarce, expensive and have addictive potentials among other adverse effects. Therefore, this study attempts to address and provide a better natural alternative to the already existing remedies. This is based on the belief that natural drugs are cheap, readily available, effective and devoid of serious adverse effects. This is evident in many herbal drugs been used locally for the treatment of insomnia. These drugs are been advertised by the users to be effective and safe. Also, many users have testified the effectiveness of these herbal drugs. This study therefore evaluated the effects of *Rauwolfia vomitoria* - a medicinal plant found growing in the wild in most parts of Nigeria - in the treatment of insomnia as well as its anti-oxidative potentials due to the fact that reactive oxygen species (ROS) were directly associated with insomnia.

2. Materials and methods

2.1. Plant Materials

Rauwolfia vomitoria leaves were collected from Nsukka, Enugu State Nigeria. The plant material was authenticated by a trained taxonomist, Mr. Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimen was deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka for future reference. The plant material was shredded then air-dried under room temperature for 3 weeks and pulverized with a mechanical grinding machine (GX160 Delmar 5.5HP).

2.2. Chemicals

Chemicals and experimental reagents used include: Methanol, Glacial acetic acid and diethyl ether (Guangdong Guanghua Chemical Factory Co., Ltd, China), Tween-80, Ascorbic acid, Fehlings solution (A&B, China), Ammonia solution, Millions reagent, ferric chloride (Griffin & George, England), HCL, Potassium dichromate, Potassium ferricyanide (Hopkin and Williams Ltd, England). All solvent/reagent purchased were of analytical grade. All laboratory reagents were freshly prepared and freshly distilled water was used when required.

2.3. Animal Source

Swiss Albino rats (120 – 130 g) of both sexes were used for the study. All the animals were obtained from the animal house of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu state, Nigeria. The animals were housed in standard laboratory conditions of 12 hours light, room temperature, and 40-60% relative

humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (National Institute of health (NIH) (2011) Pub No: 85-23) and animal protocol was approved by Animal care and ethics committee of Enugu State University of Science and Technology with approval number ESUT/AEC/0522/AP 427. Ethical approval was also gotten from Nnamdi Azikiwe ethical committee (approval number is NAU/ AREC/ 2024/ 0024).

2.4. Extraction

Rauwolfia vomitoria 1,600 g leaf powder was weighed using a weighing balance (Camry EK5350 Model, China) and extracted using cold maceration in methanol for 72 hours with intermittent shaking. The resulting solution was filtered using Whatman filter paper and the filtrate concentrated to dryness *in vacuo* using rotary evaporator (RE300 Model, United Kingdom) at 40 °C. The extract was stored in refrigerator between 0-4 °C.

2.5. Phytochemical analysis

The qualitative phytochemical analysis of the extract and fractions were carried out using standard methods described by Odoh *et al*., [19] as described by Oraekei *et al*., [20].

2.5.1. Test for alkaloids

The plant extract and fractions (0.2 g) was heated in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 minutes. The resulting solutions were allowed to cool and then filtered; then 5 mL of the filtrates used for the following tests:

- **Dragendorff's test**: To each labeled test tube, 5 mL of the sample was added, followed by 1 mL of Dragendorff's reagent. Formation of orange or red precipitates indicated the presence of alkaloids.
- **Hager's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate indicated the presence of alkaloids.
- **Wagner's test**: The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. A reddish brown precipitate indicated the presence of alkaloids.
- **Mayer's test**: A quantity of 5 mL of each of the samples was placed in labeled test tubes and a few drops of the Mayer's reagent (potassium mercuric iodide solution) were added. Formation of cream colored precipitate indicated the presence of alkaloids.

2.5.2. Test for glycosides

The samples were extracted with 1% H_2SO_4 solution in hot water bath for about 2 minutes. The resulting solution was filtered and made distinctly alkaline by adding 4 drops of 20% KOH (confirmed with litmus paper). One milliliter of Fehling's solution (equal volume of A and B) was added to the filtrates and heated on hot water bath for 2 minutes. Brick red precipitate indicated the presence of glycosides.

2.5.3. Test for saponins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the following test:

- **Frothing test:** The samples (5 mL) were placed in labeled test tubes and 5 mL of distilled water was added and the mixtures shaken vigorously. The test tubes were observed for the presence of persistent froth.

2.5.4. Test for tannins

The plant extracts and fractions (0.2 g) were dissolved in methanol individually and the resulting solutions were used for the test. To 3 mL of each of the samples a few drops of 1% Ferric chloride was added and observed for brownish green or a blue-black coloration.

2.5.5. Test for flavonoids

Using methanol, 0.2 g of the plant extracts and fractions were dissolved individually and resulting solutions were used for the following test:

- **Ammonium hydroxide test**: A quantity of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicated the presence of flavonoid.
- **Sodium hydroxide solution test**: A quantity of 10 mL of 10% sodium hydroxide solution was added to a portion of each of the samples and observed for color changes in the lower alkaline layer. Yellow color (flavones), Blue to violet color (anthocyanins), yellow to orange color (flavonones) were indicated accordingly.
- **Concentrated sulphuric acid test**: A portion of each of the samples were mixed gently with conc. sulphuric acid and observed for color change, yellowish orange color (anthocyanins), yellow to orange color (flavones), orange to crimson (flavonones) were indicated.

2.5.6. Test for steroids and terpenoids

- **Salkowski test:** The plant extracts and fractions were dissolved in methanol individually and the resulting solutions were used for the test. A 5 mL of each of the samples was mixed in 2 mL of chloroform and concentrated H2SO4 was carefully added to form a layer. A reddish brown coloration at the interface indicated a positive test.
- **Liebermann-Burchard test**: Acetic anhydride (2 mL) was added to 0.5 g of each of the fractions and methanol extracts. Concentrated H2SO4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.

2.6. Acute Toxicity Studies

Acute toxicity analysis of the extract was performed using Lorke's method [21]. This method has two phases (Phase 1 and Phase 2).

- **Phase 1:** Nine adult albino mice were weighed, marked and randomized into three groups of three mice each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of the extract. The mice were observed for 24 hours for signs of toxicity as well as mortality.
- **Phase 2**: Four mice were weighed, marked and randomized into four groups of one mouse each. They received 2000, 3000, 4000 and 5000 mg/kg body weight of the extract. Observation for 24 hours for obvious signs of toxicity and death was recorded accordingly. The LD50 was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

2.7. *In vitro* Antioxidant Assays

2.7.1. DPPH Free Radical Scavenging Activity Assay

The DPPH free radical scavenging activities of the extract and fractions were evaluated with modification of the method described by Patel and Patel [22]. Freshly prepared DPPH solution (25 µl, 0.6 mmol) was added to 25 µl of different concentrations of the extract (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 µg/mL). The volume of the solution was adjusted with methanol to a final volume 200 µl. The control tube contains 175 µl methanol and 25 µl of DPPH. After incubation in the dark for 30 minutes at room temperature, absorbance of the mixtures was obtained at 490 nm using micro plate reader. All the tests were performed in duplicate and ascorbic acid was used as standard. The DPPH radical percentage scavenging potentials of the extract and standard (ascorbic acid) were calculated from the equation below.

$$\% \text{ Inhibition of free radical} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the test/standard. The EC_{50} was determined from a plot of percentage scavenging potentials against concentration.

2.7.2. Total Phenolic content of the extract and fractions by folin-Ciocalteu's assay

The total phenolic content of the extract was determined using the method described by Kim *et al*. [23]. One milliliter of the extracts (100 µg/ml) was mixed with 0.2 ml of Folin-Ciocalteu's phenol reagent. After 5 minutes, 1 ml of 7.6% $N_{a2}CO_3$ solution was added to the mixture followed by the addition of 2 ml of distilled water. The mixture (in duplicate) was incubated at 40 °C for 30 minutes, after which the absorbance were read at 760 nm using UV-VIS spectrophotometer against blank (containing every other component of the mixture except sample). The total phenolic content was estimated from the calibrated curve which was made by preparing Gallic acid solution and expressed as milligrams of Gallic acid equivalent (GAE) per gram of the extract.

2.7.3. Linoleic acid peroxidation assay

The procedure was performed according to modified method of Choi *et al*., [24]. The extract at various concentrations (0.1 – 1.6 mg/mL) was mixed with 550 µL linoleic acid solution (0.28 mg linoleic acid and 0.28 mg Tween-20 in 100 µM phosphate buffer,pH 7.4), 500 µL of phosphate buffer (100 µM, pH 7.4) and 150 µl of ascorbic acid (10 µM). Trolox at same concentrations was used as reference standard while the blank contained the vehicle (2% DMSO) in place of sample/standard. The linoleic acid peroxidation was initiated by the addition of 0.1 mL $FeSO_4$ (10 µM) and incubated at 37 °C for 60 minutes. The mixture of reaction was cooled and added 1.5 mL trichloroacetic acid (10% in 0.5% HCl). Then, 3 mL TBA (1%, in 50 ml MNaOH) was added. The reaction mixture and TBA solution were heated in the water bath at 90 °C for 60 minutes. After cooling down, 2 mL aliquots were taken from each sample and vortexed with 2 mL butanol and centrifuged at 1000 x g for 30 minutes. The upper layer solution was separated for the spectrophotometric measurement. The absorbance of the solution was read at 532 nm and the percentage of linoleic acid peroxidation inhibition calculated using the following equation:

$$\text{Linoleic acid peroxidation inhibition (\%)} = ((A_{control} - A_{sample})/ A_{control}) \times 100$$

Where;
$A_{control}$ = Absorbance of control (without extract/standard), A_{sample} = Absorbance of extract/standard.

2.8. Sedative activity (potentiation of phenobarbital sodium-induced sleep)

2.8.1. Experimental design

The rats were divided into five groups of 5 animals each. Group 1 served as the vehicle control and received 10 ml/kg 5% Tween 80. Three groups (2, 3 and 4) were administered the methanol leaf extract at 100, 200 and 400 mg/kg body weight doses orally. Diazepam (0.4 mg/kg intraperitoneal (IP) injection) was used as reference drug (positive control). Thirty minutes after the administration of the test and control samples, each animal was injected with 40 mg/kg IP phenobarbital sodium. The animals were observed for the latent period (time between phenobarbital sodium administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) as explained by Moniruzzaman *et al*., [25].

2.9. Statistical analysis

Each experiment was repeated in triplicate and was measured as the mean value of the replicate trials ± standard error mean (SEM) (mean value ± SEM). For the plant in all three methods, the mean value and standard deviation (SD) and the standard deviation variance (SDV) were calculated to check the percentage inhibition of the antioxidant activity. ANOVA was performed to compare data and the data were analyzed using statistical package for social sciences (SPSS) version 20.

3. Results

Table 1 Results of phytochemical analysis of *Rauwolfia vomitoria* methanol leaf extract

Phytochemical	Availability
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Steroids and terpenoids	+
Glycosides	-

Key: + = Present; - = Absent

Yield: 72 g was recovered from the 1600 g powder extracted which represented 4.5% Yield

3.1. Results of acute toxicity study

No mortality was observed in both phases of the acute toxicity test. Reduced animal activity and sedation were observed in all the groups especially at second phase which received higher doses of the extract. The LD_{50} was estimated to be greater than 5,000 mg/kg body weight.

3.2. Results of anti-oxidation assay

Mean total phenolic content = 120.95 ± 1.31 mgGAE/g

Table 2 Results of DPPH scavenging activity of *Rauwolfia vomitoria* methanol leaf extract

Concentration of extract (µ/ml)	Extract %inhibition of free radicals	Ascorbic acid %inhibition of free radicals
1000	88.04	99.32
500	81.94	98.76
250	76.64	98.42
125	52.14	98.19
62.5	37.47	97.86
31.25	30.36	96.84
15.625	25.85	51.02
7.8125	19.64	29.46

Table 3 Results of lipid peroxidation assay of *Rauwolfia vomitoria* methanol leaf extract

Concentrations of extract and Trolox (mg/ml)	Extracts %inhibition of peroxidation	Trolox %inhibition of peroxidation
0.1	23.55	48.52
0.2	30.42	54.57
0.4	43.78	64.01
0.8	57.03	70.03
1.6	58.13	71.94

Table 4 Results of phenobarbital induced sleeping time

Groups	Treatments /kg body weight	Mean sleep onset ± SEM (minutes)	Mean sleep duration ± SEM (minutes)
1	10 ml 5% Tween 80	30.90 ± 1.44	64.80 ± 0.86
2	100 mg of extract	ns 30.00 ± 1.14	ns 88.40 ± 2.16
3	200 mg of extract	* 17.60 ± 1.03	* 148.60 ± 1.57
4	400 mg of extract	** 10.80 ± 0.97	** 176.80 ± 2.60
5	Diazepam 0.14 mg	** 9.80 ± 0.66	** 163.20 ± 3.09

Key: ns = not significant, * = significant, ** = moderately significant

4. Discussion

The phytochemical analysis of the leaves extract of *Rawoulfia vomitoria* showed the presence of alkaloids, saponins, tannins, flavonoids, steroids and terpenoids in the plant extract. This might be a reason why the herb *Rauwolfia vomitoria* methanol leaf extract exhibited good antioxidative effects. Phytochemicals have been shown to ameliorate diseases through attenuation of oxidative stress, inflammation, lipid peroxidation, causing tissue regeneration by regulating signaling systems and neuroprotective processes [26]. No mortality was observed in both phases of the acute toxicity test. The LD_{50} being greater than 5,000 mg/kg body weight was an indication that *Rauwolfia vomitoria* methanol leaves extract showed high safety profile. Herbs have LD_{50} values that depend on their margin of safety. As an illustration to this, a study done on methanol extract of *Languas galanga* rhizomes was investigated for antimalarial activity against *Plasmodium berghei* (NK65) infections in mice. The median lethal dose was determined to ascertain the safety of the extract in ICR mice of both sexes. The acute oral toxicity (LD_{50}) of *Languas galanga* extract in mice was established to be 4,998 mg/kg [27]. In another study that investigated the actual LD_{50} *Zingiber officinale* and *Allium sativum* ethanol extracts, *Zingiber officinale* had an actual LD_{50} of 8,660 mg/kg body weight because it took a very high dose of 10,000 mg/kg body weight to cause mortality of mouse. On the other hand, *Allium sativum* had actual LD*50* of 4,472 mg/kg body weight [28].

For the antioxidant assay, the DPPH scavenging test and lipid peroxidation assay were done. For the DPPH, ascorbic acid was used as the standard drug to assess the percentage inhibition of the free radicals and compared with the methanol extract. The same doses of the ascorbic acid and methanol extract was given to the test animals, and the lower doses of ascorbic acid had more percentage inhibition than the methanol extract but at higher doses of 250, 500 and 1000 µ/ml, the inhibitory activity of the methanol extract was almost at the same range with the ascorbic acid. Also in the lipid peroxidation assay, the trolox was used as the standard drug, and the same doses of it and the methanol extract was given to the test animals, and at doses of 0.1, 0.2, and 0.4 mg/ml, the trolox showed higher percentage inhibition than the methanol extract, and at doses of 0.8 mg/ml, 1.6 mg/ml, the methanol extract was at the same range of percentage inhibition with the standard drug. This potential of *Rawoulfia vomitoria* was buttressed in a certain study on *Maytenus royleanus* which is traditionally used in gastro-intestinal disorders. The methanol extract of leaves and its fractions were evaluated in various antioxidant assays and for its potential against lipid peroxidation and hemolytic activity. Parameters tested in the assays include scavenging of free-radicals (DPPH, ABTS, hydroxyl and superoxide radical), hydrogen peroxide scavenging, Fe3+ to Fe2+ reducing capacity, total antioxidant capacity, anti-lipid peroxidation and anti-hemolytic activity were investigated. According to the researchers, methanol extract of the herb showed the presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids, flavonoids, phenolics and phytoestrogens. Methanol extract, its ethyl acetate and n-butanol fractions showed a strong correlation coefficient with the IC50 values for the scavenging of DPPH, hydrogen peroxide radicals, superoxide radicals, anti-lipid peroxidation and anti-hemolytic efficacy. Moreover, n-butanol fraction showed the highest scavenging activity for ABTS radicals and for reduction of Fe3+ to Fe2+ [29].

Diazepam is a drug which acts as an anxiolytic and belongs to the benzodiazepine family which is a central nervous system depressant, it is used in the management of sleep disorders such as, insomnia. Benzodiazepines have a binding

site on GABA receptor type-ionophore complex. They decrease activity, moderate excitement and calm the recipient. Substance like diazepam, which was used as the reference drug in these study, reduces onset of sleep and increase the duration of phenobarbitone-induced sleep and also reduce exploratory activity possessing potential as sedative [25]. The methanol extracts were given to the test animals at doses of 100, 200, and 400 mg/kg body weight orally, 30 minutes before phenobarbitone was administered. It also showed reduced mean sleep onset similar with diazepam but more potent at higher doses of methanol extract, showing that the methanol extract of *Rawoulfia vomitoria* has anxiolytic and sedative potential. A study done in Iran reported an increasing tendency to prevent insomnia by herbal medicines throughout the world. The study gave an account of previously published research on sedative and hypnotic effects of medicinal herbs used for treatment of insomnia in Iranian traditional medicine. Various herbs have been used for reliving insomnia in Iranian traditional medicine including but not limited to *Valeriana officinalis*, Blue violte, *Salix aegyptiaca*, lotus flower, lettuce, and *Echium amoenum* [30].

5. Conclusion

The findings of this study strongly validate the rapid, long-lasting significant anti-oxidant and sedative activities of *Rawoulfia vomitoria* which is however dose dependent. Further research is needed to isolate the active principles of the plant and to understand the underlying mechanism behind the pharmacological activity of this plant.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes (ethical approval number is NAU/ AREC/ 2024/ 0024).

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Authors short Biography

Daniel Ikechukwu Oraekei is Pharmacist who graduated from the University of Benin, Edo State, Nigeria where he obtained the Bachelor of Pharmacy degree (BPharm). He also studied in Nnamdi Azikiwe University, Awka, Anambra State, Nigeria and acquired Masters of Pharmacy degree (MPharm) and he is now about to graduate with Doctor of Philosophy degree (PhD) in the Department of Pharmacology and toxicology, Faculty of Pharmaceutical Sciences of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Daniel had worked in Pharmaceutical industry in Nigeria as a production manager and he is now working as a lecturer in the department of Pharmacology and Toxicology, in Nnamdi Azikiwe University. He is a researcher who has been working on herbal drugs development and their effects on the various organs of the body including the reproductive, cardiovascular and central nervous systems.

Chukwuka Benjamin Uzodinma is a Pharmacist who graduated from the University of Nigeria, Nsukka, Enugu State, Nigeria where he obtained Bachelor of Pharmacy degree (BPharm). He proceeded to University of Benin and got Pharm D degree before studying for Masters of Pharmacy degree (MPharm) in Chukwuemeka Odimegwu Ojukwu University, Igbariam, Anambra State, Nigeria. He is currently a staff of Nnamdi Azikiwe teaching Hospital while lecturing in the department of Pharmacology and Toxicology of the University. Dr Chukwuka is a researcher in the field of herbal drugs use and drug development.

Dr Macellus Ejike Nnamani is a Pharmacist. He graduated from the University of Nigeria Nsukka, Enugu State, Nigeria (UNN) with a Bachelor of Pharmacy degree (BPharm). He also obtained both Masters and PhD Degrees from the same institution (UNN). Dr Nnamani is currently working for Nigerian Government as the Deputy Director of National Agency for Food and Drug Administration and Control (NAFDAC). He also lectures in the department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. He is a researcher in the area of Clinical Pharmacology especially in the development of herbal drugs.

Cynthia Onyonyechi Ezeugwu is an upcoming researcher who has obtained the Bachelor of Pharmacy degree from Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. She has the interest and she is enthusiastic to research in the area of Pharmacology and Toxicology where she hope to commence a study for Masters of Pharmacy degree.