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Exploring the possibility for neurobehavioural impairment of aqueous ethanolic extracts of leaf/seed of asparagus racemosus, Anogeissus latifolia Roxb, and Phyllanthus niruri growing on ipomoea carnea tree in mice

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ABSTRACT

This study aimed to investigate the liability and extracts of some medicinal plants for some neurobehavioural toxicities in mice. The results indicate, compared to negative control (distilled water) treatment mean values of 4.69±0.95 % locomotory activity reduction, 430.71±16.80 sec. sleep onset and 168.43±10.56 min. duration, 5.00±0.00 balance/motor co-ordination performance, and 54.41±1.99 novel object recognition, treatments with high oral doses of Phyllanthus niruri and Asparagus racemosus (1500 mg/kg each) did not significantly negatively impact these behavioural indices but even enhanced novel object recognition. High oral doses of Anogeissus latifolia Roxb and Ipomoea carnea (750 mg/kg each), and tramadol (133 mg/kg) caused significant ($p<0.05$) 42.24±2.64, 27.73±2.17, and 36.74±4.44, mean % locomotory activity reductions, 196.86±10.12, 193.88±15.39, and 189.14±18.31 sec. mean sleep onsets and 319.71±18.85, 309.57±20.27, and 356.00±26.01 min. mean sleep durations, 1.67±0.42, 1.30±0.40, 1.833±0.48 mean balance/motor co-ordination performances, and 40.49±5.45, 31.33±5.23, 19.37±3.96 mean novel object recognitions, respectively. Diazepam (2 mg/kg) treatment caused 33.71±2.19 mean % locomotory activity reduction, 1.33±0.49 mean balance/motor co-ordination performance, and 29.91±2.81 mean novel object recognitions. Additionally, most mouse groups exposed to tramadol, Anogeissus latifolia Roxb leaf and Phyllanthus niruri displayed unusual (hallucination-like, predator-like) fearful trepidations when in proximity with the novel objects. The results of this study suggest that extracts of Ipomoea carnea and Asparagus racemosus may not possess sedative, hypnotic, myo-relaxant, or anti-cognitive properties. However, extracts of tramadol, diazepam, Anogeissus latifolia Roxb, and Phyllanthus niruri may exhibit notable sedative, hypnotic, myo-relaxant, and anti-cognitive effects. The results of this study provide support for the historical use of Asparagus racemosus seed and Anogeissus latifolia Roxb leaf extracts in the management of memory impairments and associated neurological conditions.

1. Introduction

When developing new medications, scientists compare potential psychoactive agents to the current gold standards in terms of safety, effectiveness, and side effects, including those that may limit the drugs' therapeutic potential, like the benzodiazepines' neurobehavioral side effects. Even though the plant extracts used in this study have been studied for their anti-anxiety, anti-depressant, anti-Parkinson's, and abuse-potential effects, there aren't many scientific

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papers that talk about their possible neurobehavioral side effects.[1–4]

Asparagus racemosus, often known as shatavari, is a key plant in Ayurveda since it has the potential to treat or prevent hundreds of different diseases. It reigns supreme among herbs and earns the title "herb's queen." Steroidal glycosides, saponins (most notably Shatavarins I, II, III, and IV), polyphenols, flavonoids, alkaloids (racemosol), and vitamins are some of its bioactive components. Sapogenin, the precursor to several pharmacologically active steroids, is found in shatavari, a common plant in folk and Ayurvedic medicine.[5] The roots, stems, and leaves of this plant are the most vital, although all of them have therapeutic properties. The "Rasayanas" made from shatavari are excellent for warding off illness. The phytochemicals in it are used to treat a wide range of conditions. Phytochemicals in it are used to treat a wide range of conditions. Antispasmodic, antioxidant, anti-diabetic, anti-allergic, anti-malarial, protective, anti-neoplastic, immune response-enhancing, anti-arthritic, anti-inflammatory, anti-periodic, anti-ulcerogenic action, immune modulatory, antistress, anti-diarrheal, antidepressant, infections, tuberculosis, and so on are just some of the many medicinal properties of shatavari. Medications made from Shatavari extract on the market have been shown to have beneficial effects against leprosy, abortion, infection, fever, and pain. Diseases of the digestive tract, brain, lungs, and cervix can all be alleviated using shatavari's extracts.[6]

Asparagus racemosus, Anogeissus latifolia Roxb, and Phyllanthus niruri, all of which grow on Ipomoea carnea, have been shown to exhibit antioxidant, anti-inflammatory, and neuroprotective potential. Therefore, more research on this element of the medicinal plant is required. Combretaceae is a family of flowering plants in the Myrtales order, has over 600 species in 23 genera. Mostly found in tropical and subtropical Africa, but also in central and southern America, southern Asia, and northern Australia, it is made up of trees, shrubs, and lianas. Anogeissus is a very big genus in the Combretaceae family and is found all over the world in tropical and subtropical regions as trees, shrubs, and small trees. It has been used to cure a variety of ailments in traditional medicine, including diarrhoea, colic, stomach sickness, cough, and numerous skin conditions like ulcers, boils, psoriasis, and itching. A. latifolia (Roxb. ex-DC.) is a medium-sized deciduous tree that is indigenous to Sri Lanka, India, Myanmar, and Nepal. It is also referred to as an axlewood tree, a button tree, a dindiga tree, a gum-ghatti tree, a baklee tree, a dhaura tree, and an Indian gum tree. The herb has been employed in conventional medicine to treat liver issues, UTI infections, heart disorders, and anaemia.[7] It has been stated that the bark extract can be used to cure snake and scorpion bites, stomach disorders, colic, cough, diarrhoea, fever, and a number of skin conditions, including ulcers, boils, and itching. Also, the bark and the leaf extracts showed demulcent and astringent properties6 alongside a wide range of biological activities, e.g. antiulcer, antimicrobial, antioxidant, anthelmintic, antiplasmodia, antidiabetic, anticonvulsant and hepatoprotective activities.[8]

The cactus-like plant known as Chanka piedra (Phyllanthus niruri Linnaeus, Euphorbiaceae) is found in isolated populations across the world's tropical and subtropical regions. This annual plant may be found all along the Indian coastline. Although it has been utilized in Indian ayurvedic systems for over two thousand years, turmeric has a rather short shelf life. The Phyllanthus genus, which includes P. niruri and another 600–700 species of similar field weeds, is quite diverse. Phyllanthus niruri plant extract is used as a medication in the Indian ayurveda system and is indicated for a wide variety of conditions, including but not limited to: bronchitis, anemia, leprosy, asthma, urinary diseases, etc. According to the ancient Indian medical text Chraka Samhita, P. niruri can be used to effectively cure asthma, stimulate the liver, improve digestion, enhance appetite, and cause laxative effects. The Ayurvedic physician Maharshi Charaka classified it as Kasahara, which treats coughs; Swasahara, which treats asthma; mootrarogahara, which treats urinary tract illnesses; Kaphapittahara, which treats the kaphapitta dosha; Kaamalaahara, which treats jaundice; and Bhava prakasa Nighantu, which treats coughs and blood problems. Although its flavor is harsh, its aftertaste (vipaka) is sweet, and it may be used as an astringent.[9,10]

The Convolvulaceae family is home to Ipomoea carnea. Glycosides, reducing sugars, alkaloids, flavonoids, esters, fatty acids, alcohol, and tannins are only some of the phytochemicals found in this plant. There are phenolic substances, alkaloids, hexadecanoic acid, saponins, stearic acid, 1, 2 diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetracontane, 3-diethylamino-1-propanol, xanthoproteins, and flavonoids found in the leaves. It has been found that several of these chemicals have anti-cancer, anti-inflammatory, anti-bacterial, hepatoprotective, and hypolipidemic effects. DNA laddering has been seen in MCF-7 breast cancer cell lines after being exposed to Ipomoea carnea extracts.[11,12]

2. Materials and Methods

2.1. Materials

2.1.1. Plant parts

Asparagus racemosus seeds purchased from the Vatika Agro store in Jaipur, 302020, Rajasthan, India. The herbal garden at the Maharana Pratap College of Pharmacy in Kanpur provided us with fresh leaves of Phyllanthus niruri, Ipomoea carnea, and Anogeissus latifolia Roxb. The eminent botanist confirmed the authenticity of the plant by identifying every component of it. The specimen was placed at the

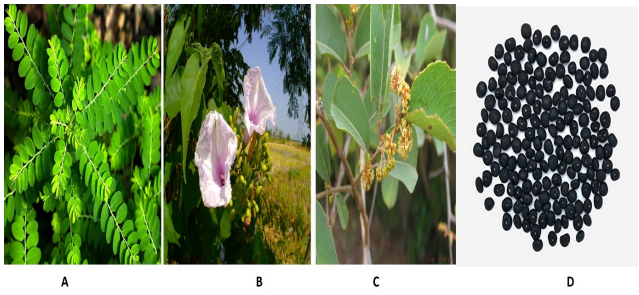

Figure 1: A: (Phyllanthus niruri); **B:** (Ipomoea carnea): **C:** Anogeissus latifolia Roxb; **D:** (Asparagus racemosus seeds)

university's herbarium house at Janta Postgraduate College, A.P.S. University, Rewa (486001), M.P. India.

2.1.2. Experimental animals

A pool of about two hundred 12-16 weeks old Swiss Albino mice of both sexes was obtained from the Maharana Pratap College of Pharmacy Kanpur. The mice were kept in a well-ventilated animal housing of the Behavioural laboratory of the Department of Pharmacology & Therapeutics, Faculty of Basic Clinical Sciences, for about three weeks under a 12-hour light/dark environment with free access to food and water before the commencement of the behavioural studies.

2.1.3. Drugs and reagents

Absolute ethanol (analytical grade), diazepam injection (Calmpose 2 mg/ml), tramadol tablets (50 mg) and injection 50 mg/ml) were obtained from Sun Pharmaceutical Industries Ltd. And Tween 80 universal solvent obtained from college laboratory.

2.1.4. Venue of the study

All experiments – plant preparations and extractions, acute toxicity, and the neurobehavioral studies took place in the Department of pharmacology, Maharana Pratap College of Pharmacy Kanpur.

2.2. Methods

2.2.1. Plant preparation and extraction

Following authentication, all plant parts were subjected to drying under early morning sunlight for hours at the initial phase of the drying process followed by air-drying until constant weights were achieved. They were then kept in non-transparent dry plastic containers until use. Asparagus racemosus seeds were roasted at 110ºC for 30 minutes, allowed to cool off and then powdered. All plant parts were each powdered with a mechanical blender. The collected plant parts were briefly rinsed in water, air-dried, then powdered and stored dry in non-transparent plastic containers for subsequent use. One hundred (100) g of fine powder of each plant part was soaked in 200 ml of aqua/ethanol (V/V: 30/70) for 18 hours. They were then each separately Whatman's paper-filtered and the filtrates subjected to electric fan-assisted air-drying. Asparagus racemosus yielded 23.7 g rich brown, Phyllanthus niruri, 10. 4 g glittering green, Anogeissus latifolia Roxb, 14.5 g deep green, and Ipomoea carnea, 9.3 g greenish brown dry extracts.

2.2.2. Acute toxicity study

A preliminary acute toxicity testing to determine the respective lethal doses (LD50s) of each extract and tramadol was carried out using the limit dose tests for Asparagus racemosus and, and toxic class toxicity protocols for Anogeissus latifolia Roxb and Phyllanthus, and tramadol. The choice of what protocol for the extracts was made based on previous toxicity reports on the plants and drugs. Oral LD50 value > 5 000 mg/kg/10 ml was found for aqueous ethanol Ipomoea carnea and Asparagus racemosus extracts, oral LD50 > 2, 000 mg/kg/10 ml for aqueous ethanol Anogeissus latifolia Roxb and Phyllanthus niruri extracts, and oral LD50 > 400 mg/kg/10 ml for tramadol. [13,14]

2.2.3. Behavioural studies

One third of the preliminary LD50s of extracts and drugs were selected as sub-lethal single high oral doses likely to produce neurobehavioural toxic effects in the experimental mice in the anticipated neuro-behavioural studies. Accordingly, 1500 mg/kg for Ipomoea carnea and Asparagus racemosus extracts, 750 mg/kg for Anogeissus latifolia Roxb, and Phyllanthus niruri extracts, and 133 mg/kg for tramadol, and experimental protocol-relevant diazepam doses were used for the subsequent behavioural assays. Diazepam was used as a positive control since most of the neurobehavioural toxicities to be evaluated are benzodiazepine related and tramadol for the diazepam-induced sleep potentiation assay. [15]

*2.2.4. Evaluation of the effect of acute high oral doses of extracts on **l**ocomotory **a**ctivities of Mice*

The evaluation of the effect of single high oral doses of extracts and drugs on the locomotory activity (an index of wakefulness/alertness of the mental state) of mice was carried out using a digital actophotometer according to the protocol adopted by Sugumaran et al., 2008 [10] with minor modifications. An actophotometer is a square or circular chamber which consists essentially of 5-6 horizontal light beams from one side of the chamber onto a set of photocells situated on the opposite side, a floor embedded with rod grid lines through which varying degrees of electric (0 – 100 volts) currents are passed, and a glass seal at the top of the device for introducing the experimental animals into the chamber.

The photocells are coupled to a digital counter which records a count when each animal interrupts the horizontal

light beams as it moves within the closed chamber either spontaneously or as facilitated by the non-injurious electric currents in the chamber's floor rod grid lines. A current of 20 volts was used throughout this assay.

Figure 2: Department of pharmacology maharana pratap college of pharmacy digital actophotometer

Briefly, healthy mice (20 – 25 g; both sexes) were randomized into 7 experimental groups I – VII (n = 6) and were each individually introduced into the test chamber of the actophotometer for 10 minutes and a basal activity was recorded for each mouse. An experimental group was subsequently given oral treatment of distilled water 10 ml/kg, aqueous ethanol Ipomoea carnea 1500 mg/kg, aqueous ethanol Asparagus racemosus 1500 mg/kg, aqueous ethanol Anogeissus latifolia Roxb 750 mg/kg, aqueous ethanol Phyllanthus niruri 750 mg/kg, aqueous tramadol 133 mg/kg, or aqueous diazepam 2 mg/kg. 1 hour following their respective treatments mice were each re-exposed to the test for 10 minutes and their activities were recorded by the device's digital counter. The counter was stopped at the end of each trial for tested mice and was re-set before the next trial. The basal and post-treatment activities for each mouse were compared and the percentage reduction or increase in activity was recorded in a way that each animal acted as its own control.

2.3. Evaluation of the hypnotic effect of acute high oral doses of extracts in mice

This evaluation was done using a diazepam-induced sleep test according to the procedure of Rakotonirina et al. (2001) adopted in brief, experimental mice (22±0.5 g; both sexes) were randomized into 6 groups (n=7) with a group given an oral treatment by gavage with distilled water (DW) 10 ml/kg, Ipomoea carnea 1500 mg/kg, Asparagus racemosus 1500 mg/kg, Anogeissus latifolia Roxb 750 mg/kg, Phyllanthus niruri 750 mg/kg, or tramadol 133 mg/kg followed 30 minutes later with oral treatment of diazepam (DZ) 40 mg/kg. Sleep onset was taken as the time gap between lastoral treatment and total loss of righting reflex, while sleep duration was taken as time gap between the total loss and full return of the righting reflex in the experimental mice (Figure 3).[16–18]

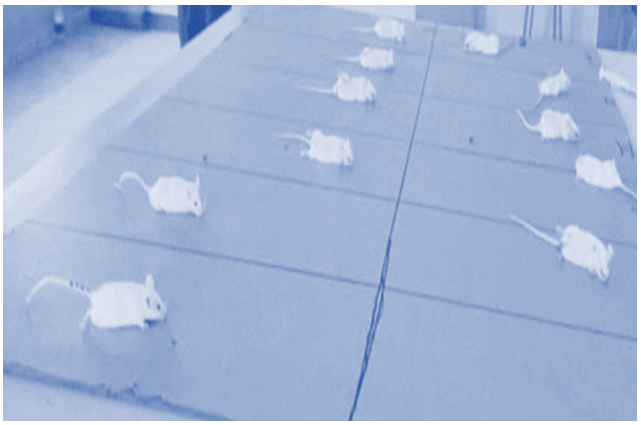

Figure 3: Experimental mice at different phases of hypnosis

2.4. Evaluation of the effect of acute high oral doses of extracts on motor balance and coordination in mice

Degree of loss of motor balance and coordination is directly proportional to level of CNS depression any myo-relaxation – two of the well-established toxicities of the benzodiazepine anxiolytics. This evaluation was accomplished by using a single horizontal bar variant of the beam (rod)-walking paradigm of Stanley, 2005 (Figure 4) [13] as described in Deacon, 2013, albeit with minor modifications.

Figure 4: Two units of Beam (rod) walking test apparatuses with 2-mm and 4-mm iron rods

The principle underpinning this test is the instinct of rodents to grip/grab objects in their proximity when suspended/floated loosely in space. On day one, mice for the main test the next day were each subjected to a screening trial by being exposed to a 2 mm-rod on the beam balance. Mice that were able to stay or hold onto the rod without falling off for at least 5 seconds were instantly selected for the main test. Mice falling off on their first attempts were given 2 additional trials. Mice that passed the test on their second attempts were not tried the 3rd time. All mice which passed the trial on 1^{st}, 2^{nd}, and 3^{rd} attempts were deemed to have qualified for inclusion in the main test the following day.

The beam balance essentially comprises of a 60 cm-long iron rod horizontally suspended (balanced) on two 50 cm-high vertical wooden beams (poles) set 50 cm apart (Figure 5).

Briefly, on the screening day – with no treatments given, and on the main test day 1 hour following oral treatments of randomized groups (n = 7, both sexes) of mice (23±0.7 g) with DW 10 ml/kg, Ipomoea carnea 1500 mg/kg, Asparagus racemosus 1500 mg/kg, Anogeissus latifolia Roxb 750 mg/kg, Phyllanthus niruri 750 mg/kg, tramadol 133 mg/kg, or diazepam 2 mg/kg were each subjected to the test but this time on the 4-mm iron rod.

Time spent on the beam balance by each mouse was determined using a watch timer.

Scoring for the times spent by the mice staying on/holding onto the rod was done as described by Deacon, 2013 with modifications, as follows: Falling off between within 5 seconds = 1, within 6-10 seconds = 2, within 11-20 seconds = 3 and within 21- 30 seconds = 4. Mice that stayed on top of the rod for/longer than 30 seconds = 5; holding onto the rod by placing one or both forepaws on it without falling for 30 sec = 5, and climbing onto the top of the rod with all 4 paws on it at any time within 30 seconds = 5. Reaching any of the vertical support beams at any time within test duration = 5.

The scores were collated and recorded for each mouse group.[13]

2.5. *Evaluation of the cognitive effect of acute high oral doses of extracts in mice*

The novel object recognition test (NORT) was adopted to assess acute high oral doses of aqueous methanol Ipomoea carnea, Asparagus racemosus, Anogeissus latifolia Roxb, Phyllanthus niruri extracts, and Tramadol on short-term memory in mice in accordance with a method previously used. Our NORT apparatus consists of a walled square open field containing no objects on the training day, but with similar or dissimilar objects placed at opposite corners of the test apparatus on test day, day 2.[14]

Figure 5: Novel object recognition test experimental set-up

The test relies heavily on the natural proclivity of rodents for novelty – which is created when one of the initial similar objects at the first mouse re-exposure is replaced with a comparatively dissimilar object at the second and final re-exposure.

Briefly, on the first day of the 2-day protocol, experimental mice were each trained to familiarize with the test environment and device by being gently dropped in the middle of the apparatus and allowed to freely explore the test environment for 10 minutes, and then returned to the home cage. The second day i.e., the test day mice were each individually re-exposed to the apparatus, but this time around with two similar colourless plastic containers cut in half - with the cut ends plastered to the floor at opposite corners within the test apparatus. The mice were allowed to freely explore the environment for 10 minutes - with only those which explored each of the plastic objects for a minimum of 20 seconds of the test period deemed to have met inclusion criteria for the main cognitive test. Subsequently, mice (21.0±0.3 g) that qualified for the definitive cognitive test were randomized into groups (n = 12) and a group receiving oral treatments of DW 10 ml/kg, Ipomoea carnea 1500 mg/kg, Asparagus racemosus 1500 mg/kg, Anogeissus latifolia Roxb, 50 mg/kg Phyllanthus niruri 750 mg/kg, tramadol 133 mg/kg, or diazepam 1 mg/kg. One hour following treatments and 4 hours after the first re-exposure, mice were again exposed to the test for a 5-minute period during which experimental subjects were allowed to freely explore the test environment, but this time around with one of the plastic containers replaced with similarly shaped but red-coloured plastic container. Times spent by each mouse exploring the objects were recorded.

Recognition or preference index (d3) = [b/e2] X 100.

Where b = time spent by the mice exploring the new object

e2 = time spent by the mice exploring both novel and old objects.

2.6. *Statistical analysis*

Means and standard error of the mean (SEM) were used to describe all experimental results. The data was analyzed using analysis of variance (ANOVA) and the Turkey post hoc test in IBM SPSS version 2.0. P-values below 0.05 were considered statistically significant.

3. Results

The effect of acute high oral doses of aqueous ethanol Ipomoea carnea, Asparagus racemosus, Anogeissus latifolia Roxb, Phyllanthus niruri extracts, and Tramadol on locomotion in mice

Compared to only 4.69±0.95 mean % locomotory activity reduction in mice exposed to distilled water treatment (Table 1), Ipomoea carnea or , Asparagus racemosus (each at 1500 mg/kg) did not significantly ($p>0.005$) alter, but Anogeissus latifolia Roxb (750 mg/kg), Phyllanthus niruri (750 mg/kg), tramadol (133 mg/kg), and diazepam (2 mg/kg) treatments caused significant ($p<0.05$) 42.24±2.64, 27.73±2.17, 36.74±4.44, and 33.71±2.19

reductions, respectively, in the mean percent locomotory activity of experimental mice.

3.1. The hypnotic effect of acute high oral doses of extracts and drugs in mice

Compared to the mean sleep onset (430.71±16.80 sec.) and duration (168.43±10.56 min.) of distilled water-treated mice, high oral Ipomoea carnea and Asparagus racemosus treatments at 1500 mg/kg neither significantly ($p>0.05$) shortened diazepam-induced mean sleep onset (398.00±23.57 sec. and 400.71±41.07 sec.) nor increased mean sleep duration (174.00±10.28 min. and 162.57±8.44 min.), respectively, whereas Anogeissus latifolia Roxb, and Phyllanthus niruri (each at 750 mg/kg), and tramadol at 133 mg/kg significantly ($P<0.05$) shortened mean sleep onset to 196.86±10.12, 193.88±15.39, and 189.14±18.31 sec., respectively, as well as increased mean sleep durations to 319.71±18.85, 309.57±20.27, and 356.00±26.01 min., respectively, in mice.

3.2. The effect of acute high oral doses of extracts and drugs on balance and motor co-ordination in mice

Our results show, compared with distilled water treatment mean performance of 5.00±0.00, Ipomoea carnea (4.83±0.17) and Asparagus racemosus (5.00±0.00) extract treatments did not ($p>0.05$) alter but Anogeissus latifolia Roxb (1.67±0.42) and Phyllanthus niruri (1.30±0.40), tramadol (1.833±0.48), and diazepam (1.33±0.49) did significantly ($p<0.05$) reduce the mean performances of the mice exposed to them on this test.

3.3. Effect of acute high oral doses of extracts and drugs on short-term memory in mice

Compared to the mean value of 54.41±1.99 of mice treated with the negative control, single high oral doses of both Ipomoea carnea (60.191±1.81) and Asparagus racemosus (59.19±2.18) did not reduce but instead caused insignificant improvements in the mean recognition indices exposed to them. In contrast, compared to the control mouse group, high oral doses of Anogeissus latifolia Roxb (40.49±5.45), Phyllanthus niruri (31.33±5.23), tramadol (19.37±3.96), and diazepam (29.91±2.81) all significantly ($p<0.05$) caused reductions in the recognition performance in mice exposed to them. In addition, most of the mice treated with tramadol, Anogeissus latifolia Roxb, and Phyllanthus niruri extracts displayed unusual fearful trepidations (? hallucinatory), like when faced with a predator, when in proximity with the novel objects.

4. Discussion

This research shows that there are other approaches to assessing whether or not actual or hypothetical psychoactive

Table 1: Effect of acute high oral doses of extracts and drugs on locomotory activity in mice

Treatments	Mean % reduction
Distilled water	4.69±0.95
Aqueous and ethanol, Asparagus racemosus seed	2.98±0.57
Aqueous and ethanol Ipomoea carnea leaf	7.45±1.83
Aqueous and ethanol Anogeissus latifolia Roxb leaf	42.24±2.64*
Aqueous and ethanol Phyllanthus niruri leaf	27.73±2.17*
Tramadol	36.74±4.44*
Diazepam	33.71±2.19*

Data presented as mean ± S.E.M. * Statistically significant ($p < 0.05$).

Table 2: The hypnotic effect of acute high oral doses of extracts and drugs in mice

Extracts/Drugs	Mean sleep onset (Seconds)	Mean sleep duration (Minutes)
Distilled water+Diazepam	430.71±16.80	168.43±10.56
Ipomoea carnea+Diazepam	398.00±23.57	174.00±10.28
Asparagus racemosus+Diazepam	400.71±41.07	162.57±8.44
Anogeissus latifolia Roxb+Diazepam	196.86±10.12*	319.71±18.85*
Phyllanthus niruri+Diazepam	193.88±15.39*	309.57±20.27*
Tramadol+Diazepam	189.14±18.31*	356.00±26.01*

Data presented as mean ± S.E.M. * Statistically significant ($p < 0.05$)

Table 3: Effect of acute high oral doses of extracts and drugs on balance and motor co-ordination in mice

Treatments	Mean performaces
Distilled water	5.00±0.00
Aqueous and ethanol, Ipomoea carnea leaf	4.83±0.17
Aqueous and ethanol, Asparagus racemosus seed	5.00±0.00
Aqueous and ethanol, Anogeissus latifolia Roxb leaf	1.67±0.42*
Aqueous and ethanol, Phyllanthus niruri leaf	1.30±0.40*
Tramadol	1.833±0.48*
Diazepam	1.33±0.49*

Data presented as mean ± S.E.M. * Statistically significant ($p < 0.05$)

Table 4: Effect of acute high oral doses of extracts and drugs on short-term memory in mice on the novel object recognition test

Treatments	Mean recognition indices (d3)
Distilled water	54.41±1.99
Aqueous and ethanol, Ipomoea carnea leaf	60.191±1.81
Aqueous and ethanol, Asparagus racemosus seed	59.19±2.18
Aqueous and ethanol, Anogeissus latifolia Roxb leaf	40.49±5.45*
Aqueous and ethanol, Phyllanthus niruri leaf	31.33±5.23*
Tramadol	19.37±3.96*
Diazepam	29.91±2.81*

Data presented as mean ± S.E.M. * Statistically significant ($p < 0.05$)

substances could share the neurobehavioral downsides of benzodiazepines. Locomotor activity is a measure of mental alertness or wakefulness in humans and other animals and is commonly affected by medications and substances that operate on the central nervous system (CNS). Since the experimental animals serve as their own controls and at least two trials are required for a complete locomotory activity profile, the actophotometric evaluation of this property in this study has some advantages over the more commonly used open-field-based evaluation, such as semi-automatic recording of the activity by a digital counter. To determine whether or not a plant extract possesses central nervous system-depressing properties, actophotometers have been used in the past. We found that large oral dosages of aqueous ethanol extracts of Ipomoea carnea leaf and Asparagus racemosus seed did not have any significant CNS depressant or stimulating effect in mice, as measured by the locomotory activity index. Although various investigations have demonstrated that Asparagus racemosus extracts have anxiolytic effects.[15] Our results may be the first to reveal that they do not have a depressive impact. Although Ipomoea carnea extracts have been shown to have anxiolytic function, this study is only the second to find no deleterious effect on locomotory activity in mice. It's possible that the anxiolytic pharmacophores in these plants are acting on a neural pathway very different from the benzodiazepine GABAergic mechanism(s), given that extracts of these medicinal plants have been reported to exert significant anxiolytic activity without a significant sedative effect that may impact locomotion. Our results reveal that diazepam-like effects on mouse locomotion are likewise produced by the Anogeissus latifolia Roxb leaf extract, the Asparagus racemosus seed extract, and tramadol. When people eat preparations of Anogeissus latifolia Roxb leaf and Asparagus racemosus seed, they usually feel sleepy and their walking is affected in a bad way. This psychoactive effect has been observed before [16,17], and it is thought to be caused by the presence of gallic acid, quinic and shikmik acids, gallotannin and tannin, ellagic acid, steroidal saponins, and alkaloids. According to a report on substance addiction, this quality is a major draw for the misuse of various preparations of the Anogeissus latifolia Roxb leaf and the Asparagus racemosus seed.The hypnotic effects of the extracts and medications were studied by examining their ability to enhance the diazepam-induced sleep technique. Anogeissus latifolia Roxb leaf and Phyllanthus niruri extracts, as well as tramadol, sped up the start of sleep and made it last longer, but Ipomoea carnea and Asparagus racemosus at 1500 mg/kg did not have any effect. An aqueous ethanol extract of Ipomoea carnea has been shown to have a similar non-hypnotic effect in mice. The fact that neither Ipomoea carnea nor Asparagus racemosus have a hypnotic effect despite being anxiolytic suggests their anxiolytic action may be based on a mechanism(s) other than benzodiazepines. The hypnotic effect of tramadol seen in this research is consistent with its reputation as a strong central nervous system depressant. Despite its well-known general and neurobehavioral toxicity, tramadol's capacity to change sensorium and mood is also suggested to be connected to its misuse liability. It is the hypnotic impact of Anogeissus latifolia Roxb and Phyllanthus niruri extracts, in addition to their sedative action, that causes us to worry. People who are subjected to accidental or purposeful overdoses (like those utilized in this study) of the various portions of Ipomoea carnea sometimes suffer both psychotropic effects as well as profound, extended slumber with accompanying mortality. Cases of prolonged anti-cancer, anti-inflammatory, anti-sleeping, anti-cardiovascular, and anti-inflammatory jaundice, gonorrhea, frequent menstruation, and diabetes, as well as topical use as a poultice for skin ulcers, sores, swelling, and itchiness, after intentional or accidental ingestion of Ipomoea carnea extracts, have been reported.[18,19]

The central nervous system (CNS), the peripheral musculo-nervous system, and the bidirectional neural modulatory interaction between these three systems are all necessary for normal balance and motor coordination in humans and other animals. This work employing the beam (rod) walking assay suggests that the CNS depressive and myorelaxant effects of prospective and current psychoactive drugs can lead to deficits in balance and motor coordination. These advantages include operational simplicity, cost-effectiveness, improved sensitivity, and predictive capability for clinically relevant sedative benzodiazepine doses,[12,13] making this assay preferable to the previously standard test for elucidating balance and motor coordination deficits in experimental animals.

When tested for their effect on balance and motor coordination, neither Ipomoea carnea nor Asparagus racemosus extracts showed any signs of CNS depression

or myo-relaxation. This finding is consistent with previous research on Ipomoea carnea, but it may be the first to show that Asparagus racemosus extract does not cause CNS depression or myo-relaxation even at high doses. Ipomoea carnea, Phyllanthus niruri, tramadol, and diazepam all cause severe impairments in this behavioral parameter in mice.[20–23] This is due to the drugs' powerful CNS depression, myo-relaxation, and neurotoxicity.

The novel object recognition test (NORT) used in this study has greater face validity for human memory and is easier, more sensitive, less time-consuming, and less stressful on the experimental subjects than other cognition-assessing protocols like Morri's water maze and Barnes tests. The cognitive abilities of mice treated with Ipomoea carnea and Asparagus racemosus extracts were on par with or even better than those of mice treated with distilled water on the NORT. This indicates that neither extract has any immediate detrimental effects on cognition. These results are consistent with those of earlier studies in which Ipomoea carnea and Asparagus racemosus extracts were given to experimental mice and shown to have a beneficial effect on their cognition.[24,25] The recognition indices of mice given Anogeissus latifolia Roxb, Phyllanthus niruri, or tramadol were considerably lower than those of animals given only water. This confirms the deleterious effect of Anogeissus latifolia Roxb and Phyllanthus niruri on cognition found in other research in human subjects. Mice given diazepam or tramadol had worse motor skills than water-treated mice. Acute doses of diazepam and tramadol in human and experimental subjects have been shown to elicit acute cognitive impairments, which is consistent with our findings.

5. Conclusion

This study has shown aqueous ethanol extracts of Ipomoea carnea and Asparagus racemosus seed did not, those of Anogeissus latifolia Roxb, Phyllanthus niruri leaf as well as tramadol, did exhibit sedative, locomotory, cognitive, and hypnotic effects associated with the benzodiazepines.

6. Conflict of Interest

The authors declare no conflict of interest.

7. Source of Funding

None.

8. Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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