Behavioural studies on the ethanol leaf extract of Grewia carpinifolia in Wistar rats.

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Abstract

Background: *Grewia carpinifolia* is a plant commonly used in the tropics to manage various central nervous system (CNS) disorders. However, despite its widespread use no scientific work has been reported to validate these claims.

Objectives: To evaluate the activity of *G. carpinifolia* as it affects behaviour using animal model.

Methods: Twenty five adult Wistar rats were randomly divided into five groups (A-E). Group A served as control (given only distilled water), Groups B,C, D and E were administered with single oral dose of ethanol extract of *G. carpinifolia* leaf at 100, 200, 400 and 800 mg/kg body weight respectively for twenty eight days consecutively. Subsequently, open field test, negative geotaxis and hanging wire test were performed. Body and brain weights were measured and histological examination of the brain was also performed.

Results: At the tested doses, the extract significantly increased the time spent on the hanging wire and decreased locomotor activity at 800 mg/kg. No significant difference was observed in body and brain weights of extract treated groups when compared with the control. No visible histological lesion was also observed.

Conclusion: The plant extract may improve muscular strength at tested doses and possess CNS depressant activity at 800 mg/kg.

Keywords: *Grewia carpinifolia*, negative geotaxis test, locomotor activity, Wistar rats.

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Introduction

Recently herbs and extract of plants have been extensively studied in search of new medicine beneficial in improving medical disorders related to the central nervous system (CNS) such as Parkinson's disease, neuromuscular weakness etc. These plants which are used in both herbal and conventional medicine offer benefits that pharmaceutical drugs lack and thus provide a safe alternative[1]. In spite of the huge benefits derived over the years from the use of these medicinal plants, substantial research has shown the risk involved in the application of some of these plants due to lack of proper dosing, method of preparation and duration of usage[2]. Therefore, scientific evaluation of these medicinal plants is not only important to the discovery of new drugs but also to assess toxicity associated with the use of these herbal preparations.

Grewia carpinifolia belongs to the family Tiliaceae. This genus comprising shrubs and trees is distributed in the warmer parts of the world. Nearly 40 species of this genus are found throughout the globe. Different parts of species of the *genus Grewia* are used as folk medicine in different part of the globe. *Grewia carpinifolia* has been reported to have antiparasitic and antioxidant activities[3]. Members of this genus are known to elicit various CNS activities. For example, *Grewia bicolor* used in the treatment of skin lesions is also used as tranquilizer[4]. Ethanolic extract of stem bark of *Grewia elastic, Grewia tenax* and *G.tiliaefolia* have been reported to possess CNS depres-

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sant activity. The aerial parts of *G. umbellifera* exhibited CNS depressant, also hypotensive and diuretic receptors in the brain have been isolated from an extract of *Grewia villosa* used in treatment of tuberculosis[5].

However, despite the widespread use of *Grewia carpinifolia* in the management of various ailments, an extensive literature search revealed that no scientific work has been reported to evaluate and provide information on the CNS activity of the plant as well as its effect on behaviour using animal models.

Materials and methods

Plant material and authentication

Fresh leaves of *Grewia carpinifolia* were collected from the botanical garden of the University of Ibadan. It was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) where herbarium specimen (voucher number FHI 109693) was deposited.

Experimental animals

Adult Wistar rats of both sexes were purchased and housed at the Laboratory Animal House, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan. The animals were housed under standard conditions of temperature (25 ± 2°C), and light, (approximately 12/12 h light-dark cycle), fed on standard diet and given water ad libitum. All the animals were acclimatized to laboratory conditions for two weeks before the commencement of the experiment. All experiments performed on the laboratory animals in this study followed the Organization for Economic Cooperation and Development (OECD) approved Standard Operation Procedures[6].

Extract preparation

The leaves were cleaned to remove adhering dirt, air-dried for eight weeks and crushed into coarse powder using a pestle and mortar. Extraction was carried out by cold maceration of 500 g of the coarse powder with 2.5L of 100% v/v ethanol for 72 h, with constant shaking using the GFL shaker (no. 3017GBh, Germany).

The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated to dryness in vacuo at 40°C using rotary evaporator to give a yield of 20% w/w of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in this study.

Preliminary phytochemical screening

Chemical tests were carried out for preliminary phytochemical screening of the leaf extract of *Grewia carpinifolia* using standard procedures to identify the constituents as described[7,8,9,10].

Experimental design

Twentyfive rats of both sexes were divided into five groups of five rats each. Group A, received distilled water and served as the control while rats in groups B, C, D and E were given 100, 200, 400 and 800 mg/kg bw of ethanol leaf extract of *Grewia carpinifolia* respectively in a single oral dose daily for 28 days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality.

Body weight change

Rats in all the groups were weighed on the first day of the experiment and thereafter weekly during the period of extract administration and on the last day of the study. Doses of the extract administered were adjusted accordingly.

Behavioural tests

Twenty four hours after the administration of the last dose of the extract, the following behavioural tests were carried out; open field test, the forelimb support (hanging wire) test, and for negative geotaxis as described[11, 12,13, 14].

Open-field test (OFT)

- Each rat was placed in the centre of a square cage (120x120cm). The floor was divided into 20cm squares drawn in black ink. At the beginning of each test, every animal was introduced to the same spot (the centre square) of the arena and was allowed to explore the arena freely for 5 minutes. The following observations were recorded:
- Locomotion, that is, number of times a rat crossed from one square to another entering with at least its two front paws
- Number of rearing that is, number of times rat stood on its hind legs
- Number of grooms that is, sets of heterogeneous constituents comprising face washing, body licking, paw licking, head and body shaking, scratching and genital licking.

The open field box was washed with 30% alcohol solution before placing the subsequent animals in it in order

to avoid possible biasing effect due to odour clues left by previous rats[11].

Forelimb support (hanging wire)

The testing procedure was as previously described[13] and was used to assess the muscle strength and balance. Each rat was suspended with both forepaws on a horizontal steel wire 80 cm long, diameter 2 mm. The animal was held in a vertical position when its front paws were placed in contact with the wire. When the rat grasped the wire, it was released, and the latency to fall was recorded with a stopwatch. Rats were randomly tested and each animal was given three trials with a 30 min inter-trial rest interval[14].

Negative geotaxis

Each rat was placed in the middle of a slab, 30° inclined to the surface plane, in a head down position and latency to turn 180° to a head up position was observed and recorded[15].

Animal sacrifice

The rats were sacrificed 24 hours following the last day of extract administration and their brains were excised, the weight were taken and recorded. Brains were quickly transferred to sample bottles containing 10% formol calcium and fixed for two days and processed for Haematoxylin and Eosin (H&E) staining technique.

The sections were mounted and examined with a light microscope (Olympus, Japan). Image acquisition was done using cameroscope 5.5 connected to a computer interface and mounted on the microscope.

Relative brain-body weight ratio: The relative brain-body weight ratio (RBW) of each rat was calculated as follows:

$$RBW = \frac{\text{Absolute brain weight (g)}}{\text{Body weight of rats on sacrifice day (g)}}$$

Statistical analysis

All data are presented as mean ± Standard Error of mean (SEM). Data was subjected to one-way ANOVA and subsequently to the Bonferroni post-test using the Graph pad Prism version 5 (Windows® Graphpad software) to perform multiple comparisons in order to assess statistical significance of differences between all possible pairs of groups. The level of significance was $P \leq 0.05$.

Results

Phytochemical screening

Chemical tests were carried for preliminary qualitative phytochemical screening of the leaves extract. Tannins, saponins, flavonoids and alkaloids were strongly present in the extract, phlobatinins, terpenoids, cardiac glycosides and anthraquinones were only present in trace amount, however coumarin was found to be absent (Table 1).

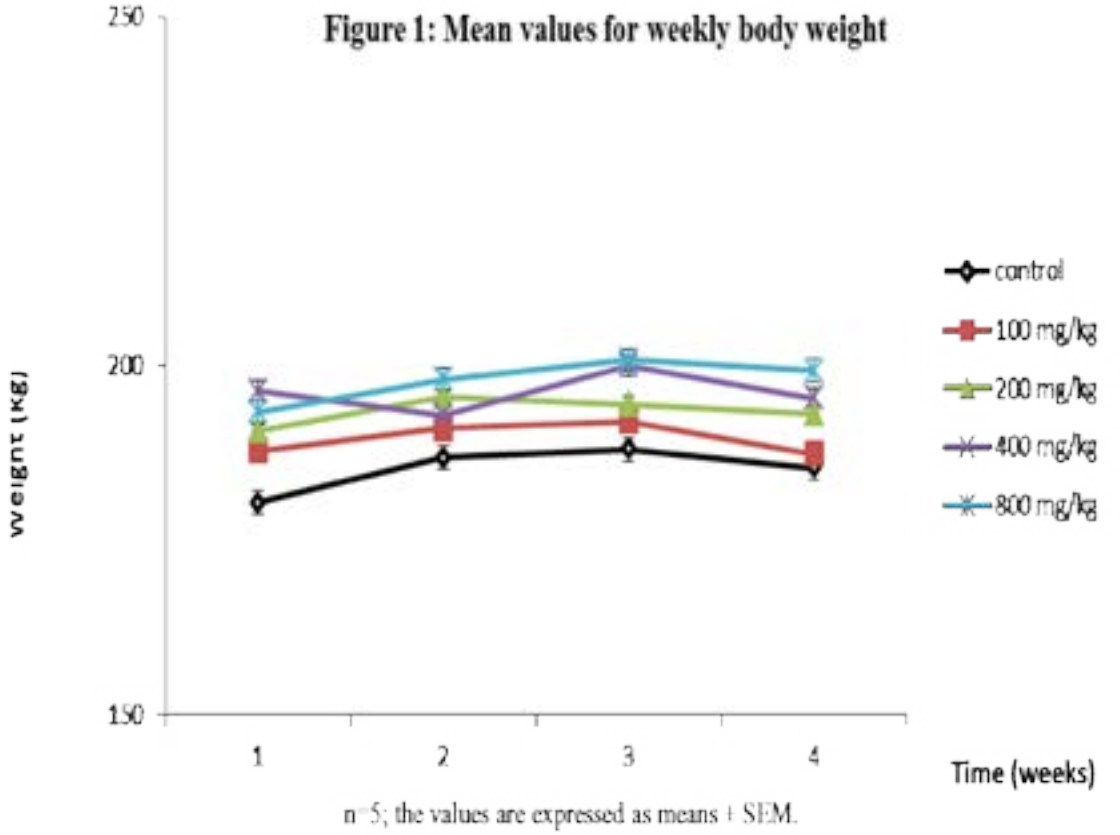
Table 1 Qualitative phytochemical analysis of ethanol leaves extract of *Grewia carpinifolia.*

Phytochemical	Qualitative
Tannins	++
Phlobatinins	+
Saponins	++
Flavonoids	++
Terpenoids	+
Cardiac glycosides	+
Coumarin	-
Alkaloids	++
Anthraquinones	+

++ = strongly positive + = trace - = not detected

Effect of Grewia carpinifolia leaves ethanol extract on body weight of Wistar rats

There were increases in the mean body weight in all the groups throughout the experimental period. The final mean body weight at the beginning of the study and point of autopsy did not differ significantly (P>0.05) in *Grewia carpinifolia* treated rats when compared with control group (Figure 1).



Effect of sub-chronic administration of Grewia carpinifolia leaves ethanol extract on behaviour

In the Open field test (OFT) there was a significant ($p \leq 0.05$) decrease in the number of new squares crossings in the 800 mg/kg group when compared to control group (Table 2), no significant difference was however observed at other tested doses. There was also a statistically significant ($p \leq 0.05$) increase in the time spent on the hanging wire in all the treated groups when compared with the control. Numbers of rearing, grooming, and faecal boli while in the open field box were not statistically different in the control and test groups.

Table 2: Mean values for the behavioural tests on day 28 following administration of *Grewia carpinifolia* leaves ethanol extract

	Control	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Rearings	20.60±2.30	30.80±1.04	26.7±1.90	31.78±1.90	19.30±2.30
Groomings	10.10±1.40	9.20±1.08	6.40±2.08	10.03±1.72	9.97±1.48
Number of new squares	25.40 ± 4.99[a]	18.80 ± 5.56	18.00 ± 6.43	17.60 ± 5.38	13.75 ± 2.66[b]
Faecal bolus	3.00 ± 1.14	5.00 ± 1.45	4.67 ± 2.91	4.00 ± 1.27	3.75 ± 1.38

n=5; Mean ± S.E.M (Standard Error of Mean); Means with different superscripts within rows are significantly different at $p<0.05$

Effect of Grewia carpinifolia leaves on relative brain weights of Wistar rats

There was no significant difference (P>0.05) between the relative brain weights at all tested doses; they were similar in the control and *Grewia carpinifolia* treated rats (Table 3).

Table 3: Effect of *Grewia carpinifolia* leaves on relative brain weights of Wistar rats treated for 28days

	Relative brain weights ($x10^{-3}$)
Control	7.60 ± 0.08
100 mg/kg	7.72 ± 0.08
200 mg/kg	7.96 ± 0.02
400 mg/kg	7.85 ± 0.05
800 mg/kg	7.88 ± 0.02

n=5; Mean ± S.E.M (Standard Error of Mean)

Histopathology

The result of the histopathology of rats treated with the ethanol extracts of *Grewia carpinifolia* leaves on the brain of rats is shown in Figure 2. No visible lesion was observed in the brain section of the control and treated groups.

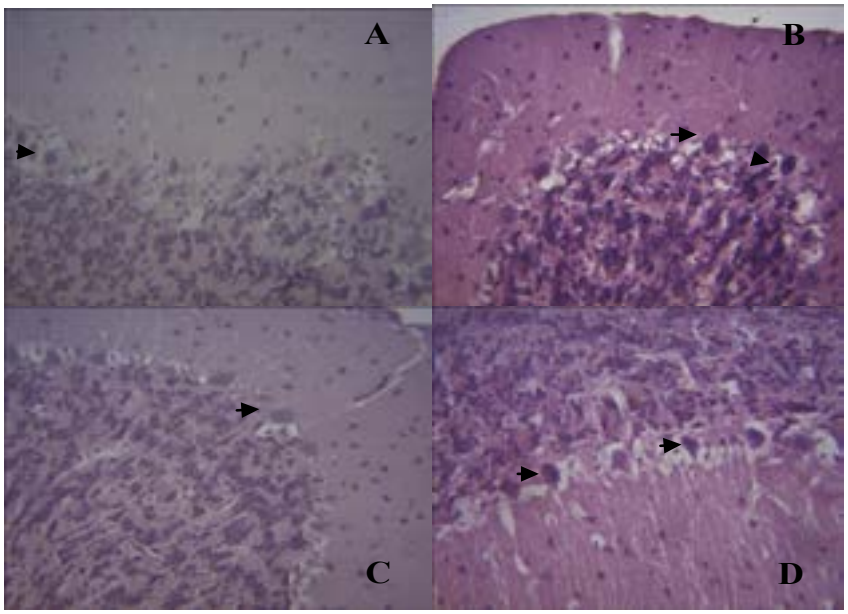


Figure 2 The brain section (Purkinje cells of the cerebellum) of rat (H&E stain x100 magnification).

A: the control group showing no visible lesion

B: The brain section (Purkinje cells of the cerebellum) of rat in the group B administered with 100 mg/kg of ethanol leaves extract of *Grewia carpinifolia* showing no visible lesion (H&E stain x100 magnification).

C: The brain section (Purkinje cells of the cerebellum) of rat in the group C administered with 200 mg/kg of ethanol leaves extract of *Grewia carpinifolia* showing no visible lesion (H&E stain x100 magnification).

D: The brain section (Purkinje cells of the cerebellum) of rat in the group E administered with 800 mg/kg of ethanol leaves extract of *Grewia carpinifolia* showing no visible lesion (H&E stainx100 magnification).

Discussion

Body weight changes serve as a sensitive indication of the general health status of animals[16]. The results of this study showed that the administration of leaf extract of *G. carpinifolia* did not interfere with growth irrespective of dose. Although the effect of extract of *G. carpinifolia* on body weight has not been earlier reported, the present observation suggests that *G. carpinifolia* did not interfere with body weight.

According to Moore et al[17], an increase in absolute/ relative organ weight is an indication of inflammation while a reduction in the same parameter can be adduced to cellular constriction. Oral administration of 100, 200, 400 and 800 mg/kg bw of *G. carpinifolia* leaves extracts also revealed no significant ($P<0.05$) difference in brain weight of extract treated animals when compared with controls.

The open field test is used to evaluate the emotional state and locomotor activity of an animal. The open field model examines anxiety related behaviour characterized by the normal aversion of the animal to an open area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters[18].

Locomotor activity has been used to represent a broad class of sensory, motor and integrative processes[19], Rang et al.,[20] reported that locomotor activity is mediated through dopamine and other neurochemical pathways. A wide variety of agents have the capacity to excite the function of the CNS, such that calming or drowsiness (sedation) is inhibited[21]. In addition, central nervous system (CNS) depressants are known to inhibit locomotor activity of animals[22].

Furthermore, inhibition of acetylcholinesterase (AchE), CNS activation of cholinergic motor inhibitory system and damage to the peripheral muscle due to necrosis of skeletal muscle fibres has been reported to reduce locomotor activity in animals[23]. The effects of *Grewia carpinifolia* on spontaneous activities were studied by rearing and the number of lines crossed. The rearing (vertical movement) is an index of the locomotor activity[24] while the increased number of line crossed (horizontal movement) is an indication of the central nervous system stimulant or depressant properties. Rats treated with extract at varying doses showed no significant increase in number of rearings, groomings and time spent in the centre.

This indicates that the plant extract did not induce anxiety in treated rats. The decreased motor activity obtained at 800 mg/kg in the present study may suggest that the ethanol extract of *Grewia carpinifolia* had some CNS depressant activities at this dose. This type of activity profile can be compared with opium alkaloid which possesses stimulant effect at low dose initially and marked sedative effect after cumulative effect or after high dose. The phytochemical constituents may be responsible for this activity[25]. Gamma-aminobutyric acid ($GABA_A$) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs acts through $GABA_A$ receptors, therefore it is possible that extracts of *G.carpinifolia* may act by potentiating GAB_Aergic inhibition in the CNS via direct activation of GAB_A receptor or indirectly by membrane hyperpolarization which led to a decrease in the firing rate of critical neurons in the brain[26]. Polyphenolic compounds, like flavonoids, tannins, saponins and phenolic acids, commonly found in plants have been reported to possess multiple biological effects[27] and are useful in many CNS disorders[28]. Many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which led to the assumption that they can act as benzodiazepine- like molecules[29]. The presence of alkaloids, flavonoids, saponins and tannins in the extract in this study might thus be responsible for its CNS depressant activity. This is in consonance with investigations on other species of the genus; Jaspers et al.[30], Hyo et al.[31] Tijani et al[32] found that extract from *Grewia bicolor, G umbellifera* and *G. lasiodiscus* respectively possesses central depressing activities.

The length of time a rat was able to hold the hanging wire is considered as an indirect measure of grip, muscle strength and co-ordination[33] which was significantly increased ($p<0.05$) at all the tested doses. This may suggest an increase grip strength which could be due to increased motor coordination and muscle tone[34] that occurred following the sub chronic administration of *Grewia carpinifolia.*

Hypertrophy of an organ is an indication of toxicity of chemical or biological substance[35]. However, no hypertrophy of the brain was observed in this study amongst

all the groups studied. In addition, the microscopic examination revealed that the cerebellum from the extract treated rats showed no alteration in cell structure when viewed under the light microscope using multiple magnification powers. The cerebellum plays a significant role in behaviour, movement control and as well as sensory perception[36]. Anxiety, stress, neurotoxic agents amongst other factors have been reported to cause loss or damage of Purkinje cells leading to cerebellar damage which consequently affects movement coordination and other cerebellum-dependent cognitive function[37]. The intact (lack of lesion) structural integrity of the cerebellum in this study further corroborate the result from the behavioural tests. This demonstrates the safety of the plant extract on cerebellar functions at tested dosages.

Conclusion

From the findings of this study we conclude that subchronic oral administration of ethanol leaf extract of *Grewia carpinifolia* appear to be safe at tested doses and may increase muscle strength, it may also possess CNS depressing activity at a dose of 800 mg/kg. Therefore, this plant merits further attention. It is suggested that further study be carried out to isolate the bioactive principle(s) responsible for the observed activities and an in-depth study to determine the mechanism by which *G. carpinifolia* may act on the CNS.

Conflict of interest statement

We have no conflict of interest to declare.

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