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Comparative Neuropharmacological Activities Methanolic Extracts of Leaves and Roots of *Cissus Cornifolia* in Mice

Yaro A. H. [1*], Anuka J. A[3], Salawu O. A[3], Hussaini I. M[2], Usman H [4] and Musa A. M[5]

[1]*Department of Pharmacology, Faculty of Medicine, Bayero University Kano Nigeria.*
Departments of [2]Pharmacology & Therapeutics and [5]Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria –Nigeria.
[3]*Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu – Abuja.*
[4]*Department of Chemistry, Faculty of Science, University of Maiduguri – Nigeria.*

ABSTRACT: Comparative neuropharmacological efficacy of the leaf and root 70 % methanol extract of *Cissus cornifolia* was studied in mice. The extractive values of the leaf and root methanol extract was found to be 31.5 g with yield of 12.6 %($^{w}/_{w}$) and 37.8 g with the yield of 15.12 %($^{w}/_{w}$) respectively. The acute toxicity (LD_{50}) values in mice were found in leaf and root extracts as 2154.1 and 1131.4 mg kg^{-1} bd. wt. (i.p.) respectively. The sedative properties on the CNS of both the leaf and root extracts were studied employing diazepam-induced sleep, motor coordination, and exploratory behavioural test in mice. Both extracts potentiated the diazepam-induced sleeping time with markedly higher duration of sleep at 600 mg kg^{-1} bd. wt. (213.8 ± 27.5) exhibited by leaf extract. There was generally appreciable variation in the activities expressed by the leaf extract compared to that of the root in all the other tests conducted. Thus, at 300 mg k^{-1} bd. wt. the leaf extract revealed 5.3 ± 0.7 while the root had 8.0 ± 0.8 as mean number of head-dips in mice. The mean duration of beam walk was found to be 6.88 ± 0.71 and 4.72 ± 0.28 expressed by the leaf and root extract respectively at the same dose of 300 mg k^{-1} bd. wt. in mice. This work further confirms our earlier report on sedative effects of this plant as used traditionally against mental problems.

Key Words: *Cissus cornifolia*, Neuropharmacological activity, leaf, root, diazepam.

INTRODUCTION

Traditional and folklore medicines play an important role in health services around the globe (Usman *et al.*, 2009). About three quarters of the World's population relies on plants and its extracts for health care (Premanathan *et al*., 2000; Gabhe *et al*., 2006). A good number of our population particularly those living in the villages depend largely on herbal remedies. Most of these herbal remedies have stood the test of time, particularly for the treatment of allergic, metabolic and cardiovascular diseases (Igoli *et al.*, 2005). Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001).

Cissus cornifolia (Baker) Planch belongs to the family Vitaceae. It is an annual herb of 1.3 M high found widely distributed in the rocky suburbs and Savannah zones of Northern Nigeria and Ghana. The plant is locally called among the Hausas as *Tsùwààwùùn birii* -robe of the monkey (Burkill, 2000). Among the various uses of the plant in the African traditional medicine is as aremedy towards gonorrhoea when taken with native natron; the leaf-sap is used by the Tanganyika as a sedative in case of mental derangement, root decoction on the other hand is used for malaria, septic tonsil and pharyngitis (Burkill,

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E-mail: yaroabdulng@yahoo.com

2000). Our earlier studies (Musa *et al*., 2008) on the leaf extract of this plant revealed the presence of Alkaloids, saponins, flavonoids, steroids/terpenoids, tannins and some sedative effects at lower doses. This study aims to further confirm the sedative action of the leaf and root extract at higher doses employing conventional neuropharmacological models in mice.

MATERIALS AND METHODS

Collection, preparation and extraction of plant materials

The leaves and roots of *Cissus cornifolia* (Baker) Planch for this study were collected from Basawa village, Sabon gari Local Government Area, Kaduna-Nigeria in the month of September 2007 from a mature plant. The plant specimen was authenticated by a Taxanomist Musa, M. of the Herbarium Unit, Biological Sciences Department Ahmadu Bello University, Zaria-Nigeria by comparing with voucher specimen No.024.

The air-dried samples were thoroughly cleaned free from soil and other unwanted particles and then ground into fine powder. 250 g each of the samples were independently macerated for 72 hours to exhaustion utilizing 2.5 L (1:10) 70 % v/v methanol in H_2O. The combined leaf and root methanol extracts were independently concentrated *in vacuo.*

Experimental animals

Healthy adult Swiss Albino mice of weight 18-25 g were used. They were obtained from the Animal house, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria. The mice were kept in a well ventilated cages at room temperature in a 12 hour light/dark cycle (6am-6pm), maintained on processed animals feeds obtained from Excel Feeds Plc, Kaduna and water *ad libitum.* Animals were acclimatized in the laboratory environment one week prior to commencement of the experiment. All experimental procedures were approved by the Ahmadu Bello University Animal Right Ethics committee.

Acute toxicity study

The acute median lethal doses (LD_{50}) of the two extracts (leaf and root) were determined using Lorke (1983) method. Briefly, the experiment was divided into two phases: phase 1 involves nine mice in three divided groups of three each; group A were treated with 10 mg kg^{-1} bd. wt. (*i.p.)*, group B had 100 mg kg^{-1} bd. wt. (*i.p.)* while group C animals were administered 1000 mg kg^{-1} bd. wt. (*i.p.).* Phase 2 involves only 3 mice grouped into three with a mouse per group. The dosages to be administered to the animals in the second phase were determined by the result of the phase 1 experiment. The final LD_{50} was then evaluated as the square root of the product of the lowest lethal dose and that of the highest non lethal doses.

Diazepam-induced sleep in mice

The method of Rokotonirina *et al.,* (2001) as adopted by Musa *et al.,* (2008) was employed for this study. The sleep potentiation time of the plant extracts was assayed in group of mice that received diazepam (DZ) at a dose of 20 mg kg^{-1} bd. wt. one hour post *i.p.* administration of root bark and leaf extract at 300, 150, 75, 37.5 and 600, 300, 150, 75 mg kg^{-1} bd. wt. respectively. Normal saline was given as vehicle at a dose of 10 mg kg^{-1} bd. wt. five mice were used in each test group.

Exploratory behaviour in mice

The experiment was conducted using wooden apparatus of dimension 40x40 cm with 16 evenly spaced holes (Perez *et al.,* 1998). The mice were divided into six groups of six each for each of the extract. In the root and leaf extract, group I and group VI respectively received Normal saline at a dose of 10 ml kg^{-1} body wt. *i.p* and diazepam at 0.5 mg kg^{-1} bd. wt. *i.p.* Groups II-V in the root treated mice received 300, 150, 75, 37.5 mg kg^{-1} bd. wt. while the leaf extract treated mice in group II-V received respectively 600, 300, 150, 75 mg kg^{-1} bd. wt. Thirty minutes post treatment, mice were singly placed on the constructed board and the number of head dips into the holes during the 5 minutes period was recorded. The results of the two extracts were expressed as Mean ± SEM.

Motor co-ordination assay in mice

The beam walking assay method of Stanley *et al.,* (2005) was adopted. Mice were trained to walk from a platform along a ruler measuring 80 cm long, 3 cm wide elevated 30 cm above a bench by a metal support to a goal box. Three test training trials was performed for each mouse; mice that successfully passed the test trials were selected and grouped randomly into six groups of six mice for each of the extract under study. In the root and leaf extract, group I and group VI respectively received Normal saline at a dose of 10 ml kg^{-1} body wt. *i.p* and diazepam at 0.5 mg kg^{-1} bd. wt. *i.p.* Groups II-V in the root treated mice received 300, 150, 75, 37.5 mg kg^{-1} bd. wt. while the leaf extract treated mice in group II-V received respectively 600, 300, 150, 75 mg kg^{-1} bd. wt. Thirty minutes after treatment, each mouse from a particular group was placed at one end and allowed to walk onto the goal box. Mice that fell were returned to the position they fell from with a maximum allowable time of 60 seconds on the beam. The number of foot slips which is

a measure of motor co-ordination deficits was recorded using a tally counter.

RESULTS

Phytochemical contents

The extractive values of the leaf and root methanol extract was found to be 31.5 g with yield of 12.6 %($^{w}/_{w}$) and 37.8 g with the yield of 15.12 %($^{w}/_{w}$) respectively. Earlier report on the phytochemical constituents by Musa *et al.*, 2008; revealed the presence of alkaloids, flavonoids, saponins, terpenoids/steroids and tannins in the leaf methanol extract. All the above secondary metabolites were also determined in the root extract except for alkaloids that was not present in the root.

Acute toxicity

The acute toxicity studies revealed that the mean lethal doses (LD_{50}) for the root and leaf methanol extracts were 1131.4 mg kg^{-1} bd. wt. *(i.p.)* and 2154.1 mg kg^{-1} bd. wt. *(i.p.)* respectively.

Effects of the extracts on the diazepam-induced sleep in mice

The 70 % methanol extracts of both the root bark and leaf studied significantly (P<0.05) enhanced the duration of sleep between a normal saline group and all the dosages of the root extract; also between leaf extract at 600 and 75 mg kg^{-1} bd. wt.; while P<0.025 was observed when the normal saline group was compared with the 300 and 150 mg kg^{-1} bd. wt. for the sleep latency assay. The mean sleep time significantly (P<0.025, 0.005 and 0.0005) increased from 49.0±17.0 in the control group to 130.3±22.3 and 154.2±15.0 in the root extract in a dose dependent manner; a significant (P<0.025 to 0.0005) dose dependent (117.2±23.7 to 213.8±27.5) increase in mean duration of sleep was noted with the leaf extract. These data were presented in Table 1.

Effect on exploratory behaviour in mice

The exploratory bahaviour studies of the root bark extract of *C. cornifolia* revealed a dose –dependent reduction at lower doses (between 150-75 mg kg^{-1} bd. wt.) in the head dip test compared to the normal saline group as shown in Table 2. Also, significant (P<0.05, 0.025) dose-dependent head dip reduction (between 300-150 mg kg^{-1} bd. wt.) was observed among the leaf extract treated groups compared with the control group; no significant difference (P>0.05) was noted at dosages of 75 and 37.5 mg kg^{-1} bd. wt. in the root extract tested groups.

Table 1:
Diazepam-induced sleep of root bark and leaf methanol extract of *Cissus cornifolia* in mice

Treatment (mg/kg)	Mean sleep latency (sec)	Mean duration of sleep (min)
NS (10 ml kg^{-1})	6.48 ± 1.8	49.0 ± 17.0
CCRE 300	2.5 ± 0.56[a]	134. 0 ± 24.1[b]
CCRE 150	2.5 ± 0.29[a]	154.2 ± 15.0[d]
CCRE 75	3.8 ± 0.20[a]	148.2 ± 21.4[c]
CCRE 37.5	2.8 ± 0.36[a]	130.3 ± 22.3[c]
CCLE 600	2.7 ± 0.21[a]	213.8 ± 27.5[d]
CCLE 300	2.0 ± 0.26[b]	126.7 ± 22.7[b]
CCLE 150	2.2 ± 0.17[b]	127.3 ± 24.5[b]
CCLE 75	2.8 ± 0.16[a]	117.2 ± 23.7[b]

Data presented as Mean ± Standard Error of Mean; a, b, c, and d represents p< 0.05, 0.025, 0.005 and 0.0005 respectively; Student t – test n = 6;
CCRE – *Cissus cornifolia* root extract; CCLE – *Cissus cornifolia* leaf extract, NS- Normal saline

Table 2:
Effect of methanol leaf extract of *Cissus cornifolia* on exploratory behaviour (head dip test) in mice

Treatment (mg/ kg)	Mean number of head dips in 5 minutes	
	Leaf extract	Root extract
NS 10ml kg^{-1}	15.5 ± 2.8	-
CCLE 600	6.0 ± 0.6[c]	13.8 ± 2.0
CCLE 300	5.3 ± 0.7[c]	8.0 ± 0.8[b]
CCLE 150	8.3 ± 1.2[b]	9.6 ± 1.0[a]
CCLE 75	9.3 ± 1.8[a]	10.3 ± 0.9
CCRE 37.5	-	13.5 ± 1.2
Diazepam 0.5	5.2 ± 0.4[c]	6.6 ± 0.6[c]

Data presented as Mean ± Standard Error of Mean; a, b and c represents. P< 0.05, 0.025 and 0.005 respectively; Student t – test n = 6;
CCRE – *Cissus cornifolia* root extract; CCLE – *Cissus cornifolia* leaf extract; NS- Normal saline

Table 3:
Effect of root and leaf methanol extract of Cissus cornifolia on motor coordination (beam walk assay) in mice

Treatment (mg/kg)	Mean duration of beam walk (sec.)	Mean number of foot slips
Normal / saline 10 ml kg^{-1}	4.25 ± 0.38	0.00 ± 0.00
CCLE 600	8.33 ± 0.62[d]	0.00 ± 0.00
CCLE 300	6.88 ± 0.71[c]	0.00 ± 0.00
CCLE 150	6.10 ± 0.80[a]	0.00 ± 0.00
CCLE 75	5.32. ± 0.69	0.00 ± 0.00
CCRE 300	4.72 ± 0.28	0.00 ± 0.00
CCRE 150	5.95 ± 0.70[a]	0.00 ± 0.00
CCRE 75	4.83 ± 0.67	0.00 ± 0.00
CCRE 37.5	4.82 ± 0.67	0.00 ± 0.00
Diazepam 0.5	8.02 ± 0.76[c]	3.83 ± 0.60

Data presented as Mean ± Standard Error of Mean.
a, c and d represent P≤0.05, 0.005 and 0.0005 respectively; student t-test n=6

Effect on motor co-ordination in mice

The methanol extract of *C. cornifolia* studied showed dose dependent effect in both extract though with little variation as shown on Table 3. The data revealed a non significant difference (P>0.05) mean duration of the beam walking between the N/S group and that of leaf extract at 75 mg kg^{-1} bd. wt. and also between the dosages at 37.5, 75, 300 mg kg^{-1} bd. wt. root extract. The mean duration of the beam walk of the N/S group varied significantly (P<0.005 and 0.0005) when compared between the leaf; and root extract (P<0.05) at 150 mg kg^{-1} bd. wt., the comparison was equally significant (P<0.005) at leaf extract dose of 300 mg kg^{-1} bd. wt. (P<0.05; while the mean duration of the beam walk of the N/S group was significant when compared with the leaf treated group at 600 mg kg^{-1} bd. wt.

DISCUSSION

The earlier report on the qualitative phytochemical components of the leaf extract of *C. cornifolia* revealed the presence of alkaloids, flavonoids, saponins, terpenoids/steroids and tannins (Musa *et al.,* 2008); these substances were reported to have curative effects (Hassan *et al.,* 2004). The sedative properties of flavonoids and saponins have been documented (Viswanatha Swamy *et al.,* 2006, Dubois *et al.,* 1986; Amos *et al.,* 2001; Musa *et al.,* 2008).

The root extract of *C. cornifolia* was found to significantly potentiate the diazepam-induced sleep in mice at high dose (150 mg kg^{-1} bd. wt.), this result is in line with earlier report by Musa *et al.,* (2006; 2008); on the other hand the leaf extract dose dependently potentiated the sedative action of diazepam significantly (P<0.0005).; this effect contradicts the pattern reported by Musa *et al*., 2008. These results suggest that the sedative activity may be observed at even lower doses of the root extract than the leaf, hence root stands to be a better candidate.

The neuropharmacological action of the plant extract as demonstrated using the head dip test revealed that the leaf extract is in line with earlier work by Rokotonirina *et al.,* (2006); Musa *et al.,* (2008). We were able to confirm that the extract possessed sedative properties at higher doses particularly 300 mg kg^{-1} bd. wt. with no significance difference in the number of head dips expressed by DZ at 0.5 mg kg^{-1}. The root extract on the other hand significantly (P<0.025) reduce the number of head dips in 5 minutes at 300 mg kg^{-1} compared to the N/S group treated with significant difference compared to DZ. This effect could not be unrelated to the presence of alkaloids in the leaf which is absent in root extract. Supportive evidence to this was the report by File and Wardill, (1975) that, a reduction in the number of head dips is a measure of sedative properties. This study further confirms that the plant extract reduced number of head dips in the exploratory test which indicates CNS depressant activities as reported by Adzu *et al.,* (2002); Viswanatha Swamy *et al.,* (2006); Musa *et al.,* (2008).

Both the leaf and root extract did not show any observable effects on motor co-ordination test. Thus, the sedative action of the leaf extract could behave centrally and not peripherally due to neuromuscular blockade (Perez *et al.,* 1998). The root extract did not show any significant difference (P>0.05) with the N/S group indicative of lack of motor in coordination deficit.

In conclusion, the leaf of this plant could stand a better choice due to its dose-dependent sedative effects and moderate toxicity level compared to the root extract. Therefore, this study further corroborates the claim by the traditional healers for the use of the leaf sap as a cure for the mental disturbances; the root can be used for similar cases but at lower doses.

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