Central nervous system depressant effects of fractions of methanol leaf extract of *Cissus cornifolia* (Baker) Planch

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Abstract*: Cissus cornifolia* is an annual herb used in the treatment of mental derangement in the African Traditional Medicine. As part of a continuous research on this medicinal plant to scientifically validate its use in mental derangement, the fractions of the leaf extract were investigated for central nervous system (CNS) depressant effects. Successive fractionation of the methanol leaf extract of *C. cornifolia* was utilized to obtain the chloroform fraction (CLF), ethyl acetate fraction (EAF) and the residual aqueous fractions (RAF). These fractions were investigated for CNS-depressant effects in mice using diazepam-induced sleep, head-dip and motor-coordination tests. CLF, EAF and RAF significantly ($p<0.01$) prolonged the duration of sleep in mice. EAF significantly ($p<0.05$) reduced the mean head-dips in mice at 75 and 150 mg/kg. Similarly, a significant decrease in the mean head-dips ($p<0.05$, $p<0.01$ and $p<0.005$) was produced by RAF at 150, 300 and 600 mg/kg respectively. In the beam-walking assay test, all the fractions did not produce motor coordination deficit in mice. The data obtained revealed the fractions of methanol leaf extract of *Cissus cornifolia* possess remarkable central nervous system depressant effects.

Keywords: *Cissus cornifolia*, CNS depressant activity, diazepam, exploratory behaviour, motor coordination.

INTRODUCTION

Medicinal plants have a long history of use in therapy globally and still make an important component of traditional medicine. Medicinal plants and other herbal products of folk medicine have served as major sources of pharmaceuticals and are still alternative sources for discovery of lead compounds (Qurishi *et al*., 2010). This is because they have remarkable biodiversity of naturally occurring secondary metabolites, chemical structures and biological activities with acclaimed better safety and efficacy profiles. In developing countries like Nigeria, the use of medicinal plants in the management of neurologic and psychiatric diseases have also gained wide patronage. This is due not only to unmet therapeutic needs but also improvements in the safety, quality and efficacy of herbal medicines with the development of science and technology (Okor, 2014).

Cissus cornifolia (Baker) Planch. is a plant that belongs to the family Vitaceae. The plant is distributed widely in Ghana and Nigeria, and is locally called among the Hausa's as "Tsuwawun biri" in Northern Nigeria. In African Traditional Medicine, *Cissus cornifolia* is used by the Fulanis in combination with potash for the treatment of gonorrhoea. The leaves and roots are used as sedative among the Tanganyikas to manage mental derangement, while decoction of the root is used for the treatment of malaria, tonsillitis and pharyngitis (Burkill, 2000).

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Scientific studies on *Cissus cornifolia* had shown that the plant possesses sedative (Yaro *et al*., 2009), antidiarrhoeal (Tanko *et al*., 2011), hypoglycaemic (Jimoh *et al*., 2013) and anticonvulsant activities (Yaro *et al*., 2015). The therapeutic efficacies of medicinal plants are mostly attributed to their bioactive constituents; as an effort to characterize the bioactive constituents of *Cissus cornifolia* leaves, this study therefore, was designed to evaluate the CNS-depressant activities of the chloroform, ethyl acetate and residual aqueous fractions of the plant in order to provide scientific evidence supporting further development of the bioactive principles as CNS depressant.

MATERIALS AND METHODS

Drugs and chemicals

Diazepam (Roche, France), Methanol, Chloroform and Ethyl acetate solvents (Sigma Aldrich, USA).

Experimental animals

Albino mice of either sex (18-24g) were obtained from the Animal Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University (A.B.U.), Zaria. The mice were maintained in well ventilated cages at room temperature and fed on standard animal feed (Vital Feed®, Bukuru, Jos) and water *ad libitum.* Animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved institutional Research and Ethical Committee (Protocol Number: DAC/IW-OT/2234-09).

Plant material

Fresh *Cissus cornifolia* leaves were collected in October 2008 in Basawa, Kaduna State, Nigeria. The plant was authenticated at the Herbarium of Department of Biological Sciences, A.B.U., Zaria, and a voucher specimen number (024) was assigned. The collected leaves were air dried under shade and then size-reduced using a pestle and mortar.

Extraction and preparation of fractions

About 1200g of the powdered leaves of *C. cornifolia* was extracted in 2 liters of aqueous methanol (methanol 70%, water 30%) for 2 weeks using the cold maceration method. The macerate was filtered and evaporated to dryness at 40^{o}C *in vacuo* to yield 115.51g of residue referred to as methanol leaf extract of *C. cornifolia*. About 100g of the crude methanol leaf extract was dissolved in water and then filtered. Subsequently, the filtrate was partitioned successively with chloroform and ethyl acetate (Deng *et al*., 2007). The chloroform, ethyl acetate and aqueous residual fractions were obtained and labeled as CLF, EAF and RAF respectively.

Phytochemical screening

The CLF, EAF and RAF obtained from leaf extract of *C. cornifolia* were subjected to standard qualitative phytochemical screening (Evans, 2002) to ascertain the constituents present.

Acute toxicity studies

The acute toxicity of CLF, EAF and RAF of *C. cornifolia* leaf were independently determined in mice using the intraperitoneal route. The study consisted of two phases; in phase 1, three groups of three mice each were used. The 1st, 2nd and 3rd groups of mice were administered the fractions at a doses of 10, 100 and 1000 mg/kg respectively and observed for 24hrs. The phase 2 involved only three mice and were administered specific doses of the extract (which depended on phase 1 results) and also observed for 24hrs (Lorke, 1983). Thereafter, the geometric mean of the highest non-lethal dose and the lowest lethal dose was calculated.

Behavioural studies

Diazepam-induced sleeping test

Sleep potentiating effects of CLF (150, 300 and 600 mg/kg), EAF (75, 150 and 300 mg/kg) and RAF (150, 300 and 600 mg/kg) were studied in four groups of 6 mice each that received diazepam (20 mg/kg *i.p.*), 30 minutes after the intraperitoneal administration of the fractions. The four groups consist of a control group (administered normal saline, 10 ml/kg) and three other test groups (fractions). The mice were monitored for onset and duration of sleep (measured as the time the animals lost their righting reflex and the time they regained the righting reflex respectively) (Rakotonirina *et al*., 2001).

Hole-board test

The influence of the fractions on the exploratory activity of mice was investigated using the head-dip test as described by File and Wardill, (1975). Six groups of six mice were used for each of the extract. The mice in the 1st group received 10 ml/kg of normal saline, *i.p.* (negative control). The 2nd, 3rd and 4th groups received the fractions (150, 300 and 600 mg/kg for CLF), (75, 150 and 300 mg/kg for EAF) and (150, 300 and 600 mg/kg, *i.p.* for RAF) respectively. Group 5 and 6 served as positive control and received diazepam at doses 0.25 and 0.5 mg/kg, *i.p.* respectively. Thirty minutes after treatment, each mouse was singly placed on a board (a 40cmx40cm wooden board with sixteen evenly spaced quholes) and then allowed to explore the board for 5 mins. The number of head-dips made by each mouse within the allowed period was recorded.

Beam-walking test

In this test, thirty mice that walked successfully along a 30cm high elevated ruler (80cm long, 3cm wide) to an enclosed goal box (hamster house) were selected (Stanley *et al*., 2005). They were grouped into five groups of six mice for each fraction under study. The 1st and 5th groups were administered 10ml/kg of normal saline and 0.25mg/kg of diazepam (negative and positive control groups respectively). The 2nd, 3rd and 4th groups were treated with the fractions (150, 300 and 600mg/kg for CLF), (75, 150 and 300mg/kg for EAF) and (150, 300 and 600mg/kg for RAF) respectively). Thirty minutes after treatment, each mouse was placed at one end of a wooden beam (60cm long, 8mm diameter and elevated 30cm above a bench) and allowed to walk to the enclosed hamster house. Each mouse was allowed a maximum of 1min. on the beam and the time to reach the hamster house, number of foot slips and number of falls made by each mouse was recorded.

STATISTICAL ANALYSIS

Data generated from the experiments were entered into SPSS software (Version 20). Descriptive statistics was carried out to obtain the mean± standard error of the mean (S.E.M.). One-way analysis of variance (ANOVA) was used in testing the differences in means between the control group and other treatment groups. Where a significant difference was obtained with ANOVA, Dunnett's post hoc test was carried out and statistical significant differences were considered at 95% confidence interval ($p<0.05$).

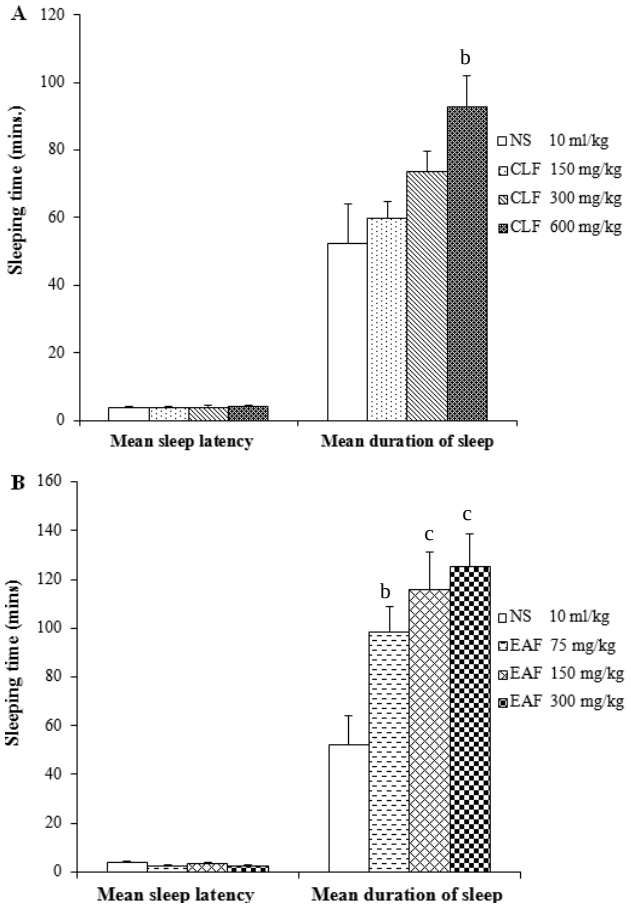
RESULTS

Successive fractionation of the methanol extract of *C. cornifolia* yielded 2.08%$^{w}/_{w}$ chloroform, 2.76% $^{w}/_{w}$ ethyl acetate and 10.04% $^{w}/_{w}$ residual aqueous fractions. Their respective phytochemical constituents are presented in table 1.

Table 1: Phytochemical constituents of fractions of methanol leaf extract of *Cissus cornifolia*

Constituents	CLF	EAF	RAF
Alkaloids	–	–	+
Anthraquinones	–	–	–
Flavonoids	+	+	+
Saponins	–	–	+
Tannins	–	+	+
Triterpenoids	–	+	–
Steroids	+	–	–

Key: Absent–, Present +, CLF=Chloroform fraction, EAF=Ethyl acetate fraction, RAF=Residual aqueous fraction



Median lethal dose (LD_{50}) determination

Acute toxicity studies revealed that the intraperitoneal LD_{50} of CLF, EAF and RAF in mice were >5000, 1264.9 and >5000 mg/kg respectively. No signs of toxic effects were produced by the chloroform and residual aqueous fractions following intraperitoneal administration in the mice. However, a mouse died at a dose of 1600 mg/kg of the EAF.

Behavioural studies

Effect of Cissus cornifolia fractions on diazepam-induced sleep in mice

The CLF did not show a significant difference ($p>0.05$) in the mean sleep onset at the doses tested (150, 300 and 600mg/kg), but significantly ($p<0.01$) increased the mean sleep duration from 52.3±11.7mins. (normal saline) to 93.0±8.8mins (highest dose of 600 mg/kg) (fig. 1). EAF decreased sleep latency significantly ($p<0.05$) at 75 and 300mg/kg when compared with control. It also prolonged the sleep duration significantly from 52.3±11.7mins. (Control group) to 98.5±10.5, 115.8±15.3 and 125.3±13.4 mins. (75, 150 and 300mg/kg of EAF respectively).The residual aqueous fraction also prolonged the sleep duration significantly ($p<0.001$) at 150 and 300mg/kg (fig.1).

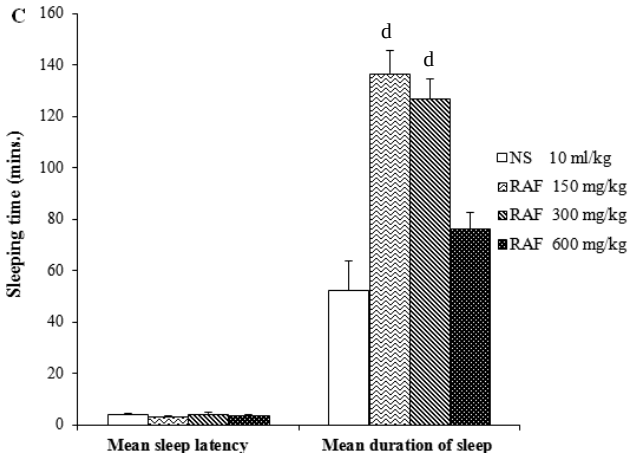
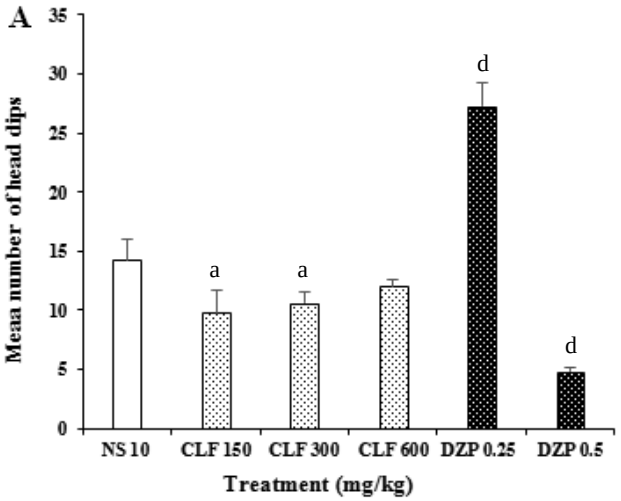


Fig. 1: Effect of fractions of methanol leaf extract of *Cissus cornifolia* on onset and duration of diazepam-induced sleep in mice. (A) Chloroform fraction (CLF); (B) Ethyl acetate fraction (EAF); (C) Residual aqueous fraction (RAF). Sleeping time presented as Mean ± S.E.M.; a, b, c and d represent $p<0.05$, $p<0.01$, $p<0.005$ and $p<0.001$ compared to control respectively – One way ANOVA and Dunnett's test, n = 6, NS = Normal saline



Effect of Cissus cornifolia fractions on hole-board test in mice

The overall results revealed a reduction in exploratory behaviour in mice with all the fractions studied. CLF significantly ($p<0.05$) reduced the mean head-dips at 150 (9.8±0.9) and 300mg/kg (10.6±1.0) as compared to the

control group (14.20±1.80) in a non-dose-dependent manner (fig. 2). Similarly, a non-dose-dependent but significant decrease ($p<0.05$) in exploratory behavior was produced by EAF. The RAF however, significantly ($p<0.05$) reduced the mean head-dips in a dose dependent manner from 14.2±1.8 in control group to 8.8±0.9, 8.2±0.9 and 6.5±0.7 at 150, 300 and 600 mg/kg respectively (fig. 2).

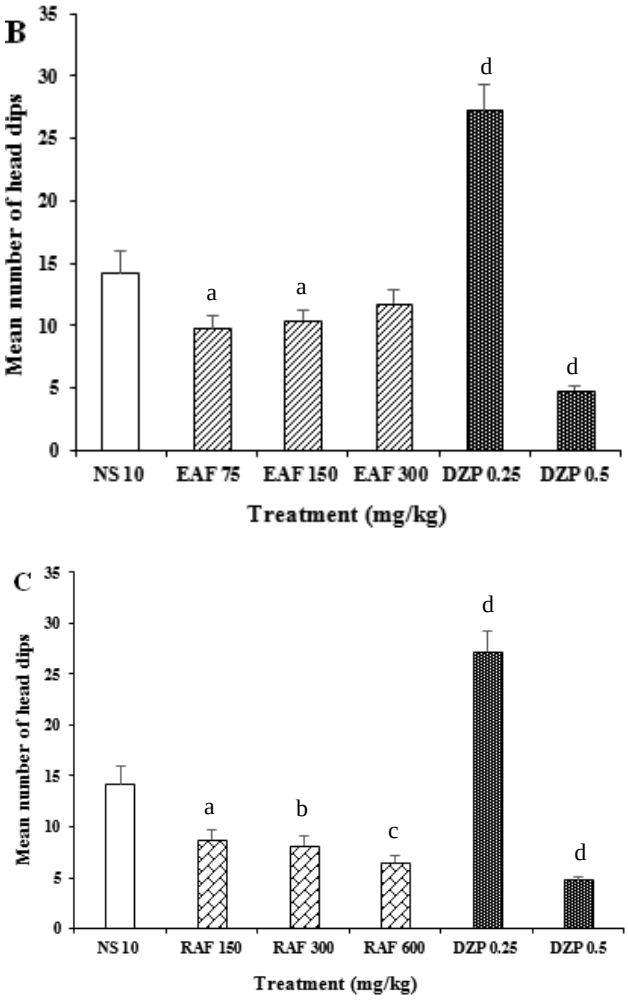


Fig. 2: Effect of fractions of methanol leaf extract of *Cissus cornifolia* on exploratory behaviour in mice. (A) Chloroform fraction (CLF); (B) Ethyl acetate fraction (EAF); (C) Residual aqueous fraction (RAF). Head dips presented as Mean ± S.E.M., a, b, c and d represent $p<0.05$, $p<0.01$, $p<0.005$ and $p<0.001$ compared to control respectively – One way ANOVA and Dunnett's test, n = 6, NS = Normal saline

Effect of Cissus cornifolia fractions of on motor coordination in mice

The CLF and EAF produced a non-significant difference ($p> 0.05$) in duration of beam walk and mean foot slips when compared control. RAF however, significantly ($p<0.001$) delayed the time to reach goal box at 600mg/kg without any effect on the number of foot slip. The standard drug used (diazepam) produced a significant ($p<0.001$) increase in number foot slips as well as the time to reach goal box when compared with control group (table 2).

DISCUSSIONS

Alkaloids, flavonoids, saponins, tannins and terpenoids were among the secondary metabolites previously reported to be biologically active in the crude methanol leaf extract of *C. cornifolia* (Musa *et al*., 2008, Yaro *et al*., 2009). In the present studies, CLF, EAF and RAF were also found to contain some of these phytochemical constituents. Thus, the observed CNS depressant properties in this study might be attributed to the different bioactive constituents found present in the fractions.

The LD_{50} of *C. cornifolia* fractions were determined and according to the classification of LD_{50} values (Lu, 1996), CLF and RAF were slightly toxic (reflected by their high LD_{50} values) while EAF was moderately toxic. However, the doses of the fractions used in this study were lower than 30% of the LD_{50} and according to Vongtau *et al*. (2004), these doses are relatively safe for ethnopharmacological research.

Diazepam-induced sleeping time is considered a very sensitive way to assess CNS-active properties of drugs (Vogel, 2008). All the fractions of *C. cornifolia* studied potentiated diazepam-induced sleeping time in mice, but the potentiation was dose dependent only with CLF and EAF. In addition, RAF also significantly reduced the sleep latency (fig.1) and this suggest that the extracts possess sleep-inducing effects. The results obtained in this study are therefore in agreement with previous reports on the plant (Yaro *et al*., 2009).

All the fractions studied (CLF, EAF and RAF) significantly reduced the mean head-dips at all the doses tested. Nose-poking is a usual behaviour of mice indicating a kind of curiosity (Vogel, 2008). A decrease in the number of nose-poking signifies sedative behaviour (File and Pellow, 1985) and a measure of CNS depressant effect (Adzu, 2002). The results obtained therefore suggest that the fractions of *C. cornifolia* methanol leaf extract possess CNS-depressant effects as indicated by the significant decrease in exploratory behaviour. The head-dip test is also used for evaluating anxiety conditions in animals (Crawley, 1985), and anxiolytic drugs have been reported to produce an increase the mean number of head-dips (Vogel, 2008). Thus, diazepam at a lower dose significantly increased hole exploration and is anxiolytic whereas the high dose diazepam decreased hole exploration and was sedating.

The beam-walking test determines motor coordination deficits induced by benzodiazepines and is also a better predictor of clinically sedating doses of the

Table 2: Effects of fractions of methanol leaf extract of *Cissus cornifolia* on motor coordination in mice

Treatment	Dose (mg/kg)	Mean duration of beam walk (Sec.)	Mean number of foot slips
Normal saline	10 ml/kg	3.8 ± 0.3	0.0 ± 0.0
CLF	150	3.3 ± 0.3	0.0 ± 0.0
CLF	300	3.6 ± 0.4	0.0 ± 0.0
CLF	600	2.7 ± 0.2	0.0 ± 0.0
EAF	75	4.1 ± 0.3	0.0 ± 0.0
EAF	150	4.2 ± 0.4	0.0 ± 0.0
EAF	300	4.3 ± 0.4	0.0 ± 0.0
RAF	150	4.1 ± 0.4	0.0 ± 0.0
RAF	300	4.2 ± 0.6	0.0 ± 0.0
RAF	600	6.3 ± 0.4[d]	0.0 ± 0.0
Diazepam	0.25	9.7 ± 0.9[d]	4.6 ± 0.6

Data are presented as Mean ± S.E.M.; [d] represents $p<0.001$ compared to control, - One way ANOVA and Dunnett's test, n= 6, CLF=Chloroform fraction, EAF=Ethyl acetate fraction, RAF=Residual aqueous fraction

benzodiazepine-like agents (Stanley *et al*., 2005). The results obtained showed that the fractions did not produce any effect on motor coordination (no effect on number of foot slips). The observed CNS-depressant effects of the fractions are thus due to central actions and not peripheral neuromuscular paralysis (Perez *et al*., 1998; Magaji *et al*., 2012; Chindo *et al*., 2014).

CONCLUSION

The results obtained demonstrated that the fractions of methanol leaf extract of *Cissus cornifolia* possess remarkable central nervous system depressant effects and further supports its use in the traditional management of mental derangements.

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