



Neuropharmacological Activities of Ethanolic Extract of *Cola millenii* Dried Leaf in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAO initiated and designed the work, supervised the research work and analysed all the results. Authors FK and LA collected and processed the plant's material, carried out all the animal experiments and prepare the first draft of the paper. Authors AOO and IAO performed the phytochemical screening, processed the LC-MS analysis of the ethanolic extract, revised and processed the paper for publication. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/28718

Editor(s):

(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.

Reviewers:

(1) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.
(2) Eliton da Silva Vasconcelos, Federal University of São Carlos– UFSCar, Brazil.
Complete Peer review History: <http://www.sciencedomain.org/review-history/15976>

Original Research Article

Received 1st August 2016
Accepted 11th August 2016
Published 27th August 2016

ABSTRACT

Objective: The leaf of *Cola millenii* is used in ethnomedicine to treat several ailments including; infections, fever and pain among several other diseases in Southwest Nigeria. Preliminary report indicates that the ethanolic leaf extract of this plant exhibits some central nervous system (CNS) activities; hence, this study investigated the acute toxicity and some central effects of the ethanolic dried leaf extract of *C. millenii* in rats.

Materials and Methods: Ethanolic dried leaf extract of *C. millenii* (CME) was obtained by soaking dried powdered leaf of the plant in 70% ethanol for 72 h and the filtrates recovered was dried *in vacuo*. The extract was tested for acute toxicity (LD₅₀) through the oral (*p.o.*) and intraperitoneal (*i.p.*) routes; dose-dependently evaluated for novelty-induced behavioural activities, sedative and

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anticonvulsant activities in rats. Diazepam (1-2 mg/kg, *i.p.*) and 5% Tween 80 served as positive and negative control groups respectively (n=5).

Results and Discussion: LD₅₀ values obtained were 5000 mg/kg, *p.o.*, and 2154 mg/kg, *i.p.* The CME (250, 500 and 1000 mg/kg, *i.p.*) significantly ($p < 0.05-0.01$) reduced rearing, grooming, locomotion and head-dips dose-dependently compared to the vehicle, signifying CNS depression; significantly ($p < 0.05$) reduced sleep latency and prolonged total sleeping time on the ketamine-induced hypnosis indicating sedative activity; prolonged the latency to convulsion, delayed time of death and offered between 20-40 percent protections against the pentylenetetrazole (PTZ)-induced convulsions, suggesting anti-convulsant potentials.

Conclusion: It is concluded that the ethanolic dried leaf extract of *C. millenii* is non-toxic orally but slightly toxic intraperitoneally; demonstrated significant depression of the CNS, possess sedative and anticonvulsant activities in rats.

Keywords: Sterculiaceae; monkey cola; LC-MS; acute toxicity; behavioural; sedative; anticonvulsant.

1. INTRODUCTION

The use of plants as sources of remedies for the treatment of diseases date back to prehistoric times and people of all continents are used to this old tradition [1]. Traditional medicine has been practiced for centuries and is still the most affordable and accessible healthcare system. Global demand for medicinal plants is continuously growing, as well as the bio-prospecting activities searching for sources of new drugs [2]. Universally, medicinal plants constitute a veritable source of semi-synthetic and traditional herbal medicine [3], while different parts of medicinal plants have been used to cure specific ailments [4]. Adjanohoun et al. [5] and Kokwaro [6] also noted that ethnobotanical studies carried out throughout Africa confirm that indigenous plants are the main constituent of traditional medicine in the region. However, in recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries. This trend has been linked with the belief that herbal medicines are safe, affordable, readily sourced, amenable to the nature of illnesses in the localities and exhibit minor adverse effects when compared with synthetic drugs [7-9]. Although little progress has been reported in terms of notable discoveries of biomolecules in the last two decades [10], plants are still recognized as sources of leads for discovery and development of novel drug compounds even as plant-derived medicines have made large contributions to human health and well-beings [11-13].

Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized developed countries are derived directly or indirectly from plants [14]. Many non-natural, synthetic drugs cause several side effects that

were not acceptable except as treatments of last resort for terminal diseases such as cancer [15]. In addition, high cost and adverse effects that are commonly associated with popular synthetic CNS drugs are a major burning global issue in managing neuropharmacological disorders.

Cola millenii K. Schum is one of the 125 species from the genus *Cola* Schott & Endl and family Sterculiaceae [16]. Also, the genus was formerly classified into the family Malvaceae and subfamily Sterculioideae, but has been transferred into the separate family Sterculiaceae containing 70 genera and 1500 species of tropical trees and shrubs [17,18]. *Cola* species are found mostly in the relatively dry parts of the rain forest, although *Cola millenii* and *Cola gigantea* are widely distributed in wet and dry forest environments [19,20]. The commonest edible species (*C. nitida* and *C. acuminata*) has a bitter flavor and high caffeine content and are chewed in many West African cultures individually or in a group setting. It is often used ceremonially, presented to tribal chiefs or presented to guests for entertainment. Kolanuts are used mainly for their stimulant and euphoriant qualities and possess effects similar to other xanthine-containing plants like cocoa, tea and coffee, however, the effects are distinctively different, producing a stronger state of euphoria and wellbeing [21] with resultant stimulant effects on the CNS and heart. Kolanuts are used as a source of alkaloids in pharmaceutical preparations [22,23].

Cola millenii K. Schum (commonly known as Monkey Cola by the Igbos and *Obi edun* by the Yorubas) is among over one hundred species belonging to the genus *Cola* Schott & Endl and family: Sterculiaceae [16]. *Cola* species are found growing wild in the forest, along the major

roads and within the campus of Obafemi Awolowo University, Ile-Ife specifically, along Road 1 (Fig. 1). Various parts of this plant species are used as food and herbal medicines [24]. There are several species under the genus *Cola* which are scattered throughout this region and identified ones include *C. lateritia*, *C. lepidota*, *C. pachycarpa* etc. It has been reported that local people of southern Nigeria and Cameroon relish the fruits including wild primate animals such as monkeys, baboons and other species which probably led to the name 'Monkey Cola' [25]. Leaves of *Cola lateritia* are used in traditional medicine to treat febrifuge (fever), pulmonary problems, infections and related ailments [26]. Preliminary phytochemical report on leaf extract of *C. millenii* showed the presence of alkaloids, saponins, tannins and cardenolides [18]. The in-vitro antioxidant activities of the leaf extract and fractions of *Cola lateritia* have been reported to show significant activity [27]. Orisakeye & Ojo [24] also reported antimicrobial and antioxidant potentials of fractions from various parts of *C. millenii*. Odugbemi [28] reported that the leaves of *C. millenii* are used in the treatment of ringworm, scabies, gonorrhoea, dysentery and as ophthalmic agent; while Sonibare et al. [18] reported the phytochemical and antimicrobial activities of this species along with others. There is paucity of literatures on this species and as part of the on-going effort to detect central nervous system activity from natural sources (medicinal plants inclusive), this plant was investigated for acute toxicity and behavioural effect in rats. Therefore, the aim of this study was to investigate the acute toxicity profile and evaluate some central effects of the ethanolic dried leaf extract of *C. millenii* in rats.

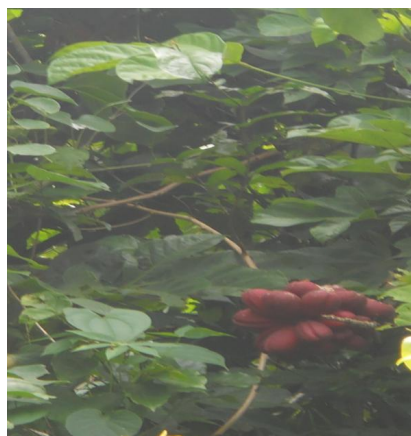


Fig. 1. Photograph of *Cola millenii* showing bunch of ripe fruits

2. MATERIALS AND METHODS

2.1 Plant Identification and Authentication

Identification and authentication of the fresh leaf of the plant was done by Mr. G.A. Ademoriyo, Herbarium Officer, Department of Botany, OAU, Ile-Ife and a specimen sample was deposited and voucher NO. IFE 17425 issued.

2.2 Plant Collection, Preparation and Extraction

Fresh leaves of *C. millenii* were collected along Road 1, Obafemi Awolowo University campus, Ile-Ife, Osun state between October and November 2014. Fresh leaves of the plant collected were air-dried in the laboratory for two weeks, ground into coarse powers before soaking in 70% ethanol for 72 h. The filtrate was then concentrated to dryness in vacuo using the rotatory evaporator to obtain semi-solid ethanolic extract of *C. millenii* (CME). The extract was stored in the refrigerator until use.

2.3 Phytochemical Screening of the Ethanolic Extract of CME

Phytochemical tests were carried out to detect the presence of phytochemical components in the ethanolic extract of the *C. millenii* [29]. Secondary metabolites screened include tannins, saponins, flavonoids, terpenoids, alkaloids, phenols, phytosterols, glycosides, anthraquinones, phylobatannins and proteins/ amino acids. All the chemicals used in this study for qualitative and quantitative phytochemical screening were of analytical grade.

2.4 LC-MS Analysis of the CME

In order to obtain a fingerprint of the crude ethanolic extract, LC-MS analysis was carried out on the extract was analysed at Central Analytical Facilities, Stellenbosch University, Stellenbosch, South Africa. The analysis was performed on Waters Synapt G2 Waters UPLC with PDA detection source was by electrospray positive, Capillary voltage 3 kV, Cone Voltage 15 V and Lock mass was Leucine encephalin.

2.5 Laboratory Materials

Observation cage, weighing balance (Mettler, Toledo), methylated spirit, stop watch, methanol,

Tween 80, oral cannulas, syringes and needles and other materials available in the laboratory.

2.6 Drugs

Diazepam (Valium® Roche, Switzerland), pentylene tetrazole (PTZ), ketamine HCL (Rotex Medica, Tritau, Germany), normal saline (Juhel Pharm., Nigeria) were purchased from the Pharmacy shop.

2.7 Laboratory Animals

Young adult male and female albino rats (Vom strain) weighing between 70 and 110 g was obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The females and males were caged in a separate home cages to prevent mating and pregnancy. The animals were kept at the same Animal House before and after experiment each day and maintained under the 12 h day/12 h night circle. The animals were supplied with regular food and water ad libitum. All tests were carried out between 10.00 a.m. and 5.00 p.m. EU Directive 2010/63/EU for animal experiment was adopted as being implemented by the University Research Committee through the Faculty of Pharmacy Postgraduate Committee, OAU, Ile-Ife, Nigeria. Ethical Approval for the study was given by the Faculty of Pharmacy Postgraduate Committee on behalf of the Obafemi Awolowo University Research Committee (URC).

2.8 Acute Toxicity Test of the Ethanolic Leaf Extract of *C. millenii* (CME) in Rats

The method used was as described by Lorke, [30]. The model involves 2 phases, first phase used 3 animals for each dose levels of 10, 100, 1000 mg/kg and the second phase involves 4 dose levels of 1000, 1600, 2900 and 5000 mg/kg (n=1). Rats were observed for the first 2 h after administration and thereafter, mortality within 24 h was recorded. Both oral and intraperitoneal routes of administration were used in order to determine the relative toxicity of the extract in the two different routes. The LD₅₀ of the ethanolic extract was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to the formula below:

$$LD_{50} = \sqrt{(A \times B)}$$

Where A is the maximum dose producing 0% death and B is the dose that producing 100% death.

2.9 Novelty-induced Behaviour (NIB): Rearing, Grooming and Locomotion

Novelty induced behaviour was assessed by methods described by Onigbogi et al. [31]. Five groups (n=6) were randomly selected. Group I was administered with the vehicle (5% Tween 80, 10 ml/kg, i.p.). Groups II-IV was injected with the extract at 3 different dose levels, group V was injected with diazepam (1 mg/kg, i.p.) to serve as positive control. Rats in all the groups were pre-treated for 30 min prior to each test. Each animal was placed inside the observation cage and assessed for rearing and grooming (20 min) and locomotive behaviour (10 min).

2.10 Head Dipping Behaviour

The effect of the CME on head-dipping behaviour was tested on hole board according to the method of Takeda et al. [32] and Yadav et al. [33]. Rats were randomly distributed as described in previous section. After 30 min pretreatment, each rat was placed in the center of the hole board. The number of head-poking demonstrated by each mouse in 5 min was recorded.

2.11 Sedative Activity of the Ethanolic Extract on the Ketamine-induced Hypnosis

Ketamine (100 mg/kg, i.p.) was used to induce sleep in rats [34]. Rats in different groups (n=6) was pre-treated with vehicle, extract at 3 different doses, while the last group was injected diazepam (1 mg/kg) 30 min prior to the administration of ketamine. Each animal was observed for sleep latency (SL) or the onset of sleep (time from injection to time of loss of righting reflex) and the duration of sleep or total sleeping time-TST i.e. time from loss and regain of consciousness [35,36].

2.12 Anticonvulsant Assessment on the Pentylene tetrazole (PTZ)-induced Convulsions

Pentylene tetrazole (85 mg/kg, i.p.) was used to induce tonic-clonic convulsion [37] to different groups of rats pre-treated with vehicle (normal saline), different doses of extract (250-1000

mg/kg) and diazepam (1 mg/kg, i.p.). The anticonvulsant activity assessment was carried out after 30 min pre-treatment before injection of PTZ (85 mg/kg, i.p.) and observed for the onset of convulsion, time of death and mortality. Animal that survived beyond 30 min post PTZ injection was assumed to be protected in this model.

2.13 Statistical Analysis

The results were expressed as Mean $\pm$ SEM. All parametric tests were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for comparison between the treated groups and negative control group. The level of significance was set at 95% confidence interval ($P < 0.05$). The statistical softwares used were GraphPad Instat3.0 and GraphPad Prism 5 (Copyright (c) 2007 GraphPad Software Inc.).

3. RESULTS

3.1 Phytochemical Screening of the CME

The presence of alkaloids, flavonoids, saponins, tannins, phenol, glycosides and steroids were detected in the crude ethanolic extract of the dried leaf of *C. millenii*. However, anthraquinones, phylobatannins, proteins and amino acids were not detected.

3.2 LC-MS Analysis of the CME

The results obtained from the LC-MS analysis of the crude ethanolic extract of *C. millenii* showed the presence of several compounds with 29

peaks indicating 29 compounds which were not distinctively characterized (Fig. 2).

3.3 Acute Toxicity Effect of the CME

In the first phase of the experiment, three dose levels (10, 100, 1000 mg/kg) were used and no mortality was observed for both intraperitoneal (i.p.) and oral (p.o.) routes of administration. In the second phase of the experiment, four (1000, 1600, 2900 and 5000 mg/kg) dose levels were used for both intraperitoneal and oral routes. After administration of 2900 mg/kg, i.p., the animal convulsed and died after 30 min which is the lowest dose that caused death in the animal, the highest dose that did not kill the animal was 1600 mg/kg, i.p., and LD₅₀ was calculated to be 2154 mg/kg, i.p. However, no death was recorded in the orally treated rats in the second phase; hence the LD₅₀ value was estimated to be ≥ 5000 mg/kg, p.o. (Table 1).

3.4 Effect of the CME on Novelty Induced Behaviour in Rats

3.4.1 Effect of CME on rearing activity

The CME (250, 500 and 1000 mg/kg, i.p) caused significant [$P < 0.01$, $F_{(4,20)}=14.6$] decrease in the number of rearing while diazepam (1 mg/kg) caused significant ($p < 0.05$) decrease when compared with the vehicle group. The mean number of locomotion for 250, 500 and 1000 mg/kg were 3.2, 2.2, and 1.8 respectively compared to 8.6 for diazepam 1 mg/kg and 15.4 for the vehicle (Fig. 3).

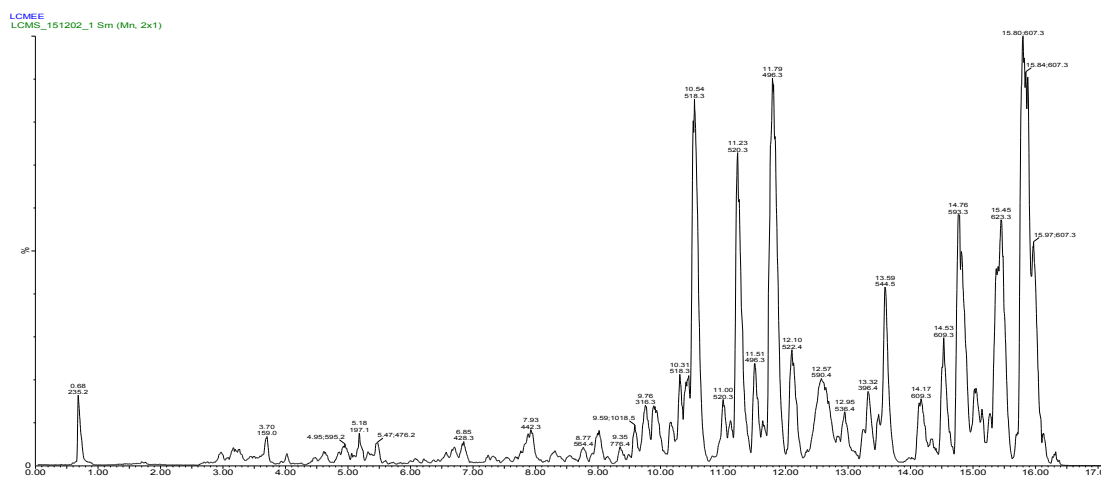
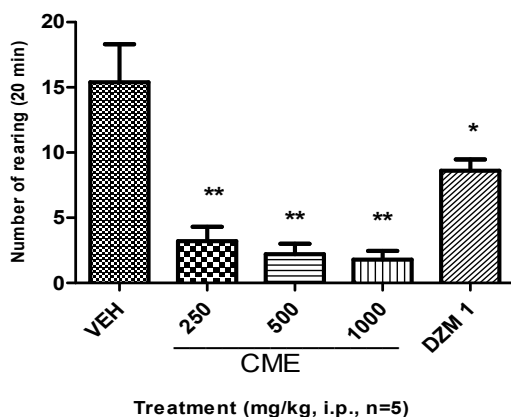


Fig. 2. LC-MS Chromatogram of ethanolic extract of *C. millenii* analysed

Table 1. Acute toxicity profile of the ethanolic leaf extract of *Cola millenii*

Dose mg/kg	Death patterns after 24 h	
	Intraperitoneal route (<i>i.p.</i>)	Oral route (<i>p.o.</i>)
Phase 1 (n=3)		
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
Phase 2 (n=1)		
1000	0/1	0/1
1600	0/1	0/1
2900	1/1	0/1
5000	1/1	0/1
LD ₅₀	(1600 x 2900) ^{1/2} =2154 mg/kg, <i>i.p.</i>	≥5000 mg/kg, <i>p.o.</i>

**Fig. 3. Effect of ethanolic leaf extract of *Cola millenii* on rearing behaviour in mice**

Each bar represents Mean±SEM. VEH, CME and DZM represent vehicle (normal saline), *Cola millenii* extract and diazepam respectively.

* $P < 0.05$; ** $P < 0.01$; statistically lower than control (ANOVA, Dunnett's test)

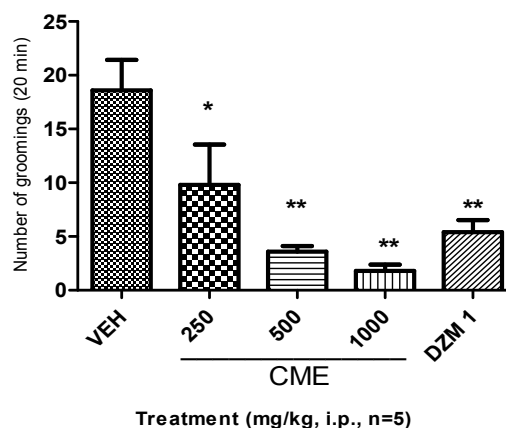
3.4.2 Effect of CME on grooming activity

The CME (250, 500 and 1000 mg/kg, *i.p.*) and diazepam (1 mg/kg) caused significant [$P < 0.01$, $F_{(4,20)}=9.39$] decrease in the number of grooming when compared with the control group. The mean number of locomotion for 250, 500 and 1000 mg/kg, *i.p.*, were 9.8, 3.6, and 1.8 respectively compared to 5.4 for diazepam and 18.6 for the vehicle (Fig. 4).

3.4.3 Effect of CME on locomotor activity

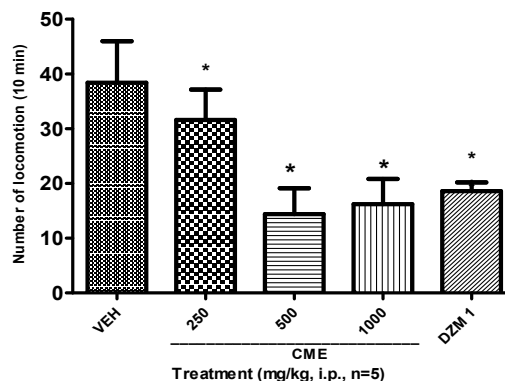
The CME at 250- 1000 mg/kg and diazepam (1 mg/kg) caused significant reduction [$P < 0.05$,

$F_{(4,20)}=4.66$] in the number of locomotion compared to the vehicle (Fig. 5).

**Fig. 4. Effect of ethanolic leaf extract of *Cola millenii* on grooming behaviour in mice**

Each bar represents Mean±SEM. VEH, CME and DZM represent vehicle (5% Tween 80), *Cola millenii* extract and diazepam respectively.

* $P < 0.05$; ** $P < 0.01$; statistically lower than negative control (ANOVA, Dunnett's test)

**Fig. 5. Effect of ethanolic leaf extract of *Cola millenii* on locomotion behaviour in mice**

Each bar represents Mean±SEM. VEH, CME and DZM represent vehicle (5% Tween 80), *Cola millenii* extract and diazepam respectively.

* $P < 0.05$; ** $P < 0.01$; statistically lower than control (ANOVA, Dunnett's test)

3.5 Effect of the CME on Head Dipping Behaviour

The ethanolic leaf extract of *C. millenii* (250, 500 and 1000 mg/kg *i.p.*) and diazepam (1 mg/kg, *i.p.*), caused significant [$P < 0.01$, $F_{(4,20)}=19.68$] decrease in the number of head-dips by the rats (Fig. 6).

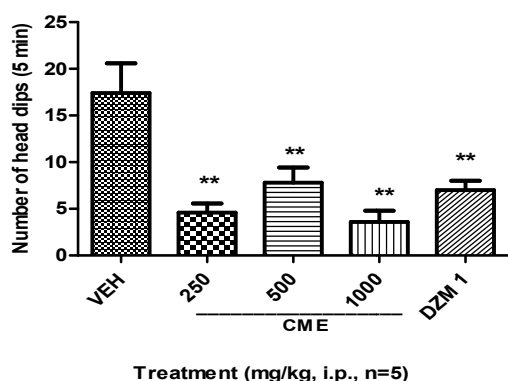


Fig. 6. Effect of ethanol leaf extract of *Cola millenii* on head-dipping behaviour in mice

Each bar represents Mean±SEM. VEH, CME and DZM represent vehicle (normal saline), *Cola millenii* extract and diazepam respectively.

** $P < 0.01$; statistically lowers than vehicle (ANOVA, Dunnett's)

3.6 Effect of the CME on Sedative Activity

3.6.1 Sleep latency (SL)

The CME dose-independently caused a decrease in the SL time compared to control (Fig. 7A). The CME (250 mg/kg) produced a slight increase in sleep latency compared to the vehicle, but at 500 mg/kg and 1000 mg/kg it significantly [$(P < 0.05; F_{(4,20)}=6.503]$ reduced the SL, with 1000 mg/kg producing the most reduction in SL time. The standard drug (diazepam (1 mg/kg) also caused a marked reduction in the sleep latency time compared to the vehicle. The extract at 250, 500 and 1000 mg/kg, gave mean SL of 112.6, 73.0 and 68.4 s compared to vehicle's 110 and diazepam's 78 s respectively.

3.6.2 Total sleeping time (TST)

The result for the TST is presented in Fig. 7B. The CME dose-dependently caused a marked prolongation in the TST. At a dose of 250 mg/kg, CME produced no prolongation of the TST, however, 500 and 1000 mg/kg it produced significant [$P < 0.01; F_{(4,20)}=40.28]$ prolongation of the TST compared to vehicle. The standard drug, diazepam (1 mg/kg) also significantly ($P < 0.05$) produced a longer TST with mean value of 72.4 min. The means TST of CME at 250, 500 and 1000 mg/kg, *i.p.*, were 49.2, 76.0 and 120 min respectively compared to negative control's 52 min.

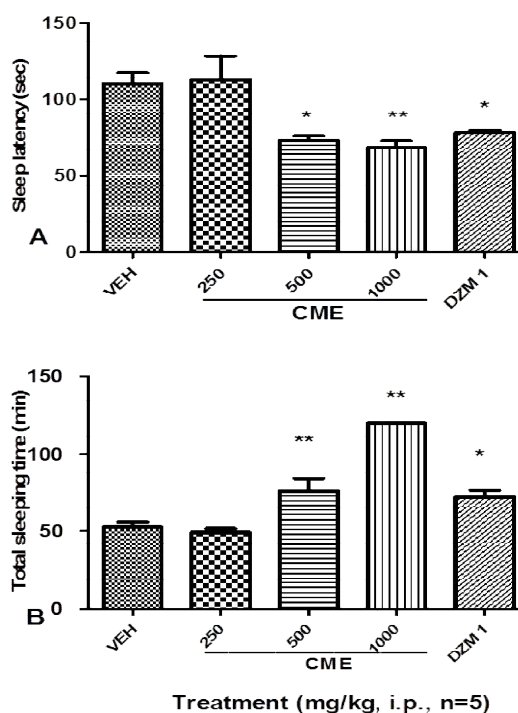


Fig. 7. Effect of ethanol leaf extract of *Cola millenii* on sleep latency [A] and total sleeping time [B] on the ketamine (100 mg/kg, i.p.)-induced hypnosis in rats

Each bar represents Mean±SEM. VEH, CME and DZM represent vehicle (5% Tween 80), *Cola millenii* extract and diazepam respectively.

* $P < 0.05$; ** $P < 0.01$; statistically lower than control (ANOVA, Dunnett's test)

3.7 Effect of CME on PTZ-induced Convulsions

3.7.1 Latency to convulsion (s)

The CME dose-independently produced an extension in the time taken for the rats to convulse. At 250 mg/kg, CME produced a mild prolongation in the latency of the rats to convulsion compared to the vehicle and at 500 mg/kg, CME significantly ($P < 0.05$) prolonged the latency to convulsion in the rats. However, CME (1000 mg/kg) and the standard drug (diazepam 1 mg/kg) also produced a prolongation in the latency to convulsion in the rats but in a non-significant way. The mean time of latency to convulsion for the CME at 250, 500 and 1000 mg/kg, *i.p.* were 41.6, 87.8 and 76.2 s respectively, compared to diazepam's 58 s and vehicle's 41.6 s (Table 2).

3.7.2 Death time (min)

The results (Table 2) showed that CME produced an increase in the death time of the animal compared to the vehicle. At 500 mg/kg, it produced the longest delay in death time with a mean death time of 33.0 min followed by the dose of 250 mg/kg (30.2 min). However, at 1000 mg/kg, CME gave the shortest death time among the doses of the extract administered with a mean death time 17.6 min. The standard drug (diazepam, 1 mg/kg) produced a longer delay in death time with mean value of 41.0 min.

3.7.3 Protection against mortality

It was observed that all the rats in the negative group died within 30 min post-PTZ injection with 0% protection and 100% mortality. However, CME at 250, 500 and 1000 mg/kg offered 40, 20 and 20% protection against PTZ-induced mortality compared to diazepam's 80% (Table 2).

4. DISCUSSION

Research into medicinal plants is gaining a new momentum in recent times because it might provide useful sources for therapy and prevention of diseases or alternatively, as simple dietary adjuncts to existing therapies [38]. The main aim of researching into natural products such as medicinal plants is to discover and develop new compounds as potent drugs to manage existing and emerging diseases [39]. Acute toxicity was carried out orally and intraperitoneally; novelty induced behaviours (locomotor, rearing, grooming and head dripping activity) was evaluated in the open field and on the hole board respectively; sedative activity was tested on ketamine-induced hypnosis and anticonvulsant effect assessed on the pentylenetetrazole-induced convulsion model. The outcome of this present study indicates that the extract of this plant demonstrated significant effect on the CNS.

The acute toxicity (LD_{50}) profile for both oral (*p.o.*) and intraperitoneal (*i.p.*) routes was carried out in this study to comparatively evaluate the toxicological profile of the CME in the two routes according to Lorke [30]. Consequently, the LD_{50} was determined to be 2154 mg/kg, *i.p.*, and ≥ 5000 mg/kg, *p.o.* in rat (Table 1). Accordingly, the CME was classified to be non-toxic and slightly toxic through the oral and intraperitoneal routes respectively [30,40]. The value of the oral route was higher than that of the intraperitoneal route because of some pharmacokinetic parameters such as rate of absorption, distribution, metabolism and excretion which differ in the two routes. Metabolism of drug in the gastrointestinal tract (GIT) by the acidic and enzymatic content and slower absorption rate can lead to lower or reduced bioavailability [41,42] and consequently, decreased the amount of the drug that is finally absorbed into the blood stream leading to low LD_{50} value. The intraperitoneal route was chosen in this study in order to bypass the possibility of metabolism if the extract was administered through the oral route. Also, it has been suggested that for studies involving the central nervous system (CNS), the intraperitoneal route is to be preferred because the effect are easily detected and the test readily reproducible [43]. The LD_{50} value obtained for the intraperitoneal route indicate that the extract is slightly toxic through this route and according to Rodricks [40] the extract could be regarded as relatively safe at the doses used in this study. Thus the doses of the extract used in this study (250, 500 and 1000 mg/kg, *i.p.*) were far below the LD_{50} value for the intraperitoneal route (2154 mg/kg, *i.p.*).

The CME at all the doses used significantly ($P < 0.01$) decreased the number of rearings dose-dependently when compared to the vehicle (Fig. 2), signifying CNS depressant activity [44]. Diazepam (1 mg/kg) did not cause significant decrease in rearings compared to vehicle in this study. The CME and diazepam

Table 2. Summary of the effect of ethanolic dried leaf extract of *C. millenii* on PTZ-induced convulsion in rats

Treatment (mg/kg, <i>i.p.</i> , n=5)	Latency to convulsion (s) Mean $\pm$ SEM	Time of death (min) Mean $\pm$ SEM	Ratio of rats that survived beyond 30 min	% protection
Vehicle	36.60 $\pm$ 1.36	14.80 $\pm$ 1.77	0/5	0
CME 250	41.60 $\pm$ 4.01	30.20 $\pm$ 5.41	2/5	40*
CME 500	87.80 $\pm$ 23.14*	33.00 $\pm$ 11.05	1/5	20*
CME 1000	76.20 $\pm$ 4.45	17.60 $\pm$ 8.03	1/5	20*
DZM 1	58.6 $\pm$ 4.24	41.00 $\pm$ 6.47	4/5	80*

Vehicle, CME and DZM represent normal saline (10 ml/kg), *Cola millenii* ethanolic leaf extract and diazepam

caused significant ($P < 0.01$) decrease in the number of grooming compared to the vehicle (Fig. 3), indicating low arousal which may be related to absence of stimulation or CNS depression. Grooming has been used as biomarkers of stress in rodents and decreased grooming probably indicate de-arousal in this study [45]. It has been suggested that drugs that have depressant effect are known to suppress grooming in experimental animals, while those that have stimulatory effects increase grooming behaviour [46].

It is generally believed that locomotor activity results from brain activation, which is manifested as an excitation of central neurons involving different neurochemical mechanism and an increase in central metabolism. Reports have showed that typical CNS depressant drugs have inhibitory effects on exploratory behaviours of animals [35]. The CME caused significant ($P < 0.05$) reduction in the locomotion of the rats when compared with the vehicle. The inhibition of spontaneous locomotion in rats treated with the CME in this study further showed that the extract has significant inhibitory effect on the CNS.

The effect of the CME on the head-dipping behaviour of the rats was depression when compared to the vehicle (Fig. 5). The CME at all the doses used caused significant ($P < 0.01$) reduction in the head dipping behaviour of the rats signifying both sedative and CNS depression [46]. The general effect of the CME on the behavioural patterns of the rats is suggestive of CNS depression. It is possible that the inhibitory effect of the extract on novelty-induced behaviour could be mediated through the GABAergic pathway [47]. The novelty induced behaviours are regulated by multiple neurotransmitter systems. They include gamma-amino butyric acid (GABA), acetylcholine, dopamine, opiod, serotonin, benzodiazepines and many others yet to be ascertained. Increased rearing behaviours are mainly modulated by enhanced dopamine neurotransmission [48] or amino butyric acid-A (GABA-A) receptor potentiation [49]. It has been reported however that most of the behavioural effect of dopamine transmission in CNS are somehow modulated by serotonin [50].

Ketamine is an antagonist of N-Methyl-D-Aspartate (NMDA)-type of excitatory receptor system, which have very significant role in seizures and been reported to cause sedation with additional study, the ethanolic extract of

C. millenii potentiated ketamine-induced sedation, since a significant increase in sleeping time was observed. The ethanolic leaf extract of this plant at 500 and 1000 mg/kg caused significant ($P < 0.05$) decrease in sleep latency (SL) induced by ketamine (100 mg/kg, *i.p.*) when compared to vehicle group (Fig. 2). Also, the extract at 500 and 1000 mg/kg caused significant ($P < 0.01$) prolongation in the total sleeping time (TST) induced by the ketamine compared to the vehicle (Fig. 3). Diazepam (standard sedative drug) at 1 mg/kg, *i.p.*, caused in-significant decrease in SL but caused significant ($P < 0.05$) prolongation in the TST induced by ketamine in this study. Decrease in SL and prolongations of TST on ketamine-induced hypnosis indicate sedative activity [34,35], hence, these sedative results confirmed that ethanolic dried leaf extract of *C. millenii* possess significant sedative activity in rats. Hence, the potentiation of the ketamine-induced sleeping time by the ethanolic extract may be due to modulation of GABA or NMDA system in the mediation of this observed sedative effect.

The results obtained for the anticonvulsant activity of this extract indicate weak activity against the PTZ-induced convulsion model. All the animals in all the groups showed signs and manifestations of tonic seizures in this study. The extract at all the doses used delayed the onset (latency) to convulsion induced by the PTZ but only significantly ($P < 0.05$) at 500 mg/kg (Table 2). There was no statistical difference in the time of death for all the treatment groups even though 80% of the rats in the positive group (diazepam-treated group) survived beyond the 30 minutes cut-off time (Table 2). The extract at all the doses used (250-1000 mg/kg) offered 20-40% protections against the PTZ-induced convulsion while diazepam offered 80% protection in this model. In a previous study [51] diazepam (1 mg/kg, *i.p.*) was reported to protect mice against PTZ-induced convulsion with 0% mortality and 100% protection. The results obtained in this study indicate that the extract of this plant exhibited slight anticonvulsant activity in this model. Further study is suggested to evaluate this extract for anticonvulsant activities using other models in mice.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, phenol, glycosides and steroids in the crude ethanolic extract of the dried leaf of this plant some of which were earlier reported [18]. However, anthraquinones, phylobatannins,

proteins and amino acids were not detected in this species. Furthermore, the ethanolic extract was subjected to LC-MS analysis in order to obtain its fingerprint. The spectra on the chromatogram showed the presence of 29 compounds (Fig. 2). These compounds could not be distinctively identified due to the fact that it is an ethanolic crude extract, which comprise various classes of polar compounds including alkaloids, saponins, flavonoids etc. The fingerprint presented here serves as the preliminary data which necessitates further fractionation followed by column chromatography to permit isolation and correct identification or characterization of the individual compounds. Several reports suggest that some of these constituents especially alkaloids, saponins and flavonoids have potent antiepileptic effect in various models [52]. In addition, Tsang et al. [53] reported that saponins have also been able to modulate the neurotransmitter levels in the brain and to possess anti-convulsant activity. Therefore, one or more of these constituents might be responsible for the neuropharmacological activities observed in this study. Consequently, it is imperative that additional studies be carried out on fractions and isolates of this plant in order to determine which of these compounds or metabolites are responsible for these various activities.

In summary, the ethanolic leaf extract of *C. millenii* evaluated in this study demonstrated significant CNS depression and sedative activity but exhibited mild anticonvulsant potentials which definitely necessitate further probing. The findings in this study are the first report on the central activity of this plant and we therefore recommend that future research effort be focused on fractionation, isolation and characterization of the extract to identify component(s) and mechanism(s) of action as vital steps towards finding a lead in the discovery of novel and potent drugs from natural products.

5. CONCLUSION

This study demonstrated that *C. millenii* ethanolic leaf extract was slightly toxic intraperitoneally but non-toxic orally; depress the CNS and possesses significant sedative activity but exhibited mild anticonvulsant potentials in rats. Finally, it is concluded that the results from this study provide supporting evidence on the potential use of the plant in ethnomedicine in addition to providing preliminary data on central activity of the plant. Thus, these findings provide new data

on the activity of the plant which may serve as lead in drug discovery from natural sources.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the Obafemi Awolowo University, Ile-Ife, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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