Original Research Article

Neuromodulatory effects of hydro-ethanol extract of *Parinari curatellifolia* leaf in mice

Nnamdi Henry Okereke[1], Rita Ijeoma Udegbunam[1], Nkiruka Obianuju Okoroafor[2]*, Kelechi Okoro[1], Augustine C Onuba[1], Sunday Ositadinma Udegbunam[1]

[1]Department of Veterinary Surgery, [2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka

*For correspondence: **Email:** obianuju.okoroafor@unn.edu.ng; **Tel:** +234-8034246087*

Abstract

Purpose: *To investigate the neuromodulatory activities of the hydro-ethanolic extract of Parinari curatellifolia (HEPC) leaves of the family Chrysobalanaceae in mice.*
Method: *The open field, hole cross, hole board, climbing, elevated plus-maze, tail suspension and ketamine-induced sleeping time tests were conducted on mice groups (n = 3) to which HEPC 200, 400, and 800 mg/kg (per os) or standard central nervous system drugs (20 mg/kg imipramine and 20 mg/kg fluoxetine) intramuscularly were administered 30 min earlier.*
Results: *Imipramine, HEPC, and fluoxetine significantly decreased (p < 0.05) the frequency of hole crossing, head dipping and locomotory activities in the animals. The immobility period during tail suspension was significantly increased (p < 0.05) in the group treated with 400 and 800 mg/kg HEPC but was significantly decreased (p < 0.05) in the imipramine group. The duration of chain climbing significantly increased (p < 0.05) after treatment with 400 and 800 mg/kg HEPC in comparison to the climbing time observed in the imipramine group. The frequency of open arm entry was significantly higher (p < 0.05) in the imipramine group compared to open arm entries in the 400 and 800 mg/kg HEPC-treated groups. Ketamine-induced sleep time was not prolonged with 200, 400 and 800 mg/kg HEPC, when compared with the standard drugs used.*
Conclusion: *P. curatellifolia extract causes sedation and muscle relaxation but does not abolish anxiety behaviour in mice. The findings, therefore, provide some scientific evidence validating the use of Parinari curatellifolia extract in the folkloric management of insomnia.*

Keywords: *Parinari curatellifolia, Imipramine, Fluoxetine, Anxiolytics*

INTRODUCTION

Some drugs that affect the central nervous system include sedatives, hypnotics, anesthetics and muscle relaxants. Anxiolytics diminish tension and anxiety [1] without altering the degree of consciousness, while hypnotics cause drowsiness or sleep [1]. According to the American Society of Anesthesiologists [1,2], sedation can be minimal (anxiolysis), moderate

---

(conscious sedation), or deep (hypnosis). Therefore, the distinction between sedation, hypnosis and anxiolysis is not consistently clear-cut and certain drugs could induce the features of these three states [1,4].

Drugs that alter the functioning of the central nervous system, notably anxiolytics, are mainly used in humans to treat insomnia and anxiety disorders [1]. Drugs used as sedative-hypnotics include benzodiazepines, barbiturates and Z-drugs namely zalephan, ezsopiclone, and ramelteon [2]. Despite their acclaimed benefits, sedative-hypnotics such as barbiturates and benzodiazepine induce several side effects overtime on their users. For instance, barbiturates have a narrow safety margin [2,4] and may cause both psychological and physiological dependence [2]. Furthermore, prolonged benzodiazepine use leads to tolerance and physical dependence [2]. In addition, the use of benzodiazepines is contra-indicated in some disease states such as myasthenia graves, sleep apnea, and bronchitis [1,2]. Based on these limitations, the use of medicinal plants and herbs with CNS depressant effect with probable reduced side effects may be preferred.

In traditional medicine practice, medicinal plants serve as a reliable and acceptable alternative to drugs in the management of anxiety and insomnia in humans [3]. Parinari *curatellifolia* (Family: Chrysobalanaceae) has been one of such plants used as a drug alternative by traditional healers in the Nsukka area, in the south eastern part of Nigeria. The leaves of this plant are soaked in 70 - 80 percent ethanol and used in the treatment of insomnia. Earlier scientific reports demonstrated that the plant possesses anticonvulsive characteristics [3]. Other reported pharmacological activities of this plant include antioxidant, anti-inflammatory and hypoglycaemic effects [3,4].

The main objective of this work was to investigate the activities of the hydro-ethanolic extract of Parinari curatellifolia (HEPC) leaves on the cerebrospinal axis and thus provide scientific evidence to authenticate the folkloric claim of its effects in the treatment of insomnia.

EXPERIMENTAL

Plant sample

Fresh whole plant materials of Parinari *curatelli-folia* were sourced from Orba, which is a town within Udenu Local Government Area (LGA) of Enugu State, Nigeria, in August 2020. The geographical coordinates of the location are 6.8645 °N and 7.4083 °E. Identification of the plant sample was carried out at International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Nigeria by Mr A Ozioko, a plant taxonomist. A voucher specimen of the sample was entered into the inventory, assigned a voucher number SDMN/16/14 and consigned to the center's herbarium.

Extract preparation

One kilogram of P. *curatellifolia* leaves was sliced, and air-dried for 2 weeks at ambient temperature and subsequently pulverized. Maceration of pulverized roots was done using 80 % ethanol accompanied by intermittent vigorous shaking of the mix at 2 h intervals. After 48 h of maceration, the mix was filtered, and a rotary evaporator, which was set at 40 °C, was used for concentration of the filtrate. The dried HEPC extract was weighed and the percentage yield was calculated. Thereafter, the HEPC extract was stored in a refrigerator maintained at 4 °C pending its usage.

Animal handling

This study was done following ethical approval. The procedures for animal handling followed the standards in the National Institute of Health (NIH) revised guidelines for laboratory animals' use and care [5]. Six to seven weeks old Swiss albino male mice of weight 35 – 40 g were used for this study. The animals were housed in poly-carbonated cages at ambient temperature of 25 °C, relative humidity of 55 – 56 % and a 12 h daylight/darkness cycle. Free access to proprietary feed (Chukun® Kaduna) and water was provided ad libitum. The animals were gavaged orally with HEPC or standard drugs as follows: group 1 (200 mg plants extract), group 2 (400 mg plant extract), group 3 (800 mg plant extract), group 4 (imipramine, 20 mg/kg) and group 5 (flouxetine, 20 mg/kg).

Acute toxicity test

This was done as initially reported by Akhila et al [6]. One control group that was administered with normal saline per os and five test groups, each composed of three randomly assigned mice were used for the study. Graded doses (200 – 3200 mg/kg) of P. *curatellifolia* extract was administered per os to the animals, which were also allowed unlimited access to feed and water. Mortality and signs of toxicity were subsequently monitored for a period of 72 h.

Neuromodulatory investigations

Hole cross test

This was done as initially reported by Takagi et al [7]. Briefly, a wooden box of 30 x 20 x 14 cm in dimension with a middle partition was used. A hole of 1.5 cm radius was created on the middle partition. After drug administration, the number of times each mouse could move across the hole to another chamber was monitored for three min at 30 min intervals for up to 2 h.

Open field test

The apparatus is made up of a wooden field measuring 0.5 square meters with staggered painted black and white squares. The field was kept in a poorly lit room. Procedures of the open field test earlier reported by Gupta et al [8] were followed. Following treatment of the animals, they were placed at the center of the open field apparatus. The number of squares voluntarily visited by the mice was monitored for 3 min at 30 min intervals for 2 h post-treatment.

Hole-board test

This was done as initially reported by Ozturk et al [9]. Briefly, a flat board of 60 × 30 cm in dimension, having 16 evenly distributed holes was used. After treatment, the frequency of head dips into the hole, made by each mouse as they walked on the platform was monitored for 3 min at 30 min interval for 2 h.

Elevated plus-maze test

The presence of anxiolytic attributes of the extract was tested using the plus-maze test. The device is made up of a pair of open and closed arms with each having a dimension of 16 x 5 x 12 cm and an elevated open roof of 50 cm height. Following administration of the test agents and standard drugs, mouse from each group was placed at the center of the apparatus with its face overlooking the open or closed arms. The behaviors of each mouse were monitored for 5 min. This includes the number of entries made into the open and closed arms, and the length of time spent within each arm [10]. Images were captured with an android phone and subsequently transferred to a laptop for clear interpretation.

Climbing test

Climbing test was done as initially reported by

Cintia et al [11]. Participating mice were pre-trained to climb a 6 cm long chain hanging from a retort stand placed 100 cm above ground. Any mouse that could climb the chain in 10 sec was selected for the test. This test started 30 min following treatments with standard drugs and plant extract.

Tail suspension test

This was done as initially reported by Steru et al [12]. The mice were randomly classified into 3 groups. After acclimatization and depending on the assigned group, mouse received 400 and 800 mg/kg of plant extract orally or imipramine (20 mg/kg orally). After one-hour post-treatments, each mouse was suspended on its tails by gripping it at about 1 cm away from the tail tip by means of an adhesive tape attached to a horizontal bar kept 50 cm above the tabletop. The behaviours of the mice were noted for a period of 5 min and the times of quietness and agitation were recorded.

Ketamine-induced sleep test

The ketamine induced sleep test was done as initially reported by Mimura et al [13]. The mice were randomly assigned to seven groups (n = 3) which received treatments as follows: group 1 (ketamine alone 200 mg/kg I.P), group 2 (200 mg of plant extract orally and ketamine 200 mg/kg), group 3 (400 mg of plant extract orally and ketamine 200 mg/kg), group 4 (800 mg of plant extract orally and ketamine 200 mg/kg), group 5 (diazepam 10 mg/kg orally and ketamine 200 mg/kg), group 6 (imipramine 20 mg/kg orally and ketamine 200 mg/kg) and group 7 (flouxetine, 20 mg/kg orally and ketamine 200 mg/kg). Except in group 1, the ketamine was administered with test drugs or extract and it was done 30 min later and via the intraperitoneal route. The disappearance of righting reflex and its restoration were monitored in the animals and the time between the two events was recorded as the sleeping time.

Thiopentone induced sleeping time

Thiopentone induced sleeping time test was done as earlier reported [14]. The mice which were randomly assigned to seven groups (n = 3) received treatments as follows: group 1 (200 mg of plant extract orally and thiopentone 50 mg/kg), group 2 (400 mg of plant extract orally and thiopentone 50 mg/kg), group 3 (800 mg of plant extract orally and thiopentone 50 mg/kg), group 4 (thiopentone alone 50 mg/kg), group 5 (Diazepam 10 mg/kg orally and thiopentone 50 mg/kg), group 6 (imipramine 20 mg/kg orally and

thiopentone 50 mg/kg) and group 7 (flouxetine 20 mg/kg orally and thiopentone 50 mg/kg. When combined, thiopentone was administered 30 min after administration of extracts or standard drugs via the intraperitoneal route. The time spent before loss of righting reflex (latent period), the sleeping time and the recovery time was noted.

Data analysis

Data generated were analyzed with one-way analysis of variance (ANOVA) statistic using the computer software, SPSS v. 20. Variations in mean across the groups were separated using the Duncan multiple range test (DNMRT). Probability (p) values below 0.05 were deemed statistically significant.

RESULTS

Acute toxicity

Within the 72 h of observation, no apparent sign of acute toxicity, change in behavior or death was observed in the animals treated with HEPC.

Hole cross results

At 0.5, 1, 1.5 and 2 h observation points, the extract (200, 400 and 800 mg/kg) significantly decreased ($p < 0.05$) the frequency of hole crossing within the group with non-significant decrease ($p > 0.05$) between groups (Table 1). The frequency of hole crossings observed at 1, 1.5 and 2 h in the HEPC-treated group was similar to the number of crossings in the imipramine and flouxetine groups. Within groups findings of the extract and the standard drugs showed that by 0.5-hours post-administration of the extract, frequency of hole crossings was significantly decreased ($p < 0.05$) till 2-hours.

Hole board

The number of head dip/poking into holes was significantly decreased ($p < 0.05$) after administration of 200, 400 and 800 mg/kg of HEPC (Table 2). A sharp decline in the incidence of head dips into holes occurred at 1.5 and 2 h after HEPC administration. The number of head-dipping counted in the HEPC treated group was similar to the number of dips in mice exposed to standard CNS depressant (imipramine and flouxetine). Group 2 (400 mg of HEPC) recorded significantly higher ($p < 0.05$) number of head dip than group 4 but non-significant change ($p > 0.05$) from other groups. However, non-significant changes were observed at 1, 1.5 and 2 h monitoring. Within groups result revealed significant drop ($p < 0.05$) from the baseline finding in all the groups from 0.5 to 2 h post administration of the extracts and the standard drugs.

Table 1: Number of hole crossings by mice treated with graded doses of HEPC

Group	Treatment	Number of crossings				
		Baseline	0.5 hour	1 hour	1.5 hour	2 hours
1	200 mg HEPC	8.33 ± 0.33^a	$5.00\pm0.59^a*$	$1.33\pm0.88^a*$	$0.67\pm0.67^a*$	$1.00\pm0.58^a*$
2	400 mg HEPC	7.00 ± 3.21^a	$4.00\pm2.08^a*$	$2.67\pm1.76^a*$	$1.33\pm1.33^a*$	$1.00\pm0.58^a*$
3	800 mg HEPC	11.00 ± 1.00^a	$4.33\pm1.20^a*$	$1.00\pm1.00^a*$	$1.33\pm0.88^a*$	$0.33\pm0.33^a*$
4	Imipramine (20 mg/kg)	8.00 ± 3.93^a	$5.67\pm2.33^a*$	$1.33\pm1.00^a*$	$4.67\pm2.91^a*$	$4.33\pm2.96^a*$
5	Flouxetine (20 mg/kg)	8.67 ± 3.93^a	$4.67\pm2.60^a*$	$1.33\pm0.88^a*$	$2.00\pm1.53^a*$	$0.33\pm0.33^a*$

Same superscripts [a] in a column indicate no significant differences between means of the groups. *Significantly different ($p < 0.05$) from values recorded at baseline to different time intervals within the group (0.5 - 2 h)

Table 2: Number of head-dips in mice treated with graded doses of HEPC

Group	Treatment	Number of dips				
		Baseline	0.5 hour	1 hour	1.5 hours	2 hours
1	200 mg HEPC	30.33 ± 2.73^a	$10.33\pm1.86^{ab}*$	$6.00\pm2.08^a*$	$2.33\pm1.45^a*$	$3.00\pm1.73^a*$
2	400 mg HEPC	29.33 ± 4.33^a	$14.33\pm1.33^b*$	$7.33\pm3.38^a*$	$5.00\pm2.65^a*$	$4.67\pm3.28^a*$
3	800 mg HEPC	40.67 ± 088^a	$9.00\pm2.00^{ab}*$	$13.67\pm4.26^a*$	$6.33\pm1.76^a*$	$5.33\pm0.67^a*$
4	Imipramine (20 mg/kg)	31.67 ± 2.33^a	$6.00\pm2.52^a*$	$3.00\pm1.15^a*$	$5.67\pm0.88^a*$	$4.67\pm1.33^a*$
5	Flouxetine (20 mg/kg)	26.67 ± 7.69^a	$12.00\pm3.51^{ab}*$	$8.33\pm3.84^a*$	$5.67\pm1.86^a*$	$3.67\pm0.88^a*$

Different superscripts [a,b,c] in a column indicate significant differences between means of the groups. * indicates significant different ($p < 0.05$) from values recorded at baseline versus different time intervals within the group (0.5 - 2 h)

Table 3: Number of movements in mice treated with graded doses of HEPC

Group	Treatment	Number of movements				
		Baseline	0.5 h	1 h	1.5 h	2 h
1	200mg HEPC	55.67±15.90[a]	46.67±20.92[a]	35.00±18.15[ab]*	40.00±28.16[a]	53.00±27.07[a]
2	400mg HEPC	57.67±17.94[a]	42.67±18.56[a]	26.33±12.47[a]*	33.33±21.70[a]*	20.33±12.03[a]*
3	800mg HEPC	96.67±3.93[a]	61.67±30.18[ab]*	77.00±19.04[bc]	50.67±14.81[a]	42.33±15.59[a]*
4	Imipramine (20mg/kg)	77.00±15.37[a]	33.00±2.31[a]*	14.00±9.02[a]*	30.67±16.76[a]*	45.00±15.57[a]*
5	Flouxetine (20mg/kg)	88.00±22.55[a]	112.00±9.81[b]*	99.00±11.02[c]*	55.67±16.33[a]*	60.67±13.32[a]*

Different superscripts ([a,b,c]) in a column indicate significant differences between means of the groups. *Significantly different ($p < 0.05$) from values recorded at baseline versus different time intervals within the group (0.5 - 2 h)

Open field results

Open field test showed that group 5's number of movement was significantly higher ($p < 0.05$) than groups 1, 2, and 4 but the number of movements was not significantly different ($p > 0.05$) from that of group 3 at 0.5 and 1 h post administration of the extracts and the standard drugs as shown in Table 3. However, by 1.5 and 2 h, non-significant changes ($p > 0.05$) were observed between groups. In group I, significant decrease ($p < 0.05$) in the number of movements was recorded by 1 h post administration of the extract while in group 2, significant decrease ($p < 0.05$) from the baseline was recorded by 1, 1.5 and 2 h post administration

In Group 5, the number of movements significantly increased ($p < 0.05$) from the baseline reading by 0.5 and 1 h post administration with corresponding significant decrease ($p < 0.05$) from the baseline value by 1.5 and 2 h post administration.

Tail suspension

The period of immobility on tail suspension was significantly decreased ($p < 0.05$) in mice treated with 400 and 800 mg/kg HEPC (Table 4). In the imipramine treated group, the period of immobility was significantly increased ($p < 0.05$).

By 0.5 h post administration of the extracts and the standard drug, significantly longer ($p < 0.05$) immobility was recorded in the two HEPC treated groups in comparison to the value recorded in the imipramine group.

Chain climbing

The duration of chain climbing (sec) increased following treatment with 400 and 800 mg/kg HEPC by 0.5 h post administration of the extract and imipramine (Table 5). The duration of chain climbing in both extract-treated groups was significantly longer ($p < 0.05$) when compared to the climbing time observed in the Imipramine group.

Elevated plus-maze

The number of entries into the open arm was significantly more ($p < 0.05$) in the imipramine group when compared to the 400 and 800 mg/kg HEPC treated groups (Table 6). In the closed arm, no significant change ($p > 0.05$) was observed at baseline and 0.5 h post administration.

Ketamine-induced sleep time

Ketamine-induced sleep time was prolonged with 200, 400 and 800 mg/kg HEPC though not

Table 4: Duration of immobility in mice treated with graded doses of HEPC

Group	Treatment	Immobility time	
		Baseline (0 h)	0.5 h
1	400mg HEPC	92.00±28.36[a]	185.67±24.92[a]
2	800mg HEPC	64.00±9.2.4[b]	104.00±43.31[b]
3	Imipramine (20mg/kg)	125.67±32.57[c]	93.33±4.48[c]

Different superscripts ([a,b,c]) in a column indicate significant differences between means

Table 5: Duration of chain climbing in mice treated with graded doses of HEPC

Group	Treatment	Duration of chain climbing (sec)	
		Baseline	0.5 hour
1	400 mg HEPC	5.67±0.33[a]	25.33±17.34[a]
2	800 mg HEPC	9.0.±2.00[a]	57.000±29.55[a]
3	Imipramine (20mg/kg)	10.33±0.88[a]	7.33±2.49[c]

Different superscripts ([a,b,c]) in a column indicate significant differences between means

Table 6: Number of open and closed arm entries in mice treated with graded doses of HEPC

Group	Treatment	Open arm (s)		Closed arm (s)	
		Number of entries		Number of entries	
		Baseline	0.5 hour	Baseline	0.5 hour
1	400mg HEPC	00.00±00.00ᵃ	00.00±00.00ᵃ	2.3±1.33ᵃ	268.67±31.33ᵃ
2	800mg HEPC	00.00±00.00ᵃ	00.00±00.00ᵃ	0.33±0.83ᵃ	282.33±11.41ᵃ
3	Imipramine (20mg/kg)	2.67±0.67ᵇ	43.00±19.86ᵇ	4.33±0.88ᵃ	195.33±32.46ᵃ

Different superscripts (ᵃ,ᵇ) in a column indicate significant differences between mean

Table 7: Duration of ketamine-induced sleep in mice

Group	Treatment	Sleep duration (min)
1	Ketamine alone (200mg/kg)	49.33±7.31ᵃᵇ
2	200mg HEPC + ketamine (200mg/kg)	51.33±8.84ᵃ
3	400mg HEPC + ketamine (200mg/kg)	69.67±6.01ᵃᵇ
4	800mg HEPC + ketamine(200mg/kg)	68.33±7.62ᵃᵇ
5	Diazepam (10mg/kg)	174.33±16.67ᵈ
6	Imipramine (20mg/kg +ketamine 200mg/kg)	118.67±24.10ᶜ
7	Flouxetine (20mg/kg + ketamine 200mg/kg)	91.00±13.80ᶜ

Different superscripts (ᵃ,ᵇ,ᶜ,ᵈ) in a column indicate significant difference between means

Table 8: Duration of thiopentone-induced sleep time in mice

Group	Treatment	Duration of sleep (min)
1	200mg HEPC + thiopentone (50mg/kg)	179.00±145.25ᵃᵇ
2	400mg HEPC+thiopentone (50mg/kg)	42.33±7.80ᵃ
3	800mg HEPC+thiopentone (50mg/kg)	17.33±4.63ᵃ
4	Thiopentone (50mg/kg)	82.00±19.08ᵃ
5	Diazepam 10mg/kg+thiopentone (50mg/kg)	385.33±130.93ᵃᵇ
6	Imipramine 20mg/kg+thiopentone (50mg/kg)	93.33±11.33ᵃ
7	Flouxetine 20mg/kg+thiopentone (50mg/kg)	154.67±68.10ᵃᵇ

Different superscript (ᵃ,ᵇ) in a column indicate significant differences between means

significantly. However, diazepam (10 mg/kg), imipramine and flouxetine significantly prolonged ($p < 0.05$) the ketamine-induced sleeping time in the study animals as shown in Table 7.

Thiopentone-induced sleep time

The administration of 200 mg HEPC before thiopentone significantly prolonged sleeping time in mice ($p < 0.05$). However, the sleeping time was significantly ($p < 0.05$) shortened in mice pre-treated with 400 and 800 mg/kg extract before thiopentone administration (Table 8). Diazepam (10 mg/kg) and flouxetine significantly ($p < 0.05$) prolonged thiopentone-induced sleep time.

DISCUSSION

In this study, HEPC significantly decreased hole crossing frequency, the number of locomotion and head dips in mice. The ability of Citrus maxima, Cola *millenii*, Commelina *diffusa* and Thuja *occidentalis* extracts to individually depress the cerebrospinal axis (CSA) have been previously studied using the aforementioned models [15]. According to their various reports, a decrease in the number of locomotion, hole crossing and head dipping suggests a CSA

depressant or sedative property of the extract studied. Therefore, the finding of this study suggests that HEPC possesses CSA depressant effect.

The uneasiness produced by tail suspension is a stress factor in the test. Tail suspension is considered a derivative of the behavioral despair test [16]. The aggregate duration of the test could be partitioned into period of agitation followed by period of immobility [15,16].

It is hypothesized that normal animals subjected to an insoluble repugnant situation swaps between two types of behaviours namely agitation and fixity. In tests used to assess anti-depressant activities of drugs such as forced swim and tail suspension experiments, the diminishing of immobility duration indicates anti-depressant activity while prolongation of immobility time reflects CNS depression-like effect [15,17]. Furthermore, diazepam had been shown to increase the duration of immobility while anti-depressant such as imipramine decreases the duration of immobility [10,18]. The observation of increased immobility time in HEPC-treated mice further suggests that this plant possesses a CNS depressant effect.

Elevated plus-maze technique is an extensively utilized behavioural assay for rodents to validate the anxiolytic effect of pharmacological agents [10]. In this test, animals that have some degrees of anxiety show preference for the closed arm of the apparatus [10]. The classic parameters for evaluation of anxiety are the aggregate time spent on the open arms and the frequency of entry into open arms which reflect avoidance of open arms [10,18]. Elevated latencies, fewer numbers of visits, and the briefness of time spent in the undesirable open arms (that is evasion of the open arms) indicate a significant level of anxiety in rodents [10,18]. In this study, mice in HEPC groups showed more preference for the closed arms of the plus-maze. This finding suggests that the administration of HEPC did not abolish anxiety in mice and therefore indicates that HEPC has no anxiolytic effect.

The duration of chain climbing was prolonged in mice after administration of HEPC. In an earlier study, increased time of chain climbing by Citrus maxima extract was attributed to the muscle relaxant property of the extracts [13]. Therefore, it can be concluded that HEPC administration produced muscle relaxation in mice.

The prolongation of ketamine-induced sleep time in mice by HEPC showed that it potentiated the effect of ketamine. According to the report of Mimura et al [13], prolongation of ketamine-induced hypnosis by the plant extract studied, suggests that the extract has sedative activity. Thus, it can be concluded that potentiation of ketamine effect by HEPC may be due to its modulation of the N-methyl-D-Aspartate system. In this study, sleep was also induced by thiopentone a well-known ultra-short acting barbiturate. The shortening of thiopentone sleep time observed in HEPC pre-treated mice suggests that the extract may have bound to GABA receptor, therefore, preventing the activity of thiopentone on these receptors.

CONCLUSION

The use of P. *curatellifolia* produces sedation and muscle relaxation, with no ability to abolish anxiety in mice. The results obtained, therefore, provide scientific evidence validating the use of P. *curatellifolia* extract in the folkloric management of insomnia.

DECLARATIONS

Ethical approval

Ethical approval for the use of laboratory animals in this research was obtained from the Experimentation Ethics Committee on Animal Use, Faculty of Veterinary Medicine, University of Nigeria (approval no. UNAEC/19/8992).

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. Stahl SM. Don't ask, don't tell, but benzodiazepines are still the leading treatments for anxiety disorder. J Clin Psychiatry 2002; 63(9): 756–757.
2. Schlatter J, Sitbon N, Saulnier JL. Drugs and drug abusers. Presse Med 2001; 30(6): 282–287.
3. Magassouba FB, Diallo A, Kouyaté M, Mara F, Mara O, Bangoura O, Camara A, Traoré S, Diallo AK, Zaoro M, et al. Corrigendum to "Ethnobotanical survey and antibacterial activity of some plants used in Guinean

traditional medicine". J Ethnopharmocol 2010; 114: 44-53.

4. *Diehl MS, Atindehou KK, T'er'e H, Betschart B. Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria. J Ethnopharmacol 2004; 95: 277-284.*

5. *National Institute of Health. Guide for the Care and Use of Laboratory Animal (Revised). 8th Edition. National Academies Press, 500 Fifth Street, NW, Lockbox 285, Washington, DC, United States of America 1985; pp: 83-123.*

6. *Arnold HJ, Gulumian MJ. Pharmacopoeia of traditional medicine in Venda J Ethnopharmacol 1984; 12: 35-74.*

7. *Akhila JS, Deepa S, Alwar MC. Acute toxicity studies and determination of median lethal dose. Current Sci 2007; 93: 917-920.*

8. *Takagi K, Watanabe M, Saito H. Studies of the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethanol and its acyl esters on the central nervous system. Japan J Pharmacol 1971; 21(6): 797–810.*

9. *Gupta BD, Dandiya PC, Gupta ML. A psycho-pharmacological analysis of behaviour in rats. Japan J Pharmacol 1971; 21(3): 293–298.*

10. *Ozturk Y, Aydine S, Baser KHC, Berberoglu H. Effects of Hypericum perforatum L. and Hypericum calycinum L. extracts on the central nervous system in mice, Phytomed 1996; 3(2): 139–146.*

11. *Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacol 1987; 92(2): 180-185.*

12. *Cíntia V, Thereza CMD, Antonio PC, Cilene L. Frequency of climbing behavior as a predictor of altered motor activity in rat forced swimming test. Neurosci Lett 2008; 445: 170–173*

13. *Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol 1985; 85(3): 367-370.*

14. *Mimura M, Namiki A, Kishi R, Ikeda T, Miyake H. Antagonistic effect of physostigmine on ketamine-induced anesthesia. Psychopharmacol 1990; 102: 399-403.*

15. *Sultana T, Mannan A, Ahmed T. Evaluation of central nervous system (CNS) depressant activity of methanolic extract of Commelina difusa Burm in mice. Clin Phytosci 2018; 4(5): 2-7 https://doi.org/10.1186/s40816-018-0063-1.*

16. *Rodgers RJ, Cole JC. Anxiety enhancement in the murine elevated pluz-maze by immediate prior exposure to social stressors. Physiol Behaviour 1993; 53(2): 383-388*

17. *Steru L, Chermat B, Thierry B, Simon P. Tail suspension test, A new method for screening antidepressant in mice and psychopharmacology. Psychopharmacol 1985; 85, 367-370*

18. *Rodger RJ, Johnson NJ. Factors analysis of spatiotemporal and ethological measure in murine elevated plus-maze test of anxiety Pharmacol Biochem Behav 1995; 52(2): 297-303.*