Original Research Article

Role of Gabanergic and, or serotonergic in the anti-sezure activity of methanol extract of *Cranium jagus* (J. Thomps) in experimenral mice.

ABSTRACT

Objective: Cranium jagus is used in traditional African medicine in the treatment of epilepsy, pain and inflammations.

Methods: Anticonvulsant activity was investigated using picrotoxin (PCT) and strychnine (STR) tests in experimental paradigm.

Results: *Cranium jagus* leaf methanol extract (200, 400, and 800 $mg\,kg^{-1}$) potentiated hexobarbitone-induced sleeping time and significantly ($p<0.05$) shortened the duration of convulsions in PCT-induced seizures. Delay in the onset of convulsions in PCT-induced seizure were very significant ($p<0.05$). Reduction in the frequency of seizures was also significant ($p<0.05$) in the test. Diazepam (1 $mg\,kg^{-1}$) was used as reference anticonvulsant drugs in the experimental models. While, CJB failed to protect the animals against picrotoxin-induced convulsion. Neither the extract of CJL nor CJB confer significant effect on STR-induced convulsion. Flumazenil (GABA receptor antagonist) and cyproheptadine (5-HT2 receptor antagonist) blocked the effect of CJL extract in the PCT tests significantly suggesting that *Cranium jagus* may be acting by enhancing the effects of the GABAergic and serotonergic systems.

Conclusion: The data obtained suggest that methanol extract of C. jagus possessed significant anticonvulsant effect, thereby confirming the traditional uses of C. jagus in the treatment of epilepsies; mechanisms of which may involve interaction with GABAergic and serotonergic pathway.

Keywords *Cranium jagus*, hexobarbitone, picrotoxin, strychnine, flumazenil, and serotonin

1 INTRODUCTION

The plants used as herbs in the treatment of different kind of ailment was originally in form of concoction, infusion, decoction, etc., which were crude means of extraction prior the advent of scientific means of extraction and eventually lead to purification, identification and characterization of the precise compound(s) that may be responsible for the reported pharmacological properties [1]. Very recently, many phytoconstituents whose pharmacological properties were not known have been extensively discovered to have significant medicinal important. Screening of natural products from plants, animals, minerals and microorganism have led to isolation of compounds used today for the treatment of various diseases [2]. Plants, of the four sources of drug mentioned earlier, were found as the most abundant, diversified and constitutive. Although the modern synthetic methods of drug discovery have revolutionized drug production, plants remain a valuable source of drugs as many important drugs used in medicine today can be traced to plants [3].

Crinum jagus (Thomps.) Dandy [family AMARYLLIDACEAE], is a common plant distributed in the swampy locations with tulip-like white flowers that appear in the dry season [4]. It is a tender perennial bulb that is native to tropical Africa. Its stalk grows up to about 1m tall from a clump of strap-shaped green leaves. Morphologically, it is an erect and herbaceous plant with bulbous underground rootstock of fleshy leaf bases. Leaves are linear, and the widest point is nearer to the tip than to the base. When arising from the ground level, it is pale green on the abaxial but glossy green on the ad axial surface. Leaf tip is obtuse, with length of leaves (63.2-105.3cm) being seven times the width (9.0-14.1cm) [5]. Flowering shoot is one per plant, pale green, usually about half, but sometimes as long as the leaf blade. It is flattened laterally, and forms a receptacle at the tip with two opposing bracts that enclosed the flowers when young. Flowers range from 4 to 12 per flowering shoot, sometimes including the aborted flowers, and a flower having six petals, white on both surfaces. Stem is a much reduced corm, and roots are numerous with a fibrous system [3]. The medicinally useful part of the plant in this regard is the bulb [6, 5]. It is used as an active rubafacient and anthelmintic [7]; in the treatment of memory loss associated with old age in Southwestern Nigeria [8]; in the treatment of open sores and anticonvulsant preparations [9]. *C. jagus* leaf is used in the treatment of convulsion and asthma [10]. Cold infusion of the fresh leaves of the plant is being used for bathing young children suffering from general body debility and rickets in Sierra Leone [11]. A decoction is given as a vermifuge in the Gold coast (Ghana). In East Africa, the decoction of *C. jagus* is used for the treatment of sores [12]. Some of the traditional uses of this plant have been scientifically authenticated [13, 14, 15]. Chemical investigations of the plant revealed the presence of phenolic compounds in large quantity, which include, tetrahydro-1, 4-oxazine (morpholine), crinamine, lycorine, psuedolycorine, hamayne, bowdensine, and demethoxy-bowdensine, 6-hydroxycrinamine [16,17]. Phytoconstituents such as saponins and tannins, also a mineral compound like calcium oxalate and calcium tetrata, were reportedly present [18, 19]. Some of the phenolic compounds obtained from the plant extracts were reported to have exhibited enzymatic and non-enzymatic antioxidant effects [18, 20]. Considering the extensive uses of the plant in folkloric medicine, we, therefore, set out to assess some other pharmacological properties of *Cranium jagus* (Leaf and Bulb) using mice.

2.0 MATERIALS AND METHODS

2.1 *Animal material*

Healthy Swiss male albino mice (20-30 g) were obtained from the Animal House of the Ladoke Akintola University of Technology, Ogbomosho, Oyo state Nigeria were used for this study. Animals were maintained under favourable environmental conditions at a temperature of 22.5 ± 2.0 °C and relative humidity in the range of 56–63 % with 12 hours light/ dark cycle. Their cages were clean by removing husk and excreta every day. The foods in Pellets form obtained from Ladokun feeds, Nigeria Limited was given to the mice with fresh water *ad libitum* during the period of acclimatization. Animals were left to acclimatize to the laboratory environment for 14 days' period before performing the experiments. Animals were allowed to fast overnight before the operations. In the process of conducting the investigation, mice were randomly assigned into five groups: the control, standard drug, and three test groups (200, 400, and 800 mg/kg). Animals in each group were administered through the intraperitoneal route of drug administration.

All experimental rules applying to animal safety and care were observed, and approval was obtained from the Institutional Animal Ethical Committee of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

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2.2 ***Plant materials***

The fresh leaves and bulbs of Cranium *jagus* (Thomps) were used for this study

Figure 1 . The fresh leaves and bulbs of Cranium jagus (Thomps)

2.2.1 ***Plant source and identification***

The fresh leaves and bulbs of *C. jagus* (Thomps) were collected locally from their natural habitat in the Obafemi Awolowo University (OAU) campus, along Road 7, University Staff Quarters. The plant was identified by Mr. I. Ogunlowo, Department of Pharmacognosy Herbarium unit, Faculty of Pharmacy, OAU, where a voucher specimen (IFE HERBARIUM, 17533) was deposited.

2.2.2 ***Extraction of plant material***

The fresh leaves and bulbs of *Cranium jagus* were both air-dried separately and then made into powder using the electric machine. Extraction was performed by adding 1.1 g of *Cranium jagus* leaf (CJL) or 1.5 g of the bulb (CJB) to 5 liters of absolute methanol in two separate sterile flasks with a stopper (to prevent loss of volatile liquid), the mixture was extracted by agitation. After 24 hours, it was decanted and filtered using filter paper No. 1 (Whatmann London, UK). The filtrate was evaporated to dryness using a rotary evaporator (Buchi Rota Vapour R110) and freeze-dried until a solid mass was obtained. The dried residue of extract of CJL or CJB was sealed tightly in a separate glass vial, and each stored in a refrigerator at 4^{o} C until used.

2.3 ***Acute toxicity test and determination of*** LD_{50}

Acute toxicity test was carried out using the method of Lorke's (1983) [21]. A total of 12 mice were used; in the initial phase, animals were assigned randomly into three groups of 3 mice each. Animals in each group were administered an intraperitoneal injection of extract at 10, 100, and 1000 mg/kg body weight following which they were observed for signs of toxicity and death in the first 24 hours [22]. In the second phase, another set of 4 mice were randomly assigned into three groups of 1 mouse each and administered the extract intraperitoneally at 1600, 2900, and 5000 mg/kg based on the result of the first phase. The LD_{50} was then calculated as the square root of the product of the maximum dose for all surviving and minimum treatment dose for all mortality using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no death,
D_{100} = Lowest dose that produced mortality.

2.4 ***Anticonvulsant Studies of the leaf and bulb extracts of C. jagus.***

2.4.1 ***Effect of leaf and bulb extracts of Cranium jagus on hexobarbitone-induced Sleeping Time***

The sleep evaluation method was based on prolongation of hexobarbitone-induced sleeping time [23]. The mice were randomly divided into five groups (n=6). Group 1 received normal saline (10 ml/kg, i.p), group 2 received the standard (Diazepam, 5 mg/kg, i.p) while CJL extract (200-800 mg/kg, i.p) was given to groups (3-5). Thirty minutes later, each mouse received hexobarbitone sodium (85 mg/kg, i.p) Duration of sleep was calculated as the interval between the loss and recovery of the righting reflex [24]. The same procedure was repeated for CJB

2.4.2 ***Effect of leaf and bulb extracts of Cranium jagus on Picrotoxin- or strychnine-induced convulsions***

Picrotocin-induced convulsion models are used to investigate the anticonvulsive activities of drugs, natural compounds, or chemical substances in the animal. Mice were randomly divided into eight groups of five mice in each group and intraperitoneally pretreated with vehicle or normal saline at 10 ml/kg, phenobarbital (40 mg/kg, i.p.) a standard anticonvulsant agent, and CJB extract (200, 400 and 800 mg/kg). Thirty minutes later, the seizure was induced by intraperitoneal administration of picrotoxin (10 mg/kg i.p) or strychnine (2 mg/kg, i.p) as described by Elisha et al., (1988) [25]; following which each mouse was placed in an observer box. Convulsion was scored as clonic contractions and anticonvulsant activity measured as the onset and duration of convulsive episodes' increases [26]. The same procedure was repeated for CJL extract.

***2.4.3 GABAergic pathway involvement*:** Thirty mice were randomly assigned into 3 groups (n = 10). Flumazenil (3 mg kg^{-1}) was administered intraperitoneally and fifteen minutes later CJL (1200 mg/kg, i.p) or vehicle (10 ml/kg, i.p) or phenobarbitone (40 mg kg^{-1}) were administered. Thirty minutes later, the seizure was induced by intraperitoneal administration of picrotoxin (10 mg/kg i.p). Convulsion was scored as clonic contractions and anticonvulsant activity measured as the onset and duration of convulsive episodes' increases [25] (Nogueira and Vassilieff, 2000)..

2.4.4 Serotonergic pathway involvement: Thirty mice were randomly assigned into 3 groups (n = 10). Cyproheptadine (4 mg kg^{-1}) was administered intraperitoneally as described by Michael, (2006).; fifteen minutes later CJL (1200 mg/kg, i.p) or vehicle (10 ml/kg, i.p) or phenobarbitone (40 mg kg^{-1}) were administered. The seizure was induced by intraperitoneal administration of

picrotoxin (10 mg/kg i.p) after 30 minutes pre-treatment with extract. Convulsion was scored as clonic contractions and anticonvulsant activity measured as the onset and duration of convulsive episodes' increases [25].

2.5 *Statistical Analysis*

Data were analyzed using a one-way analysis of variance (ANOVA) followed by posthoc tests (Student Newman Keul's), which was used to determine the source of a significant effect. Results were expressed as Mean ± SEM., while $p<0.05$ was taken as an acceptable level of significant difference from control or vehicle.

3.0 RESULTS

3.1 *Extraction*

Appropriately 1.5kg of powdered *C. jagus* bulb and 1.1 g of powdered *C. jagus* leaf were extracted by maceration with 5litre of absolute methanol and then dried using rotary evaporator afforded 45.6 g, 3.04 %w/w and 40.9 g, 3.72 %w/w respectively.

3.2 *Acute toxicity of C. jagus*

There was no mortality observed in the First phase of acute toxicity studies of both extracts, while in the second phase of acute toxicity studies of both extracts (CJL and CJB), mortality was observed in 2900 and 5000 mg in both extracts extract treated group respectively as stated in Tables 1 and 2. The lethal doses (LD_{50}) of CJL and CJB extracts were calculated as 2154 and 3808 mg/kg body weight, respectively, as shown below.

Table 1: Acute toxicity test (Phase 1) of methanol leaf and bulb extract of *C. jagus* in mice

Groups	Doses (mg/kg)	Mortality	Mortality (%)
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0

Table 2: Acute toxicity test (Phase 1I).

Groups	Doses (mg/kg)	Deaths		Mortality (%)	
		CJL	CJB	CJL	CJB
1	1600	0/1	0/1	0	0
2	2900	1/1	0/1	100	0
3	5000	0/1	1/1	0	100

Calculation of LD_{50}

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = 1600 mg/kg

D_{100} = 2900 mg/kg
LD_{50} of CJL = 2154 mg/kg body weight
D_0 = 2900 mg/kg for CJB
D_{100} = 5000 mg/kg for CJB
LD_{50} of CJB = 3808 mg/kg

3.3 ***Anticonvulsant activities***

3.3.1 ***Effects of Cranium jagus leaf and bulb extracts on hexobarbital-induced sleeping time in mice***

The extract of CJL significantly prolonged the duration and shortened the onset of hexobarbitone-induced sleeping time in a dose-related manner, while, the effect of *Cranium jagus* bulb was statistically non-significant in relation to control (Figure 2).

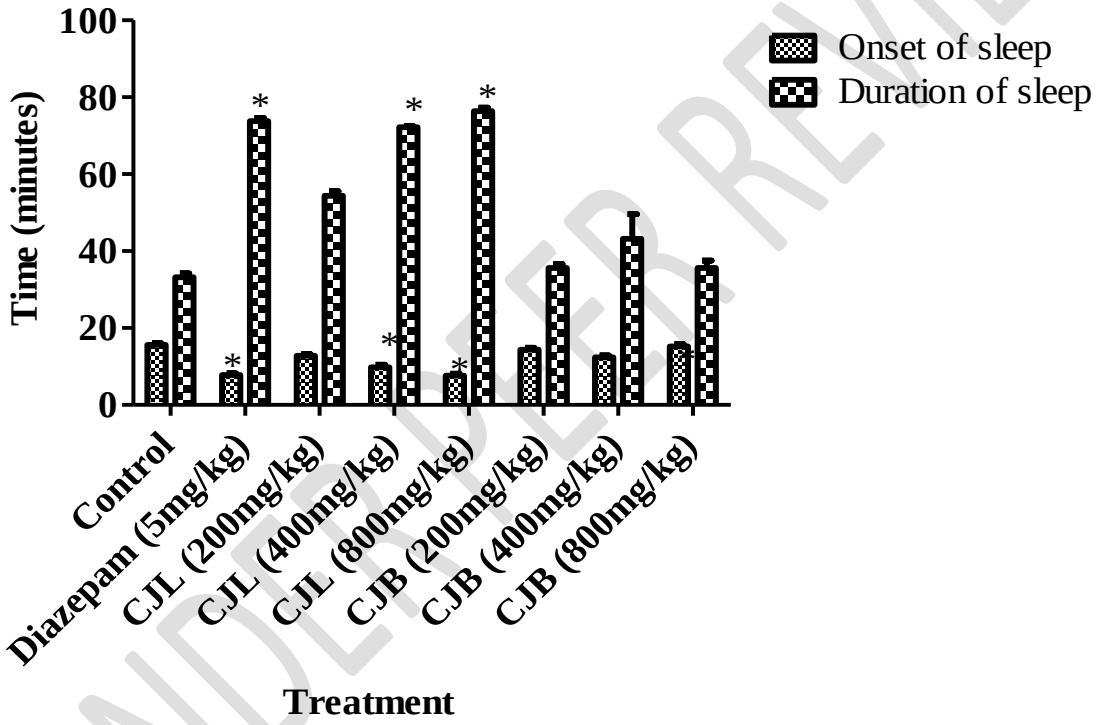

Figure 2: Effects of Cranium jagus leaf and bulb extracts on hexobarbital-induced sleeping time in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

3.3.2 ***Effect of C. jagus leaf and bulb extract on strychnine- or picrotoxin-induced convulsions***

The extract of CJL at all doses although significantly ($p<0.05$) increased the onset of seizure but failed to protect the animals against strychnine, except at a dose of 800 mg/kg, where the duration of convulsion was significantly prolonged with 20% of mice survived (not shown), while the CJB extract did not confer any significant protection (figure 3). Phenobarbital sodium did not have any significant effect on strychnine-induced convulsion as shown in figure 3.

However, the extract of CJL significantly reduced the seizure severity in a dose-dependent manner against picrotoxin-induced death as it prolonged the onset of action and duration of convulsion, significant increase was observed at 400 and 800 mg/kg doses (Figure 4). While, CJB extract, although brought about prolongation of onset of seizure but failed to protect the animal against mortality as the duration of convulsion remained relatively the same as negative control. Phenobarbital sodium (a standard drug) significantly and completely protected the animals against the effect of picrotoxin-induced seizure (Figure 4).

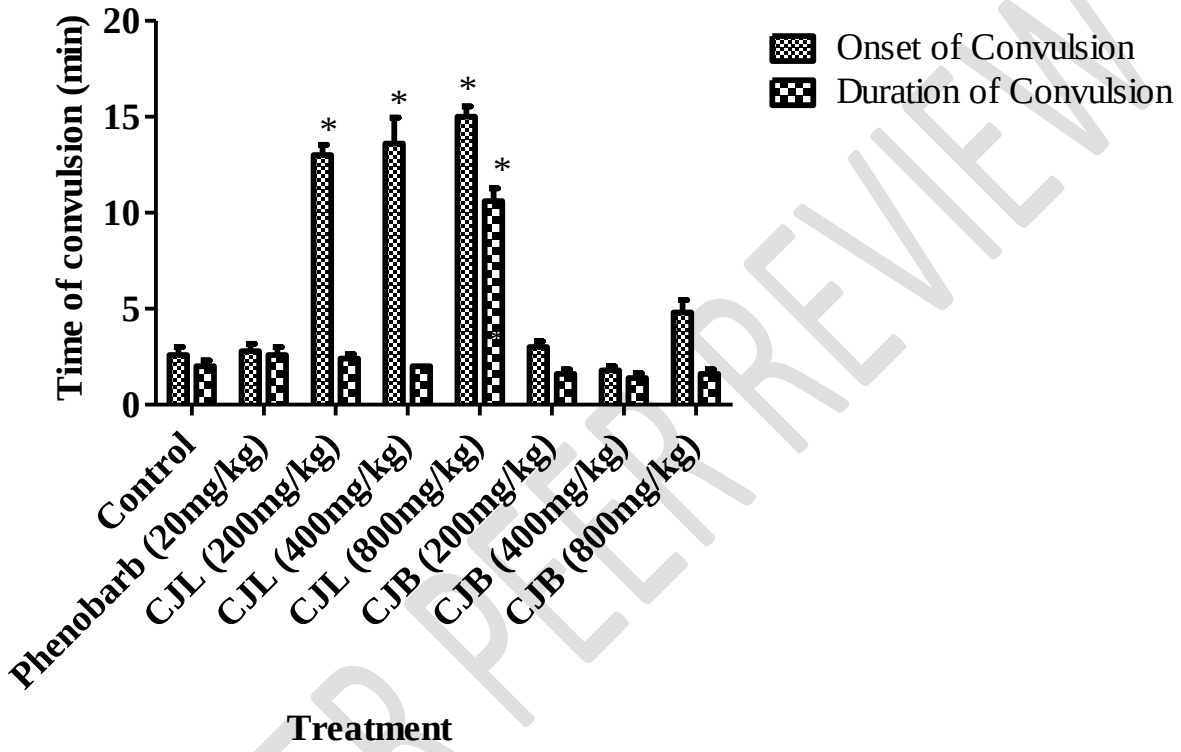

Figure 3: Effect of *C. jagus* leaf and bulb extract on strychnine-induced convulsions. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

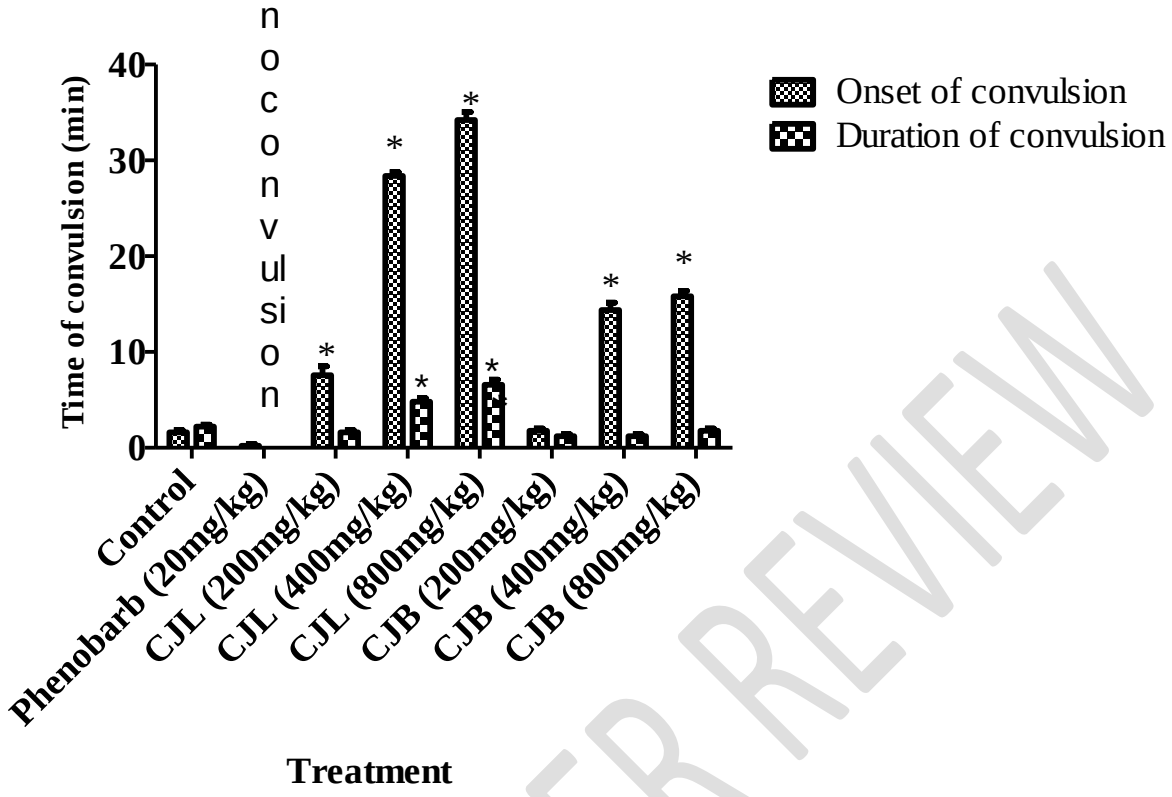

Figure 4: Effect of *C. jagus* leaf and bulb extract on picrotoxin-induced convulsions
Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

Possible involvements of GABAergic and serotonergic pathway in the mechanism of action of *C. jagus.*

The flumazenil, a GABA receptor antagonist and cyproheptadine a 5-HT_2 receptor antagonist were used in this study. Flumazenil acts by competitively inhibit the activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex. From the results obtained, flumazenil significantly nullified the effect of *C. jagus* and the standard (phenobarbitone) when compared to control. Similarly, cyproheptadine a 5-HT_2 receptor antagonist reversed the effect of *C. jagus* but not the standard drug (phenobarbitone) by reducing the onset and duration of convulsion significantly and consequently resulting in 0% protection of the mice from seizure. This is an indication of possible GABAnergic and serotonergic receptors antagonism (Table 3).

Table 3: Elucidation of mechanism of Anticonvulsant effect of stem bark extract of *Cranium jagus* leave extract

Treatments	Doses(mg/kg)	Onset of seizure(sec)**	Duration (sec)**	%protection	%Mortality
Control	0	221.00±12.21	221.01±23.21	0	100
CJL	800	378.13±17.51	98.12±16.01	40	60
Phenob	40	301.80±14.35	65.41±15.21	40	60
FLU+Contr	3	257.31±10.01	218.22±16.03	0	100
FLU+CJL	5+800	357.01±17.03	295.10±01.21	0	100*
FLU+Phenob	5+40	365.34±31.01	487.23±10.73	0	100*
CYP+CJL	4+800	332.54±42.37	435.17±53.17	0	100*

**Value are recorded as mean±SEM (n=5). *Value are statistically significant ($p<0.05$) in relation to control. One-way ANOVA follows by Neuman-Keuls multiple comparison test
Flu: Flumazenil, Cyp: Cyproheptadine,

DISCUSSION

The claims of therapeutic successes on central nervous system disorders by traditional medicine practitioners using *C. jagus* leaf and bulb have not been exhaustively subjected to scientific investigation. In this study, the pharmacological profile of *C. jagus* leaf and bulb extracts were determined using animal (mice) as a model.

Extraction is a fundamental step in drug discovery of a new drug from the medicinal plant and is a process of separation of medicinally active constituents from their sources [28]. For extraction purposes, classical techniques like maceration, decoction, percolation, soxhlet, microwave-assisted extraction, ultrasound extraction, and supercritical fluid extraction have been used [29, 28, 30]. A different solvent such as methanol, ethanol, acetone alone, or with water, ethyl-acetate, has usually been used for classical extraction [30]. The use of an appropriate extraction method, plant material, and solvent ensures a good quality extract [26]. Methanol was used for extraction in this study because it has a very high extractive value as it can separate both the polar and non-polar compound present.

The *C. jagus* exhibited sedative effects, as shown by their abilities to prolong hexobarbitone-induced sleeping time. Several neurotransmitters and endogenous molecules are involved in regulation of sleep and wakefulness. The neurons that promote sleep is found to be located in the anterior hypothalamus which bring about the release of gamma-aminobutyric acid (GABA) suppressing or inhibiting the activity of wake-inducing areas of the brain [31]. Hexobarbital is known to act at GABA receptors ionophore complex and favour the binding of GABA. Also, benzodiazepine agonists such as diazepam enhance the affinity of GABA for its receptor and hence prolong pentobarbital-induced sleep duration [32]. Similarly, some medicinal plants interact with the GABAergic system to induce their hypnotic effect [33]. The hypnotic activity of medicinal plants has been attributed to different phytochemical compounds such as flavonoids, terpenes, and saponins [34, 35]. Studies have also shown that the potentiation of barbiturate hypnosis is an index for central nervous system depression and that drugs with sedative properties prolonged the time of sleep produced by barbiturate [36]. Two parameters were measured in this experiment, onset (sleep latency) and duration of sleep. Sleep latency is defined as the time in a minute from injection time to loss of righting reflex (unconsciousness), while the duration of sleep is defined as the total time in a minute from loss of righting reflex to regain of righting reflex (recovery of consciousness) [37]. Therefore, it may be suggested that the ability of the extracts to prolong barbiturate-induced sleeping time indicates that it possesses central

nervous system depressant property. Diazepam belongs to the benzodiazepine group which has a binding site on GABA receptor type ionophore complex (GABAA) [38] and this mechanism can be useful in the onset of sleep and increase of sleep duration. *C. jagus* leaf administration at doses of 200-800 mg/kg produced sedative effect similar to that observed with 5 mg/kg of diazepam. This effect observed may be mediated via GABA-ergic system. Therefore, the drugs that influence these systems can be important in insomnia disorder.

Glycine and gamma amino acid (GABA) are amino acids which act as inhibitory neurotransmitters in the CNS, the inhibition of which has been implicated in convulsions. Strychnine is a potent spinal cord convulsant, which blocks glycine receptors selectively to induce excitatory response in the CNS. GABA is the main inhibitory neurotransmitter substance in the brain and is generally implicated in convulsion. Inhibition of GABA-ergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABA-ergic neurotransmission is known to inhibit or attenuate seizures [39]. Moreover, some studies indicated that picrotocin diminishes the GABAergic tone (Ahmadiani et al., 2003) [40], probably by a competitive antagonistic action on the BZD receptors. Similarly, drugs that enhance GABAA-receptor neurotransmission, such as BZDs [38] can block seizures induced by picrotoxin. The antagonism of STR -induced convulsions suggests the presence of anticonvulsant effect through glycine-STR-sensitive receptors. Picrotoxin, a GABA-A receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA-A receptors, thus preventing the entry of chloride ions into the neurons. This leads to decreased GABA transmission and activity in the brain. Thus, convulsions arising from picrotoxin are due to the decreased GABA-A receptors-mediated inhibition which tips the balance in favour of glutamate-mediated excitatory transmission [41]. The abilities of the extracts of *C. jagus* leaf (CJL) to attenuate seizures induced by picrotocin may possibly be due to an interaction with GABA-A receptors and /or GABA transmission. Phenobarbitone, a reference anticonvulsant, known to enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride channels of GABA-A receptors produced similar effects on picrotoxin-induced seizures. Since *C. jagus* extracts mimicked, to some extent, the anticonvulsant actions of phenobarbitone, it is possible that *C. jagus* leaf antagonize picrotoxin-induced seizure by opening the chloride channel associated with GABA-A receptors. The findings of this study indicated that *C. jagus* leaf was more potent in delaying myoclonic and clonic seizures and more effective in protecting animals from picrotocin-induced death than was the extract of *C. jagus* bulb. This may be due to the type and/or amounts of chemical compositions in the *C. jagus* leaf.

To further investigate the possible involvement of GABAergic pathway in the mechanisms of action of *Cranium jagus* leaf extract, flumazenil (GABA receptor antagonist) was used. The effect of flumazenil in this study revealed that it acts by competitively inhibiting the activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex. From the results, flumazenil antagonized the effect of *Cranium jagus* leaf extract significantly when compared to both vehicle control and phenobarbitone. Similarly, cyproheptadine ($5\text{-}HT_2$ receptor antagonist) antagonized the effect of *Cranium jagus leaf extract* but not the standard drug (phenobarbitone) by significantly reducing their onset and duration of convulsion and consequently causing 0% protection from seizure. This is an indication of possible serotonergic, $5HT_2$ receptor antagonism by the extract.

CONCLUSION

This study showed that *C. jagus* leaf extract possessed anticonvulsant activity; it may have potential clinical use in absence seizure in humans. Our findings support the acclaimed usefulness of *Cranium jagus* (leaf and bulb) in folkloric medicine. The anticonvulsant mechanisms of the plant might involve its interaction with GABAergic and serotonergic pathway based on our study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol of this study was approved by the Research Ethics Committee, Faculty of Basic Medical Sciences Ladoke Akintola University of Technology Ogbomoso, Nigeria (Approval no. LAU/FBMS/ETHICS/2020/011) and conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85-93 revised in 1985)

HUMAN AND ANIMAL RIGHTS

No humans were involved in this study. All the animals' procedures were followed in accordance with the ethical standards of Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology.

CONSENT FOR PUBLICATION

We all agreed that the article be published if found suitable for publication in .

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

ABBREVIATIONS

ANOVA = Analysis of variance
BZD = Benzodiazepine
CJB = Cranium jagus bulb
CJL = Cranium jagus leaf
CNS = Central nervous system
GABA = Gamma amino butyric acid
LD 50 = Lethal dose at 50%
OAU = Obafemi Awolowo University
SEM = Standard error of means
STR = Strychnine

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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