EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 10
43-50
Year: 2020

ANTI-NOCICEPTIVE POTENTIAL OF METHANOL EXTRACTS OF *CRANIUM JAGUS* (J. THOMPS) IN MICE

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Article Received on 03/08/2020 **Article Revised on 23/08/2020** **Article Accepted on 13/09/2020**

ABSTRACT

Objective: The study investigated the antinociceptive potential of methanol leaf and bulb extracts of *Cranium jagus* in mice. **Methods:** The leaf and bulb extracts of *Cranium jagus* (CJL and CJB) were prepared as per standard procedures and evaluated at three different doses (200, 400, and 800 mg/kg, i.p), they were screened for anti-nociceptive (hot plate test, acetic acid-induced pain, formalin-induced paw lick). **Results**: The extract of CJL significantly ($p<0.05$) increased the latency time against the thermal stimulus-induced by hot-plate, inhibited neurogenic and inflammatory pain induced by formalin. Similarly, CJB produced a significant ($p<0.05$) reduction in licking time in inflammatory pain, but not in neurogenic pain. Both extracts significantly ($p<0.05$) inhibited the abdominal constriction due to irritation of the stomach in acetic acid-induced pain, dose-dependently. **Conclusion:** This study showed that *Cranium jagus* leaf and bulb extracts possessed analgesic activity. *Cranium jagus* leaf is centrally and peripherally mediated, while, *Cranium jagus* bulb is peripherally mediated. Our findings support the acclaimed usefulness of *Cranium jagus* in folkloric medicine.

KEYWORDS: *Cranium jagus*, anti-nociceptive, formalin, hot-plate, analgesic.

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.

2.2 *Plant materials*

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

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 *Analgesic Studies of the leaf and bulb extracts of C. jagus*

2.4.1 *Effect of crude methanol leaf and bulb extracts of C. jagus on the acetic acid-induced writhing test*

Writhing in mice was induced according to the method described by Koster *et al*., (1959).[23] The mice were randomly divided into eight groups, and group 1 received normal saline (10ml/kg, i.p), CJL (200-800mg/kg, i.p) was given to groups (2-4) and CJB at the same doses was given to mice in group (5-7), while Acetylsalicylate (ASA, 150 mg/kg, i.p), was administered to group 8 as reference drug. Each mouse was given freshly prepared 0.6% aqueous solution of acetic acid and then placed in an observer box. The animals were pretreated for 30minutes before acetic acid administration. Nociception was evaluated by counting the number of abdominal constrictions for 20minutes after the administration of acetic acid. Decrease in the number of writhes or increase in percentage inhibition against abdominal constriction was taken as an index of analgesia.

2.4.2 ***Effect of crude methanol leaf and bulb extracts of C. jagus on Hot plate***

In this experiment, the pain episode was induced by thermal stimulus as described by Hunskaar *et al.*, (1986).[24] The mice were randomly divided into eight groups, and group 1 received normal saline (10ml/kg, i.p), CJL or CJB (200-800mg/kg, i.p) was given to groups (2-4 or 5-7), while morphine (5 mg/kg, i.p), was administered to group 8 as reference drug. The animals were pretreated for 30minutes; each mouse was placed in a hot plate maintained at 55±0.5º Nociception was evaluated when the animal began to lick its hind paw or attempt to jump out of the hot plate. The time taken to lick the hind paw was taken as reaction time. Anti-nociceptive activity was expressed as the increased in reaction time.

2.4.3 ***Effect of crude methanol leaf and bulb extracts of C. jagus on Formalin-induced paw lick***

In this experiment paw lick in mouse was induced by formalin according to the method described by Hunskaar and Hole (1987).[25] The mice were randomly divided into eight groups, and group 1 received normal saline (10ml/kg, i.p), CJB or CJL (200-800mg/kg, i.p) was given to groups (2-4 or 5-7), and the group eight received the standard drug (morphine sulfate, 5 mg/kg). Each mouse was injected at right hind paw with freshly prepared formalin (1%, 2ul). The animals were pretreated with extracts for 30minutes before the injection of formalin. Nociception was evaluated when the animal began to lick its paw at 0-5 minutes (early phase) and 20-30 minutes (late phase). The anti-nociceptive activity was expressed as the reduction in duration of paw lick.

2.7 ***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 ***Analgesic activities***

3.3.1 ***Effects of C. jagus (Leaf and Bulb) on Acetic Acid-Induced Writhing***

The result for the test of analgesic activity showed that CJL and CJB caused significant reduction ($p<0.05$) in abdominal constriction induced by acetic acid (Figure 1), a dose-dependent effect was observed with percentage inhibition of 36.4, 46.4, 75.6 and 15.4, 39.3, 49.4 % for the doses of 200, 400 and 800mg/kg of the extracts respectively. Aspirin (ASA), a reference drug, produced 83.6% inhibition (Table 3).

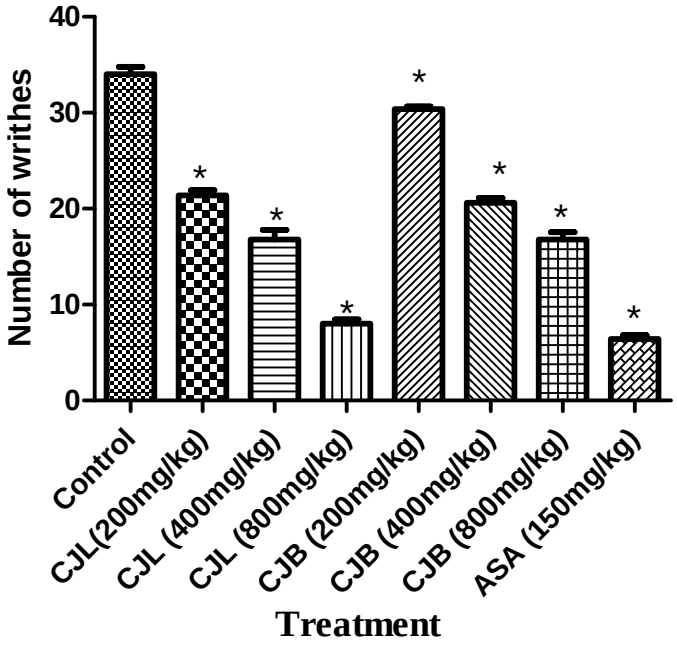

Figure 1: Effects of C. jagus (Leaf and Bulb) on Acetic Acid-Induced Writhing.

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

Table 3: Effect of extracts of CJL and CJB on acetic acid-induced writhing.

Pretreatments	Doses (mg/kg)	% inhibition
Control	0	0
CJL	200	36.4*
CJL	400	46.4*
CJL	800	75.6*
CJB	200	15.4*
CJB	400	39.4*
CJB	800	49.4*
ASA	150	83.6*

**Values are recorded as means±SEM (n=5).
*Values are statistically significant (p<0.05) in relation to control. One-way ANOVA follow by Newman-Keuls Multiple Comparison tests.

3.3.2 Effect of *C. jagus* on a hot plate

CJL significantly (p<0.05) increased the latency time against the thermal stimulus-induced by the hot plate in a dose-dependent manner in all the three doses. While CJB did not produce a statistically significant effect (Figure 2).

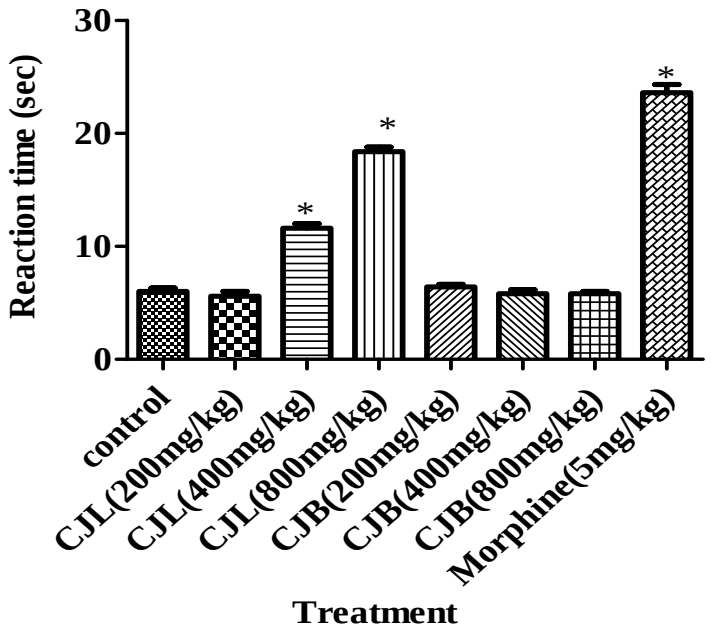

Figure 2: Effect of C. jagus on a hot plate.

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

3.3.3 ***Effect of C. jagus (Leaf and Bulb) on formalin-induced paw lick***

In figure 3, it was shown that pre-treatment with the extract of CJB produced significant changes of paw licking time in the second phase of pain response but not in the first phase. *Cranium jagus* leaf (CJL) produced a dose-dependent and significant (p<0.05) reduction in licking time in both the first and second phases of formalin-induced paw lick similar to morphine (standard drug).

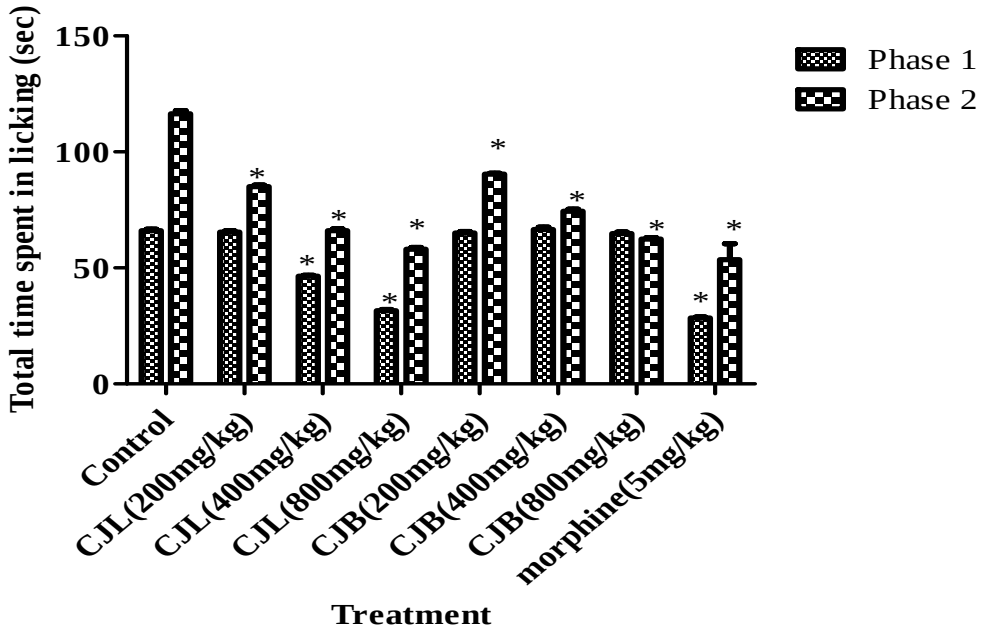

Figure 3: Effect of C. jagus (Leaf and Bulb) on formalin-induced paw lick.

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

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 antinociceptive 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.[26] For extraction purposes, classical techniques like maceration, decoction, percolation, soxhlet, microwave-assisted extraction, ultrasound extraction, and supercritical fluid extraction have been used.[27], [26], [28] A different solvent such as methanol, ethanol, acetone alone, or with water, ethyl-acetate, has usually been used for classical extraction.[8] 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.

Acetic acid mouse writhing test is a widely used animal model for routing screening of compounds with peripheral analgesic activity.[29], [30], [31] The writhing response is considered to be a visceral inflammatory pain.[32], [33] Acetic acid is a chemical irritant that produces tissue necrosis of the peritoneal region accompanied by the release of chemical mediators such as bradykinin, prostaglandin, histamine, substance P, vasoactive polypeptide, which cause pain either by activation or sensitization of nociceptors that encode tissue injury[34], [35], [31], while the hot plate or tail immersion model of pains is generally used to detect centrally acting analgesics.[25] The extracts of CJL and CJB produced significant ($p<0.05$) anti-nociceptive effects in chemically induced nociceptive pain stimuli in mice. The inhibitory effect exhibited by the extract of *C. jagus* against nociceptive action of acetic acid in mice may suggest the presence of phytochemically active substances with analgesic property. This suggestion further supported by the finding that CJL extract inhibited the nociceptive behaviour in both neurogenic and inflammatory pain produced by formalin in mice. While CJB extract significantly inhibited inflammatory pain. The anti-nociceptive effect of extracts of CJL and CJB in acetic acid-induced writhing might be due to the blockade or inhibition of release of endogenous substances that excites pain nerve endings similar to that of non-steroidal anti-inflammatory drugs (NSAIDs).[36]

Formalin is used as chemical noxious stimuli to trigger pain. This test was normally used to study both central as well as peripheral analgesic activity.[37] Injection of formalin is associated with two phases; the neurogenic pain (first phase) or early phase due to stimulation of nociceptors, followed by the pain due to inflammation during the late phase (second phase).[38], [25] The neurogenic pain is centrally mediated and is attributed to the direct stimulation of nociceptive primary afferents nerve fibers and the release of pain mediators (kinin, histamine and serotonin). The inflammatory pain is peripherally mediated and it is due to peripheral release of chemical pain mediators (such as prostaglandin) that sensitize nociceptors as previously reported.[39] The peripherally analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are only effective against inflammatory pain produced by formalin.[40], [37] In contrast, the centrally acting analgesic drugs such as morphine inhibit both the neurogenic and inflammatory pains caused by formalin. The inhibitory effect demonstrated by CJL against neurogenic and inflammatory pains may suggest peripheral and central analgesic actions similar to morphine. The inhibitory effect demonstrated by CJB against inflammatory (phase) pain may suggest peripheral analgesic effect similar to NSAIDs.

Hot plate is made up of a transparent cylindrical glass used to keep the animal on the heated surface of the plate.[24] The temperature of hot plate is set using a thermos-regulated water-circulated pump. This hot plate test is also considered to be sensitive to drugs acting at the supra-spinal modulation level of the pain response[41] suggesting a modulatory effect of the investigated extracts. The reaction time is defined as the time when the animal is placed inside the hot plate surface, and the time when the animal began to licks its paw or appear to jumps off to avoid painful effect of hot plate.[42], [43] Hot plate test is universally accepted model used to evaluate substances or analgesics with central origin.[44] *C. jagus* bulb did not show any promising analgesic activity in hot plate test, whilst CJL did exhibit significant analgesic activity. Hence, the result of hot plate test supported the result of formalin-induced paw licks and affirmed the presence of centrally acting analgesic activity of the extract of CJL. However, it is not known whether the analgesic action is opioid-like in nature and or involves dopaminergic or other mechanism. The use of selective antagonist like Naloxone or metoclopramide might help in understanding the mechanism involved.

CONCLUSION

Both extracts possessed analgesic activity. *Cranium jagus* leaf is both centrally and peripherally mediated, while, *Cranium jagus* bulb is only peripherally mediated. Our findings support the acclaimed usefulness of *Cranium jagus* (leaf and bulb) in folkloric medicine; however, the responsible phytochemical(s), the underlying mechanism of its action, and the safety profile of the plant as a medicinal remedy should be clarified in future studies.

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 European Journal of Biomedical and Pharmaceutical Sciences.

AVAILABILITY OF DATA AND MATERIALS

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

FUNDING

None.

CONFLICT OF INTEREST

We declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENT

We acknowledged the Technical staff of the Department of Pharmacology and Therapeutic, Ladoke Akintola University of Technology, Ogbomoso, and Drug Research and Production Unit of Obafemi Awolowo University, Ile-Ife for their unwavering support throughout the study.

ABBREVIATIONS

ANOVA = Analysis of variance
ASA = Acetyl salicylatic acid
CJB = Cranium jagus bulb
CJL = Cranium jagus leaf
LD 50 = Lethal dose at 50%
NSAIDs = Non-steroidal anti-inflammatory drugs
OAU = Obafemi Awolowo University
SEM = Standard error of means

REFERENCES

1. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. *Journal of Natural Products*, 2001; 75: 311-335.
2. Fabricant DS, Famswort NR. The value of plant used in traditional medicine for drug discovery. *Environmental Health Journal*, 2001; 109: 69-75.
3. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. *Life Sciences*, 2005; 78: 431.
4. Olorode O. Taxonomy of West African Flowering Plants. *Longman Publishing Company, London* 1984; 121.
5. Ogunkunle ATJ, Olopade OR. Studies on the Asthma Coughs Plant Crinum jagus L. (Amaryllidaceae) in Nigeria. *African Journal of Plant Science*, 2011; 5(2): 108-114.
6. Omobuwajo OR, Alade GO, Sowemimo A. Indigenous Knowledge and Practices of Women Herb Sellers of Southwestern Nigeria. *Indian Journal of Traditional Knowledge*, 2008; 7(3): 505-510.
7. Odugbemi TA. Textbook of Medicinal plants from Nigeria. 2008; 99.
8. Houghton PJ, Agbedahunsi JM, Adegbulugbe A. Choline esteraee inhibitory properties of alkaloids from two Nigerian Crinum species. *Phytochemistry*, 2004; 65: 2893-2896.
9. Adesanya SA, Olugbade TA, Odebiyi OO, Aladesanmi JA. Antibacterial alkaloids in Crinum jagus. *International Journal of Pharmacognosy*, 1992; 30(4): 303-307.
10. Borokin TI, Ighere DA, Clement M, Ajiboye TO, Alowonle AA. Ethnobiological survey of traditional medicine practice for the treatment of piles and Diabetes mellitus in Oyo State. *Journal of Medicinal Plants Studies***,** 2013; 1(5): 30 – 40.
11. Dalziel JM. The useful plants of West Tropical Africa. *The Crown Agents for the Colonies, London.* 1937; 528-560.
12. Kokwaro JO. Medicinal Plants of East Africa. General printers, Kenya, 1976; 230.
13. Ode OJ, Asuzu IU. The anti-snake venom activities of the methanolic extract of the bulb of Crinum jagus (Amaryllidaceae). *Toxicon*. 2006; 48(3): 331-342.
14. Ode OJ, Nwaehujor CO, Onakpa MM. Evaluation of antihaemorrhagic and antioxidant potentials of Crinum jagus bulb. *Int J Appl Biol Pharm Tech*. 2010; 1: 1330–1336.
15. Shorinwa OA, Ebong OO, Obianime AW, Siminialayi IM. The effect of acetone extracts of Crinum jagus bulbs on the histology of the kidney, liver and testis of albino rats. Peak *J Med Plant Res*. 2013; 2: 38–44.
16. Thi Ngoc Tram N, Titorenkova TV, St Bankova V, Handjieva NV, Popov SS. Review Crinum L. (Amaryllidaceae). *Fitoterapia*, 2002; 73(3): 183-208.
17. Nwaehujor CO, Nwinyi FC, Ode JO. Liver protective activity of the methanol extract of Crinum jargus bulb against acetaminophen-induced hepatic damage in rats. *Asian J Biochem*. 2012; 7: 182–193.
18. Goncalves RS, Battistin A, Pauletti G, Rota L, Serafini LA. Antioxidant properties of essential oils from Mentha species evidenced by electrochemical methods. *Rev Bras Pl Med Botucatu.* 2009; 11: 372–382.
19. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complement Altern Med*. 2012; 16(12): 221-225.
20. Ekren S, Yerlikaya O, Tokul HE, Akpınar A, Açu M. Chemical composition, antimicrobial activity and antioxidant capacity of some medicinal and aromatic plant extracts. *Afr J Microbiol Res*. 2013; 7: 383–388
21. Lorke D. A new Approach to Acute Toxicity Testing. *Archives of toxicology*, 1983; 54: 275-287.
22. Desi I, Nagymajtenyi L, Schulz H. Behavioral and neurotoxicological changes caused by cadmium treatment of rats during development. *J Appl Toxicol*. 1998; 18(1): 63–70.
23. Koster R, Anderson M, Debeer. Acetic acid induced analgesic screening. *Federation Proceeding*, 1959; 18: 418-420.

24. Hunskaar S, Berge OG, Hole K. Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test. *Pain*, 1986; 25: 125–132.
25. Hunskaar S, Hole K. The formalin test in mice: Dissociation between inflammatory and non-inflammatory pain. *Pain*, 1987; 30: 103-114.
26. Handa SS. Extraction technology from medicinal and aromatic plant. *Trieste, Italy International center for sciences and technology*, 200; 1-143.
27. Ghani, A. Medicinal plants of Bangladesh with chemical constituents and uses. *Dhaka, Bangladesh, Asiatic Society of Bangladesh*, 2003; 34.
28. Biesaga M. "Influence of extraction methods on stability of flavonoidz". *Journal of Chromatography A*, 2011; 1218: 2505-2512.
29. Nunez-Guillen ME, Silva-Emini JA, Lmdala AJ, Souccar C. *International Journal of Pharmacolognosy*, 1997; 35: 99-104.
30. Khan H, Saeed M, Gilani AU, Khan MA, Dar A, Khan A. The antinociceptive activity of Polygonatum verticillatum rhizomes in pain models. *Journal of Ethnopharmacology*, 2010; 127: 520-527.
31. Ibrar M, Muhammed N, Barkatullah P, Khan H, Jahan F, Asrha N. Antinociceptive and Anticonvulsant activities of essential oils of Zanthoxylum armatum. *Phytopharmacology*, 2012; 3: 191-198
32. Collier HDJ, Dinnin LC, Johnson CA, Schneider C. The abdominal response and its suppression by analgesic drugs in the mouse. *British Journal of Pharmacology and chemotheraphy*, 1968; 32: 295-310.
33. Venessa ST, Jucelia BS, Maria SA, Antonia R, Celia HY, Mariam AOP, Glauciermar D, Orlando VS. Antinociceptive and Antiinflammatory Effects of Ethanol Extract from Vernonia polyanthes Leaves in Rodents. *International Journal of Molecular Science*, 2012; 13: 3887-3899.
34. Gene RM, Segura L, Adzet T, Marin E, Iglesia J. Heterotheca inuloides: anti-inflammatory and analgesic effect. *Journal of Ethnopharmacology*, 1998; 60: 157-160.
35. Mazid M, Datta BK, Nahar L, Rashid MA, Bachar SC, Bashar SA. Analgesic and diuretic properties of alpha-santalone from Polygonum flaccidum, *Phytotherpy Research*, 2010; 24: 1084-1087.
36. Mamun-Or-Rashid MD, Ashiqul Islam MD, Shah A. Evaluation of Analgesic Activity by Acetic Acid Induced Writhing Method of Crude Extracts of Acacia nilotica. *Scholars Academic Journal of Pharmacy*, 2017**;** 6(4): 126-138.
37. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain*, 1992; 51: 5-14.
38. Dubuisson D, Dennis SG. "The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats," *Pain*, 1978; (4)2: 161–174.
39. Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y. Nocistatin, a peptide that blocks nociceptin action in pain transmission. *Nature*, 1998; 392: 286-289.
40. Shibata M, Ohkubo T, Takahashi H, Inoki R. "Modified formalin test: characteristic biphasic pain response," *ain*, 1989; 3(38): 347–352.
41. Yaksh TL, Rudy TA. "Studies on the direct spinal action of narcotics in the production of analgesia in the rat," *Journal of Pharmacology and Experimental Therapeutics*, 1977; 2(202): 411–428.
42. Ripoll N, Hascoet M, Bourin M. The four-plates test: anxiolytic or analgesic paradigm? *Progress in neuro-psychopharmacology and biological Psychiatry*, 2006; 30: 873-880.
43. Tzschentke TM, Christoph T, Kogel B. A novel opioid receptor agonist/norepineprine reuptake inhibitor with broad spectrum analgesic properties. *Journal of pharmacology and experimental therapeutics*, 2007; 323: 265-276.