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Evaluation of the Analgesic Activity of the Aqueous and Hydroethanolic Extract from *Crinum scillifolium* Bulbs (Amaryllidaceae)

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Authors' contributions

This work was done as team with all authors. Authors KKBD and EKE designed the study, performed the different tests and write this article. Author KSL performed the statistical analysis and managed literature. Authors DGC and YHF checked results and managed the scientific research of study. All authors read and approved the manuscript.

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ABSTRACT

Aims: *Crinum scillifolium* is a plant in the family Amaryllidaceae. The present study aimed to evaluate a possible analgesic activity of an aqueous and hydroethanolic extract of *Crinum scillifolium* bulbs.
Study Design: This is an experimental study involving the extraction of the bioactive agents from *Crinum scillifolium* bulbs using water and hydroethanolic solution and the evaluation of the analgesic activity.
Place and Duration of Study: Study was carried out in Laboratory of Biochemical pharmacodynamics and laboratory of clinical and therapeutic pharmacology, University Felix Houphouet Boigny between September and October 2017.

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Methodology: For this purpose, two methods were used for the evaluation of the analgesic activity. The acetic acid-induced writhing test was used for peripheral analgesic activity and tail immersion for central analgesic activity. Swiss albino mice were used as an animal model. Extracts were administered orally at 100 and 200 mg/kg.
Results: The extracts and aspirin (150 mg/kg) produced a significant ($P \leq 0.001$) inhibition in acetic acid-induced writhing test. The hydroethanolic extract produced significant dose-dependent ($P \leq .001$) reduction the number of writhes with peak effect (91.16 % inhibition) produced at the highest dose of 200 mg/kg. This effect was comparable with that produced by aspirin (86.61 % inhibition). The aqueous extract at a dose range of 100 and 200 mg/kg respectively decreased abdominal writhing induced by the acetic acid at a rate of 59.83 % and 58.83 %. For the evaluation of central analgesic activity, the administration of both extracts increased tail stretch reflex time but the results were not statistically significant compared to the control.
Conclusion: These results demonstrate that *Crinum scillifolium* bulbs possess peripheral analgesic properties, supporting the traditional use of those plants in pain.

Keywords: Crinum scillifolium; analgesic; peripheral; central; aqueous and hydroethanolic extract.

1. INTRODUCTION

Pain is a complex multidimensional neurophysiological phenomenon that is subjective and individual. The main objective of pain is to prevent a potential danger for the human body [1]. There are two major mechanisms of pain which correspond different therapeutic strategies: Nociceptive pain is an inflammatory response to painful or noxious stimuli (tissue damage), and neuropathic pain caused by nerve damage [2].

Common drugs for pain relief such as aspirin and morphine have been widely used in recent decades. In most instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), can only relieve 50% of the pain in about 30% of patients [3]. In addition, many of these drugs cause serious side effects. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders renal failure, liver and cardiovascular toxicity [4,5]. In this context, the use of natural substances and more particularly medicinal plants becomes an important alternative way to discover drugs with fewer side effects [6].

Bulbs of various *Crinum* species are used to treat ailments such as renal and hepatic condition [7]. They are also used in the treatment of sores [8], sexually transmitted diseases and backache [9]. There have been several studies on crinum species. Among this studies, Authors demonstrated that crinum bulbs have analgesic, anti-inflammatory, anticonvulsivant activities [10, 11] and [12]. In the present study, we have evaluated the analgesic effect of aqueous and hydroethanolic extract using the writhing and tail flick assays.

2. MATERIALS AND METHODS

2.1 Plant Material

Bulbs of *Crinum Scillifolium* were collected in May 2017 from Sikensi, at 80 km to Abidjan (Ivory Coast). The plant was identified and authenticated by the Laboratory of Botanic, University Félix Houphouët Boigny. After collection, the Bulbs of *Crinum scillifolium* were washed with distilled water. They were dried under the shade at 23°C and the dried bulbs were ground into fine powder.

2.2 Preparation of Plant Extract

2.2.1 Aqueous extract

50 g of the plant powder was macerated in 50 ml of distilled water for 48 hours with stirring. The liquid extract obtained after filtration through hydrophilic cotton followed by Whatman filter paper was evaporated to dryness in an oven at a temperature of 40°C. The extract was stored in the refrigerator (4°C) until ready use. From this various concentration were reconstituted in a known volume of distilled water before administration.

2.2.2 Hydroethanolic extract

The Guédé-Guina 1990 method modified was used to obtain 90% *Crinum scillifolium* hydroethanolic extract [13]. A 90% hydroethanolic solution (ETOH / H2O, 90:10) was used for extraction. The plant powder was extracted with hydroethanolic solution (50 g of powder in 500 ml of hydroethanolic solution) by cold maceration for 48 h with stirring to obtain

hydroethanolic extract (EHE) of *Crinum scillifolium*. The macerate was filtered through Whatman filter paper and evaporated to dryness in an oven at a temperature of 40°C. The extract was stored in the refrigerator (4°C) until ready use. From this various concentration were reconstituted in a known volume of distilled water before administration.

2.3 Material Animal

Female Swiss albino mice weighing (22 – 30 g) were used in this study which were bred in the Laboratory Animal (UFR Pharmaceutical and Biological Sciences; University Félix Houphouët Boigny). The animals were maintained in standard laboratory conditions (25°C) and light/dark cycles, i.e. 12/12h and fed with standard food and water. In all the experimental studies, each group consisted of six animals. Each animal was used only once. The investigation conforms to the recommendation of OECD in 2008 [14]. Before the experiment, the mice were divided into homogeneous lots by weight.

2.4 Methods of Peripheral Analgesic Study

Assessment of analgesic activity took into account two components: peripheral and central analgesic activity.

2.4.1 Peripheral analgesic activity: Acetic acid-induced writhing

The acetic acid-induced writhing test was carried out using the previously reported method (Korster *et al.,* 1959) with slight modification [15]. Female mice weighing (22-30g) were divided into six groups of six mice each, the group 1 served as control and was administered distilled water, group 2 was pre-treated with the standard drug aspirin at a dose of 150 mg/kg, group 3 and 4 were administered the aqueous extract respectively at the dose of 100 and 200 mg/kg and group 5 and 6 were administered the hydroethanolic extract respectively at the dose of 100 and 200 mg/kg. Writhing was induced by intraperitoneal injection of acetic acid solution 1% at a dose of 10ml/kg 30 min after the pre-treatment. The mice were placed individually in transparent cages and then, the number of writhes was counted during continuous observation for 20 min beginning at 5 min after acetic acid injection. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

$$\%\, inhibition = \frac{Wc - Wt}{Wc} \times 100$$

Wt and Wc represent the number of writhing in treated groups, and control group respectively.

2.4.2 Central analgesic activity: Tail immersion test

The method described by D'Amour & Smith (1941) was used for experiment [16]. This method consists to soak the tail of the mouse in hot water at 55°C and study the tail retraction reflex of the animal, before and after the administration of the extracts. It has been established that the tail retraction normal time is 2 seconds. Thereby, only mice whose reflex time is less than or equal to 2 seconds, will be retained for the experimentation. In this test, mice were divided into six groups of six mice each, the group 1 served as control and was administered distilled water, group 2 was pre-treated with the standard drug morphine at a dose of 10 mg/kg, group 3 and 4 were administered the aqueous extract respectively at the dose of 100 and 200 mg/kg and group 5 and 6 were administered the hydroethanolic extract respectively at the dose of 100 and 200 mg/kg. The test was carried out warm water bath set a temperature of 55° C, where 2 cm of the animal tail was immersed into the warm water. The time between tail submersion and tail deflection was recorded at 30 min, 60 min, 90 min and 120min after the treatment by extracts using a digital stopwatch. A cut-off time of 15 s was maintained to avoid tail tissue damage in rodents.

2.5 Drugs and Chemicals

The drugs used were acetylsalicylic and morphine (Sigma, France). Other chemicals used were purchased locally.

2.6 Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test using the Graph Pad Prism 5.0 software package. The level of significance was determined in comparison with the control group. Statistical significance was accepted for $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

3. RESULTS

3.1 Peripheral Analgesic Activity: Acetic Acid-induced Writhing

In the writhing test, acetic acid was used to induce pain of peripheral origin in mice. A writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Analgesic activity of the test samples is inferred from a decrease in the frequency of writhing. As summarized in Table 1, intraperitoneal injection of acetic acid (1%) induced an average of 18.67 ± 4.367 writhes in a period of 20 min. Oral administration the plant extracts produced a significant (P < .001) and dose-dependent inhibition of acetic acid-induced abdominal constriction in mice. When the animals are treated with 200 mg/kg of the hydroethanolic extract, a greater reduction number of contortions is observed with a percentage inhibition of 91.96%. These results were comparable to standard drug aspirin that produced 86.61% inhibition at a dose of 150 mg/kg of body weight. The aqueous extract at a dose of (100 and 200 mg/kg) respectively showed writhing inhibition percentage of (59.83 % and 58.83 %) compared to control group.

3.2 Central Analgesic Activity: Tail Immersion Test

The effects of the aqueous and hydroethanolic extract of *crinum scillifolium* bulbs and morphine on the tail withdrawal latencies were represented on Fig. 1. Administration of morphine (10 mg / kg) significantly increased the withdrawal time of the tail of the mouse up to 13.49 ± 2.18 s (*P* < .001) against 3.053 ± 0.61 s for the control group at T 30 min; A significant difference is also observed after 60 and 90 min compared to the control group. The differences observed between the extract doses and control groups were not statistically significant.

There would be no central analgesic effect of the extracts at the doses used. However, the administration of the two extracts made it possible to increase the retraction time of the tail of the mice during the experiment.

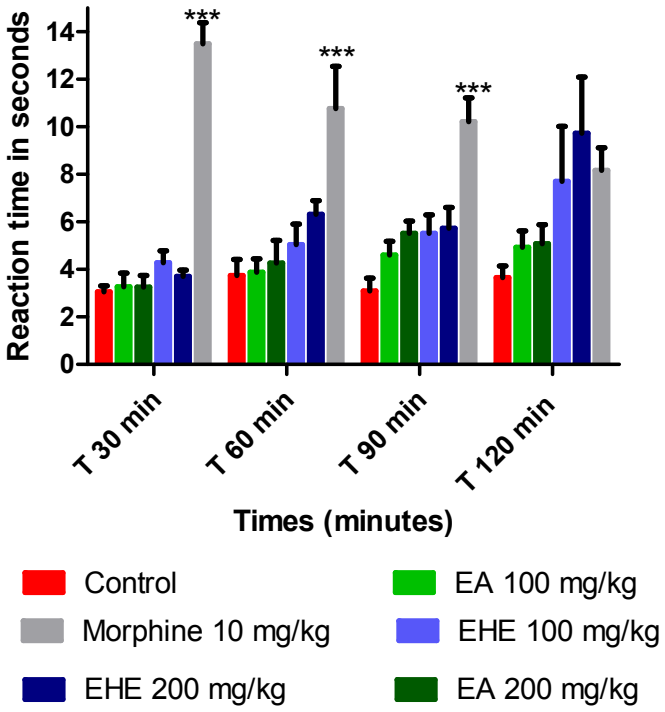

Fig. 1. Effect of Crinum scillifolium aqueous (EA) and hydroethanolic (EHE) extract on nociceptive responses in tail immersion test

*Values are expressed as mean ± SD (n = 6). ***P < .001; **P <. 01 significant from control*

Table 1. Effect of *Crinum scillifolium* aqueous and hydroethanolic extract on acetic acid-induced writhing in mice

Treatment	Dose mg/kg	Number of writhes	% inhibition
Control	-	18.67 ± 4.367	-
EA	100	7.5 ± 2.074***	59.83
EA	200	7.667 ±2.16***	58.94
EHE	100	8.33 ±4.633***	55.38
EHE	200	1.5 ± 2.811***	91.96
Aspirin	150	2.5 ± 2.258***	86.61

*Each value is mean ± SEM, N= 6 mice, the data was analyzed by using One Way ANOVA followed by Dunnett's *** (P < .001) significant from control*

4. DISCUSSION

The objective of this study was to evaluate analgesic activity of the aqueous and hydro-ethanolic extract of *Crinum scillifolium* bulbs. The present study demonstrated *Crinum* extracts possess peripheral analgesic activity. The writhing test was used because of its sensitivity, although it was not specific to the study of analgesic activity [17,18]. Collier et al., 1968 studies showed that an IP injection of acetic acid, into the peritoneal cavity of animals causes a tissue damage responsible for the release of a number of chemical mediators such as histamine, bradykinin, serotonin, acetylcholine and prostaglandins (PGE2α, PGF2α) [19]. These latter sensitize nociceptors to painful stimuli and cause late and diffuse pain [18,19]. This pain is manifested in mice by abdominal contractions, stretching movements of the body and hind legs, torsion of the dorso-abdominal muscles associated with decreased activity and motor in coordination. Aspirin which is a peripheral analgesic, tested at 150 mg/kg causes a pain inhibition of 86.61% which is similar to the hydroethanolic extract at dose of 200 mg/kg. It is well known that the purely analgesic action of aspirin is essentially related to the decrease of prostaglandin synthesis at the inflammatory site [20]. The reduction in number of abdominal writhes caused by aqueous and hydroethanolic suggests the peripheral analgesic activity of both extracts which could be related to the reduction in the release of inflammatory mediators. The genus *Crinum* have also been documented to present huge number alkaloids [21,22]. While alkaloids are well known for their ability to inhibit the perception of pain [23,24]. The presence of these chemical compounds in the extract could be responsible for the peripheral analgesic activity.

Regarding the results with the tail flick test, morphine (10 mg/kg) produced the most significant central analgesic activity during all observation time points, except at 120 min. In comparison with control, no significant analgesic effect was observed for extracts. The tail-flick method is based on the observation that morphine-like compounds are selectively able to prolong the reaction time of typical tail-withdrawal effect in mice. This method is also useful in differentiating central opioid-like analgesics from peripheral analgesics [25]. The analgesic activity of the aqueous and hydroethnolic extract of *Crinum scillifolium* might be peripheral.

5. CONCLUSION

In conclusion, the aqueous and hydroethanolic extract of the bulbs of *Crinum scillifolium* displayed analgesic activity and supported the traditional use of this plant in pain relief. Further study is warranted to identify the active compounds present in this extract and to elucidate the mechanisms involved in its analgesic properties.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Félix Houphouet-Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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