GVU JOURNAL OF SCIENCE, HEALTH AND TECHNOLOGY
Vol. 9(1), 2024; 47-52

p-ISSN: 2536-6866
e-ISSN: 2659-1529
UWAYA (2024)

EVALUATION OF THE ANALGESIC EFFECT OF AQUEOUS ROOT EXTRACT OF *Combretum platypterum* (Welw) Hutch and Dalziel ON RODENTS

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DOI: https://doi.org/10.5281/zenodo.13334654

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Abstract: Pain management is essential in many clinical cases, and an interdisciplinary approach eases suffering and improves the quality of life of those living with pain. The aim of this study was to test the analgesic effects of an aqueous root extract of *Combretum platypterum*. Three animal models were used: the acetic acid-induced writhing test, the hot plate model, and the tail flick model. In the acetic acid-induced writhing test, both the test extract and the standard drug reduced the number of abdominal constrictions and increased the percentage inhibition of writhing in a dose-dependent manner compared to the control. In the Hot Plate and Tail Flick models, the test extract and the standard drugs increased the pain threshold compared to the control group (****$p<0.0001$, ***$p<0.001$, **$p<0.01$, and *$p<0.05$). The results of pharmacological tests performed in this study suggest that the plant extract has significant analgesic activity and confirms its ethno-medical use to treat back pains.

Keywords: Analgesic, *Combretum platypterum*, writhing, Ethno-medical, Plant extract

1 INTRODUCTION

The management of pain is important in many clinical cases, and it is an interdisciplinary approach for easing the suffering and improving the quality of life of those living with pain (Akande and Ologe, 2007; Erah *et al.,* 2003). Analgesics, or painkillers, are a group of drugs used to achieve analgesia or relief from pain (Akande and Ologe, 2007; Zeruesenay*et al.,* 2002). These analgesic drugs are useful against pain but exhibit many adverse effects; this led to an interest in the search for safer and more available drugs for relieving pain (Erah *et al.,* 2003). Plants are used as painkillers today (Ahmadiani *et al*., 2000; Ovuru *et al*., 2023). The populations of developing countries worldwide continue to rely on the use of traditional medicines as their primary source of healthcare (Adesina *et al.,* 2013). Ethnobotanical studies carried out throughout Africa confirm that native plants are the main constituents of traditional African medicines (Sandhu and Heinrich, 2005; Gupta *et al*., 2005; Ovuru *et al*., 2023). Using plants as a source of relief from illness is as old as mankind, with record practices dating back at least 4000 years (Christophersen *et al*., 1991). *Combretum platypterum* belongs to the family Combretaceae. In Nigeria, it is commonly called “mmanyanza” (palm wine of the sunbird) or “achichanza” by the Igbos; the Yorubas refer to it as “OganOgandudu” or “Oganibule” (Bredenkamp, 2000). While the Binis refer to it as ‘ove” “oven” or “ovben-ome” (Aigbokhan, 2014), It is used in ethnomedicine to treat lower backaches, fever, eye problems, malaria, swellings, lumps, conjunctivitis, coughs, sexually transmitted diseases, helminthiasis, and diarrhea. They also use it as a tonic, febrifuge, and to stop postpartum bleeding (Bongerset et *al*., 2005; Liben, 1983; Watt and Breyer-Brandwijk, 1962). Although this herb has been traditionally used, there have been no scientific investigations into its usage for pain therapy. Therefore, the aim of this study was to examine the pain-relieving effect of the

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water-based root extract of *Combretum platypterum*.

Materials and Methods

Plant Collection and Extraction

Fresh leaves of *Combretum platypterum* were collected from Idumiru in Igbanke West in Orhionmwon, Edo State. The plant was identified and authenticated by Dr. H. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. The plant was deposited in the University of Benin Herbarium with voucher number UBHc063. The fresh leaves were air-dried for 14 days in the Department of Pharmacognosy at the University of Benin, Benin City. Afterwards, they were ground to powder form with an electric mill. The powdered plant material was extracted with water over 24 hours using the maceration method and then filtered over a clean, fine white cloth. The filtrate was concentrated to dryness over a water bath, and the dried extract was stored in a glass container in a refrigerator until use.

Experimental Animals

Male and female Swiss albino mice (25–35 g) and adult albino rats of both sexes weighing 100–250 g were purchased from a commercial farm in Benin City and housed in the animal facility of the Department of Biochemistry, University of Benin, Benin City. The animals were allowed to acclimatize for 2 weeks with a 12-hour light/dark cycle at room temperature. They were fed with standard rodent pellets and water *ad libitum*. The animals were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Experimental Protocol

Acetic Acid-Induced Writhing in Mice

The method described by Akor et al. (2015) was used. Twenty mice were randomly divided into five groups of four animals per group. Group 1 served as the control and was administered distilled water (10 ml/kg orally); Groups 2, 3, and 4 were administered the test substance, which was an aqueous root extract of *Combretum platypterum* orally at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. Group 5 served as the standard and was administered pentazocine (3 mg/kg). Thirty minutes later, acetic acid (0.6%) was injected intraperitoneally into each mouse. The number of writhings, which comprised constriction of the abdominal muscle together with stretching of the hind limbs, was counted for 30 minutes after the acetic acid injection. Inhibition of pain was expressed as a percentage of protection using the formula:

$$\% \text{ Inhibition of pain} = \frac{\text{mean writhing (control) - mean writhing (treated)}}{\text{mean writhing (control)}} \times 100$$

where mean writhing (control) is the mean writhing of the distilled water-treated animals, and mean writhing (treated) is the mean writhing of the animal given the plant extract, standard drug, or each dose of *C. platypterum* leaf extract.

Hot Plate Test

The method described by Badilla et al. (2003) was used. The hot plate temperature was maintained at 55 ± 1 °C. A cut-off time of 60 seconds was adopted to prevent tissue damage. Licking, biting of the hind paw, or jumping were taken as signs of pain perception. Adult Swiss mice (25–35 g) of both sexes, screened for a suitable reaction time 24 hours before the experiment, were used. The animals were randomly divided into five groups of four mice each. Group 1 served as the control and was administered distilled water (10 ml/kg). Groups 2, 3, and 4 were administered the test substance of aqueous root extract at doses of 50, 100, and 200 mg/kg, respectively. Group 5 served as the standard and was administered pentazocine (3 mg/kg). Thirty minutes later, each animal was placed on the hot plate, and the index of the response latency (time between placement and licking, biting the hind paws, or jumping) was recorded. Response latencies were taken at 30, 60, and 90 minutes after treatment (Debasis et al., 2011). The reaction time was recorded, and the percentage increase in the pain threshold was calculated.

$$\%\text{Increase in pain threshold} = \frac{\text{Ta - Tb}}{\text{Tb}} \times 100$$

where Ta = mean reaction time at each time after treatment with either pentazocine or each dose of the *C. platypterum* leaf extract, and Tb = mean baseline latency.

Tail-Flick Test

The experiment was carried out by measuring tail withdrawal time from a heat source (Ezeja et al.,

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2011). Twenty rats were randomly divided into five groups of four rats per group and fasted for 12 hours. Rats were placed in the tail flick apparatus, and their pain threshold was determined. The rat in group 1 served as the control and was administered distilled water (2 ml/kg). Groups 2, 3, and 4 were administered the test substance, an aqueous root extract of *C. platypterum*, at doses of 50, 100, and 200 mg/kg, respectively. Group 5 served as the standard and was administered pentazocine (3 mg/kg). After 30 minutes, each rat was placed on the tail flick apparatus containing heat, and the time taken for the rat to flick the tail, known as pain reaction time (PRT), was recorded for all the rats.

Statistical Analysis

Data were expressed as the mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical analysis was performed using GraphPad Prism V.6.01, where pi0.05 was considered significant.

Results

An aqueous root extract of *C. platypterum* (200 mg/kg) reduced abdominal constrictions in mice. The reduction was significant ($*P < 0.05$) when compared with the control. The standard drug aspirin (100 mg/kg) produced a better analgesic effect than the extract (Table 1). The extract and standard (pentozacine, 3 mg/kg) increased the latency time of pain compared to the control ($* * * * p < 0.0001$, $* * *p < 0.001$, $* * p < 0.01$, and $*p < 0.05$). The extract at all doses increased the threshold of pain (Table 2). The extract and standard (pentozacine, 3 mg/kg) increased the latency time of pain compared to the control ($p < 0.0001$, $*\$p < 0.001$, $\$p < 0.01$, and $*p < 0.05$). The extract at all doses increased the threshold of pain, but the standard drug produced a better analgesic effect than the extract at 60 and 90 minutes (Figure 1).

Discussion

Certain noxious stimuli are painful, and reflex movements or behaviors resulting from such stimuli show a pain threshold. The stimulus may be thermal, electrical, mechanical, or chemical, according to Ezeja et al. (2011), and this informed the three analgesic models: acetic acid-induced writhing, tail flick, and hot plate methods used in this study.

In the acetic acid-induced writhing test, the aqueous root extract of *Combretum platypterum* dose-dependently reduced the number of abdominal constrictions. The effect was significant at 200 mg/kg when compared to the control. The percentage inhibition of writhing was also dose-dependent, increasing from 0 in the control group (distilled water) to 67% in the group that received 200 mg/kg of the extract. The standard drug, aspirin (100 mg/kg), however, produced a better analgesic effect than the extract. Acetic acid acts by inducing the release of prostaglandins and lipoxygenase products into the peritoneum (Yongna et al., 2005), which activate the pain-sensing neurons that are responsive to the non-steroidal anti-inflammatory medications, making the test suitable for evaluating mild pain-relieving non-steroidal anti-inflammatory substances (Ngulde, 2010).

The inhibition of writhing in mice by the aqueous extract suggested a peripheral mechanism of action via inhibition of prostaglandins, serotonin, histamine, bradykinins, and substance P endings (Eekankopf et al., 1988; Ngulde, 2010). Pain sensation in the acetic acid-induced writhing method elicits a localized inflammatory response resulting from the release of free arachidonic acid from tissue phospholipids (Ahmed et al., 2006) via cyclooxygenase (COX) and prostaglandin biosynthesis (Adiukwu et al., 2013). Acetic acid-induced writhing has been associated with an increased level of PGE2 and PGF2 in peritoneal fluids and lipoxygenase products (Dhara et al., 2000). An increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria et al., 2008). The acetic acid-induced writhing method has been found effective for testing peripherally active analgesics. The significant pain reduction of the plant extract might be due to analgesic principles acting within the prostaglandin pathways (Ukwuani and Hassan, 2014). The abdominal writhing induced by acetic acid has been reported to be less selective (Trongsakulet et al., 2003) and has been proposed to act indirectly by releasing endogenous mediators that stimulate neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma et al., 2003).

In order to confirm the anti-nociceptive effect of the extract, the tail flick and the hot plate tests were

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e-ISSN: 2659-1529
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Table 1: Effects of aqueous root extract of *C. platypterum* on acetic acid induced writhing test in mice

Group	Number of Writhing	% Pain Inhibition
Control	96.40 ± 11.00	-
50 mg/kg	85.60 ± 27.14	11.44
100 mg/kg	61.84 ± 7.99	35.85
200 mg/kg	31.80 ± 3.31*	67.01
Aspirin (100 mg/kg)	19.20 ± 40**	80.08

The extract at 200 mg/kg and standard (aspirin, 100 mg/kg) reduced the number writhing in mice compared to control ($**p < 0.01$ and $*p < 0.05$). Values are represented as mean ± SEM, n = 5 per group.

Table 2: Effects of aqueous root extract of *C. platypterum* on latency time in hot plate test in mice

Group	30 mins	60 mins	90 mins
Control	5.20 ± 0.59	5.78 ± 0.41	5.78 ± 0.20
50 mg/kg	6.86 ± 0.85	11.14 ± 9.91****	7.56 ± 0.58*
100 mg/kg	8.60 ± 0.55**	9.82 ± 0.63**	7.56 ± 0.32*
200 mg/kg	7.54 ± 0.20*	9.68 ± 0.44**	7.54 ± 0.14*
Pen (3 mg/kg)	7.54 ± 0.15*	11.26 ± 0.72****	8.78 ± 0.57***

The extract and standard (pentozacine, 3 mg/kg) significantly increased latency time of pain compared to control ($****p < 0.0001$, $***p < 0.001$, $**p < 0.01$ and $*p < 0.05$). The extract at all dose increased the threshold of pain. Values are represented as mean ± SEM, n = 5 per group.

carried out. In both tests, the extract and standard (pentozacine, 3 mg/kg) significantly increased the latency time of pain compared to the control. The extract at all doses increased the threshold of pain. Thermal nociceptive tests are more sensitive to opioid receptors, and non-thermal tests are more sensitive to opioid receptors (Akor et al., 2015). The tail-flick test is considered selective for the drugs acting centrally. It measures the complex response to a non-inflammatory, acute nociceptive input and is one model used for studying central nociceptive activity (Sabina et al., 2009). The effects of the extract on the tail flick and hot plate methods confirmed its analgesic action. This further suggests a central mechanism of action for the extract. Pentazocine is known to elevate the pain threshold of albino rats or mice towards heat and pressure (Hollander et al., 2009). In the hot plate and tail flick experiments, pentazocine's effect as an analgesic had a higher effect than that of the extract. The analgesic superiority is expected since pentazocine is a narcotic analgesic used to ease deep-seated pain (Akor et al., 2015). Since there were significant activities recorded in both methods (tail flick and hot plate), the extract could be said to act both peripherally and centrally in producing analgesia. Noxious stimuli cause the release of chemicals such as prostaglandins and decarboxylated amines (histamine and serotonin), thereby inducing pain locally (Nunez et al., 1997; Yongna et al., 2005). Peripherally acting analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs), act by inhibiting the release of prostaglandins (Wagner et al., 2004; Ngulde, 2010). Centrally acting analgesics such as pentazocine act through their receptors in the central nervous system (CNS) by increasing the threshold response to pain stimuli (Hollander et al., 2009). Opiod analgesics inhibit both peripheral and central mechanisms of pain, while NSAIDs inhibit only peripheral pain (Akoret al., 2015).

Conclusion

In conclusion, the aqueous root extract of *Combretum platypterum* was found to have analgesic effects. In accordance with the present study, it was observed that *Combretum platypterum* analgesic effects may be centrally and peripherally pain-acting. Further studies should be initiated to study the exact mechanism of action and phytochemical investigations to find out

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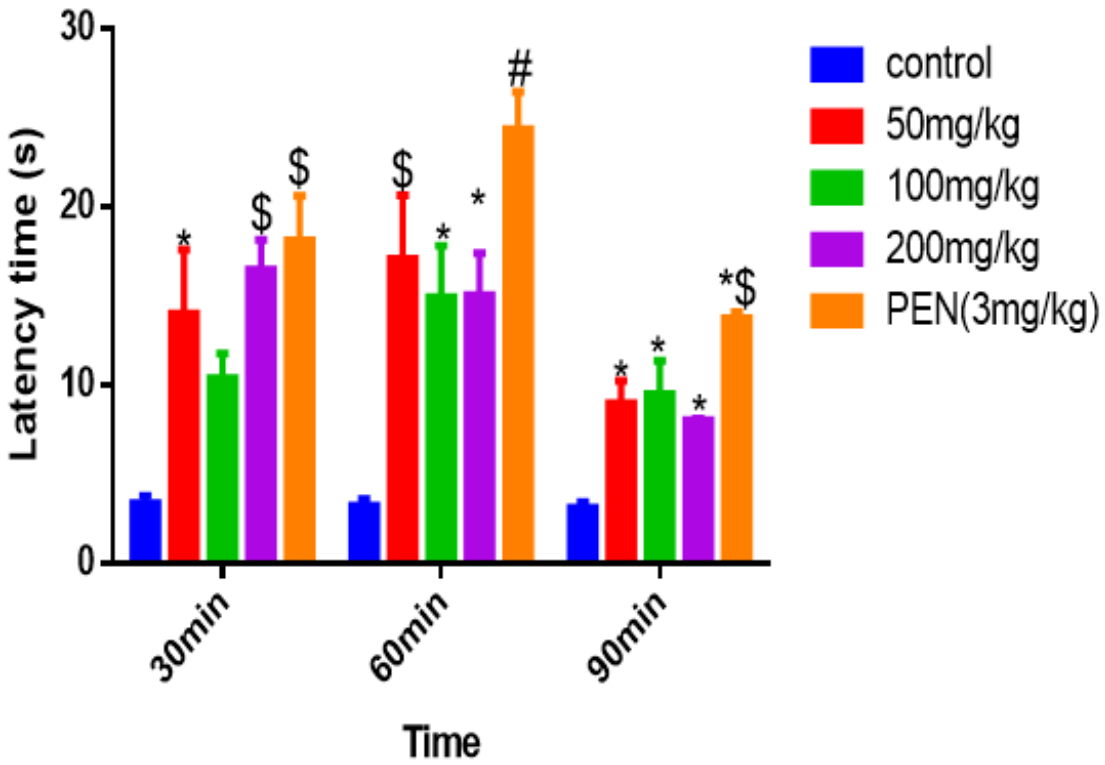

Figure 1: Effect of aqueous root extract of *Combretum platypterum* on tail flick induced pain in rats. The extract and standard (pentozacine, 3 mg/kg) increased latency time of pain compared to control ($p < 0.0001$, $*\$p < 0.001$, $\$p < 0.01$ and $*p < 0.05$). The extract at all dose increased the threshold of pain. Pen: pentozacine, values are represented as Mean ± SEM, n = 4 per group.

which active constituent is responsible for its analgesic activity.

Acknowledgments

The authors would like to acknowledge Mr. S. Okorese, Chief Technologist of the Department of Animal and Environmental Biology (AEB), Faculty of Life Sciences, for helping to house and take very good care of the animals, and Mr. Barnabas, Chief Technologist of the Department of Science Laboratory Technology, for his assistance during the experiment.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This research did not receive any specific grants from funding agencies.

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