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Original Research Article

Antinociceptive Effect of Methanol Extracts of *Parquentina nigrescens* (afzel) bullock (Periplocaceae) Fruit Bark

Lucky O Okunrobo*, John O Uwaya and Precious E Ehimhen
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, university of Benin, Benin City. Nigeria.

*For correspondence: *Email:* okunrobo@uniben.edu Tel: +234-8034725416

Abstract

Purpose: To investigate the antinociceptive activity of the methanol fruit bark extract of *Parquetina nigrescens.*
Methods: The methanol fruit bark extract of *Parquetina nigrescens* was screened for antinociceptive activity using formalin and acetic acid-induced pain method at doses 50mg/kg, 100mg/kg and 200mg/kg in Swiss albino mice.
Results: The results obtained shows significant ($p< 0.05$ and $p< 0.01$) dose dependent antinociceptive activity of the extract of the fruit bark.
Conclusion: The study has demonstrated that the methanol extract of fruit bark of *Parquetina nigrescens* exerts potential dose-dependent analgesic effect in animal models both centrally and peripherally.

Keywords: *Parquentina nigrescens*, fruit bark, antinociceptive action, formalin induced pain.

Indexing: Index Copernicus, African Index Medicus

Introduction

Parquetina nigrescens (Periplocaceae), a shrub found in equatorial West Africa [1,2], has been used in traditional medicine practice for centuries. It is a perennial plant with twining stems and a base tapering 10-15cm long and 6-8cm broad. It has smooth long stems on which the leaves are located. Its flower grows from its side with branches having whitish outside and inner reddish colouration. The fruit which have th featherlike seed [3] is composed of two parts; an outer woody and an inner softer part. *Parquetina nigrescens* is known as kwankwanin in Hausa, Mgbidim gbe in Igbo, Ewidun in Yoruba, Inuwu elepe in Yoruba (Ife) [3], and Olilia Or Ovie ukpakoma in Avianwu (Etsako) languages in Nigeria. The leaves, roots and the latex are the parts of the plants commonly used for traditional medicine [4]. It is a constituent of a commercial herbal preparation (Jubi formula $^{(R)}$) in Nigeria used in the treatment of anaemia in humans [5]. Investigations have revealed many properties of the plant including haematinic properties [5, 6], cardiotonic and catecholamine-like effects [7], uterine spasmogenic action [8], antisickling activity [9,10]. Antioxidant [11], increase in erythrocyte indices [12], antibacterial and antifungal activity [13, 14], hypoglycemic activities [3], anti-inflammatory and antipyretic effects [15] etc. We recently reported phytochemical constituents of *Parquetina nigrescens* fruit as containing reducing sugars,alkaloids, saponins and cardiac glycosides and the proximate analysis [16].

In most of the scientific investigation carried out on the plant, the leaves were mainly used. This might be because the leaves are mostly used traditionally. Currently, there is little or no report on the activity using the fruit bark. The purpose of this study therefore was to screen for the antinociceptive activity of the methanol fruit bark extract of *Parquetina nigrescens.*

Experimental

Collection and processing of plant material

The fruits of *Parquetina nigrescens* were collected from Iraokhor, Etsako central Local Government Area of Edo state in December 2011. The fruits were identified by Dr A Bamidele of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city. They were air dried under a shade for 3 weeks and the featherlike seeds were separated from the fruit bark. The air dried fruit bark was thereafter reduced to powder using an electric mill and the resultant powder was weighed and stored in an air tight container until used.

Extraction of plant material

200g of the powdered fruit bark of *Parquetina nigrescens* was extracted with methanol using a soxhelt apparatus. The extract obtained was concentrated using rotary evaporator and the yield of 28g (14%) obtained. The crude extract was stored in a refrigerator at 4 ^{o}C pending the time for further investigations.

Animals

Swiss albino mice weighing between 25-30 g of both sexes (pregnant females excluded) were used for this study. The animals were obtained from the animal house, department of anatomy, Faculty of Basic Medical Sciences university of Benin. The animals were maintained under standard environmental conditions and allowed free access to feed (growers mash from Bendel feed and flour mill) and water.
The animals were allowed to acclimatize for 2 weeks and were fasted overnight, (but allowed fresh water) prior to commencement of antinociceptive study. Five animals per group were used for the study. Ethical approval was obtained for the study from the Animal Use and ethics committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Formalin Induced Pain in Mice

The method of Okunrobo *et al* [17] was used. Formalin (20 µL of 2 %) was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting of the injected paw was taken as an indicator of pain response. Responses were taken for 5 minutes after formalin injection (first phase) and 15-30 minutes after formalin injection (second phase). The test extract (50, 100 and 200 mg/kg), acetylsalicylic acid (100 mg/kg) or 5% Tween (0.2ml) was administered orally by gavage 60 minutes before formalin injection. 5 % Tween 80 was used as negative control. All data were expressed as mean ± SEM and the percentage inhibition of pain was calculated thus:

$$\%inhibition = \frac{(N - Nt)}{N} \times 100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where N = average time (in seconds) of licking and biting in control group, and Nt = average time (in seconds) of licking and biting in test group.

Acetic Acid Induced Writhing Response in Mice

The method of Okunrobo *et al* [18] was used. Tween 80 (5%, 0.2ml), acetylsalicylic acid (100 mg/kg) or the extract (50, 100 and 200 mg/kg) were administered orally 60 minutes before inducing pain. 10 ml/kg of 0.6 % acetic acid saline was then injected intraperitonally. The number of writhings (indicated by the stretching and aching of the back of the animal) was counted for 30 minutes after acetic acid injection. Acetylsalicylic acid (100 mg/kg) was used as a reference drug while 5 % Tween 80 was used as negative control. The percentage inhibition (% analgesic activity) was calculated as in equation 1 where N is the mean number of writhing in control group and Nt is the mean number of writhing in test group.

Statistical analysis

All values were expressed as mean % response of 5 experiments ±SEM (standard error of mean). Comparisons were made using one-way ANOVA with Dunnett's Multiple Comparison Test and Student t-test, where appropriate $P<0.05$ indicated statistical significance in all cases.

Results

The methanol extract of the fruit bark of *Parquetina nigrescens* inhibited both the early and late phase of the formalin test at doses of 50 – 200 mg/kg in a dose dependent manner. Acetic acid induced model revealed that the crude methanol extract of the fruit bark of *Parquetina nigrescens* inhibited the writhing response in mice at doses of 50 – 200 mg/kg, with the highest activity at 200 mg/kg.

Discussion

Test for Analgesia
Pain is a subjective symptom that is affected by psychological factors. A wide variety of chemical agents could be used to alleviate or ameliorate pain. These agents mediate their effect through central or peripheral mechanisms. The complex nature of the chain of central and peripheral mechanisms that underline pain sensation and the fact that no simple test is good enough to predict the efficiency of a test agent in humans makes the use of various experimental models imperative when screening a drug for pharmacologic activity [19].

The mechanism for testing analgesic was selected such that both centrally and peripherally mediated effects were investigated. The acetic acid induced abdominal constriction (writhing) method elucidated peripheral activity, while the formalin test investigated both peripheral and central activity [20].

Formalin induced pain in mice

In the formalin test there is distinctive biphasic nociceptive response termed neurogenic and inflammatory phases. Drugs that primarily act on central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [21].The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive

Table 1: Effect of the methanol extract of the fruit bark of *Parquetina nigrescens* on formalin induced pain.

Treatment (p.o)	Licking time		% Inhibition	
	Early phase (0 – 5 mins)	Late phase (15 – 30 mins)	Early phase	Late phase
5% Tween 80 10ml/kg	97.50 ± 25.25	34.00 ± 20.46	-	-
P. nigrescens 50mg/kg	65.50 ± 16.30	21 ± 13.57	32.82	38.24
P. nigrescens 100mg/kg	50.00 ± 12.68*	13.00 ± 4.44*	48.71	61.76
P. nigrescens 200mg/kg	41.75 ± 5.45*	7.25 ± 3.75**	57.18	78.68
Acetyl salicylic acid 100mg/kg	77.25 ± 8.78	6.75 ± 2.10**	20.77	80.15

*Results expressed in mean ± SEM, n = 5, *p < 0.05, p < 0.01*

Table 2: Effect of the methanol extract of the fruit bark of *Parquetina nigrescens* on acetic acid induced writhing test

Treatment (p.o)	Mean number of Writhes per 30 min	Percentage Inhibition of Pain (%)
5% Tween 80 10ml/kg	39 ± 5.87	-
P. nigrescens 50mg/kg	26.2 ± 13.2	32.82
P.nigrescens 100mg/kg	23.2 ± 4.75	40.51
P.nigrescens 200mg/kg	18.6 ± 6.45*	52.31
Acetylsalicylic acid 100mg/kg	5.6 ± 1.08**	85.64

*Results expressed in mean ± SEM, n = 5, *p < 0.05, **p < 0.005*

neurons [22-25]. Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the late phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [23,25].

From the results obtained, the methanol extract of the fruit bark of *Parquetina nigrescens* inhibited both the early and late phase of the formalin test at doses of 50 – 200 mg/kg in a dose dependent manner. This indicates that the methanol extract inhibits both the peripheral and the central pain mechanism. The highest inhibition (57.18 % early phase and 78.68 % late phase) was observed when 200 mg/kg of the extract was used, although acetylsalicylic acid (positive control) at a dose of 100 mg/kg inhibited the late phase strongly (80.15 %) than the extract at the 200 mg/kg dose. Acetylsalicylic acid had a low inhibition (20.77 %) at the early phase. This may likely be as a result of its peripherally mediated mechanism. The aqueous extract of *Parquetina nigrescens* at doses of 50 – 200 mg/kg produced significant antinociception in the formalin test [15].

Acetic acid induced writhing in mice

The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesic. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels [26, 28]. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE_2 and $PGF_{2\alpha}$ and their levels increase in the peritoneal fluid of the acetic acid induced mice [29]. The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins.

From the results obtained using the acetic acid induced model, the crude methanol extract of the fruit bark of *Parquetina nigrescens* inhibited the writhing response in mice at doses of 50 – 200 mg/kg. The highest inhibition was seen at the 200mg/kg dose (52.31 %). This value was low when compared with results obtained for second phase of the formalin induced test (78.68 %). Although acetylsalicylic acid had a higher inhibition of 85.64 % for the acetic acid induced test. This may therefore imply that the mechanism of analgesia of the extract may not be solely by prostaglandin inhibition but rather a combination of both central and peripheral mechanisms as seen. The result obtained is comparable to that of Owoyele et al [30] which shows that the ethanol leaf extract of *Parquetina nigrescens* significantly inhibited acetic acid-induced irritation of paw of rats at (50, 100 or 200 mg/kg BW).

Conclusion

Results of this study have demonstrated that the methanol extract of fruit bark of *Parquetina nigrescens* exerts potential dose dependent analgesic effect in animal models both centrally and peripherally.

Conflict of Interest

There is no conflict of interest associated with this work

Contribution of Authors

We 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 the authors. LO Okunrobo conceived and designed the study, JO Uwaya analysed the data

while PE Ehimhen collected the data. All authors approved the final manuscript for publication.

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