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Antinociceptive and antipyretic properties of ethanol extract of *Oryza bathii* (Poaceae) in wistar rats

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Abstract
Ethanol extract of *Oryza barthii* obtained by cold maceration was investigated for antinociceptive and antipyretic activities using the hot plate and brewer's yeast-induced hyperthermia methods, respectively in adult wistar rats. The medications used as positive control were piroxicam at 20 mg/kg intra-peritoneal (i.p) for the antinociceptive study and aspirin at 100 mg/kg i.p. for the antipyretic study and both induced significant delay in the reaction time of the rats to thermal stimulus and hyperthermia respectively. *Oryza barthii* (Poaceae) extract administered at dosages of between 125 – 500 mg/kg i.p, significantly delayed the reaction time of rats to thermal stimulus produced by the hot plate and reduced the hyperthermia in a dose-dependent manner. The results showed that *O. barthii* possesses antinociceptive and antipyretic activities, thus justifying the folklore use of the plant in traditional medicine for the control of fever and can be an alternative medicament in the management of pyrexia.

Keywords: Analgesic, Antinociceptive, Antipyretic, intraperitoneal, *Oryza barthii* extract
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Introduction

The use of medicinal plants in curing diseases is as old as man (Odugbemi & Akinsulire, 2006). According to the World Health Organisation (WHO), about 80% of the world's population living in developing countries rely essentially on plants for their primary health care (Owolabi *et al.,* 2007). At present, it is easier to determine the efficacy and safety of herbal remedies because their beneficial or adverse effects can be traced to their respective constituent compounds (Rodriguez-Fragoso *et al.,* 2008).

Oryza barthii is an annual, erect to semi-erect wild rice that grows up to 150cm tall in tufts. The stem is erect or geniculately. It ascends with roots from the lower nodes, spongy, striates glaborous. This wild rice is native to sub-Saharan Africa, and is found in savanna woodland, or fadama. The plant is endemic in inland areas of West Africa (Odugbemi & Akinsulire, 2008). It is found growing abundantly across tropical Africa from Mauritania east, to Ethiopia and south to Bostwana and Zimbabwe (Odugbemi & Akinsulire, 2006) and follows the Niger river banks to Nigeria. *O. barthii* grows in shallow or deep water, seasonally flooded land, stagnant water, and slowly flowing water or pools; it prefers clay or black cotton soils, and is found in open habitats (Kamatenesi-Mugisha & Oryem-Origa, 2005).

The seed from this plant if collected in reasonable quantity is said to be edible especially during famine periods. Before now the plant had been believed to be of no medicinal value as there are only paucity of information on its medicinal properties.

It is, therefore, in the light of the increasing importance of the plant and their products as sources of alternative therapeutic remedies that this study was carried out, in order to validate its uses in the management of pain in traditional phytomedicine.

Materials and Methods

Collection of plant sample and identification

The plant was obtained from a swampy area along a river bank in Unguwan Romi village Kaduna, Kaduna state, Whole plant of *Oryza barthii* were collected and submitted for identification at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and the voucher number of 1611 was obtained.

Plant extraction procedure

The fresh whole plant specimen collected was cured in the sun for 12 hours and, thereafter, dried in an empty, but airy room. The dried specimen was collected and grounded to a coarse powder. The total weight of the powdered plant obtained was 156 g.

Cold extraction was carried out using maceration technique as described by Handa (2008). One hundred and fifty grams of the coarse powder of *O. bathii* was soaked in 2 litres of 97% ethanol solution, and allowed to stand for 72 hrs (during this period the solution was agitated twice daily) collection was via decantation and filtration. This procedure was repeated twice before discarding the plant material. The filtrate was concentrated using a rotary evaporator in a water bath at 67^{o}C and dried to a green residue using a desiccant. The total weight of the green residue was 6.7g which was equivalent to 4.46 % yield. The extract was then stored in the refrigerator at 4^{o}C until the time of use.

Phytochemical screening

The ethanol extract of *Oryza barthii* was evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, glycosides, sterols and triterpenes using standard procedures as described by Trease & Evans (2002) and Tiwari *et al. (*2011).

Storage of the extract

The extract was stored by wrapping it in foil paper and kept in an air-tight container to minimize absorption of water from the environment.

Experimental animals

Adult Wistar rats of both sexes, weighing 197 – 210 g were obtained from the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria and housed in the Theriogenology animal room of the Faculty of Veterinary Medicine at room temperature (26 ± 3°C). They were kept for two weeks to acclimatize during which the rats were screened for parasites, and treated accordingly. They were given access to growers' mash with maize bran and water *ad-libitum*. The animals were deprived of feed overnight before the test, but were given free access to water during the day. The experiment was conducted with the approval of the research ethics and animal use committee of the Department of Veterinary Surgery and Radiology of Ahmadu Bello University Zaria, Nigeria, with the approval number VSR/14/ 0012.

Acute toxicity (LD_{50}) studies in rats

Acute toxicity (LD_{50}) study of the extract was determined by intraperitoneal administration of the extract to rats (Lorke, 1983). The rats were fasted overnight and the LD_{50} evaluation carried out in two phases as follows: In the first phase, three groups of three rats each were treated with the extract at 10, 100 and 1000 mg/kg weight by weight (w/w), respectively administered intraperitoneally (i.p) and were monitored for 24 hours for signs of toxicity such as paw licking, sedation, convulsion, decreased locomotor activity, salivation and mortality. In the second phase, three groups of one rat each were treated with the extract at 1600, 2900 and 5000 mg/kg b.w respectively. The rats were also monitored for 24 hours for the development of signs of toxicity. The LD_{50} value was then calculated as the geometric mean of the highest non-lethal dose that is the highest dose that did not result in mortality and the lowest lethal dose, which was the lowest dose that resulted in mortality.

Antinociceptive activity

Behavioural testing to evaluate the antinociceptive activity was carried out using hot-plate method. The hot-plate test was carried out in two phases following intra-peritoneal (i.p) administration of the extract (Le Bar *et al.,* 2001). In the first phase, the rats were divided into three groups of four animals each. Normal saline 10 ml/kg i.p was given to the negative control group, piroxicam 20 mg/kg i.p was given to the positive control group, while the extract (500 mg/kg) was also administered i.p to the test group. In the second phase, graded dosages of the extract were administered i.p to three groups of four animals each, at 125 mg/kg, 250 mg/kg and 500 mg/kg.

Thirty minutes after treatment, the animals were placed in a beaker, which was placed on the hot plate (Bioseb®) with the temperature maintained at 52.5^{o}C ± 5^{o}C. The reaction time was the time taken

by the rats to begin the licking of their front paws or jump out of the beaker. The duration of endurance of the heat stimulus (heat endurance) was recorded in seconds by taking readings at 30 minute intervals; from 30 minutes, up till 180 minutes after administering the drugs (Vogel, 2008).
Ceiling time that is, the maximum time rats are allowed in the beaker in the absence of any reaction was 20 seconds, in order to avoid tissue damage to the rat's paws (Agbaje & Ajidahun, 2011).

Antipyretic activity
Brewer's yeast-induced pyrexia method was used to evaluate the antipyretic activity. The rectal temperature of the rats was recorded before the administration of the extract or standard drugs using an electronic digital thermometer (Kruuse® inc Germany). Hyperthermia was induced in the rats by subcutaneous injection of 20% brewer's yeast, suspended in normal saline at 10 ml/kg (Vogel, 2008; Archana *et al.,* 2005). Food was immediately withdrawn and the temperature rise was monitored 24 hours after yeast injection. Rats showing a rise in rectal temperature below 0.5°C above their initial readings were removed as this were counted as not significant. The experiment was carried out in two phases.
In the first phase, the rats were divided into three groups of four rats each. Normal saline at 10 ml/kg was administered to the negative control group; aspirin (Unicure® India) at 100 mg/kg i.p was administered to the positive control group; while the extract at 500 mg/kg was also given i.p to the study group. In the second phase, graded dosages of the extract were administered to three groups of four rats each at 125 mg/kg, 250 mg/kg and 500 mg/kg. All the drugs and extracts were administered i.p. rectal temperatures of the rats were recorded at 30 minute intervals from 30 minutes, up till 180 minutes after administering the various treatments.

Statistical analysis
Data were expressed as mean ± SD. Results were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 by one-way Analysis of Variance (one-way ANOVA). Statistical significance was determined and values with $P < 0.05$ considered significant (Tello & Crewson, 2003).

Results
Phytochemical screening
Preliminary phytochemical analysis of the ethanol extract showed the presence of sterols, flavonoids, triterpenoids, tannins, carbohydrates, saponins and alkaloids.

Acute toxicity test
No death was recorded in both phases of investigation. However, signs of toxicity that was observed included dizziness, pruritus, jerky extension of the head and neck, paw licking, decreased locomotor activity and laboured breathing. The LD_{50} of the ethanol extract of *Oryza barthii* was greater than 5000 mg/kg.

Antinociceptive test
Hot-plate method: The administration of the crude extract (*O. bathii*) at 125mg/kg failed to elicit any significant changes, but at 250 mg/kg the crude extract exhibited a significant ($p<0.001$) increase in the heat endurance time at 90 mins of 1.45 ± 0.12 seconds, while *O. bathii* at 500 mg/kg induced significant increases compared with the negative control group at 90, 120, 150 and 180 minutes as 1.59 ± 0.14, 1.43 ± 0.11, 1.24 ± 0.09 and 1.11 ± 0.07

Table 1: Antinociceptive effect on different treatment groups at the respective observation times (in seconds)

Time post treatment (mins)	Normal saline (10 ml/kg)	Piroxicam (20 mg/kg)	Extract (125 g/kg)	Extract (250 mg/kg)	Extract (500 mg/kg)
30	0.50 ± 0.08	0.89 ± 0.12	0.54 ± 0.12	0.54 ± 0.06	0.71±0.08
60	0.60 ± 0.09	1.05 ± 0.18*	0.64 ± 0.10	0.66 ± 0.08	0.86±0.17
90	1.07 ± 0.17	1.59 ± 0.21***	1.12 ± 0.15	1.45±0.24**	1.59±0.29***
120	0.94 ± 0.13	1.45± 0.17***	0.96 ± 0.16	1.24±0.21	1.43± 0.22***
150	0.80 ± 0.10	1.45 ± 0.14***	0.84 ± 0.13	1.05±0.26	1.24± 0.17**
180	0.70 ± 0.11	1.19 ± 0.16**	0.73 ± 0.17	0.84±0.20	1.11± 0.13**

Data values shown represent mean ± SD (n =4). Where ** indicated $p < 0.01$ and *** indicated $p < 0.001$ when compared with the negative control using hot plate test (Two-way ANOVA followed by Bonferroni multiple comparison test). Extract = ethanolic extract of *Oryza barthii*

Table 2: Effect of the extract of Oryza *bathii* on rectal temperature (°C) of wistar rat

Time interval (Mins)	Normal saline (10ml/Kg)	Aspirin (100 mg/kg)	Extract (125 mg/kg)	Extract (250 mg/kg)	Extract (500 mg/kg)
0	37.10 ± 0.08	37.23 ± 0.33	37.18 ± 0.17	37.10 ± 0.14	37.20 ± 0.32
30	38.63 ± 0.22	38.80±0.34	38.48±0.22	38.60±0.18	38.85± 0.31
60	38.88± 0.25	39.23±0.44	38.75±0.24	38.93±0.21	39.25± 0.25
90	39.20± 0.29	38.85± 0.64	39.00±0.10	39.35±0.27	39.10± 0.52
120	39.58± 0.25	38.48± 0.60***	39.43±0.12	39.45±0.37	38.75± 0.21**
150	39.60± 0.21	38.08± 0.48***	39.73± 0.06	39.15± 0.45	38.30± 0.22***
180	39.35± 0.29	37.65± 0.38***	39.47± 0.38	38.63± 0.38*	37.90±0.08***

Data values shown represent mean ± SD (n = 4). Where * indicated $p < 0.05$, ** indicated $p < 0.01$, and *** indicated $p < 0.001$ when compared with the negative control (normal saline) using the brewer's yeast treated test (Two-way ANOVA followed by Bonferroni multiple comparison test). Extract = ethanolic extract of *Oryza barthii*

seconds, respectively (Table 1, Figure 1). The standard drug which was piroxicam also elicited changes in the heat endurance time throughout the course of the experiment.

Antipyretic test

The xtract at 125 mg/kg, the extract *O. barthii* had no significant antipyretic effect. At 250 mg/kg, it exhibited significant ($p<0.05$) antipyretic effect at 150 minutes' post-administration of 38.63 ± 0.19^{o}C. However, *O. barthii* at 500 mg/kg, exhibited significant ($p< 0.01$ and $p< 0.001$) decreases in rectal temperature that were observed at 120, 150 and 180 minutes' post administration of 38.75 ± 0.10, 38.30 ± 0.10 and 37.90 ± 0.04^{O}C respectively (Table 2). Similar effects to that of the plant extract were observed in the standard test group of aspirin at 100mg/kg.

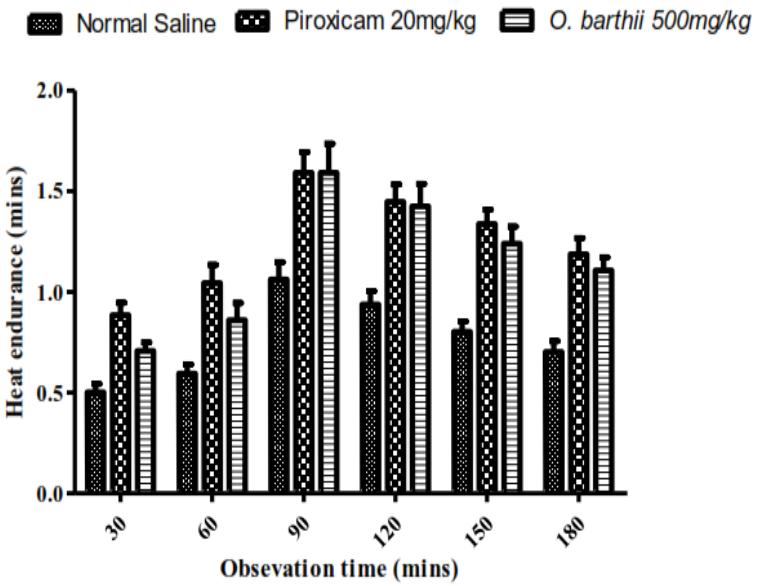

Figure 1: Comparative analgesic effects between the control groups and the extract at 500mg/kg using the hot plate test

Discussion

The analgesic effect of the extract was achieved by inhibiting the cyclooxygenases and prostaglandins, which are mediators of both pain and pyrexia. This is attributable to the fact that the hot-plate assay is a somatic pain model that does not produce inflammation. The repetitive afferent input enhances the response of the animal to noxious stimulus (radiant heat on a hot-plate). The hot plate-induced pain also indicates the presence of narcotic involvement (Flores *et al.,* 2004). Activation of prostanoid receptors by this stimulus increases the opening of voltage sensitive Ca^{2+} channels and enhances primary afferent peptide release. It may be possible that the extract exerts its therapeutic action by antagonizing prostanoid receptors, or inhibiting the synthesis of prostaglandins (Tamma *et al.,* 2003; Kumar *et al.,* 2011). Piroxicam which is a potent long-acting NSAID and a short-acting analgesic, is known to exert its analgesic activity by reversibly inhibiting the cyclooxygenase enzymes, as well as by lowering the concentration of prostaglandins (Candelario-Jalil *et al.,* 2005).

The significant analgesic effect exhibited by the extract indicates that the extract contains high amount of active constituents that can inhibit both the centrally acting pain stimulus and tonic pain induced by the hot plate test (Kumar *et al.,* 2011). The significant antinociceptive activity demonstrated by the extract might therefore be due to the presence of flavonoids and phenolic substances in the extract (Taesotikul *et al.,* 2003; Trapero-Mozos *et al.,* 2012). In both experiments, the ethanol extract showed significant analgesic and antipyretic effects, which was comparable to those observed for the non-steroidal anti-inflammatory drugs piroxicam and aspirin, respectively. This was observed in both tests when the highest dosage of 500 mg/kg of the extract was administered.

The ethanolic extract of *Oryza barthii* showed antipyretic effect that compared favorably with aspirin. Although no direct evidence exists to support the claim that the extract exerts its effects by interfering with prostaglandin synthesis in the hypothalamus, it may possess components that are responsible for this effect. The observation of the effect of the extract as compared to that of aspirin agrees with the fact that aspirin exerts its antipyretic effect by inhibiting prostaglandin synthesis in the hypothalamus. In a related study it was reported that *Dalbergia odorifera* extract inhibit the synthesis of prostaglandin (Bonazzi *et al.,* 2000; Hajare *et al.,* 2000; Simmons *et al.,* 2004).

The mechanism of action of aspirin involves the covalent modification of cyclooxygenase (COX) as a result of the interactions of aspirin with COX isoforms. Aspirin irreversibly inhibits COX-1 by acetylating an active site serine, which resides in the arachidonic acid-binding pocket (Bonazzi *et al.,* 2000). This reaction appears to cause steric hindrance to the binding of arachidonic acid, thereby blocking COX-1 activity (Parihar *et al.,* 2010).

Unlike other Non-steroidal anti-inflammatory drugs (NSAIDs), aspirin does not influence the peroxidase activity of COX-2 (Candelario-Jalil *et al.,* 2005), which converts prostaglandin G2 (PGG2) into prostaglandin H2 (PGH2). Therefore, after aspirin treatment, the unchecked peroxidase activity of COX-2 can continue to generate free radical species and PGH2. At low doses of aspirin, which are known to be more effectively inhibit COX-1, rather than COX-2 (Demasi *et al.,* 2000), large amounts of PGG2 (formed by the cyclooxygenase activity of COX-2) are then converted into PGH2 by the peroxidase activity of COX-2 and further metabolized by the terminal prostaglandin synthases.

It is concluded that the analgesic and antipyretic effects observed, may be as a result of the presence and activity of flavonoids and terpenoids which are known to target prostaglandins, the mediators of pyrexia and pain. In addition, the antipyretic activity of the extract may be achieved by way of enhancing the production of the body's own antipyretic substances like vasopressin and arginine as reported by Chandrasekharan *et al.* (2002).

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