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Anti-nociceptive Activity of Combined Methanol Extract of Edible Fruit Pulps of some Medicinal Plants

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ABSTRACT

The fruit pulps from *Adansonia digitata*, *Hyphaene thebaica*, *Ziziphus mauritiana* and *Ziziphus spina-christi* are consumed locally in North-East Nigeria and are regarded as analgesics singly and as a recipe in folkloric use. This study investigated the anti-nociceptive properties of the combined methanol extract of the fruit pulps from these plants. Equal amount (200 g) of each fruit pulp was used to prepare methanol extract of the recipe. Standard methods were used for phytochemical analysis and LD_{50} determination. Acetic acid-induced writhing, analgesiometer and formalin test were used to study the anti-nociceptive activities in rats and mice at doses of 200, 400 and 800 mg/kg body weight. The phytochemical analysis revealed the presence of saponins, glycosides and terpenoids. The LD_{50} values were greater than 5000 mg/kg in both rats and mice using oral and intraperitoneal routes respectively. There was significant ($p < 0.05$) dose dependent anti-nociceptive activity at 200, 400 and 800 mg/kg in acetic acid induced writhing, analgesiometer and second phase of formalin test. The recipe's activity was greater than that of diclofenac (control) in writhing test at 200 mg/kg and analgesiometer test at 400 mg/kg. The methanol extract of the fruit pulps recipe was safe and had dose-dependent anti-nociceptive activities in laboratory animals supporting the folkloric use of these fruits and recipe in the treatment of pain.

Keywords: Anti-nociception, fruit pulps, methanol extract, recipe, rats, mice.

Introduction

Pain, inflammation and fever are hallmark symptoms of infection or physical injury. These symptoms can manifest simultaneously or independent when provoked. Treatment involve the use of certain drugs such as non-steroidal anti-inflammatory drug (NSAID) e.g. salicylate, narcotics (opioids) e.g. morphine or corticosteroids e.g. hydrocortisone.[1,2] Toxic and unwanted side effects of these drugs limit their use.[3,4] The use of plants and plant products as source of medication is as old as human existence. Although crude approach is often imbibed in using them, many of these applications yielded positive results. Well above 50 % of the world's population depends on plants for their medication.[5] Different parts of plants have been put to use as medicines. Traditionally, plants are used singly or in combination as recipe following a set of instructions on its preparation.

Adansonia digitata L., *Hyphaene thebaica* L., *Ziziphus mauritiana* L. and *Ziziphus spina-christi* L. are plants whose different parts have been reported to have wide range of medicinal benefits, which include anti-microbial, anti-oxidant, anti-inflammatory, analgesic, ant-diabetic and lots of other uses.[6-11] These four plants are respectively called in Hausa in Northern Nigeria as "Kuka", "Goruba", "Magarya" and "Kurna" while their respective English names are baobab, doum palm, jujube/Chinese date and Christ's thorn. People in North East Nigeria consume the fruit of these plants and believed to have nutritional and analgesic benefits. However, there is paucity of scientific data on the medicinal benefit of the edible parts of these plants especially the fruit pulps in their single form or in combination with each other. Scanty literature on the acute toxicity of the different fruit pulps in rodents showed LD_{50} values of greater than 5000 mg/kg.[12-14] The present study was aimed at determining the acute toxicity and anti-nociceptive activities of a methanol extracted recipe from the edible fruit pulps of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* plants.

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Materials and Methods

Sample collection

The fruit pulps of *A. digitata*, *Z. mauritiana* and *Z. spina-christi* were collected in dried form from Gamboru market, Maiduguri, Borno State, Nigeria and *H. thebaica* was collected from Geidam, Yobe State, Nigeria. The plants were authenticated by Prof. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri. Voucher specimens were deposited at the Research laboratory, Department of Chemistry, University of Maiduguri, Nigeria. The voucher numbers are CHM/12/032 (*H. thebaica*), CHM/12/006 (*A. digitata*), CHM/12/023 (*Z. mauritiana*) and CHM/12/028 (*Z. spina-christi*).

Experimental animals

Albino rats (53) (102-182 g) and mice (28) (15.3-19.4 g) of both sexes were purchased from the Animal House, Department of Biochemistry and Department of Veterinary Pharmacology and Toxicology,

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University of Maiduguri. They were kept in plastic cages at the Veterinary Pharmacology Laboratory, University of Maiduguri for a minimum period of one week before the commencement of the experiments. They were fed with rat pellets (Vital Feeds Nig Ltd Jos, Nigeria) and water ad libitum. The experiments were conducted in compliance with the international guiding principles for biochemical research involving animals.[15] Ethical clearance was obtained from the Research and Ethics Committee, Faculty of Pharmacy, University of Maiduguri with approval number FP/022018/TETFP03

Extract preparation

The air-dried fruit pulps of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* were pulverized using wooden mortar and pestle. Equal amount (200 g) of each powdered sample were combined together in a container and shaken to form uniform mixture. The powdered plant mixture (800 g) was extracted exhaustively with methanol using Soxhlet apparatus as previously described and adopted.[16,17] The resultant extract which was regarded as a recipe was filtered using Whatman filter paper (No. 1) and the filtrate was concentrated to dryness in-vacuo at 40 °C using rotary evaporator (R201D PEC Medicals U.S.A.)

Phytochemical analyses

Equal amount of powered samples of each fruit pulp was thoroughly mixed together. The qualitative phytochemical analyses of the recipe were carried out according to standard methods.[18-20] The methods include Molisch's, Barfoed's, Fehling's and Selivanoff's tests for carbohydrates; Shinoda's, ferric chloride, lead acetate and sodium hydroxide tests for flavonoids; test for terpenoids with sulphuric acid; Lieberman Burchard and Salkowski tests for cardiac glycosides; Dragendorff's reagent and Mayer's reagent tests for alkaloids; frothing test for saponins; and Borntrager's test for anthraquinones.

Acute toxicity study

The median lethal dose (LD_{50}) was carried out in rats (oral) and mice (i.p.) based on modified Organization for Economic Co-operation and Development (OECD) guidelines.[21] The extract was dissolved in distilled water (300 mg/mL and 100 mg/mL for rats and mice, respectively). A total of three rats and three mice were dosed with 5000 mg/kg of the extract orally and intraperitoneally, respectively. They were observed closely for 24 h and then kept for 14 days for signs of toxicity such as change in behaviour, feeding, coat condition and mortality.

Determination of anti-nociceptive properties

Acetic acid-induced writhing test

Twenty-five adult mice (15.3-19.4 g) of both sexes were randomly separated into five groups of five mice each. The mice in group A received distilled water (0.1 mL) to serve as control group while the mice in groups B, C and D received 200, 400 and 800 mg/kg body weight (BW), respectively, of the extract, and the mice in group E received diclofenac 25 mg/kg BW to serve as the positive control group. The route of administration was intra-peritoneal (i.p) on the right side of the abdomen. Thirty minutes after treatment, 0.1 mL of 1% acetic acid solution was administered (i.p, on the left side of the abdomen) to all mice to induce writhing. After five minutes of acetic acid administration, writhing movement was counted, stopped at ten minutes.[22] A reduction in the number of writhing as compared with the negative control group was considered as evidence of analgesia and the percentage protection was determined using the formula below.[23]

$$\% \text{ inhibition} = \frac{W_C - W_T}{W_C} \times 100$$

Where:

W_C = Mean number of writhes in control group

W_T = Mean number of writhes in test group

Analgesiometer test

Twenty-five rats (102-182 g) of either sexes were randomly divided into five groups of five rats each and the modified methods of Randall-Selitto and Adzu were used.[24,25] Administration was by oral route. Group A rats received distilled water (0.1 mL) to serve as control group while rats in groups B, C and D received 200, 400 and 800 mg/kg body weight (BW), of the extract, respectively, rats in group E received diclofenac 50 mg/kg BW to serve as the positive control group. The rats' left hind paw was placed between the plinth and plunger of the Analgesiometer (Ugo Basile, Italy, model 37215) and then pressure exerted on the middle dorsum of the paw until signs of pain were noticed. Stimulus was terminated and force threshold readings in grams were taken as soon as nociceptive response was elicited. Readings were taken at pre-treatment, 30 min and 60 min post treatment. Increase reading of the analgesiometer at 30 and 60 min compared to pre-treatment was indicative of anti-nociception.

Formalin test

Twenty-five (102-182 g) rats of both sexes were separated at random into five groups of five rats. Treatment was the same as for the Analgesiometer test above. After 30 minutes each rat in all the groups was injected 0.8 µL of 1% formalin into the plantar surface of the left hind paw.[26] The rat was observed for the first five minutes and then from 20 to 30 minutes after formalin administration. The time spent in licking and lifting the injected paw was recorded in seconds during these periods.

Statistical analysis

The data were expressed as mean ± standard error of the mean (S.E.M.) and analysed statistically by one-way analysis of variance (ANOVA) and Dunnett post hoc-test. Graphpad Prism Computer statistical software package was used for the analysis and $P < 0.05$ was considered significantly different.[27]

Results and Discussion

Results of phytochemical screening

The extract yield was 21% w/w water soluble brown gummy mass. The phytochemical constituents detected are presented in Table 1. The results showed that extract contained cardiac glycosides, saponins, terpenoids, flavonoids and carbohydrates while alkaloids, soluble starch and anthraquinones were absent in the extract. These phytochemicals are very important in mediating pharmacological activities of the extract. For instance, flavonoids have free radical scavenging, anti-oxidant and anti-inflammatory activities.[28]

Acute toxicity activities of the recipe

The administration of the extract for determination of median lethal dose (LD_{50}) did not cause mortality at 5000 mg/kg dose up to 14 days' post treatment. Hence the LD_{50} values were greater than 5000 mg/kg in both rats and mice based on OECD method.[21] Depression was observed on the first day of administration. This indicates that the extract is safe for up to 5000 mg/kg body weight making it a suitable lead for drug development and discovery. Acute toxicity values (LD_{50}) of the different fruit pulps have been reported to be above 5000 mg/kg.[12-14] This implies interactions among different constituents in the recipe may not result in the formation of toxic principles since the recipe is safe up to 5000 mg/kg.

Anti-nociceptive activity of the recipe

The result of the effect of the extract on acetic acid-induced writhing in mice is shown in Figure 1. The extract showed an inhibition of the number of writhing at the doses of 200, 400 and 800 mg/kg with dose-dependent percentage inhibitions of 56.96%, 83.50%, and 89.97%, respectively while the standard drug, diclofenac (25 mg/kg) showed inhibition of 48.22%. Number of writhes in treated and positive control groups were significantly ($p < 0.05$) lower than the negative control group. The percentage inhibition by the recipe was all higher than that of the standard drug, diclofenac.

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Table 1: Phytochemical of the methanol extracts of a recipe from fruit pulps of *A. digitata, H. thebaica, Z. mauritiana* and *Z. spina-christi*

Phytochemical constituent	Result
Flavonoids	+
Cardiac glycoside	+
Terpenoids	+
Alkaloids	-
Carbohydrate	+
Saponins	+
Soluble starch	-
Free anthraquinones	-
Combined anthraquinones	-

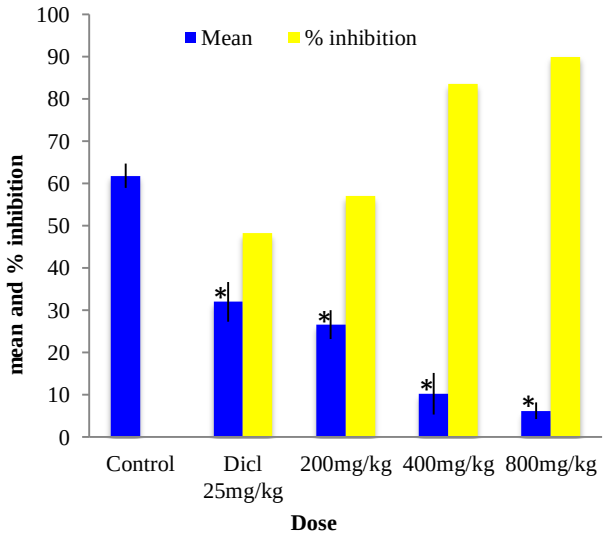
+ indicates presence; - indicates not detected

The result of the formalin test is shown in Figure 2. At doses of 400 and 800 mg/kg, the extract showed significant inhibition ($p < 0.05$) in the second phase of the formalin test from 341.20 ± 71.98 sec in the negative control group to 248.00 ± 64.34 sec and 233.00 ± 43.59 sec, respectively. There was significant inhibition ($p < 0.05$) in the positive control (diclofenac) group during both phases with respective values of 132.40 ± 15.06 and 213.00 ± 11.42 sec.

The route to inflammation, pain and pyrexia often involve the cyclooxygenase (COX) enzymes that catalyze the synthesis of prostanoids such as pro-inflammatory prostaglandins PGE_2, and $PGF_{2\alpha}$.[29,30] Acetic acid-induced writhing is employed in investigating peripheral anti-nociceptive drug leads.[31,32] The painful sensation associated with irritation of peritoneal cavity by intraperitoneal injection of acetic acid is characterized by abdominal contractions, movements of the body as a whole, twisting of the abdominal muscles, and a reduction in motor activity. Moreover, in this method, pain is generated by stimulating peripheral nociceptive neurons via endogenous mediators such as bradykinin, serotonin and capsaicin.[33] Thus, the peripheral nociceptive response of abdominal writhing induced by acetic acid mainly involves the release of arachidonic acid metabolites via COX, and prostaglandin PGE_2 and $PGF_{2\alpha}$ biosynthesis.[31,34,35] The methanol extract of the recipe showed a significant ($p < 0.05$) dose-dependent significant ($p < 0.05$) inhibition of the number of writhing at the doses of 200, 400 and 800 mg/kg with percentage inhibition of 56.96%, 83.50%, and 89.97%, respectively when compared with the standard drug diclofenac (25 mg/kg) that had inhibition of 48.22%. The possible mechanism for this success could be as a result of inhibiting the pro-inflammatory prostanoid synthetic pathway leading to the synthesis of PGE_2 and $PGF_{2\alpha}$. The extract also significantly ($p < 0.05$) inhibited the second phase of the formalin-induced pain at doses of 400 and 800 mg/kg. This indicates that the extract can suppress inflammatory mediators such as serotonin and prostaglandins since it inhibited the second phase or inflammatory phase.[36] Formalin-induced pain is biphasic. The first phase is due to direct local irritation and stimulation of afferent sensory nerve endings by the chemical (formalin) which occur during the first 5 minutes of exposure. The second phase occurs during 20-30 minutes of exposure. It is due to combined effect of direct local stimulation of the nerve endings and central stimulation in the dorsal horn of the Central Nervous System (CNS). Several studies have shown the anti-nociceptive properties of different parts of these plants in this recipe at doses similar to the adopted doses in this present study.[6,7]

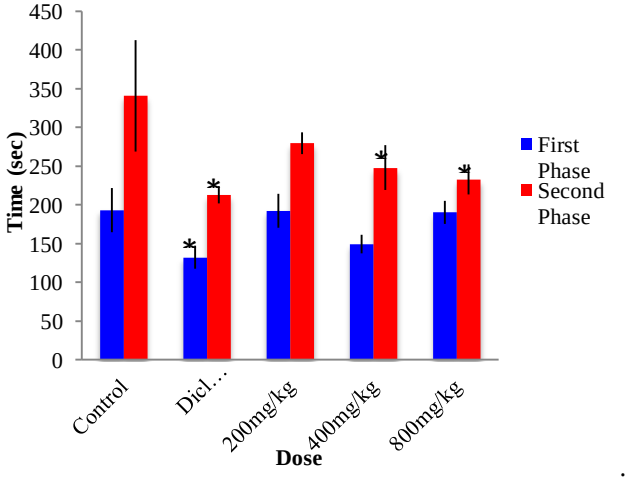
The activity of the extract of the recipe on analgesiometer test is shown in Figure 3. The extract induced significant ($p < 0.05$) increase in threshold to pain at all the doses when compared to the initial pretreatment readings. Diclofenac (positive control) increased the threshold to pain significantly ($p < 0.05$) from 2.88 ± 0.61 x20 g to 6.32 ± 0.68 x20 g and 6.18 ± 0.79 x20 g at thirty minutes and sixty minutes' post administration, respectively. There was a dose-dependent increase in threshold to pain. The highest activity was recorded at the 800 mg/kg dose with pretreatment value of 3.12 ± 0.43 x20 g and increased significantly ($p < 0.05$) to 7.92 ± 0.91 x20 g and 10.36 ± 1.32 x20 g at 30 and 60 mins post treatment, respectively.

The analgesiometer study further confirmed the anti-nociceptive ability of the extract as it exhibited a dose-dependent time bound rise in threshold to pain compared with the standard drug, diclofenac. Both the drug and the recipe produced significant ($p < 0.05$) anti-nociceptive activity compared to the control. At higher doses of 400 and 800 mg/kg, the effects of the recipe were higher than that of the standard drug. Phytochemical screening of the extract revealed the presence of secondary metabolites like flavonoids, carbohydrates and saponins. Flavonoids are known to exhibit different pharmacological effects such as analgesic, anti-inflammatory and antimicrobial.[1] Thus in this present study the pharmacological effect observed could be attributed to the flavonoids present.



* P < 0.05 compared to the control, values are in Mean ± SEM

Figure 1: Effect of methanol extract of a recipe from fruit pulps of *H. thebaica*, *A. digitata*, *Z. spina-christi* and *Z. mauritiana* on acetic acid induced writhing in mice



*P < 0.05 compared to the control, values are in Mean ± SEM

Figure 2: Effect of the methanol extract of a recipe from fruit pulps of *A. digitata*, *H. thebaica*, *Z. mauritiana* and *Z. spina-christi* on formalin test in rats

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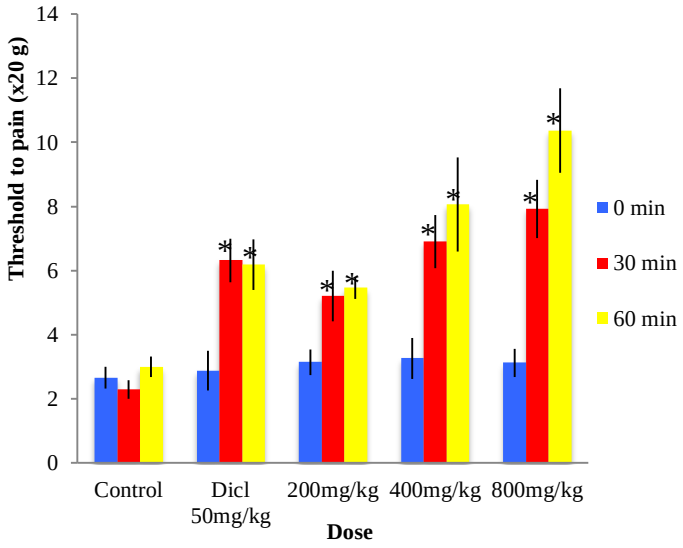

* P < 0.05 compared to the zero value within the same row, values are in Mean ± SEM

Figure 3: Effect of methanol extract of a recipe from fruit pulps of *H. thebaica, A. digitata, Z. spina-christi* and *Z.mauritiana*on analgesiometer in rats

Conclusion

The methanol extract of the recipe from *A. digitata* L. *H. thebaica* L., *Z. mauritiana* L. and *Z. spina-christi* L. possesses anti-nociceptic properties that could as well be developed into a potential source of drug that is safe and affordable. The extract of the recipe recorded significant activities in both chemical and mechanical model of anti-nociceptive study and a dose of 400 mg/kg is effective in the animal models studied. It is recommended that further study be carried out to isolate and identify possible compounds responsible for the anti-nociceptive activity in the different models.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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