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Antiinflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals

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ABSTRACT

Objective: To scientifically verify the claims of our traditional healers on the anti-inflammatory activity of *Carica papaya (C. papaya)* and possibly deduce its activities. **Methods:** 0.1 mL of fresh egg albumin was injected into the right hind-paw of adult white Wistar rats to induce inflammation an hour post intraperitoneal (IP) administration of 50-200 mg/kg doses of the extract to 3 groups of 5 rats per group. The 4th group of 5 rats was used as negative control and received 2 mL/kg (IP) of physiological saline, while the 5th group of 5 rats was used as positive-comparative control and received (IP) 150 mg/kg of aspirin. Increases in diameter of the paw were measured with the aid of Veneer Calipers before extract administration and at interval of 30 minutes post administration for further 2 hours. Percentage inhibition of oedema was calculated for each dose group and results were subjected to statistical analysis using student *t*-test and analysis of variance (ANOVA). **Results:** All doses of extract showed a dose and time dependent inhibition effects of oedema ($P<0.05$). **Conclusions:** This work is at present though limited to animals, the anti-inflammatory activity of the seeds of *C. papaya* is perhaps proven.

1. Introduction

Inflammation can be defined as a reaction of a living cell or tissue to injury, infection or irritation/infiltration. Inflammation is characterized by pains, swelling, redness and heat/fever. Inflammation could be induced by conditions that bring about the release of inflammatory mediators such as histamine, prostaglandins, nitric oxide, serotonin, cytokines, leukotrienes, platelet activating factor and substance P[1]

Plants form good sources of cheap and affordable drugs and medicinal plants possess therapeutic efficacy like their orthodox drugs counterpart yet, they exhibit less or no side/adverse unwanted effects[2-5].

Carica papaya L *(C. papaya)* commonly known as pawpaw is easily recognized by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal of cluster of large and long-stalked leaves. It is rapidly growing and can grow up to 20 m tall. It is widely distributed throughout West Africa and may be cultivated or it can come up spontaneously on a fertile soil and occurs as monoccious or dioccious plant[6].

Traditional medicine healers use every part of the plant for treatment of a wide range of ailments[7]. Its leaves are boiled together with those of lemon grass and guava for treatment of malaria. Its leaves also possess sedative, muscle relaxant, antioxidant and anticonvulsant effects, the roots extracts are purgatives, the seeds and pulp possess antibacterial activity [6]

C. papaya has its purified active constituents as proteolytic enzymes, of which papain is found in most part of the plant and chymopapain is found in the latex. Earlier works on *C. papaya* have shown the clinical and pharmacological importance of these proteolytic enzymes[8]. Higher levels of these enzymes are found in younger plants than the older ones.

Anti inflammatory, antipyretic and analgesic activities of the leaves extracts have also been demonstrated[6]. Animals are not left out in the dividends from *C. papaya*. In Indonesia and Philippines, *C. papaya* is used as de-wormers, reduction in body-weight and as "after-feed" in post parturition animals perhaps to encourage lactation.

This study was designed to verify the therapeutic use of

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this plant as an anti-inflammatory agent, by traditional medicine healers and also aid in the search for new drugs development from plants.

2. Materials and methods

The matured but unripe fruits of *C. papaya* were harvested within the campus of the University of Port Harcourt, Nigeria. The taxonomy of the plant has been done earlier[6]. The fruits were cut open and the black seeds were collected into a clean dry metal plate.

Fresh eggs were bought from a dealer in Port Harcourt, Nigeria. While aspirin tablets (300 mg) were purchased from University of Port Harcourt's Hospital Pharmacy. All animals were bought from University of Port Harcourt's Animal House.

2.1. Seeds extraction

The seeds were air dried for 5 days and ground into a fine powder using a coronal manual grinder. 80.65 g of the fine powder was immersed into 300 mL of methanol and allowed to stand for 48 hours at room temperature. Filtration was then carried out using a WhatMan No.1 filter paper. The filtrate was poured into a clean-dry conical flask and evaporated to semi-solid on a hot plate of 30 ℃. The semi-solid filtrate was poured into an evaporating dish and further evaporated to dryness at 30 ℃ for 2 days. The dry solid was scraped out, weighed and refrigerated until required. The percentage extract yield was calculated out from the initial fine powder weight.

2.2. Phytochemistry study

A preliminary phytochemical study was done by re-dissolving the appropriate amount of the dry extract in distilled water and appropriate test reagent added[9]. Phytochemical screening was carried out to test for the presence of alkaloids, flavonoids, proteins, sugars/reducing substances, glycosides, saponins, resins, fats/oils and starch.

2.3. Animal experiment: LD_{50} study

With the approval of the University's ethical committee on the use and handling of animals, 36 Swiss albino male mice of 18-25 g weight were divided into 6 groups (A-F) of 6 animals per group for the LD_{50} study.

The animals were allowed to acclimatized to their new mates and cages overnight. They were fed on mice pellets (Pfizer Feeds PLC) and had no restricted access to both feeds and good drinking water throughout the experiment. An initial pilot study was carried out to determine the minimum dosage that killed all animals in the study group and the maximum dosage that killed no animal.

The animals in their cages of 6/cage (A-E) received crude drug extract administered intraperitoneally, (IP) in the ranges of 50, 100, 200, 400 and 800 mg/kg respectively. The negative control group (F) received 0.25 mL/kg of normal saline IP.

The symptoms of toxicity and, or death were observed within 24 hours and recorded. Any dead animal was removed from cage as soon as possible. The LD_{50} was calculated as the probit of the minimum dose of the extract that killed half the number of the animals in the study group or the mean of two doses where applicable[10].

2.4. Animal experiments: anti-inflammatory study

A total of 20 adult white Wistar albino rats of both sexes and of average weight of 220 g were used. They were placed in cages and grouped into five (A-E) of 4 per group. They were then left to acclimatize to the laboratory environment for four days. The animals were deprived of feed for 12 h prior to the experiment but were allowed access to pure drinking water. They were not allowed access to both feed and pure drinking water during the experiment.

Fresh egg albumin was injected into Sub-Planter of the right hind-paw of adult white Wister rats to induce inflammation was adopted for this work.

0.1 mL of the egg albumin was injected into Sub-Planter of the right hind-paw of 3 groups (A-C) of rats an hour post intraperitoneal (IP) administration of 50-200 mg/kg doses of extract. 150 mg/kg of aspirin was given to group D and group E was used as negative control and received 2 mL/kg (IP) of physiological saline.

Increases in diameter of the paw were measured with the aid of Veneer Calipers before extract administration and at intervals of 30 minutes post administration for further 2 hours. Percentage inhibition of oedema was calculated for each dose group and results were subjected to statistical analysis using student *t*-test and analysis of variance (ANOVA). All results were compared to negative control at time t and the below stated formula applied.

$$\frac{\text{Average inflammation of negative control at time t}}{\text{Average inflammation of treated group at time t}} \times 100$$

%inhibition of inflammation= 100 − %inflammation[5]

3. Results

3.1. Phytochemistry study

From the starting ground seeds weight of 80.65 g, 2.40 g was obtained as extraction yield. Thus, giving a 2.53% yield. Glycosides was averagely present in the seeds of *C. papaya*, alkaloids was abundantly present. Resin, carbohydrates, fats and fixed oils were detected in low values.

3.2. LD_{50} study

Lethal dose was calculated as the mean of the probit of the highest dose (400 mg/kg) that killed less than half of the number of animals in its group and the lowest dose (800 mg/kg) that killed half of the number of animals in its group dosage[10]. Our LD_{50} was thus estimated to be 620 mg/kg.

The effects of methanolic seeds extract of *C. papaya* on fresh egg albumin induced inflammation in rats are shown in Table 1 and Table 2.

There was a significant inhibition of oedema (anti-

Table 1
Various sizes of oedema vs doses of aspirin and extract (mm).

Treatment	20 min later	40 min later	60 min later	80 min later	100 min later
Extract 50 mg/kg	0.9	0.9	0.8	0.7	0.6
Extract 100 mg/kg	0.8	0.8	0.7	0.7	0.6
Extract 200 mg/kg	0.8	0.7	0.7	0.7	0.6
Aspirin 150 mg/kg	0.8	0.6	0.4	0.3	0.2
Normal saline mL/kg	1.4	1.4	1.5	1.5	1.4

inflammatory activity) at all doses of extract ($P<0.05$), which was dose and time dependent. There was however no observable difference in effect between 200 mg/kg and 100 mg/kg at 60–120 minutes. Inhibition effects of aspirin was sharply different as there was observable changes at all time interval ($P<0.01$).

Inhibition of inflammation for the crude extract ranged between 57.1% and 64.2% unlike that of aspirin which stood at 85.7%. Anti-inflammatory activity of aspirin is therefore higher than that of the crude extract of the seeds of *C. papaya*. Perhaps with characterization and purification of the active components, this disparity shall be corrected.

Table 2
Inhibition of inflammation *vs* doses of aspirin and extract (%).

Treatment dose	Sizes in paw	Percentage inhibition of inflammation
Extract 50 mg/kg	0.80±0.08	57.1
Extract 100 mg/kg	0.90± 0.11	64.2
Extract 200 mg/kg	0.90± 0.11	64.2
Aspirin 150 mg/kg	1.20±0.13	85.7
Normal saline 2.0 mL/kg	0.00 ±0.00	0.0

Sizes in paw=Mean ±SEM

4. Discussion

Inflammation can be defined as a reaction of a living cell or tissue to injury, infection or irritation/infiltration. It is characterized by pains, swelling, redness and heat/fever. Prostaglandins and leukotrienes are released by a host of mechanical, thermal, chemical, bacterial and other insults, and they contribute importantly to the signs and symptoms of inflammation[11]. Mast cell which is very rich in histamine has membrane receptors both for special class of antibody (IgE) and for complements components– C3a and C5a. Mast cell can be activated to secrete inflammatory mediators through these receptors and also by direct physical damage. A transmembrane protein CD40, expressed on monocytes, neutrophils and platelets also plays roles in inflammation [12].

Our results clearly show anti-inflammatory activity of the seeds of *C. papaya*. Generally, the effect of the methanolic extract of the seed with inhibition ranging from 57.1% to 64.2% is however lower than 85.7% of aspirin, a standard anti-inflammatory drug. Mechanism that possibly underline this anti-inflammatory activity include inhibition of the actions of inflammatory mediators such as histamine, prostaglandins, nitric oxide, cytokines, platelet activating factor and substance P, effect on adrenocorticoid hormone and immunosuppression.

This work is at present though limited to animals, the anti-inflammatory activity of the seeds of *C. papaya* is perhaps proven.

Conflict of interest statement

We declare that we have no conflict of interest.

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