International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 9 (1), pp. 001-005, January, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antinociceptive and anti-inflammatory effects of the methanol seed extract of *Carica papaya* in mice and rats

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Accepted 11 September, 2019

The methanol seed extract of *Carica papaya* (CPE) was investigated for antinociceptive and anti-inflammatory activities in mice and rats. The brine shrimp lethality test of the extract showed LC_{50} of 106.94 ppm at 95% confidence interval. The extract (5, 10 and 20 mg/kg, p.o.) significantly ($p < 0.05$) increased pentobarbitone-induced sleeping time by more than 280% compared with the control. Like pethidine (9.1 mg/kg, i.p.), CPE (10 and 20 mg/kg, p.o.) significantly ($p < 0.05$) decreased the paw licking time in early (0 - 5 min) and late (25 - 30 min) phases of formalin-induced nociception. This antinociceptive effect was more in the late phase than early phase. Also, CP (5, 10 and 20 mg/kg, p.o.) significantly ($p < 0.05$) decreased the number of acetic acid-induced abdominal contortions by 25, 60, and 64%, respectively. Indomethacin (10 mg/kg, p.o.), CPE (5, 10 and 20 mg/kg, p.o.) showed a typical biphasic anti- inflammatory effect in carrageenin-induced paw oedema in rats. The anti-inflammatory effect though moderate, was dose-dependent and higher in 2 h than 4 h after administration of the phlogistic agent. In conclusion, CPE contains potent bioactive compounds (alkaloids, flavonoids and polyphenols) which showed antinociceptive effect probably mediated centrally and peripherally; and also involving mild anti-inflammatory mechanisms.

Key words: *Carica papaya*, brine shrimps, antinociception, anti-inflammatory.

INTRODUCTION

Research in plants with medicinal properties and the identification of their chemical components responsible for their activities have corroborated the traditional uses of ancient healing wisdom and lore (Kamanyi et al., 2009). A review of plants exhibiting analgesic and anti-inflammatory activities showed that different species of 96 *genera* belonging to many families (Handa et al., 1992) have been reported, of which the family Caricaceae was not listed. It is believed that current analgesia-inducing drugs as opioids and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are not useful in all cases because of their side effects and low potency (Ahmadiani et al., 1998). For instance, morphine causes acute morphine poisoning, hypotension, dependence etc, while the NSAIDs are associated with gastric irritation, bleeding, ulcers and perforation (Hardman et al., 1996). As a result searches for other alternatives became necessary and beneficial.

Carica papaya (Caricaceae) is a medicinal plant that originated from Central America, which has spread to different parts of the world including African and Nigeria in particular. It is known for its plethora of folkloric uses and pharmacological activities. It contains two major bioactive compounds, namely, papain and chymopapain, which are used in brewing, wine- making, textile and tanning industries (Brocklehurst and Salih, 1985). Other compounds are alkaloids, flavonoids and other phenolic compounds. It is used by the natives in treating malarial fever, diabetes mellitus, and bacterial infection, as a dewormer and as an ecbolic agent (Lohiya et al., 1992; Kinyuy, 1993; Chinoy et al., 1995). It is also used in improving digestion.

The anthelmintic effect of the latex of *C. papaya* against *Ascaris suum* in naturally infected pigs and *Ascaridia galli* infection in chicken has been reported

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(Satyanarayanana et al., 1982, Satrija et al., 1995). In ethno-veterinary medicine, the seeds of *C. papaya* have used as dewormer in Indonesia and Philippines (IRR, 1994). The extract of the pulp and seeds showed bacteriostatic properties against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, and Bacillus subtilis in vitro*. D (+) glucosamine isolated from the latex has been shown to have antifungal activity by inhibiting the growth of *C. albicans* in culture media (Giordiani et al., 1996). The central nervous and cardiovascular effects of the methanol leaf extract of *C. papaya* have been documented (Gupta et al., 1990). The fruits can be directly applied topically to skin sours (Chinoy and Padman, 1996). Recently, Nsukka indigenes chew the dry seed of *C. papaya* to alleviate nagging head ache (migraine) and in reducing swollen wounds and reduce high blood pressure.

Therefore, the main purpose of this study is to investigate the analgesic and anti-inflammatory activities of the methanol seed extract of *C. papaya* (CPE), in order to confirm the rationale for its folkloric use.

MATERIALS AND METHODS

Plant material and extraction

The fruits of *C. papaya* were harvested from the zoological garden of University of Nigeria, Nsukka in February, 2008. It was identified as *C. papaya* by A.O. Ozioko of Biodiversity and Conservation Programme (BDCP), Nsukka, Enugu State and a voucher sample (UNN/DBH/CP08/s07) was kept in the herbarium of Department of Botany, University of Nigeria, Nsukka.

They were cut open and the seeds were collected, dried on top of laboratory bench and pulverized into coarse powder using a hammer mill. About 200 g of the coarse powder was extracted by cold maceration with 1.0 litre of 70% aqueous methanol and intermittent shaking for 48 h. The extract was filtered with Whatmann #1 filter paper and concentrated *in vacuo* to dryness using rotary evaporator. The yield was calculated and the extract stored in a refrigerator at 4°C. The extract yielded about 3.61 w/w dry matter which was light brown in colour.

Experimental animals

Eighty five (85) white albino Wistar mice of either sex (30 - 45 g) and twenty five (25) white albino Wistar rats (86 - 100 g) of either sex were procured from the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were kept in stainless steel cages and were fed *ad-libitum* with standard laboratory animal feed (Guinea Feed®), except in situations, where fasting was required. They were also provided with clean tap water. They were maintained in accordance with the recommendation in the Guide for the Care and Use of Laboratory Animals (DHHS, NIH Publication No. 85-23, 1985). They were allowed 2 weeks to acclimatize before the start of the experiments.

Brine shrimps lethality test

The toxicity of CPE was determined by the modified method of McLaughlin et al. (1991). Briefly, ten 48 h-hatched nauplii of *Artermia salina* obtained from a pet shop in Davis, California, USA were incubated (10 nauplii in a well) in 10, 100, 1000 ppm CPE. The experiment was performed in triplicate. Equal volume of distilled water, which was used in dissolving the extract, was added to the control group. The nauplii were incubated at room temperature (37°C) for 24 h and the surviving nauplii were determined. The data was analyzed using probit analysis of Minitab Program (Minitab for Windows Release 12.21) to determine the LC_{50} at 95% confidence interval.

Effect on pentobarbitone-induced narcosis

The effect of CPE on narcosis was determined by intraperitoneal administration of pentobarbitone sodium (35 mg/kg, b.w.). Briefly, 30 mice were randomly grouped into five (A – E) of six mice each. Group A mice (control) received distilled water (10 ml/kg) orally, while groups B - E were treated with increasing doses (5, 10, 20, 40 mg/kg, b.w.) of the extract by the same oral route (Chinoy and Padman, 1996). Thirty minutes later, the mice were given sodium pentobarbitone (35 mg/kg) intraperitoneally. The time of injection, time of sleep (loss of righting reflex) and the time of awakening (regain of righting reflex) were recorded. The sleeping time was calculated as the interval between the loss and recovery of the righting reflex (Shetty and Anika, 1982).

Effect on formalin-induced nociception

The experiment was conducted according to the method of Marchioro et al. (2005) . Thirty mice (30 - 35 g) of both sexes were randomly grouped into five of 6 mice each. Group A of the mice received distilled water (10 ml/kg), which served as the negative control. Group B mice were treated with pethidine (9.1 mg/kg, i.p.), which was the positive control. Groups C-E received graded doses (5, 10, 20 mg/kg, b.w.) of CP by oral administration respectively.

Thirty minutes post administration of the drugs and CPE; the mice were injected with 50 µl of 5% formalin into the sub plantar area of the hind limb (Oliviera and Barros, 2006). The paw licking time (PLT) was recorded using a stop watch after the administration of formalin. The time the mice remain licking or biting the paws during the first phase (0 - 5 min) and second phase (20 - 25 min) of the reaction was recorded.

Effect on acetic acid-induced abdominal writhing

The method of Vale et al. (2003) was used. Five groups of mice consisting of 6 mice each were fasted for 12 h but given adequate amount of water. Group B received pethidine (9.1 mg/kg) intraperitoneally, while groups C - D received 5, 10, 20 mg/kg of CP by oral administration respectively. Mice in group A received distilled water (10 ml/kg) and served as control. Forty five minutes later, the mice received 10 ml/kg of 0.7% acetic acid intraperitoneally. The number of writhing or abdominal stretches produced in each mouse was counted for 30 min.

Effects on carrageenin-induced paw odema

The anti-inflammatory effect of the extract of CPE was conducted using carrageenin-induced paw oedema in rats (Winter et al., 1962). Briefly, 30 rats (143 – 165 g) of both sexes were randomly divided into 5 groups of six rats each. Group A rats were given distilled water (10 ml/kg), which served as the control, while group B rats were treated with indomethacin (10 mg/kg, p.o.) suspended in 1% carbonated buffer solution. The remaining C - E groups were treated with graded doses (5, 10, 20 mg/kg, b.w.) of CP by oral administration respectively. Before the treatment, the volume

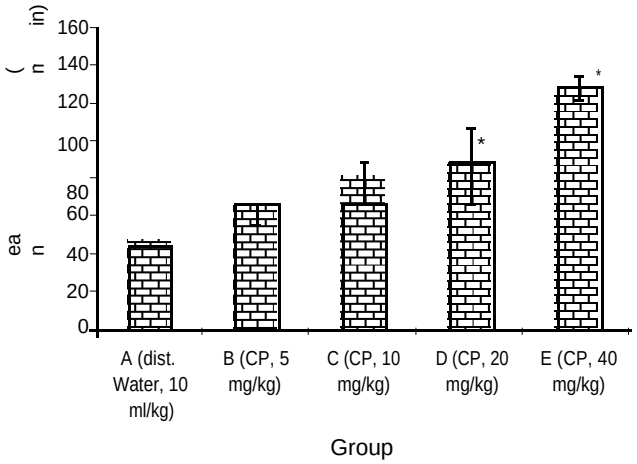

Figure 1. Effect of methanolic seed extract of *Carica papaya* on pentobarbitone-induced sleeping time in mice.

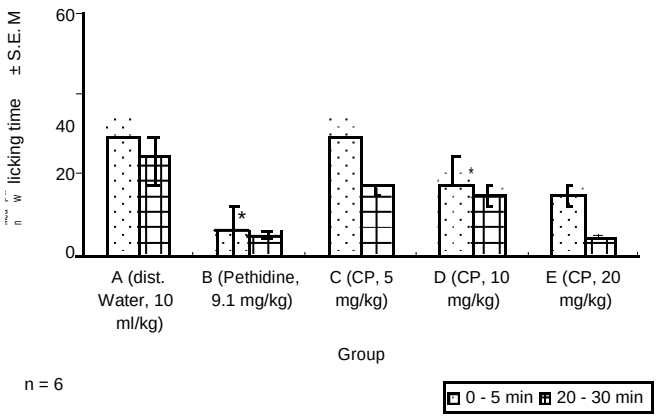

Figure 2. Effect of methanolic seed extract of *C papaya* on formalin-induced nociception in mice.

displacement by the normal paw (V_o) was measured for each rat. Forty five minutes post administration of the extract and indomethacin, 0.5 ml of carrageenin (1 %) in normal saline was injected into the sub plantar area of the hind paw. The change in volume due to carrageenin-induced paw swelling (V_t) of the paw was measured at 1, 2, 3, 4 and 5 h after, using plethysmographic method.

The percent inhibition was calculated using the modified formula (Oyewole. 2004; Garcia et al., 2004) below:

$$\text{Percent inhibition} = \frac{(V_t\text{-}V_o)_{\text{control}} - (V_t\text{-}V_o)_{\text{treated group}} \times 100}{(V_t - V_o)_{\text{control}}}$$

Statistical analysis

The results were presented as mean ± S.E.M and were subjected to one way ANOVA. P values of 0.05 or less were considered significant. Except, where the data were expressed as percent of the control or zero time reading.

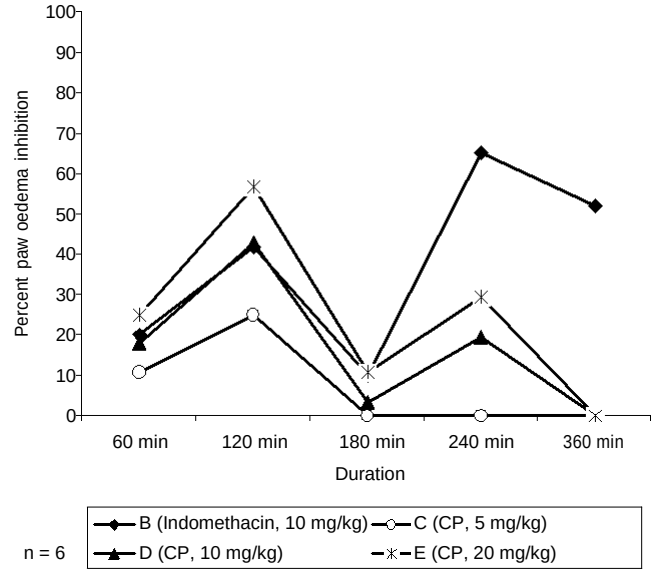

Figure 3. Effect of metrhanolic seed extract of *C. papaya* on carrageenin-induced paw oedema in rat.

RESULTS

The plant extract gave a yield of 3.16% w/w solid matter, which was light brown in colour and pasty in consistency. The brine shrimps lethality test of CP gave LC_{50} of 106.94 ppm and EC_{50} of 10.69 ppm at 95% confidence interval. The surviving shrimps were dull and weak, when compared with the untreated shrimps.

The result of the effect of the extract on pentobarbitone-induced sleeping time was presented in Figure 1. The result showed that, CP (5, 10, 20 mg/kg) produced significant ($p < 0.05$) increase in sleeping time with increase in dose. The highest dose of 40 mg/kg induced more than 280% increase in sleeping time compared to the control (group A).

The antinociceptive effect of *C. papaya* extract using formalin-induced nociception model was presented in Figure 2. Pethidine (9.1 mg/kg) significantly ($p < 0.05$) inhibited both early (0 - 5 min) and late phase (25 - 30 min) of formalin-induced nociception. Similarly, CP (10, 20 mg/kg) significantly ($p < 0.05$) decreased paw licking time in formalin-induced nociception. The antinociceptive effect of CP (20 mg/kg) was not significantly ($p > 0.05$) different from that of pethidine.

The result of the analgesic effect of *C. papaya* extract using acetic acid-induced abdominal writhing was presented in Table 1. Pethidine (9.1 mg/kg) and CP (5, 10, 20 mg/kg) significantly ($p < 0.05$) decreased the number of acetic acid-induced abdominal stretches. There was no significant difference between CP (20 mg/kg) and pethidine (9.1 mg/kg).

The anti-inflammatory effect of CPE in carrageenin-induced paw edema was presented in Figure 3. Both indomethacin and CPE produced typical biphasic anti-inflammatory effect. CP induced dose-dependent inhibition of paw edema at 2 h after administration of carrageenin

Table 1. The effect of the methanol extract of *C. papaya* seed on acetic acid- induced writhing reflex in mice.

Groups	Mean number of abdominal stretches ± S.E.M	% Pain inhibition
Distilled water (10 ml/kg, p.o.)	78.6 ± 5.66	0.0
Pethidine (9.1 mg/kg, i.p.)	28.8 ± 3.0*	63.4
CP (5 mg/kg, i.p.)	58.6 ± 6.55*	25.4
CP (10 mg/kg, i.p.)	31.4 ± 1.55*	60.1
CP (20 mg/kg, i.p.)	28.4 ± 3.13*	64.0

N = 5 mice.
CP, *C. papaya* extract.
Significant at $p < 0.05$ compared with the control (group A).

and this effect was higher than that of indomethacin. Indomethacin (10 mg/kg) induced the highest anti-inflammatory effect at 4 h post administration of the phlogistic agent, which was about 3 times higher than CPE.

Discussion and Conclusion

Medicinal plants are believed to be important source of new chemical substance with potential therapeutic effect and plant species that traditionally have been used as pain killers should be seen as a strategy in research for new antinociceptive drugs ((Haejazian et al., 2008). The brine shrimps lethality test (BSLT) showed that CPE contains very potent bioactive compounds and the bioactivity is rated high with LC_{50} of 106.94 ppm.

McLaughlin et al. (1991) stated that, EC_{50} value for general toxicity of natural product is approximately one tenth of the value of the LC_{50} in BSLT; therefore, the EC_{50} of CPE should be approximated to 10.69 ppm. The low LC_{50} and EC_{50} values showed that the extract contained very potent bioactive compounds which may be cytotoxic and affect the nervous system and should be taken in low dose. The results of this study show that, the methanol extract from the seed of *C. papaya* possesses anti-nociceptive activities against pains induced by acetic acid and formalin. The extract presented a moderate anti-inflammatory effect on the acute oedema induced by sub plantar injection of carrageenin.

According to Nguelefack et al. (2004), acetic acid induces writhing through the release of pain mediators such as histamines, serotonin, and prostaglandins or by direct stimulation of acid sensitive receptors. Active substances against this pain model may interfere with one of these mediator systems or may act on the central nervous system (CNS) by blocking the pain influx transmission. CPE significantly reduced the number of acetic acid-induced writhing. Therefore, it could be acting peripherally on the mediator system or on the CNS.

In order to assay whether CPE possesses central or peripheral analgesic effect, it was further tested on pain induced by formalin. Injection of formalin under the sub-aponeurotic space of the hind paws causes pain with two phases. The first phase or neurogenic phase, is due to the release of substance P and it is followed by a second phase or inflammatory phase, which is characterized by the release of serotonin, histamine, bradykinin, and prostaglandins (Murray et al., 1988; Tjolsen et al., 1992; Gaertner et al., 1999).

Indomethacin, a well known NSAID inhibits only the 2nd phase of this pain model, while central analgesics inhibit both phases (Stai et al., 1995). CPE significantly inhibited both phases, which suggests that, it possesses central analgesic activity, but could possess peripheral activity since the effect was higher in the 2nd phase. The effect of CPE (20 mg/kg) on formalin-induced nociception was similar to pethidine (9.1 mg/kg), the reference drug used. CPE-induced central analgesia was further confirmed by the fact that, the extract significantly increased pentobarbitone-induced narcosis by more than 260% compared with the control. It also markedly reduced the induction time in pentobarbitone-induced narcosis (data not provided). The increase in narcosis could be due to inhibition of the microsomal enzymes responsible for the biotransformation of pentobarbitone or as a result of receptor site potentiation; which is an important factor in drug synergism or both (Hardman et al., 1996). Due to deep narcosis observed in mice that receive CPE (40 mg/kg), this dose was further dropped for further experiments.

The acute anti-inflammatory of CPE was evaluated using carrageenin-induced oedema model. The extract showed moderate and typical biphasic anti-inflammatory activity on carrageenin model. This model, presents three phases with production of various mediators. The first phase (0 - 2 h) is due to the release of serotonin and histamine; the second phase (3 - 4 h) is predominantly due to kinins and the third phase (> 4 h) is due to the release of prostaglandins (DiRosa et al., 1971; Holsapple et al., 1980). CPE (20 mg/kg) caused 57% inhibition (moderate) of carrageenin-induced paw oedema in the first phase and weak activity in the second and third phases.

This finding suggests the inhibition of serotonin and histamine synthesis as observed in formalin-induced nociception. *C. papaya* contains alkaloids, flavonoids and polyphenolic compounds (Tona et al., 1998), and alkaloids,

flavonoids and saponins have been found in other natural products with analgesic and anti- inflammatory properties (Kerber, 1999; Fernanda et al., 2002). Therefore, the antinociceptive and anti-inflammatory activities of CPE may be due to the presence of alkaloids, flavonoids and other polyphenols.

In conclusion, this study has shown that, the methanol extract of *C. papaya* seed showed significant anti-nociceptive effect, which justified its use in managing headache/migraine. It also showed moderate and acute anti-inflammatory effect, which may be responsible for its use in treating skin sours and swollen wounds. The results suggest the presence of very potent bioactive compounds which must be taken in very low dose. Efforts are being made to isolate and possibly characterize the active principle(s) from the crude extract.

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