ORIGINAL RESEARCH article

Cucurbita pepo used in the folkloric treatment of malaria, mediates anti-inflammatory, anti-nociceptive, antipyretic, and immunomodulatory effects in murine models

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Abstract: Malaria is an infectious disease that presents fever, inflammation of joints, vomiting, arthralgia, abdominal cramps, splenomegaly, hepatomegaly, and severe anemia in children. This study was undertaken to investigate the anti-inflammatory, anti-nociceptive, antipyretic, and immunomodulatory properties of *Cucurbita pepo* and its use as an adjunct in the treatment of malaria in Nigeria. The dried plant material (aerial parts) was extracted by cold maceration using 70.0% ethanol. The extract was characterized by GC-MS analysis and subjected to pharmacological evaluation in systemic and topical inflammation, acetic acid-induced nociception, and lipopolysaccharide-induced pyrexia. Its effect on the primary humoral immune response to sheep red blood cells was also assessed. *Cucurbita pepo* extract (CpE) revealed its major components to be palmitic acids (47.4%), 9-octadecanoic acid (10.8%), and 4-coumaric acid (5.3%). The extract suppressed paw inflammation in a dose-dependent manner, producing a significant effect at a dose of 900 mg/kg. CpE at 0.25, 1.25, and 2.5 mg inhibited topical inflammation by 17.2% to 39.0% and lymphocyte infiltration associated with the inflammatory response. At a dose of 900 mg/kg, CpE significantly reduced hyperthermia, and its effect was comparatively higher than that of indomethacin. The extract inhibited writhing response to abdominal pain, although its effect was statistically insignificant. Treatment with the extract also stimulated the immune response, evidenced by an increase in hemagglutination antibody titer and relative spleen weight in CpE-treated groups. Particularly, the extract evoked a stronger immunostimulatory response compared to levamisole, a standard immunostimulatory agent. These findings provide evidence to support the use of *C. pepo* in the treatment of symptoms associated with malaria.

Introduction

The immune system is the defense system found in vertebrates that protects against invading agents or substances alien to the system. The immune system can generate a variety of cellular and chemical mediators to recognize and eliminate a variety of noxious substances. Immunomodulation connotes changes in the immune system that

involve the induction, expression, amplification, or inhibition of any phase of the immune system [1]. Most disease states are intertwined with the immune system and usually present as inflammation, analgesia, or fever signifying disturbed immune and biological systems. Inflammation and pain associated with fever often, signify underlying ailments and have become crucial in research in recent years [2]. Fever proceeds with inflammation as a secondary impact caused by enhanced production of prostaglandins [3]. The pathophysiology of diseases such as malaria portrays an interplay between the host immune system and the organism's virulent factors, which most times leads to an imbalance in the immune system and brings about a cascade of immune reactions. Inflammation is mediated by signaling molecules produced by the immune system, such as leukocytes, macrophages, and mast cells, undergoing cellular responses to inflammatory mediators [4, 5].

Malaria is a mosquito-borne disease caused by the intracellular *Plasmodium* parasite, typified by *Plasmodium falciparum,* which accounts for more than 90.0% of the world's malaria mortality and morbidity [6, 7]. Malaria usually presents with fever, inflammation of joints, headaches, lethargy, malaise, anorexia, vomiting, arthralgia, and abdominal cramps, and in children, there are possibilities of splenomegaly, hepatomegaly, and severe anemia [8]. The patient's immune status is crucial in curbing the disease and reducing the public health threat in children and pregnant women. Medicinal plants have the capability of affecting the biological and immune systems positively to bring about improved quality of life [9]. They exert an immunomodulatory effect in the treatment of diseases either by inhibition or stimulation of the immune cascade caused by antigens [10]. Medicinal plants remain vibrant candidates for novel drugs in the modern treatment of diseases, with accessibility, affordability, and good safety profile being salient [11-14]. Every part of medicinal plants is crucial in the discovery of new and active pharmacological compounds. Old discoveries are being rediscovered with new chemical applications because of a new understanding of molecular applications and clinical observations. Important diseases such as malaria, Alzheimer's, and others, have their main therapies partly from medicinal plants [11, 12]. *Cucurbita pepo* (*C. pepo*) belongs to the Cucurbitaceae family, which consists of 130 genera and about 800 species. They are popularly referred to as cucurbits (gourd family), mostly used as food and medicine. *C. pepo* is one of the oldest known cultivated species of the Cucurbitaceae family. The plant is native to Northern Mexico and the southwestern and eastern USA. It also exists in the wild in Europe and Asia. *C. pepo* is used by traditional folklore healers in the south-east of Nigeria, Enugu State, for the treatment of fever, and its extract was recently reported to possess antimalarial activity [15, 16]. The leaves can be used in the treatment of alcohol-induced liver toxicity and oxidative stress in rats [17]. Despite the traditional claims on its use in the treatment of malaria, there is a paucity of scientific and pharmacological evidence in the literature validating these claims. This study aims to investigate the immunomodulatory, anti-inflammatory, analgesic, and antipyretic activities of *C. pepo*, based on its acclaimed use as an adjunctive malaria remedy.

Material and methods

Plant collection: Fresh aerial parts of *C. pepo* were collected in August 2024 from Orba, Enugu State (6.8541° N, 7.4625° E). The plant was identified and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicines and Drug Development (InterCEDD), Nsukka, Enugu state. Voucher specimen [InterCEDD/ 27648] was deposited in the InterCEDD herbarium.

Preparation of plant extract: Leaves and twigs of the plant were collected, cleaned, and air-dried in the shade. The dried plant material was pulverized using an electric hammer mill, and extraction was by cold maceration. A 3.0 kg quantity of dried plant material was extracted using 70.0% ethanol by soaking the leaves of *C. pepo* in the solvent for 48 hrs with gentle shaking. The mixture was filtered using a muslin cloth and subsequently a Whatman filter paper (size 1, 125 mm diameter). The filtrate was evaporated to dryness on a water bath maintained at

55.0ºC, to obtain the dried *C. pepo* extract (CpE). The extract was stored at 4.0°C and freshly reconstituted in distilled water before use.

Elemental analysis: A 1.0 g amount of CpE was placed in a small beaker, and 10.0 ml of concentrated HNO_3 was added and allowed to stand overnight. The mixture was heated on a hotplate until the release of NO_2 fumes ceased, and the beaker was allowed to cool. Perchloric acid (4.0 ml, 70.0%) was added and warmed over a water bath to obtain a concentrate. Afterward, the concentrated sample was filtered using Whatman filter paper, and the filtrate was transferred to a volumetric flask and diluted with ionized water to 50.0 ml. From this stock, a 40 ppm solution was prepared and diluted with deionized water, and serial dilutions of 20-0.625 ppm were prepared. The appropriate elements and their detection wavelengths were selected when the machine was turned on and gas was allowed to flow with an ignited flame. A calibration curve was plotted using the appropriate standard. The absorbance of the test sample was taken in triplicate and the average was calculated.

GC-MS analysis of CpE: GC-MS analysis was carried out using the Agilent 6890-5975 GC-MS system. It consists of a gas chromatography instrument, a mass spectrophotometer, and a mass Hunter software. A 5.0% phenyl methyl siloxane HP-5MS capillary column was used. 100 mg of the extract was extracted in equal volumes of a mixture of ethyl acetate, methanol, acetonitrile, and chloroform for 48 hrs after reconstituting with distilled water. The extract was mixed with silica gel and subjected to column purification with methanol as eluting solvent under an isocratic condition. The eluate was concentrated with trimethylsilate to 2.0 ml as previously described [18]. An injection volume of 1.0 µL was taken from the concentrated 2.0 ml and introduced to the GC-MS system in a split-less mode using ultra-highly purified Helium as a carrier gas at a 2.0 mL/min flow rate. Injector temperature and oven temperature were maintained at 250 ºC and 100 ºC respectively, and held for five minutes, and ramped at 8.0 ºC/min for 7.5 min. The transfer line temperature of GC-MS was maintained constantly at 240 ºC. MS operated in electron impact mode at 70 eV and the ion source operated at a temperature of 200 ºC while the detector temperature was 300 ºC. Compounds were identified based on information in the National Institute of Standards and Technology (Gaithersburg, USA) standard reference library (https://webbook.nist.gov/) and data obtained on Agilent Mass Hunter software.

Experimental animals: Swiss Albino mice of both sexes weighing between 15-25 g and Wistar Albino rats of both sexes weighing 100-150 g were acclimatized for two weeks under laboratory conditions in the animal facility center of the Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The mice were housed in plastic cages, while the rats were housed in steel cages in a ventilated room at a temperature of (20.0 ºC), fed with standard rodent chow, and allowed free access to potable water.

Ethical approval: All experiments were carried out following the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals; NIH publication No. 85-23, and NIPRD's standard operating procedures [19]. The approval has been obtained from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Egg albumin-induced inflammation: This test was performed according to the method described by Adzu *et al* [20]. Twenty-five rats weighing 100-150 g were weighed and coded. Baseline paw volume was recorded using a digital plethysmometer (LE 7500, Letica Scientific Instruments). The rats were randomized based on baseline paw volume into five groups of five animals each. Groups II-IV were pre-treated with 100, 300, and 900 mg/kg CpE, while groups I and V were given distilled water (10.0 ml/kg) as a negative control and indomethacin (10.0 mg/kg) as a positive control, respectively. One hour after pre-treatment, the animals were injected with 0.1 ml of egg albumin subcutaneously in the left hind paw of each rat, and paw volume was taken at one-hour intervals

over a five- hour period. Edema was quantified as the difference in paw volume at baseline and at the different time points post-induction of inflammation.

Xylene-induced topical inflammation: Thirty-six mice were randomly divided into six groups (n=6). 5.0 µL of various concentrations of CpE (500, 250, and 50 mg/ml) corresponding to 2.5, 1.25, and 0.25 mg of the extract was applied topically on the anterior surface of the right ear of each mouse. Two untreated control groups (sham-inflamed and inflamed) were topically administered with an equivalent volume of the vehicle, while the positive control group was treated with 5.0 µL indomethacin (100 mg/ml) corresponding to 0.5 mg of indomethacin. Topical inflammation was instantly induced on the posterior surface of the same ear by the application of xylene (5.0 µL). One set of control animals, the sham group, was induced with distilled water in place of xylene. Two hours after the induction of inflammation, mice were euthanized under deep chloroform anesthesia, and circular sections (6.0 mm diameter) of the right (treated) and left (untreated) ears were punched out using a cork borer and weighed. Ear edema in each animal was quantified as the weight difference between the two ear plugs. The anti-inflammatory activity was evaluated as a percentage edema inhibition in the treated animals relative to control animals. After weighing, the ear samples of the right ear for each group were fixed in 10.0% formalin for histopathological examination. The ears were fixed in formalin for one week, and fixed tissues were embedded in paraffin and afterward, cut into 3-4 µm sections. The sections were mounted on glass slides, stained with eosin and hematoxylin stains, and examined under a microscope.

Acetic acid-induced abdominal pain: The study was carried out according to the method described by Adzu *et al* [20]. 30 mice weighing 15-30 g were weighed, randomized, and divided into five groups of six mice per group. Group I- control, groups II-IV were given 100, 300, and 900 mg/kg CpE orally. Group V was given the indomethacin (10 mg/kg) while the control was fed with normal saline. After one hour, dilute acetic acid (0.065%) was injected intraperitoneally into the experimental mice. Each mouse was individually observed for five minutes, and the number of abdominal writhings was counted for ten minutes and recorded accordingly.

LPS-induced pyrexia: The lipopolysaccharide (LPS)-induced fever method described by Santos *et al* [21] was used for this assay. This experiment was performed during the light phase of the circadian cycle, between nine am and five pm. 36 rats were selected based on body temperature below 38 ºC. The animals were fasted overnight in the experimental room and basal body temperature, and body weight were measured using a rectal thermometer and a weighing balance, respectively. The rats were coded and randomized into six groups (n=6) according to basal body temperatures. Rats in groups I-III received 100, 300, and 900 mg/kg body weight of *Cp*E by gavage, while group IV was given indomethacin orally. Rats in groups V and VI served as hyperthermic and normothermic controls, respectively, and received pyrogen-free sterile water orally. After one hour, the rats in group VI received pyrogen-free sterile water for injection via intraperitoneal injection (10 ml/kg), while the other groups (I-VI) were injected intraperitoneally with LPS (100 µg/kg) dissolved in pyrogen-free sterile water for injection. Body temperatures were taken every hour for four hours after LPS administration. The body temperature was measured by gently inserting a small digital thermometer three cm into the rectum until a beep sound was heard. During the temperature measurements, each rat was held gently during the experiment to avoid changes in rectal temperature secondary to handling.

Immunomodulatory assay: Rats were divided into five groups of six rats each. Group I (control) served as an untreated, immune-challenged control; group II was the positive control (Levamisole 2.5 mg/kg) used as the standard immunostimulatory agent in the study, while groups III, IV, and V were the test groups of concentrations of 100, 300, and 900 mg/kg of CpE. The humoral immune response study is a 28-day study following the method described by Hueza *et al*. with some modifications [22]. Rats in all the groups were treated with either normal saline for control at 10 mg/ml, ethanol extract of *C. pepo,* or levamisole once daily for 21 days by gavage and

were all antigenically challenged by intraperitoneal injection of 0.1 ml of 20.0% sheep red blood cells (SRBC) in 0.9% normal saline on day 21 of the assay. The body weights of all the rats were checked weekly, and doses of agents were adjusted accordingly. Treatment continued until day 28, when the rats were euthanized under chloroform anesthesia. Blood samples were collected in plain serum tubes and allowed to clot at room temperature for 45 min. The spleen was harvested from each rat and weighed.

Determination of primary humoral response (hemagglutination titer): The blood samples were centrifuged at 3,200 g for 10.0 min, and serum was collected using micropipettes. Serum was diluted in a two-fold fashion with normal saline. Fresh SRBC suspension (25.0 µL) of 20.0% was introduced into 25.0 µL of two-fold diluted serum in a microtiter plate. The microtiter plates were incubated at 37 °C for one hour and checked for hemagglutination. The lowest dilutions with hemagglutination were taken as the antibody titers.

Statistical analysis: Data obtained were analyzed using one-way analysis of variance (ANOVA) to compare the means between groups using GraphPad Prism Version 6.0 software for Windows (San Diego, California, USA). Data were expressed as Mean±SEM. Differences between the means of the treated and control groups were considered significant at $P<0.05$.

Results

The ethanol extract of *C. pepo* yielded elements in varying concentrations. The highest occurring element was magnesium (2247.1 µg/g; 41.8%), while the lowest was copper (6.65 µg/g; 0.12%). The other elements detected included calcium (1360.85 µg/g; 25.3%), iron (159.95 µg/g; 2.98%), zinc (135.15 µg/g; 2.52%), and sodium (1461.75 µg/g; 27.2%). Chromium, lead, and manganese were undetected. The GC-MS fingerprint revealed the presence of various fatty acids notably palmitic acids (17.61; 47.42%), 9-octdedenoic acid (18.995; 10.83%), 4-coumaric acid (16.839; 5.28%), propanoic acid (16.009; 5.30%) and some fatty acids also occurring in smaller quantities such as hexadecenoic acid (16.63; 1.63%), and stearic acid (19.199; 1.73%) (**Figure 1**).

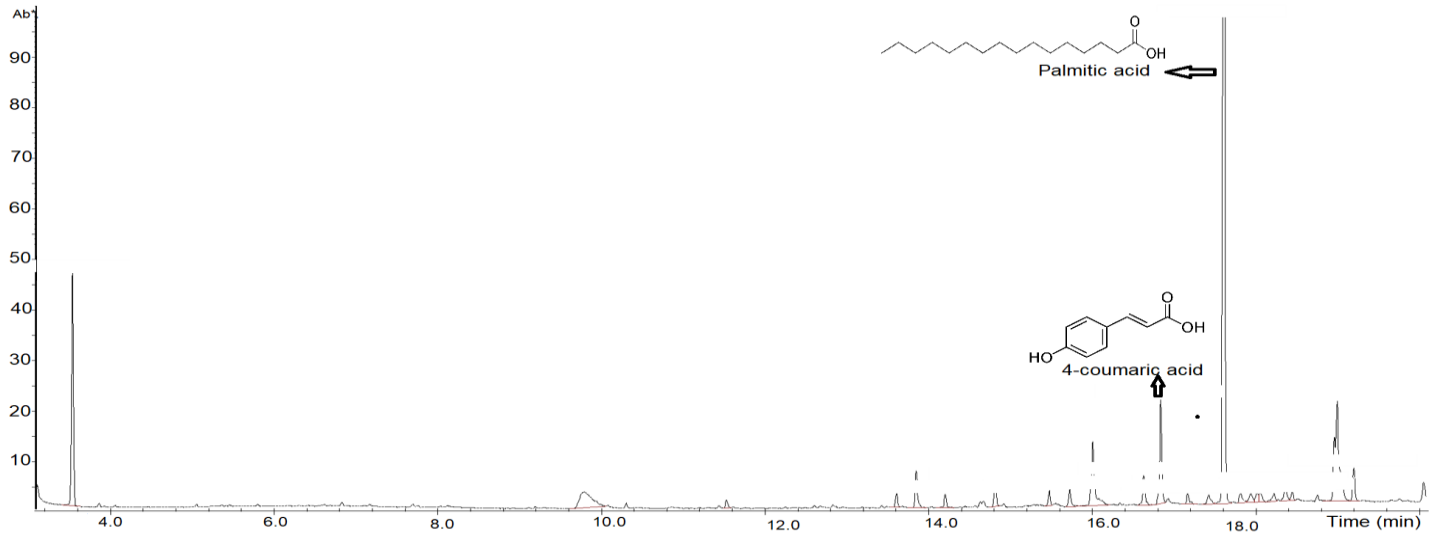

Figure 1: GC-MS fingerprint of *Cucurbita pepo* extract

Egg albumin-induced inflammation is observed to occur in two phases. A pronounced increase in paw volume was observed in the early phase (one hour). Paw volume was found to continually rise into the second phase in the egg albumin-inflamed control group. Pre-treatment of animals with CpE (100, 300, and 900 mg/kg) suppressed edema formation after the early phase, and the 900 mg/kg dose of CpE significantly (51.0%)

suppressed edema formation and reversed it at three hours (**Figure 2**). An analysis of the global edematous response (area under the curve) revealed that CpE at a dose of 900 mg/kg exhibited a significant anti-inflammatory effect (**Table 1**).

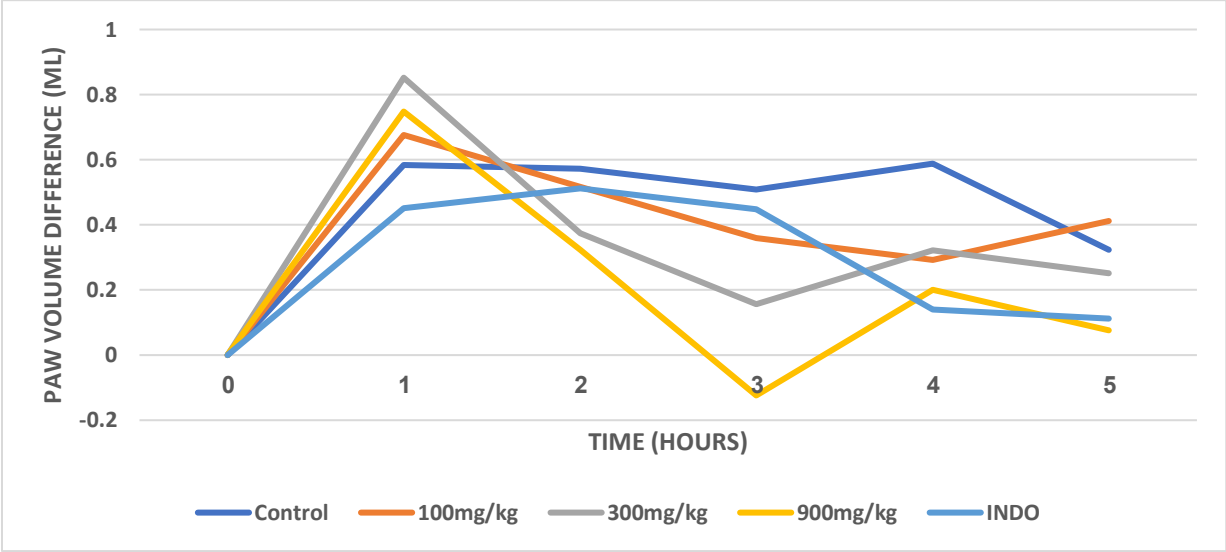

Figure 2: Effect of *Cucurbita pepo* extract on egg albumin-induced paw edema

Table 1: Effect of *Cucurbita pepo* leaf extract on global edematous response in egg albumin-induced paw edema

Treatment	Dose (mg/kg)	AUC	Inhibition (%)
Vehicle	-	2.414±0.288	
CpE	100	2.050±0.395	15
CpE	300	1.830±0.305	24
CpE	900	1.186±0.397*	51
Indomethacin	10	1.662±0.1714	31.2

Data shown are the mean±standard error of mean, n=5, *p<0.05 vs vehicle

Effect of CpE on xylene-induced topical edema: Topically administered xylene on mice ears induced inflammation characterized by reddening, fluid accumulation, and an increase in the weight of the inflamed ear tissue. The weight difference between the left and right ear tissue showed that the extract at different amounts caused inhibition of edema compared with the untreated, inflamed control group. CpE at 0.25 mg revealed the highest inhibition percentage (39.0%). CpE (1.25 and 2.5 mg) revealed the least degree of cellular edema and inflammation (**Figure 3**). The inhibition is dose-unrelated, and indomethacin (0.5 g/ml) exhibited profound inhibition (56.0%) of ear edema (**Table 2**). Histopathological examination also revealed that at the highest amount of CpE applied topically, there was a lower number of inflammatory cytokines observed, with a thinned-out epithelial area and evidence of tissue regenerating adnexa structures (**Figure 3**).

Table 2: Effect of *Cucurbita pepo* extract on xylene-induced topical ear edema

Treatment	Ear tissue weight difference	Inhibition (%)
Positive control	0.00048±0.005	-
Xylene	0.0153±0.0014	-
Indomethacin	0.0067±0.0012	56.3
CpE-2.5	0.0118±0.0020	22.8
CpE-1.25	0.0127±0.0022	17.2
CpE-0.25	0.0093±0.0025	39.0

Data shown are the mean±standard error of mean, n=6.

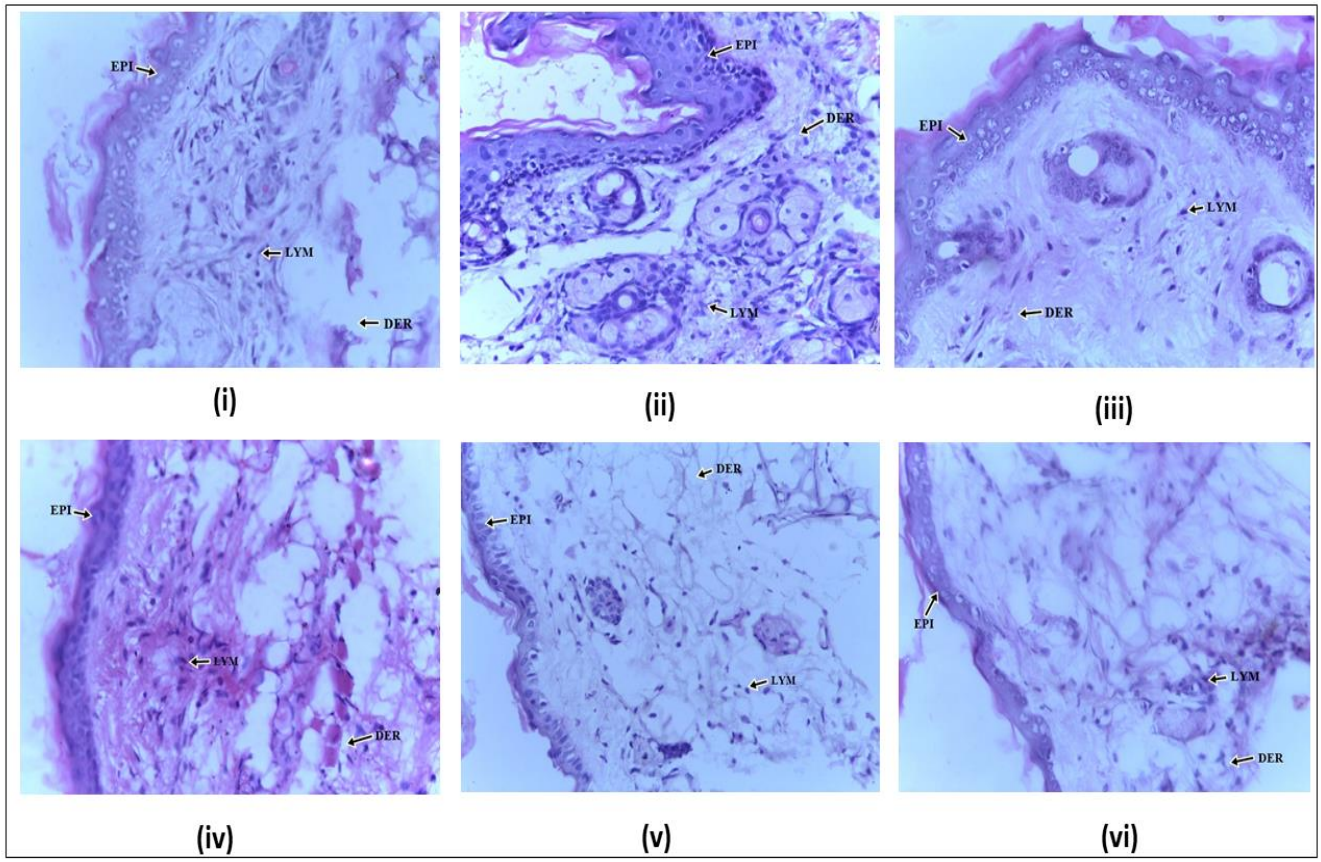

Figure 3: Sections (×100 magnification) of mouse ear skin tissue, showing **(i)** sham-inflamed group, **(ii)** untreated inflamed control group with epidermal thickening; loose, edematous dermis (++) with intense (++++) collection of inflammatory infiltrates, mainly lymphocytes and scanty polymorphs were observed. Mild destruction of adnexal structures was also noted **(iii)** 0.25 mg/ear CpE: consists of moderate (+++) amount of inflammatory infiltrates mainly lymphocytes and dermal edema (+) **(iv)** 1.25 mg/ear CpE: thinned-out epidermal layer with dermal edema, collagenous fibers and sparse (+) amount of lymphocytic infiltrates **(v)** 2.5 mg/ear CpE: thinned-out epidermal layer with a loose (+) edematous cellular dermis consisting infiltration (++) of mature adipocytes, collagenous fibers and a sparse amount of lymphocytic infiltrates and regenerating adnexa structures **(vi)** 0.5 mg/ear indomethacin: thinned epidermis with loose (+) edematous cellular dermis consisting of mature adipocytes, sparse (+) amount of lymphocytic infiltrates and regenerating adnexa structures.

Effect of CpE on acetic acid abdominal writhing: Abdominal contraction caused by the injection of dilute acetic acid was inhibited by CpE at all doses in a dose-independent manner, though this effect was not statistically significant. At 100 mg/kg, CpE revealed a reduced number of abdominal writhes than at other concentrations. However, indomethacin significantly reversed abdominal writhing caused by acetic acid (**Table 3**)

Table 3: Effect of *Cucurbita pepo* extract on acetic acid-induced abdominal writhing

Treatment	Dose (mg/kg)	Number of writhes	Inhibition (%)
Vehicle	-	40.5±7.8	-
CpE	100	23.8±4.7	41.2
CpE	300	37.0±4.6	08.6
CpE	900	29.0±5.8	28.4
Indomethacin	10.0	12.33±4.6**	69.6

Data shown are the mean±standard error of mean, (n=6), $^{**}p<0.01$

Effect of CpE on LPS-induced pyrexia: LPS from *E. coli* provoked a pyretic response following injection in all the LPS-injected groups. At three hours post-injection of LPS, there was a drop in body temperature for the

indomethacin group and CpE groups, and this trend was sustained to the 4th hour in all the CpE-treated groups (**Figure 4**). The reduction in body temperature was, however, not dose-dependent, although an analysis of the global fever response revealed that CpE (900 mg/kg) produced a significant effect (**Table 4**).

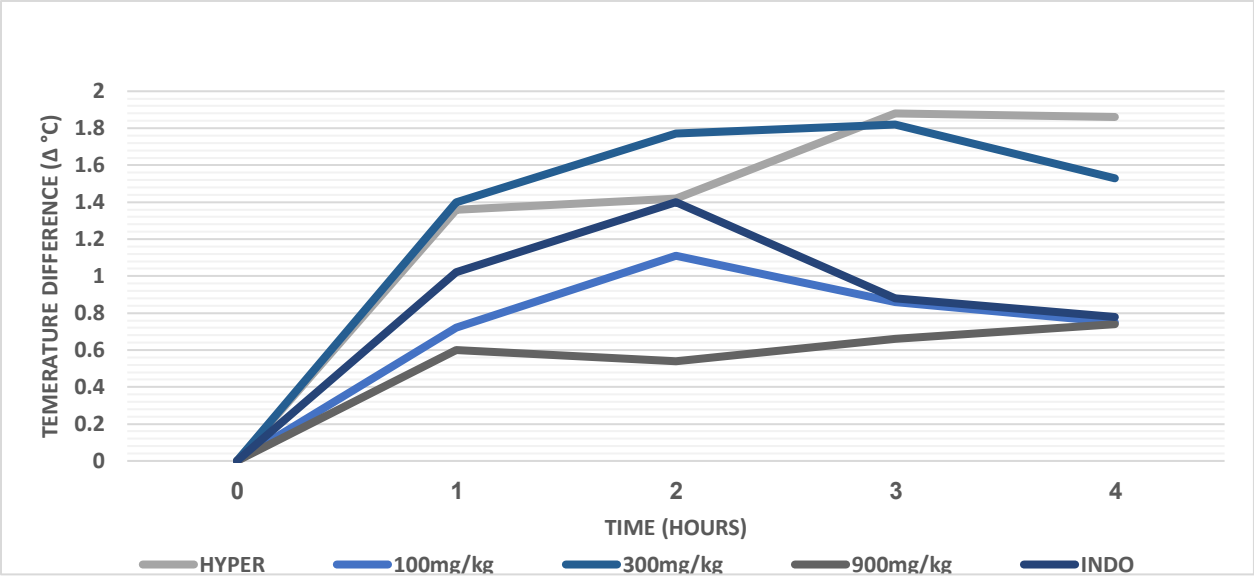

Figure 4. Effect of *Cucurbita pepo* extract on LPS-induced pyrexia

Table 4: Effect of *Cucurbita pepo* extract on global fever response in LPS-induced pyrexia in rats

Treatment	Dose (mg/kg)	AUC
Vehicle	-	5.59±1.068
CpE	100	4.8±1.207
CpE	300	5.83±0.822
CpE	900	2.17±0.83*
Indomethacin	10	2.79±0.30

AUC- Area under Curve, *p<0.05 (n=6) Data shown are the mean ± standard error of mean

Immunomodulatory activity of C. pepo (primary humoral response): The haemagglutination antibody titer was used to assess the humoral immune response in rats after challenging the immune system with sheep red blood cells. CpE-treated groups at all doses exhibited an increase in haemagglutination titer activity when compared to the control. At 900 mg/kg, CpE displayed the highest titer value of haemagglutination. Furthermore, the titer value correlated with a corresponding increase in relative spleen weights (**Table 5**).

Table 5: Immunomodulatory effect of *Cucurbita pepo* extract

Treatment	Dose (mg/kg)	HA-titer	Relative splenic weight
Vehicle	-	5.75±0.48	0.39±0.040
CpE	100	6.17±0.31	0.48±0.049 (+ 23.0%)
CpE	300	6.17±0.17	0.51±0.041 (+30.77%)
CpE	900	6.33±0.21	0.48±0.038 (+23.0%)
Levamisole	2.5	5.83±0.31	0.415±0.045 (+.6.4%)

Data shown are the mean±standard error of mean, (n=6)

Discussion

In the search for plants with bioactive constituents used against inflammation and fever, *C. pepo* was selected based on the literature review of being used in folklore medicine by traditional healers for the treatment of fever-

related malaria in southeast Nigeria [15]. Before this study, the ethanol CpE has been established to possess antimalarial activity [16]. Plants that demonstrate antimalaria activity may do so via parasite-specific and non-parasite-specific mechanisms, and may elicit toxicity [16]. From our previous study of the plant, there was no form of acute toxicity recorded when it was administered to mice [16]. Thus, the present study was focused on the evaluation of the anti-inflammatory, analgesic, antipyretic, and immunomodulatory activities of the ethanol extract of *C. pepo* aerial parts using different animal models, in addition to its GC-MS profiling and elemental analysis. The GC-MS analysis of CpE revealed the presence of long-chain fatty acids notably, 4-coumaric acid, palmitic acid, hexadecenoic acid, and stearic acid as bioactive principles. These phytoconstituents are known to possess important pharmacological activities. Fatty acids act directly on T cells to suppress PGE_2 IL-6, IL-1β, and TNF-α, thereby modulating immune responses in various disease conditions [22]. 4-coumaric acid also possesses anti-inflammatory properties by inhibiting phospholipase A_2 and antioxidant activity *in vitro* [23, 24]. Fatty acids may work synergistically with other phytoconstituents to exhibit anti-inflammatory, antipyretic, and immunomodulatory activities.

The *in vivo* anti-inflammatory activity was investigated using two common inflammatory animal models, egg-albumin-induced paw edema in rats, and xylene-induced ear edema in mice. Inflammation is a complex biological response of body tissues to harmful stimuli such as irritants and pathogens [25]. It is protective and involves immune cells, blood vessels, and molecular mediators [10]. Inflammation is a biphasic reaction; the first phase is the direct effect of the irritants, which bring about neurogenic pain. The second phase involves the release of inflammatory mediators and vasodilation, resulting in increased red blood cell perfusion to the tissues as well as increased permeability. There is the exudation of plasma proteins and fluid, bradykinins, and prostaglandins. Increased sensitivity to pain, altering blood vessels to allow migration of leukocytes into the affected tissues. Injection of egg albumin is known to induce the release of inflammatory mediators such as prostaglandins and other oxygenase products (bradykinins, lysosomes) [26]. CpE significantly inhibited the paw edema from the 2^{nd} hour post-induction of egg albumin for all the concentrations of CpE. CpE at 900 mg/kg produced the highest anti-inflammatory activity. The result suggests that CpE might act as a peripheral analgesic and anti-inflammatory agent by blocking the first and second phases of inflammation. Edema caused by the application of xylene on the right ear resolved on the application of CpE at all the concentrations, probably by inhibition of release of the prostaglandins and other mediators, and vasoconstriction of the affected parts, and decreasing the permeability of blood fluid to the area. The anti-inflammatory activity of *C. pepo* ethanol extract can also be attributed to one or more phytochemical constituents discovered in the extract. Flavonoids are known to be effective in acute inflammation and block prostaglandins involved in pain perception on exposure to irritants, and chemical and thermal stimuli [27]. Flavonoids are found to inhibit phosphodiesterase, seen in cell activation, and the effect is dependent on the biosynthesis of protein cytokines that mediate the adhesion of circulating leukocytes to the injured tissues. Hence, the significant anti-inflammatory activity of CpE recorded can be attributed to the bioactive substance. Acetic acid is an irritating agent that stimulates local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into the peritoneal cavity, and it acts peripherally on the mediators [28]. An increase in mast cell counts and their degranulation is implicated in pain-associated pathologies. Mast cells reside in tissues connected with the external environment and upon exposure to allergens release their mediators, which include histamine, serotonin, heparin, nitric oxide, lipid-derived prostaglandins, tumor necrosis factor (TNFα), leukotrienes, and neuropeptides [29, 30]. The anti-nociceptive effect of CpE using the acetic acid-induced writhing test is indicative of the peripheral analgesic activity of the extract. Any agent that lowers the writhing number demonstrates analgesia by inhibiting prostaglandin synthesis (PGE_2 and $PGF_2\alpha$), released from inflamed tissues or by inhibition of the enzyme cyclo-oxygenase, which prevents the synthesis of PGE2 production is considered to possess a peripheral mechanism of pain inhibition [12, 31-345]. *In vitro* studies

of phytochemical constituents of some plants in the past have revealed the analgesic properties of saponins and flavonoids through inhibition of the synthesis of enzymes involved in the production of chemical mediators such as prostaglandins [35, 36]. The combination of bioactive compounds known and unknown may be responsible for the anti-inflammatory and analgesic activities of *C. pepo*. Lipopolysaccharide (LPS) from *E. coli* was used to induce fever in rats. Exogenous pyrogens such as bacterial LPS from the cell wall of gram-negative bacteria trigger fever and result in the release of prostaglandins, which act on the hypothalamus to generate a systemic response in the body. LPS binds to the immune system protein, lipopolysaccharide-binding protein (LBP). The LBP-LPS complex binds to the CD14 receptor on macrophages and results in the release of endogenous cytokines- IL-1, IL-6, and TNFα, and the arachidonic acid pathway. Subcutaneous injection of LPS takes one hour to induce fever [37]. This is considered the most important method for screening medicinal plants for antipyretic activity. The oral administration of CpE significantly attenuated pyrexia four hours post-induction of fever in Wistar rats. There are many mediators of fever, and their endogenous or exogenous inhibition leads to attenuation of fever. In response to fever, the hypothalamus signals the release of cytokines into circulation, and this stimulates the release of local prostaglandins, mainly prostaglandin E_2 (PGE_2). This acts to elevate the hypothalamic thermal set point and is the ultimate mediator of febrile response [38].

Macrophages are cells that undergo physiological changes in response to infections or pathogen-derived products or antigens. They are the first line of host defense. Macrophages are the main source of cytokines [39]. Macrophages bring about the production of interleukin (IL)-1B, a pro-inflammatory cytokine that mediates inflammation, inducing fever and acute phase response. Overproduction of pro-inflammatory cytokines may cause immunopathology, while underproduction of cytokines results in uncontrolled infection. CpE caused increased hemagglutination, in response to the antigen- sheep red blood cells (SRBCs). This may be as a result of inhibition of IL-1β or the production of IL-10, an anti-inflammatory cytokine, or inhibition of translation and transcription of inflammatory cytokines. Macrophages can also reduce antigen presentation or inhibit T-cell activation [40]. The spleen readily provided the source of macrophage cells that easily eliminated the SRBC. The immunomodulatory effect of CpE may also be due to the presence of flavonoids in the plant. This is supported by a previous report, which showed that the administration of flavonoids showed significant inhibition of SRBC-specific hemagglutination titer and increased the production of antibodies [41].

Conclusion: This study indicates that the ethanolic extract of *Cucurbita pepo* has a potential protective immunomodulatory effect by the humoral immune mechanism. The analgesic effect of *Cucurbita pepo* extract relied on peripheral mechanisms and exerted anti-inflammatory and antipyretic effects. These activities are probably attributed to the presence of bioactive compounds in the plant.

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يُستخدم نبات القرع العسلي في العلاج الشعبي للملاريا، وله تأثيرات مضادة للالتهابات، ومضادة للألم، وخافضة للحرارة، ومعدلة للمناعة في نماذج الفئران

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ملخص: الملاريا مرض مُعدٍ يُسبب الحمى، والتهاب المفاصل، والقيء، وآلام المفاصل، وتقلصات البطن، وتضخم الطحال، وتضخم الكبد، وفقر الدم الشديد لدى الأطفال. أُجريت هذه الدراسة لدراسة خصائص نبات القرع (Cucurbita pepo) المضادة للالتهابات، والمضادة للألم، والخافضة للحرارة، والمُعدِّلة للمناعة، واستخدامه كعلاج مساعد في علاج الملاريا في نيجيريا. استُخلصت المادة النباتية المجففة (الأجزاء الهوائية) عن طريق النقع البارد باستخدام 70% من الإيثانول. وُصف المستخلص باستخدام تحليل كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS)، وخضع لتقييم دوائي في حالات الالتهاب الجهازي والموضعي، وشعور الألم الناتج عن حمض الأسيتيك، والحمى الناتجة عن عديد السكاريد الدهني. كما قُيِّم تأثيره على الاستجابة المناعية الخلطية الأولية لخلايا الدم الحمراء للأغنام. أظهر مستخلص نبات القراص (CpE) أن مكوناته الرئيسية هي أحماض البالمتيك (47.4%)، وحمض 9-أوكتاديكانويك (10.8%)، وحمض 4-كوماريك (5.3%). وقد كبح المستخلص التهاب القدمين بشكل يعتمد على الجرعة، محققًا تأثيرًا ملحوظًا عند جرعة 900 ملغ/كغ. كما ثبط مستخلص CpE، بتركيزات 0.25 و1.25 و2.5 ملغ، الالتهاب الموضعي بنسبة تراوحت بين 17.2% و39.0%.وتسلل الخلايا الليمفاوية المرتبط بالاستجابة الالتهابية. بجرعة 900 ملغ/كغ، خفّض CpE بشكل ملحوظ ارتفاع الحرارة، وكان تأثيره أعلى نسبيًا من تأثير الإندوميثاسين. ثبّط المستخلص الاستجابة المؤلمة لألم البطن، على الرغم من أن تأثيره كان ضئيلًا إحصائيًا. كما حفّز العلاج بالمستخلص الاستجابة المناعية، ويتجلى ذلك في زيادة عيار الأجسام المضادة للتراص الدموي والوزن النسبي للطحال في المجموعات المعالجة بـ CpE. وبشكل خاص، أثار المستخلص استجابة مناعية أقوى مقارنةً بالليفاميزول، وهو عامل منبه مناعي قياسي. تُقدّم هذه النتائج أدلة تدعم استخدام C. pepo في علاج الأعراض المصاحبة للملاريا.