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Anti-inflammatory Activity of *Cucumis sativus* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author UMMA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors UMMA and OFCN managed the literature searches, analyses of the study performed the spectroscopy analysis and authors UMMA and CAA managed the experimental process and author UMMA identified the species of the plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the anti-inflammatory activity and acute toxicity of *Cucumis sativus* L.
Study Design: Animal model assays of anti-inflammatory.
Place and Duration of Study: Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, 410001, Nigeria between April 2014 and November 2014.
Methodology: To evaluate anti-inflammatory activity, test substances that included whole *Cucumis sativus* L. homogenate were administered to four groups of Wistar rats. A control group received normal saline; a reference group received a standard anti-inflammatory drug, Diclofenac® while 2 test groups received whole *Cucumis sativus* L. fruit homogenate, respectively. Inflammation of the right hind paw of rats was induced by subplantar injection of 0.1 ml of 2% agar-agar suspension and increases in paw volumes, which relate to anti-inflammation, were measured using the volume displacement method. To evaluate the acute toxicity of *Cucumis sativus* L. fruit homogenate, 20 albino mice grouped into five groups of four mice each with animals were used. Animals in different groups were orally administered with different amount of the whole fruit homogenate. The animals were monitored for dullness, nervousness, uncoordinated movement, and death within 24 hours after administration.

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Results: Paw volume progressive decreased within 5.5 hours in test groups after administration of *Cucumis sativus* L. The administered *Cucumis sativus* L did not induce adverse effects on the mice within the concentration range of 0.5 mL/kg body weight to 5 mL/kg body weight test animals.
Conclusion: The whole fruit homogenate of *Cucumis sativus* L. had anti-inflammatory activity and no dose-dependent side effects.

Keywords: Cucumis sativus L.; inflammation; anti-inflammation; antioxidant; acute toxicity.

1. INTRODUCTION

Inflammation is a public health concern as it is a central attribute of biological response towards infections or injuries. When chronic or deregulated, this beneficial response may retard healing, and ultimately trigger diseased conditions in many organisms including humans [1,2]. To mitigate the adverse effects of inflammation in humans, effort is focused on the use of nonsteroidal anti-inflammatory drugs, [2-6] and disease-modifying antirheumatic drugs [2,7-9]. Although these drugs are efficacious in treating inflammation-triggered disorders, dose-dependent side effects are evidenced [2]. In addition, none of these drugs are primary preventive measures [2]. For instance, increased risk of upper gastrointestinal disorder is associated with the use of nonsteroidal anti-inflammatory drugs [6,10,11]. Consequently, inflammation-management platforms with attenuated side effects are critically needed.

An important component of these platforms are dietary mixes that incorporate variety of fish oil, tea, cocoa and chocolate [2] as well as fruits, vegetables, whole grains, and nuts [12-15]. Intake of a variety of fruits, and vegetables lowers risk of inflammation, and relates inversely with inflammation biomarkers, such as C-reactive protein, interleukin-6, tumour necrosis factor-α [16,17]. The inverse relation of fruits and vegetable with these biomarkers is due to the presence of bioactive phytochemicals, such as polyphenols, omega-3 fatty acids, and dietary fibre in fruits and vegetables.

Cucumber (*Cucumis sativus*, L.) is an edible fruit that belongs to the Cucurbitaceae family [18], and is rich in some of the aforementioned bioactive phytochemicals [19,20]. As an example, bioactive phenolic compounds are present in methanol/water extracts of fresh *Cucumis sativus* L. [21]. Further, methanol extract of the leaves of *Cucumis sativus* L. contains *C*-glycosyl flavonoids, phytochemicals that are linked in the defense mechanism of the plant [22]. Given the plethora of bioactive phytochemicals in *Cucumis sativus* L., its use in folk medicine in the management of several health disorders that include diabetes mellitus, hypertension and inflammation is understandable [21]. Indeed, extracts of *Cucumis sativus* L. exhibit anticancer [20], antioxidant [23-25], antimicrobial [24,26], antidiabetic [27], analgesic [28] and antiulcer [25] properties. In Africa, *Cucumis sativus* L. is applied in the treatment of tropical sprue, a malabsorption disease characterized by flattering of the villi and inflammation of the linings of the small intestine [29]. Indeed, *Cucumis sativus* L. fruits is recommended as a dietary treatment for tropical sprue [30]. Despite the recommendation on the use of *Cucumis sativus* L. in the management of tropical sprue, an inflammatory-related disease as well as the anti-inflammatory activity of its seeds [31], to the best of our knowledge, the anti-inflammatory properties of the whole fruit is yet to be empirically established [19]. Here, we show the anti-inflammatory activity of whole *Cucumis sativus* L. fruit homogenate using animal models. We also examined the antioxidant activity as well as the acute toxicity of the whole fruit homogenate. Our results highlight the potential pharmaceutical function of the whole fruit homogenate in the treatment of inflammation.

2. MATERIALS AND METHODS

2.1 Chemicals, Plant Materials and Animal Models

All the chemicals and reagents were of analytical grade, and were used as obtained from the suppliers. Fresh whole *Cucumis sativus* L. fruits were purchased from Nsukka main market, Nsukka, Nigeria and were identified at the Bioresources Development and Conservation Programme Research Center, Nsukka, Nigeria. The fruits were homogenized using high-speed blender and administered without dilution. Twenty albino mice of Swiss strain (22–28 g) were used for index of acute toxicity study while sixteen Wistar rats (120–200 g) were used for anti-inflammatory.

2.2 Anti-inflammation Assay

The rat paws oedema method of Winter et al. [32] as modified previously [33] was used to evaluate the anti-inflammatory activity of the whole *Cucumis sativus L*. fruit homogenate. Prior to the studies, the rats were grouped into four groups (1-4) of four rats each, were acclimatized for seven days, fasted and deprived of water for 18 hours. Anti-inflammatory test substance was orally administered 1 h before inducing inflammation. The control group 1 received normal saline (5 mL/kg body weight (b.w.)), the reference group 2 received a standard anti-inflammatory drug, Diclofenac®, (150 mg/kg b.w.) while test groups 3 and 4 received 2 mL/kg b.w. and 4 mL/kg b.w. of whole *Cucumis sativus* L. fruit homogenate, respectively. Inflammation of the right hind paw was induced by subplantar injection of 0.1 ml of 2% agar-agar suspension. The changes in paw volumes were measured using the volume displacement method [33] immediately before agar-agar injection and 1.5, 3.0 and 5.5 hours after injection. The percentage inhibition of oedema was calculated as described [34].

2.3 Determination of Phenolic, Flavonoid and Anthocyanin Contents

The phenolic, flavonoids and anthocyanin content of the homogenate were determined using reported protocols [35]. As an example, total phenols was determined as follows: 2 g of the homogenate was macerated in 20 ml of 80% ethanol for 5 minutes, centrifuged for 10 minutes and 1 mL of the supernatant was transferred into a test tube. To this was added 4 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent, followed by 2 mL of 20% Na_2CO_3. The mixture was allowed to stand for 90 minutes, and the absorbance was taken at 760 nm using SP 500 Spectrophotometer, Pye Unicam. The measurements, which were taken in triplicates, were compared against a standard curve of prepared gallic acid solutions and expressed as milligram of gallic acid equivalent (GAE) per gram of homogenate. To ascertain the flavonoid content, 5 mL of the supernatant was transferred to a test tube. To this was added 0.3 mL of 5% of sodium nitrite solution and the mixture was allowed to stand for 5 minutes. Thereafter, 0.3 mL of 10% ferric chloride was added and the mixture was allowed to stand for another 6 minutes. Then 4 mL of 4% sodium hydroxide was added and the absorbance was measured immediately and after 15 minutes of incubation at room temperature. For the anthocyanin content, 2 g of the homogenate was macerated in 20 mL of sodium citrate pH 3.4 buffer, centrifuged for 5 minutes and 2 mL of supernatant was transferred into two different test tubes. Then, 4 mL of citrate buffer was added to one test tube and a 1:1 $HCl:H_2O$ mixture was added to the other test tube. The absorbance of the mixtures was taken at 500 nm after 1 hour using water as blank. The flavonoid and anthocyanin contents were determined in triplicates and expressed as milligram of catechin equivalents and cyanidin 3-glucoside equivalents per gram of homogenate, respectively [34].

2.4 DPPH Radical Scavenging Assay

The radical scavenging activity of whole *Cucumis sativus* L. fruit homogenate and a standard, vitamin E, was determined spectrophotometrically using the stable radical scavenger, 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) as reported [36] but with slight modifications. A quantity, 2 g, of fruit homogenate was macerated with 20 mL of ethanol, centrifuged and 0.1 mL of the supernatant measured into a test tube. This was followed by the addition of 0.9 mL with absolute ethanol and 0.5 mL of 0.3 mM DPPH. The mixture was kept in the dark for 30 min at room temperature. Thereafter, decrease in absorption was measured at 518 nm using SP 500 Spectrophotometer, Pye Unicam. The absorption of a blank that contained the same amount of absolute ethanol and DPPH was also measured and the percentage inhibition was calculated as reported previously [32]. All measurements were carried out in triplicates.

2.5 Index Acute Toxicity Studies

The method of Lorke [37] was used to ascertain the acute toxicity of *Cucumis sativus* L homogenate. Twenty albino mice were used in this study. Prior to the study, the animals were acclimatized for seven days, starved of food for 18 hours but allowed access to water. The animals were grouped into five groups (5-9) of four mice each with animals. Animals in groups 5,6,7,8 and 9 were orally administered with 0.5, 1.01.5,3.0,5.0 mL/kg body weight of the whole fruit homogenate, respectively. The animals were frequently observed after administration of the homogenate for adverse effects that included dullness, nervousness, uncoordinated movement, and death within 24 hours.

2.6 Statistical Analysis

All analyses were run in triplicate and results are expressed as mean±SEM. Tests of statistical significance were carried out using two-way analysis of variance (ANOVA). The Statistical Product and Service Solutions (SPSS) using IBM version 20 was used and P = .05 were considered significant.

3. RESULTS AND DISCUSSION

A growing number of scientific investigations support food-based strategy in the management and treatment of diseases including inflammation [2] and this approach is popular in folk medicine. For instance, Africa folk medicine, *Cucumis sativus* L. is used to attenuate and mitigate tropical sprue, a disease characterized by the flattering of the villi and inflammation of the linings of the small intestine. Again, in Puerto Rico, the consumption of *Cucumis sativus* L. is a recommended remedy for the treatment of tropical sprue [30]. Despite the ethnopharmaceutical applications of this fruit, specifically in the treatment of tropical sprue, scientific research is yet to verify this potential. As tropical sprue is accompanied by the inflammation of the lining of the small intestine, it would, therefore, be interesting and worthwhile to investigate the role of *Cucumis sativus* L. in the mitigation of chronic inflammation. In this report, we focus on the role of whole *Cucumis sativus* L. fruit homogenate in the mitigation of inflammation using a modified rat paw oedema method.

Similar to carrageenan-induced inflammation, agar-induced inflammation in rat paw is biphasic. The early phase, which lasts up to 2 hours after administration of the agar-agar irritant, is prompted by the release histamine, 5-hydroxyl tryptamine, kinin and serotonin [38,39]. The later phase commences after 2 hours of irritant administration and last till 5 hours and is triggered by bradykinin, protease, prostaglandins, and lysosome [38,39]. Thus, in this study, the paw volume was monitored within 5.5 hours. A progressive decrease in paw volume within this time frame was observed in test groups 3 and 4 that received whole *Cucumis sativus* L. fruit homogenates (Fig. 1). In addition to the decrease in paw volume, these groups also exhibited lower paw volume as compared to animals in control group 1 (Fig. 1). Also increasing the amount of *Cucumis sativus* L. homogenate from 2 mL/kg body weight (b.w.) to 4 mL/kg b.w. reduced paw volume in the test groups (Fig. 1). This suggested that *Cucumis sativus* L is an anti-inflammatory food-based pharmaceutical. The anti-inflammatory activity is due to the presence of bioactive phytochemicals that inhibits the release or mitigates the action of pro-inflammation mediators [2,15,16].

To gain an insight into the levels of phytochemicals in the assayed *Cucumis sativus* L. we determined the amount of total polyphenols, flavonoids and anthocyanin in the whole fruit homogenate (Table 1). Appreciable amounts of anthocyanin (1.21±0.39 mg of cyanidin 3-glucoside equivalent/g of homogenate), flavonoids (2.14±0.56 mg of catechin equivalents/g of homogenate) and polyphenols (8.51±0.50 mg of GAE/g of homogenate) were found in the fruit homogenate. The result of phytochemical screening contrasted those of Jony and Roksana [39] who reported the absence of flavonoids in the ethanol extract of *Cucumis sativus.* It could be that flavonoids were not detected as a result of the extraction method used, as Kumar et al., [28] reported the presence of flavonoids in the aqueous extract, thus correlating the findings of this investigation. Polyphenols exhibit anti-inflammatory activities *via* inhibiting the action of pro-inflammatory enzymes, modulating the production of pro-inflammatory molecules, inhibiting pro-inflammatory cell adhesion molecules, and/or scavenging reactive oxygen species (ROS) [2]. As radical species, ROS enhances inflammation *via* the activation of inflammatory genes that include nuclear factor κB, and polyphenols regulates these genes by scavenging ROS or increasing the antioxidant activities. The antioxidant activities of the homogenate were also determined using the DPPH radical scavenging assay. The essence of DPPH method is the reaction of antioxidants with 1, 1-diphenyl-2-picryl hydrazyl (DPPH), resulting in discoloration of the latter. The degree of discoloration at 518 nm is a measure of the antioxidant activity [40]. Good antioxidant activities as evidenced from the decrease in the mean absorbance value and increase in the percentage inhibition of DPPH (Table 2) was found in the assayed *Cucumis sativus* L. This observation on the DPPH radical scavenging activity of the homogenate of *Cucumis sativus* L fruit was in agreement with that of Agarwal et al. [41].

Table 1. Quantitative phytochemical constituents of the homogenate of *Cucumis sativus* L. fruit

Phytochemical	Composition (mg/g)[a]
Polyphenol	8.51±0.50
Flavonoid	2.14±0.56
Anthocyanin	1.21±0.39

[a]*Polyphenol, flavonoid, and anthocyanin contents are expressed as milligram of gallic acid equivalent, milligram of catechin equivalents, and cyanidin 3-glucoside equivalents per gram of homogenate, respectively*

To compare the anti-inflammatory activity of our homogenate with commercially available anti-inflammatory drugs, Diclofenac® a commercially available anti-inflammatory drug was administered to animals in reference group 2. The administration of 4 mL/kg b.w. of the whole *Cucumis sativus* L. whole fruit homogenate to test group 4 animal models suppressed oedema to an extent similar to that of Diclofenac® after 5.5 hours (Fig. 1). This result demonstrates the potential of *Cucumis sativus* L. as a comparable platform to commercially available synthetic anti-inflammatory drugs for treating inflammation. Unlike these synthetic drugs, the whole fruit homogenate of *Cucumis sativus* L. does not have dose-dependent side effects. To test this hypothesis, acute toxicity studies of the whole fruit homogenate were conducted using a modified Lorke method [36]. This method, which applies to agricultural produce as well, provides acceptable information on the lethal dose (LD_{50}) using small number of experimental animals [37]. No mortality or uncoordinated movements was observed with gradual increase in the administered dose of *Cucumis sativus* L from 0.5 mL/kg b.w. to 5 mL/kg b.w. (Table 2). This findings support our hypothesis, demonstrating the absence of dose-dependent side effects.

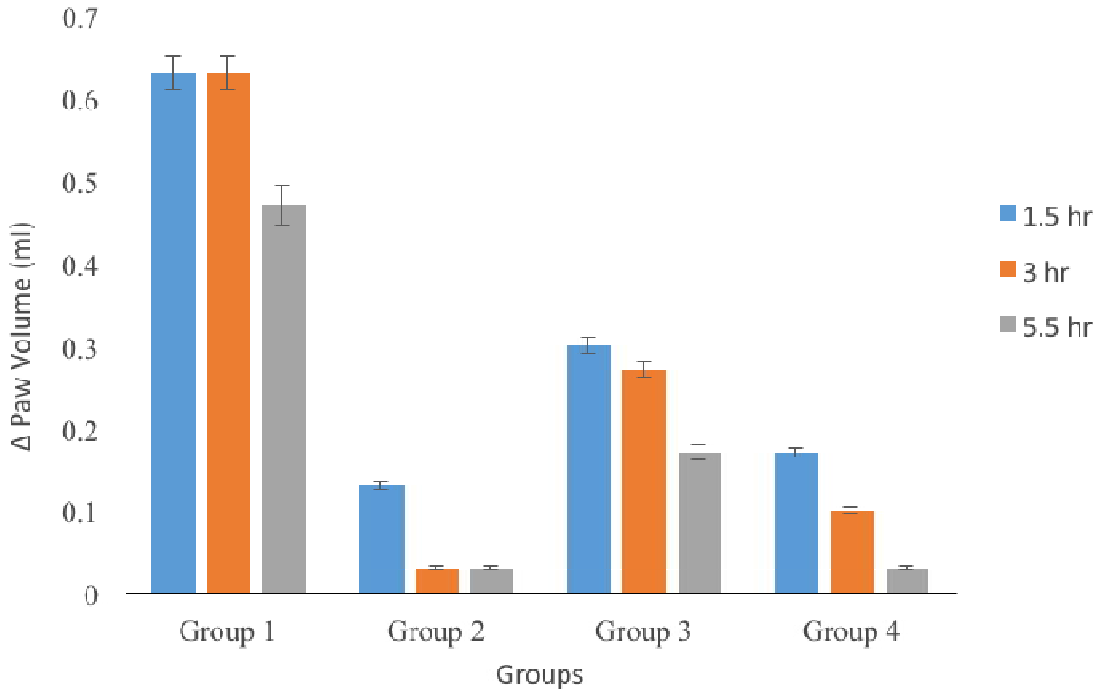

Fig. 1. Change in rat paw volume in the different groups. Control group 1, administrated with 5 mL/kg b.w. normal saline; reference group 2, administrated with 150 mg/kg b.w. Diclofenac®; test group 3, administered 2 mL/kg b.w. whole *Cucumis sativus* L. fruit homogenates; and test group 4, administered 4 mL/kg b.w. whole *Cucumis sativus* L. fruit homogenates

Table 2. DPPH radical scavenging activity of the whole *Cucumis sativus* L. homogenate fruits and vitamin E.

Test sample (mL)	Δ Mean absorbance value (mean±SEM[a])	% Inhibition[b]
0.1	0.421±0.001	50.8
0.2	0.411±0.003	52.0
0.3	0.380±0.005	55.6
0.4	0.349±0.004	59.6
0.5	0.340±0.002	60.3
Vitamin E[c]	0.321±0.001	62.5
Control	0.857±0.003	-

[a]SEM was significant at P = .05 compared to control. [b]Percentage inhibition of radical formation was calculated relative to control. [c]The concentration and volume of vitamin E used were 0.1 mg/mL and 0.5 mL, respectively

Table 3. Result of acute toxicity test

Group	Dosage (mL/kg b.w)	Number of dead animals/total number of animals
5	0.5	0/4
6	1.0	0/4
7	1.5	0/4
8	3.0	0/4
9	5.0	0/4

4. CONCLUSION

Cucumis sativus L. is used in folk medicine as a treatment of tropical sprue, an inflammatory-related disease. We investigated the anti-inflammatory activity of *Cucumis sativus* L. using animal models. Our results suggest that the whole fruit homogenate of *Cucumis sativus* L. had anti-inflammatory activity and unlike synthetic drugs, had no dose-dependent side effects. This presents *Cucumis sativus* L. as a functional food for the management of inflammation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental protocols including the use of animal models were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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