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Research Article

Inhibition of Phospholipase A_2 and Prostaglandin Synthase Activities as Possible Mechanisms for the Anti-Inflammatory Effect of *Cucumis sativus* Fruit Homogenate

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Abstract

Research on inflammation has become the focus of global scientific study because of its implication in virtually all human and animal diseases. *Cucumis sativus* has been reported to have anti-oxidant activity, anti-inflammatory and analgesic effect. The fresh fruit of *Cucumis sativus* was homogenized and used for all experimental analysis without dilution in doses of 0.5ml and 1.0ml. In this work, the effects of the fruit homogenate on phospholipase A_2 and prostaglandin synthase activities were studied. Data were analysed using two-way ANOVA; the acceptance level of significance was $p < 0.05$. The homogenate exhibited a significant ($p < 0.05$) dose dependent inhibition of prostaglandin synthase activity compared to indomethacin, standard drug. The fruit homogenate significantly ($p < 0.05$) inhibited phospholipase A_2 activity in all the doses. The results indicate that the homogenate may exert its anti-inflammatory effect by sequential inhibition of phospholipase A_2 and prostaglandins synthase activities.

Keywords: *Cucumis sativus*; Phospholipase A_2; Prostaglandin synthase; Inflammation

Statement of problem

Despite the established anti-inflammatory properties and the ethnopharmaceutical applications of *Cucumis sativus* fruits, scientific research is yet to ascertain the biochemical mechanism for the anti-inflammatory effect of *Cucumis sativus* fruit. It was therefore worthwhile investigating the mechanism of action of *Cucumis sativus* fruit's anti-inflammatory properties. In this study, we focus on the effect of whole *Cucumis sativus* fruit homogenate on the activities of Phospholipase A_2 and Prostaglandins synthase, determining the percentage inhibition of the enzymes' activities.

Introduction

Cucumis sativus is an edible fruit that belongs to the Cucurbitaceae family [1] and is rich in some bioactive phytochemicals [2-3]. Bioactive phenolic compounds are present in methanol and water extracts of fresh *Cucumis sativus* [4]. Furthermore, methanol extract of the leaves of *Cucumis sativus* contains C-glycosyl flavonoids, phytochemicals that are linked to the defense mechanism of the plant [5]. Given the plethora of bioactive phytochemicals in *Cucumis sativus*, its use in folk medicine in the management of several health disorders (diabetes mellitus, hypertension and inflammation) is understandable [4]. Indeed, extracts of *Cucumis sativus* exhibit anticancer [3], antioxidant [6-8], antimicrobial [7,9], antidiabetic [10], analgesic [11], antiulcer [8] and anti-inflammatory [12-13] properties. Indeed, cucumis sativus fruit is recommended as a dietary treatment for tropical sprue [14].

Inflammation is a normal protective response to tissue injury that is caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the result of concerted participation of large number of vasoactive, chemotactic and proliferative factors at different stages and there are many targets for anti-inflammatory action [15]. The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It (Inflammation), a dynamic process elicited in response to mechanical injuries, burns, microbial infections and other noxious stimuli that threaten the well-being of the host, involves changes in blood flow, increased vascular permeability, destruction of tissues via the activation and migration of leucocytes with synthesis of reactive oxygen derivatives (oxidative burst) and the synthesis of local inflammatory mediators, such as prostaglandins (PG), leukotrienes[16] and platelet-activating factors induced by phospholipase A_2, cyclooxygenases (COXs) and lipoxygenases. Arachidonic acid is a key biological intermediate that is converted

into a large number of eicosanoids with potent biological activities. The two major pathways of arachidonic acid metabolism are the COX pathway, which results in the formation of both PGs and thromboxanes and the 5-lipoxygenase pathway, which is responsible for the formation of leukotrienes and 5S-hydroxy-6E, 8Z, 11Z, 14Z- eicosatetranoic acid (5-HETE) [17].

Phospholipase A_2 (PLA_2) (or phosphatide acylhydrolase 2) is an enzyme that catalyzes the hydrolysis of acyl group attached to the 2-position of intracellular membrane phosphoglycerides. This hydrolysis releases arachidonic acid from membrane phosphoglycerides [18]. Arachidonic acid is the major precursor of pro-inflammatory eicosanoids – Prostaglandins and Leukotrines [18-19]. This enzyme is the main component of snake venom and it has been investigated for its similarity to mammalian phospholipase [20]. Due to the role of PLA_2 in the inflammatory process, PLA_2 have been considered as potential targets in anti-inflammatory drug discovery, resulting in an interest in PLA_2 inhibitors. Plant species that are traditionally used as snake-bite antidotes because haemolytic and myolytic phospholipase A_2 are often present in snake venom, which results in damage to cell membranes, endothelium, skeletal muscle, nerves and erythrocytes are consider inhibitors of phospholipase A_2[17].

Prostaglandins (PG) are important lipid mediators derived from arachidonic acid that control not only numerous physiological events such as blood pressure, blood clotting and sleep but also inflammation [21]. Prostaglandin E_2 is a key player in pyresis, pain and inflammatory responses and the beneficial therapeutic effects of non-steroidal anti-inflammatory drugs (NSAIDs) are essentially attributed to the suppression of prostaglandins E_2 [22]. The biosynthetic pathway to prostaglandin E_2 includes the release of arachidonic acid from membrane phospholipids by phospholipases A_2 followed by conversion via Cox-1 and -2 to prostaglandins H_2 and its subsequent isomerization by prostaglandins E_2 synthases (PGEs). Upon appropriate stimulation of neutrophils, arachidonic acid is cleaved from membrane phospholipids and can be converted to leukotrienes and prostaglandins through 5-lipoxygenase (5-Lox) or cyclooxygenase (Cox) pathways respectively [23].

This work was aimed to assay the effect of the fruit homogenate of *Cucumis sativus* upon PLA2 and PG activity as possible anti-inflammatory mechanism.

Materials and methods

Plant material

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 used without dilution.

Chemicals

All the chemicals and reagents used for this study include analytical grade of methanol, hydroquinone, ethylacetate, sucrose, ethylene diamine tetracetate (EDTA), hydrochloric acid, sulphuric acid (BDH chemicals, Poole England), sodium chloride, tri-sodium citrate (May and Baker, England), glutathione, and haemoglobin (Sigma, U.S.A). Other reagents and solvents were also of analytical grade.

Assay of phospholipase a2 activity

The effect of the homogenate of *Cucumis sativus* fruit on phospholipase A_2 activity was assayed according to the methods of Vane [24] and Morimoto *et al* [25].

Enzyme preparation

The enzyme preparation was obtained from Bacillus cereus strain culture. The organism was cultured in nutrient broth for three days. The culture was transferred into a test tube containing normal saline and centrifuged at 3000 x g for 10 min. The cells settled at the bottom while the supernatant contained the exuded enzyme. The supernatant was decanted and used for enzyme assay.

Assay of enzyme activity

Aliquots (0.5ml) of re-suspended erythrocytes were mixed with normal saline containing 2mM calcium chloride and the enzyme preparation and incubated either in the absence or presence of the Cucumis sativus fruit homogenate, as shown in (Table) below.

The reaction was initiated by the addition of 0.2 ml of the enzyme preparation. The reaction mixture was incubated at 37^0C for 1 hr., and the incubates centrifuged at 3000 x g for 10 min and the absorption of the supernatant was read against the blank (containing the enzyme and the homogenate, but without RBC) at 418 nm. Prednisolone, a known inhibitor of the enzyme was used as standard. The percentage inhibition of the enzyme activity was calculated with the relationship:

HRBC (ml)	Homogenate (ml)	Normal saline (ml)	Enzyme preparation (ml)
0.5	-	2.0	0.2
0.5	0.4	1.5	0.2
0.5	0.6	1.5	0.2
0.5	0.8	1.5	0.2
0.5	1.0	1.5	0.2
0.5	Prednisolone (1ml/mg)	1.5	0.2

Table: Reaction medium for assay of phospholipase A_2 activity

$$\% \text{ Inhibition} = 1 - \frac{\text{Absorbance of test sample}}{\text{Absorbance of control (Tube without homogenate)}} \times 100$$

Assay of prostaglandin synthase activity

Prostaglandin synthase activity was assayed by a modification [26] of the methods of Yoshimoto., *et al.* [27] and Flower., *et al* [28].

Isolation of the enzyme-containing fraction

The enzyme - prostaglandin synthase was isolated from beef seminal vesicle by the method of Nugteren., *et al* [29]. The frozen beef seminal vesicle obtained from the local slaughterhouse (Ikpa Commodity Market, Nsukka) was thawed and freed of fat and adhering connective tissues. A known quantity was weighed out, sliced and homogenized in 5 volumes of sucrose – EDTA for 1 min at 0.4^{0}C. The homogenate was centrifuged at 6000 x g for 10 min, the supernatant decanted. The pellet was centrifuged for 10 min at 15000 x g and the supernatant was again decanted, centrifuged at 18000 x g for 10 min, and the supernatant used as the crude enzyme preparation.

Procedure

The reaction mixture contained 1.5 ml cofactor solution (33 mM hydroquinone, 21 mM glutathione and 40 µM haemoglobin, 0.3 ml buffer), 8 mg of enzyme preparation and 0.5 ml arachidonic acid as substrate. After incubating at 37^{0}C for 2 min, the reaction was stopped by adding 1.5 ml of 0.2 M citric acid. The incubate was extracted twice with 5 ml ethylacetate and centrifuged at 2,500 g for 10 min. Each time, a4 ml aliquots of the top organic layer was pipetted into a clean test tube. The combined ethylacetate extract was evaporated to dryness under a stream of nitrogen gas. The residue was dried overnight in vacuum and then dissolved in 2 ml methanol and 0.5 ml 3 M KOH solution was added to the solution and allowed to stand for 15 min. The absorbance of tests against blank (the blank contained everything in the reaction mixture and a boiled (denatured) enzyme in place of the active enzyme sample at 37^{0}C) was read at 278 nm. Triplicate determinations were made with the assay mixture containing 0.1, 0.5, 1.0 ml of the fruit homogenate. Indomethacin (0.4 mg/ml) was used as standard drug and control.

Enzyme activity

Enzyme activity was quantified using the relationship:

Unit g-1 enzyme preparation =

$\{(Abs_{278}\ min^{-1} \times 10 \times 2.5 \times 1000) / (25.6 \times 9 \times mg_{\ enzyme}\ test^{-1})\}$

The percentage inhibition of the enzyme activity was calculated with the relationship:

$$\% \text{ Inhibition} = 1 - \frac{\text{Enzyme activity of test sample}}{\text{Enzyme activity of control}} \times 100$$

Results

The homogenate of *Cucumis sativus* fruit significantly ($p < 0.05$) inhibited phospholipase A2 activity in a dose related manner provoking inhibition comparable to that of prednisolone. High percentage inhibition of activity (81.5 and 82.4) were obtained for doses of 0.8 and 1.0 ml of the fruit's homogenate as compare to 82.8 % inhibition of activity for that of the standard drug, prednisolone (1 ml/mg) (Table 1).

Test sample	Volume (ml)	Δ Mean Absorbance	% Inhibition
Control	-	0.571 ± 0.003	00.0
Homogenate	0.4	0.299 ± 0.001	47.6
	0.6	0.216 ± 0.002 ^{A}	62.3
	0.8	0.106 ± 0.002 ^{A}	81.4
	1.0	0.101 ± 0.002 ^{A}	82.3
Prednisolone (1mg/ml)	1.0	0.098 ± 0.001 ^{A}	82.8

Table 1: Effect of the homogenate of *Cucumis sativus* fruit on phospholipase A_2 activity

Values of absorbance shown are Mean ± SD (n=3) *P < 0.05 compared to control. Percentage inhibition of phospholipase A_2 activity was calculated relative to control.

The homogenate of *Cucumis sativus* fruits evoked significant ($p < 0.05$) concentration dependent inhibition of prostaglandin synthase activity. The activity of the prostaglandin synthase enzyme preparation from beef seminal vesicle was 5.59, 2.87, 2.71 and 2.56. The standard anti-inflammatory drug, indomethacin (0.4 mg/ml) inhibited the enzyme activity by 82.0%. *Cucumis sativus*

Test sample	Amount (ml)	Δ mean absorbance	Enzyme activity (IU/L)	% Inhibition
Control	-	0.513 ± 0.001	14.26	00.0
Homogenate	0.1	0.201 ± 0.007 [A]	5.59	60.8
	0.5	0.103 ± 0.001 [A]	2.87	79.9
	1.0	0.098 ± 0.004 [A]	2.71	81.0
Indomethacin (4mg/ml)	1.0	0.092 ± 0.002 [A]	2.56	82.0

Table 2: Effect of the homogenate of Cucumis sativus fruit on prostaglandin synthase activity

Values of absorbance shown are Mean ± SD (n=3). [A]P < 0.001 compared to the control. Percent inhibition of enzyme activity was calculated relative to the control.

homogenates inhibited the enzyme activity by 79.9 and 81.0 % respectively at 0.5 and 1.0 ml concentration which is comparable to the inhibition exhibited by the standard drug.

Discussion

A growing number of scientific investigations support food-based strategy in the management and treatment of diseases including inflammation [30]. This approach is popular in folk medicine. For instance, in African folk medicine, *Cucumis sativus* 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. Also, in Puerto Rico, the consumption of *Cucumis sativus* is a recommended remedy for the treatment of tropical sprue [14]. Many scientific reports have established the anti-inflammatory properties of *Cucumis sativus* fruit [12].

Phospholipase A_2 cleaves free fatty acid from erythrocyte phospholipids. The enzyme activity assayed using its action on erythrocyte membrane, creates leakage thus causing haemoglobin to flow out into the medium. *Cucumis sativus* was highly effective in inhibiting phospholipase A_2 activity. The inhibition of phospholipase A_2 may either be directly or by an action of *Cucumis sativus* on the membrane [31]. Shah., *et al* [17] reported that the inhibition of Phospholipase A_2 is mediated via lipocortine or by direct interaction with the enzyme itself. The former mechanism utilizes the protein, lipocortine, the synthesis of which is commanded by steroidal hormones and steroid like plants known as triterpenoids. Example of lipocortine mediated phospholipase A_2 inhibitors that are of therapeutic value and potent anti-inflammatory drugs are cortisone, prednisolone and betamethasone [32]. In this study, *Cucumis sativus* fruit inhibition of phospholipase A_2 activity compare significantly with the standard drug, prednisolone. Inhibition of phospholipase A_2 therefore implies that the homogenate of *Cucumis sativus* fruit may suppress the mobilisation of free fatty acids from membrane phospholipids. It was reported that anti-inflammatory and immunosuppressive steroids inhibit arachidonic acid and its metabolites (prostaglandins) by induction which inhibits phospholipase A_2 [33-34], thereby suggesting that *Cucumis sativus* may be an immunosuppresor.

Prostaglandins released from membrane phospholipids in response to hormones and other signals, functionally vary in a tissue-specific manner, but several of them trigger pain, fever or inflammation [35-36]. They are synthesized *de novo* by the action of prostaglandin synthase from the free fatty acid precursor, arachidonic acid which is released from membrane phospholipids by the action of phospholipase A_2. In this study, the synthesis of prostaglandin was effectively inhibited by the homogenate of *Cucumis sativus* fruit. Vane [24] considers inhibition of prostaglandin synthesis as the mechanism by which aspirin-like drugs produce anti-inflammatory effects. By implication, the mobilization of substrate was inhibited, thereby accounting for decreased availability of the mediators-prostaglandins. The sequential inhibition of prostaglandin synthesis leads to potent suppression of prostaglandins synthesis and possible amplification of the anti-inflammatory activity of the homogenate of *Cucumis sativus* fruit.

Thus, the homogenate may exert anti-inflammatory effect by sequential inhibition of phospholipase A2 and prostaglandins synthase.

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