EFFECT OF CUCUMBER(*CUCUMIS SATIVUS*) FRUIT HOMOGENATE ON HYPOTONICITY – INDUCED HAEMOLYSIS OF RED BLOOD CELL

Short Research Article

ABSTRACT

Background: Several findings and reports show that people with high intake of*Cucumis sativus*(Cucumber) have relief in pains, swelling and other inflammatory signs. Human red blood cell membrane stabilization has been used as a method to investigate the mechanism of action of anti-inflammatory drugs. The anti-inflammatory activity of ~~C~~cucumber has been demonstrated in previous study. In this study, we aimed at assessing the effect of cucumber(*Cucumis sativus*) fruit homogenate on hypotonicity – induced haemolysis of red blood cell.

Method: Whole fresh blood (3ml) was collected from healthy volunteer into plastic tubes containing 0.1 volume of 3.8% trisodium citrate and used within 8 hr. The blood sample was centrifuged at 3000 x g for 10 min and the supernatant (plasma) discarded. The pellet was washed twice by resuspending ~~it~~ in a volume of normal saline equal to the volume of the supernatant (plasma) and centrifuged at 3000 x g for 10min. The pellet (0.1 ml) was resuspended in 2.5 ml of normal saline and used as the red blood cell (RBC).

Results: The results revealed that ~~C~~cucumber (*Cucumis sativus*) fruit homogenate significantly ($p < 0.05$) inhibited hypotonicity-induced red blood cell haemolysis when compared to indomethacin (a known standard drug).

Conclusion: Cucumber has membrane stabilization effect on the red blood cell.

Keywords:Hypotonicity, Membrane stabilization, Red blood cells, Inflammation, Homogenate,*Cucumis sativus*

Introduction

Red blood cells (RBCs) along with its membrane have always been a salient approach for the investigation of various physiological and metabolic characteristics [10].During inflammation, the lysosomes lyse ~~to~~and release their component enzymes which produce a variety of disorders. Since human red blood cell membranes are similar to lysosomal membranes [4], human red blood cell membrane stabilization has, therefore, been used as a method to investigate the mechanism of action of anti-inflammatory drugs [3]. Stabilization of lysosomal membranes is

key in limiting inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release [16]. Some non-steroidal anti-inflammatory drugs(NSAIDs) like indomethacin and acetylsalicylic acid are known to possess membrane stabilization properties [7, 20-21] which may contribute to the potency of their anti-inflammatory effect.

Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and lysis, resulting in the release of haemoglobin, hence haemolysis. Haemolysis is an indication of the stability of red blood cell membrane [9, 17]. The vitality of cells depends on the integrity of their membranes. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This notion is consistent with the observation that the breakdown of biomolecules leads to the formation of free radicals which in turn enhance cellular damage [26, 22-23]. The progression of bone destruction seen in rheumatoid patient for example, has been shown to be due to increased free radical activity [14]. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances [18].

Cucumis sativus (~~C~~cucumber) is a widely cultivated plant in the gourd family of Cucurbitaceae, which also includes important crops such as melon, water melon, and squash [5]. There is increased consumption of *Cucumis sativus* fruits possibly because of their high nutritional value. The nutritional composition of *Cucumis sativus* include protein, fat, and carbohydrate as primary metabolites~~;~~, and dietary fibre which is important for the digestive system. *Cucumis sativus* contains some essential vitamins and ~~**anti-oxidants**~~**antioxidants**which are effective in human health [19].The anti-inflammatory activity of ~~**C**~~cucumber has been demonstrated in previous study [1]. In this study, we aimed to evaluate the *in vitro* membrane stabilization activities of *Cucumis sativus* fruit homogenate on hypotonicity – induced haemolysis of red blood cell (RBC).

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, Nigeriaby Mr Alfred Ozioko, an ethnobotanist of the herbarium unit with voucher number 13201400. The fruits were homogenized using high-speed blender and used without dilution.

Hypotonicity – induced haemolysis of red blood cell of *C. sativus* fruit

The effect of *Cucumis sativus* fruit homogenate on hypotonicity – induced haemolysis of red blood cell was investigated using the method of Murugesh [27] with ~~a~~**minor**modification.

Principle: Hypotonicity-induced haemolysis of red blood cells occurs due to osmotically coupled water uptake by the cells, and leads to swelling and subsequently lysis. This results in the release of haemoglobins which absorbs maximally at 418nm. Hence the optical density at 418nm is reflection of haemoglobin concentration. Reflection of the stability of red blood cell membrane is thus measured by changes in optical density, changes in haemoglobin concentration in the medium.

Preparation of erythrocyte suspension: Fresh whole blood (3ml) was collected from healthy human volunteer(after consenting to participate in the research, following the signing of the consent form and guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria), into plastic tubes containing 0.1 volume of 3.8% trisodium citrate and used within 8 hr. The blood sample was centrifuged at 3000 x g for 10 min and the supernatant (plasma) discarded. The pellet was washed twice by resuspending it in a volume of normal saline equal to the volume of the supernatant (plasma) and centrifuge at 3000 x g for 10min. The pellet (0.1 ml) was resuspended in 2.5 ml of normal saline and used as the red blood cell (RBC).

Procedure: A set of seven tubes were used for the analysis. The reaction medium is shown in Table 1 below.

Table 1: Hypotonicity-induced haemolysis of RBC reaction medium

Tube	RBC (ml)	Distilled Water (ml)	Normal Saline (ml)	Fruit Homogenate (ml)
1	0.1	0	1.9	0
2	0.1	1.0	0.9	0
3	0.1	1.0	0.5	0.1
4	0.1	1.0	0.5	0.2
5	0.1	1.0	0.5	0.4
6	0.1	1.0	0.5	0.6
7	0.1	1.0	0.5	Indomethacin (0.4mg/ml)

The reaction medium was incubated at 37 ^{o}C for 1 hour. After incubation, each of the incubates was centrifuged at 3000 x g for 10 min to terminate the reaction. The absorptions of the respective supernatants were measured at 418 nm as a measure of extent of haemolysis. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde *et al*. (1999).

$$\% \text{ Inhibition of haemolysis} = 100 \times \frac{OD1 - OD2}{OD1}$$

Where:

OD1 = Optical density of hypotonic-buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution

Blank reaction medium contained 1.2ml normal saline and 0.8ml water.

Ethical Approval

All experimental protocols including the involvement of human participants were approved and followed the guidelines of the Faculty of Biological Sciences Ethical Committee of the University of Nigeria, Nsukka, Nigeria.

Results:

Table 2 shows that the homogenate of *Cucumis sativus* fruits significantly inhibited lysis induced by water in dose dependent manner. When erythrocytes were suspended in water and later centrifuged, the supernatant was found to have a mean absorbance of 1.367 at 418nm. On the other hand, suspension of the erythrocytes in normal saline, given the same treatment as in the case of water gave an absorbance of 0.280. The result showed that in the hypotonic (water) environment, there was liberation of haemoglobin and hence the high absorbance reading. Table 2 shows that when the homogenate was introduced, there was decreases in absorbance readings. This inhibition of haemolysis was found to be dose dependent, increasing with increased concentration of the extract in the medium. High percentage inhibition of haemolysis (64.9 and 94.4) was obtained at 0.4 and 0.6ml doses of the homogenate respectively, comparable to that of the standard drug, indomethacin (81.4).

Table 2: Inhibition of hypotonicity-induced haemolysis bythe homogenate of *Cucumis sativus* fruit

Treatment	**Mean O.D at 418nm**	**% Inhibition of Haemolysis**
Isotonic solution	0.140	-
Hypotonic solution (Control)	0.684	-
Test sample (0.1ml)	0.610	10.8
Test sample (0.2ml)	0.428	37.4
Test sample (0.4ml)	0.240 [A]	64.9
Test sample (0.6ml)	0.038 [A]	94.4

Indomethacin (0.4mg/ml)	0.127 ^{A}	81.4

Mean values having **uppercase**letters as superscripts down the column are considered significant ($p < 0.05$) compared to control. Percentage inhibition of haemolysis was calculated relative to control.n = 3.

Discussion:

Compounds with membrane-stabilizing effects are widelyknown for their abilities to inhibit the early phase of inflammation reactions[8, 24]. The stabilization of the red blood cell membrane prevents the release of lytic enzymes and active mediators of inflammation, such as 5-hydroxytrptamine, histamine and kinins [15].

The homogenate of *Cucumis sativus* fruit was found to exhibit high membrane stabilization effect against hypotonicity induced haemolysis of the red cells as is shown by the level of inhibition of haemolysis. Protection against hypotonicity-induced haemolysis is related to membrane stabilization which is an anti-inflammatory index [8, 12]. This inhibition of haemolysis was found to be dose dependent, increasing with increased amount of the homogenate in the medium and was comparable with that of indomethacin, a standard anti-inflammatory drug. Hypotonicity-induced haemolysis of human red blood cells (HRBC) occurs due to water uptake by the cells and leads to the release of haemoglobin which absorbs maximally at 418 nm. Hence, the reduced optical density at 418 nm obtained for the various *Cucumis sativus* test samples was a reflection of the stabilization of the red cell membrane caused by the fruit homogenate. The fruit may also inhibit processes which stimulate or enhance the efflux of intracellular components. The erythrocyte membrane is analogous to the lysosomal membrane [11, 25]. Its stabilization implies that *Cucumis sativus* may as well stabilize lysosomal membranes against the release of lytic enzymes.

Lysosomal enzymes play an important role in the development of acute and chronic inflammation. Most of the anti-inflammatory drugs exert their beneficial effects by either inhibiting the release of the enzymes or ~~by~~ stabilizing the lysosomal membranes [11]. Stabilization of lysosomal membranes is important in preventing the leakage of serum protein and fluids into the tissue during the period of increased permeability caused by inflammatory

mediators. The anti-haemolytic properties of *Cucumis sativus* fruit homogenate may be due to the presence of some active constituents such as flavonoids, tannins and saponins. It has been reported that flavonoids exert profound stabilizing effects on lysosomes both *in-vitro* and *in-vivo* in experimental animals [6, 24] while tannins and saponins have the ability to bind cations and other biomolecules, and are able to stabilize the erythrocyte membrane [13]. The high membrane stabilizing activity of the homogenate of *Cucumis sativus* fruit observed in this study may be due to its flavonoids and tannins contents, which has been reported in previous studies [1, 2].

Conclusion

The result of this study indicates that cucumber has the ability to~~inhibits~~**inhibit**hypotonicity – induced haemolysis of red blood cell, thereby confirming the membrane stability properties of cucumber.

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