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Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of *Momordica charantia* in rats

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ABSTRACT

*This study reports the anti-inflammatory and membrane-stabilizing property of an aqueous extract of *Momordica charantia* (MC) leaves in rats. The carageenin-induced rat paw oedema was utilized as the model for acute inflammation, whilst formaldehyde-induced rat paw oedema was used as the model for sub-chronic inflammatory states. The probable mode by which MC mediates its effects on inflammatory conditions was studied on rat blood cells (RBC) exposed to hypotonic solution. The results of the study revealed that the extract possesses anti-inflammatory property. MC was found to significantly ($p < 0.05$) reduced the oedema swellings induced by the phlogistic agents in rats in a dose-related manner. However, the extract did not exhibit membrane-stabilizing property, as it failed to significantly ($P < 0.05$) reduced the levels of haemolysis of RBC exposed to hypotonic solution. The acute toxicity studies of oral doses of aqueous extract of *Momordica charantia* leaf in mice revealed that it has a high safety profile, as the extract was well tolerated by the animals. The results of the study suggest that the anti-inflammatory activity demonstrated by *Momordica charantia* leaf may not be related to membrane-stabilization. (*Afr. J. Biomed. Res.* 9:119 - 124, May 2006)*

Keywords: *Momordica charantia*, anti-inflammatory, membrane-stabilizing property

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INTRODUCTION

Momordica charantia (Cucurbitaceae) or bitter melon is widely cultivated in Africa, Asia and South America for its valuable medicinal properties (Lotlikar and Rajarama, 1966). Indigenous people all over the world employ the fruit juice or leaf tea for diabetes, colic, wounds, infections, hepatitis, laxative and stimulant (Lotlikar and Rajarama, 1966; Plattel and Srinivasan, 1997). Bitter melon is also useful in the treatment of inflammatory conditions such as rheumatism and gout. It is supposed to purify blood and dissipate melancholia (Lotlikar and Rajarama, 1966).

The juice of bitter melon is usually taken 2 or 3 doses over the course of the day for its therapeutic purposes (Lotlikar and Rajarama, 1966). The only problem is that the juice of this plant tastes extremely bitter. In folk medicine, it is generally believed that the bitterer this plant is, the more medicinal value it has (Lotlikar and Rajarama, 1966). Phytochemical studies revealed that *Momordica charantia* contained alkaloids, saponins, glycosides, phenolic constituents, reducing sugars and free acids. The presence of 5-hydroxytryptamine in bitter melon has also been reported (Dhalla *et al.*, 1996).

The extract from the leaves of *M. charantia* was reported to exhibit hypoglycemic activity comparable to that of tolbutamide (Plattel and Srinivassan, 1999; Lotlikar and Rajarama, 1966). Treatment with bitter melon was found to lower blood glucose levels in animal and human studies (Lotlikar and Rajarama, 1966). The extract of this plant has been shown to exert anti-bacterial, antineoplastic, antiviral and anti-mutagenic properties (Jilka *et al.*, 1983; Guevara *et al.*, 1990). The extract also demonstrated potent purgative effect and produced contractions of the guinea ileum (Sofowora, 1979). Literature survey however, revealed scanty information that suggests the usefulness of the leaf extract of *M. charantia* in the control of inflammation. This study therefore, reports the anti-inflammatory and membrane stabilizing properties of the aqueous leaf extract of *Momordica charantia* in rats.

MATERIAL AND METHODS

Plant material

The leaves of *M. charantia* were purchased from Mushin market, Lagos and authenticated by Prof. J. D.

Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Nigeria.

Laboratory animals

Sprague-Dawley rats (150-200 g) and Swiss albino mice (18-25 g) of either sex obtained from the Laboratory Animals Center, College of Medicine, University of Lagos, Lagos, Nigeria were used for the various studies. They were kept in a well-ventilated environment, had free access to food and water *ad libitum*.

Preparation of plant extract

The leaves of *M. charantia* were dried in the oven at 40°C. The dried materials were ground into fine powder. Two hundred grams (250 g) of the powdered material was soaked in 500 ml of distilled water for 24 hours. The solution was filtered and the filtrate was evaporated to a sticky residue in an oven at 40°C. The yield of the extract was 10.5% with reference to the powdered material. A portion of the dried extract was weighed and dissolved in distilled water to make an appropriate concentration for the study.

Experimental paradigms

Carrageenin induced oedema: Acute inflammation was induced utilizing Carrageenin-induced rat paw oedema model as previously described (Winter *et al.*, 1962). Rats (6 per group) were divided into five groups. The first 3 groups received oral doses of 300, 500 and 1000 mg/kg of the extract. The 4th and 5th groups were treated orally with indomethacin (10 mg/kg) as a reference drug and saline (10 ml/kg) as control respectively. Thirty minutes later, 0.1 ml of 1.0 % carrageenin was injected into the right hind paw of each rat. The linear circumference of the injected paw was measured at the third hour induction of inflammation and the percentage inhibition of oedema was calculated (Jain and Khanna, 1981).

$$\% \text{ Inhibition of oedema} = \frac{l_0 - l_1}{l_0}$$

l_0 = change in paw circumference in saline-control group

l_1 = change in paw circumference in drug treated group

Formaldehyde-induced sub-acute inflammation:

Formaldehyde induced sub chronic inflammatory state was carried out as previously described (Jain and Khanna, 1981). Rats (8 per group) were pretreated orally with the extract (300-1000 mg/kg, i.p), indomethacin (10 mg/kg, p.o) and saline (10 ml/kg, p.o) respectively. Thirty minutes later, 0.1 ml of 2% formaldehyde was injected into the right hind paw of each rat. The linear circumference of the injected paw was measured at 48 hours after formaldehyde injection and the percentage inhibition of oedema was calculated as described above.

Membrane stabilizing activity

Preparation of erythrocyte suspension: Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 minutes at 3000 g.

Hypotonic solution-induced rat erythrocyte haemolysis: Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis (Shinde *et al.*, 1999). The test sample consisted of stock erythrocyte (RBC) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (0.25-2.0 mg/ml) or indomethacin (0.1 mg/ml). The control sample consisted of 0.5 ml of RBC mixed with hypotonic -buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde *et al.*, (1999).

$$\% \text{ Inhibition of heamolysis} = 100 \times \{OD_1 - OD_2 / OD_1\}$$

Where:

OD_1 = Optical density of hypotonic-buffered saline solution alone

OD_2 = Optical density of test sample in hypotonic solution

Acute toxicity study

Acute toxicity study was carried according to the method previously described (Miller and Taniter,

1944). Mice (6/group) were divided into five groups. The first 4 groups received oral doses of 1, 2, 4, and 8g/kg of the extract. The fifth group received saline (10 ml/kg) orally. Mortality was assessed 24 hours after administration. The animals were also observed for toxic symptoms and mortality was determined 24 hours after treatment.

Data analysis

Data obtained from this study were expressed as mean \pm SEM. Statistical analysis was performed using Student's t test. P-values less than 0.05 were considered statistically significant.

RESULTS**Acute toxicity studies**

Acute toxicity study showed that the extract possessed high safety profile as no death was observed at oral doses of 1.0-8.0 g/kg in mice. The behavioural changes observed at these doses, were reduced motor activity, ataxia and hyperventilation.

Effect on carrageenin-induced oedema

The oedema swellings induced by carrageenin in rats was significantly ($p < 0.05$) inhibited by the extract (300-1000 mg/kg, p.o) dose-dependently and by indomethacin (10 mg/kg, p.o). As shown in table 1, the extract (1000 mg/kg) reduced the oedema swellings by 54.2% as compared with 68.80% reduction produced by indomethacin (10 mg/kg, p.o).

Table 1:

Effect of aqueous leaf extract of *Momordica charantia* on carrageenin-induced oedema in rats

Sample	Dose (mg/kg)	Difference in paw diameter (cm)	% Inhibition of oedema
Saline	-	0.48 \pm 0.02	-
<i>M.charantia</i>	300	0.34 \pm 0.05*	29.17
<i>M.charantia</i>	500	0.24 \pm 0.01*	50.00
<i>M.charantia</i>	1000	0.22 \pm 0.07*	54.17
Indomethacin	10	0.15 \pm 0.01*	68.75

Each value represents the mean \pm SEM from 10 animals in each group. All values are significant at $P < 0.05$ compared to normal saline-control group (Student's t test).

Table 2:Effect of the aqueous seed extract of *Momordica charantia* on formaldehyde-induced oedema in rats

Sample	Dose (mg/kg)	Difference in paw diameter (cm)	% Inhibition of oedema
Saline	-	0.75±0.01	-
<i>M.charantia</i>	300	0.65±0.03*	13.33
<i>M.charantia</i>	500	0.62±0.05*	17.34
<i>M.charantia</i>	1000	0.53±0.08*	29.33
Indomethacin	10	0.37±0.02*	50.67

Each value represents the mean ± SEM of 6 animals in each group. * $p < 0.05$ compared with saline-control group (Student's *t* test).

Effect on formaldehyde-induced oedema swellings

The oedema swellings produced by formaldehyde at the 24th hour post-administration, was significantly ($p < 0.05$) reduced by the extract in a dose-dependent manner and by indomethacin (10 mg/kg, p.o.). The extract (1000 mg/kg, p.o) reduced the size of the oedema by 29.30%, as compared with 50.70% produced by 10 mg/kg of indomethacin (Table 2).

Effect on erythrocyte membrane stability

The extract at concentration range of 0.50-2.0 mg/ml did not significantly protect the rat erythrocyte membrane against lysis induced by hypotonic solution.

In contrast, indomethacin (0.10 mg/ml) offered a significant protection of the rat RBC against the

damaging effect of hypotonic solution. At a concentration of 2.0 mg/ml, the extract produced 24.50% inhibition of RBC haemolysis as compared with 48.8% produced by indomethacin (Table 3).

DISCUSSION

The results of the study showed that *Momordica charantia* leaf extract possesses anti-inflammatory property, as it significantly inhibited oedema induced by carrageenin and formaldehyde in rats. However, the extract did not show membrane stabilizing effect, as it failed to offer significant protection of the erythrocyte against lysis induced by hypotonic solution.

The inflammatory condition induced by carrageenin involves step-wise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase and prostaglandins in the acute late phase (Dirosa *et al.*, 1971; Heller *et al.*, 1998). These chemical substances produced increase in vascular permeability, thereby promoting accumulation of fluid in tissues that accounts for the oedema (Williams and Morley, 1973; White, 1999). Similarly, formaldehyde, a potent oedematous agent, produced inflammation through the release of several inflammatory mediators including prostaglandins (Tjolsen *et al.*, 1992). The ability of the extract to reduce the size of oedema produced by carrageenin and formaldehyde, suggests that it contained chemical component (s) that may be active against inflammatory conditions.

Table 3:Effect of the aqueous leaf of *Momordica charantia* rat erythrocyte haemolysis

Sample	Concentration	Optical density (OD)	%Inhibition of heamolysis
Hypotonic medium	50 mM	0.72±0.05	-
<i>M.charantia</i>	0.25 mg/ml	0.64±0.04	11.11
<i>M.charantia</i>	0.50 mg/ml	0.63±0.06	12.50
<i>M.charantia</i>	1.0 mg/ml	0.62±0.05	13.88
<i>M.charantia</i>	2.0 mg/ml	0.55±0.08	23.61
Indomethacin	0.10 mg/ml	0.36±0.20*	50.00

Each value represents the mean ± SEM of 6 experiments. * $p < 0.05$ compared with hypotonic medium (Student's *t* test).

It is well known that the vitality of cells depends on the integrity of their membranes (Ferrali *et al.*, 1992). Exposure of red blood cell to injurious substances such as hypotonic medium and phenylhydrazine results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin (Augusto *et al.*, 1982; Ferrali *et al.*, 1992). 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 (Augusto *et al.*, 1982; Ferrali *et al.*, 1992).

This notion is consistent with the observation that the breakdown of bio-membranes leads to the formation of free radicals which in turn enhance cellular damage (Halliwell *et al.*, 1988; Maxwell, 1995). The progression of bone destruction seen in rheumatoid patients for example, has been shown to be due to increased free radical activity (Cotran *et al.*, 1999; Pattison *et al.*, 2004). It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances (Maxwell, 1995; Shinde *et al.*, 1999; Liu *et al.*, 1992; Perez *et al.*, 1996).

Compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators (Aitadafoun *et al.*, 1996). However, the extract of this plant did not demonstrate significant membrane stabilizing property, which suggests that its anti-inflammatory activity observed in this study, may be related to the inhibition of the late phase of inflammatory events, namely the release of chemical mediators.

In conclusion, the results of the study suggest that *Momordica charantia* leaf extract may offer some beneficial effects in the management of inflammatory conditions.

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