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The anti-inflammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin-induced oedema and granuloma tissue formation in rats

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ABSTRACT

Objective: To evaluate the possible anti-inflammatory effect of garden egg [*Solanum aethiopicum* (*S. aethiopicum*)] using experimentally induced inflammatory models in rats. **Methods:** Oedema was induced on the rat hind paw by the injection of 0.1 mL undiluted fresh egg albumin (phlogistic agent) into the subplantar surface of the rat paw. Tissue granuloma was induced in the rats by the implantation of two autoclaved cotton pellets (30 mg) under the flank of previously shaved back of anaesthetised rats. Garden egg extract doses were administered to the rats for seven consecutive days. On day 8, the animals were killed and the pellets surrounded by granuloma tissue were dissected out and dried. **Results:** Extracts of garden egg significantly ($P < 0.05$) reduced the fresh egg albumin-induced rat paw oedema and also significantly ($P < 0.05$) reduced the granuloma tissue formation in the treated groups when compared to the control. **Conclusions:** This study shows that the fruits of garden egg (*S. aethiopicum*) have anti-inflammatory activity in the models studied.

1. Introduction

Garden egg [*Solanum aethiopicum* (*S. aethiopicum*)] also known as African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family Solanaceae. The genus *Solanum* includes both the edible and non-edible species. The family is one of the largest and most important families of vegetable grown for their edible fruits[1]. They are native to sub-Saharan Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality. The fruits can be eaten fresh without cooking and have a long history of consumption in West Africa[2]. Depending on the type, either the leaves and young shoots or the fruits or both are eaten; they may be consumed raw, dried, cooked or in salad form. The fruit is berry; the seeds have large endosperm, and are grown mainly for food and medicinal purposes.

Inflammation is a complex biological response of vascular tissues to invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage. Although

inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases[3]. The non-steroidal anti-inflammatory drugs (NSAIDs) possess anti-inflammatory action for treating several inflammatory conditions including rheumatoid arthritis, osteoarthritis and musculoskeletal disorders, but prolonged use of NSAIDs has some associated adverse effects such as nausea, fluid retention, bleeding and gastric lesion[4]. Therefore, new anti-inflammatory drugs devoid of these side effects are being researched on as alternatives to NSAIDs.

Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats. They are used to treat colic; severe pain resulting in periodic spasm in an abdominal organ and blood pressure[5]. Other reports on the pharmacological activity of the plant show that it has purgative[6], sedative and anti-diabetic effects[7], but none have reported on its anti-inflammatory activity. Some observations and oral reports though, show that people with high consumption of garden egg have relief in arthritic pains and swelling. Lack of scientific data to support these claims prompted this study which was therefore aimed at assessing the possible anti-inflammatory activity of garden egg (*S. aethiopicum*) in both acute and chronic inflammatory models using rats.

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2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of analytical grade. They were products of Sigma Aldrich, Germany.

2.2. Plant material

Fresh fruits of garden egg (*S. aethiopicum*) were obtained from the Agric Farm of the Faculty of Agricultural Sciences of the University of Nigeria, Nsukka and were identified by Mr. Ugwuozor, a taxonomist of Botany Department, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium unit of the Department of Botany, University of Nigeria, Nsukka. The plant was chopped into tiny bits, air-dried for 2 weeks and milled with a mechanical grinder. The ground plant (500 g) was macerated in methanol for 24 h, filtered with a white cloth and the filtrate concentrated using a rotary evaporator (IKA, Germany) at an optimum temperature of 40–50 °C. The yield of the dried extract was 5 g and was designated methanol extract of *S. aethiopicum* (MESA).

2.3. Plant incorporation into diet

The milled garden egg fruit was incorporated into the normal diet of the animals in varying concentrations of 5%, 10%, 20%, and were fed to the rats for fourteen (14) days.

2.4. Animals

Swiss albino mice (22–28 g) and adult Wistar rats (120–200 g) of both sexes obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka were used. They were housed in metal steel cages and acclimatised in the laboratory for seven days before the experiments. They were given free access to water and fed with growers mash (Niger Feeds, Nigeria) bought from the local market. The research was conducted in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85–23, revised 1985).

2.5. Acute toxicity study

The acute toxicity test of the plant extract was carried out by the method of Lorke to define the range of lethal dose and safe dose for the extract. Eighteen Swiss albino mice starved of food for 18 h but allowed access to water were used for the study. There were grouped (three mice per group) treated intraperitoneally (i.p.) with the plant extract at varied dose levels (50, 100, 400, 600, 1 000, and 1 500 mg/kg). The animals were then observed for nervousness, dullness, incoordination and or mortality for 24 h.

2.6. Anti-inflammatory test

Three methods were used to assay the anti-inflammatory

activity of the garden egg. Twenty five adult wistar rats of either sex (120–200 g) randomly divided into five groups of five rats each were used for each method. They were fasted and deprived of water for 18 h before the experiment.

2.7. Egg albumin-induced paw oedema in rats

The rat paw oedema method of Winter *et al* was used. Garden egg extract (100, 200 and 400 mg/kg, i.p.) or indomethacin (10 mg/kg, i.p.) suspended in normal saline were administered to the rats. Control group received normal saline (1 mL/kg, i.p.). One hour post treatment, inflammation of the hind paw was induced by injecting 0.1 mL of undiluted fresh egg albumin into the subplantar surface of the right hind paw of rats. The right hind paw volumes of the rats were taken on the principle of volume displacement using LETICA Digital Plethysmometer (LE 7500) immediately before the experiment (zero time) and at 1hr intervals after the injection of egg albumin for a period of 5 h. The average oedema at every interval was assessed in terms of difference in volume displacement after injecting the egg albumin and zero time volume displacement of the injected paw ($V_1 - V_0$). Percent inhibition of oedema was also calculated for each treated group.

2.8. Egg albumin-induced paw oedema in rats fed with garden egg incorporated diet

Twenty (25) adult Wistar rats divided into 5 groups of 5 rats each were fed milled garden egg fruits incorporated into the normal diet of the animals in varying amounts of 5%, 10% and 20% for 14 d. Fresh egg albumin was used to induce paw oedema in the rats on the 14th day and the oedema formation was monitored in the groups for 5 h. Average oedema and its inhibition were assessed as above.

2.9. Cotton-pellet induced granuloma

This experiment was done according to the method of Winter and Porter. Twenty five adult Wistar rats divided into five groups of five rats each were also used. The animals were fasted but had free access to water. Two autoclaved cotton pellets weighing 30 mg each were aseptically implanted (s.c.) one on each side, under the flank of anaesthetised rats. The garden egg extract (100, 200 and 400 mg/kg, p.o.) or indomethacin (10 mg/kg, p.o.) suspended in normal saline were administered to the rats for 7 d consecutively starting from the day of cotton pellet insertion. The control group received normal saline (1 mL/kg, p.o.). On day 8, the animals were killed and the pellets were dissected out from the extraneous tissues. The wet pellets were dried in a hot air oven at 40 °C till a constant weight was obtained. Increases in the dry weight of the pellets were taken as a measure of the granuloma tissue formed around each pellet. The granuloma tissue formation was calculated thus:

$$\left[\frac{T_c - T_t}{T_c} \times 100 \right]$$

Where T_c = weight of granuloma tissue of control group; T_t = weight of granuloma tissue of treated group.

2.10. Statistical analysis

This was done using SPSS version 15.0 (SPSS Inc. Chicago, IL, USA). All values are expressed as mean \pm SEM. Data were analysed by two-way ANOVA and difference between means was assessed by Duncan's new multiple range. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity study

Result of acute toxicity study showed that there was no mortality or any significant change in the behaviour of the mice recorded up to the dose of 1 500 mg/kg of the plant extract. Based on the results of the preliminary toxicity testing, the doses for further studies were decided to be 100, 200 and 400 mg/kg bodyweight of the rats.

3.2. Effect of the methanol extract of *S. aethiopicum* (MESA) on egg albumin-induced paw oedema in rats

The methanol extract of garden egg (*S. aethiopicum*) (100, 200 and 400 mg/kg b.w) significantly ($P < 0.01$) inhibited the oedema formation induced by egg albumin. Table 1 shows the average oedema for the various groups at different time

intervals and the percent inhibition of oedema. The oedema inhibition shown by the garden egg treated groups was found to be comparable to that obtained for indomethacin (10 mg/kg).

3.3. Effect of the garden egg (*S. aethiopicum*) incorporated diet on egg albumin-induced paw oedema in rats

For the animals that were fed with garden egg (*S. aethiopicum*) incorporated diet for fourteen days and then challenged with egg albumin, there were significant reductions ($P < 0.01$) in the rat paw oedema and hence, increase in percentage inhibition of oedema. The oedema reduction occurred progressively in a dose related manner, with the group fed with 20% garden egg having the lowest paw oedema and highest inhibition of oedema relative to the other groups (Table 2).

3.4. Effect of the methanol extract of *S. aethiopicum* (MESA) on cotton-pellet induced granuloma in rats

The methanol extract of *S. aethiopicum* (100, 200 and 400 mg/kg b.w) significantly reduced the cotton pellet induced granuloma tissue formation in the rats, though, not in a dose dependent manner (Table 3). The reduction in granuloma tissue formation recorded for the garden egg treated groups was found to be lower than that obtained for indomethacin (10 mg/kg).

Table 1

Effect of the methanol extract of *S. aethiopicum* (MESA) on egg albumin-induced paw oedema in rats.

Treatment	Dose (mg/kg)	Δ paw oedema (mL) and % inhibition of oedema				
		1 h	2 h	3 h	4 h	5 h
Normal saline (Control)	1 mL/kg	0.86 \pm 0.07	0.76 \pm 0.05	0.62 \pm 0.04	0.42 \pm 0.04	0.30 \pm 0.04
MESA	100	0.70 \pm 0.05* (18.60)	0.56 \pm 0.04** (26.32)	0.42 \pm 0.03** (32.26)	0.26 \pm 0.03** (38.10)	0.13 \pm 0.01*** (56.67)
MESA	200	0.72 \pm 0.05* (16.28)	0.60 \pm 0.03** (21.05)	0.46 \pm 0.05** (25.81)	0.28 \pm 0.04** (33.33)	0.18 \pm 0.04** (40.00)
MESA	400	0.60 \pm 0.07*** (30.23)	0.54 \pm 0.05*** (28.95)	0.40 \pm 0.06*** (35.48)	0.31 \pm 0.09* (26.19)	0.18 \pm 0.04** (40.00)
Indomethacin	10	0.66 \pm 0.06** (23.26)	0.52 \pm 0.08*** (31.58)	0.40 \pm 0.05*** (35.48)	0.32 \pm 0.04* (23.81)	0.20 \pm 0.07* (33.33)

*Reduction in oedema significant at $P < 0.05$ compared to control, ** $P < 0.01$, *** $P < 0.001$. Values of oedema shown are mean \pm SEM ($n=5$). Values in parenthesis () are percentage inhibition of oedema calculated relative to control.

Table 2

Effect of garden egg incorporated diet on egg albumin induced rat paw oedema.

Treatment	% of garden egg	Δ paw oedema (mL) and % inhibition of oedema				
		1 h	2 h	3 h	4 h	5 h
Control	–	1.16 \pm 0.10	1.12 \pm 0.08	1.08 \pm 0.06	1.10 \pm 0.08	1.10 \pm 0.08
Garden egg	5	0.72 \pm 0.08** (37.93)	0.60 \pm 0.07** (46.43)	0.58 \pm 0.09** (46.30)	0.50 \pm 0.07*** (54.55)	0.46 \pm 0.07*** (58.18)
Garden egg	10	0.88 \pm 0.06* (24.14)	0.72 \pm 0.06** (35.71)	0.66 \pm 0.08** (38.89)	0.46 \pm 0.07*** (58.18)	0.40 \pm 0.07*** (63.64)
Garden egg	20	0.60 \pm 0.07* (48.28)	0.52 \pm 0.06** (53.57)	0.46 \pm 0.08*** (57.41)	0.40 \pm 0.07*** (63.64)	0.20 \pm 0.07*** (81.82)
Indomethacin (10 mg/kg)	–	1.06 \pm 0.09 (8.60)	0.90 \pm 0.07 (19.60)	0.64 \pm 0.09** (40.70)	0.46 \pm 0.07*** (58.20)	0.28 \pm 0.08*** (74.50)

*Reduction in oedema significant at $P < 0.05$ compared to control, ** $P < 0.01$, *** $P < 0.001$. Values of oedema shown are mean \pm SEM ($n=5$). Values in parenthesis () are percentage inhibition of oedema calculated relative to control.

Table 3Effect of the methanol extract of *S. aethiopicum* (MESA) on cotton pellet granuloma.

Treatment	Dose (mg/kg/d)	Granuloma wt (mg)±SEM	% reduction
Normal saline (Control)	1 mL/kg	157.00±9.15	–
MESA	100	111.00±7.41***	29.30
MESA	200	136.00±6.40***	13.38
MESA	400	121.00±9.62***	22.93
Indomethacin	10	102.00±3.97***	34.39

Level of significance *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Percentage reduction of granuloma tissue was calculated relative to control.

4. Discussion

Research on inflammation has become the focus of global scientific study because of its implication in virtually all human and animal diseases. Plant based drugs used in the practice of traditional treatment of diseases including inflammation have become the focus of current research because they are cheap and have great therapeutic potential without much of the side effects associated with synthetic drugs[8].

The present study evaluated the anti-inflammatory activity of the methanol extract of *S. aethiopicum* on experimentally induced paw oedema and granuloma tissue formation in rats and showed its effectiveness in reducing inflammation in both acute and chronic inflammatory models. In the acute inflammation tests, MESA showed good anti-inflammatory activity, suppressing the egg albumin induced rat paw oedema both at the early and later phases of oedema. The oedema reduction obtained was comparable to that obtained for indomethacin, the standard drug used. Oedema formation results from the synergistic action of inflammatory mediators such as histamine, serotonin and bradykinin at the site of a local inflammatory insult leading to increased vascular permeability and blood flow[9]. Oedema formation due to egg albumin in the rat paw is a biphasic event. The early phase of oedema which begins immediately after the administration of the irritant and lasting up to 2 h is probably due to the release of histamine and serotonin, while the later phase, occurring from 3 to 5 h after the administration of the irritant is induced by bradykinin, protease, prostaglandins and lysosome[10]. Subcutaneous injection of egg albumin into the rat paw produces oedema resulting from plasma extravasation, increased tissue water and plasma protein exudation along with neutrophil extravasation[11]. The reduction of oedemogenesis in the first phase evinced by both the garden egg extract and the garden egg incorporated diet in this study suggests that the plant is effective in inhibiting the release or action of the early phase mediators that arrive first at the site of injury thereby reducing vascular permeability, fluid exudation and thus, suppressing oedema. Suppression of oedema in the second phase of inflammation suggests that the anti-inflammatory activity of *S. aethiopicum* may also be due to the suppression of the prostaglandins and kinnin formation induced by the egg albumin within this period. The significant progressive reduction in the rat paw volume of groups fed with the

garden egg incorporated diet suggests that continuous intake of the plant could confer protection against inflammatory oedema and thus could be useful in the treatment and management of inflammatory conditions. Report by Mutalik *et al*[12] showed that *S. melogena* species of garden egg inhibited experimentally induced swelling of the hind paw of rats. The reduction of egg albumin induced rat paw oedema by garden egg (*S. aethiopicum*) in this present study correlates with the report of Mutalik *et al* of the reduction in experimentally induced rat paw oedema by garden egg.

The inhibitory effect of MESA on cotton pellet granuloma may be due to an inhibitory effect on granulocyte infiltration and the release of inflammatory mediators that promote cell proliferation and angiogenesis. The cotton pellet granuloma is widely used to evaluate the transudative and proliferative components of chronic inflammation and can serve as a subchronic and chronic inflammatory test model for the study of anti-arthritis substances[13]. The moist weight of the pellets correlates with transude, the dry weight of the pellet correlates with the amount of granulomatous tissue formed[14]. Chronic inflammation occurs by means of the development of proliferate cells which can be either spread or in granuloma form. Granulomas form in response to immune mediation when macrophages and lymphocytes accumulate around inert foreign particles that have not been eliminated, together with epithelioid and giant cells derived from macrophages to form a ball of cell. In chronic inflammatory states, the efficacy of anti-inflammatory agents can be indicated by the inhibition of fibroblasts and infiltration of neutrophils and exudation[15]. *S. aethiopicum* inhibited the cotton pellet induced granuloma in this study at rates comparable to that of indomethacin. We however can not give specific reasons why the lowest dose (100 mg/kg) of the extract produced a higher inhibition of granuloma tissue formation than the intermediate and highest doses (200 and 400 mg/kg). The significant inhibition of cotton pellet induced granuloma by MESA suggests an efficacy of the plant in inhibiting granulocyte infiltration and increase in the number of fibroblast during granuloma tissue formation and thus, a potential for its use in the treatment of chronic inflammatory conditions. Non-steroidal anti-inflammatory drugs such as indomethacin decrease the size of granulomas by the inhibition of granulocyte infiltration, prevention of the generation of collagen fibres and suppression of mucopolysaccharides[14], but prolonged use of the NSAIDs has some associated side effects which

include gastric lesions and ulcers^[4].

We reported in an earlier study that MESA has anti-ulcerogenic properties and confers protection against indomethacin and aspirin induced ulcers. This simultaneous anti-inflammatory and anti-ulcerogenic property of garden egg suggests a dual usefulness of the plant and its promise as an alternative anti-inflammatory therapy devoid of deleterious effects on the integrity of gastric mucosa. The plant could thus be used for the treatment of chronic inflammatory pains and swelling such as seen in rheumatoid arthritis without the risk of gastric ulcer development after prolonged intake.

Phytochemical study of the plant showed abundance of flavonoids, alkaloids, glycosides and terpenoids. Flavonoids have been reported to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid^[16–20]. Many anti-inflammatory plants and agents modify inflammatory responses by accelerating the destruction or antagonising the action of the mediators of inflammatory reaction. Foods and fruits rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases^[21,22], thus suggesting that the flavonoids in garden egg might be part of the active anti-inflammatory constituents in the plant.

We conclude that this study has shown that the fruits of garden egg (*S. aethiopicum*) have anti-inflammatory activity in the models studied.

Conflict of interest statement

We declare that we have no conflict of interest.

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