Evaluation of Anti-Diarrhoeal Action of a Hot-Water Infusion of *Ocimum gratissimum,* Linn

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Abstract

Hot-water infusion of the leaf of Ocimum gratissimum was tested for anti-diarrhoeal action using animal models. Protection of the animals from castor oil-induced diarrhoea and reduction of the number and frequency of faecal output were used as criteria for anti-diarrhoeal activity. The infusion significantly decreased the number of faecal droppings as compared to a normal saline control; it also reduced the propulsive movement of the gastrointestinal contents. The infusion caused a reduction of acetylcholine-induced contraction of isolated guinea pig ileum in a dose dependent manner. There is indication that the anti-diarrhoeal action of the plant infusion may be due to a blockage of muscarinic receptors.

Keywords: *Ocimum gratissimum*, Anti-diarrhoeal action, Inhibition, Smooth muscle contraction

Introduction

The diversity of biologically active substances of plant origin and their bioactivities are of current interest among biomedical researchers. *Ocimum gratissimum*, Linn (Family, Labiaceae), widely distributed in tropical and warm temperate regions, has been of research interest in recent times. The leaf is utilised in ethnomedicine to treat a variety of disease conditions (Said, *et al*., 1969). In tropical Asia, especially India, it is used as aromatic baths in the treatment of rheumatism and paralysis (Ephraim, *et al.,* 2003). In the West African sub-region, the plant is found around settlements and cultivated specifically for its folkloric uses (Offiah and Chikwendu, 1999). A survey of the literature suggests that *O. gratissimum* has potentials for various therapeutic uses including the treatment of bacterial (Nakamura, *et al*., 1999, Jedlickova *et al*., 1992, Ofokansi *et al*., 2003) and fungal (Nwosu and Okafor, 1995, Lima *et al*., 1993) infections. There is also indication that *O. gratissimum* has antimutagenic activity (Obaseke-Ebor *et al*., 1993) as well as immunomodulatory effect (Said *et al*., 1969). The leaves of the plant are added in cooking for their aromatic flavour.

The leaf of *O. gratissimum* contains a wide range of essential oils to which much of the biological activities and the flavour are attributed (Silva *et al*., 2004). The oils are more readily obtained using heat extraction methods such as steam or microwave distillation (Silva *et al*., 2004) than by cold extraction. If the active principles are not thermolabile, then it would be expected that the extracts obtained with the aid of heat should demonstrate more biological activity than the cold extracts.

In an effort to examine the anti-diarrhoeal actions of *O. gratissimum* leaves, Offiah and Chikwendu (1999) adopted the cold method of extraction. These investigators paid no attention to the possible effect of heat on the activity of the extract. Heat has very important influence on the activity of drugs generally and, in particular, those of natural origin. In most of the ethnomedical uses indicate above, hot water extracts of the plant are often employed. It is necessary to find out whether there could be any deleterious consequence of exposing the plant material to excessive heat in the process of extraction or processing. The present study, therefore, examines the effect of heat on the anti-diarrhoeal action of a hot water infusion of *O. gratissimum* leaves. Where necessary a direct comparison has been made between the current results and those obtained using cold water extracts.

Materials and Methods

Plant material: Fresh leaves of *O. gratissimum* were collected in the early morning hours from the botanical gardens in our University. They were authenticated as *Ocimum* leaves by Mr. A. Ozioko, a plant taxonomist in the Department of Botany of our University.

Animals: Sixteen adult albino rats of weight ranging between 200 – 250 g were used. Also fifteen albino mice of an average weight of 22.54 g were employed in some aspects of the study. One adult guinea pig was obtained for the isolated ileum study. Each group of animals was housed in separate metal cages, and fed freely on standard animal feeds and water.

Chemicals and reagents: The chemical compounds and reagents used in the study included, atropine sulphate (Merck, GDR), castor oil (Well's brand produced in Spain), acetylcholine, Ach (Sigma Chemical Co. USA) and diphenoxylate sodium (RFG Life Sciences Ltd, Germany). The Tyrode's solution was freshly prepared in our laboratories using a standard formula (Akah and Odita, 2001).

Extraction of plant material: Fresh leaves weighing a total of 300 grams were rinsed clean under running tap water. The leaves were then placed in 100 ml of distilled water in a beaker. The beaker was placed in a boiling water bath, in order to ensure indirect heating of the plant materials. The beaker was securely covered to minimize

Table 1: Effect of *O. gratissimum* leaf infusion on the number of droppings by rat over 24 h period

Doses of administered drugs or extract	Mean number of droppings (±SEM) at different time intervals after castor oil administration				
	1 h	2 h	4 h	8 h	24 h
Diphenoxylate (5 mg/kg)	0	0	0	0	0
***O. gratissimum* (20 ml/kg)**	0	5 ±1.75	2 ± 1.00	0	0
***O. gratissimum* (40 ml/kg)**	0	3 ±1.78	0	0	0
Normal saline (25 ml/kg)	0	7 ±1.30	8 ±1.63	6 ±1.73	0

evaporation of water and essential oils. The application of heat was continued for one hour during which time active principles infused into the water from the leaves. The resultant infusion was clearly filtered, allowed to cool and stored in a refrigerator at about 4 ^{o}C until used.

Induction of diarrhoea in rats: The rats were placed in four groups, each group in a separate cage lined with white linen. Prior to the induction of diarrhoea, animals in each group received orally by means of polythene cannula, as follows: Group 1 – 20 ml/kg of *O. gratissimum* infusion; Group 2 – 40 ml/kg of *O. gratissimum*; Group 3 – 5 mg/kg of diphenoxylate sodium and Group – 25 ml/kg of normal saline. Groups 3 and 4 acted as positive and negative controls respectively. One hour after the above treatments, 3 ml of castor oil were administered orally to each rat in order to induce diarrhoea. The animals were then observed at regular intervals for the number and frequency of faecal output.

Gastrointestinal motility test: In this test, the mice were separated into three groups of five each. The animals were fasted overnight prior to the test, but had free access to water. Animals in the various groups received orally 20 ml/kg of *O. gratissimum* infusion, 1 mg/kg of atropine sulphate and 25 ml/kg of normal saline respectively. Five minutes following drug administration, 1 ml of a 5 % of charcoal (suspended in 10 % aqueous solution of tragacanth powder) was administered orally to each animal. The animals were sacrificed 30 min later, the peritoneum was opened and the entire lengths of intestines (pylorus to caecum) were carefully stretched out and then cut open. The distance travelled by the charcoal meal was measured.

Tests on isolated guinea pig ileum: Freshly isolated segments (2 cm long) of guinea pig ileum were suspended in an organ bath containing aerated Tyrode's solution, maintained at 37 ^{o}C. A period of 30 min was allowed for tissue equilibration with bathing liquid. Responses of the tissue to varying doses of Ach or *O. gratissimum* infusion were recorded on kymographic paper attached to a rotating drum. Contact time of each drug was 15 sec with a 5-minute cycle washing and drug inoculation.

Statistical analysis: Empirical results were presented as mean ± SEM (standard error on the mean). The Student's t-test was used to evaluate the data at $p<0.05$.

Results and Discussion

At the end of 60 minutes of hot-water extraction of the fresh leaves of *O. gratissimum*, a volume of about 105 ml of the infusion was obtained as against an original volume of 100 ml of water. Since the container was securely covered during the heating process, it would be expected that much of the essential oil content of the leaves as well as other phytochemical constituents might have dissolved in the extracting fluid, thereby increasing the final volume. Some of the biological actions of aqueous extracts of *O. gratissimum* leaf have been attributed to the essential oils (Ndouga and Ouamba, 1997). Since the leaf contains a wide variety of essential oils (Silva *et al*., 2004), various biological activities would reside in the different oils.

The effect of the *O. gratissimum* aqueous infusion on the number and frequency of faecal droppings following castor oil-induced diarrhoea is shown in Table 1. Diphenoxylate, a classical anti-diarrhoeal agent, protected the rats from induced diarrhoea for the duration of the test. Effect of the crude *O. gratissimum* infusion can also be evaluated vis-à-vis the negative control typified by normal saline. There is evidence that the *O. gratissimum* infusion at the two dose levels used produced a significant reduction in the frequency and duration of faecal output. It is also noted that the diarrhoea-reducing effect of the infusion is dose-dependent. The trend recorded in this study points to the possibility of higher doses of the infusion producing a result similar to that of diphenoxylate. The exploration of higher doses of the aqueous infusion of *O. gratissimum* must, however, be considered alongside its possible histopathological effects on tissues and organs (Ephraim *et al*., 2003). Such effects may be transient as no major toxicities have been associated with the various ethnomedicinal uses of the plant. It is noteworthy that in addition to controlling diarrhoea, higher doses of the plant extract may also possess hepatoprotective activity (Ephraim *et al*., 2003).

It may be necessary, at this stage, to conjecture the mechanism of anti-diarrhoeal action of the infusion. Reference is made to the Ach-induced contraction of the isolated guinea pig ileal tissue, as shown in Figure 1. Ach is a neurotransmitter with a potent agonist effect on muscarinic receptors (Hardman and Gilman, 2001). Points "a" to "e" in Fig. 1 indicate that the tissue was actively responsive to the agonist effect of Ach. Points "f", "g", and "h" show the Ach-induced contraction after the tissue was exposed to 3 ml of *O. gratissimum* infusion. There is a visible reduction in the levels of tissue contraction.

Table 2: Effect of *O. gratissimum* leaf infusion on propulsive movement of intestinal contents in mice

Doses of administered drugs or extract	Hot water infusion *Mean % distance	Cold water infusion *Mean % distance
Normal saline (25 ml/kg)	77.10 ± 2.46	76.45 ± 0.14
***O. gratissimum* (20 ml/kg)**	60.2 0 ± 2.66	43.72 ± 0.14
Atropine sulphate (1mg/kg)	48.80 ± 1.04	32.90 ± 0.14

**The distance travelled by charcoal meal in small intestine of mice expressed as percentage of total length of intestine (pylorus to caecum). Source: Offiah and Chikwendu (1999).*

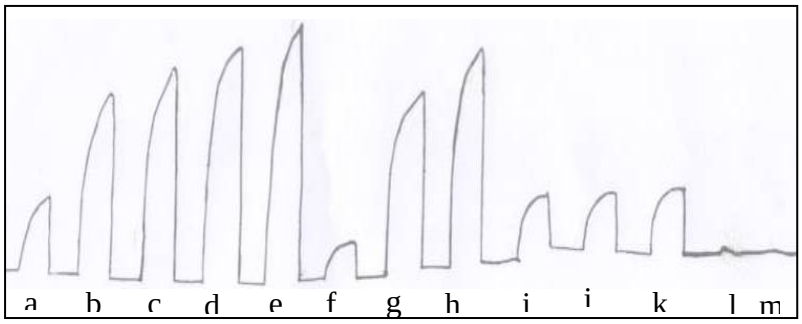

Fig. 1: Effect of *O. gratissimum* infusion on isolated guinea pig ileum.
Key: a, b, c, d, e = 0.1, 0.2, 0.3, 0.4, 0.5 ml of Ach (25 µg/ml);
f, g, h = 0.1, 0.3, 0.5 ml of Ach (25 µg/ml) following 3ml of *O. gratissimum* extract;
i, j, k = 0.3, 0.4, 0.5 ml of Ach (25 µg/ml) following 6ml of *O. gratissimum* extract;
l, m = 0.5 ml (in duplicate) of Ach (25 µg/ml) following 15 ml of *O. gratissimum* extract.

The effects were more discernible when higher doses of *O. gratissimum* infusion were used. Points "i", "j", and "k" represent the effect of 6 ml of the infusion, while points "l" and "m" illustrate the effect of 15 ml of the infusion. The entire result demonstrates a systematic blockage of the muscarinic receptors of the ileum by the *O. gratissimum* infusion. This portrays the fact that some constituent(s) of a hot-water infusion of *O. gratissimum* leaves may act as muscarinic receptor antagonists. Recent findings by Madeira *et al*. (2000) suggest that the antimuscarinic action of *O. gratissimum* extract may reside in the essential oil constituents, while Sofowora (1993) has specifically attributed the action to eugenol. This accounts for about 73 % of the essential oil composition of *O. gratissimum* (Silva *et al*., 2004).

Inhibition of the contractility of the intestinal tissue would be expected to normally affect the peristaltic movement of intestinal contents. This can be verified from the results shown in Table 2, which illustrate the effect of the *O. gratissimum* infusion on the movement of a charcoal meal along the intestinal lumen. We found it appropriate to use this parameter to evaluate the effect of heat on the active principles of the aqueous infusion. While the present study utilises a hot water-based infusion, a similar study reported by Offiah and Chikwendu (1999) makes use of a cold water-based extract. In evaluating the effect of heat, therefore, we have selected the results of comparable doses of the drugs used in both studies. Normal saline, a physiologically inert fluid has been used as a negative control and atropine sulphate, a classical anticholinergic agent serves as the positive control. Although the aqueous infusion of *O. gratissimum*, in its crude form, is not comparable to atropine sulphate, the shorter distances traversed by the charcoal meal due to the plant extract, as compared to that effect of normal saline, is evident of the reduction of the propulsive movement of the intestinal contents. This activity was present in both the hot water-based infusion and the cold water-based infusion but quantitatively more in the latter. Relaxation of the intestinal smooth muscle would cause decreased movement of the intestinal contents. The disparity in the quantitative effects of the hot and cold water-based infusions is most probably due to the method of extraction. Ephraim *et al.* (2003) have suggested that the application of heat in the extraction process may reduce the eugenol content of the extract, which is believed to largely impart antimuscarinic, hence, anti-diarrhoeal property, to the extract.

Conclusion: The anti-diarrhoeal property of the aqueous infusion of the leaf of *O. gratissimum* is not in doubt. But the actual constituent of the infusion responsible for the effect is yet a matter of speculation. This obviously opens a challenge for further investigation on this extract. The mechanism of anti-diarrhoeal action is by inhibition of intestinal tissue contractility through possible blockage of muscarinic receptors. In as much as the hot water-based infusion of *O. gratissimum* is convenient to ingest and more appealing to taste, there is evidence that some of the biological activities of the plant is lost when exposed to heat. The major inconvenience associated with the cold-water extract, however, derives from its organoleptic effects, due to the colouring of the extract by chlorophyll pigments. Other methods of extraction devoid of heat and which eliminates the greenishness associated with cold water extraction of the fresh leaves would be preferable. For this reason, an investigation is underway to determine the bioactivity of cold-water extracts of dried leaves of *O. gratissimum*.

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