Evaluation of Anti-Diarrhoeal Property of Crude Aqueous Extract of *Ocimum gratissimum* L. (Labiatae) In Rats.

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Abstract

The anti-diarrhoeal property of aqueous extract of *Ocimum gratissimum* was investigated in Wistar albino rats. Aqueous leaf extract of this plant, at various doses tested (25, 50 & 100mg/kg body weight) displayed remarkable anti-diarrhoeal activity evidenced by the reduction in the rate of defecation and consistency of faeces in albino rats. The protective role of *Ocimum gratissimum* extract at 100mg/kg body weight was comparable to that of the reference drug, diphenoxylate (50mg/kg body weight). *Ocimum gratissimum* extract mimicked the action of adrenaline and noradrenaline on isolated guinea pig ileum by abolishing the acetylcholine – induced contraction of the smooth muscles of ileum and also exhibited anti-inflammatory action against agar – induced rat paw oedema in the dose range of 100 to 400mg/kg body weight. Like phenylbutazone, the ability of the extract to block oedemogenesis was more manifest at the second phase (5 – 6hrs) after induction of inflammation) of the reactions.

Key words: Anti-diarrhoeal, *Ocimum gratissimum*, Oedemogenesis, Anti-inflammatory, Diphenoxylate

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INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel sound and movement, wet stool and abdominal pain. It is the leading cause of malnutrition and death among children in the developing countries of the world today¹.

Several pharmaceuticals such as diphenoxylate and anti-microbial agents are available for the treatment and management of both adult and infantile diarrhoea. In recent times, emphasis has been focused on the use of oral rehydration solution (ORS) as a replacement therapy to replenish the lost fluid and electrolytes in diarrhoeic cases¹. However, there is still need for a continuing search for more effective anti-diarrhoeal agents with probably minimal side actions.

The perennial plant *Ocimum gratissimum* (family Labiatae) is widely distributed in the tropics particularly in Nigeria. It is the most abundant specie of *Ocimum*. Nutritional importance of this plant centers on it's usefulness as a seasoning because of its aromatic flavour². In folk medicine, *Ocimum gratissimum* is extensively used throughout West Africa as a febrifuge, anti – malarial, anti – convulsant and against cough³. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves has been found to possess antiseptic, anti-bacterial and antifungal activities^{3,4,5,6}. Some phytochemical constituents of *Ocimum gratissimum* have been isolated and identified. The volatile aromatic oil from the leaves consists mainly of thymol (32 – 65%) and eugenol². It also contains xanthenes, terpenes and lactone⁷.

In the Southern part of the country, Nigeria, crude aqueous extract of *Ocimum gratissimum* is commonly employed in the treatment of diarrhoea. The scientific basis for the application of this plant as an anti-diarrhoeal drug has not been investigated *in vivo*. In the present study we investigated the anti-diarrhoeal potential of this aqueous extract of *Ocimum gratissimum* in albino rats using inhibition of frequency of defecation and wetness of faeces as indices of anti-diarrhoeal action. Some possible mechanisms of action were also elucidated.

MATERIALS AND METHODS

Experimental Animals:

Male Wistar albino rats (120 – 180g wt.) and guinea pigs of either sex (300 – 400g) were purchased from the animal house of Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were kept in the animal house of Department of Applied Biochemistry, Nnamdi Azikiwe University Awka for four weeks to acclimatize. They were provided with adequate Guinea animal feed and water. Prior to their use for experimentation, the animals were starved for 12 – 16 hours.

Plant Materials and Chemicals:

The leaves of *Ocimum gratissimum* were purchased from Awka Main market, Anambra State, Nigeria and authenticated in the Federal Ministry of Agriculture and Forestry, Enugu. Adrenaline, noradrenaline and acetylcholine were purchased from Sigma Chemicals Company, St Louis, U.S.A. Agar – agar, potassium chloride, magnesium chloride and glucose were obtained from BDH chemicals Limited, Poole, England. Sodium chloride, Sodium hydrogen phosphate and sodium hydrogen carbonate were from Janssen Chemica, Belgium. Diphenoxylate was obtained from Searle (India) Limited. Phenylbutazone was from Kawala Overseas while castor oil was purchased from Bellson & Co. (Druggists) Southport, England.

Extraction Procedure:

The leaves were air – dried and ground into powder. A total of 200g of the powdered leaves were macerated in 500ml of distilled water for 8 hours and filtered through cheese cloth, glasswool and Whatman No. 1 filter paper. The filtrate was evaporated to dryness by heating in a water bath at a temperature of 100°C. The dry aqueous extract was stored in the refridgerator and used as the crude extract or crude drug preparation. The extract and the reference drugs, diphenoxylate and phenylbutazone, used were each dissolved in normal saline (vehicle).

Assay of Castor oil – induced Diarrhoea:

The rats were grouped into five groups

of five animals each according to their weights and housed in separate locally fabricated metabolic cages. Two control groups were employed. The first control group received only normal saline (1ml/kg body wt.) whereas the second control animals were treated with the standard anti-diarrhoeal drug diphenoxylate (50mg/kg body wt.). The three test groups of animals were given graded doses of the extract (25, 50 & 100mg/kg body wt. respectively).

The extract and diphenoxylate were administered intraperitoneally 1 hour before the oral administration of the cathartic agent castor oil (1ml per rat). Oral administration of castor oil was facilitated by the use of a stomach tube. The animals were monitored for 12 hours for consistency of stool and the frequency of defecation.

At the end of this period, the total number of the fecal matter for each group and the number of diarrhoeic (wet) faeces were recorded and the mean value for each group calculated. Values for the treated groups were compared with that of control rats that received normal saline.

The mean number of diarrhoeic faeces – pooled by the group that received normal saline (1ml/kg body wt.) and Castor oil was considered as 100%.

Percentage inhibition of wetness of faeces and frequency of stooling caused by the extract and diphenoxylate were obtained by comparing them with castor oil (see box).

associated with increased rate of contraction of the smooth muscles of the ileum. The effect of *Ocimum gratissimum* extract on intestinal motility, determined by the magnitude of contraction of isolated guinea pig ileum caused by the extract, was studied.

Guinea pigs of either sex weighing between (300 – 400g) were used. The animals were starved for 12hr before used so that the gut would be clean enough. They were killed by stunning on the head. The abdomen was exposed and a (2 cm) length of ileum was cut and suspended in an organ bath containing Tyrode's solution of the following composition in g/litre (NaCl, 8.0; KCl, 0.2; MgCl, 0.2; NaHPO, 0.5; NaHCO, 1.0; and glucose, 1.0). The solution was quickly and carefully aerated and was allowed time to relax fully in the solution at 37°C. One end of the tissue was tied to a hook on the aerator and the other end to the chymograph.

After 30 minutes of equilibration, viability of the tissue was tested by recording its response to different concentrations (16, 32 & 50µg/ml) of acetylcholine (Ach) – which increases the amplitude of intestinal smooth muscle contraction. The effects of graded doses (2 to 16mg/ml) of the extract were then recorded and compared with those of adrenaline and noradrenaline which are known physiological antagonists to acetylcholine action. Viability was also confirmed at intervals during the course of the experiment.

$\begin{aligned} \text{\% inhibition of defecation} &= \frac{\text{Mean no. of defeaction caused by castor oil} - \text{Mean no. of defeaction caused by drug or extract}}{\text{Mean no. of defeaction caused by castor oil}} \times \frac{100}{1} \end{aligned}$
$\begin{aligned} \text{\% inhibition of diarrhoeic faeces} &= \frac{\text{Mean no. of diarrhoeic faeces produced by castor oil} - \text{Mean no. of diarrhoeic faeces produced by drug or extract}}{\text{No. of diarrhoeic faeces produced by castor oil}} \times \frac{100}{1} \end{aligned}$

Assay of Isolated guinea pig ileum contraction:

Altered intestinal motility resulting in rapid transit through the lumen has been identified as one of the major causes of diarrhoea. Increased motility is usually

Agar – induced rat paw oedema:

A modified method⁸ of Winter et al (1962)⁹ was used to study the effect of *Ocimum gratissimum* extract on agar – induced rat paw oedema. The animals were assigned into five groups of five rats per group.

Normal saline (10ml/kg body wt.) was injected intraperitoneally into the control group of rats whereas the reference animals were administered the reference drug, phenylbutazone (150mg/kg body wt.). The test groups received different doses of the extract (100mg/kg, 200mg/kg and 400mg/kg wt., respectively). The extract and phenylbutazone were dissolved in normal saline and administered intraperitoneally.

Rat paw volumes were measured by the mercury displacement method⁹. One hour after treatment, all the rats received subplantar injections of 2% suspension of agar agar (1ml/kg body wt.) at their right hind paw. Agar agar was suspended in normal saline. Changes in paw volumes, 90mins, 150mins, 210mins, 270mins, 330mins and 360mins after agar injections were determined.

The results of the experiment were recorded as mean $\pm$ SEM, and the level of significance was tested using the students' t-test (see box)

Inflammation	=	Average paw vol. At time t	–	Average paw vol. at zero time
% inflammation	=	$\frac{\text{Average inflammation of treated group at time t}}{\text{Average inflammation of control at same time}} \times 100 / 1$		
% Inhibition	=	100 – % inflammation.		

RESULTS

Inhibition Of Castor Oil – Induced Diarrhoea.

Results obtained in the evaluation showed that castor oil increased the mean number of defecation of rats that received normal saline (from 2.25 to 6.00). This increase was regarded as maximum (100%). It also increased the mean number of diarrhoeic faeces (from 0.00 to 5.75). As revealed in Table 1 (A&B), though the administration of 25mg/kg body wt. Of extract did not cause any significant change in the frequency of defecation, reduction (82%) in the wetness of the drops at this dose was statistically significant ($p < 0.05$) (see Table 2). A higher dose of extract, 50mg/kg body wt., afforded greater protection against castor oil induced diarrhoea. This was manifested in the significant reduction (at $p < 0.05$) of frequent

stooling. It was interesting to note that 100mg/kg body wt. of *Ocimum gratissimum* extract gave 100% protection ($p < 0.05$) since no wet faeces was recorded (Table 2).

Relating the antidiarrhoeal activity of *Ocimum gratissimum* extract to that of the standard drug diphenoxylate (at the same dose, 50mg/kg body wt.), both drugs may be regarded as nearly equipotent demonstrating 91.3% and 100% inhibition of wetness of faeces respectively.

Effect of *Ocimum gratissimum* Extract on Isolated guinea – pig ileum:

Fig. I shows that acetylcholine (16 μ g/ml) induced contractility of relaxed guinea pig ileum. The tone of contraction increased with increasing concentration of acetylcholine, 32 & 50 μ g/ml, (see peaks 1, 2 & 3 respectively). When *Ocimum gratissimum* extract, 2mg/ml, was allowed to perfuse the ileum the response to acetylcholine was drastically reduced (peak 4). At higher doses, 8

& 16mg/ml, of the extract contraction was completely abolished since no peaks were observed (responses 5 & 8 respectively). At intervals peaks 6,7 and 9 recorded with 100, 10 and 100 μ g/ml of acetylcholine still confirmed the viability of the tissue. Adrenaline (32 μ g/ml) and noradrenaline (32 μ g/ml) relaxed the smooth muscles of the ileum i.e. no peaks were shown (responses 10 and 11 respectively). All the other peaks 12 – 16 obtained with 80 μ g/ml of acetylcholine were used to show that the tissue was viable even at the end of the experiment.

Inhibition of Agar – Induced Rat paw oedema:

Results indicated that *Ocimum gratissimum* extract caused a dose – dependent antagonism of agar induced rat paw – oedema. Marked reduction in the percentage of inflammation (from 100% to 22%) was observed

TABLE 1A: Inhibition of frequency of defecation by *Ocimum gratissimum* extract.

Treatment	Mean no. of defecation per rat	% inhibition of defecation	P value
Normal saline alone (1ml/kg wt.)	2.25 $\pm$ 0.22	0.00	-
Castor oil	6.00 $\pm$ 1.35	* 100.00	P < 0.05
OG, 25mg/kg wt.	5.00 $\pm$ 1.30	16.67	Not significant
OG, 50mg/kg wt.	3.00 $\pm$ 0.25	50.00	P < 0.05
OG, 100mg/kg wt.	1.25 $\pm$ 0.51	79.17	P < 0.05
Diphenoxylate, 50mg/kg wt.	0.50 $\pm$ 0.17	91.67	P < 0.05

Values represented mean $\pm$ SEM

* 100.00 means % increase in defecation.

TABLE 1B: Inhibition of wetness of faeces by *Ocimum gratissimum* extract.

Treatment	Mean no. of diarrhoeic faeces	% inhibition of diarrhoeic faeces	P value
Normal saline alone (1ml/kg wt.)	0.00	0.00	-
Castor oil	5.75 $\pm$ 0.60	0.00	P < 0.0005
OG, 25mg/kg wt.	1.50 $\pm$ 0.45	82.60	P < 0.05
OG, 50mg/kg wt.	0.50 $\pm$ 0.20	91.30	P < 0.025
OG, 100mg/kg wt.	* 0.00	100.00	P < 0.005
Diphenoxylate, 50mg/kg wt.	* 0.00	100.00	P < 0.0005

Values represented mean $\pm$ SEM

* 0.00 means no defecation.

Table 2: Changes In Paw Volumes After Administration Of Agar:

DOSE	90MIN	150MIN	210MIN	270MIN	330MIN	360MIN
150mg/kg P.B.	0.067	0.034	0.034	0.034	0.034	0.034
100mg/kg Extract	0.099	0.066	0.066	0.066	0.066	0.066
200mg/kg Extract	0.030	0.00	0.00	0.00	0.00	0.00
400mg/kg Extract	0.10	0.10	0.067	0.067	0.034	0.034
10ml/kg Normal Saline (control)	0.133	0.30	0.30	0.30	0.30	0.30

Fig. 1: Effect of *Ocimum gratissimum* extract on guinea-pig ileum smooth muscle.

KEY:

1, 2 & 3 = Ach (16, 32 & 50 μ g/ml respectively).

4, 5 & 8 = OG extract (2, 8 & 16 μ g/ml respectively).

6, 7 & 9 = Ach (100, 10 & 100 μ g/ml respectively).

10 & 11 = Adrenaline (32 μ g/ml) & Noradrenaline (32 μ g/ml).

12 – 16 = Ach (80 μ g/ml)

at 100mg/kg body wt. of extract. Larger dose of 200mg/kg wt. did not produce any change in paw volumes at the second phase of the inflammatory reactions i.e. 5 – 6hr after induction of inflammation with agar agar (Table 2).

The effect of the extract was also seen to be biphasic in that a higher dose of 400mg/kg body wt. was less potent than 200mg/kg wt. The percentage inhibition of inflammation were 88.67% and 100 % respectively when the extract (200mg/kg body wt.) was compared with the standard anti-inflammatory drug, phenylbutazone at both phases of inflammation (Table 2, Figure 2 and 3).

DISCUSSION

The castor oil test has been an extensively used pharmacological test to screen and evaluate antidiarrhoeal properties of drugs in rats. Within 1hr of oral administration of the oil the animals begin to evacuate watery stools¹⁰. The aqueous leaf extract of *Ocimum gratissimum* offered a dose – related protection against castor oil – induced diarrhoea within the dose-range (25 to 100mg/kg body wt.) tested. This was evidenced by the significant reduction in the frequency of defecation (Table 1A) and wetness of faeces (Table 1B).

Consequent on oral administration of the cathartic agent, castor oil, there is immediate and

Fig. 2: Percentage (%) Inflammation after administration of agar.

Fig. 3: Percentage (%) Inhibition of Inflammation after administration of agar.

efficient hydrolysis of the oil by intestinal lipase resulting in the release of free ricinoleic acid¹⁰. Ricinoleic acid produces an irritating action that enhances the peristaltic activity of the small intestine seen in diarrhoea^{10,11}. From fig. 1, the peaks revealed that leaf extract also inhibited the

intestinal motility caused by contact of the tissue with acetylcholine, a substance that has been known to contract smooth muscles of ileum¹². Recent findings by Madeira *et al*¹³ showed that the essential oil of *Ocimum gratissimum* reversibly and concentration - dependently

reversed the tonic contractions induced by acetylcholine. Therefore, the relaxant property of *Ocimum gratissimum* extract portrayed in this study may be ascribable to these essential oils. On the other hand, this relaxant activity was similar to that observed with adrenaline and noradrenaline. Hence, analysis is being undertaken to ascertain the chemical nature of the fraction responsible for this action.

The second physiological effect of released ricinoleic acid is to cause inflammatory swelling of the intestinal mucosa¹¹. In the course of this study, the extract elicited strong anti-inflammatory activity against agar – induced oedemogenesis. Therefore, another mechanism of action of this aqueous extract could be by blocking the swelling of the mucosa. However, the biphasic nature of the anti-inflammatory response may indicate the presence of a substance with inflammatory property in this same extract. The anti-inflammatory and inflammatory substances are yet to be isolated and characterized using silica gel G – 60 packed column chromatography.

Conclusion

Since the doses administered in this study were chosen to reflect the traditional use of *Ocimum gratissimum*, and our results show that this plant possesses anti-diarrhoeal activity against castor – oil induced diarrhoea in rats, it may therefore be concluded that the findings in this *in vivo* work are consistent with the popular use of the plant, *Ocimum gratissimum*, in the treatment of gastrointestinal disorders particularly diarrhoea.

The antidiarrhoeal action of the medicinal herb from our study may be ascribed to its anti – inflammatory activity and ability to inhibit the peristaltic movement of the intestine by relaxing the intestinal smooth muscles. The results also suggest that this plant may not only serve in the treatment of varying types of diarrhoeal diseases but could also be used in inflammatory disorders such as arthritic conditions as an alternative to the non – steroidal anti-inflammatory drugs (NSAID).

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