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Establishing a preliminary basis for the use of *Ocimum gratissimum* in the traditional management of haemorrhoids

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Abstract
The ethanolic leaf extract of *Ocimum gratissimum* was tested for anti-inflammatory, analgesic and muscle relaxant properties following reports of its use in the traditional management of haemorrhoids. The isolated rabbit jejunum and phrenic nerve-diaphragm preparations were used to screen for smooth and skeletal muscle relaxant effects respectively. Anti-inflammatory and analgesic activities were evaluated using rat-paw oedema method and hot plate methods respectively. The results show that the crude extract ($2.0 \times 10^{-4} - 3.2 \times 10^{-3}$g/ml) inhibited both the twitch responses to nerve stimulation and the rhythmic contractility of the rabbit jejunum in a graded manner. The extract (100-1000mg/kg) also dose-dependently inhibited egg white-induced increase in rat paw oedema and prolonged the reaction time of mice subjected to thermal stimuli. The study suggests a scientific basis for the use of *Ocimum gratissimum* in the traditional management of haemorrhoids.

Keywords: *Ocimum gratissimum*, haemorrhoids, anti-inflammatory, analgesic

INTRODUCTION

Haemorrhoids are fibrovascular cushions with arteriovenous connections which protrude into the lumen of the anal canal (Ramakrishna, 2009). Haemorrhoidal columns are a common cause of anal pathology due to their rich vascular supply and have a tendency to engorge and inflame (Rosen, 2006). Perianal venous congestion develops rapidly with chronic elevation of intra-abdominal pressure as seen in defecation of hard stool, physical exertion and portal hypertension.

Both gender of all races are affected, however, prevalence increases with rising socio-economic status and peaks between 45 and 65 years (Johnson and Sonnenberg, 1990). A haemorrhoid is usually associated with other conditions such as Crohn's disease, inflammatory bowel disease and ulcerative colitis. Others include anal fissure, chronic constipation and diarrhoea (Ramakrishna, 2009), and pregnancy (Rosen, 2006).

The most common presenting symptoms are itching, burning, bleeding, pain, seepage, prolapse and thrombus formation. The incidence of anaemia arising from bleeding haemorrhoids is rare (Ibrahim *et al*, 2008). Spasms of the *levator ani*, internal and external anal sphincter muscles result in tenderness of the perianal region (Ramakrishna 2009). Symptoms are non-specific; definitive diagnosis involves thorough history and physical assessment.

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Haemorrhoids are divided into internal and external haemorrhoids depending on whether they occur above or below the anorectal line.

Dietary, medical and surgical measures are major treatment approaches. Systemic and topical analgesics, antihistamines, anti-inflammatory agents and stool softeners are commonly used drugs. However, these agents are associated with uncomfortable side effects, and worsening of prognosis, in the case of steroids in an immuno-compromised patient.

Ocimum gratissimum (Linn) is a free growing shrub that reaches 1-2m in height. It is used commonly as a spice in cooking. It has extensive use in West Africa as a febrifuge and anticonvulsant. Juice derived from the crushed leaves are claimed to be useful in the management of intestinal disorders (Ezekwesili *et al.*, 2004). The essential oils extracted from plants grown in Brazil have anti-inflammatory and analgesic properties (Rabelo *et al.*, 2003). Vieira *et al.* (2001) have isolated eugenol, thymol and geraniol from the leave of *O. gratissimum*.

Recently, one of the authors encountered the use of leaves of *O. gratissimum* for the traditional treatment of haemorrhoids. This study was therefore carried out to establish preliminary basis for the use of locally grown *O. gratissimum* in the traditional management of haemorrhoids using *in vitro* and *in vivo* pharmacological models in experimental animals.

EXPERIMENTAL

Animals. Albino rats (100-150g) and mice (20-25g) of the Wister strain and hybrid (New Zealand White) rabbits (1.3kg) were procured from the animal house facility of the University of Jos. Animals for *in vitro* studies were fasted overnight to increase tissue sensitivity and eliminate fecal matter from the gut. All animals were fed with standard diet and given water *ad libitum*, and kept in neat cages under standard conditions until required for use.

Drugs. Pentazocine injection (Mercury Laboratories Ltd, India); Absolute ethanol (Laboratory Chemical Ltd, United Kingdom); Pancuronium injection (Rotex Medical, Germany); Suxamethonium chloride injection BP (Rotex Medical, Germany); Neostigmine methyl sulphate (Duopharma, Malaysia); Potassium chloride (BDH, Poole England); Prazocine (Pfizer Inc, USA); Propranolol (Sigma Co., Germany); Isoprenaline (Sigma Co., Germany).

Instruments. Recording microdynanometer and isometric/isotonic transducer (Ugo-Basile, Milan Italy 13177); students kymograph (Bioscience, Sheerness Kent, UK), Thermocirculator (Churchill UK)

Plant Material. The leaves of *Ocimum gratissimum* were collected in June 2010 within Jos metropolis and identified by Mr. Azila Joseph of the Federal College of Forestry Jos. The leaves were shade-dried for seven days after which they were pounded to coarse powder. Two hundred grams of the powdered leaves was cold extracted with 700ml of absolute ethanol for 48hours. The ethanol was subsequently evaporated off to give a dark green crude extract (yield = 13.8%). This was stored at 4^0C until needed.

Effect on rabbit jejunum. A New Zealand White rabbit weighing 1.3kg was sacrificed, and through a lower abdominal incision, the jejunum was identified and isolated. A 3cm length was sectioned and mounted in a 50ml organ bath containing Tyrode physiological salt solution (composition: NaCl 8.0g, KCl 0.2g, $MgCl_2$ 0.1g, $CaCl_2$ 0.2g, $NaHC0_3$ 1.0g, D-Glucose 1.0g in 1litre of freshly distilled water). The tissue was maintained at 37^0C and aerated with air. The preparation was allowed to equilibrate for 45minutes before the effects of the extract and standard drugs were tested.

Effect on skeletal muscle. The phrenic nerve and diaphragm from an adult Wistar Albino rat was isolated and mounted according to the method described by Williamson et al (1996).

Anti-inflammatory effect. Fifteen rats (100-150g) were divided into five groups of three rats each. The ethanolic leaf extract of *O. gratissimum* (100-1000mg/kg) was administered to three groups intra-peritonealy, while the two other groups received acetyl salicylic acid (10mg/kg) as reference drug and normal saline respectively. The method described by Ratheesh and Helen (2007) for screening anti-inflammatory activity was modified, where 0.1ml of egg white was used to induce inflammation rather than carrageenan. The percentage inhibition in oedema size was used as a measure of anti-inflammatory activity.

Analgesic activity. Fifteen mice were divided into 5 groups of 3 mice each. The test groups received the extract (100-500mg/kg) intra-peritoneally. Positive control group was administered pentazocine (0.5mg/kg) while the negative control group was administered normal saline. Analgesic activity was evaluated according to the method described by Williamson et al (1996). Analgesic activity was measured as prolongation in reaction time to heat-induced stimulus.

Fig. 1: Effect of the Ethanolic Leaf Extract of *O. gratissimum* on the Rat Phrenic Nerve-Diaphragm Preparation

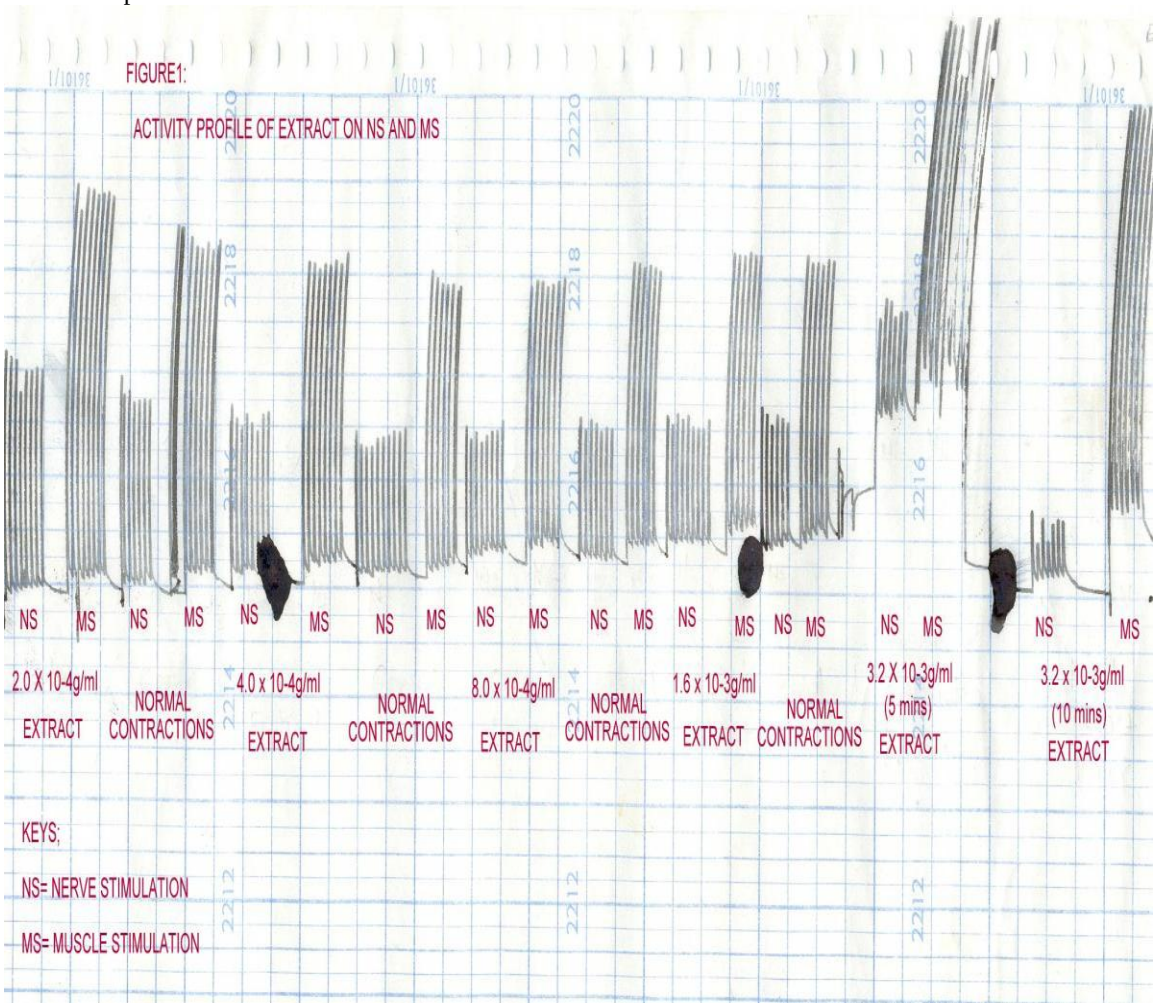

Fig. 2: Effect of the Ethanolic Leaf Extract of *O. gratissimum* on Rabbit Jejunum

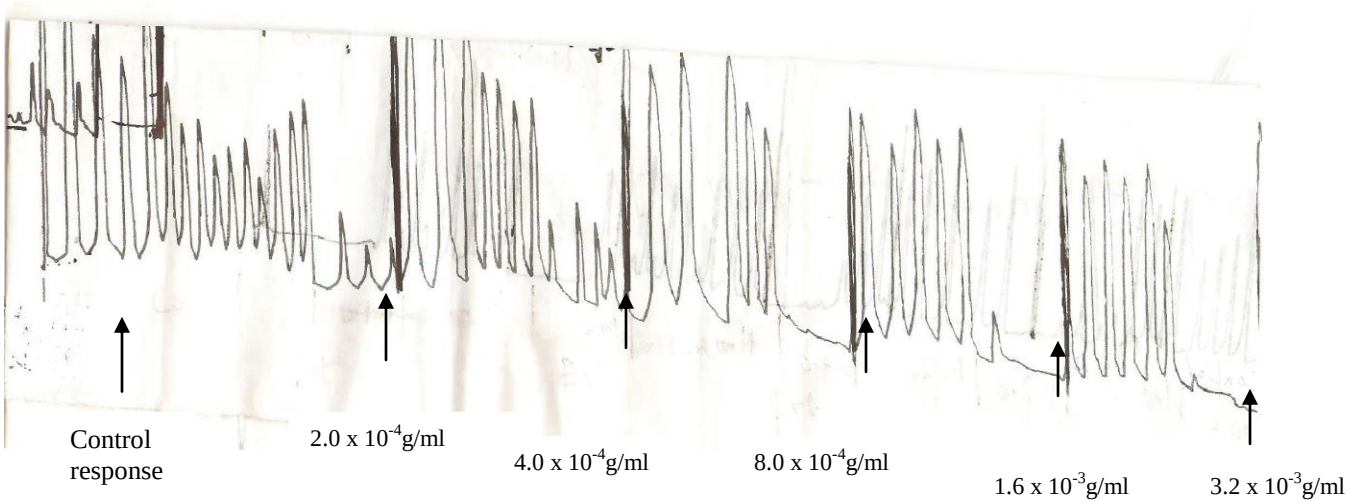

Table 1: Anti-inflammatory effect of the ethanolic leaf extract of *O. gratissimum* in egg white-induced rat paw oedema

Treatment	Dose(mg/kg)	Mean difference	Percent inhibition in paw size
Normal saline	0.1 mL	0.15 ± 0.03	0
O. gratissimum	100	0.12 ± 0.03	20.0
O. gratissimum	500	0.08 ± 0.02[a]	46.0
O. gratissimum	1000	0.04 ± 0.02[a]	73.0
Aspirin	20	0.06 ± 0.02[a]	60.0

Values are mean ± S.E.M. $p < 0.05$; n = 3

Table 2: Analgesic Effect of the ethanolic leaf extract of *O. gratissimum* on thermal-induced nociception in rats

Treatment	Dose(mg/kg)	Mean reaction time (s)
Normal saline	0.1 mL	19.30 ± 1.00
O. gratissimum	100	27.91 ± 1.24[a]
O. gratissimum	500	53.81 ± 4.49[a]
O. gratissimum	1000	98.94 ± 2.85[a]
Pentazocine	0.5	59.39 ± 0.66[a]

Values are mean ± S.E.M. $p < 0.05$; n = 3

RESULTS

The extract dose-dependently suppressed the contractions of the rat diaphragm to nerve stimulation, with a maximal response observed at 3.2 x 10^{-3} g/ml (Figure 1). The spontaneous rhythmic contractions of the isolated rabbit jejunum were also abolished at 3.2 x 10^{-3} g/ml (Figure 2). *In vivo* studies showed that the ethanolic leaf extract of *O. gratissimum* dose-dependently inhibited egg white induced oedema in rat paw (Table 1) while exhibiting significant analgesic action (Table 2). Both actions were comparable to standard drugs.

DISCUSSION

The results show that ethanolic leaf extract of *O. gratissimum* dose-dependently suppressed skeletal muscle neurotransmission (Figure 1), an effect qualitatively similar to that induced by tubocurarine. Competitive blockade of post-synaptic acetylcholine receptors interferes with development of the end plate potential that is essential for skeletal muscle contraction. The *levator ani* is the

muscle that enables defecation and spasms of the muscles of the perianal region contributes the tenderness that is classical symptom of perianal pathology.

As shown in Figure 2, the extract has smooth muscle relaxant effect in much the same way as adrenergic agonists. However, this effect was not blocked by known adrenoceptor blockers indicating that non-adrenergic mechanisms may be responsible.

Tables 1and 2 show that the ethanolic leaf extract of *O. gratissimum* has significantly potent anti-inflammatory and analgesic activities. Acute inflammation is induced by mediators such as histamine, serotonin, bradykinin and the prostaglandins (Katzung, 1998). These mediators are also potent stimulants of sensory nerve endings (Burkhalter *et al*, 1998). The results suggest that there are principles in the crude extract that modify the physiological response to inflammation and nociception, as seen in animal models of inflammation and pain.

This study establishes a scientific basis for the use of *O. gratissimum* in the management of symptoms of anal pathologies by traditional medical practitioners.

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