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# Healing Effects of the Methanol Extracts of *Vernonia amygdalina* (Asteraceae) *Ocimum gratissimum, (Lamiaceae)* and their Combination on Croton Oil-Induced Hemorrhoid in Rats

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**Abstract**

Plant decoctions have been used since ancient times for various diseases. It is usually believed that combining different plants from different sources ensures efficacy. *Vernonia amygdalina* (Asteraceae) and *Ocimum gratissimum* (Lamiaceae) and their combination have been used in treating various illness such as cold, growth, immunity and infections. The study aimed to determine the effects of methanol extracts of *Vernonia amygdalina* (VA) and *Ocimum gratissimum* (OG) leaves and their combination on croton oil-induced hemorrhoid in rats. We adopted the histological procedures of recto-anus portion of Wistar rats before and after treatment, determine the rectal temperature, measure the weights of rectum (rectum-anus portion measuring 2 cm), the % reductions in the swelling of the rectum of the treated animals in relation to the control, the anus swelling coefficient (the recto-anus coefficient- RAC) and the amount of NO release from homogenized rectum as an index of inflammation and protection from treatment. One hundred and ten (110) rats group into 10 groups (10/gp) were treated orally as follows; Groups I - negative control, Group II -Hemorrhoid inducer as negative control, Group III, (*Vernonia amygdalina* (VA*)* 400 mg/kg extract, Group IV (*Ocimum gratissimum* (OG) 400 mg/kg extract). Group V -combined 400 mg/kg extracts of both VA + OG. Group VI - Cortisol 200 mg/kg as positive control. The remaining 4 groups were treated rectally as follows; Group VII - Starch as negative control, Groups VIII - VA 400 mg/kg extract, Group IX- *OG* 400 mg/kg extract and. Group X - combined 400 mg/kg extracts of both VA + *OG*. Groups II – X also received 0.16 ml of hemorrhoid inducer inserted into rat anus for 10 seconds. Quantitative estimation of edema was done by determining the weight of the recto-anal tissue. The recto-anal coefficient was estimated as a direct measure of inflammation. Biochemical parameters such as NO were estimated in each group after the therapy. Histopathological variations among the groups were analyzed. Statistical analysis of the data affirms the curative effect of VA and OG on Hemorrhoids. The results demonstrated that VA and OG were effective when compared to cortisol. The results also showed that there was a significant decrease in NO production compared to negative controls and that there was no remarkable derangement in the cytoarchitecture of the anorectum tissues. It was therefore concluded that methanol leaf extracts of VA and OG may possess cytoprotective potential.

**Keywords***:* Hemorrhoids, *Vernonia amygdalina and Ocimum gratissimum*, nitric oxide, histological procedures.

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## Introduction

Hemorrhoids are defined as the symptomatic enlargement and distal displacement of the normal anal cushions. The most common symptom of hemorrhoids is rectal bleeding associated with bowel movement. The abnormal dilatation and distortion of the vascular channel, together with destructive changes in the supporting connective tissue within the anal cushion, is a paramount finding of hemorrhoids (Loder *et al.,* 1994). Also associated with hemorrhoids are dilated, twisted (varicose) veins located in the wall of the rectum and anus. Hemorrhoids occur when veins in the rectum or anus become enlarged and may eventually bleed. Hemorrhoids may also become inflamed or may develop a blood clot (thrombus). Hemorrhoids are one of the most common forms of varicose veins, manifesting as painful and or itchy, and can bleed, typically after or during defecation

Internal hemorrhoids that form above the boundary between the rectum and anus (anorectal junction) are the most common refs. It appears in grades I, II, III and IV. Sometimes it protrudes through the anus outside the body, becoming irritated and painful, known as protruding hemorrhoid (Ugwu *et al*., 2024). External hemorrhoids forms below the anorectal junction, and may include painful swelling or a hard lump around the anus that results when a blood clot forms refs. It is externally painful and usually requires minor surgery. Hemorrhoids can also result from increased pressure in the veins of anorectal area caused by pressure from pregnancy, frequent heavy lifting, repeated straining during bowel movements probably due to constipation, and increased blood pressure in the portal vein. Hemorrhoids can be prevented with appropriate diet and life style modifications, and can be treated.

Hemorrhoidal disease is one of the most frequently encountered anorectal conditions in general practice (Sheikh *et al* 2020). However, its true prevalence in the general population is not well known. Prevalence have been reported in various countries around the world. In Africa, the reported incidence ranged from 7% to 56.2% in Bamako, Ethiopia, Brazzaville and Nigeria (Okafor *et al* 2023). The treatment of hemorrhoids is aimed at correcting the underlying cause, which may be a variety of factors. In cases where the condition is relatively mild, eating a diet high in fiber, drinking plenty of water, and applying local anesthetic creams to relieve itching and pain is advisable. In symptomatic cases, treatments containing analgesics, non-steroidal anti-inflammatories, or anesthetics are employed to address hemorrhoidal attacks refs. Another potential treatment option is the application of a ligature to the hemorrhoidal artery. In the event of disease progression, surgical intervention is the definitive treatment for severe and thrombosed external hemorrhoids (Ma, et al 2020, Stratta and Trompetto, 2021, Devi, *et al* 2023)

Taking stool softeners or laxatives to relieve straining during bowel movements, soaking anus in warm water (sitz bath), or tying hemorrhoid protrusions off with rubber band (rubber band ligation) and laser destruction (infrared photocoagulation) or an electrical current (electrocoagulation) may be helpful.

Ethnobotanical studies (Muya, *et al*., 2014, Bashige-Chiribagula *et al*., 2017) targeted on pathologies have yielded insights into the treatment of hemorrhoidal diseases by medicinal plants. *Vernonia shirensis and Aframomum angustifolium* leaves or stem bark are used locally against hemorrhoids. The leaves of *Asparagus africanus* and *Pericopsis angolensis* and the roots of *Piliostigma thonningii* and many more are employed as anti-hemorrhoidal agents in non-conventional medicine. (Muya, *et al*., 2014, Bashige-Chiribagula *et al*., 2017, Amuri, *et al* 2018, Ugwu *et al*., 2024). Existing treatments are supportive, and do not address the cause, thus relapse of hemorrhoid is frequent. Currently no single agent with anti-hemorrhoid effect from natural sources has regulatory agency approval, The aim of this study was to evaluate the anti-hemorrhoid properties of the crude methanol leaf extracts of *Vernonia amygdalina* (VA) and *Ocimum gratissimum* (OG) in Wistar albino rats

## Materials and Methods

### *Materials*

The materials used in this study included methanol, sohxlet extractor, croton oil, diethyl ether, pyridine, cotton swabs, plastic gavage, hydrocortisone injection powder, Bouin fluid, paraffin oil, hemotozolin and eosin stain, Olympus microscope (CS21).

### *Methods*

#### *Collection of plants and identification*

Freshly leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were collected from the main University campus of Obafemi Awolowo University, Ile-Ife, Nigeria. The plants were identified by Mr. O.A Oladele of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The voucher numbers for *Ocimum gratissimum* UIH-22617 (University. of Ibadan) and *Vernonia amygdalina* FHI - 110415 (Forestry Research Institute of Nigeria), and IFE-16885 (Obafemi Awolowo University).

#### *Preparation of Extracts*

The methanol extract of *Ocimum gratissimum* (OG) was prepared by soaking 200 g of dried powdered OG leaf in a 5 L of methanol in a round bottom flask and shaken at intervals over 48 hours, filtered with cotton wool in a funnel and evaporated in vacuo on a rotary evaporator to dryness. *Vernonia amygdalina (*VA) extract was prepared by soaking 325 g of powdered dried VA leaf in the Soxhlet extractor with 5L methanol and extracted over 48 hours, the extract was evaporated in vacuo on a rotary evaporator to dryness.

*Formulation of Granules*

The dried crude methanol extracts of VA and OG, hydrocortisone powder and dry cassava starch powder were incorporated into granules using ratio 1:2, 1:3, 1:4, 1:5 respectively. One part of the extract, or the positive control or negative control was triturated with required parts of dry cassava starch, and few drops of water were added to make some irregular granules which were passed through a 70 micro micron sieve to get fine granules and spread out to dry under room temperature.

*Animals*

One hundred and ten healthy Wistar rats (150-200 g) of either sex bred under standard conditions (temp. 27±3° C, relative humidity 65 %) at the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used. The animals were maintained at 25 $\pm$1°C under natural 12 h daylight/ night conditions for at least 1 week before the experimental procedures. All the animals were fed with standard diet. The "principle of laboratory animal care" (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985). All experiments followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and received approval from the University Postgraduate School with student registration number (UI/PGS/2015/159238)

*Induction of hemorrhoids in rat anus*

The hemorrhoid was induced with a mixture of water, pyridine, diethyl-ether and 6% croton oil in diethyl-ether in the ratio of 1:4:6:10. Cotton swabs were soaked in 0.16 ml of hemorrhoid inducer and inserted into rat anus for 10 sec (Alajasimi, *et al* 2013, Nallajerla and Ganta 2022; Budi Kusumo, *et al* 2024). The rats were fasted from food for 24 hours but water was allowed *ad libitum* The extracts and positive control were administered by two routes oral and rectal routes, at 400 mg/kg. The animal groupings and treatment for the two routes were;

*Oral route*

Group I Distilled water as negative control, Group II Untreated Hemorrhoids, Group III VA extract 400 mg / kg, Group IV OG extract, 400 mg / kg, Group V VA (200 mg/kg) + OG (200 mg/kg). = 400 mg/kg), Group VI Cortisol (200 mg/kg)

*Rectal route*

Group VII Starch as negative control, Groups VIII VA extract (400 mg / kg), Group IX *OG* extract (400 mg / kg), Group X *VA* (200 mg/kg) + *OG*. (200 mg/kg) = 400 mg/kg)

The parameters evaluated include the rectal temperature measured with rectal thermometer and the weight of the rectum.

The animals were later sacrificed with an overdose of diethyl ether. The rectum was isolated and cleared of any adhering tissue. The rectum-anus portion measuring 2 cm was cut from each rat starting from the circular hairline on the anus epithelium using a pair of compasses. The cut piece was then opened longitudinally, blotted on a piece of tissue- paper and weighed immediately. The weight of tissue corresponding to 150 g body weight was then calculated in mg, and the percentage reductions in the weights of the rectum of the treated animals in relation to the control weights were calculated as:

$$\frac{\text{Weight of treated group – weight of control}}{\text{Weight of treated group}} \times \frac{100}{1}$$

The recto-anus coefficient (RAC) was calculated using the formula:

$$\frac{\text{weight of recto-anus (mg)}}{\text{body weight (g)}}$$

*Nitric Oxide Determination* (NO)

Nitrite determination was done on 50 µL aliquots of homogenized rectal sample mixed with 200 µL of the Griess reagent (Abhaypratap *et al*., 2019). The absorbance was read at 540 nm after 10 min of reaction and $NO_2$-concentration was determined with reference to a standard curve using concentrations from 1 to 250µM sodium nitrite in culture medium.

*Histological studies*

The recto-anal portion was harvested and fixed in Bouin fluid (5% picric acid + 10% formalin +5%acetic acid) embedded in liquid paraffin and stained with hemotoxylin and eosin (HE). Preparations of the slides were evaluated with a light field microscope Olympus, CS21)

*Statistical Analysis*

The results were expressed as mean ± S.E.M and analyzed using Student t-test followed by one way analysis of variance (ANOVA) for comparing pairs of data. The significant level was set at $P < 0.05$.

**Results**

*Extraction Yield*

The % yield of OG and VA was 9.79 and 33.10 respectively

*Effect of oral OG and VA on hemorrhoid*

The weight of 2 cm recto-anus portion of the hemorrhoids and the weight of the recto-anus portion equivalent to 150 g were not significantly different in the groups treated with the

extracts and cortisol. There was a consistent increase and decrease in RAC values and the amount of nitric oxide (NO) released respectively (Figure 1). The largest amount of NO was released in Hemorrhoid untreated control. Down-ward trend decreases in NO by the treated groups showed that VA 400 mg/kg < OG 400 mg/kg < VA + OG 400 mg/kg combination < 400 mg/kg (Figure 1).

*Effect of oral OG and VA on rectal temperature*

Hemorrhoid induction resulted in an increase in temperature (Figure 2) as a result of inflammation. Treatment with the extracts alone or in combination reversed the elevated temperature

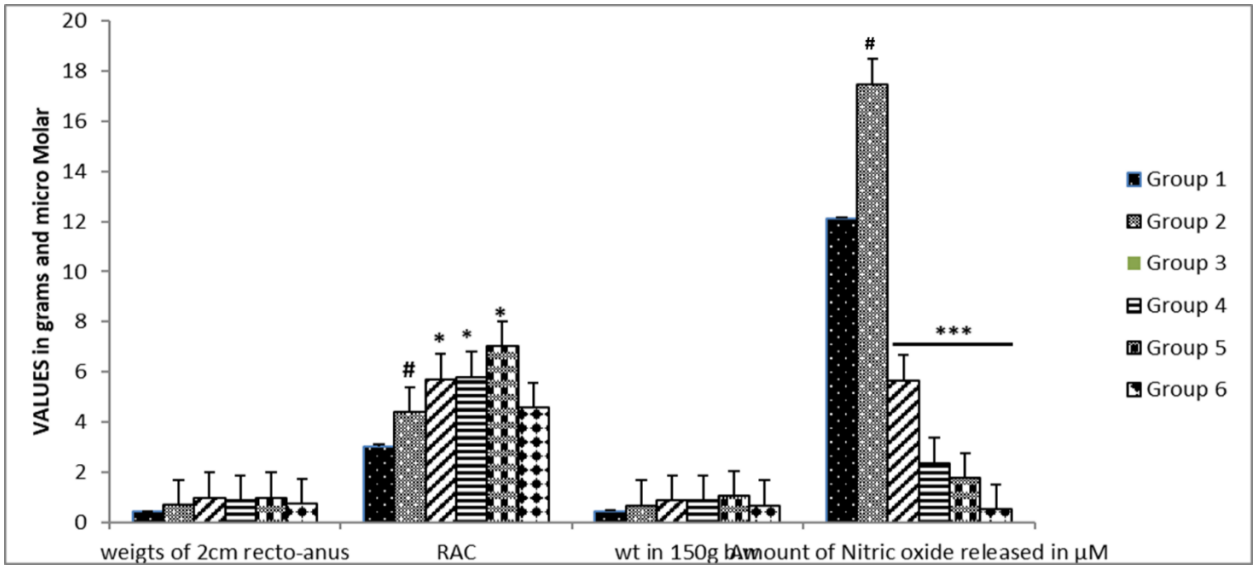


**Figure 1:** The weights of 2cm recto-anus portion of the hemorrhoids, the recto-anus coefficients (RAC), the weights of the recto-anus portion equivalent to 150 g and the amount of nitric oxide (NO) measured from the homogenate of the recto-anus portion. Group 1Control and no hemorrhoid induction, Group. 2- Hemorrhoid induction + normal saline, Group. 3-Hemorrhoid +*VA* 400 mg/kg, Group.4- Hemorrhoid +*OG* 400 mg/kg, Group.5-Hemorrhoid + VA + OG 400 mg/kg Group. 6-Hemorrhoid + cortisol 400 mg/kg

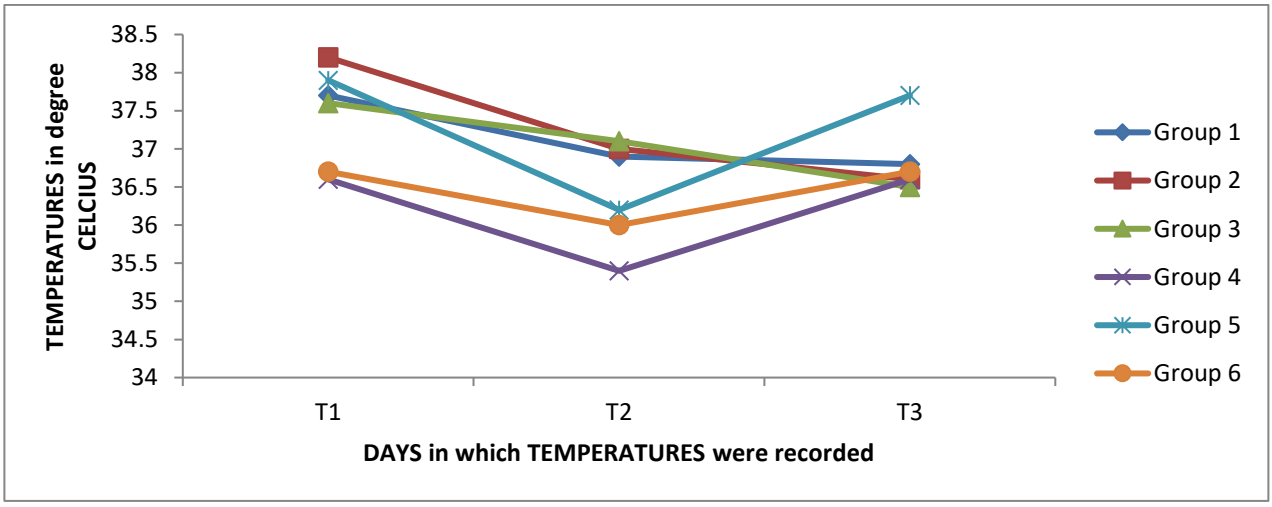


**Figure 2:** Effects of the extracts on temperature, T1, before induction; T2 before treatment and T3 after 24 hours after treatment. Group 1 Control and no hemorrhoid induction, Group. 2- Hemorrhoid induction + normal saline, Group. 3-Hemorrhoid +*VA* 400 mg/kg, Group.4- Hemorrhoid +*OG* 400 mg/kg, Group.5-Hemorrhoid + VA + OG 400 mg/kg Group. 6-Hemorrhoid + cortisol 400 mg/kg

*Effects of rectal VA and GO on Hemorrhoid*

Treatment with the extracts did not significantly affect the weight of 2cm recto-anus portion of the hemorrhoids and the weight of the recto-anus portion equivalent to 150 g. These weights were not significantly higher than those of the control group (Figure 3). There was a significant ($P < 0.5$) increase in RAC values and decrease in the amount of nitric oxide (NO) released, respectively (Figure 3). The release of NO is an indication of inflammation, and the decrease in the amount of NO indicates an index/degree of wound healing.

*Effects of rectal VA and OG on rectal Temperature*

There was a consistent increase in the rectal temperature as a result of hemorrhoids (Figure 4). Treatment with the extracts alone or in combination resulted in a downwards reduction in the rectal temperature, with the extract combination exhibiting most reduction ($P > 0.05$).

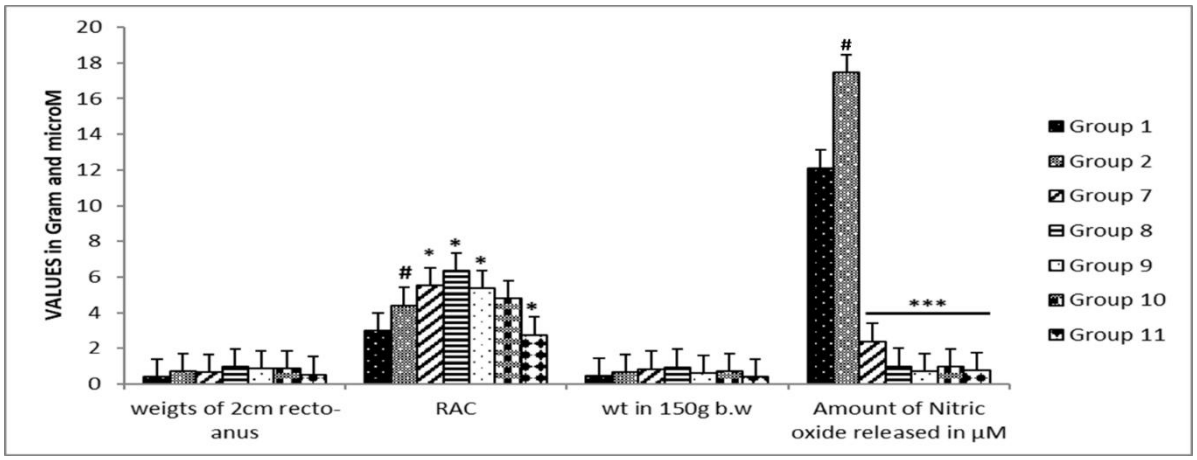


**Figure 3:** The effect of the extracts on the weights of 2cm recto-anus portion of the hemorrhoids, the recto-anus coefficients (RAC), the weights of the recto-anus portion equivalent to 150 g and the amount of nitric oxide (NO) measured from the homogenate of the recto-anus portion in after rectal administration. Group.1 No treatment and no hemorrhoid induction, Group 2 Hemorrhoid induction + normal saline, Group 7 Hemorrhoid+ starch, Group. 8 Hemorrhoid +*VA* 400 mg/kg, Group 9 Hemorrhoid +*OG* 400 mg/kg, Group.10 Hemorrhoid + VA + OG 400 mg/kg and Group Hemorrhoid + 11 cortisol 400 mg/kg

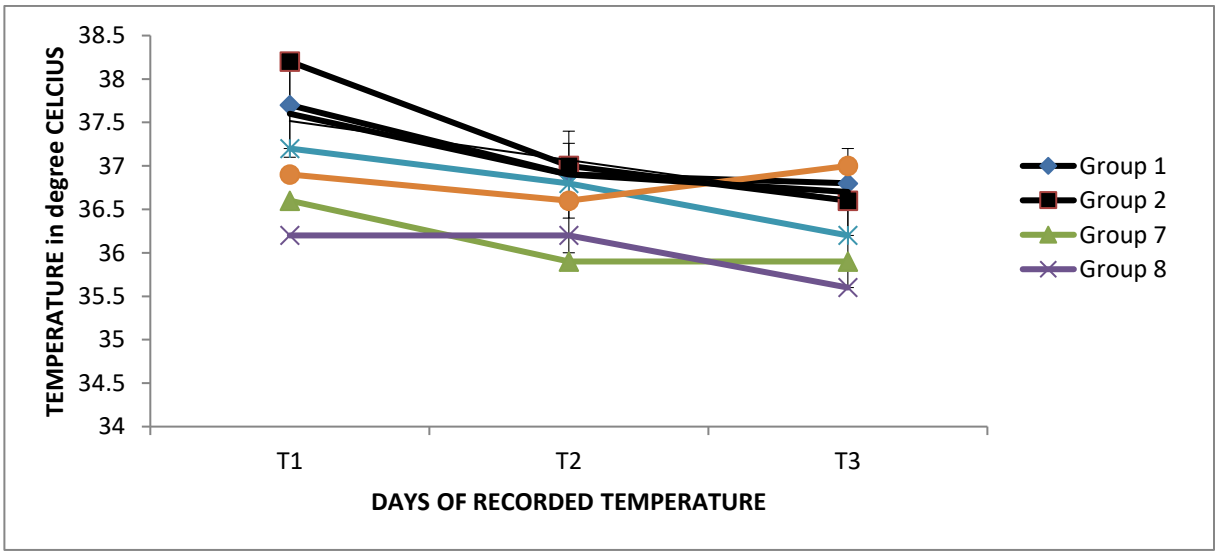


**Figure 4:** The figure indicates the various pattern of temperature taken through the rectum of the animal used before the induction of hemorrhoid (T1), before the treatment of various agents (T2) and finally after the treatment 24 hour after induction of hemorrhoid (T3) in groups 1-6 when the agents were administered rectally. Group.1 No treatment and no hemorrhoid induction, Group 2 Hemorrhoid induction + normal saline, Group 7 Hemorrhoid+ starch, Group. 8 Hemorrhoid +*VA* 400 mg/kg, Group 9 Hemorrhoid +*OG* 400 mg/kg, Group.10 Hemorrhoid + VA + OG 400 mg/kg

Results in Table 1, and Figures 6 and 7 showed the comparative effects of the extracts on recto-anus coefficients (RAC), % inhibition of oedema and the percentage (%) swelling of the rectum-anus portions by the 2 routes of administration. The activity of the extracts and their combination were more pronounce in rectal route than oral route

**Table 1:** The Comparative effects of the extracts in the oral and rectal routes.

| Groups A | RAC | % swelling of the recto-anus | % inhibition of oedema | Groups B | RAC | % swelling of the recto-anus | % inhibition of oedema |
|---|---|---|---|---|---|---|---|
| Negative control | 3.09 ± 0.12 | - | - | Negative control | 3.13 ± 0.00 | - | |
| Hemorrhoid control (normal saline) | 4.39± 0.84# | 100 | 0 | Hemorrhoid control (starch) | 5.03 ± 0.38# | 100 | 0 |
| Hemorrhoid + V.a(400 mg/kg) | 5.51± 1.11* | 20.82 | 14.0 | Hemorrhoid + V.a(400 mg/kg) | 5.48 ± 0.44* | 8.21 | 76.30 |
| Hemorrhoid + O.g(400 mg/kg) | 5.80± 0.70* | 24.35 | 8.40 | Hemorrhoid + O.g(400 mg/kg) | 6.24 ±1.17* | 19.39 | 36.30 |
| Hemorrhoid + V.a + O.g (400 mg/kg) | 6.84± 0.73* | 35.82 | 88.40 | Hemorrhoid + V.a + O.g (400 mg/kg) | 5.07 ± 0.79* | 0.79 | 97.80 |
| Hemorrhoid + Cortsol(400 mg/kg) | 4.58± 0.15* | 4.15 | 85.40 | Hemorrhoid + Cortsol(400 mg/kg) | 2.77 ± 0.28* | -81.59 (0) | 218.90 |

Group A represents orally treated animals, while Group B represents the rectally treated groups. (n=5) (* and # P < 0.05)

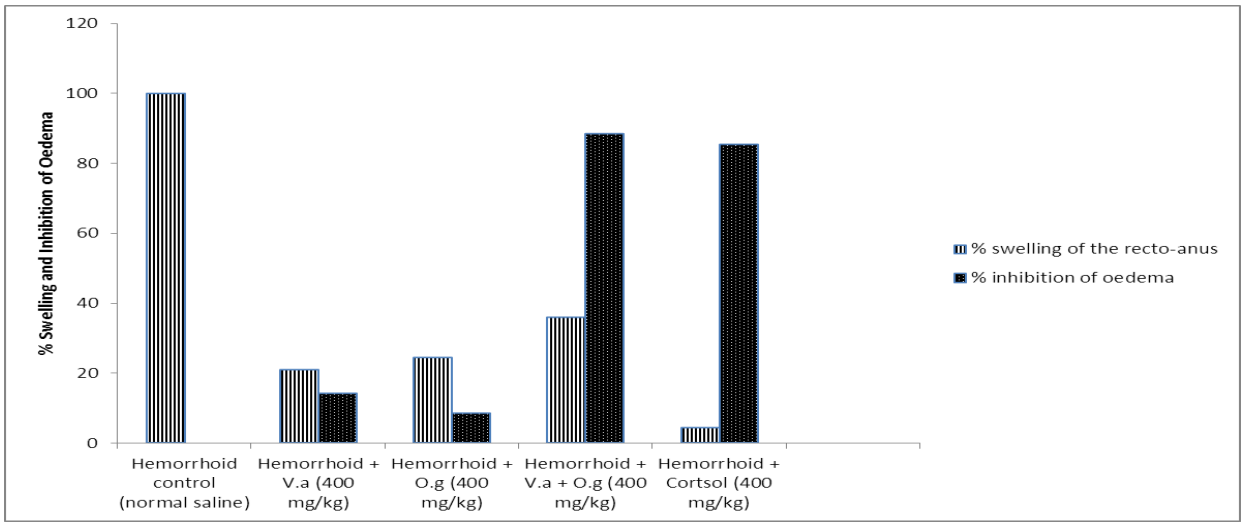


**Figure 6:** The percentage (%) swelling of the rectum-anus portions as an index of oedema and percentage (%) inhibition of oedema of the treated animals in relation to the hemorrhoid induced control in oral treated groups.

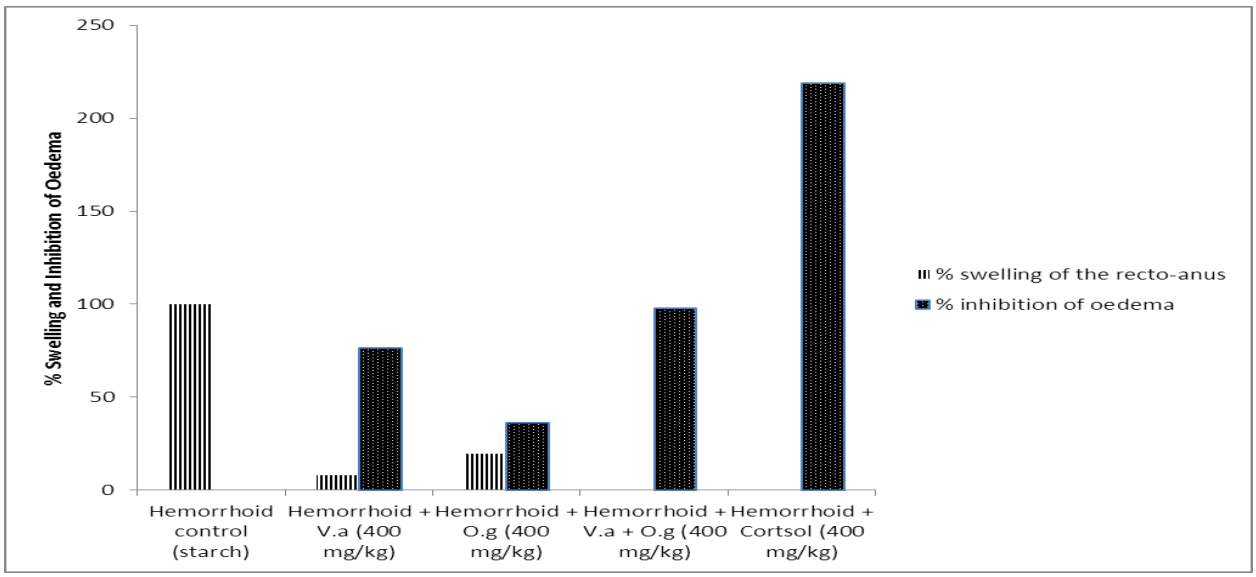


**Figure 7:** The percentage (%) swelling of the rectum-anus portions as an index of oedema and % inhibition of oedema of the treated animals in relation to the hemorrhoid induced control in rectal treated groups.

*Histological studies*

Histological assessment of the wound healing effect of *Vernonia amydaglina* and *Ocimum gratissimum* methanol extract and, their combination on croton oil -induced hemorrhoid revealed that the anus induced with croton oil but not treated showed localized area of epithelial denudation marked with necrosis and exposure of the basement membrane after 24 hours of induction. In extract-treated groups, there is gradual epithelial regeneration, which was coupled with angiogenesis after treatment (Figures not shown)

**Discussion**

Hemorrhoids is a pathological condition, which is characterized by a severe vasodilatation at the recto-anal region, which leads to inflammation of the surrounding tissues, this will further lead to secondary complications such as extravasation of fluid into interstitial space mainly due to increased vascular permeability, migration of large quantity of inflammatory cells (granulocytes and monocytes). In the present study, croton oil has been used as an inducer/phlogistic agent to induce experimental hemorrhoids. Croton oil causes inflammation due to the release of soluble factors involving inflammatory lipid metabolites (prostaglandins, leukotrienes, lipoxins), kinins (bradykinins, chemokines), nitric oxide, and cytokines (TNF-α, IL-6) refs. These factors, alone and/or in combination, regulate the activation of resident cells (fibroblasts, endothelial cells, macrophages, and mast cells) and newly recruited inflammatory cells (monocytes, lymphocytes, neutrophils, and eosinophils) leading to systemic response to inflammation (fever, pain, swellings and loss of functions) (Carol and Timothy 1997).

The natural products and medicinal plants extracts has become the most explored sources of new medicine for various gastrointestinal disorders, especially in the treatment of hemorroids in various experimental models for evaluating anti-inflammatory and anti-hemorroidal drugs. (Ugwu, *et al*., 2024, Shaleh 2026). In hemorrhoidal tissue, it has been reported that there is very intense inflammation on both the vascular system and the supportive connective tissue, hence, inflammation can lead to the pathological changes observe in hemorrhoids leading to mucosal ulceration, thrombosis, vessel dilation, and the distortion of the smooth muscle layer, together with the surrounding supportive tissue (Lohsiriwat, Varut 2012, Serra*, et al*., 2016, Wang, 2023).

Hemorrhoids are said to occur when the veins in or around the anus, or in the lower rectum, are swollen and often also inflamed. There are lots of irritants that can be used to induce hemorrhoids, some of which are: exogenous stimuli (such as phorbol-12-myristate 13-acetate, ultraviolet B radiation, and lipopolysaccharide), non-sensitizing contact irritants (croton oil, sodium lauryl sulfate, methyl salicylate, ethyl phenylpropiolate, sodium aescinate), sensitizing irritants (oxazolone, dinitrofluorobenzene), and ulcerative agents (phenol, benzalkonium chloride, chromium trioxide) (Di Hu, *et al*., 2024).

Croton oil, an irritant agent, induces an inflammatory response when applied locally/topically. This response is through activation of phospholipase $A_2$ and by initiation of arachidonate metabolism (Simon, 1999, Porwal, et al., 2024) The released arachidonic acid, in turn, is metabolized by COX and LOX enzymes, promoting the production of eicosanoids and leukotrienes. As a result, there is an increase in vascular permeability and hydrostatic pressure, leading to edema formation and migration of neutrophils to the damaged area.

This process occurs with the activation of T-lymphocytes, macrophages, neutrophils, monocytes, mast cells, and dendritic cells (Porwal, *et al*., 2024a, Porwal, *et al*., 2024b), which are responsible for secreting pro- and anti-inflammatory cytokines like RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted, CCL-5), TNF-α, and VEGF (vascular endothelial growth factor), as well as interleukins IL-1β, IL-6, IL-8, IL-17, and interferon gamma (IFN-γ)—these cytokines were found to be significantly overexpressed in hemorrhoids. IL-10, an anti-inflammatory cytokine, is under expressed (Zhou, *et al* 2023). Studies have shown that some substances (e.g., polyherbal formulation Anoac-H, turmocin) help to downregulate the expression of those cytokines in fibroblasts and macrophages of hemorrhoid tissues, which leads to a reduction in inflammation, and, due to VEGF's primary function, to a decrease in vascular density, which is increased in hemorrhoids (Porwal, *et al*., 2024a, Porwal, *et al*., 2024b).

The recto-anus edema reaches its peak in the 6th hour, as a result of the formation of the arachidonic acid metabolites and other pro-inflammatory mediators (Swingle *et al.,* 1981; Porwal*, et al*., 2024a,). The local irritation caused by topical administration of croton oil, unleashes the release of several mediators, such as bradykinin, substance P, Prostaglandins (COX and LOX), Nitric oxide and proinflammatory peripheral mediators as well as cytokines such as IL-1β, TNF-α and IL-8. (Shinwan *et al*; 2019; Porwal, et al., 2024b,). This experimental model is used to detect nitric oxide in vivo inhibition activity, being very sensitive to steroidal antiinflammatory drugs (SAIDs) and to NSAIDs (Young, *et al,* 1984; Zhou, *et al* 2023;).

Nitric oxide (NO) is a molecule produced in many different cells, and it contributes to vascular dilatation and the development of varicose veins (Haviarová, *et al*., 2011). It was found to be overly present, together with two Nitric Oxide Synthases (NOS), endothelial (eNOS) and neuronal (nNOS) (Gokce, *et al*., 2020) in hemorrhoids, while asymmetric dimethylarginine, a molecule that inhibits NOS, was under expressed in those tissues (Lohsiriwat, *et al*., 2018). The high expression of NOS in hemorrhoids leads to excessive amounts of NO being synthesized, and that causes an increase in blood flow and the twisting of veins, which adds to the varicose vein's theory of the pathogenesis of hemorrhoids.

In this model, both oral and rectal application of *Vernonia amygdalina* and *Ocimum gratissimum* leaves extracts produced a significant anti-edematogenic effect, similar in intensity to the result observed with cortisol as positive control. Although the present study established the efficacy of *Vernonia amygdalina* and *Ocimum gratissimum* leaves as anti-inflammatory agents, its exact mechanism and the mediators involved in the inhibition of one mediator (nitric oxide NO) in inflammation were also explored.

The histological procedures of the recto-anus portion of Wistar rats before and after treatment to see the intensity of tissue damage and repair were adopted. From the percentage (%) reductions in the oedema and swelling of the rectum of the treated animals in relation to the control weights, it is clearly shown that both *Vernonia amygdalina* and *Ocimum gratissimum* leaves produced anti-hemorrhoid/anti-inflammatory activity either as mono or combination therapy, when orally or rectally administered. These two plants have been shown to demonstrate the following activities: GIT relaxant effect, antidiarrhoeal, wound healing, antibacterial, antifungal, antioxidant activities which are related to inflammatory conditions (Orafidiya *et al.,*2004, Orafidiya *et al.,*2005, Prabhu, *et al.,* 2009).

Apart from the fact that these two plants have been identified to inhibit COX and LOX enzymes activities in their anti-inflammatory effects (Sahouo *et al* 2003), a contributory inhibition of nitric oxide (NO) in this study is a clear indication of their anti-hemorrhoid effect. NO and COX as mediators have been implicated in hemorrhoids (Lohsiriwat, Varut 2012). In hemorrhoids, nitric oxide synthase, an enzyme which synthesizes nitric oxide from L-arginine, has been reported to increase significantly (Stankevicius *et al*., 2003; Han *et al*., 2005). Constituents in the leaves of *Ocimum gratissimum* responsible for biological activities were essential and volatiles oils identified as: eugenol, methyl eugenol,cis-ocimene,trans-ocimene,pinene, camphor,germacrene- D, trans-carypohyllene, farnesene and l-bisabolene (Orafidiya *et al* 2006). However, *Vernonia amygdalina*, has the following active constituents which include saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones and sesquiterpenes. (Ighile, *et al*.,1995; Akinpelu, 1999; Tona, *et al*., 2004; Erasto, *et al*., .2006). These constituents claimed to be responsible for various biological activities could be suggested to produce the effects observed in this study. Orafidiya *et al* (2006) explained the vascular permeability-increasing effect of the essential oil of *Ocimum gratissimum* as a mechanism for its wound healing property. These two plants have been identified to inhibit COX and LOX enzymes activities in their anti-inflammatory effects (Sahouo *et al*.; 2003). Just like *Euphorbia prostrate,* these plants may exhibit phlebotomid activity, vasculoprotective effects, and antagonism of the biochemical mediators of inflammation (Singla, and Pathak 1989, 1990). Another evidence of their anti-hemorroid effect is indicated from the levels of nitric oxide release. The downward trend of inhibition of NO released points to the fact that the constituents of these two extracts prevented further inflammation and ensured the process of wound healing. (Young, *et al*., 1984; Shinwan *et al*. 2019, Yu *et al* 2024). One of the cardinal signs of inflammation is the changes in temperature. There was a decrease in temperature of the recto anus after croton oil administration and the induction of hemorrhoids. Heat directly causes hemorrhoids and contributes to their development or make them worse. For example, the increase in summer heatwave temperatures can cause hemorrhoids, as heat promotes vasodilation in the hemorrhoid cushions, causing itching, swelling, inflammation and pain (Branisteanu *et al*., 2018). In this study, the effects of VA, OG and their combination demonstrated a reversal in

rectum temperature. Contrary to the results obtained for orally administered groups, the temperatures pattern in rectally treated rats showed a downward decrease in temperature of the recto anus except the group that received the extract combination, this is an indication that oral administration had a better response to the temperature pattern than rectal administration.

Histological observation of the recto-anal tissue showed presence of inflammatory cells, fibrin, congestion, hemorrhage, vasodilatation, and medium to high degrees of necrosis. The results of present studies showed very severe extravasation of croton oil induced hemorrhoid and increased levels of proinflammatory nitric oxide (NO). These pathological changes were supported by histological changes of the recto-anal portion exhibiting severe vasodilatation, inflammatory cells infiltration, along with hypertrophy of the mucosal cells and hemorrhagic spots. The normal control group showed normal cytoarchitecture of the recto-anal region. *Vernonia amygdalina* and *Ocimum gratissimum* leaf extract could be said to down regulate extravasation, cytokines inflammatory factors, and improved the histopathological evaluations (reduced the presence of inflammatory cells and fluid) which were involved in the development of hemorrhoids due to croton oil application (Nallajerla and Ganta 2022; Budi Kusumo, *et al* 2024). Interestingly, a single day treatment with VA, OG, and their combination significantly ameliorated the pathological hallmarks of croton oil-induced hemorrhoids in rats compared to positive control. This may be due to the potent anti-inflammatory activity of the herbs. The molecular mechanism behind the anti-hemorrhoidal activity of these extracts may be through the inhibition of NO release.

**Conclusion**

The findings of this study suggest that l OG, VA, alone or in combination ameliorated the croton oil-induced hemorrhoids in rats. Notably, the combination of VA with OG was found to be more effective than single administration of either VA or OG by oral or rectal administration. The anti-hemorrhoidal action of these plants can be attributed to the possibility of their interference with iNOS enzymes involved in the production of nitric oxide implicated in inflammation of the hemorrhoids.

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