

ORIGINAL CONTRIBUTION

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# Anti-nociceptive and anti-inflammatory effects of an aqueous extract of blended leaves of *Ocimum gratissimum* and *Psidium guajava*

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## Abstract

**Background:** To investigate the antinociceptive and anti-inflammatory activities of aqueous extract of a blended mixture of dried leaves of *Ocimum gratissimum* and *Psidium guajava*, a traditional analgesic drug polyherbal (TADP) used as a remedy for pain-related conditions.

**Methods:** Antinociceptive activity of TADP (100, 200 and 400 mg/kg) was evaluated in the hot plate test and acetic acid-induced nociception in mice while the anti-inflammatory was evaluated in carrageenan-induced paw oedema in rats. Levels of nitrite, myeloperoxidase, glutathione and malondialdehyde were assayed in carrageenan-induced paw tissue.

**Results:** TADP (200 and 400 mg/kg) significantly prolong the latency time in the hot-plate test. TADP (100–400 mg/kg) produced a dose-dependent significant inhibition of the acetic-acid induced abdominal constriction. The antinociceptive activity of TADP in the presence of naloxone and atropine was not reversed whereas yohimbine and glibenclamide significantly reversed it. TADP (100, 200 and 400 mg/kg) significantly reduced the swelling in the carrageenan-induced oedema model and also produced a reduction in the nitrite and myeloperoxidase level. TADP (400 mg/kg) significantly reduced malondialdehyde concentration and increase glutathione level in the carrageenan-induced rat paw. TADP significantly decrease the number of cellular infiltrates in the histopathological assessment.

**Conclusion:** These results indicate that polyherbal product containing blended leaves of *Ocimum gratissimum* and *Psidium guajava* possess antinociceptive and anti-inflammatory properties, hence represents a promising alternative remedy in inflammation-induced pain.

**Keywords:** Analgesic, Polyherbal, *Ocimum gratissimum*, *Psidium guajava*

## Introduction

Pain is an unpleasant experience associated with actual or potential tissue damage [1] and it is also known to be sensory and therefore it is experienced emotionally. It is unarguably one of the serious problems globally and as such has lost its defensive mechanisms [2]. The intensity, duration and nature especially of chronic pain is

incomparable to the extent of tissue damage that occurs [2]. It has been estimated to affect 1 in 5 adults in a population, also 1 in 10 adults is diagnosed with chronic pain each year [3]. Although, pain is a protective mechanism by which the body responds to harmful stimulus, improperly managed pain can lead to decreased productivity and diminished quality of life as it has been reported to affect the psyche, behaviour, social life of the individual and also present with socio-economic burden to the society [2, 4]. Pain associated with nociception is the feeling whereby there is the warning against the danger of damage to tissue or it shows an already

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inflicted damage as a result of illness or injury. Patients therefore presents decreased pain threshold and increased response to stimulus resulting in increased nociception [5, 6].

Inflammatory pain has been reported to produce mechanical allodynia and thermal hypersensitivity which results from the release of inflammatory mediators from inflammatory or adjacent tissues. Acute inflammatory pain causes much suffering and turned to be intractable as a result of poor and ineffective treatment [7]. Inflammatory pain though a common clinical symptom still lacks effective treatment as the most frequently used agents such as non-steroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors are associated with adverse effects [6, 8]. Hence, there is the need to search for alternative therapy of plant origin that could ameliorate pain resulting from inflammation.

*Ocimum gratissimum* belongs to the genus *Ocimum* belonging to the family Lamiaceae, a widely grown plant found in tropical and subtropical regions. *Ocimum gratissimum* belongs to the group of plants known as spices, a sub-shrub and perennial plant [9]. The plant is economically important due to their essential oils and utilization as medicine for many disorders, including pain, fever and central nervous system. *O. gratissimum* leaf is reported to be rich in polyphenolic compounds (rutin, caffeic acid, cirsimaritin, xanthomicrol) and essential oils (thymol, eugenol and geraniol) [9, 10]. The antinociceptive activity has been reported of the essential oils [10] and aqueous extract [11].

*Psidium guajava*, is an important food crop and medicinal plant in tropical and subtropical countries that is widely used for food and in folk medicine around of world. *Psidium guajava* Linn. belongs to the genus *Psidium* belonging to the family Myrtaceae [12]. More recent ethnopharmacological studies show that *P. guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, dental caries, wounds, pain relief and reducing fever. The leaf of *P. guajava* is used locally in Nigerian ethnomedicine to manage, control, and/or treat a plethora of human ailments, including diabetes mellitus and hypertension [12]. The leaf is rich in essential oil with main components being  $\alpha$ -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, cardinene and curcumene. The flavonoids found in the leaves include myricetin, quercetin, luteolin and kaempferol. The essential oil from *P. guajava* leaves and its constituent,  $\alpha$ -pinene have been shown to produce a significant antinociceptive effect [13].

Due to the abundance of the ethnopharmacological reports of *O. gratissimum* and *P. guajava*, careful selection of blend of the two leaves was prepared and sold as polyherbal formulation for the management of pain

associated conditions, hence, the need to validate the anti-nociceptive and anti-inflammatory activities of this formulation.

## Methods

### Experimental animals

Swiss mice of either sex weighing 18-25 g were obtained from laboratory animal farms around University of Ibadan. The animals were housed in polypropylene plastic cages and maintained under environmental conditions of light and humidity and were fed with pelletized feed (Vital Feeds Ltd., PLC, Jos, Nigeria) and received water ad libitum. The animals were acclimatized for 5 days in the Pharmacology laboratory at the University of Ibadan prior to carrying out the experiments. All experiments were carried out with strict compliance to The "Principle of Laboratory Animal Care" (NIH Publication No. 85-23) and ethical guidelines for investigation of experimental pain in conscious animals.

### Extraction of herbal mixtures

Dried leaves of *O. gratissimum* and *P. guajava* blended together at a ratio of 2:1 and known as traditional analgesic drug polyherbal (TADP). 100 g of the powdered mixture was soaked in 500 mL of distilled water for 24 h as described by Awodele et al. [14]. The extract was separated by filtration in white muslin cloth and doubly filtered through a cotton wool fixed in a funnel. The recovered extract was concentrated to dryness in an oven for 4 days at 50 °C, giving a dark brownish color with a yield of 10.17%. The extract denoted as TADP was stored in a refrigerator and daily prepared in distilled water for pharmacological assays.

### Evaluation of antinociceptive activity of TADP in hot plate test

A quick and relatively inexpensive method of testing acute thermal is in using the hot plate test, it measures reaction time to lick paw or jump when mice are placed on hot surfaces [15]. In this experiment, five groups of mice ( $n = 6$ ) each were selected. Group 1 served as control and received the vehicle (distilled water, 0.1 mL/10 g b.w.). TADP at the concentration of 100, 200 and 400 mg/kg b.w. was administered orally to groups 2, 3 and 4, respectively and group 5 received Pethidine (5 mg/kg b.w.). The mice were placed singly in the Ugo Basile hot plate (35,100, Italy) maintained at  $55 \pm 1$  °C and observed for the reaction time due to the thermal stimulation. Reaction time was recorded when the animals licked their fore-and hind paws or jumped; at before (0) and 60, 90, 120 and 150 min after administration of test drugs. The mice which reacted within 20 s were selected for the studies.

The mean percentage maximum possible effect (% MPE) was calculated as:

$$\%MPE = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Cut-off time} - \text{Post-drug latency}} \times 100$$

#### Evaluation of antinociceptive activity of TADP in acetic acid-induced nociception in mice

Writhing was induced by intraperitoneal injection 0.6% acetic acid (0.1 mL/ 10 body weight) [16]. Five groups of mice ( $n = 5$ ) were used in this study comprising the vehicle (0.1 mL/10 g distilled water), indomethacin (10 mg/kg), or TADP (100, 200 and 400 mg/kg). Vehicle, indomethacin and TADP were administered to overnight fasted mice for 1 h before acetic acid (0.6%) was administered intraperitoneally, mice were placed singly in a transparent observation box. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. The number of writhings occurring between 5 and 20 min were recorded. Percentage of inhibition of writhing was calculated and evaluated statistically.

#### Evaluation of the possible mechanism of action of TADP (400 mg/kg) on acetic acid-induced writhes in mice

To investigate the participation of the opioidergic, cholinergic, adrenergic, and nitric oxide pathway in the antinociceptive effect of the extract. Mice were pretreated with naloxone (1 mg/kg), an antagonist of opioid receptors for 15 min before they received TADP (400 mg/kg). Also, atropine (2 mg/kg), a muscarinic receptor antagonist, yohimbine (2 mg/kg), an  $\alpha_2$  adrenergic receptor antagonist, glibenclamide (10 mg/kg), a blocker of ATP-sensitive  $K^+$ -channels, and L-NAME (10 mg/kg), a non-specific inhibitor of nitric oxide synthase enzyme. The animals received acetic acid (0.6% i.p.) 1 h later, the writhing response was recorded as described above.

#### Assessment of effect of TADP on locomotory activity in *Open-field test*

To assess the possible nonspecific muscle relaxants or the sedative effects of TADP, the motor performance of the mice was evaluated on the open-field apparatus [17]. Groups of mice ( $n = 5$ ) were treated with vehicle (0.1 mL/10 g, p.o.), TADP (400 mg/kg, p.o.) 1 h before the performance of the test. The mice were placed in the center of the UgoBasile activity cage apparatus and allowed to have free ambulation for 5 min of observation of the locomotion frequency (horizontal activity and vertical activity). The numbers of horizontal or vertical beams breaks were counted automatically by the activity meter.

#### Evaluation of anti-inflammatory properties of TADP in carrageenan-induced paw oedema model

Inflammatory oedema was induced in the right hind paw of female rats according to the method described by Winter et al. [18]. The animals were divided into five groups ( $n = 5$ ); group 1 was negative control group that was orally pre-treated with vehicle (10 mL/kg distilled water), groups 2–4 were orally pre-treated with TADP (100, 200 and 400 mg/kg) and group 5 received indomethacin (10 mg/kg). One hour after oral pre-treatment, 0.1 ml of 1% carrageenan was injected into the right hind paw of each rat under the subplantar aponeurosis. Measurement of paw volumes was done before and at 1, 2, 3, and 4 h after injection of carrageenan using the UgoBasile (7134) digital plethysmometer. The increase in paw volume was calculated and plotted against time (hour). The area under the curve for the increase against time was computed from 0 to 4 h. The percentage of inhibition of total oedema formation was calculated with the formula below

$$\%inhibition = \left[ \frac{(\text{AUC of control} - \text{AUC of treatment})}{\text{AUC of control}} \right] \times 100\%$$

#### Collection and homogenization of inflamed paws

The animals were euthanised by deep ether anaesthesia, paw were removed just below the ankle joints, cleaned in sodium phosphate buffer and weighed. The wet paws were weighed and recorded. The paws were frozen at  $-20^\circ\text{C}$ . The frozen paws were homogenized in cold phosphate buffer by grinding with mortar and pestle placed in an ice bucket. The homogenized paws were centrifuged at 10,000 rpm for 10 min at  $4^\circ\text{C}$  in refrigerated centrifuge. Aliquot of the supernatant was used to assay for levels of nitrite, reduced glutathione, malondialdehyde (MDA) and myeloperoxidase enzyme activities in carrageenan-injected paws.

#### Biochemical analysis

Aliquots of supernatant were used to measure the production of nitric oxide in the carrageenan-injected paws. Nitrite level was measured using Griess reagent assay [19]. Myeloperoxidase enzyme activity as index of neutrophil activation in paw was determined according to the method of Bradley et al. [20]. MDA levels as a marker of lipid peroxidation was determined using the thiobarbituric acid reactive substance (TBARS) assay [21]. The method as described by Sin et al. [22], was adopted in the determination of reduced glutathione concentration in carrageenan-injected paw.

### Evaluation of the effect of TADP on the histopathological changes in carrageenan-induced rat paw

Carrageenan-injected paws were fixed in 10% buffered formalin. For histological evaluation, the paw samples were embedded in paraffin and sectioned; 4- $\mu$ m-thick sections were deparaffinized, stained with hematoxylin and eosin (H & E), and then examined under a light microscope by an experienced pathologist without knowledge of the treatments.

### Statistical analysis of data

Data obtained from animal studies were expressed as mean  $\pm$  standard error of mean (SEM). Data were analysed for significant main effect using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc multiple comparison test. Values were considered statistically significant when  $p < 0.05$ .

## Results

### Effect of TADP in hot plate test

Oral treatment of TADP at 100 mg/mL did not significantly change the latency time in the hot plate test at 55 °C compared with the vehicle-treated animals whereas TADP 200 and 400 mg/kg and Pethidine (5 mg/kg) significantly ( $p < 0.05$ ) increased the maximal possible effect (Fig. 1a). The area under the curve for prolonged latency at 60–150 min is as shown in Fig. 1b below. TADP (200 and 400 mg/kg) significantly prolonged latency by 68.6% and 69.3%, respectively. Pethidine (5 mg/kg) significantly prolonged latency by 77.7%.

### Effect of TADP in acetic-acid induced writhing test

As shown in Table 1 below, oral administration of TADP (100–400 mg/kg) produced a dose-dependent

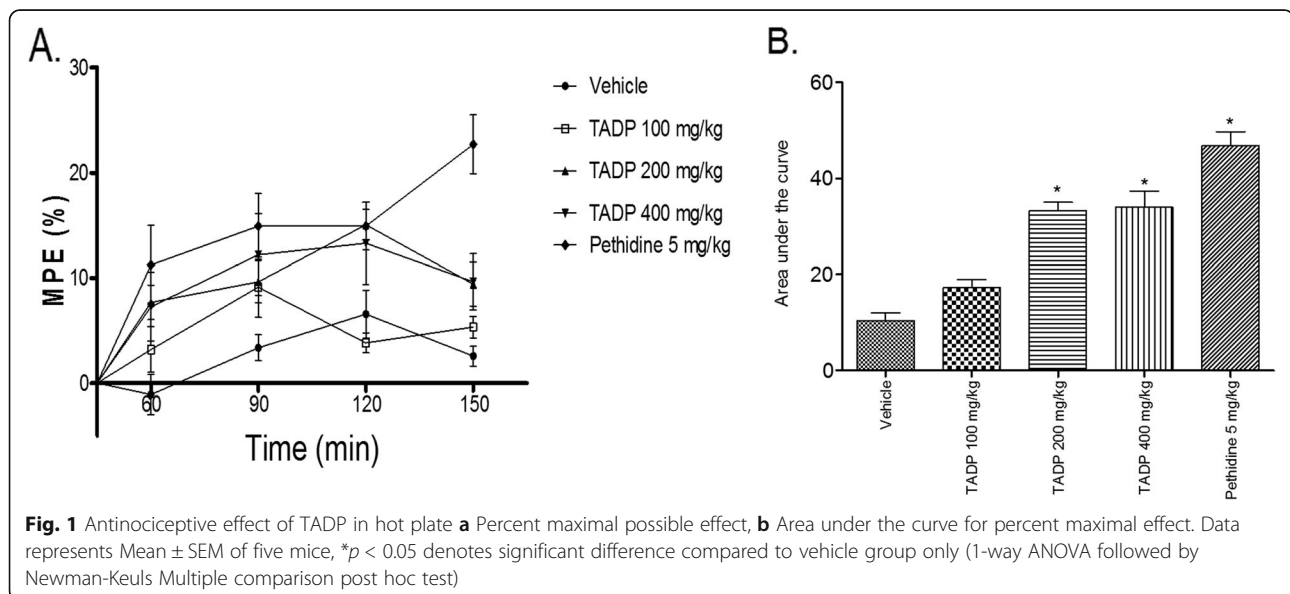
and significant ( $p < 0.05$ ) inhibition of acetic-acid-induced abdominal constrictions in mice. Maximum inhibition of 54.1% was observed at TADP 400 mg/kg while indomethacin treatment produced 84.6% inhibition of writhes.

### Effect of TADP on locomotory performance in mice

Treatment with TADP 400 mg/kg caused an insignificant ( $p > 0.05$ ) increase in horizontal beam breaks representing spontaneous locomotory activity but significantly reduced the rearing activity. Pethidine did not cause a significant reduction in the number of vertical beam breaks representing rearing (Table 2).

### Possible mechanism of antinociceptive activity of TADP in acetic acid-induced writhing

As shown in Fig. 2 below, the antinociceptive activity of TADP was not reversed by naloxone (1 mg/kg). This shows that TADP is not acting via the opioid receptors. Atropine (2 mg/kg), a muscarinic receptor antagonist did not reverse the antinociceptive activity of TADP (400 mg/kg), howbeit insignificantly ( $p > 0.05$ ) enhanced the activity in the acetic acid-induced writhing test. Yohimbine (2 mg/kg), an  $\alpha_2$ -adrenoceptor antagonist significantly reversed the anti-nociceptive activity of TADP (400 mg/kg) in the acetic acid-induced writhing test (Fig. 2). Glibenclamide (10 mg/kg), given 15 min prior to TADP (400 mg/kg) significantly ( $p < 0.05$ ) reversed the antinociceptive activity of TADP when assessed in the acetic acid-induced writhing test. L-NAME (10 mg/kg), an inhibitor of nitric oxide synthase did not modify the antinociceptive activity of TADP (400 mg/kg) in the acetic acid-induced writhing test.



**Table 1** Effect of TADP on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	N <sup>o</sup> of writhings	Inhibition of writhing (%)
Vehicle	10 mL/kg	53.2 ± 2.3	–
TADP	100	42.2 ± 3.6	20.7*
TADP	200	40.0 ± 3.8	24.8*
TADP	400	24.4 ± 4.4	54.1*
Indomethacin	10	8.2 ± 0.7	84.6*

Data represents Mean ± SEM of five mice

\*denotes significant value at  $p < 0.05$ **Effect of TADP on carrageenan-induced rat paw oedema**

Induction of acute inflammation in vehicle (distilled water) rats resulted in a prominent increase in paw volume beginning at 1 h post carrageenan injection and reached a peak at 4 h (Fig. 3a). The degree of swelling of the carrageenan-injected paw was significantly ( $p < 0.05$ ) reduced in animals that were pretreated with TADP (100, 200 and 400 mg/kg) and indomethacin (10 mg/kg). As shown in Fig. 3b, pretreatment with TADP extract (100, 200 and 400 mg/kg) significantly decrease ( $p < 0.05$ ) the paw inflammatory response measured as area under the curve (AUC) by 35.1%, 52.7% and 57.9%, respectively. Indomethacin (10 mg/kg) significantly reduced the total inflammatory response by 75.7% (Fig. 3b).

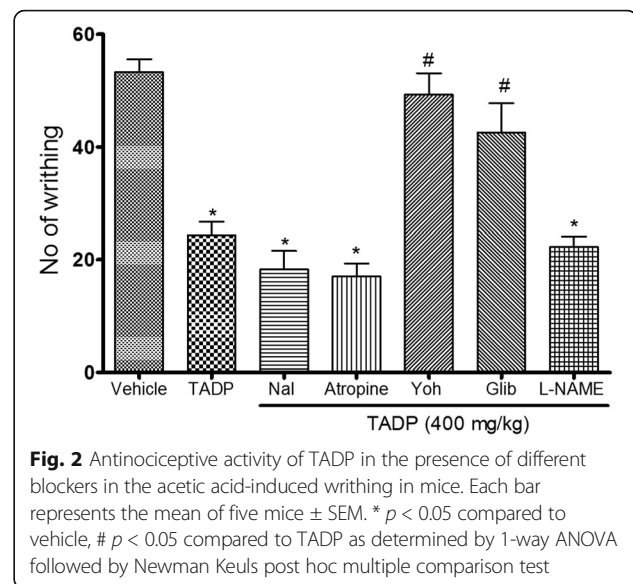
**Effect of TADP on nitrite level in carrageenan-induced rat paw**

As shown in Fig. 4a, injection of carrageenan into the rat hind paw induced a marked increase in the nitrite level 4 h after injection. The treatment of rats with TADP (100, 200 and 400 mg/kg) and indomethacin (10 mg/kg) caused a significant decreased of the carrageenan-induced increased NO in the paw tissue. Four hours after injection of carrageenan in rat paws, there was an increase in MPO activity as a marker of neutrophil infiltration in paw tissue of vehicle treated rats (Fig. 4b). This effect was significant ( $p < 0.05$ ) at TADP (100, 200 and 400 mg/kg) by 49.7%, 42.8% and 31.1%, respectively. Indomethacin also showed a significant ( $p < 0.05$ ) reduction of paw MPO activity.

**Table 2** Effect of TADP on Locomotory activity in mice in the open field test

Treatment	Dose	Spontaneous locomotor activity (horizontal beam breaks/5 min)	Rearing (vertical beam breaks/ 5 min)
Vehicle	10 mL/kg	398.3 ± 16.3	21.2 ± 1.7
TADP	400 mg/kg	413.0 ± 10.4	8.00 ± 4.0*
Pethidine	5 mg/kg	618.5 ± 27.4*	16.0 ± 2.3

Data represents Mean ± SEM of five mice

\*denotes significant value at  $p < 0.05$ **Fig. 2** Antinociceptive activity of TADP in the presence of different blockers in the acetic acid-induced writhing in mice. Each bar represents the mean of five mice ± SEM. \*  $p < 0.05$  compared to vehicle, #  $p < 0.05$  compared to TADP as determined by 1-way ANOVA followed by Newman Keuls post hoc multiple comparison test**Effect of TADP on carrageenan-induced oxidative stress in rat paw**

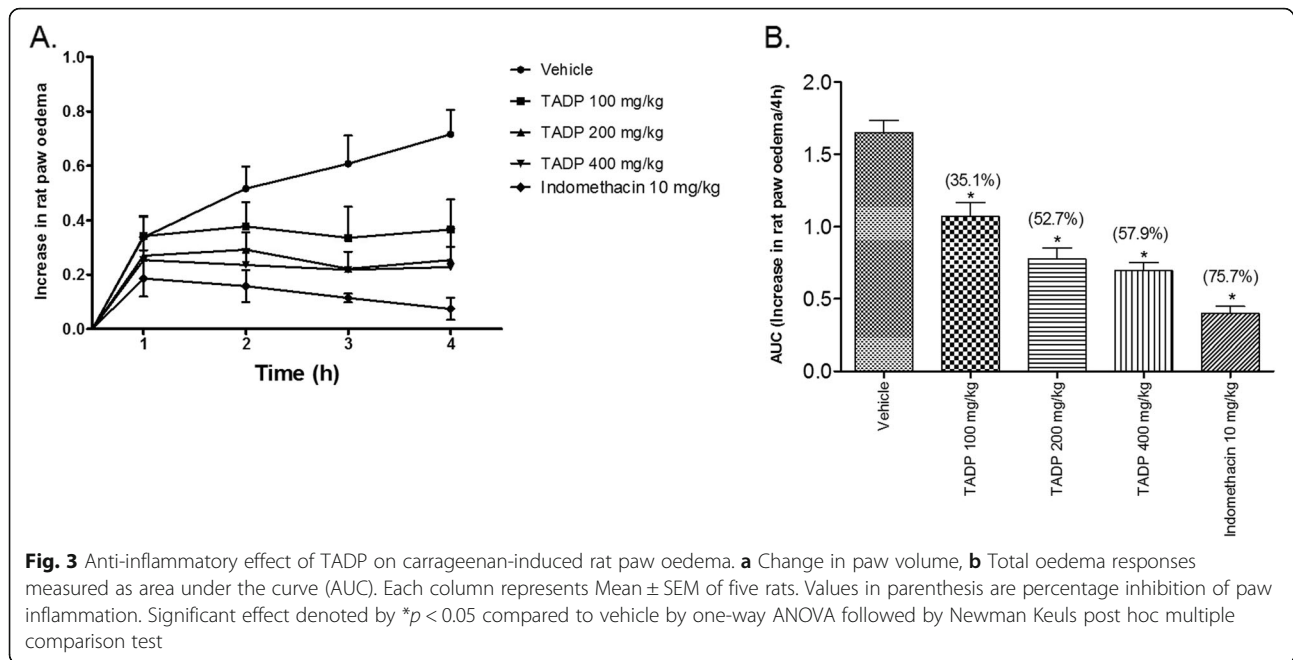
The protective role of TADP on carrageenan-induced oxidative stress in rat paw was assessed by measuring lipid peroxidation and reduced glutathione level. As shown in Table 3, TADP (400 mg/kg) and indomethacin (10 mg/kg) significantly ( $p < 0.05$ ) reduced the malondialdehyde level when compared to vehicle treated animals. Carrageenan-induced a depletion of GSH levels in control rats (Table 3), which was prevented by TADP (400 mg/kg) and indomethacin (10 mg/kg).

**Effect of TADP on Histopathological examination of carrageenan-induced paw tissue oedema**

As shown in Fig. 5, paw tissue of vehicle-treated rats showed an acute inflammation with extensive extravasations, there are widespread dense amounts of mononuclear inflammatory cells just below the epidermis and extending throughout the dermis to the hypodermis. Blood vessels are markedly congested (Fig. 5). As observed in Fig. 5, treatment of rats with TADP at 100, 200, 400 mg/kg and indomethacin (10 mg/kg) exhibited a significant decrease in the number of cellular infiltrates.

**Discussion**

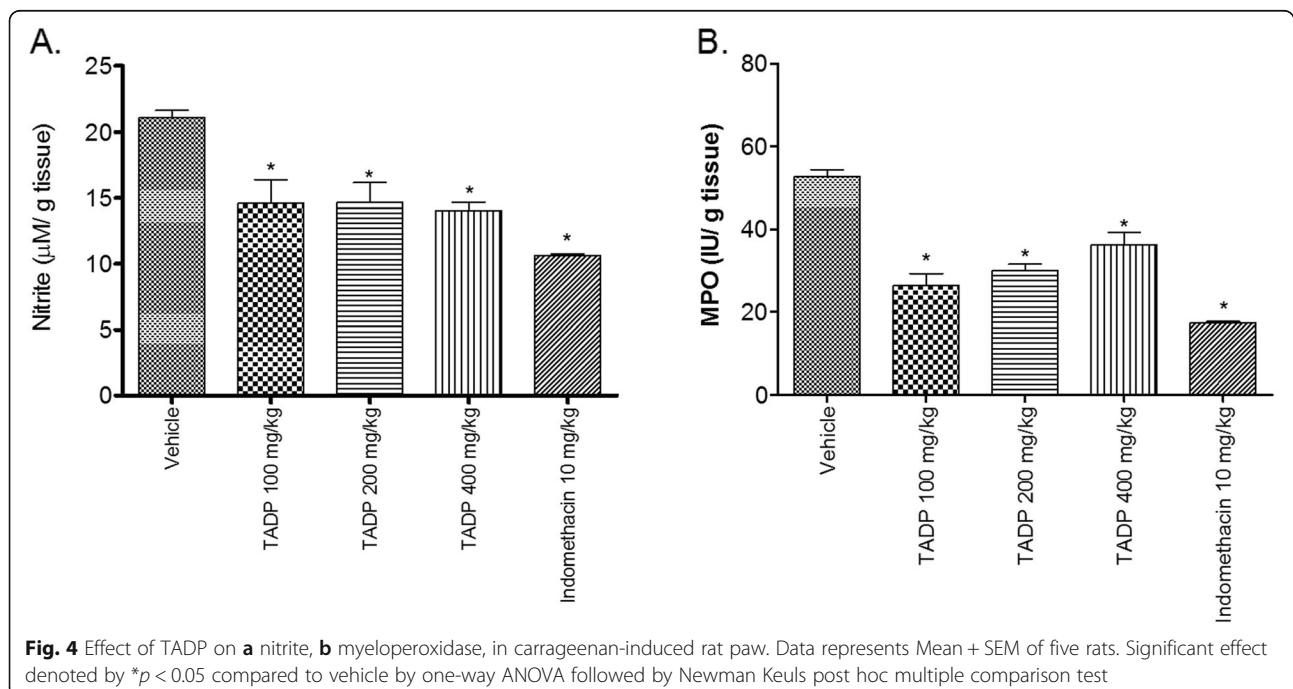
This study was carried out to establish the antinociceptive and anti-inflammatory effects produced by the oral administration of an aqueous extract of polyherbal formulation of blended leaves of *Ocimum gratissimum* and *Psidium guajava* in rodents. The results show that TADP at higher doses showed pronounced anti-noceptive activity as it was able to significantly prolong the latency time in the hot-plate test and also produce a significant inhibition



in the acetic-acid induced abdominal constriction. TADP at a dose of 400 mg/kg increase the horizontal beam break and a reduction in the rearing behaviour. TADP significantly reduced the swelling in the carageenan-induced oedema model and produced a reduction in the nitrite and myeloperoxidase level. TADP at a dose of 400 mg/kg significantly reduced malondialdehyde concentration and increased glutathione level in the carageenan-induced increased oxidative stress level. TADP and indomethacin

significantly decrease the number of cellular infiltrates in the histopathological assessment.

Thermally –induced nociception in the hot plate is considered to be selective for centrally acting analgesic compounds (morphine-like drugs). TADP showed positive activity in this test. On the other hand, the acetic acid-induced abdominal-writhing test is considered as a conventional animal model of pain and one of the most sensitive methods used for the screening of substances



**Table 3** Antioxidant effect of TADP in carrageenan-induced rats paw

Treatment	Dose (mg/kg)	TBARs (nM MDA/g tissue)	GSH ( $\mu$ M/ g tissue)
Vehicle	10 mL/kg	2.84 $\pm$ 0.13	2.40 $\pm$ 0.25
TADP	100	2.50 $\pm$ 0.19	2.96 $\pm$ 0.26
TADP	200	2.48 $\pm$ 0.11	2.57 $\pm$ 0.24
TADP	400	2.18 $\pm$ 0.13*	3.04 $\pm$ 0.21*
Indomethacin	10	1.89 $\pm$ 0.08*	3.24 $\pm$ 0.23*

Data represents Mean  $\pm$  SEM of five mice

\*denotes significant value at  $p < 0.05$

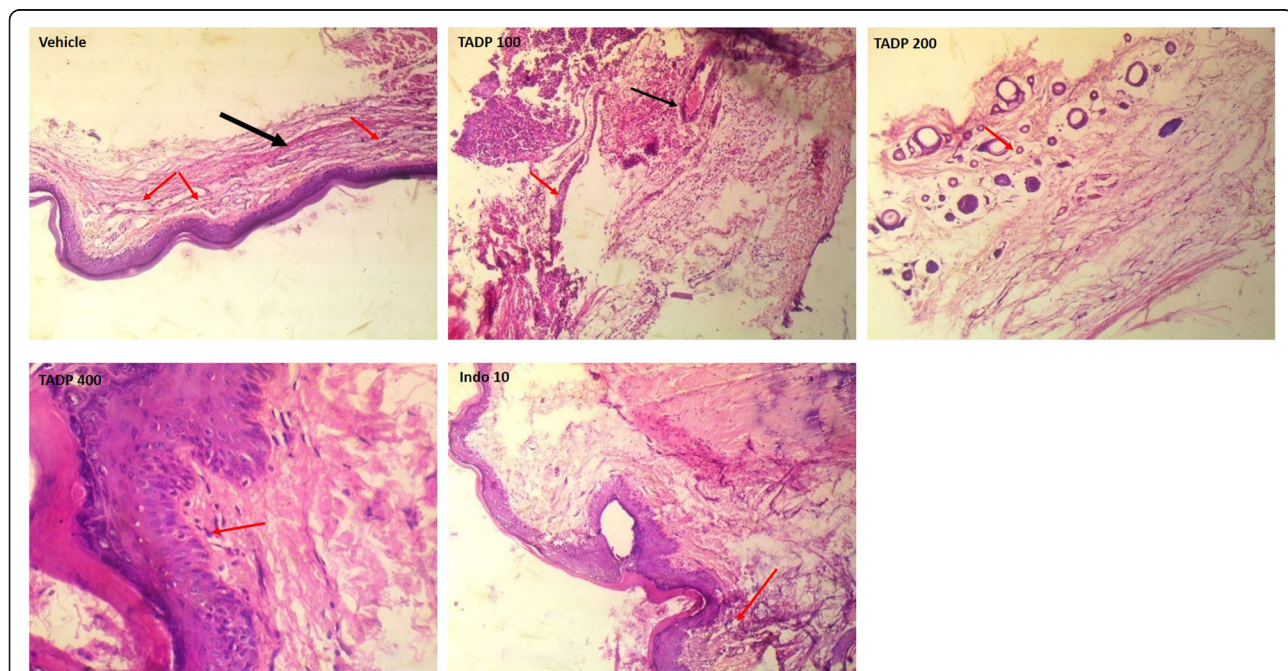
that have both analgesic origin and/or anti-inflammatory effects [23]. Injection of acetic acid into the peritoneal cavity induces nociception by directly activating non-selective cationic channels located in the primary afferent pathways or indirectly by promoting the release of various endogenous algescic mediators such as prostaglandins, cytokines, bradykinin and others, as well as increasing lipoxygenase (LOX) and cyclooxygenase (COX) production in peripheral tissues [24]. The release of these endogenous substances with subsequent stimulation and sensitization of the peripheral primary afferent C-fibers neurons in the animal peritoneum produced a viscerosomatic reflex leading to abdominal constrictions [24].

The findings of the present study indicate that the oral pre-treatment of mice with aqueous extract of polyherbal formulation of the leaves of *O. gratissimum* and *P. guajava* produced a significant dose-dependent reduction in

the number of abdominal writhing induced by acetic acid as compared to the control group. This finding suggests that the antinociceptive action of TADP in the acetic acid-induced abdominal-writhing test could be the result of inhibited release of endogenous algescic mediators or direct inhibitory activity at nerve endings of the primary afferent neurons and/or inhibition of the transmission pathway entering the dorsal horn.

The experimental models of nociception in mice are very good and sensitive models but however they are non-specific because, in the writhing test for instance, the response can be suppressed by muscle relaxants and other types of drugs, which could lead to misinterpretation of the results. In order to rule out the chances of false positive effect of TADP, we evaluated its effect on spontaneous locomotor function in the activity meter cage. TADP did not alter the exploratory activity but significantly reduced rearing activities. The results revealed that the observed anti-nociceptive effect of TADP was not as a result of sedation but slight impairment of rearing activity in mice.

The acetic acid-induced test was used in the investigation of the possible mechanism of antinociceptive activity of aqueous extract of polyherbal formulation of *O. gratissimum* and *P. guajava* leaves. Acetic acid-induced writhing represent a predictive model because it provokes a stereotyped behavior in mice. Transmission of visceral nociceptive pathway is related to the spinal cord, paravertebral nucleus and certain hypothalamic nuclei is



**Fig. 5** Photomicrograph of histopathologic examination of paw tissue of rats treated with TADP, 5 h after injection of carrageenan (Carr). Sections were stained with H and E,  $\times 400$ . Red color arrow indicates polymorphonuclear cell infiltration, black arrow indicates vascular congestion

well defined in this model [25]. Thus, it is possible to investigate the possible anti-nociceptive activity of TADP in this model.

The results showed that pretreatment with naloxone, atropine and L-NAME did not significantly reversed the antinociceptive activity of TADP in the acetic acid-induced writhing test. This suggests that TADP does not activate the opioid receptors or increases the endogenous opioid system. Also, it seems that there was no interaction with the cholinergic muscarinic receptor system or the nitric oxide synthase enzymes. Interestingly, the antinociceptive activity of TADP was significantly reversed by yohimbine, an  $\alpha_2$ -adrenoceptor antagonist. An interaction between dopaminergic, adrenergic and opioid systems has been suggested in the modulation of pain perception [26]. The reversal of the antinociceptive effect of the extract by yohimbine further confirms the interaction between the dopaminergic, adrenergic and opioid system because the systems play a vital role in regulating pain perception. The dopaminergic system has been reported as a potential target for the treatment of chronic pain because dopaminergic agonists were recently shown to promote recovery of locomotor function following an injury to the spinal cord [27]. However, the adrenergic and the dopaminergic system has shown the effects of the nociceptive responses through the modulation of presynaptic inhibition in mouse [28]. Antinociceptive effect of some flavonoids such as quercetin has been shown to involve the participation of the alpha-2 adrenergic systems in the modulation of pain [29]. Thus, analgesics drugs act through the descending circuits [30]. Furthermore, our result showed that the antinociceptive effect of TADP is related to the ATP-sensitive  $K^+$ -channels. Pretreatment with glibenclamide, a blocker of the ATP-sensitive  $K^+$ -channels decreased the antinociceptive effect of TADP.

Inflammatory reactions cause high filtration of neutrophils into the tissue and thereby increase the level of myeloperoxidase. The activity of Myeloperoxidase has been used as a good model to estimate neutrophil content in inflamed tissues and therefore it has been widely used as a biomarker of inflammation [20, 31]. MPO secretion by stimulated neutrophils acts as a host defense mechanism by mediating microbial killing. MPO is secreted in large quantity during inflammation because it is highly effective during inflammatory processes and also for its use as an indicator of polymorphonuclear leukocytes presence in tissues, MPO has been widely used as an inflammatory marker of both acute and chronic conditions [31, 32]. Moreover, MPO changes have also been associated to the severity of many pain related diseases [33–35]. Injection of Carrageenan increases the myeloperoxidase content in the paw which was reduced by the blended extract of *O. gratissimum* and *P. guajava*.

Increased oxidative stress has been reported to be associated with increased pain. Reactive oxygen species (ROS) is known to play an important role in the pathogenesis of so many disease states resulting in pains [36] that is characterized by altered metabolic conditions of cells, increased production of ROS, thus increased oxidative stress acts as a mediator of pain stimuli. Although, the body possesses antioxidant defences and repair mechanisms against free radical oxidative stress, increase level of free radicals in the body increases its oxidative stress level thereby hindering the defence mechanism [37]. Thus, the use of plants with antioxidant property could serve as a remedy to pain. The results show that TADP at a higher dose reduces the level of malondialdehyde which was increased by the administration of carrageenan and as prevent depletion of reduced glutathione.

Carrageenan-induced inflammation depends on paw tissue nitrite production [38]. Recruitment of inflammatory cells to the site are possible source of NO during inflammation, therefore a potential target of TADP effect. Reduction in inflammatory cells recruitment was observed from the histopathological examination of the paw tissue from the H&E staining. This reduction might account for the reduced level of nitrite determined in the paw tissues.

## Conclusion

In conclusion, this study provides evidence that aqueous extract of polyherbal blend of leaves of *Ocimum gratissimum* and *Psidium guajava* possesses good antinociceptive and anti-inflammatory activities in rodents. It further suggests that the antinociceptive mechanisms might partly involve interaction with adrenergic pathways, while anti-inflammatory effects might be due to inhibition of oxidative stress.

## Abbreviations

AUC: Area under the Curve; GSH: Reduced glutathione; H&E: Hematoxylin and Eosin; MPO: Myeloperoxidase; TADP: Traditional Analgesic Drug Polyherbal; TBARS: Thiobarbituric Acid Reacting Substances

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## Authors' contributions

AAO, AMA and SU conceived and designed the experiments. AAO and AMA performed the experiments; AMA analysed the data; AAO, AMA and OO wrote the paper. All authors read and approved the final manuscript.

## Funding

Not applicable

## Availability of data and materials

The datasets supporting the conclusions in the article are included within the article.

## Ethics approval and consent to participate

Authors declared that all experiments were carried out with strict compliance to The "Principle of Laboratory Animal Care" (NIH Publication No.



85–23) and ethical guidelines for investigation of experimental pain in conscious animals.

#### Consent for publication

Not applicable

#### Competing interests

The authors declare that they have no competing interests.

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