ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2025.14

The Nigerian Journal of PHARMACY
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THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

In vivo assessment of analgesic, antipyretic and anti-inflammatory effects of combined aqueous crude leaf extract of *Senna occidentalis* Linn. and *Vernonia amygdalina* Delile in rodents

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ARTICLE INFO

Article history:

Received 31st October 2024
Revised 26th March 2025
Accepted 12th April 2025
Online Published

Keywords:

Senna occidentalis,

Vernonia amygdalina,

dual therapy,

inflammation,

pyrexia

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ABSTRACT

Background: Pain is a significant global health burden. The side effects of the medications used currently to treat pain are quite serious—combination therapy guards against side effects and the development of resistance. Nigerians utilise *Senna occidentalis* and *Vernonia amygdalina* as traditional medicines for various illnesses. It has been noted that each plant has the potential to be analgesic, antipyretic, and anti-inflammatory. Thus this study evaluates the analgesic, antipyretic, and anti-inflammatory impact of the combined aqueous leaf extract of these plants.
Methods: The formalin-induced paw-licking test, the acetic acid-induced writhing test in mice, and the tail flick model test in rats were used to evaluate analgesic effectiveness. Rats' paw oedema caused by carrageenan was used to investigate the anti-inflammatory effect, while rabbits' pyrexia caused by *Escherichia coli* endotoxin was used to assess the antipyretic action.
Results: The combined effects of the extract exhibited a significant ($p<0.01$) inhibition of acetic acid-induced writhing. It also showed a significant ($p<0.05$) reduction in time spent licking the paw in the 0 – 5 min, although the greatest significance ($p<0.01$) was seen with the 400 mg/kg dose. However, in the 15 – 30 min, there was a reduction in time spent licking the paw and this was completely abolished by 200 mg/kg. In the tail-flick test, the combined effects of the extract showed a significant ($p<0.001$) increase in tolerance time at the 2nd, 3rd, and 4th hour. The combined aqueous leaf extracts (200 mg/kg) produced significant ($p<0.05$) anti-inflammatory effects at the 30th min in the carrageenan model. In *Escherichia coli* endotoxin-induced pyrexia test in rabbits, the combined effect of the extract (400 mg/kg, oral) exhibited a significant ($p<0.05$) reduction in the rectal temperature at the 60th min.
Conclusion: This study showed that *S. occidentalis* and *V. amygdalina* combined aqueous leaf extract possesses significant analgesic and antipyretic activities. However, there is no significant anti-inflammatory activity. Further studies are warranted to elucidate the mechanism by which the combined extract exerts these effects.

1. Introduction

In many disorders, pain is an uncomfortable emotional and sensory experience that is typically brought on by strong or harmful stimuli. Globally, pain affects billions of people daily[1]. Given its prevalence, and its associated morbidity and mortality, it is regarded as a major global health burden and priority[2]. According to Nahin *et al.*[3], there are 52.4 cases of chronic pain (pain occurring "most days" or "every day") per 1000 persons per year, which is a significant incidence when compared to other chronic diseases and conditions like diabetes that have known incidence in the adult population in the United States[4], depression[5], and hypertension[6]. Inflammation is part of the complex biological response of vascular tissues to harmful stimuli,

such as pathogens, damaged cells or irritants[7]. A complicated physiologic reaction of vascular tissues to damaging stimuli includes inflammation, infections, damaged cells, or irritants, among other things[7]. The traditional symptoms of acute inflammation include swelling, redness, heat, discomfort, and loss of function. Acute inflammation is defined by Cortran & Robbins[8] as a temporary process that often appears within a few minutes or hours and disappears as the inciting stimulus is removed. According to Chandrasoma & Taylor[9], five cardinal signs define it. The classical signs of acute inflammation are pain, heat, redness, swelling and loss of function. Cortran & Robbins[8] described acute inflammation as a transient process that often manifests itself within a few minutes or hours and goes away as the triggering stimulus is eliminated. It is characterised by five cardinal signs[9].

Dual therapy with a non-opioid/opioid combination offers a protective activity for adverse effects over opioids alone[10]. A therapeutic approach that combines two or more medicinal substances is called combination therapy. This enhances efficacy in contrast to the monotherapy strategy because it addresses many pathways in a way that is typically additive or synergistic. In addition to offering the intended therapeutic benefits, this strategy could mitigate drug resistance[11]. Combining two or more medications with distinct modes of action is intended to create a synergistic interaction that will lessen the severity and frequency of side effects while delivering an acceptable analgesic effect at lower dosages[12]. The widespread application of combination medication in the treatment of pain, pyrexia, and inflammation highlights the need for combination-specific research and highlights the existing limits of the many pharmacotherapies that are now accessible[13]. *Vernonia amygdalina* has been used for the treatment of malaria fever. It has been reported to have antioxidant, anti-inflammatory, anticancer[14] and analgesic[15] properties. *Vernonia amygdalina* caused a marked reduction in triglyceride [16]. It possesses cardioprotective effect [17] and has also been used for the treatment of snake bite[18]. Oyeyemi *et al.*[19] reported that the leaf of this plant is used to treat diabetes, constipation, high blood pressure.

Senna occidentalis is one of the most widely used herbal plants in the tropical and subtropical regions of the world[20]. *S. occidentalis* has been used as natural medicine in the rainforest and other tropical areas for centuries. It is used in the treatment of diabetes mellitus and diabetic nephropathy[21]. It possesses hypolipidemic[22] and antipyretic[23] activities. Its antimicrobial[24], antioxidant and hepatoprotective[25], anti-inflammatory[26], antimalarial[27], antianxiety and antidepressant[28], antiplasmodial[29], anticonvulsant and analgesic[31] properties have been reported.

Although there have been studies on the analgesic, anti-pyretic and anti-inflammatory effects of *Senna occidentalis and Vernonia amygdalina* separately, there is no known report on the combined effect of the two on analgesia, pyrexia and inflammation. Combination therapy is advised to reduce the incidence of resistance and adverse effects. Therefore, this study was undertaken to investigate the combined effect of aqueous leaf extract of *Senna occidentalis and Vernonia amygdalinaon* analgesia, pyrexia and inflammation.

Materials and Methods

Drugs and chemicals

Aspirin (Acetyl Salicylic Acid, Halewood laboratories), 3% tween 80 (Halewood laboratories), Gum Acacia (Halewood laboratories), Pentazocine (Laborate pharmaceuticals India), Formalin (Halewood laboratories), acetic Acid (Laborate pharmaceuticals, India), Indomethacin (Biochemika/Fluka, Italy), Carrageenan (Sigma, USA), Aspirin (Acetyl Salicylic Acid, Halewood laboratories), *Escherichia Colim* (Pharmaceutical microbiology laboratory, University of Benin, Benin-City).

Plant materials and extraction

Senna occidentalis: The fresh leaves of *Senna occidentalis* were collected in Okada, Ovia North East, Edo State at about 7:15 am. The leaves were authenticated by the Forest Research Institute of Nigeria, Ibadan, where the herbarium sample with voucher number FHI 109765 has been deposited. The leaves were then transferred to the Pharmacology and Toxicology Laboratory, University of Benin, Benin-City where they were washed with distilled water and air dried for about 45 min. The leaves were then

plucked and 1310 g was weighed, chopped and soaked with 2.62 L of distilled water. The blended leaves were kept in a conical flask made in U.K.

After that, it was then heated at 40^{0}C for 30 min using an electro thermal scientific heater (U.K). The essence of heating was to hasten the extraction. After heating the extract for 30 min, it was then left in the conical flask to cool for about 15 min after which it was then filtered in a glass conical flask using a glass funnel and a muslin cloth, thereafter it was further passed through Whatman filter paper. It was then poured into plastic containers and kept in the fridge overnight so as to avoid spoilage or degradation by microorganisms. The next day, the extracts of *Senna occidentalis* were then concentrated using a rotary evaporator, after which the extract was then dried in an oven at a temperature of 40 ^{0}C for 3 days so as to dry-up the remaining moisture and to make it hard for weighing. The dried extract was stored in a bottle and kept in a refrigerator at a temperature of 4 ^{0}C until when needed. The percentage yield gave us 90.7 %w/w.

Vernonia amygdalina: The fresh leaves of *Vernonia amygdalina* were collected in Urora, Benin City, Edo State at about 7:00 am. The identification and authentication of the plant were carried out by Mr. Sunday Nweke (of the blessed memory) from the Department of Pharmacognosy, University of Benin, Benin-City. The leaves were then transferred to the Pharmacology and Toxicology Laboratory, University of Benin, Benin-City where they were washed with distilled water and air dried for about 45 min. The leaves (3000 g) were weighed, chopped and then blended using a 3 in 1 Eurosonic blender (Made in China) with 6 L of distilled waters. The blended leaves were then filtered using muslin cloth, thereafter it was further passed through Whatman filter paper. The filtrate was then concentrated using rotary evaporator, and after that, it was dried for 2 days in an oven at a temperature of 40 ^{0}C to remove the remaining moisture and to make the extract hard for weighing. The dried extract was packed in a bottle and stored in a refrigerator at a temperature of 4 ^{0}C until when needed. The percentage yield was 93 $\%^{w}/_{w}$.

Experimental animals

The experiments described in this study were performed using albino mice weighing between 25 – 35 g, albino rats weighing between 100-160 g and 165-230 g, and albino rabbits weighing between 0.45 – 1.6 kg. The Department of Pharmacology and Toxicology at the University of Benin provided the animals, which were of both sexes. They were housed in plastic cages at the University of Benin's Department of Pharmacology and Toxicology's laboratory animal house in Benin-City, Nigeria. The animals were housed in a standard controlled setting before the experiment, with unrestricted access to food (Super-deluxe Animal feeds by Premier Feed Mills Co. Ltd., Nigeria, Top feeds® Grower Mash) and clean water. Before the experiment began, they had two weeks to become acclimatized to the environment. Principles of laboratory animal care" (NIH publication No. 85- 23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

Test for analgesic activity

Acetic acid-induced writhing in mice

This was based on a modification of the Koster *et al.*[32] approach. Five sets of five Swiss albino mice of both sexes were randomly selected. The standard or reference group was given 100 mg/kg of acetylsalicylic acid (ASA), while the test groups were given 100, 200, and 400 mg/kg of the combined extracts (1:1 ratio) orally. The extract doses were selected following the doses of the individual plant extracts which have been used previously[33]. The control group was given 2 mL/kg of distilled water. They were all delivered orally. The mice received an interperitoneal injection of acetic acid (0.6%) at a dose of 10 mL/kg one hour later. After each mouse had 30 min of acetic acid treatment, the number of writhes was recorded.

Formalin-induced pain test in mice

This was based on an adaptation of the procedure that Rasika & Bhalke [34] outlined. Five groups comprising five animals each were randomly selected from among Swiss albino mice of both sexes. The test groups were treated orally with 100, 200, and 400 mg/kg of the combined extracts while the standard or reference group received 10 mg/kg of pentazocine injected intra-peritoneally. The control group received distilled water (10 mL/kg) orally.

One hour later for others, and 30 min later for pentazocine administration, the animals were injected with 0.02 mL of 1% formalin at the right hind paw subcutaneously. Immediately, the time spent in licking the right hind paw for the first 5 min and the last 15 min of 30 min was noted for each animal in the five groups.

Tail flick test in rats

The method provided by Akindele & Adeyemi[35] was modified to serve as the basis for this experiment. Rats of both sexes from each of the five groups (n=5) were randomly selected. The standard or reference group received intraperitoneal injections of pentazocine at a dose of 10 mg/kg, while the test groups were administered 100, 200 and 400 mg/kg of a combined extract. Oral distilled water at a dose of 10 mL/kg is given to control group. Analgesiometer (Ugo Basile, Italy) was used to measure the reaction times of the rats in each of the five groups after their tails were placed on it. The times were recorded at 0, 1, 2, 3, and 4 hours. A constant 55 to 60 degrees Celsius were maintained. Before the animals were treated, the response time was also recorded.

Test for anti-pyretic activity

***Escherichia coli* endotoxin-induced pyrexia in rabbits**

This was based on the modification of the method described by Owolabi & Omogbai[36]. Fifteen albino rabbits were randomly divided into five groups (n=3) and used for this experiment. The original anal temperature of the animals was checked and noted before induction of pyrexia and treatment of the animals. *Escherichia coli* endotoxin (10^6 Colony forming unit/ml) was then administered at a dose of 1 mL/5kg to all the animals in the groups through intravenous route via the marginal ear vein. This was done to induce pyrexia. The anal temperature of the animals was checked and noted 15 min later. Following an hour of pyrexia induction, the test groups were given oral treatments of 100, 200, and 400 mg of the combined extracts, whilst the standard group was given 100 mg/kg of acetylsalicylic acid, and the control group was given 10 mL/kg of distilled water. The anal temperature of the animals was checked and recorded respectively at 30, 45, 60, 75 and 90 min. The temperature was measured in degrees Celsius.

Test for anti-inflammatory activity

Carrageenan-induced Paw Oedema

Swiss albino rats of both sexes were randomly divided into five groups (n=5) to determine the extent of paw oedema caused by carrageenan. The standard or reference group received indomethacin (10 mg/kg) subcutaneously at the right hind paw, while the test groups were administered 100, 200 and 400 mg/kg of both extracts orally. The oral dose for the control group was 10 mL/kg of distilled water. An hour later, the control, standard and test groups (100, 200 and 400 mg/kg groups) received subcutaneous injections of 1% carrageenan at the right hind paw[37]. The paw thickness was measured using a Venier caliper [38] at 30 min 1, 2, 3, 4, and 24 hours, 7 and 14 days, following administration of carrageenan.

Statistical analysis

The data were presented as mean SEM. Students' t test with graph pad prism version 2.05 a was used to compare the treatment groups with the control group; n is the number of animals utilised. Findings were considered significant when p was less than 0.05.

Results

Analgesic activity

Effect on acetic acid-induced writhing in mice

The result of acetic acid-induced mouse writhing is presented in Table 1. Treatment with the combined aqueous leaf extracts of *Senna Occidentalis* and *Vernonia amygdalina* significantly reduced the number of writhes ($p<0.01$) at all doses tested (100, 200 and 400 mg/kg) when compared with the control group. However, the standard group produced the lowest number of writhes when compared to the other treatment groups. It was shown that the result was not dose-dependent.

Table 1: The effects of oral administration of combined aqueous leaf extracts (100, 200 and 400 mg/kg) of *Senna occidentalis* and *Vernonia amygdalina* on acetic acid-induced mouse writhing in mice

Treatment (mg/kg)	No of writhes
Control (2 mL/kg)	62.0 ± 2.88
Aspirin (100)	11.0 ± 6.15[a]
SO & VA (100)	20.5 ± 8.11[b]
SO & VA (200)	26.8 ± 5.0[b]
SO & VA (400)	19.25 ± 5.5[b]

Values are mean ± SEM (n=5), [a]p<0.001, [b]p<0.01, significantly lower than the control, Control= Distilled water, Standard = Aspirin, SO = *Senna occidentalis*, VA = *Vernonia amygdalina*.

Effect on formalin-induced pain test

Table 2 shows the combined impact of *Senna occidentalis* and *Vernonia amygdalina* on the formalin-induced pain test in mice. In this case, paw licking time was shown to be dose-dependently shorter in the 0–5 min (neurogenic pain) group as compared to the control. In the 0–5 min (neurogenic pain), the combined impact of both extracts was most significant ($p<0.01$) at the 400 mg/kg dose. However, in the 15 – 30 min (peripheral pain), the combined effect of the extracts was still dose-dependent as it completely abolished paw-licking at the 200 mg/kg dose ($p<0.0001$). The combined effect of the extracts compares well with pentazocine which is the standard drug where significant reduction was also noticed ($p< 0.0001$).

Table 2: The effects of oral administration of combine aqueous leaf extracts (100, 200 and 400 mg/kg) of *Senna occidentalis* and *Vernonia amygdalina* on formalin-induced pain test in mice

	Time Spent Licking the Paw in Sec	
Treatment (mg/kg)	0-5 min (sec)	15-30 min (sec)
Control (2 mL/kg)	124.60 ± 13.59	108.00 ± 27.57
Pentazocine (10)	29.75 ± 11.15[a]	0.00 ± 0.00[a]
SO & VA (100)	81.00 ± 5.66[c]	33.40 ± 10.91[c]
SO & VA (200)	75.75 ± 2.09[c]	0.00 ± 0.00[a]
SO & VA (400)	54.75 ± 8.40[b]	11.20 ± 11.20[b]

Values are mean ± SEM (n = 5), [a] p<0.0001, [b]p<0,01 and [c]p<0.05, significantly lower than the control, Control = Distilled water, Standard = Pentazocine, SO = *Senna occidentalis*, VA = *Vernonia amygdalina*.

Effect on tail-flick tests in rats

The combined effects of *Senna occidentalis* and *Vernonia amygdalina* on tail flick test is presented in Figure 1. The combined effect of the extract only became significant from the second hour. At the second hour, a significant increase ($p<0.01$) in the tolerance time was produced by the 100 mg/kg dose. There was also prolongation of tolerance time at the second hour by the 200 and 400 mg/kg dose of the combined extracts. At the third and fourth hours, a significant prolongation ($p<0.01$) of tolerance time was observed at the 100, 200 and 400 mg/kg dose. Thus the combined effect of both the extract at 100, 200 and 400 mg/kg dose was able to prolong the tolerance time by producing a protective effect that inhibited the animal's response to pain. The combined effects of both extracts compares well with pentazocine where significant ($p<0.01$) increase in tolerance time was also noticed.

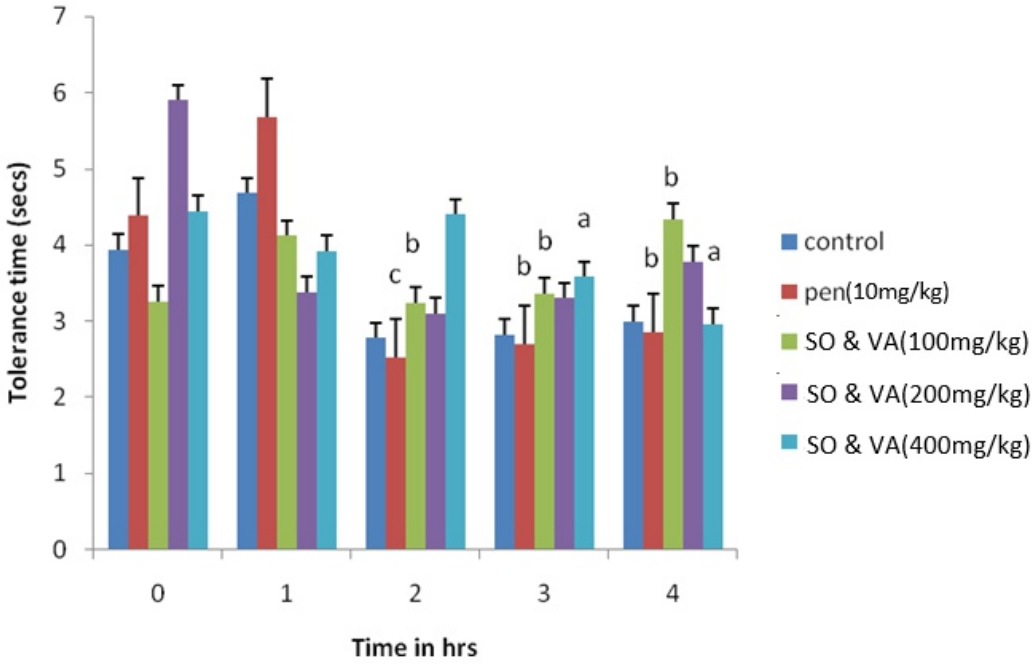

Figure 1: The effect of oral administration of combined aqueous leaf extracts (100, 200 and 400 mg/kg) of *Senna occidentalis* and *Vernonia amygdalina* on tail flick test in rats. Values are mean ± SEM (n=5), [a]$p<0.0001$, [b]$p<0.001$ and [c]$p<0.05$, significantly lower than the control, Pen = Pentazocine, SO = *Senna occidentalis*, VA = *Vernonia amygadalina*, Control = Distilled water.

Effect on *Escherichia coli* endotoxin-induced pyrexia in rabbits

The combined effect of *Senna occidentalis* and *Vernonia amygdalina* on *Escherichia coli* endotoxin induced pyrexia in rabbits is presented in Figure 2. Significant reduction ($p<0.05$) in the rectal temperature of the rabbit was only noticed at the 45th and 60th min by Aspirin (standard) and the 400 mg/kg dose respectively. Although other doses of the extract produced reduction from the 45th min, the effect was however not significant.

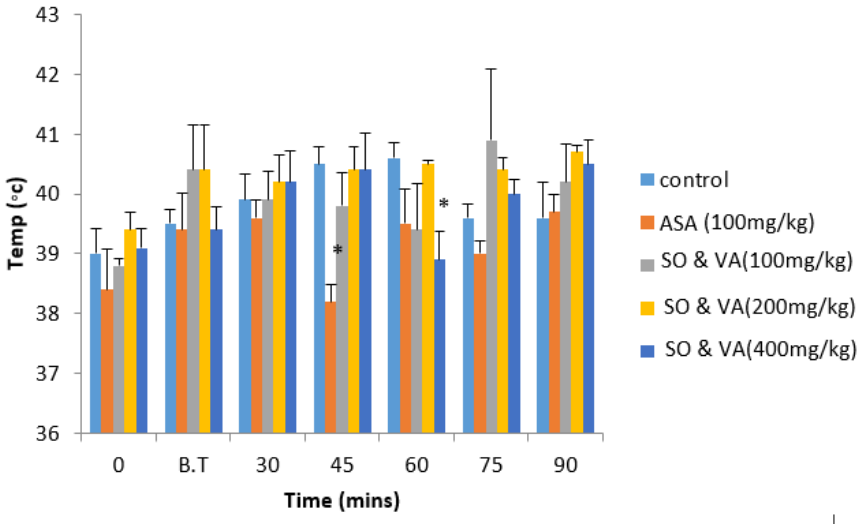

Figure 2: The effect of oral administration of combined aqueous leaf extracts (100, 200 and 400 mg/kg) of *Senna occidentalis* and *Vernonia amygdalina* on *Escherichia coli* endotoxin-induced pyrexia in rabbits. Values are mean ± SEM (n=5), *p<0.05 significantly higher than the control, B.T stands for before treatment. n= 5, ASA= Acetyl salicylic acid, SO= S*enna occidentalis*, VA= *Vernonia amygdalina*, Control = Distilled water.

Anti-inflammatory activity

Effect on carrageenan-induced paw oedema

Using the carrageenan-induced paw oedema, the anti-inflammatory effect of the combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* is shown in Figure 3. Significant inhibitory effects were produced ($p<0.05$) by the 200 mg dose at 30 min. In the second, third, and fourth hours, the conventional medication demonstrated significant anti-inflammatory benefits ($p<0.05$) in comparison to the control group. During the second and third hours, there was a significant ($p<0.05$) rise in inflammation observed with the 400 mg dosage of the combined extracts.

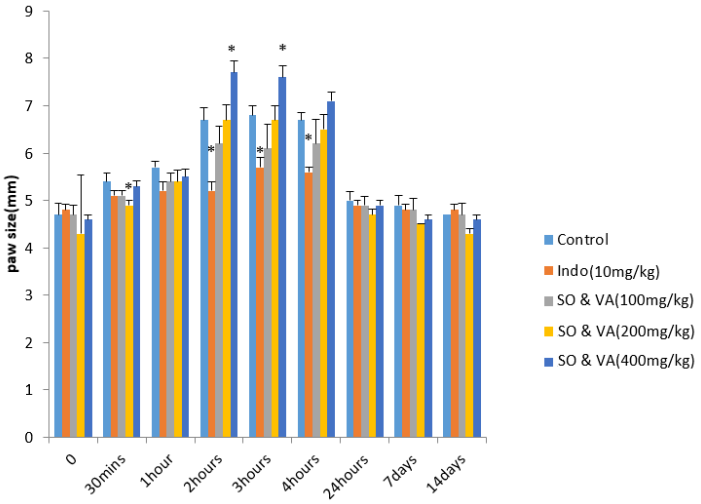

Figure 3. The effect of oral administration of combined aqueous leaf extracts (100, 200 and 400 mg/kg) of *Senna occidentalis* and *Vernonia amygdalina* on carrageenan-induced paw oedema in rats. Values are mean ± SEM (n=5), *p<0.05 significantly higher than the control. Indo= Indomethacine, SO= *Senna occidentalis*, VA= *Vernonia amygdalina*, Control = Distilled water.

Discussion

Medicinal plants are thought to be a significant source of novel chemicals, either as pure active principles or traditional preparations. They have the potentials for treating several diseases including pain, thus play a key role in human health[39]. The analgesic effect of combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* was evaluated using acetic acid-induced mouse writhing, formalin-induced paw oedema and tail-flick model test. These tests are chemical and thermal methods of evaluating nociception in rodents respectively. Thermal-induced nociception indicates narcotic involvements[40]. It is common practice to assess peripheral anti-nociceptive function in mice by inducing writhing in them with acetic acid[41]. Because of its great sensitivity, it can detect anti-nociceptive effects of medications at levels where other tests, like as the tail flick test, might not detect any effect[42]. It is believed that local peritoneal receptors partially mediate the abdominal constriction response [43].

Oral administration of combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* significantly reduced the number of writhes induced by acetic acid at all doses tested (100, 200, and 400 mg/kg dose) when compared with the control group. However, the standard group produced the lowest number of writhes. This therefore suggests that the analgesic effect of the extract could be probably peripherally mediated. The tail flick test elucidates central activity while formalin test evaluates central (first phase) and peripheral (second phase) activities[44]. The formalin test employs an adequate painful stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. The pain stimulus, a continuous rather than a transient one, may have resemblance to some kinds of clinical pain and observations are made on animals which are restrained only lightly or not at all[45]. Bradykinin and substance P release during the first phase, known as the neurogenic phase, is followed by the release of serotonin, histamine, bradykinin, and prostaglandin during the second phase, known as the inflammatory phase[46]. NSAIDs such as ibuprofen, acetyl salicylic acids inhibit just the second stage of this pain model, whereas both stages are hindered by central analgesics like morphine[47]. When compared to the control, the combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* significantly reduced paw licking during the 0–5 min period (neurogeninc pain) at all doses. However, in the 15-30 min (peripheral pain), the combined effect of the extracts also produced a dose dependent reduction in paw licking at all doses as it completely abolished paw licking at the 200 mg/kg dose. This however suggests that the combined aqueous leaf extracts of *Senna occidentalis* and *Vernonica amygdalina* may be effective in the management of both neurogenic and peripheral pain. The tail flick test is a model that is frequently used to evaluate central nociceptive activity. It measures the complex reaction to acute, non-inflammatory nociceptive input.

According to Gupta *et al.*[48] it is a well-established fact that any drug that prolongs the tail flick latency time in this test is working centrally. The combined effect of the extracts only became significant from the second hour. At the second hour, increase in tolerance time was produced by the 100, 200, and 400 mg/kg dose. At the third and fourth hour, a significant prolongation of tolerance time was observed at the 100, 200, and 400 mg/kg dose. Therefore, the combined effect of the extract exhibit central analgesic activity.

The analgesic effect of the combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* is suggested to be as a result of the presence of phytochemicals such as sesquiterpene, steroid saponin, lactones, flavonoids, tannins, terpenes[49]. *Vernonia amygdalina* has been reported to contain alkaloids, flavonoids, tannins and saponins[50]. *S. occidentalis* contains anthraquinones, flavonoids, alkaloids, saponins, terpenoids, and tannins[51].

The test model used in this study is very useful in evaluating the anti-pyretic effects of medicinal plants. From this study, significant reduction in the rectal temperature of the rabbits was only noticed at the 45th and 60th min by aspirin (standard) and the 400 mg/kg dose respectively. Although other doses of the extract produced reduction from the 45th min, the effect was however not significant. The above result suggests that the combined effect of both plant extract possesses a mild anti-pyretic activity. Fever is a complex physiologic response triggered by infection or aseptic stimuli[52]. Body temperature rises in response to an increase in prostaglandin E2 concentration in a specific area of the brain. Such an increase leads to a significant change in the rate at which neurons in the hypothalamus that control

thermoregulation process[52]. The antipyretic effect of combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina* was assessed using *Escherichia coli* endotoxin-induced pyrexia in rabbits, as anti-pyretic activities are frequently mentioned as a characteristic of drugs or compounds which have an inhibitory activity on prostaglandin biosynthesis[36]. *Escherichia coli endotoxin*-induced pyrexia is also called pathogenic fever[52]. The result of this experiment showed that significant inhibitory effect of inflammation was produced by the 200 mg/kg dose of the combined extract at 30 min. The standard drug showed significance compared to the control group at the second, third and fourth hour. The 400 mg/kg dose also showed significant increase in inflammation at the second and third hour.

Hence the above result indicates that the combined leaf extract of *Senna occidentalis* and *Vernonia amygdalina* produced inhibitory effects of chemical mediators in the 30th min. of the first phase, and not the second and continuous phases of inflammation. The extract may have antihistamine properties, as indicated by the inhibition of the first phase, which could lessen the effects of carrageenan-induced microvascular leakage[53]. According to Igbe *et al.*[54], histamine causes an increase in vascular permeability in artery endothelial cells, which causes fluid and cells to overflow. The effect of the combined extract in the second phase suggests that there is no possible inhibition of cyclooxygenase synthesis[55]. This reflects that the combined plant extracts possess no significant anti-inflammatory effect since inhibition was found only in the 30th min of the first phase of carrageenan induced inflammation. *Senna occidentalis* and *Vernonia amygdalina* contains phytochemical constituents and alkaloids such as terpenes, sesquiterpenes lactones, flavonoids, tannins, Achrosin, aloe-emodin, emodine, anthraquinones, anthrones, apigenin, chrysophanic acid and xanthorine[56,49]. The presence of these alkaloids may be the cause of the combined aqueous leaf extract of *Senna occidentalis* and *Vernonia amygdalina*'s anti-inflammatory properties, as these alkaloids have been shown to have anti-inflammatory activity[54].

Conclusion

In conclusion, the combined aqueous leaf extracts of *Senna occidentalis* and *Vernonia amygdalina* possess both significant neurogenic, peripheral and central analgesic as well as mild anti-pyretic effects. However, there is not significant anti-inflammatory activity.

Acknowledgement

Prof Ray Ozolua is appreciated for his mentorship and guidance.

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