

# Evaluation of Antipyretic and Analgesic Effects of Aqueous Extract of Leaves of *Vernonia Amygdalina* Del. (Asteraceae)

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## Research Article

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## Abstract

**Objective:** This study aims to evaluate the antipyretic and analgesic effects of leaves of *V. amygdalina* collected in Brazzaville-Congo.

**Method:** The antipyretic effect was induced using brewer's yeast induced pyrexia. The results obtained show that paracetamol and the aqueous extract of *V. amygdalina* at the doses used (400 and 800 mg/kg) increased significantly ( $p < 0.05$ ;  $p < 0.01$  and  $p < 0.001$ ) pyrexia induced by brewer's yeast from the second hour to the fifth hour of observation. Analgesic effect was evaluated by using the acetic acid-induced writhing, the pressure induced by the analgesymeter as well as the pain induced by formaldehyde. The results obtained show that the aqueous extract at the doses used (400 and 800 mg/ kg) significantly ( $p < 0.05$ ;  $p < 0.001$ ) reduces the number of abdominal writhing induced by acetic acid, significantly increased the latency time of the paw of the animal at the pressure induced by the analgesic meter ( $p < 0.05$  and  $p < 0.01$ ) and also significantly decreased the number of licking or biting of the paw compared to the control ( $p < 0.001$ ).

**Conclusion:** This result suggests that the aqueous extract of leaves of *V. amygdalina* at doses used (400 and 800 mg/kg) has an antipyretic and an antalgic effects.

**Keywords:** Antipyretic Effect; Analgesic Effect ; Vernonia Amygdalina; Immunological; Infusions

## Introduction

Herbal medicine has developed over centuries in different cultures. Written traces are proof that man has always been interested in plants as a source of food, or a means of curing his diseases [1]. In West Africa, as in the rest of the continent, more than 80 % of the population uses traditional medicine and medicinal plants for their primary health care [2]. Plants contain a molecular diversity of secondary metabolites [1,3]. This molecular diversity is of interest to scientific research for the discovery of new active ingredients or the development of improved traditional medicines to combat pathologies including fever and pain. Building on this reality, WHO's strategy since 2002 has been to commission the governments of member states to: "integrate the relevant aspects of traditional medicine into national health systems" [4]. Indeed, pain and fever can be treated in the Congo by the use of medicinal plants [5,6]. *V.amygdalina* is a medicinal plant known to have several properties as justified by its wide use in different traditional African medicines. Boiled leaves are used for the treatment of intestinal worms, fever, malaria, diarrhea, dysentery, hepatitis, cough and fertility. They are also used as a laxative, remedy for scabies, wounds, headaches and stomach upset. Root extracts are used for the treatment of malaria, gastrointestinal diseases and intestinal worms. In Congo, it is commonly used to treat cutaneous lesions [6]. In Nigeria, leaves are applied to wounds to replace iodine tincture. In Zimbabwe, root infusion is used to treat sexually transmitted diseases. Bark infusions are also taken to treat fever and diarrhea, and dried flowers are used against stomach disorders [7]. Previous pharmacological studies have shown that *V. amygdalina* has properties such as antimalarial [8]; antioxidative [9,10]; Immunological [11]; Antidiabetic [12]; anti-inflammatory and antinociceptive [10,13]. In this study, we aimed investigated antipyretic and analgesic effects of leaves of *V.amygdalina* (Asteraceae) collected in Brazzaville-Congo.

## Plant Material

Dry and sprayed leaves of *V. amygdalina* (Asteraceae) were used. These leaves were collected in Brazzaville. Botanical identification of the plant material was done by Mousamboté, botanist systematist of Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the Herbarium of the National Institute for Research in Exact and Natural Sciences (NIRENS) witch a collected sample was compared to a reference sample (No. 8331 of 13.10 1950). After that, the drying of collected leaves was done at the Laboratory of the Faculty of Science and Technology for 14 days at  $27 \pm 1$  ° C. After that, plant

material were dried and pulverized with a mortar. The powder obtained was used to prepare the aqueous extract. 250 g of powder from the dry leaves were mixed in 2500 ml of distilled water in a balloon heater. The whole was boiled for 15 minutes. After cooling and filtration, the filtrate obtained was evaporated in a water bath. Using a spatula, the filtrate was mixed continuously until complete evaporation of water. The aqueous extract obtained was preserved to evaluate the antipyretic and analgesic effects.

## Animals

Albino rats (150-200 g) and albino mice (25-30 g) of either sex obtained from the Faculty of Science and Technical of Marien NGOUABI-University were used. They were fed with a standard feed and water *ad libitum*. They were acclimatized during one week before experimentation and were housed under standard conditions (12 hours light and 12 hours dark) and at the temperature of  $27 \pm 1$  °C. The rules of ethics published by the International Association for the Study of Pain [14] have been considered.

## Methods

### Brewer's Yeast Pyrexia Test

The method described by Abdur et al, (2014) was used [15]. The animals were divided into groups of 5 rats each and their normal temperature was recorded using digital thermometer. Pyrexia was induced by injection of 10 mL/kg *s.c.* of 20 % suspension of Brewer's yeast (*Saccharomyces cerevisiae*). After 24 h rectal temperature was recorded. All the animals which did not present an increase in their rectal temperature of 0.5 °C 24<sup>th</sup> hours after the local injection of Brewer's yeast were isolated [16]. After that aqueous extract of *V.amygdalina* (400 and 800 mg/kg), paracetamol (standard drug, 100 mg/kg) and physiological solution (control group, 0.5 mL/100 g) were administered orally to groups. Rectal temperature was recorded periodically at 1, 2, 3, 4 and 5 hours after drugs administration. Antipyretic activity was defined as the ability of test drugs to reverse the induced pyrexia [16].

### Acetic Acid-Induced Pain In Mice

The pain was induced in the mice by using 0.6 % acetic acid solution [17]. The animals were divided into groups of 6 mice each. Aqueous extract of *V.amygdalina* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups, one hour prior to the local injection of acetic acid (10 ml/kg, IP). 5 minutes after

acetic acid injection, the number of writhing made by each mouse was recorded during 20 minutes [18]. A substance that has an analgesic effect will reduce the number of abdominal cramps compared to the control group. The analgesic effect was given by the inhibition (I) of the abdominal writhing.

### Analgesimeter Induced Pain Experiment

Method described by Elion Itou et al. (2017) was used [18]. The animals were divided into groups of 6 rats each. Aqueous extract of *V.amygdalina* (400 and 800 mg/kg), paracetamol (standard drug, 50 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups. One (1) hour after oral administration of the tested products, nociceptive thresholds were determined by using the analgesimeter (Cat. No. 37215 Ugo Basile, Italy). A constantly increasing pressure was applied on the right hand paw until the rats withdraw the paw. The sensitivity threshold to pain was determined and the reaction time calculated [18].

### Formaldehyde Induced Hind Pain

The pain was induced by using 2.5 % formaldehyde solution [18,19]. The animals were divided into groups of 5 rat each. Different doses of aqueous extract of *V.amygdalina* (400 and 800 mg/kg), Tramadol (standard drug, 10 mg/kg) and distilled water (control group, 0.5 mL/100 g) were administered orally to groups, one hour prior to the local injection of formaldehyde subcutaneous tissue of the plantar surface of the right paw. Animals were placed in various cages to observe the noxious effects. The frequency (Number of licking or biting paw/10 min) that the animal licks or bites its paw was monitored over 0-10 min for neurogenic pain response and 10-30 min for inflammatory pain response. The analgesic effect was given by the inhibition of the pain [18]. A central analgesic would inhibit the two phases equally, but a peripheral analgesic would only inhibit the second phase [20].

## Result

### Antipyretic Effect of Aqueous Extract of *V.Amygdalina*

The results are shown in Table 1. They show that all the experimental animals had a hyperthermia greater than 0.5°C compared to their normal rectal temperature 24 hours after subcutaneous administration of the brewer's yeast. The aqueous extract of *V.amygdalina* leaves at the doses used (400 and 800 mg/kg) and the paracetamol used as the reference molecule did not significantly decrease the rectal temperature 1 hour after induction of pyrexia ( $p > 0.05$ ). However, from 2 hours to 5 hours, the aqueous extract of *V.amygdalina* at the doses used (400 and 800 mg / kg) as well as paracetamol attenuate significantly ( $p < 0.05$ ;  $p < 0.01$  and  $p < 0.001$ ) pyrexia induced in all rats by the injection of Brewer's yeast (*Saccharomyces cerevisiae*) with a maximum at 2 and 3 hours for aqueous extract respectively at the doses of 800 and 300 mg/kg. In addition, the maximum temperature decrease is obtained at 3 h with paracetamol (100 mg/kg) used as a reference molecule.

### Effect of the Aqueous Extract of *V.Amygdalina* on Pain Induced by Acetic Acid

The administration of acetic acid to the experimental animals caused abdominal writhes (Table 2). Paracetamol and aqueous extract at the doses used significantly decreased abdominal writhes ( $P < 0.001$ ) compared to the control group (distilled water). The number of abdominal writhes developed by the experimental animals is  $109.33 \pm 0.39$  (control group); of  $99.66 \pm 0.20$  (paracetamol, standard drug); of  $72.82 \pm 3.94$  for the aqueous extract at the dose of 400 mg/kg and finally  $56.66 \pm 4.10$  for aqueous extract at the dose of 800 mg/kg. The strongest inhibition is observed with the aqueous extract at 800 mg/kg (48.17%) compared to the aqueous extract at 400 mg/kg (33.39 %) and paracetamol (8.84 %).

Treatment	Number of abdominal writhes	Inhibition %
Control group (0.5 mL/100g) +Ac. Acet (10 mL/kg, IP)	109.33±0.39	/
Para. (50 mg/kg) +Ac. Acet (10 mL/kg, IP)	99.66±0.20***	8.84
Ver. a (400 mg/kg) +Ac. Acet (10 mL/kg, IP)	72.82±3.94***	33.39
Ver.a (800 mg/kg) +Ac. Acet (10 mL/kg, IP)	56.66±4.10***	48.17

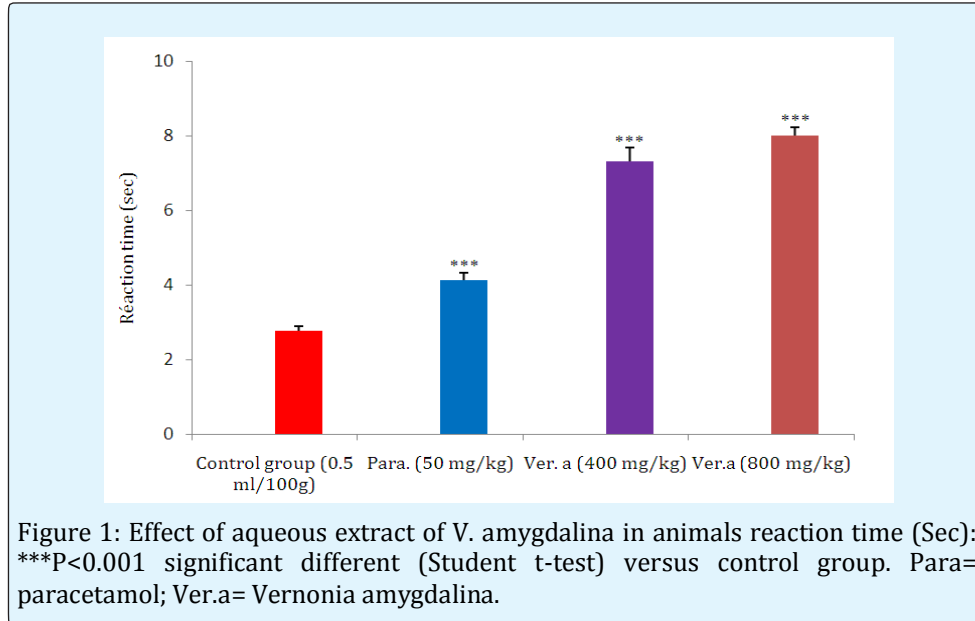
Each value represents the mean  $\pm$  ESM of abdominal writhes. \*\*\* $P < 0.001$  significant different (Student t-test) versus control group. Ac. Acet= acetic acid; Para= paracetamol; Ver.a= Vernonia amygdalina

Table 1: Effect of aqueous extract of *V.amygdalina* in abdominal writhes induced by acetic acid 0.6 % in mice.

### Effect on the Pain Induced by the Analgesy Meter

The pressure induced by the analgesymeter caused pains in the legs of the experimental animals (Figure 1). Paracetamol and the aqueous extract at the doses used (400 and 800 mg/kg) significantly increase the reaction

time ( $P < 0.001$ ) following the pain induced by the apparatus compared to the control group (distilled water). This time is  $2.76 \pm 0.13$  Sec for the control group;  $4.13 \pm 0.20$  Sec for paracetamol;  $7.32 \pm 0.37$  Sec for the aqueous extract at 400 mg/kg and  $8.02 \pm 0.20$  Sec for the aqueous extract at 800 mg/kg.



### Effect of the Aqueous Extract of *V. Amygdalina* on Pain Induced by Formaldehyde

Subcutaneous tissue of the plantar surface administration of the formaldehyde solution caused neurogenic pain response and inflammatory pain response in experimental animals (Table 3). During the neurogenic pain response and the inflammatory pain response, the tramadol (10 ml/kg) and aqueous extract at the doses used (400 and 800 mg/kg) significantly reduced the frequency of licking or biting of the paw ( $p < 0.001$ ). During the neurogenic pain response, the frequencies and

inhibitions are  $31.48 \pm 3.74$  for the control group;  $8.2 \pm 0.48$  (73.88 % of inhibition) for tramadol;  $10.8 \pm 0.37$  (63.85 % of inhibition) for the extract aqueous 400 mg/kg and  $7.33 \pm 1.94$  (76.65 % of inhibition) for the aqueous extract 800 mg/kg. During the inflammatory pain response, frequencies and inhibitions are  $66.40 \pm 1.93$  for the control group; of  $11.40 \pm 1.69$  (82.83 % of inhibition) for tramadol;  $24.00 \pm 1.38$  (63.85 % of inhibition) for aqueous extract 400 mg/kg and  $17.8 \pm 1.94$  (73.19 % of inhibition) the aqueous extract 800 mg/kg.

Treatment Doses		Temperature (T°) after pyrexia induced						
		T° normal	0h	1h	2h	3h	4h	5h
Control group	(0.5 mL/100g)	36.58 ± 0.28	37.58 ± 0.17	37.82 ± 0.23	37.688 ± 0.07	37.512 ± 0.58	37.428 ± 0.50	37.44 ± 0.22
para.	(100 mg/kg)	36.10 ± 0.21	37.12 ± 0.20	37.06 ± 0.45 <sup>ns</sup>	36.08 ± 0.35 <sup>**</sup>	35.84 ± 0.30 <sup>***</sup>	35.68 ± 0.50 <sup>***</sup>	35.68 ± 0.26 <sup>**</sup>
<i>V.amyg</i>	(400 mg/kg)	35.92 ± 0.25	37.1 ± 0.27	36.94 ± 0.23 <sup>ns</sup>	36.55 ± 0.14 <sup>*</sup>	36.352 ± 0.20 <sup>***</sup>	36.16 ± 0.52 <sup>***</sup>	36.14 ± 0.16 <sup>**</sup>
	(800 mg/kg)	35.96 ± 0.06	36.98 ± 0.22	36.66 ± 0.13 <sup>ns</sup>	36.44 ± 0.12 <sup>***</sup>	36.26 ± 0.3 <sup>***</sup>	36.16 ± 0.50 <sup>***</sup>	36.04 ± 0.27 <sup>***</sup>

Each value represents the mean ± ESM of temperature. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $P < 0.001$  significant different (Student t-test) versus control group. ns= no significant value  $p > 0.05$ ; Ver.a= Vernonia amygdalina

Table 2: Effect of aqueous extract of *V.amygdalina* in pyrexia induced by the injection of Brewer's yeast (*Saccharomyces cerevisiae*).

Treatment	Doses	Neurogenic pain response (0-10 min)		Inflammatory pain response (10-30 min)	
		Frequency (Hz) of legs lickind and of biting	Inhibition %	Frequency (Hz) of legs lickind and of biting	Inhibition %
Control group	(0.5 mL/100g)	31.40±3.74	/	66.40±1.93	/
Tramadol	(10 mg/kg)	8.20±0.48***	73.88	11.4±1.69***	82.83
<i>V.amyg</i>	(400 mg/kg)	10.80±0.37***	65.60	24,00±1.38***	63.85
	(800 mg/kg)	7.33±1.94***	76.65	17.80±1.94***	73.19

Each value represents the mean ± ESM of frequency. \*\*\*P<0.001 significant different (Student t-test) versus control group. Ver. amygd= *Vernonia amygdalina*

Table 3: Effect of aqueous extract of *V.amygdalina* in pain induced by formaldehyde 2.5 %.

## Discussion

Pyrexia was induced by subcutaneous administration of Brewer's yeast (*Saccharomyces cerevisiae*). Brewer's yeast induces hyperthermia by increasing prostaglandin synthesis [21]. The hyperthermia induced by the injection of Brewer's yeast is linked to the release of cytokines (TNF $\alpha$ , IL1 $\beta$ , and IL6) which have reached the blood vessels stimulate the biosynthesis of prostaglandins (PGE2) around the hypothalamic thermoregulatory center [22,23]. Aqueous extract of *V.amygdalina* attenuate significantly (p < 0.05; p < 0.01 and p < 0.001) pyrexia induced in all rats by the injection of Brewer's yeast. This result suggests an antipyretic effect of aqueous extract of *V.amygdalina* which could pass by the interference with one of mechanism of Brewer's yeast induced pyrexia.

In this study, we used three methods to evaluate the analgesic effect of the aqueous extract of *V. amygdalina* leaves. Acetic acid and the analgesimeter test induces peripheral pain, the formaldehyde test induces central and peripheral pain [24]. Intraperitoneally administration of acetic acid induced abdominal writhes in mice. Acetic acid is a sensory irritant that induces peripheral pain [17,25]. This induction of pain by acetic acid causes an increase in the peritoneal fluid of prostaglandins (PGE2 and PGF2 $\alpha$ ) as well as lipooxygenase products involved partly in the peritoneal receptors and cause peripheral inflammatory pain response by inducing capillary permeability [26-28]. In this study, aqueous extract at the doses used significantly decreased abdominal writhing (P < 0.001) compared to the control group (distilled water). This result suggests an analgesic effect of aqueous extract of *V.amygdalina*. The effect of the aqueous extract was evaluated on peripheral-type pain with the Analgesimeter. In fact, tissue trauma caused by mechanical pressure causes local swelling of the traumatized tissues responsible for the release of bradykinin, serotonin, potassium and hydrogen ions [29]. These substances are typically algogenic substances that

activate nociceptors [30,31]. The production of lactic acid is also responsible for the pain encountered during ischemia or muscle exercises, histamine, substance P, prostaglandins [29]. Prostaglandins and probably leukotrienes are not very algogenic but play a key role since they sensitize receptors to the action of other substances [32,33]. The fact that the aqueous extract (400 and 800 mg/kg) opposes this mechanical type of pain suggests that it interferes or inhibits the release of these algogenic mediators. These two methods are not specific; that's why the formaldehyde test was used. The formaldehyde test is a very important model used to highlight not only the analgesic effect but also to clarify the mechanisms of analgesic action. Subplantar administration of 2.5% formaldehyde in rats induces neurogenic response pain and inflammatory response pain [34]. The neurogenic pain response is a direct result of the stimulation of nociceptors in rats. The paw and reflects the central pain caused by the release of substance P at the central level. The inflammatory pain response is due to the release of histamine, serotonin, bradykinin and prostaglandins [35], which are the precursors of peripheral pain. Aqueous extract at the doses used (400 and 800 mg/kg) significantly reduced the frequency of licking or biting of the paw (p < 0.001) during the neurogenic pain response and inflammatory pain response. This result suggests that aqueous extract could have a peripheral and central analgesic effect.

## Conclusion

*V.amygdalina* is a Congolese medicinal plant known to have several properties. It appears from this study that its aqueous extract at the doses used has an antipyretic and analgesic effect what could justify its traditional use in the treatment of fever and pain.

## Conflict of Interests

The authors declare that they have no conflict of interest.

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