# The Analgesic and Antiplasmodial Activities and Toxicology of *Vernonia amygdalina*

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ABSTRACT   *Vernonia amygdalina* possesses several bioactive compounds and is used in traditional medicines of southwestern Uganda, along with other regions. Its analgesic potential has not been investigated thus far. The present study examines the antinociceptive potential of the aqueous leaf extract (50–200 mg/kg) using three models of nociception (acetic acid-induced writhing, formalin test, and tail-flick test), antiplasmodial activity, and toxicology of the extract. The results show the extract significantly inhibits acetic acid-induced writhing and the formalin test in mice but did not give a potent effect in the tail-flick test, suggesting that the extract may have peripheral and central analgesic properties. The extract also exhibited significant antiplasmodial activity in mice against *Plasmodium berghei* with 73% inhibition in the group that received a dose of 200 mg/kg i.p. daily for 4 days. Toxicology results show no clinical signs of toxicity or adverse toxicological effects in the treated groups, except for a significant decrease in red blood cell count and a dose-dependent increase in serum bilirubin. These changes were within control values based on historical reference ranges at doses of 500–2,000 mg/kg/day for 14 consecutive days as compared to the control. This study supports the traditional use of *V. amygdalina* as an alternative therapy for malaria and the symptomatic relief of pain usually associated with malaria.

KEY WORDS: • *malaria* • *pain* • *toxicity* • *traditional medicine* • *Uganda*

## INTRODUCTION

$V$ERNONIA AMYGDALINA (Family Asteraceae), also called bitter leaf, is a popular African vegetable that grows as a shrub or small tree indigenous to Central and East Africa, including Uganda.[1] It is known locally as "omubirizi" in southwestern Uganda and used traditionally for pain relief and malaria attack. It is obtained from The Medical Traditional Healer Association in Rukararwe, Bushenyi District, Uganda, in sachets containing 65.5 g of a greenish powder and sold under the name of AM (anti-malaria). Patients are instructed to soak each sachet in three mugs of about 1.5 L of hot water (80°C), to produce tea. They should then drink half a glass (about 0.25 L) two times daily for 4–7 days. Smaller doses are prescribed for children according to their weight. This plant has ethnomedical use in treating venereal diseases, gastrointestinal problems, and malaria.[2–4] There

are reports on the hypoglycemic, antineoplastic, antibacterial, and antioxidant properties of the plant.[5–8] Despite the varied use of the plant, there is no information on its analgesic properties and exact toxicology on subchronic exposure, although some reports exist on its antiplasmodial effects.[9–12] Previous phytochemical reports show the presence of steroids, saponins, and flavonoids.[13–17] This work sought to establish a basis for its use to relieve pain by evaluating the antinociceptive activity of the aqueous leaf extract of *V. amygdalina* in a mouse model. The antiplasmodial effect and subchronic toxicity of the plant were also tested.

## MATERIALS AND METHODS

### Extract preparation

Leaves of *V. amygdalina* were collected from the botanical garden of the Rukararwe Traditional Medicine Health Center, a division of the Rukararwe Partnership Workshop for Rural Development in Bushenyi District, southwestern Uganda. The plant was authenticated by Dominic Byarugaba, a botanist with the Department of Biology, Mbarara University of Science and Technology, Mbarara, Uganda. A voucher specimen (number 8-12) was retained in the De-

partment. The plant material was air-dried and grounded into a coarse powder. Three hundred fifty grams of this powder was macerated in 2 L of distilled water for 24 hours with occasional shaking (model 3017 rotary shaker, GFL, Burgwedel, Germany) and then extracted using a Soxhlet extractor (Gallemkamp, London, UK). The resultant extract was evaporated in a water bath, under controlled temperature not exceeding the one used by the healers in their plant preparation (80°C), to yield 32.3 g of semisolid residue. The semisolid residue was reconstituted in distilled water, and appropriate concentrations were used in the experiment.

### Phytochemical screening

The presence of various phytochemical constituents in the extract was determined using standard screening tests.[18]

### Animals

Adult Wistar rats (weighing 130–150 g) and Swiss albino mice (weighing 18–26 g) of either sex, maintained at the Animal Facility Centre of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, were used for the experiments. The animals were kept in plastic cages at room temperature and moisture, under a naturally illuminated environment with a 12:12-hour dark/light cycle. They were fed with standard diet (Ladokun Feeds Ltd., Ibadan, Nigeria) and had access to tap water *ad libitum*. The animal experiments were conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*[19] and ethical guidelines for investigation of experimental pain in conscious animals.[20] All efforts were made to minimize suffering and reduce the number of animals used.

### Drugs, reagents, and test agents

Acetylsalicylic acid (ASA), chloroquine (CQ), and acetic acid were from Sigma Chemical Co. (St. Louis, MO). Pethidine hydrochloride was from Bayer (Newbury, UK), and formaldehyde was from M&B (Lagos, Nigeria). Sterile normal saline (10 mL/kg. i.p.) was used as the control in all studies, while three dose levels of the extract (50, 100, or 200 mg/kg, p.o.) prepared in distilled water were used for the test groups. ASA (100 mg/kg, i.p.), pethidine (50 mg/kg, p.o.), or CQ (5 mg/kg, i.p.) was used as the reference drugs.

### Acute toxicity test

The intraperitoneal acute toxicity of the extract was evaluated in Swiss albino mice using method slightly modified from that of Lorke.[21] Briefly, this method involve the determination of 50% lethality dose ($LD_{50}$) value in a biphasic manner. The animals were starved but allowed access to water 24 hours prior to the study. In the initial investigatory step (phase 1), a range of doses of the extract producing the toxic effects was established. This was done by intraperitoneal administration of geometrically increasing doses of

the extract (10, 100, 1,000, or 1,500 mg/kg) to four groups of mice ($n = 4$). Based on the results obtained, a phase 2 investigatory step was done by giving more specific doses (200, 400, 600, or 800 mg/kg i.p) to four other groups of mice ($n = 4$).

The mice were observed for 24 hours for such signs as excitement, dullness, ataxia, or death. The $LD_{50}$ was estimated from the geometric mean of the dose that caused 100% mortality and the dose that caused no lethality. The same procedure was used in rats that received 1,000, 2,000, 3,000, or 5,000 mg/kg orally ($n = 4$) in phase 1 and 1,500, 3,000, 4,000, or 5,000 mg/kg orally in the second phase ($n = 4$).

### Acetic acid-induced writhing in mice

The abdominal writhing test induced by chemical stimulation of acetic acid was performed in mice as originally described by Siegmund *et al.*[22] The mice were divided into different groups (of five mice each) and differently pretreated with the extract (50, 100, or 200 mg/kg p.o.), aspirin (100 mg/kg i.p), or normal saline (10 mL/kg p.o.). At 30 and 60 minutes after the treatment, 0.7% acetic acid (10 mL/kg i.p.) was administered to the mice. Five minutes later each mouse was placed in a transparent cage, and the number of writhing events (a response consisting of abdominal wall contraction and pelvic rotation, followed by hind limb extension) was recorded for 10 minutes. The results of the treatment groups were compared with those of normal saline-pretreated controls. The writhing percentage was calculated as (test mean/control mean) $\times$ 100.

### Formalin test

For the formalin studies, rats were injected with 0.05 mL of formalin (2.5% formaldehyde) into the subplantar surface of the left hind paw 30 minutes after treatment with saline (10 mL/kg p.o.), extract (100 and 200 mg/kg p.o.), or ASA (100 mg/kg. i.p.). Severity of pain (for both control and test groups $n = 5$) was simultaneously observed and rated as scores using pain measurements.[23] This was rated as follows: score 0, rat can bear weight on the injected paw; score 1, light resting of the paw on the floor; score 2, partial elevation of the injected paw; and score 3, total elevation with licking and biting of the paw. These observations were recorded every minute for the first 10 minutes (early phase) and at every 5 minutes for the period between 15 and 60 minutes (late phase).

### Tail-flick test

The antinociceptive effects of *V. amygdalina* and the reference drug, expressed as the time required for rat tail flick after exposure to a source of heat, were evaluated according to the description of D'Amour and Smith.[24] Briefly, before treatment, the terminal (2 cm) of each rat's tail was immersed in hot water contained in a 500-mL beaker and maintained at 55 $\pm$ 1°C using a thermoregulated hot plate

(Socrel DS-35, Ugo Basile, Comerio, Italy), and the time (in seconds) between the onset of stimulation and tail withdrawal was measured as the tail-flick latency. Twenty-five rats that showed response within 0–4 seconds were selected and placed in groups of five ($n = 5$) for the study. Immediately after basal latency assessment, normal saline, reference drug (pethidine hydrochloride [50 mg/kg]), or the plant extract (50–200 mg/kg p.o.) was administered, and the reaction time was again recorded at 30 and 60 minutes.[25]

*Antiplasmodial activity*

The test was carried out according to the curative procedure described earlier by Adzu *et al.*[26] A donor mouse infected with rodent malaria (*Plasmodium berghei*) was anesthetized with chloroform, and the abdomen was opened. Blood was collected through cardiac puncture with a sterile needle and syringes (Zarinject, Lagos) in such a way that 0.2 mL of the blood contained about $1 \times 10^7$ infected red blood cells (RBCs). Twenty-five mice were inoculated intrapeitoneally with 0.2 mL each. The mice were then randomized, placed in groups of five ($n = 5$), and treated as follows on day 3: group 1 received normal saline, group 2 received CQ (5 mg/kg, i.p.), and groups 3–5 received 50, 100, and 200 mg/kg i.p. of extract, respectively. The treatment continued daily until day 7. Thick and thin blood smears were collected daily from tail blood, fixed with methanol, stained with 4% Giemsa at pH 7.2 for 45 minutes, and examined microscopically (model YS2-H, Nikon, Tokyo, Japan). The increase or decrease in parasitemia, defined as the number of infected and uninfected RBCs, was counted in five different fields, and mean survival time (within 30 days) was recorded.
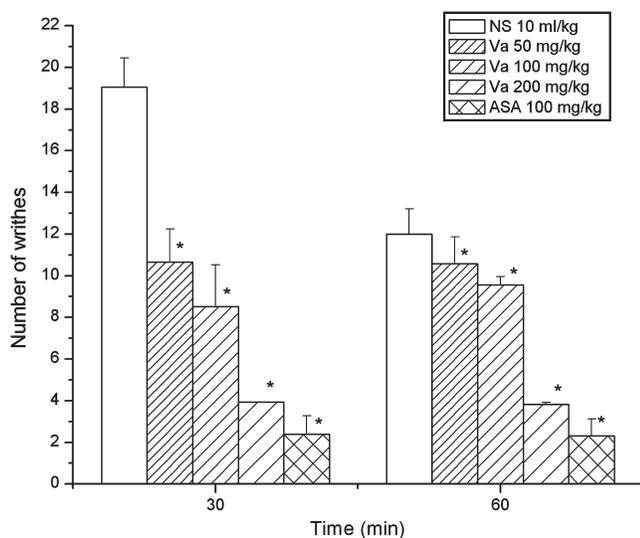


**FIG. 1.** Effect of the aqueous extract of *V. amygdalina* (Va) leaves on acetic acid-induced writhing in mice for 5 minutes. NS, normal saline. All data are mean ± SEM values (in g) ($n = 5$). *Significantly different ($P < .05$) between treated group and NS control.
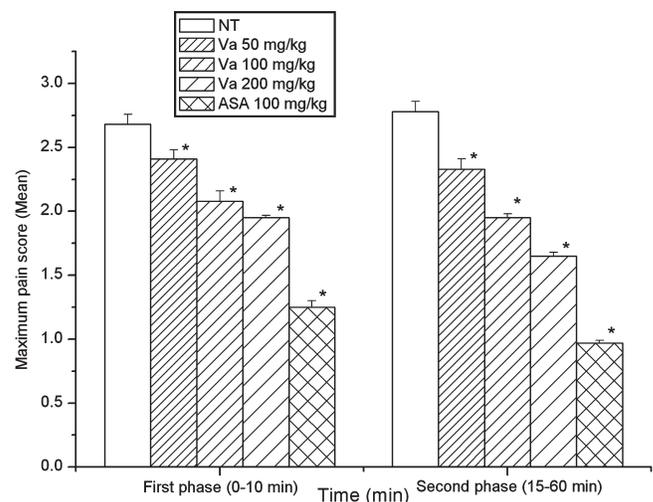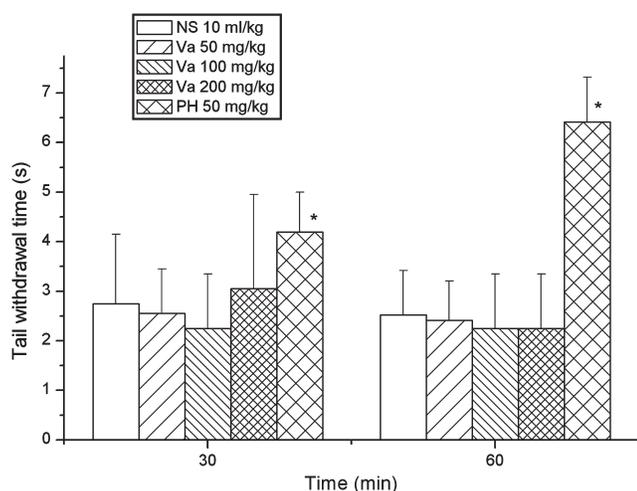


**FIG. 2.** Effect of the aqueous extract of *V. amygdalina* (Va) leaves on the formalin test in rats. NT, nontreated animals. All data are mean ± SEM (in g) values ($n = 5$). *Significantly different ($P < .05$) between treated groups and NT control. The maximum pain score was 3.

*Subchronic toxicity test*

Twenty-eight rats divided into four weight-matched groups of seven rats each (both sexes) were used for the study. Three test groups received 500, 1,000, or 2,000 mg/kg *V. amygdalina* by gavage with a biomedical needle (straight, 16 gauge, 76.2 mm long, 3 mm in diameter; Harvard Apparatus, Holliston, MA) daily for 14 days. The control group received normal saline.

Food and water intake were measured daily, while the animals' body weights were obtained every other day. All animals were observed at least once daily for clinical signs (such as lethargy, hyperactivity, depression, and diarrhea). On day 14, immediately prior to euthanasia, all animals were anesthetized with chloroform and bled via the descending aorta for hematology and clinical chemistry determination. Organs were dissected and weighed to determine absolute and relative weight. The blood for clinical chemistry was allowed to clot in Microtainer (BD, Franklin Lakes, NJ) separator tubes and centrifuged, and sera were collected and stored at $-20°C$ until ready for biochemical analyses. Commercial kits (Biosystem BTS-310 [Biosystem S.A., Barcelona, Spain] and Vitros DT systems [Ortho-Clinical Diagnostics, Summit, NJ]) were used to analyze liver function, renal function, and the electrolyte test.

The hematological tests were carried out in EDTA-anticoagulated blood. The hemoglobin (Hb) concentration was analyzed by the cyanmethemoglobin method, packed cell volume (PCV) by the micromethod, and white blood cell (WBC) (total and differential) and platelet counts by visual methods.[27] The mean cell Hb concentration (MCHC) was calculated by dividing the Hb concentration by PCV.[28]

**FIG. 3.** Effect of the aqueous extract of *V. amygdalina* (Va) leaves on the thermal stimulus-induced tail-flick test in rats. NS, normal saline; PH, pethidine hydrochloride. All data are mean ± SEM values (in g) ($n = 5$). *Significantly different ($P < .05$).

## Statistical analysis

The calculation of percentage writhing reflex for antinociception was based on the expression of the numerical data as mean ± SEM values. Fluid intake, animal weight, and antimalaria test were analyzed using Student's *t* test, while Dunnett's *t* test was used to test for differences between individual treatment groups. $P < .05$ was considered statistically significant.

## RESULTS

### Phytochemical test

The aqueous extract of *V. amygdalina* gave positive test results for tannins, saponins, phenols, flavonoids, steroids, and alkaloids.

### Acetic acid-induced writhing in mice

Figure 1 shows the numbers of the abdominal writhing episodes evoked by intraperitoneal injection of acetic acid in mice as well as the antinociceptive effect of *V. amyg-*

*dalina.* It can be seen that treatment with *V. amygdalina* (50, 100, or 200 mg/kg p.o.) could dose-dependently reduce the number of writhing episodes of mice in comparison with that of normal saline animals, while the reference drug ASA (100 mg/kg) showed a stronger analgesic effect than the extract in this nociceptive model.

### Formalin and tail-flick tests

The extract at doses of 100 and 200 mg/kg induced significant reduction in pain response in both phases (aphasic and tonic) in the formalin test in comparison with the control (Fig. 2). In all cases ASA, a positive analgesic agent, demonstrated significant antinociceptive action with a slightly stronger pharmacological intensity than *V. amygdalina* at 200 mg/kg in the late phase. The extract exerted no significant effect on nociception in the tail-flick test as values obtained correspond with those of the control saline group. On the other hand, pethidine (50 mg/kg), the reference drug, produced a significant increase in the reaction time to thermal stimuli at the two times of measurement (Fig. 3).

### Antiplasmodial activity

The extract caused a significant ($P < .05$) and dose-dependent reduction in mean parasitemia in mice infected with *P. berghei* in comparison to CQ (5 mg/kg). The extract caused a parasitemia reduction of 52% with 50 mg/kg, 64% with 100 mg/kg, and 73% with 200 mg/kg (Table 1). All the mice in the saline group died within 15 days throughout the 30-day observation period of the experiment, while the remaining mice recovered fully.

### Acute toxicity test

The $LD_{50}$ was established to be 560 ± 1.21 mg/kg i.p. in mice and 3.32 ± 0.15 g/kg p.o. in rats. Gait abnormalities, reduction in stereotypic activities, and then death were, however, seen in high doses.

### Subchronic toxicity

No treatment deaths occurred, and no treatment-related clinical signs were noted during the study. The extract did

TABLE 1. CURATIVE ACTIVITY OF THE AQUEOUS EXTRACT OF *V. AMYGDALINA* AND CQ AGAINST *P. BERGHEI* IN MICE

| Treatment | Dose (mg/kg, i.p.) | Parasitemia (day 2–day 6) | Inhibition (%) |
|---|---|---|---|
| Normal saline (10 mL/kg) | — | 14.2 ± 0.25 | — |
| *V. amygdalina* | 50 | 6.7 ± 0.17* | 52.8 |
| | 100 | 5.0 ± 0.22* | 64.8 |
| | 200 | 3.7 ± 0.17* | 73.9 |
| | 5 | 3.1 ± 0.25* | 78.2 |

Data are mean ± SEM counts ($n = 5$).
*Significantly ($P < .05$) different from the saline control group.

TABLE 2. EFFECT OF *V. AMYGDALINA* ON DAILY FOOD AND FLUID INTAKE, BODY WEIGHTS, AND ORGAN WEIGHTS OF RATS

| Treatment (mg/kg) | Food intake (g) | Fluid intake (mL) | Initial body | Final body | Liver Absolute | Liver Relative | Heart Absolute | Heart Relative | Kidney Absolute | Kidney Relative | Lungs Absolute | Lungs Relative | Spleen Absolute | Spleen Relative |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 (Control) | 131.82 ± 7.04 | 155.63 ± 11.64 | 147.92 ± 7.35 | 178.92 ± 6.51 | 7.03 ± 0.18 | 3.96 ± 0.19 | 0.65 ± 0.02 | 0.35 ± 0.01 | 1.43 ± 0.49 | 0.79 ± 0.02 | 1.42 ± 0.12 | 0.80 ± 0.07 | 0.84 ± 0.05 | 0.48 ± 0.04 |
| 500 | 131.82 ± 10.52 | 148.33 ± 19.22 | 161.38 ± 14.77 | 181.11 ± 14.11 | 6.78 ± 0.42 | 3.72 ± 0.47 | 0.83 ± 0.09 | 0.46 ± 0.08 | 1.34 ± 0.09 | 0.76 ± 0.06 | 1.66 ± 0.23 | 0.97 ± 0.20 | 0.76 ± 0.62 | 0.43 ± 0.03 |
| 1,000 | 113.49 ± 10.14 | 120.16 ± 6.51 | 137.62 ± 14.32 | 158.17 ± 17.93 | 5.37 ± 0.58 | 3.64 ± 0.52 | 0.64 ± 0.08 | 0.44 ± 0.07 | 1.26 ± 0.10 | 0.85 ± 0.11 | 1.50 ± 0.10 | 0.99 ± 0.11 | 0.63 ± 0.05 | 0.46 ± 0.05 |
| 2,000 | 129.82 ± 12.20 | 147.16 ± 12.18 | 134.58 ± 9.72 | 156.94 ± 6.85 | 6.43 ± 0.11 | 4.17 ± 0.41 | 0.74 ± 0.06 | 0.48 ± 0.06 | 1.36 ± 0.09 | 0.88 ± 0.07 | 1.33 ± 0.09 | 0.87 ± 0.07 | 0.74 ± 0.42 | 0.48 ± 0.05 |

Data are mean ± SEM values ($n = 7$). Relative organ weight = (absolute organ weight/final body weight) × 100.

not exert significant changes on mean body and organ weight or fluid and food intake (Table 2). All animals demonstrated a progressive increase in body weight during the exposure. The hematology result showed a significant decrease ($P < .05$) in RBC count at the dose of 2,000 mg/kg compared to the control (Table 3). The result of the clinical chemistry parameter showed a dose-dependent increase in direct and total bilirubin. There was also an increase in uric acid at doses of 500 and 1,000 mg/kg compared to the control (Table 4).

## DISCUSSION

As the use of herbal medicine becomes more prevalent, especially in rural areas, pharmacological evidence to understand the action of these medicines and the underlying mechanisms, to support their proper and safe use, is essential. In the current study, the analgesic effect of the leave extract of *V. amygdalina* was assessed using three nociceptive animal models: acetic acid-induced writhing, formalin test, and tail-flick test. In the writhing response model, acetic acid was injected into the peritoneal cavity of mice; the acid causes nociception in the abdomen due to the release of various substances that excite pain nerve ending.[29] According to previous reports this assay is commonly used in mice to detect both central and peripheral analgesia.[30] *V. amygdalina* showed an ability to diminish the numbers of writhing episodes, in a dose-dependent manner, indicating significant inhibition of the acetic acid-induced visceral nociception. The possible mechanism may be a blocking effect by the extract or the release of endogenous substances, including prostaglandin E$_2$ and prostaglandin F$_{2\alpha}$, that excite pain nerve endings, which are found in the writhing response test model of mice.[31]

Similarly, the extract demonstrated significant analgesic effects in initial and late phases of the formalin test. The phase 1 formalin pain responses are thought to reflect activity that is prominent in A$\beta$, A$\delta$, and high-threshold C nociceptor afferent fibers. Phase 2 behavioral responses likely reflect activity in mechanically insensitive afferent fibers and activity of A$\delta$ and C fibers.[32] Since the mechanism of the analgesic effect of *V. amygdalina* is apparent in these two models, it can be suggested that this effect may be linked to processes in the prevention of sensitization of the noci-

TABLE 3. EFFECT OF *V. AMYGDALINA* ON SELECTED HEMATOLOGICAL PARAMETERS IN RATS

| Parameter | Control | V. amygdalina 500 mg/kg | V. amygdalina 1,000 mg/kg | V. amygdalina 2,000 mg/kg |
|---|---|---|---|---|
| Hb (g/dL) | 12.17 ± 0.27 | 9.97 ± 0.73 | 11.88 ± 0.78 | 10.54 ± 0.47 |
| PCV (%) | 36.14 ± 0.79 | 29.71 ± 2.20 | 35.42 ± 2.34 | 31.42 ± 1.41 |
| MCHC (g/dL) | 32.14 ± 0.34 | 30.28 ± 0.60 | 32.00 ± 0.65 | 30.85 ± 0.40 |
| RBCs (× 10$^{12}$/L) | 4.64 ± 0.13 | 3.84 ± 0.25# | 4.30 ± 0.35# | 3.58 ± 0.14# |
| Platelets (× 10$^9$/L) | 156.42 ± 3.77 | 139.85 ± 4.38 | 153.28 ± 6.95 | 136.71 ± 3.82# |
| WBCs (× 10$^9$/L) | 6.57 ± 0.43 | 5.57 ± 0.87 | 6.68 ± 0.51 | 3.47 ± 0.89# |
| Neutrophils (%) | 20.57 ± 2.42 | 25.57 ± 1.95* | 24.00 ± 1.77* | 21.00 ± 1.67 |
| Lymphocytes (%) | 79.28 ± 2.53 | 74.42 ± 1.95 | 77.4 ± 2.47 | 83.28 ± 3.48* |

Data are mean ± SEM values ($n = 7$).
*Significantly increased ($P \leq .05$) compared to control.
#Significantly reduced ($P \leq .05$) compared to control.

TABLE 4. EFFECTS OF *V. AMYGDALINA* ON CLINICAL CHEMISTRY PARAMETERS IN RATS

| Parameter | Control | V. amygdalina 500 mg/kg | V. amygdalina 1,000 mg/kg | V. amygdalina 2,000 mg/kg |
|---|---|---|---|---|
| ALP (IU/L) | 212.1 ± 2.21 | 215.5 ± 3.77 | 218.8 ± 8.11 | 218.7 ± 3.45 |
| Total protein (g/L) | 71.5 ± 1.11 | 74.3 ± 2.69 | 77.0 ± 0.54 | 73.7 ± 2.21 |
| Albumin (g/L) | 38.3 ± 1.25 | 39.7 ± 1.04 | 40.0 ± 0.94 | 39.6 ± 0.76 |
| Bilirubin ($\mu$mol/L) | | | | |
| Direct | 1.8 ± 0.82 | 2.5 ± 1.01* | 3.2 ± 1.51* | 4.0 ± 1.78* |
| Total | 4.2 ± 2.65 | 6.28 ± 2.63* | 5.71 ± 2.53* | 6.57 ± 3.19* |
| ALT (IU/L) | 32.7 ± 5.80 | 34.60 ± 10.18 | 35.10 ± 11.04 | 33.70 ± 6.23 |
| AST (IU/L) | 42.28 ± 10.27 | 40.43 ± 10.68 | 41.29 ± 12.32 | 41.14 ± 5.52 |
| Cholesterol ($\mu$mol/L) | 1.23 ± 0.32 | 0.92 ± 0.33 | 1.25 ± 0.34 | 1.10 ± 0.28 |
| TG ($\mu$mol/L) | 0.51 ± 0.04 | 0.86 ± 0.18 | 0.60 ± 0.60 | 0.47 ± 0.06 |
| HDL (IU/L) | 0.44 ± 0.39 | 0.44 ± 0.31 | 0.36 ± 0.07 | 0.42 ± 0.44 |
| LDL ($\mu$mol/L) | 0.37 ± 0.20 | 0.53 ± 0.19 | 0.69 ± 0.20 | 0.64 ± 0.16 |
| VLDL ($\mu$mol/L) | 0.23 ± 0.02 | 0.39 ± 0.08 | 0.27 ± 0.02 | 0.21 ± 0.03 |
| Cholesterol/HDL ($\mu$mol/L) | 2.80 ± 0.84 | 1.95 ± 0.73 | 2.60 ± 0.69 | 2.72 ± 0.80 |
| $K^+$ (mmol/L) | 6.92 ± 028 | 8.04 ± 0.50 | 7.02 ± 0.52 | 7.35 ± 0.48 |
| $Na^+$ (mmol/L) | 139.14 ± 3.34 | 140.71 ± 5.84 | 147.43 ± 2.79* | 145.57 ± 1.06* |
| $Cl^-$ (mmol/L) | 107.57 ± 2.72 | 110.70 ± 5.32 | 117.40 ± 4.37* | 114.00 ± 1.46* |
| $HCO_2^-$ (mmol/L) | 23.71 ± 0.91 | 22.28 ± 1.32 | 21.14 ± 0.39 | 21.14 ± 0.40 |
| Uric acid ($\mu$mol/L) | 106.6 ± 6.74 | 164.6 ± 19.18 | 168.6 ± 8.21 | 134.8 ± 9.50 |
| Urea (mmol/L) | 6.80 ± 0.44 | 7.31 ± 0.39 | 6.82 ± 0.72 | 4.60 ± 0.41# |
| Creatinine (mmol/L) | 76.57 ± 2.80 | 73.71 ± 8.4 | 70.14 ± 2.52 | 76.85 ± 4.06 |

Data are mean ± SEM values (*n* = 7). ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; VLDL, very-low-density lipoprotein.

*Significantly increased ($P \leq .05$) compared to control.

#Significantly reduced ($P \leq .05$) compared to control.

ceptor, down-regulation of the sensitized nociceptor, and/or blockage of the nociceptor at peripheral and/or central levels.[33] However, the extract given in similar doses orally in rats failed to produce antinociception in the tail-flick test, while pethidine, a centrally acting analgesic compound, exhibited significant activity in the dose of 50 mg/kg. The reason for this finding might be as a result of a central nervous system effect that is devoid of morphine-like in action.

As preliminary phytochemical results indicate, the antinociceptive effect of the aqueous extract of *V. amygdalina* may be due to the presence of tannins, saponins, phenols, flavonoids, steroids, and alkaloids. The presence of these same phytoconstituents in another species, *Vernonia cinerea*, has been reported to have analgesic and anti-inflammatory activity *in vivo*.[34]

The aqueous extract of *V. amygdalina* was also found to have *in vivo* activities against *P. berghei* in mice. At 200 mg/kg the antiplasmodial activity was comparable to that in CQ-treated mice; this result is consistent with earlier reports.[11] Sesqiterpene lactones such as vernolepin, vernolin, vernodalin, and hydroxyvernodalin isolated from *V. amygdalina* leaves have been reported to exhibit antiplasmodial activity against *Plasmodium falciparum*.[35] Empirically, this plant is used in decoctions alone, but other plants may be added to reduce the side effect of nausea that results from the herb's bitter taste.[14]

The acute oral toxicity results from the *V. amygdalina* extract (3.32 ± 0.15 g/kg p.o.) indicate that the extract may be safe based on the chemical labeling and classification of acute systemic toxicity on oral $LD_{50}$ values, recommended by the Organization for Economic Cooperation and Development.[36] However, it has been reported that the $LD_{50}$ is not an absolute value but an inherently variable biologic parameter that cannot be compared to constants such as molecular weight or melting point.[37] Nonetheless, the adverse signs of gait abnormalities, reduction in stereotypic activities, and deaths were seen at the higher doses.

In the subchronic study, hematologically, RBC counts decreased in the treated groups. The serum chemistry parameter shows an increase in the direct and total bilirubin value. In several organs, mainly heart and liver, cell damage is followed by increased levels of a number of cytoplasmic enzymes in the blood, a phenomenon that provides the basis for clinical diagnosis of heart and liver diseases, *e.g.*, liver enzymes are usually raised in acute hepatotoxicity but tend to be decreased with prolonged intoxication because of damage to the liver cells.[38] In this study, the extract did not exert significant effects on the serum chemistry parameters; the increase in bilirubin levels was probably due to the decreased RBC values.

In conclusion, the results of this study showed analgesic activity of the aqueous extract of *V. amygdalina* with clear and significant antiplasmodial effects in mice. There was no indication of toxicity in rats. Incidental findings below or above standard reference levels were all within control val-

ues based on historical reference ranges. This finding in the mouse model supports the traditional use of *V. amygdalina* in the treatment of pain and malaria.

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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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