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# Evaluation of Antivenom Potential of *Faidherbia albida* (Delile) A. Chev Root-Bark Extract against *Bitis arietans* Venom Toxicity

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**ABSTRACT**

**Background and Objective:** The venom of *Bitis arietans* (puff adder) is a polymer of life-threatening toxins, which causes severe tissue damage. Extracts from *Faidherbia albida* may possess the potentiality in mitigating venom-induced tissue damage. Hence, this research was aimed at evaluating the antivenom potential of *F. albida* root-bark extracts against *B. arietans* venom toxicity. **Materials and Methods:** *Faidherbia albida* roots were methanol-extracted and fractionated using hexane, ethyl acetate, butanol, and water. The most active fraction (PCF18) was purified via column and thin-layer chromatography. Rats (n = 28) were divided into seven groups: Normal control, venom only, venom+antivenom, venom+PCF18, venom+vitamin C, venom+vitamin E, and venom+PCF18+vitamins+antivenom. After 24 hrs, blood samples were collected to assess hepatic, renal, hematological, and anti-hemotoxic effects. Data were analyzed using one-way ANOVA, and means were compared by Duncan's multiple range test in SPSS version 20, with $p<0.05$ considered significant. **Results:** A chromatographic fraction; PCF18 was found to be the most active fraction. *Bitis arietans* venom induced profound alterations of liver enzymes, kidney and blood parameters, treatment with PCF18 showed significant ($p<0.05$) reduction in AST, ALP, ALT and a significant ($p<0.05$) increase in TP, B and ALB compared to venom control. PCF18 treated group also showed significant ($p<0.05$) reduction in creatinine, urea and $HCO_3$. There was no significant ($p>0.05$) difference between the hematological parameters of venom control and PCF18. However, PCF18, antivenom and adjuvant-treated groups showed significant ($p<0.05$) reduction in clotting and bleeding times as well as hemolysis and fibrinogenation compared to venom control. **Conclusion:** The findings established that PCF18 exhibited potent hepatic and renal protective effects and also possessed anti-hemotoxic potential against *B. arietans* venom-induced toxicity.

**KEYWORDS**

Hepatoprotective, nephroprotective, *Faidherbia albida*, *Bitis arietans*, anti-hemotoxic



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## INTRODUCTION

The venom of *Bitis arietans* (puff adder) contains toxins that causes serious damage to the kidneys and liver and also induces a profound effect on the clotting system and integrity of blood vessels[1]. These effects are primarily due to hemotoxins, especially snake venom metalloproteinases (SVMPs)[2]. In the kidney, *Bitis arietans* venom damages renal tissues, thereby altering blood levels of kidney function markers, such as creatinine and blood urea nitrogen (BUN). These alterations also result in changes in sodium and potassium concentrations[3]. The venom interferes with the blood-clotting system due to the presence of enzymes (serine and metalloproteinases)[4]. These venom enzymes inhibit the activities of clotting factors, leading to uncontrollable external and internal bleeding (hemorrhage)[5]. The immune system recognizes venoms as antigens; thus, venoms stimulate an increase in white blood cells, lymphocytes, and neutrophils, while their hemolytic effects reduce red blood cells[6].

Medicinal plants are used to manage infectious and noninfectious tropical disease[7]. Although advancements were made in the development of antivenoms against several species of snake, these antivenoms have several limitations, which include: Failure to cure the local and systemic tissue-damaging effects of snake venom, meaning antivenom only neutralizes snake venom's toxins and thus doesn't have any impact on repairing or curing any damaging effect caused to the liver, kidney, or blood vessels. A significant number of synthesized drugs were of plant origin[8]. Regassa *et al*.[9] reported that over 40% of the synthesized drugs originated from medicinal plants. Several medicinal plants have been traditionally used to treat snakebite victims; many have been scientifically validated. Apart from their antivenom potential, some of these plants have been reported to be effective in managing hepatic, renal, and blood-related diseases[10].

*Faidherbia albida* has been traditionally used to treat various ailments, including snakebite envenoming. Recently, it has also been documented to neutralize *B. arietans* venom[11]. Therefore, isolation of active compounds from *F. albida* and determination of their potential to neutralize venom-induced liver, kidney, and hemo toxicity will be vital for understanding the mechanism of action of the isolates in neutralizing *B. arietans* venom toxins. This study aimed to investigate the antivenom potential of *Faidherbia albida* root-bark extracts and their active chromatographic fraction in protecting against *Bitis arietans* venom-induced liver, kidney, and blood toxicity.

## MATERIALS AND METHODS

**Study area and duration:** This research was conducted in accordance with guidelines governing the conduct of research involving animals in Kebbi State University of Science and Technology, Aliero, Nigeria. The study was conducted over a period of 7 months from June 2025 to December 2025.

**Ethical consideration:** ethical approval was obtained from the University Research Ethics Committee.

**Collection and authentication of plant sample:** *Faidherbia albida* root was collected within Aliero town, Kebbi State, Nigeria and authenticated at the Department of Plant Science and Biotechnology, Abdullahi Fodio University of Science and Technology, Aliero. The voucher specimen number [KSUSTA/PSB/H/319] was subsequently deposited at the herbarium of the Department, for reference purposes.

**Methanol extraction:** One hundred and fifty grams (150 g) of the semi-powder of *Faidherbia albida* root was soaked in 1 liter of methanol for 72 hrs. The sample was subsequently filtered through muslin cloth. The filtrate was concentrated in a rotator evaporator, after which the concentrated crude was exposed to allow the remaining methanol to evaporate. The solid extract was preserved in a refrigerator until needed for use[12].

**Solvent-fractionation of *Faidherbia albida* root methanol extract:** *Faidherbia albida* root crude methanol extract was separated using n-hexane, ethyl acetate, butanol and water by liquid-liquid extraction. Fifty grams (50 g) of the methanol extract was diluted in 50 mL of water in a 500 mL separating funnel. This was then sequentially mixed (partitioned) with equal volumes of hexane, ethyl acetate, butanol and water. The solvents were introduced in order of increasing polarity to obtain n-hexane, ethyl acetate, butanol and last aqueous fractions respectively. The solvents were evaporated and the fractions yielded were preserved in a refrigerator for future use[13].

**Column chromatography:** Silica gel sized (80-120 mesh) slurry was prepared using N-hexane. The slurry was carefully poured into a chromatographic column and the silica gel was allowed to settle down to form an unbroken packing. Then, the excess N-hexane in the column was collected through the stop-cock of the column. A flat bed made of cotton wool was arranged on top of silica gel. About (3 g) of dry powdered crude fractionated aqueous extract was added on top of the cotton bed and covered with another cotton bed, and allowed to be activated for 30 min. Thereafter, the column was successfully eluted with a gradient of solvent systems, including N-hexane, ethyl acetate, methanol, and water, either singly or in various ratios.

**Thin layer chromatography (TLC):** A TLC plate of 4 cm wide and 10 cm long was used. A small amount of each column chromatographic fraction (CCF1-156) collected was spotted on a TLC plate. Then, the plates were placed into a TLC chamber containing a solvent system (methanol and water in specific ratios) to a depth of 1 cm, and the chamber was covered for a few minutes. Spot was visualized by dipping the plate into a covered container containing a solution of 10% V/V H2SO4 and 5% W/V vanillin in methanol. Bottles having similar *Rf* values were pooled together[14]. The Rf value was calculated using a formula below.

$$\text{RF value} = \frac{\text{Distance moved by the molecule (spot)}}{\text{Distance moved by the mobile phase (Solvent front)}}$$

**Experimental animals:** The albino rats used in this study were purchased from Animal House, Usmanu Danfodiyo University, Sokoto, Nigeria. They were brought to Animal House, Faculty of Life Science, Abdullahi Fodio University of Science and Technology, Aliero, in well-ventilated cages. Before the trial started, the rats were kept in a clean cage for 14 days to acclimatize. The rats were fed a typical rat diet and given unlimited access to water.

**Collection, milking and preparation of *B. arietan* venom:** The collection of *B. arietans*, milking and preparation of its venom were conducted using the same procedures as reported in Sani *et al.*[11].

**Standard snake venom:** Polyvalent snake venom antisera (Batch No.: 8904012480039, Manufacture Date: November 2022, Expiry Date: October 2026) manufactured by Bharat Serums and Vaccines Limited, India, was used as standard antivenom.

**Venom toxicity induction:** *Bitis arietans* venom sub-lethal dose (60% of $LD_{50}$) was used to induce toxicity to the animals. The $LD_{50}$ was based on a previous report by Sani *et al.*[11].

Twenty-eight (28) albino rats were randomly distributed into seven (7) groups, with each group containing four (4) rats. The venom was administered intraperitoneally (i.p.), and the extract was administered orally.

| Groups | Treatments |
|---|---|
| Group 1: | Received oral administration of distilled water and served as a normal control |
| Group 2: | Venom control was injected (IP) with 0.2 mg/kg b. wt. of snake venom |
| Group 3: | Received (i.p.) injection of 0.2 mg/kg b. wt. of snake venom, 30 min later administered (i.v.) with standard antivenin (1 mL per 0.45 mg venom), and served as the standard control |
| Group 4: | Received venom (0.2 mg/kg b. wt.) and were treated 30 min later with 100 mg/kg of the PCF18 |
| Group 5: | Received venom (0.2 mg/kg b. wt.) and were treated 30 min later with 100 mg/kg of vitamin C |
| Group 6: | Received venom (0.2 mg/kg b. wt.) and were treated 30 min later with 100 mg/kg of vitamin E |
| Group 7: | Received venom (0.2 mg/kg b. wt.) and were treated 30 min later with antivenin (1 mL/0.45 mg), PCF18, vitamin C and vitamin E (100 mg/kg b. wt.) |

The same volume of preparations was administered to all the groups. After venom and treatment administrations at a specific time interval, parameters such as bleeding time, clotting time, and defibrinogenation were recorded. Twenty-four hours later, the animals were sacrificed, and blood samples were collected for biochemical analysis.

**Hepatoprotection assay:** The Bowers and McComb[15] method was used to estimate alkaline phosphatase activity. Reitman and Frankel's[16], approach was used to measure the catalytic activity of aspartate aminotransferase and alanine aminotransferase. The bromocresol green method, as modified by Doumas *et al.*[17], was used to measure albumin. The Biuret reaction method to determine total protein was employed[18]. Doumas *et al.*[19], calorimetric method was used to measure total and direct bilirubin.

**Nephroprotection assay:** Beale and Croft[20], colorimetric method was used to measure serum urea. Jaffe's approach, as outlined by Bartels and Bohmer[21], was used to measure serum creatinine. The Henry *et al.*[22] method was used to calculate the serum uric acid concentration. Flame photometry was used to assess the sodium and potassium ions in serum[23]. The titration/volumetric approach were used to test serum bicarbonate and chloride ions[24].

**Haemato-protection analysis:** Hematological parameters via, White Blood Cells count (WBC), Lymphocytes (LYM), Granulocytes (GRA), Red Blood Count (RBC), Hemoglobin (HGB), Hematocrits (HCT), Mean Cell Volume (MCV), Mean Corpuscular Heamoglobin (MCHC), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelets (PLT), and Procalcitonin, were examined using a Sysmex XS800i automated hematological analyzer (Sysmex Corporation, USA)[25].

**Determination of bleeding time:** Bleeding time was assessed using the procedure described by Mohammed *et al.*[26]. Two-hour post-treatment, the rats were carefully tail-punctured with a needle. Blood was gently blotted from the punctured area using white filter paper. Measurements were recorded every 15 sec. The final result was noted when the filter paper no longer stained with blood.

**Determination of clotting time:** The time required for fresh blood to clot on glass slides (Clotting time) was assessed using the methodology established by Ieko *et al.*[27]. Three hours after treatment of the animals, the tails of the rats were bled, and blood samples were dropped on a sterile, flat slide. Every 15 sec, the tip of an office pin was inserted into the blood until a thread-like structure became evident between the blood drop and the pin's tip. This thread-like formation indicated the presence of a fibrin clot, and the time was subsequently recorded.

**Inhibition of venom defibrinogenating activity:** The venom effect, which, when injected (*i.d.*) into a rat, causes incoagulable blood 4 hrs later, after the animal treatment defibrinogenating activity (DFA)] was examined[28].

**Inhibition of venom haemorrhagic activity:** The least amount of venom, which when injected intradermally (*i.d*) into rats, results in a 10 mm diameter haemorrhagic lesion 6 hrs later"[29]. Six hours after the animal treatment, the haemorrhagic lesions were measured and recorded.

**Inhibition of venom necrotizing activity:** The amount of venom which, when injected (*i.d*) into rats, results in a necrotic lesion of 5 mm diameter 3 days later"[29]. Three days after the animal treatment, the necrotic lesions were measured and recorded.to assess the anti-necrotizing effect of the fraction.

**Inhibition of venom haemolytic activity:** Haemolysis induced by venom and its subsequent neutralization by the fraction were assessed according to the methodology outlined by Herbert *et al*.[30]. Blood samples of 1 mL each were collected using heparin as an anticoagulant. The collected blood was centrifuged at 3000 rpm for 10 min, separating the packed cells. These cells were washed three times with phosphate buffer (0.15 M, pH 7.4) and then re-centrifuged to isolate the cells. Subsequently, 3 mL of 0.15 M phosphate buffer (pH 7.4) was added to each test tube, and the mixture was shaken thoroughly. The supernatant absorbance was measured at 540 nm. All assays were performed in triplicate. Finally, the percentage of haemolysis and protection was calculated using the following formulas:

$$\text{Inhibition (\%)} = \frac{AC - AT}{AC} \times 100$$

Where,
AT = Absorbance of treated sample
AC = Absorbance of control.

**Data analysis:** The data were analysed statistically using One-Way Analysis of Variance (ANOVA). Means were compared using the Duncan multiple comparison test with the aid of the Statistical Package for the Social Sciences (SPSS) version 20. $p<0.05$ is considered significant.

## RESULTS AND DISCUSSION

**Column and thin-layer chromatography:** One hundred and fifty-six column fractions were obtained; the fractions were pooled into 21 fractions using thin-layer chromatography. Pooled chromatographic fraction 18 (PCF18) was selected for this study as the most active fraction.

**Hepato-protective effect of PCF18:** The hepato-protective effect of the pooled chromatographic fraction 18 (PCF18) against the *B. arietans* venom-induced toxicity is presented in Table 1. The results showed significant ($p<0.05$) increases in AST, ALT, ALP, TB and DB in the induced negative control group compared to normal and all treatment groups. Adjuvant (combination) and vitamin E treatments significantly ($p<0.05$) decreased AST level, while antivenin control and vitamin C treatments significantly ($p<0.05$) decreased the level of ALT. The ALP level did not differ significantly ($p>0.05$) between treatment groups and the normal control. Induced control showed significant ($p<0.05$) reductions in total protein (TP) and albumin (ALB), with ALB levels in the PCF18, vitamin C, and adjuvant groups comparable ($p>0.05$) to those in the normal control. The TB levels were comparable in the PCF18 and adjuvant groups to the normal control, whereas the antivenin control and vitamin C treated groups significantly ($p<0.05$) reduced TB levels compared to the normal control. Only antivenin and PCF18 groups significantly ($p<0.05$) decreased in DB compared to the normal control.

Table 1: Hepatoprotective effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity in rats

| Parameter | Normal control | Venom control | Standard antivenin control | PCF18 100 mg/kg b. wt | Vitamin C 100 mg/kg b. wt | Vitamin E 100 mg/kg b. wt | Adjuvant |
|---|---|---|---|---|---|---|---|
| AST (U/L) | 31.09±0.46$^{c}$ | 106.45±0.92$^{g}$ | 45.54±0.31$^{d}$ | 53.75±0.76$^{e}$ | 64.39±0.61$^{f}$ | 28.09±0.46$^{b}$ | 24.08±1.32$^{a}$ |
| ALT (U/L) | 17.29±0.22$^{c}$ | 36.78±0.33$^{g}$ | 13.91±0.11$^{a}$ | 23.68±0.22$^{e}$ | 16.54±0.19$^{b}$ | 19.24±0.23$^{d}$ | 24.94±0.27$^{f}$ |
| ALP (U/L) | 49.68±3.19$^{b}$ | 90.06±2.36$^{c}$ | 46.92±5.75$^{b}$ | 55.20±1.59$^{b}$ | 36.80±2.43$^{a}$ | 53.36±2.43$^{b}$ | 57.04±3.32$^{b}$ |
| TP (g/L) | 5.73±0.31$^{b}$ | 3.66±0.02$^{a}$ | 6.97±0.02$^{d}$ | 7.12±0.01$^{d}$ | 6.30±0.03$^{c}$ | 6.89±0.02$^{d}$ | 6.17±0.02$^{c}$ |
| ALB (g/L) | 2.74±0.02$^{b}$ | 1.60±0.00$^{a}$ | 3.47±0.17$^{c}$ | 2.95±0.02$^{b}$ | 2.87 ±0.01$^{b}$ | 3.28±0.00$^{c}$ | 2.89±0.01$^{b}$ |
| TB (mg/dL) | 0.74±0.02$^{bc}$ | 1.07±0.01$^{e}$ | 0.66±0.02$^{a}$ | 0.67±0.01$^{ab}$ | 0.93±0.06$^{a}$ | 0.85±0.03$^{d}$ | 0.79±0.01$^{cd}$ |
| DB (mg/dL) | 0.33±0.01$^{b}$ | 0.48±0.00$^{f}$ | 0.29±0.01$^{a}$ | 0.30±0.21$^{a}$ | 0.45±0.01$^{e}$ | 0.38±0.01$^{d}$ | 0.35±0.01$^{c}$ |

Results are presented as mean SEM (n = 3), ANOVA (one-way) and Duncan's multiple comparison test were used to test for significance in SPSS version 20.0. Groups with similar alphabetical superscript in rows are not significantly different at (p>0.05). TB: Total bilirubin, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, TP: Total protein, ALB: Albumin, DB: Direct bilirubin and AST: Aspartate aminotransferase, PCF18- pooled chromatographic fraction 18 Adjuvant= antivenin+PCF18 vitamin C+vitamin E (100 mg/kg)

Table 2: Nephroprotective effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity in rats

| Parameter | Normal control | Venom control | Standard antivenin control | PCF18 100 mg/kg b. wt | Vitamin C 100 mg/kg b. wt | Vitamin E 100 mg/kg b. wt | Adjuvant |
|---|---|---|---|---|---|---|---|
| Creatinine (mg/dL) | 28.90±0.51$^{a}$ | 76.64±6.15$^{c}$ | 66.37±0.51$^{b}$ | 62.41±0.39$^{b}$ | 25.64±0.91$^{a}$ | 63.15±0.51$^{b}$ | 60.77±0.83$^{b}$ |
| Urea (mmol/L) | 8.08±0.23$^{b}$ | 8.81±0.01$^{e}$ | 9.18±0.02$^{f}$ | 8.43±0.02$^{c}$ | 8.85±0.09$^{e}$ | 7.67 ±0.03$^{a}$ | 8.66±0.01$^{d}$ |
| Uric acid (mg/dL) | 8.22±0.06$^{d}$ | 4.00±0.06$^{a}$ | 8.89±0.06$^{e}$ | 8.17±0.04$^{cd}$ | 7.56±0.04$^{b}$ | 8.35±0.06$^{d}$ | 7.99±0.10$^{c}$ |
| $K^{+}$ (mmol/L) | 9.67±1.45$^{a}$ | 21.67±1.20$^{c}$ | 16.67±1.85$^{b}$ | 21.67±2.19$^{c}$ | 21.33±0.88$^{c}$ | 19.00±0.00$^{bc}$ | 18.00±0.57$^{bc}$ |
| $Na^{+}$ (mmol/L) | 130.33±4.26$^{a}$ | 154.33±17.85$^{a}$ | 134.33±2.60$^{a}$ | 133.00±3.21$^{a}$ | 143.00±1.73$^{a}$ | 138.00 ±2.65$^{a}$ | 131.67±0.33$^{a}$ |
| $Cl^{-}$ (mmol/L) | 90.00±3.79$^{a}$ | 110.67±4.91$^{c}$ | 99.00±0.58$^{ab}$ | 104.33±3.71$^{bc}$ | 110.33±4.26$^{c}$ | 114.00±1.15$^{c}$ | 97.00±1.00$^{ab}$ |
| $HCO_3^{-}$ (mmol/L) | 43.33±2.60$^{a}$ | 64.00±2.08$^{d}$ | 48.67±3.38$^{ab}$ | 52.33±2.40$^{bc}$ | 59.00±2.00$^{cd}$ | 62.67±1.33$^{d}$ | 47.00±1.73$^{ab}$ |

Results are presented as mean SEM (n = 3), ANOVA (one-way) and Duncan's multiple comparison test were used to test for significance in SPSS version 20.0. Groups with similar alphabetical superscripts in rows are not significantly different (p>0.05). Chloride =(Cl-). Potassium =(K+), Sodium= (Na+) and PCF18- pooled chromatographic fraction 18, Adjuvant = antivenin+PCF18 vitamin C+vitamin E (100 mg/kg)

**Nephro-protective effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity:** The nephro-protective effect of PCF18 on *B. arietans* venom-induced toxicity in rats is presented in Table 2. The results revealed significant (p<0.05) increases in creatinine, urea, Cl , and $HCO_3^{-}$ in the induced control compared to the normal control and all treatment groups. There was no significant (p>0.05) difference in creatinine levels between the vitamin C-treated group and the normal control; however, the antivenin, PCF18, vitamin E, and adjuvant groups showed significant increases (p<0.05). Urea levels were significantly reduced in the vitamin E-treated group compared to the normal control, while antivenin, PCF18, vitamin A, and adjuvant groups showed significant increases (p<0.05). Only the antivenin group significantly (p<0.05) reduced K levels compared to the induced control, though not to the level of the normal control (p>0.05). Uric acid levels decreased significantly (p<0.05) in the induced control compared with the normal control and all treatment groups. However, no significant changes (p>0.05) were observed in the PCF18 and vitamin E-treated groups compared to the normal control, while vitamin C and adjuvant groups significantly decreased (p<0.05), and the antivenin group significantly increased (p<0.05) compared to the normal control. Only $HCO_3^{-}$ levels of antivenin control and adjuvant-treated groups were comparable (p>0.05) compared to normal control; meanwhile, antivenin control, PCF18, vitamin C, and vitamin E significantly (p<0.05) increased compared to normal control.

**Hematoprotective effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity:** The protective effect of PCF18 on hematological parameters of *B. arietans* venom-induced toxicity in rats is presented in Table 3. There were no significant differences (p>0.05) in WBC, GRA, HCT, MCH, MPV, PDW, and PCT of induced control, antivenin control, PCF18, vitamin C, vitamin E, and adjuvant treatment groups compared to normal control. There was a reduction in RBC in the induced control compared to the normal control and all treatment groups. However, the reduction is significant (p<0.05) only when compared with the normal control, antivenin control, vitamin C, and vitamin E-treated groups, respectively. Similarly, HGB increased in the induced control compared with the normal

Table 3: Hematoprotective effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity in rats

| Parameter | Normal control | Venom control | Standard antivenin control | PCF18 100 mg/kg b. wt | Vitamin C 100 mg/kg b. wt | Vitamin E 100 mg/kg b. wt | Adjuvant |
|---|---|---|---|---|---|---|---|
| WBC(x10$^3$/UL) | 15.14±2.21$^a$ | 18.03±0.88$^a$ | 18.72±1.11$^a$ | 15.25±0.58$^a$ | 17.38±2.19$^a$ | 18.20±1.80$^a$ | 15.75±2.85$^a$ |
| LYM (%) | 64.70±5.25$^a$ | 75.20±10.33$^{ab}$ | 77.33±10.29$^{ab}$ | 87.27±1.59$^c$ | 88.80±1.57$^c$ | 89.80±1.42$^c$ | 85.60±0.42$^c$ |
| GRA (%) | 3.97±1.40$^a$ | 6.40 ±1.29$^a$ | 4.30±1.32$^a$ | 5.50±1.17$^a$ | 4.43±1.28$^a$ | 3.03±0.23$^a$ | 5.77±0.39$^a$ |
| RBC (10$^6$/UL) | 8.87±0.43$^b$ | 5.78±1.32$^a$ | 8.41±0.35$^b$ | 7.47±0.45$^{ab}$ | 7.87±0.38$^b$ | 8.53±0.25$^b$ | 7.04±0.06$^{ab}$ |
| HGB (g/dL) | 11.77±0.69$^a$ | 16.03±0.43$^c$ | 15.07±0.78$^{bc}$ | 14.00±0.91$^{bc}$ | 14.23±0.85$^{bc}$ | 15.23±0.62$^{bc}$ | 13.50±0.36$^{ab}$ |
| HCT (%) | 46.43±0.49$^a$ | 47.47±2.05$^a$ | 52.10±2.73$^a$ | 47.40±3.42$^a$ | 48.63±3.24$^a$ | 52.80±2.06$^a$ | 45.80±1.76$^a$ |
| MCV (fL) | 55.10±1.74$^a$ | 70.80±4.65$^c$ | 61.90±0.87$^{ab}$ | 63.37±1.54$^b$ | 61.73±1.48$^{ab}$ | 61.90±0.87$^{ab}$ | 64.93±2.22$^{bc}$ |
| MCH (pg) | 18.30±0.49$^a$ | 18.83±0.58$^a$ | 17.93±0.35$^a$ | 18.70±0.31$^a$ | 18.10±0.46$^a$ | 17.90±0.39$^a$ | 19.13±0.43$^a$ |
| MCHC (g/dL) | 20.63±0.66$^a$ | 31.40±1.92$^b$ | 28.97±0.29$^b$ | 29.53±0.24$^b$ | 29.30±0.38$^b$ | 29.90±0.31$^b$ | 29.53±0.35$^b$ |
| PLT (10$^3$/UL) | 352.00±22.74$^a$ | 654.33±17.67$^c$ | 426.00±36.06$^{ab}$ | 527.33±26.19$^{bc}$ | 507.00±38.37$^{abc}$ | 506.67±96.92$^{abc}$ | 634.67±64.67$^c$ |
| MPV (fL) | 6.57±0.21$^a$ | 6.87±0.49$^a$ | 6.93±0.25$^a$ | 7.10±0.66$^a$ | 7.00±0.70$^a$ | 6.93±0.25$^a$ | 6.90±0.36$^a$ |
| PDW (fL) | 7.03±0.55$^a$ | 7.10±0.12$^a$ | 7.77±0.33$^a$ | 7.60±0.13$^a$ | 7.63±0.32$^a$ | 7.97±0.37$^a$ | 7.23±0.37$^a$ |
| PCT (fL) | 0.43±0.03$^a$ | 0.32±0.00$^a$ | 0.39±0.02$^a$ | 0.38±0.02$^a$ | 0.36±0.02$^a$ | 0.35±0.02$^a$ | 0.44±0.02$^a$ |

Results are presented as mean SEM (n = 3), ANOVA (one-way) and Duncan's multiple-comparison test were used to test for significance in SPSS version 20.0. groups with similar alphabetical superscript in rows are not significantly different at (p>0.05), White blood cells count (WBC), lymphocytes (LYM), granulocytes (GRA), red blood count (RBC), hemoglobin (HGB), hematocrits (HCT), mean cell volume (MCV), mean corpuscular heamoglobin (MCHC), mean corpuscular heamoglobin concentration (MCHC), platelets (PLT), and procalcitonin (PCT). PCF18- pooled chromatographic fraction 18. Adjuvant = antivenin+PCF18 vitamin C+vitamin E (100 mg/kg)

Table 4: Anti-hemotoxic effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity in rats

| Treatment | Bleeding time (s) | Clothing time (s) | De-fibrinogenation (s) | Hemorrhagic activity (mm) | Necrotizing Activity (mm) | Hemolysis (%) |
|---|---|---|---|---|---|---|
| Normal control | 90.54±4.91$^a$ | 89.60±9.81$^{ab}$ | 65.33±2.33$^a$ | - | - | - |
| Distilled $H_2O$ (5 mL/kg b. wt) | | | | | | |
| Negative control | 161.57±30.35$^b$ | 131.00±16.93$^c$ | 105.33±8.57$^b$ | - | - | 71.14±0.38$^e$ |
| Positive control | 75.69±18.36$^a$ | 89.40±9.10$^{ab}$ | 75.00±1.73$^a$ | - | - | 29.94±0.61$^a$ |
| PCF18 (100 mg/kg) | 72.38±21.47$^a$ | 97.60±9.48$^{abc}$ | 73.67±2.73$^a$ | - | - | 48.15±1.52$^c$ |
| Vitamin C 100 mg/kg | 90.22±23.57$^a$ | 130.20±16.57$^c$ | 105.33±1.45$^b$ | - | - | 62.19±0.15$^d$ |
| Vitamin E 100 mg/kg | 128.57±10.50$^{ab}$ | 115.00±11.96$^{bc}$ | 98.00±3.79$^b$ | - | - | 63.00±0.14$^d$ |
| Adjuvant | 82.57±12.06$^a$ | 73.00±5.27$^a$ | 71.33±0.88$^a$ | - | - | 23.38±1.31$^a$ |

Results are presented as mean SEM (n = 3). ANOVA (one-way) and Duncan's multiple-comparison test were used to test for significance in SPSS version 20.0, Groups with similar alphabetical superscript in rows are not significantly different at (p>0.05), PCF18- pooled chromatographic fraction 18, - = Not observed, Adjuvant = antivenin +PCF18 vitamin C +vitamin E (100 mg/kg)

control and all treatment groups; however, the increase was significant (p<0.05) only when compared with the normal control and the adjuvant treatment groups. A significant (p<0.05) increase in MCV was observed in the induced control compared with the normal control and all treatment groups except the adjuvant group; meanwhile, only the MCVs of the antivenin control, vitamin A, and vitamin E were comparable (p>0.05) with the normal control. An increase in MCHC was observed in the induced control compared with the normal control and all treatment groups, but the difference was significant only compared with the normal control (p>0.05).

**Anti-hemotoxic effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity:** The anti-hemotoxic effect of pooled chromatographic fraction 18 on *B. arietans* venom-induced toxicity in rats is presented in Table 4. The results revealed that induced control showed significant (p<0.05) increases in bleeding time and fribrinogenation activity compared to normal control and all treatment groups. Meanwhile, the bleeding time of all treatment groups (antivenin, PCF18, vitamin C, vitamin E, and adjuvant) was not significantly different from that of the normal control (p>0.05). However, only the fibrinogen activity of antivenin control, PCF18, and adjuvant was comparable (p>0.05) with that of the normal control. The clothing time of the induced control group significantly (p<0.05)

decreased only compared to the normal control, antivenin, and adjuvant-treated groups. A significant ($p<0.05$) increase in hemolytic activity was observed in the induced control compared to antivenin, PCF18, vitamin C, vitamin E, and adjuvant treatment groups, while only the adjuvant-treated group is comparable ($p>0.05$) to the antivenin control.

## DISCUSSION

Venom-induced liver toxicity is a serious condition that can be caused by various snake venoms, leading to enzyme (AST, ALT, and ALP) and bilirubin release in circulation and decreased serum levels of albumin and total protein[31]. In the present study, the untreated control exhibited these conditions; these effects were not observed in PCF18, standard antivenin control, vitamin C, vitamin E and adjuvant treated groups, suggesting possible hepatoprotection. The present study agrees with the findings of Direito *et al*.[32], who also reported the hepatoprotective effects of some plant-derived compounds. Similarly, Abdulkhaleq *et al*.[33] reported the hepatoprotective effect of vitamin C and E against several liver induced toxicity models. The hepatoprotection activity observed might attributed to the antioxidants properties of plant derived compounds, vitamin C and E, which directly neutralize free radicals that induced liver damage from toxins (such as venom toxin), and disease, thereby decreasing elevated liver enzymes (ALT, AST), and increasing protein synthesis.

Venom-induced nephrotoxicity, or kidney damage caused by snake venoms, can occur through various mechanisms, including direct nephrotoxicity[34]. In the present study, an alteration in serum kidney parameters was observed in the venom control, leading to elevated creatinine and urea levels, metabolic alkalosis (high $HCO_3$), hyperchloremia, hypouricemia, hypernatremia, and hyperkalemia. However, the ability of PCF18 (alphamethyl cennamoyl chloride), standard antivenin control, vitamin C, vitamin E and adjuvant to restore creatinine, urea, uric acid, $Na^+$, and $HCO_3$ indicates a possible nephroprotective effect of this fraction. Shabbir *et al*.[35] also reported the nephroprotective activity of some medicinal plants and their isolated compounds against the toxic effects of snake venom. The nephroprotective effect observed in the present study might be attributed to the strong antioxidants properties of PCF18, vitamins C and E, which might ultimately combat oxidative stress and lipid peroxidation that result in kidney cells damage from venom toxins.

Some plant extracts exhibit protective effects against venom-induced hematotoxicity, with studies showing that several plants and their isolated compounds can neutralize venom effects and reduce hematological damage[35-37]. In the present study, PCF18 showed a protective effect on some haematological parameters such as (RBC, HGB, and MCV). The increased RBC concentration and decreases in HGB level in PCF18-treated groups might be directly attributed to the fraction's ability to inhibit the haemolytic effect of *B. arietans* venom. This is supported by the findings[36] who reported that some isolated plant derivatives inhibit the hemolytic activity of *B. arietans* venom.

*Bitis. arietans* causes significant bleeding (hemorrhage), systemically and locally, because of the action of metalloproteinases that degrade capillary blood vessels and result in uncontrolled bleeding. The venom also interferes with normal physiological blood clotting mechanisms, leading to coagulopathy and hypofibrinogenemia[36,37]. The phospholipase $A_2$ present in the venom also induced red blood cell degradation (hemolysis)[4]. The facts stated above are in line with the findings of the present study, as the venom-induced control showed significant increases in bleeding time, clotting time, and fibrinogenating activity, and a decrease in red blood cells. Interestingly, treatment with PCF18 results in significant reductions in clotting time and bleeding time, fibrinogen activity, and hemolysis. These activities might be linked to the isolates' (PCF18) ability to inhibit venom enzymes (metalloproteinase and phospholipase A2). This agrees with the findings of Sittishevapark *et al*.[38] who also reported venom enzyme inhibition as a mechanism of anti-hemotoxic effect exerted by medicinal plants.

## CONCLUSION

This study documented that pooled chromatographic 18 (PCF18), standard antivenin, and adjuvant (antivenin+PCF18 vitamin C+vitamin E) treated groups exhibited potent hepatoprotective effect via decreasing elevated liver enzymes (ALT, ALP, AST), and increasing protein synthesis. Similarly, PCF18, standard antivenin, and adjuvant-treated groups showed a profound nephroprotective effect through potential normalization of creatinine, urea, uric acid concentration altered by *B. arietans* venom-induced toxicity. The present study also established the hematopoietic protective efficacies of PCF18 and adjuvant treated groups via reduced immune system components, anti-haemolytic, and reduction in both bleeding and clothing time triggered by *B. arietans* venom-induced toxicity. Hence, this study established an organ- and/or tissue-damaging inhibitory effect (hepatoprotection, nephroprotection, and hemoprotection) as one of the mechanisms conferred by the *Faidherbia albida* root chromatographic isolate (PCF18) against *B. arietans* venom-induced toxicity.

## SIGNIFICANCE STATEMENT

*Bitis arietans* (puff adder) venom is a life-threatening toxin, which causes severe tissue damage; it is among the leading causes of morbidity and fatality, especially in underdeveloped countries. The present findings established that *Faidherbia albida* root extracts exhibited potent hepatoprotective, nephroprotective and hemoprotective effect against *B. arietans* venom induced toxicity. Thus serving as a lead to develop a safe, readily available and affordable antivenoms. The present study also validates the mechanism of antivenom activity by which *Faidherbia albida* root extracts exerts its effect.

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