

# ANTI-VENOM POTENTIAL OF AQUEOUS EXTRACT OF ROOT OF *VETIVERIA ZIZANIOIDES* (L.) NASH AGAINST *ECHIS CARINATUS* (SAW-SCALED VIPER) VENOM

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## Abstract

*Vetiveria zizanioides* Nash (family: Poaceae) is a well-known medicinal plant in South India traditional medicine for the management of many diseases, but investigation concerning its pharmacological characteristics are rare. In this study, we evaluate its venom neutralizing properties against *Echis carinatus* venom in mice. Freshly collected *Vetiveria zizanioides* roots were air dried, powdered and extracted in aqueous. To study the anti-venom properties, the venom was administered intraperitoneally the mice (male) weighing between 18-25gm were randomly divided into eight (8) groups of five (5). Group 1-8 received water, plant extract, venom alone (5µg/ml; middle dose-10µg/ml; and high dose- 15µg/ml), plant extract (01 ml at four times) a respectively. After 30 minutes venom injected through i.p the extract was administered orally at a dose of 0.1ml for every 2 hours during eight hours. Later envenomation different parameters such as blood count, antioxidant enzyme activities like CAT, SOD, MDA, GPx, GSH, AST, ALP, and ALT were noted. At the end of the observation eight hours period, animals were sacrificed and dissected for adverse effects if any based on histopathology examination of their brain, heart, liver, and kidney. Our results showed that *Vetiveria zizanioides* aqueous root extract (VZRE) neutralized some biological effects of *Echis carinatus* venom (ECV). The venom increased the enzyme activities and other blood parameters. The plant extract was able to reduce these parameters in the extracted treated groups. Details of the results are discussed. From this study, it is clear that *Vetiveria zizanioides* root extract had anti-venom activity in animal model. The above result indicate that the plant extract possess potent snake venom neutralizing capacity and could potentially be used for therapeutic purpose in case of snake bite envenomation.

Keywords: *Vetiveria zizanioides*, *Echis carinatus*, Blood count, Antioxidant Enzyme activities

## 1. Introduction

In recent years the subject of plants used to treat snake bite has attracted the attention of several reviewers. Plants and their extracts have been used for the treatment of snake bite in most areas where venomous species are endemic. Due to rapid development in the field of science and technology, and vast growth in industrial and agricultural sector, poisoning due to snake bite is spreading like wild fire. It is well known that snake venom is complex mixture of enzymes, peptides and proteins of low molecular mass with specific chemical and biological activities. Although the full burden of human suffering attributable to snake bite remains obscure, hundreds of people are known to be envenomed and thousands of people were killed or maimed by snakes every year. To treat envenoming, the production and clinical use of antivenom must be improved. Since some research articles prove that snake venom contains several neurotoxic, cardiotoxic, cytotoxic, nerve growth factor, lectins, disintegrins, haemorrhagins and many other different enzymes. These proteins not only inflict death to animals and humans, but can also be used for the treatment of thrombosis, arthritis, cancer and many other diseases (Sanjay *et al.*, 2002).

Many medicinal plants are used to treat snake bite such plants are recorded in texts dealing with the Ethnopharmacology of geographical areas but in most instances there is no information on the method of use of the plant, the part of the plant used for the type of snake whose venom it is supposed to counteract. Plants and their extracts have been used for the treatment of snake bite in most areas where venomous species are endemic (Houghton and Osibogun, 1991). In spite of this wealth of Ethnopharmacological information only a relatively few species have been tested scientifically for anti-venom activity and the species where an active compound has been isolated are very few in number. The aim of the present study was to evaluate the ability of *Vetiveria zizanioides* extract to neutralize some biological effects of *Echis carinatus* venom in mice.

## 2. Materials and methods

### 2.1. Plant sample

Fresh roots of *Vetiveria zizanioides* were collected from Nehru Herbal Gardens, Nehru Arts and Science College. The plant material was identified and authenticated in the Botanical Survey of India, Coimbatore, Tamil Nadu, India (Ref no: BSI/ SRC/ 5/23/2011-12/Tech-1673). The roots were cleaned and air-dried for a week and powdered using pulverize and stored in an air tight container. The powdered roots (200 gm) were extracted with 250 ml water by using Soxhlet extractor for three days. The extraction with water was repeated three times. The obtained water extract was filtered and evaporated using a rotary and freeze drier to give the crude dried extract. The extract was stored at room temperature until use.

### 2.2. Venom sample

The freeze-dried snake venom powder of *Echis carinatus* was obtained from Irula's snake Catchers Industrial Co-operative Society Limited, Chennai and was stored at 4°C.

### 2.3. Animals

The male mice of weighing 18-25 gm were used for the study. The animals were kept in plastic cages with saw dust as bedding under condition of 12: 12 hr light and dark cycle and animals were fed on standard mice pellets, and water was supplied ad libitum. This study was approved by the Department of Animal Sciences, Bharathidasan University, Thiruchirapalli according to the institutional reference no: BDU/ IAEC/62/2013.

## 2.4. Experimental design

The male mice were randomly divided into eight groups of five mice

Group 1 (G1): Control group that received only water

Group 2 (G2): Control group that *Vetiveria zizanioides* root extract

Group 3 (G3): Envenomed mice treated with *Echis carinatus* in low dose (5µg/ml)

Group 4 (G4): Envenomed mice treated with *Echis carinatus* in middle dose (10µg/ml)

Group 5 (G5): Envenomed mice treated with *Echis carinatus* in high dose (15µg/ml)

Group 6 (G6): Envenomed mice low dose (5µg/ml) treated with *Vetiveria zizanioides* root extract (0.1 ml at 4 time)

Group 7 (G7): Envenomed mice middle dose (10 µg/ml) treated with *Vetiveria zizanioides* root extract (0.1 ml at 4 time)

Group 8 (G8): Envenomed mice high dose (15µg/ml) treated with *Vetiveria zizanioides* root extract (0.1 ml at 4 time)

## 2.5. Blood sample collection and measurement of some hematological parameters

At the end of the experimental period, the animals were made in active by chloroform anaesthization. Blood samples were collected through cardiac puncture into EDTA bottles to prevent coagulation. The blood samples were centrifuged for five minutes, results were read on the hematocrit realer for White Blood Cell (WBC), Red Blood Cell (RBC), and hemoglobin level, platelet as described by Baker and Silverton (1985).

## 2.6. Antioxidant Enzyme Activity Assays

Catalase activity (CAT), superoxide dismutase(SOD), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH), Alkaline Phosphatase (ALP), Alanine amino transferase (ALT), Asparate amino transferase (AST) activity assay were determined according to the method of Sinha (1972), Marklund and Marklund (1974), Rotruck *et al.* (1973), Moron *et al.* (1979), Ohkawa *et al.* (1979), Schmidt and Schmidt (1963), Schmidt and Schmidt (1963), and Reitman and Franked (1957), respectively.

## 2.7. Histological studies

The histology of tissue was studied adopting the routine paraffin method (Hamilton, 1977) and resin embedding method (Hayat, 1981). A section of tissue must be mounted over the slide for the microscopic examination.

## 2.8. Statistical analysis

Data were statistically analyzed using SPSS software (16.0) descriptive analyses including mean and standard error were applied to all biochemical measurements in control and treated groups. Group differences were considered statistically significant at the level of  $p < 0.05$ . One –way ANOVA was used to assess differences between the treated groups in all the biochemical measurements and hematological parameters.

## 3. Results

### 3.1. Hematological Parameters

Table 1 shows the effect of *Vetiveria zizanioides* extract on hematological parameters were significantly ( $p < 0.05$ ) reduced in group 3, 4 and 5 (envenomed mice) when compared with the extract treated group 6, 7 and 8. The WBC, RBC, HGB, PLT was most reduced in venom treated groups when compared with other hematological parameters. This therefore means that the extract neutralized the biological effect induced by the venom in the extract treated group that had increased HGB, WBC, RBC, and PLT.

WBC level was  $7.5 \times 10^9/L$  in control group, but this value reduce to 5.8, 3.3 and  $1.2 \times 10^9/L$  in G3, G4 and G5, respectively. On the other hand, VZRE applications, in low, medium and high

doses (G6, G7 and G8) alleviate adverse effect of venom and increased to 7.5, 7.6 and 4.8. Increasing ratio was the %29.31, %130.3 and %300 compared to ECV tree doses (G3, G4 and G5), respectively but this value was still lower the control treatment. RBC level was  $7.7 \times 10^{12}/L$  in control group, but this value reduce to 4.4, 2.1 and  $0.8 \times 10^{12}/L$  in G3, G4 and G5, respectively. On the other hand, VZRE applications, in low, medium and high doses (G6, G7 and G8) alleviate adverse effect of venom and increased to 7.3, 4.9 and 3.6. Increasing ratio was the %65.90, %133.3 and %350 compared to ECV treated tree doses, respectively but this value was two fold lower the control treatment. Similar results were observed for HGB and platelet and increasing ratio was the %41.2, %60.9 and %145.4 for HGB and %0.44, %3.99 and %17.3 for PLT compared to ECV treated tree doses (G3, G4 and G5), respectively (Table 1).

### 3.2. Antioxidant Enzyme Activity Assays

The result of the effect of *Vetiveria zizanioides* root extract (VZRE) on the activities of the enzymes and antioxidant assayed is as presented in Fig.1 to Fig. 24. The snake venom induced increased activity (G3, G4 and G5). The extract treated group i.e., 6, 7 and 8 had reduced enzyme activity (ALP, AST, ALT) in the Brain, Liver and Kidney. These reductions were statistically significant ( $p < 0.05$ ) when compared with the control group.

The Antioxidant activity of Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Reduced Glutathione (GSH), Malondialdehyde (MDA) show marked decrease ( $p < 0.05$ ) in venom-treated mice. These antioxidant enzyme activities increased significantly in the VZRE (Fig.1 – Fig. 24).

CAT activity of brain was  $0.28 \mu M g^{-1}$  protein in control group, and it has been the highest value compared to the other treatment. But when PEB was applied, CAT activity of brain decreased as to CB. VZRE and with plant extract application, in low, medium and high doses (G1, G2 and G3) improving adverse effect of venom and decreased to 0.26, 0.21 and 0.18 respectively. Decreasing ratio was the %7.14, %25.00 and %35.71 compared to CB, respectively (Figure 1). But CAT activity of liver was  $0.18 \mu M g^{-1}$  protein in control group, and VZRE with plant extract application, in medium and high doses (G2 and G3) alleviate adverse effect of venom and increased to 0.28, and 0.30 respectively. Increasing ratio was the %55.56, and %66.67 compared to CB, respectively. (Figure 3).

CAT activity of kidney was  $0.17 \mu M g^{-1}$  protein in control group, it has been the highest value compared to the other treatment. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 0.10, 0.07 and 0.05 respectively. Decreasing ratio was the %41.18, %58.82 and %70.59 compared to CB, respectively (Figure 5).

SOD activity of brain was  $0.088 \mu M g^{-1}$  protein in control group, it has been the highest value compared to the other groups. Second, the highest value determined in application of PEB. VZRE application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 0.075, 0.063 and 0.0559 respectively. Decreasing ratio was the %14.77, %28.41 and %32.95 compared to CB, respectively (Figure 1). Similar results were observed in SOD activity of liver and the highest SOD value was determined in CB ( $0.27 \mu M g^{-1}$  protein). VZRE with plant extract application, in high doses (G3SVB, and G3TB) improving adverse effect of venom and decreased to 0.18, and 0.14 respectively. Decreasing ratio was the %33.33 and %48.15 compared to CB, respectively (Figure 3).

SOD activity of kidney was  $0.17 \mu M g^{-1}$  protein in control group, it has been the highest value compared to the other treatment. VZRE application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 0.10, 0.07

and 0.05 respectively. Decreasing ratio was the %41.18, %58.82 and %70.59 compared to CB, respectively (Figure 5).

GPx activity of brain was  $10 \mu\text{M g}^{-1}$  protein in control group, and it has been the highest value compared to the other treatment. When PEB was applied, GPx activity of brain decreased as to CB. But this decreasing was not important statistically. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 6.2, 5.4, and  $4.2 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %38, %46 and %58 compared to CB, respectively (Figure 1). But GPx activity of liver was  $2.40 \mu\text{M g}^{-1}$  protein in control group, and VZRE with plant extract application, in medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 0.80, 0.60, and  $0.50 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %66.67, %75.00, and %79.17 compared to CB, respectively. (Figure 3).

GPx activity of kidney was  $2.90 \mu\text{M g}^{-1}$  protein in control group, it has been the highest value compared to the other treatment. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 1.60, 0.40 and 0.40 respectively. Decreasing ratio was the %44.83, %86.21, and %86.21 compared to CB, respectively (Figure 5).

GSH activity of brain was  $1.35 \mu\text{M g}^{-1}$  protein in control group, and it has been the highest value compared to the other treatment. When PEB was applied, GPx activity of brain decreased as to CB. But this decreasing was not important statistically. VZRE and with plant extract application, in medium and high doses (G2SVB, G3SVB and G3TB) improved adverse effect of venom and decreased to 0.30, 0.10, and  $0.15 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %77.78, %92.59 and %88.89 compared to CB, respectively (Figure 1). But GSH activity of liver was  $0.90 \mu\text{M g}^{-1}$  protein in control group, and VZRE with plant extract application, in medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 0.30, 0.25, and  $0.20 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %66.67, %72.22, and %77.78 compared to CB, respectively. (Figure 3).

GSH activity of kidney was  $0.18 \mu\text{M g}^{-1}$  protein in control group, it has been the highest value compared to the other treatment. VZRE and with plant extract application, in high doses (G3SVB, and G3TB) improved adverse effect of venom and decreased to 0.11 and 0.08 respectively. Decreasing ratio was the %38.89 and %55.56 compared to CB, respectively (Figure 5).

MDA activity of brain was  $120 \mu\text{M g}^{-1}$  protein in control group, and it has been the highest value compared to the other treatment. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) improved adverse effect of venom and decreased to 85, 65, and  $55 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %29.17, 45.83, and %54.17 compared to CB, respectively (Figure 2). But MDA activity of liver was  $85 \mu\text{M g}^{-1}$  protein in control group, and VZRE with plant extract application, in medium and high doses (G2SVB and G3SVB) improved adverse effect of venom and decreased to 60 and  $40 \mu\text{M g}^{-1}$  protein respectively. Decreasing ratio was the %29.41, and %52.94 compared to CB, respectively. (Figure 4).

MDA activity of kidney was  $93 \mu\text{M g}^{-1}$  protein in control group. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB, and G3SVB) improved adverse effect of venom and decreased to 72, 70 and 50 respectively. Decreasing ratio was the %22.58, 24.73 and %46.24 compared to CB, respectively (Figure 6).

Similar results were determined AST, ALP, and ALT activity of brain, liver and kidney. AST activity of brain, kidney and liver were 82, 84, and 79  $\mu\text{M g}^{-1}$  protein in control group respectively. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) alleviate adverse effect of venom and increased to 105, 120, and 150; 90, 98, and 103; 90, 110, and 118  $\mu\text{M g}^{-1}$  protein in brain, liver, and kidney respectively. Increasing ratios in G1SVB were the %28.05, 82.93, and 25.61 compared to CB, respectively in brain, liver and kidney (Figure 2, 4, 6).

ALP activity of brain, kidney and liver were 110, 105 and 112  $\mu\text{M g}^{-1}$  protein in control group respectively. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) alleviate adverse effect of venom and increased to 118, 155, and 160; 118, 130, and 140; 125, 135, and 148  $\mu\text{M g}^{-1}$  protein in brain, liver, and kidney respectively. Increasing ratios in G1SVB were the %7.27, 12.38, and 11.61 compared to CB, respectively in brain, liver and kidney (Figure 2, 4, 6).

ALT activity of brain, kidney and liver were 148, 102 and 122  $\mu\text{M g}^{-1}$  protein in control group respectively. VZRE and with plant extract application, in low, medium and high doses (G1SVB, G2SVB and G3SVB) alleviate adverse effect of venom and increased to 190, 210, and 260; 105, 112, and 118; 130, 145, and 157  $\mu\text{M g}^{-1}$  protein in brain, liver, and kidney respectively. Increasing ratios in G1SVB were the %28.38, 2.94, and 6.56 compared to CB, respectively in brain, liver and kidney (Figure 2, 4, 6).

### 3.3. Histological examination

To further confirm the effect of venom treated groups shows inflammatory in liver and kidney. The extract administrated groups were reduced the liver and kidney damage. In group 1 and 2 appeared to be normal in the brain, heart, liver and kidney.

#### Plate: 1 Experimental design used for the work

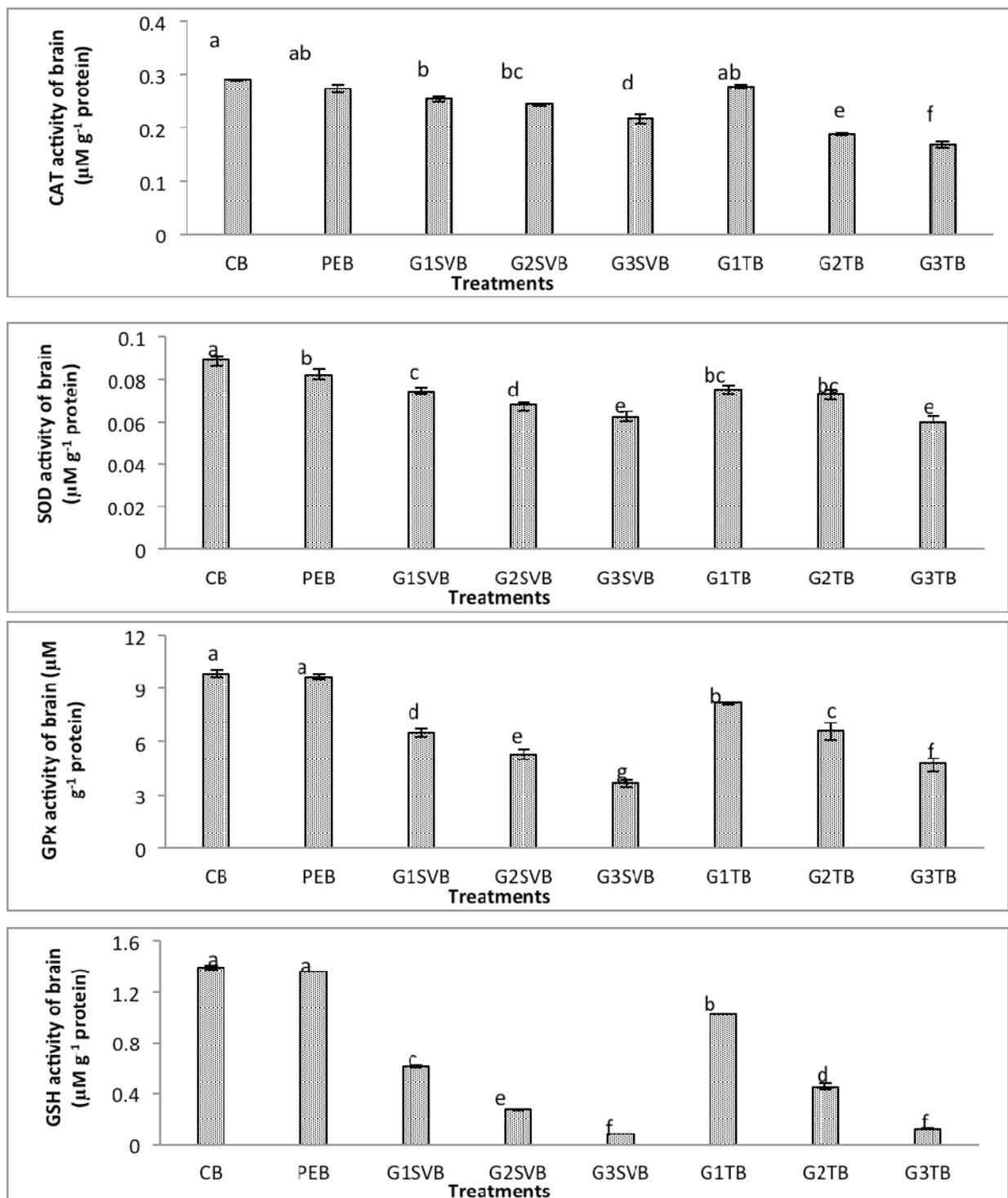


**Photo Description:** A and B: Male mice weighing between 18-25 gm were used for the study; C: Blood samples were collected for RBC, WBC, Platelet and Haemoglobin count; D: Organs like heart, Kidney, liver and brain were removed for antioxidant assays and histopathological examination

**Table.1 Effect of *Vetiveria zizanioides* extract on some hematological parameters in mice**

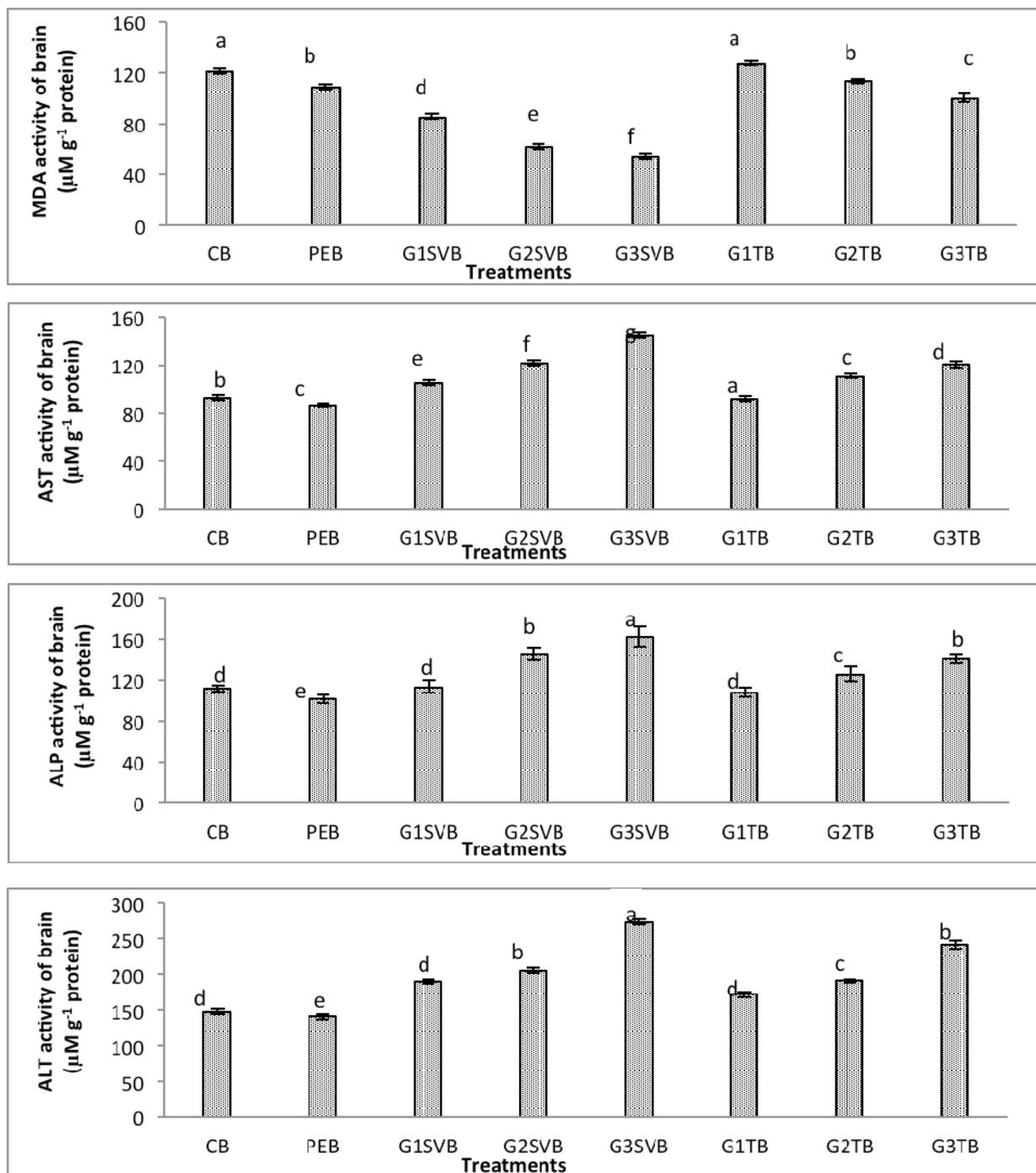
<b>Treatment groups</b>	<b>RBC (X10<sup>12</sup>/L)</b>	<b>WBC (X 10<sup>9</sup>/L)</b>	<b>HGB (g/dL)</b>	<b>Platelet (X10<sup>9</sup>/L)</b>
<b>Group 1</b>	7.7 ± 0.82	7.5 ± 0.70	15.2 ± 0.76	236.60 ± 11.8
<b>Group 2</b>	7.9 ± 1.03	6.1 ± 0.80	12.7 ± 0.79	228.40 ± 10.2
<b>Group 3</b>	4.4 ± 1.00	5.8 ± 1.39	11.9 ± 0.92	227.60 ± 5.8
<b>Group 4</b>	2.1 ± 0.82	3.3 ± 0.68	8.7 ± 0.65	180.20 ± 4.6
<b>Group 5</b>	0.8 ± 0.29	1.2 ± 0.56	5.5 ± 1.47	146.60 ± 6.2
<b>Group 6</b>	7.3 ± 0.86	7.5 ± 1.46	16.8 ± 1.02	228.60 ± 7.1
<b>Group 7</b>	4.9 ± 1.05	7.6 ± 1.10	14.0 ± 1.00	187.40 ± 15.8
<b>Group 8</b>	3.6 ± 0.62	4.8 ± 1.00	13.5 ± 1.30	172.00 ± 8.4

\* White Blood Cell (WBC), Red Blood Cell (RBC), and Hemoglobin level (HGB)



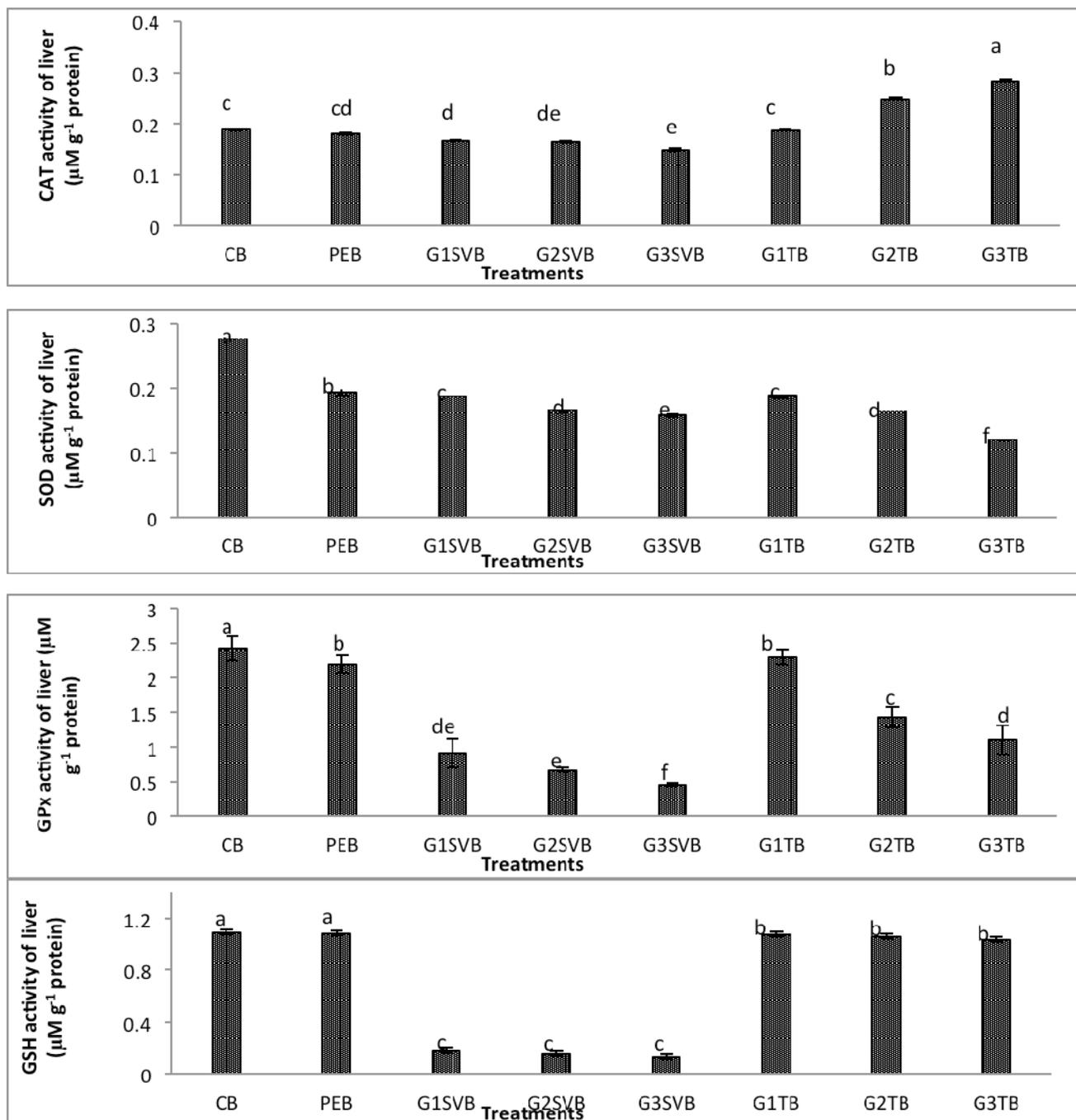
**Figure 1.** The Antioxidant activity of brain catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced Glutathione (GSH) in venom-treated mice.

\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated



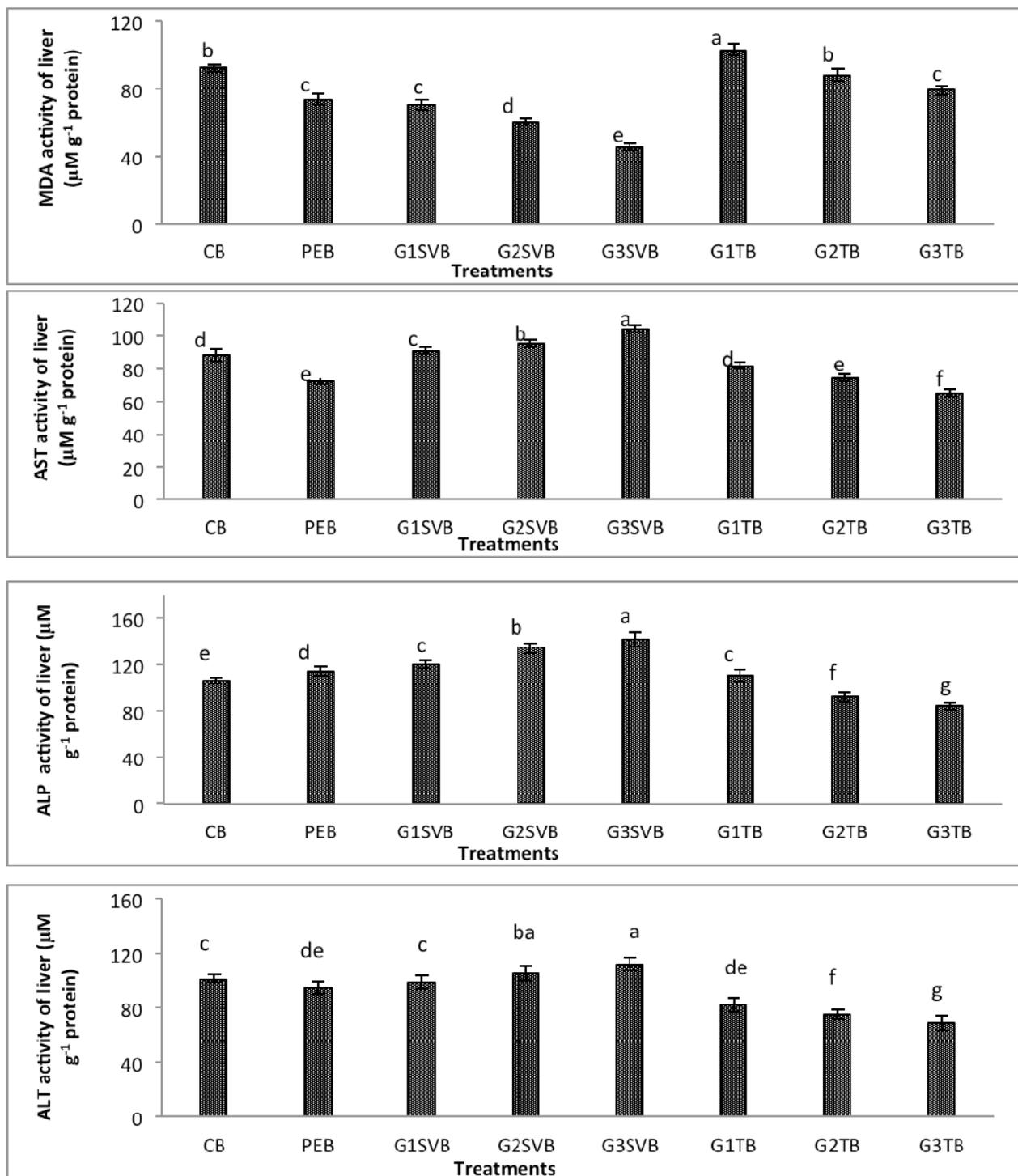
**Figure 2.** The Antioxidant activity of brain alkaline phosphatase (ALP), alanine amino transferase (ALT), asparate amino transferase (AST) activity in venom-treated mice

\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated



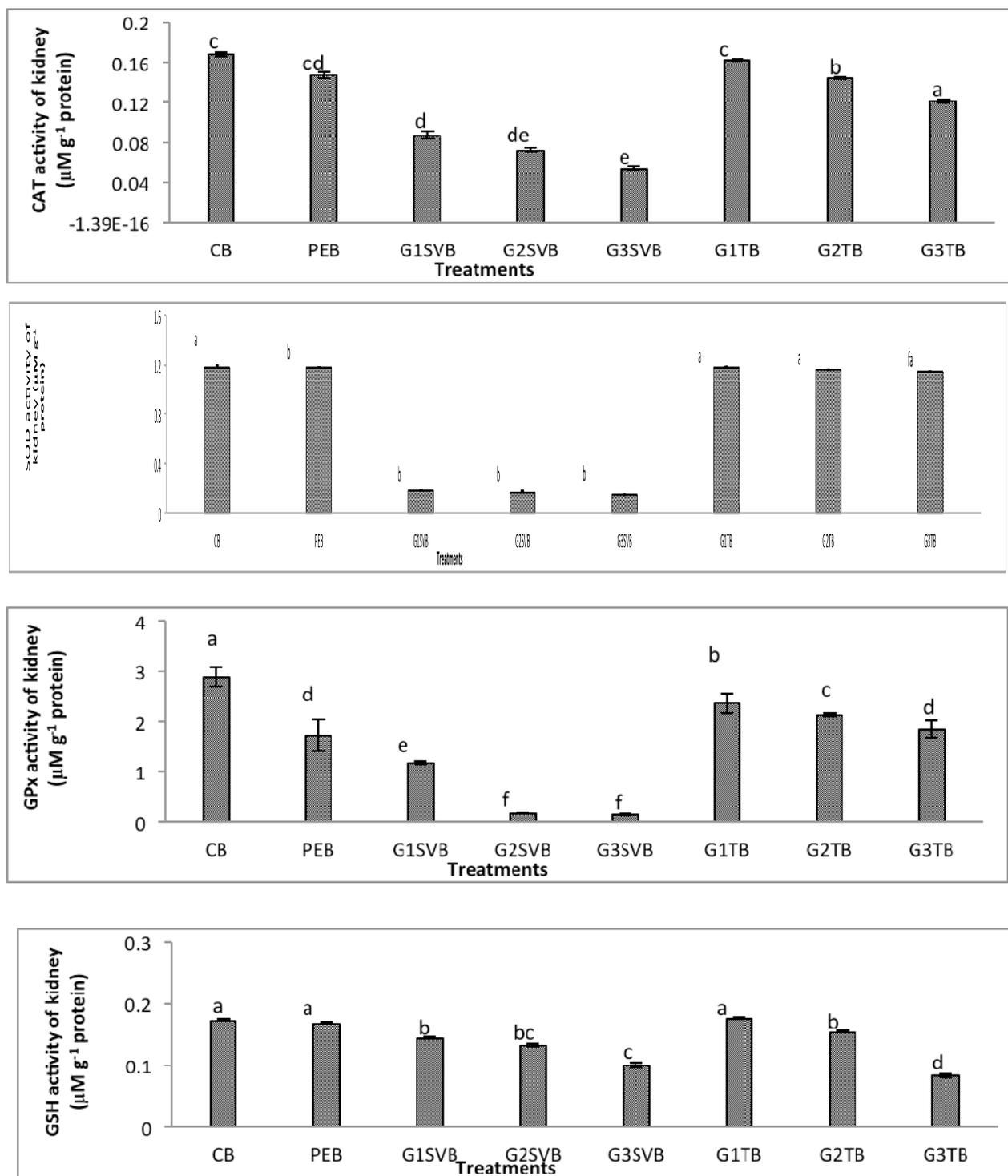
**Figure 3.** The Antioxidant activity of liver catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced Glutathione (GSH) in venom-treated mice.

\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated



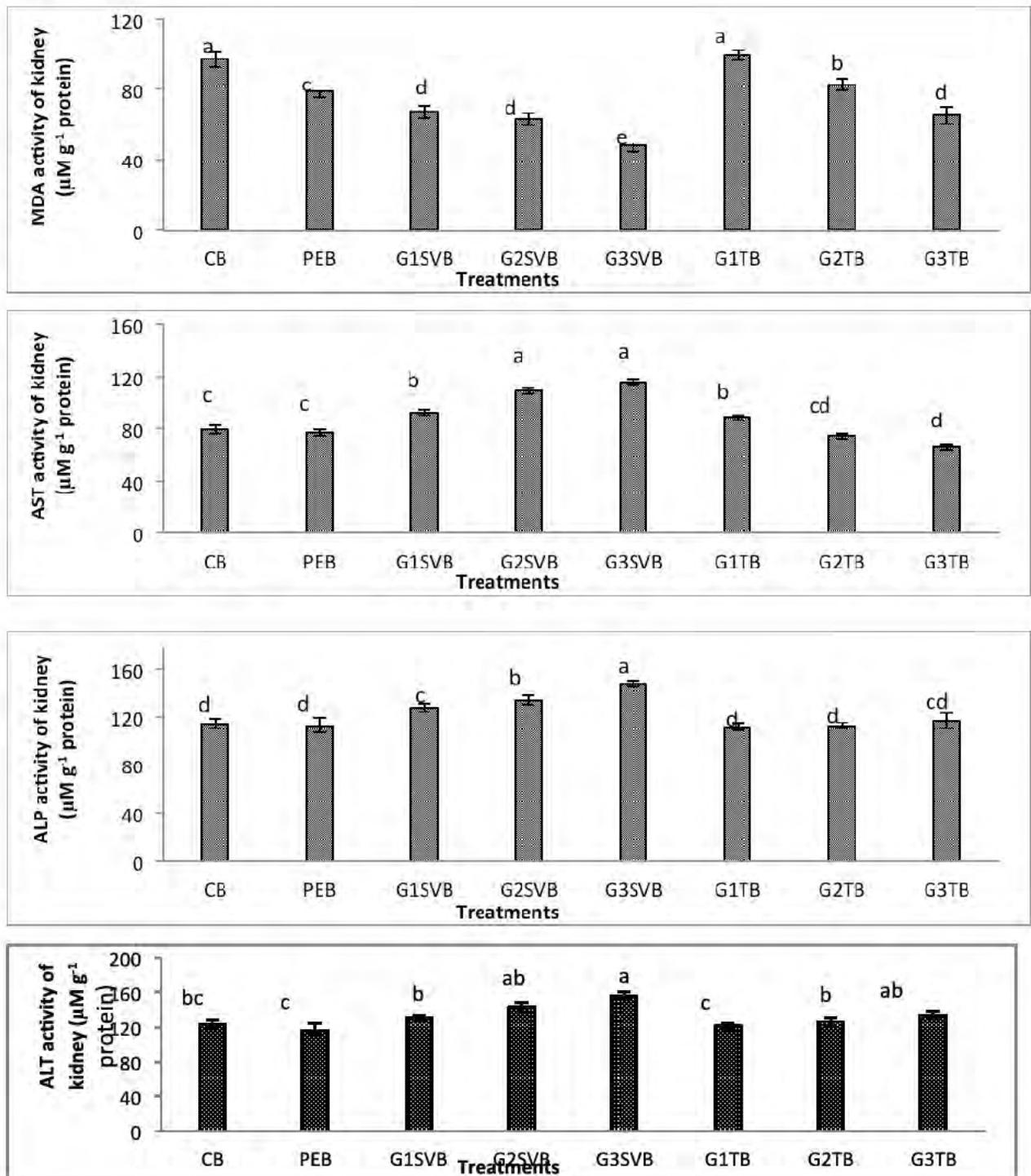
**Figure 4.** The Antioxidant activity of liver alkaline phosphatase (ALP), alanine amino transferase (ALT), asparate amino transferase (AST) activity in venom-treated mice

\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated



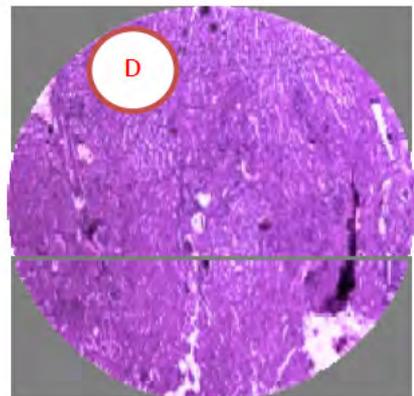
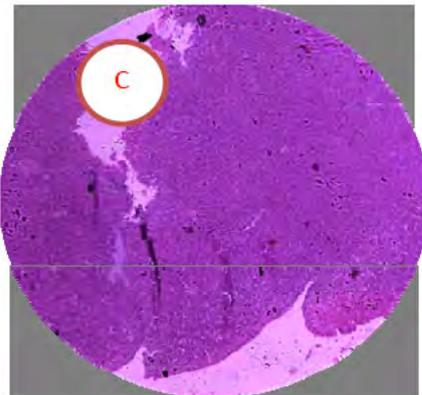
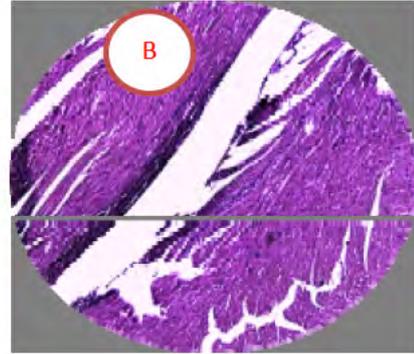
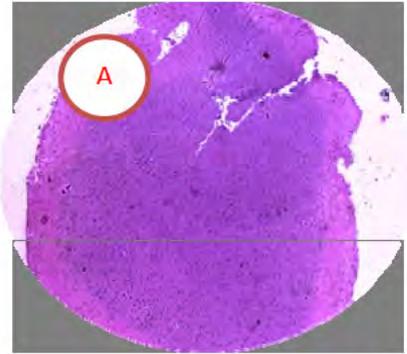
**Figure 5.** The Antioxidant activity of kidney catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced Glutathione (GSH) in venom-treated mice.venom-treated mice.

\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated

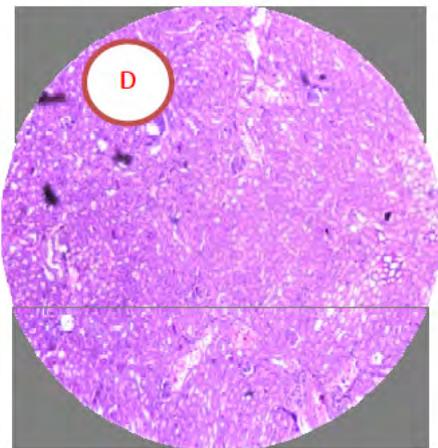
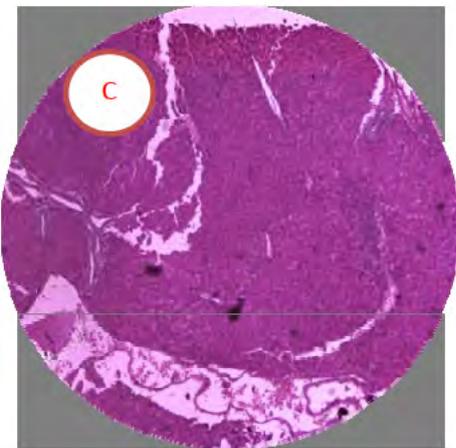
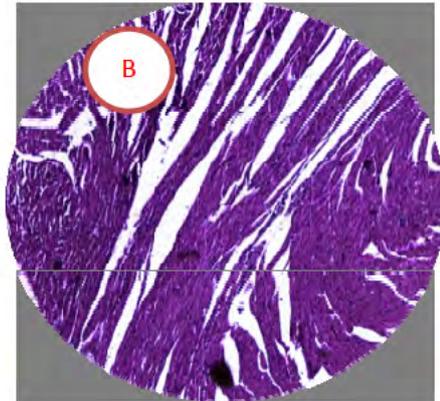
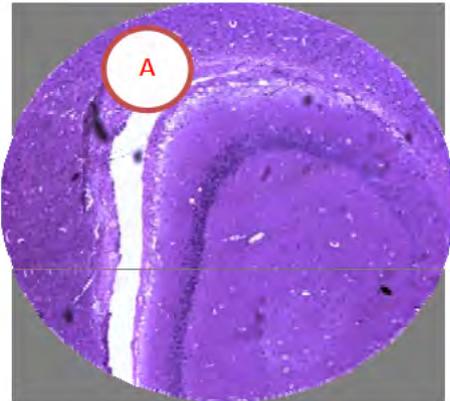


**Figure 6.** The Antioxidant activity of kidney alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST) activity in venom-treated mice

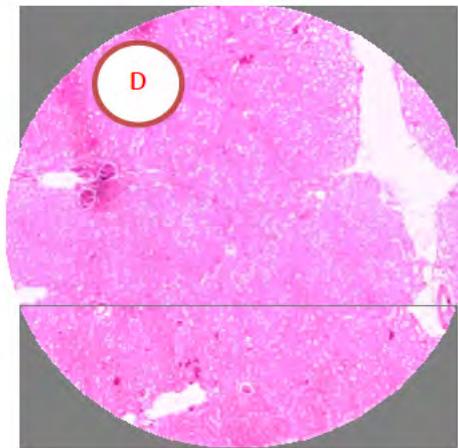
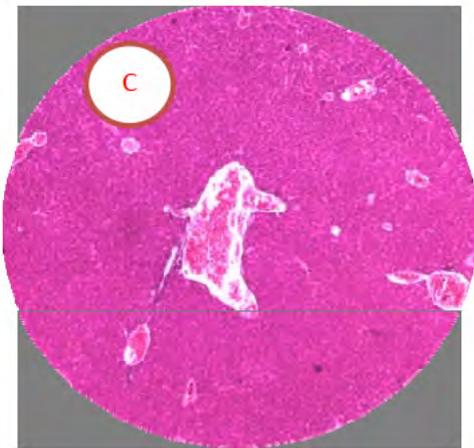
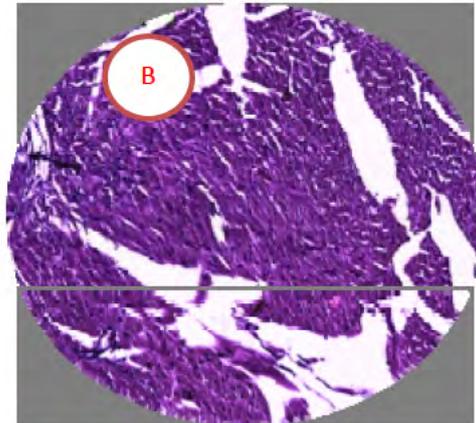
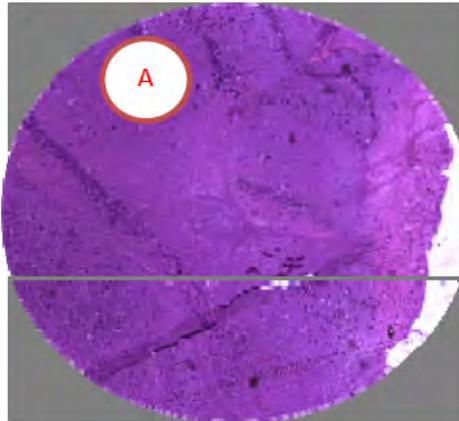
\* CB- control; PEB- plant extract treated; G1SVB- venom treated in low dose; G2SVB- venom treated in middle dose; G3SVB- venom treated in high dose; G1TB- venom low dose + plant extract treated; G2TB- venom middle dose + plant extract treated; G3TB- venom high dose + plant extract treated



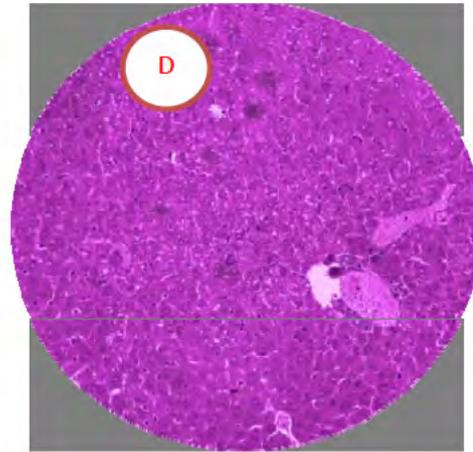
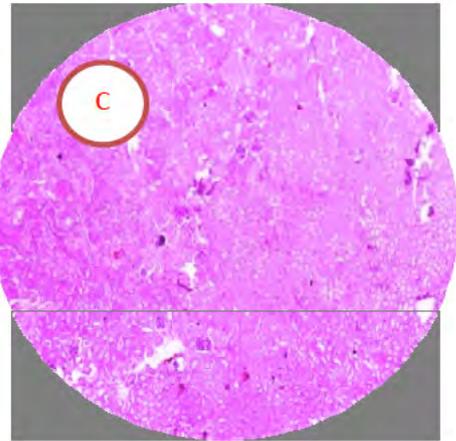
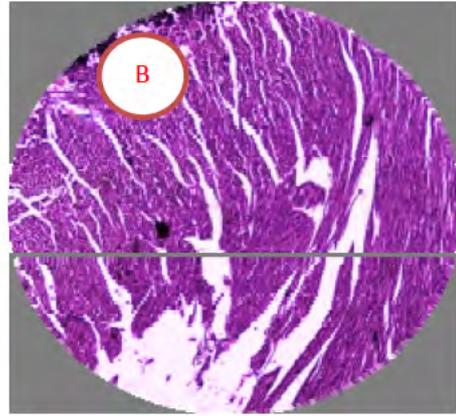
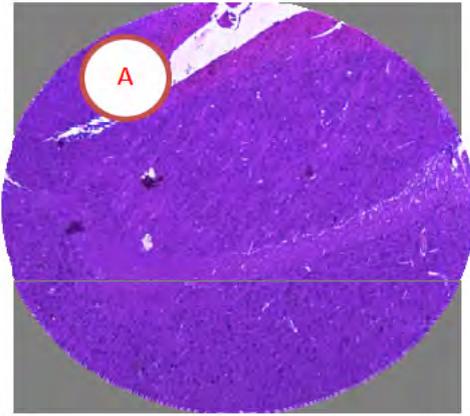
**Figure 7.**Group 1: A-Sections from the brain appear unremarkable, B- Sections from the heart appear unremarkable, C- Sections from the liver appear unremarkable; D- Sections from the kidney appear unremarkable.



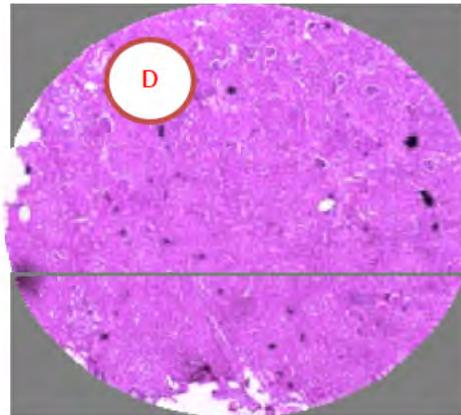
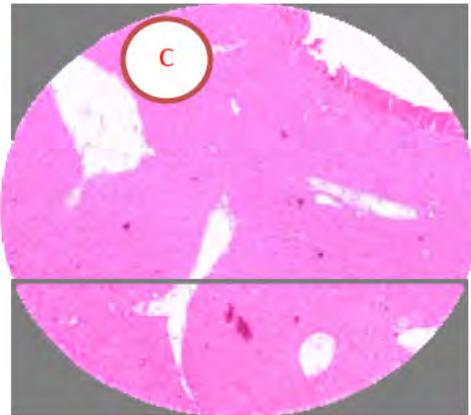
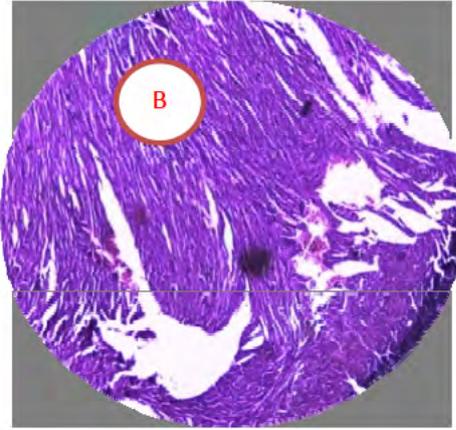
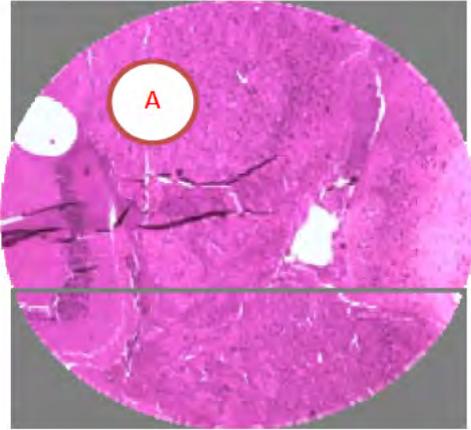
**Figure 8.** Group 2: A-Sections from the brain appear unremarkable, B- Sections from the heart appear unremarkable,C- Sections from the liver appear unremarkable,D- Sections from the kidney appear unremarkable



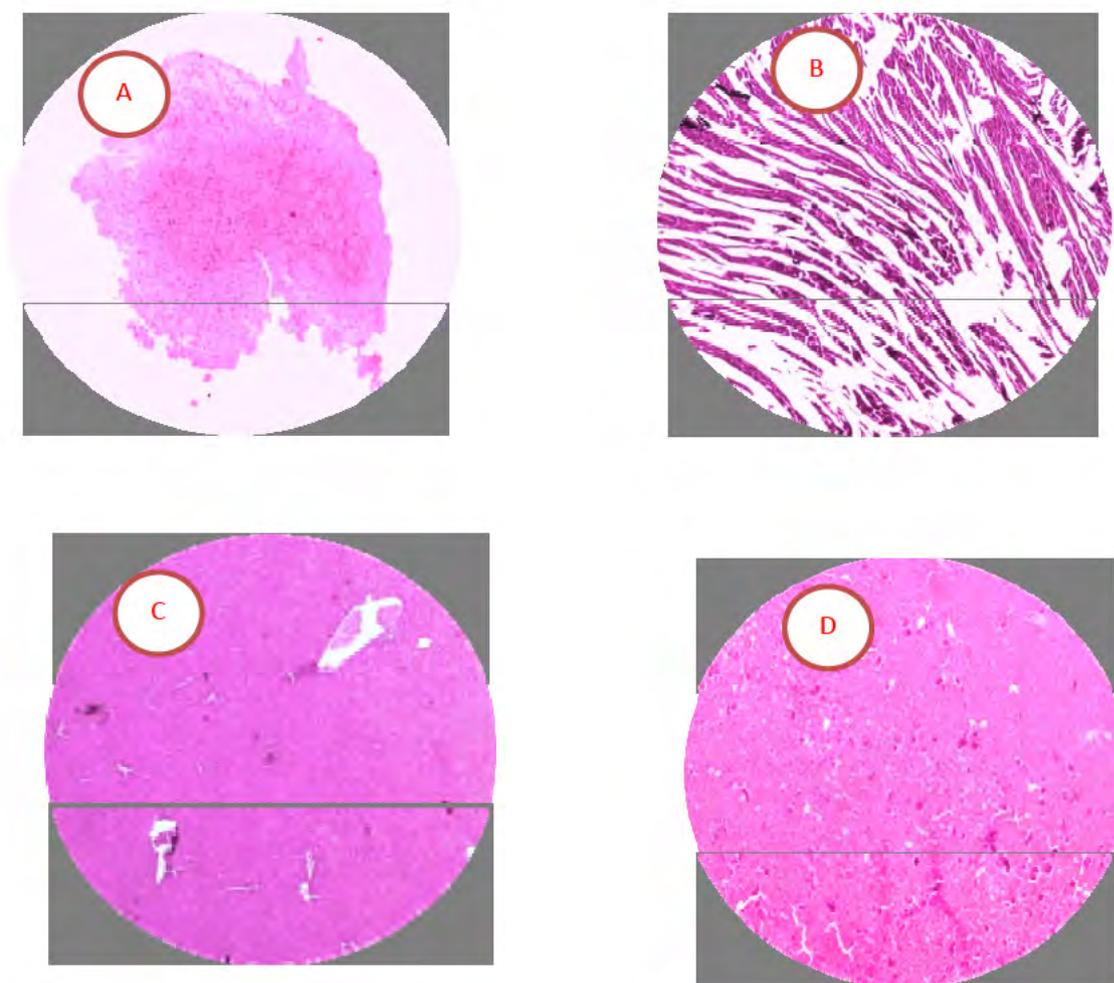
**Figure 9.** Group 3: A-Sections from the brain appear unremarkable, B- Sections from the heart appear unremarkable, C- Sections from the liver show congestion of the vessels, D- Sections from the kidney show only vascular congestion noted



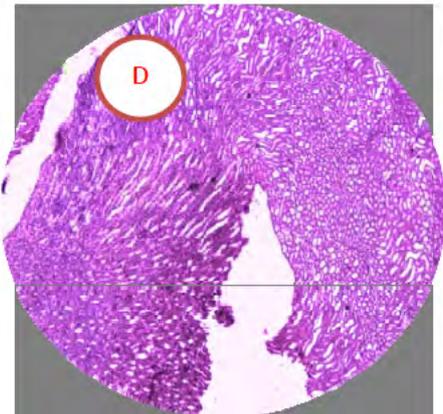
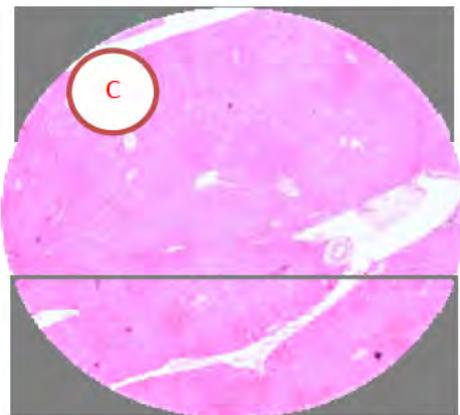
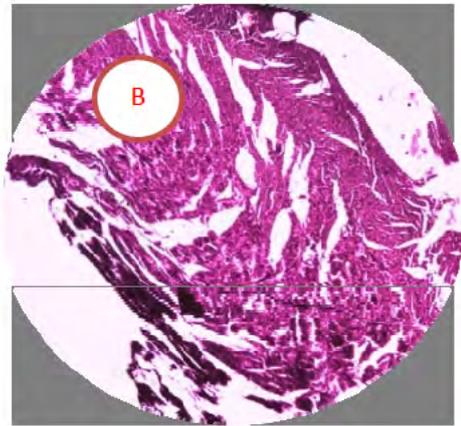
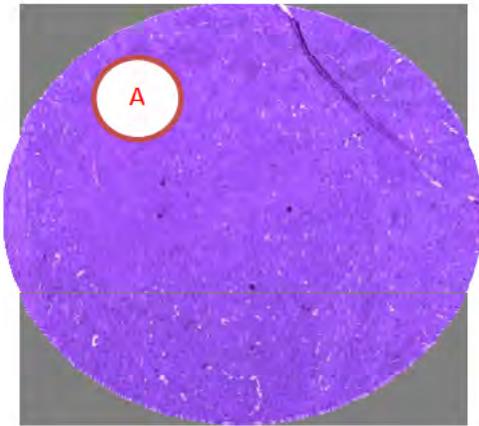
**Figure 10.** Group 4: A - Sections from the brain appear unremarkable, B - Sections from the heart appear unremarkable, C - Sections from the liver marked degeneration of the hepatocytes with congestion of the vessel, D- Sections from the kidney show the glomeruli, tubules and the interstitium appear inflammation



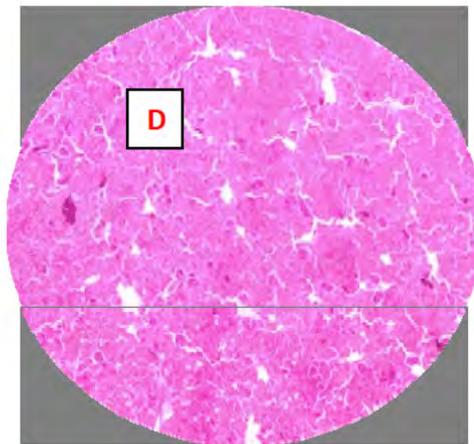
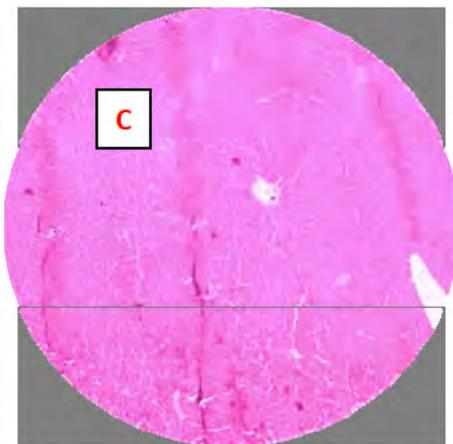
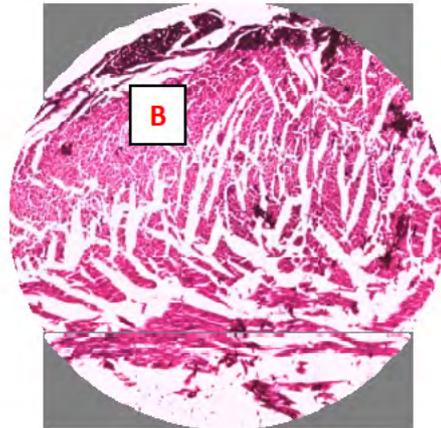
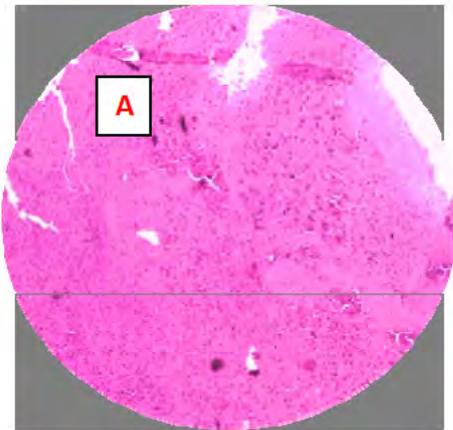
**Figure 11.**Group 5: A - Sections from the brain appear unremarkable, B - Sections from the heart appear unremarkable, C - Sections from the liver marked degeneration of the hepatocytes with congestion of the vessel and the portal tract damaged, D - Sections from the kidney show the glomeruli, tubules and the interstitium appear inflammation.



**Figure 12.**Group 6: A- sections from the brain appear unremarkable, B- Section from the cardiac muscle show no significant pathological changes, C- Congestion of the vessels appear normal, D- Vascular congestion appear normal



**Figure 13.**Group: 7A- Sections from the brain show significant pathology, B- Sections from the heart appear unremarkable, C- Sections from the liver show normal hepatocytes, portal tracts, D- The glomeruli, tubules and the interstitium appear unremarkable and are free of inflammation.



**Figure 14.**Group: 8 A- Sections from the brain show significant pathology, B- Sections from the heart appear unremarkable, C- Sections from the liver show normal hepatocytes, portal tracts, D- The glomeruli, tubules and the interstitium appear unremarkable and are free of inflammation.

#### 4. Discussion

This study was carried out to establish the scientific basis for the traditional application of *Vetiveria zizanioides* in the treatment of victims of snake bite among the mice (Durodola, 1995 and Kela, 1990). The aqueous extract of the root contains an active compound that can be subjected to bioassays as shown by the antioxidant assays.

The most effective and acceptable therapy for snake bite victims is the immediate administration of anti-venom following envenomation (Mahanta and Mukkerjee, 2001). Although, the use of plants against the effects of snake bite has been recognized, more scientific attention has been given to since last 20 years (Alam and Gome, 2003). Like plants, snake venom can also be considered a sophisticated laboratory of biotechnology. The search for bioactive molecules in plants used in folk medicine has been growing in the past few years. In this study we have reported by *Echis carinatus* venom including various parameters such as hematological parameters, antioxidant enzyme activities and histological examination which were noted.

The results suggest that *Echis carinatus* venom can disturb mice metabolism. The study showed that the extract of *Vetiveria zizanioides* was effective in neutralizing the hematological parameters and the effects of *Echis carinatus* venom in mice. The several traditional workers have studied the ability of plants as well as their purified compound to inhibit biological activities of snake venom (Melo *et al.*, 1991; maiorano *et al.*, 2005; Oliveira *et al.*, 2005; Cavalcante *et al.*, 2007; Lomonte *et al.*, 2009; De Paula *et al.*, 2010). However, only a few have investigated the neutralizing mechanism of this action. In some cases a direct interaction with catalytic sites of enzymes or which metal ions which are essential for enzyme activities may be involved (Borges *et al.*, 2005 and Nunez *et al.*, 2004)

The envenomed mice were reduced significantly ( $p < 0.05$ ), when compared with non-envenomed ones. This is consonance with the report of Mwagi *et al.*, 1995. WBC are effectors of the immune system in group 3, 4 and 5 there was significant reduction in the WBC compared to group 6, 7 and 8 that received venom and extract. This suggests that the plant extract must have combated the venom directly without cells of the immune system producing effector cells.

Haemoglobin is the principal molecule responsible for the transport of both oxygen and carbon dioxide in blood in group 3, 4 and 5 the haemoglobin level decreased due to the effect of the venom compared to group 6, 7 and 8.

In the present study CAT level in brain, liver, kidney significantly decreased in venom treated group when compared to control and plant extract treated groups. In similar study, Karahon *et al.*, 2005 observed significantly reduced activity of catalase in venom treated groups. Significant and dose dependent improvement in the activity of brain, liver, kidney catalase was observed in *Vetiveria zizanioides* treated groups when compared to envenomed groups. The plant extract also significantly increased the CAT activity when compared to envenomed treated groups shown from figures.

SOD is an important cellular antioxidant enzyme (a metal protein) which catalysis the conversion of superoxide radical to hydrogen peroxide and oxygen. In the present study, SOD activity of brain, liver, kidney was significantly decreased in group 3, 4 and 5 when compared to control and plant extract treated group (2). These observations were in correlation with the findings of Ramasammy *et al.*, 1987.

Glutathione peroxidase is selenium containing metallo enzyme, that catalyzes the oxidation of reduced glutathione by peroxide to form water and oxidized glutathione. In our study, GPx level of brain, liver and kidney significantly decreased in venom treated groups (3, 4

and 5) when compared to control. *Vetiveria zizanioides* improved the GPx, activity towards control levels in a dose dependent manner. Farombi and Ekor (2006) also reported a similar finding where in pretreatment of curcumin at 200 mg/kg for two weeks significantly resorted the GPx as compared to control group.

GSH is an endogenous non-enzymatic antioxidant that prevents damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al.*, 2003). In this study, GSH level of brain, liver and kidney was significantly decreased in venom treated group when compared to control. These observations were in concurrence with the findings of Ali *et al.*, (1992) who found significantly decreased level of GSH content in kidney homogenate treated with venom at the rate of 80 mg/kg/day for six days in male wistar rats. Similarly significant improvement in the level of brain, liver and kidney GSH was observed in *Vetiveria zizanioides* treated groups Triwedi *et al.*, (2000) have also reported that administration of *Andrographis paniculata* extract induced diminished levels of GSH which was concordant with our results.

In our study, extract of *Vetiveria zizanioides* showed significant dose dependent reduction of MDA level in brain, liver and kidney. Envenomed groups also produced significant reduction in MDA level. Triwedi and Rawal, 2000 reported that administration of *Andrographis paniculata* extract reversed induced elevated levels of MDA.

The outcome of the evaluation of the biochemical and anti-snake venom effects of *Vetiveria zizanioides* extracts revealed increases in AST, ALT and ALP in the venom treated groups. The extract treated groups show significant decrease in AST, ALP and ALT in brain, liver and kidney. Antioxidant enzymes catalyze decomposition of reactive oxygen species and maintain the normal physiological state of cells. CAT, SOD and GPx are three major antioxidant and they differ from each other in structure, tissue distribution and cofactor requirement (Shahjahan *et al.*, 2005).

The findings also gain support from the histo pathological studies. The hepatocytes and portal tracts changes observed in liver of venom treated groups in low, middle and high have been found are reserved in *Vetiveria zizanioides* treated mice. The kidney show glomeruli, tubules and the interstitium appear inflammation in venom treated groups. The kidney appears free from inflammation in extract treated groups. The brain and heart section shows unremarkable in control group (1), extract treated group (2).

This may be attributed to *Vetiveria zizanioides* induced increase in antioxidant activities, regeneration and reparative process of cellular membrane, and up regulated antioxidant enzyme status, thus restoring the functional balance between pro-oxidant and anti-oxidant pathway. In conclusion, the present experimental results indicate that *Vetiveria zizanioides* extract was effective in neutralizing the toxic effects of *Echis carinatus* venom and or has an alternative or complementary treatment strategy of envenomation by *Echis carinatus*. Further experiment could address the fractioning of the *Vetiveria zizanioides* extract in order to identify the bioactive compounds responsible for these observations, their efficacy, safety and the mechanism of action which could possibly lead to the development of pharmaceutical formulations for treating snake bite accident victims. Our study on the aqueous root extract of *Vetiveria zizanioides* has demonstrated some useful activities that support its traditional use against snake bite.

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