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The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* (Amaryllidaceae)

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Abstract

The anti-snake venom activities of the methanolic extract of the bulb of *Crinum jagus* plant (Amaryllidaceae) were investigated in vitro and in vivo against the venoms of three notable snake species: *Echis ocellatus*, *Bitis arietans* and *Naja nigricollis*. The extract was prepared by cold marceration in 50% methanol at 37 °C with intermittent shaking for 48 h. An yield of 12.8% w/w dry extract was obtained. Oral administration of *C. jagus* extract (1000 mg/kg) protected 50% of mice, while injection of a 30 min pre-incubated mixture of the same dose of extract and venom gave 100% protection against the lethal effects of *E. ocellatus* venom (10 mg/kg, i.m.). The intraperitoneal administration of the extract at 250 mg/kg, 30 min before the injection of *E. ocellatus* venom (10 mg/kg, i.m.), significantly ($p<0.05$) prolonged the death time of poisoned mice. *C. jagus* extract (500 mg/kg, *per os*), gave 50% protection against *B. arietans* venom (9.5 mg/kg, i.m.) in mice while the pre-incubation of a mixture of the same dose of venom and extract (500 mg/kg), prior to injection (i.p.) of the mixture, gave only 33.3% protection. The pre-incubation of 500 mg/kg of *C. jagus* extract with *N. nigricollis* venom (6 mg/kg) prior to i.p. injection of the mixture protected 50% of the treated mice. There were generally no significant differences in the death times of mice that were given the same dose of the extract orally 30 min before injection of the venoms and those administered with the pre-incubated mixtures of venom and extract. The pre-incubation of the extract and *E. ocellatus* venom (5 mg/kg) for 30 min, before the i.m. injection of the mixture, significantly reduced infiltration of inflammatory cells to the site of injection 4 h post treatment. The concentrations of plasma creatine kinase in poisoned mice were significantly ($p<0.01$ or $p<0.05$) reduced after the injection (i.p.) of *C. jagus* extract (1000 mg/kg) pre-incubated with *E. ocellatus* (5 mg/kg) or *B. arietans* (7 mg/kg) venom, respectively. The bulb extract of *C. jagus* blocked the haemorrhagic activity of a standard haemorrhagic dose (2.8 mg/ml) of *E. ocellatus* venom at various concentrations (1.7, 3.3 and 6.7 mg/ml). The methanolic bulb extract of *C. jagus* was therefore able to significantly protect mice from death, myonecrosis and haemorrhage induced by the lethal effects of venoms of notable snake species in Nigeria.

Keywords: *Crinum jagus*; *Echis ocellatus*; *Bitis arietans*; *Naja nigricollis*; Antihaemorrhagic activity; Creatine kinase activity

1. Introduction

In Nigeria, snakebites cause significant deaths in human and animal species. Unfortunately, conventional antivenoms currently available are not only expensive, but do not effectively neutralize venom induced haemorrhage, myonecrosis and nephrotoxicity. Some of the antivenoms cause allergic reaction in patients (Grant et al., 2000; Gutierrez et al., 1980; Ferreira et al., 1992). In humans, treatment of snakebite is normally continued until the clinical

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signs of envenomation disappear (Viejeth et al., 2000); the prolonged regime of injections imposes a lot of stress on the victims. Plants have reportedly been used locally to treat diverse cases of snakebites (Akunyili and Akubue, 1987; Wang et al., 1997; Borges et al., 2000; Asuzu and Harvey, 2003; Yang et al., 1998) but many of the studies lack systematic scientific procedures, which are necessary for the development of an antivenom agent from plants (Martz, 1992). The bulb of *Crinum jagus* plant is used traditionally for the treatment of various cases of snakebite by the *Igede* speaking tribe of Oju Local Government Area in Benue State and the *Fulani* nomads from Northern Nigeria living among them. It is an acclaimed snake venom antidote, which is effective even at advanced stages of envenomation.

C. jagus commonly called Harmattan lily, belongs to Amaryllidaceae, a heterogenous family of 86 genera and about 1310 species (Lawrence, 1951) which are widely distributed throughout the world. *C. jagus* is popularly known in *Igede* as 'Aru inyi' (Elephant's knee) or 'Okonkilo inyi' (Elephant's potato) and as gadali among the *Fulani* and *Hausa* in Northern Nigeria (Dalziel, 1937). All the species are of ornamental value. In Sierra leone, a cold infusion of the fresh leaves is used to bathe young children suffering from general body debility, rickets, etc. (Dalziel, 1937). In Gold Coast, a decoction is given as a vermifuge. In Lagos, the bulbs of several species are sold for various medicinal purposes. In East Africa, the decoction of *Crinum* is used for the treatment of sores (Kokwaro, 1976).

The present study investigates the acclaimed antivenom activities of the bulb of *C. jagus* using venoms from three notable snake species found in Nigeria, namely, *Echis ocellatus*, *Bitis arietans* and *Naja nigricollis*. There was until now, no systematic investigation of the antivenom effect of this plant using various pharmacological models. The extract of *C. jagus* will be administered through various routes so as to ascertain the most effective route of administration.

2. Materials and methods

2.1. Solutions, reagents and chemicals

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Creatine kinase kit (Quimica Clinica Applicada, Spain), freeze dried *E. ocellatus*, *B. arietans* and *N. nigricollis* venoms (Liverpool School of Tropical Medicine, UK), spectrophotometer (Spectrolab, USA) and a locally fabricated incubator were used.

2.2. Animals

Inbred Wistar mice of both sexes with average weight of 26 g were used as test animals. The animals, which were obtained from the laboratory animal facility of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were housed in stainless steel cages at room temperature of 28–32 °C and under a light period of 16–18 h daily. They were fed on standard commercial feed (Topfeeds®, Nigeria).

2.3. Preparation of plant material

Fresh bulbs of the plant were collected in April 2004 from farm locations in Ochimode village, Oju Local Government Area in Benue State, Nigeria. The plant was duly identified as *C. jagus* by Mr. Ozioko, a taxonomist with the University of Nigeria, Nsukka. The bulbs (underground stems) were cut into small pieces with a knife, dried under mild sunlight and pulverized into powder with a laboratory mill. The powder (500 g) was exhaustively extracted with 31 of 50% methanol. The extraction was by cold maceration at 37 °C with intermittent shaking for 48 h. The extract was concentrated by vacuum rotary evaporation and stored in a refrigerator at 4 °C. The concentration of the extract was determined and the percentage yield was calculated.

2.4. Acute toxicity study

Five groups of Wistar mice of both sexes with each group containing five mice were used. Four of the groups were treated orally with varying doses of *C. jagus* extract at 250, 500, 1000 and 2000 mg/kg, respectively. The fifth group was given an equivalent volume of distilled water to serve as control. The animals were observed for toxic signs like excitability, dullness, diarrhoea, inappetence and death over 24 h.

The rest of the in vivo experiments were performed separately with 24 adult Wistar mice of both sexes randomly allocated to 4 groups with each group having 6 mice. At the end of each of the experiments, the number of mice that were

dead or alive and the time of death were recorded. The mean death times of mice in the groups were analysed statistically using one-way analysis of variance (ANOVA) and Student's *t* test (Steel and Torrie, 1960).

2.5. Evaluation of *C. jagus* extract for protective activity against *E. ocellatus* venom

2.5.1. Oral administration of the extract, 30 min prior to the injection of *E. ocellatus* venom (8.5 mg/kg)

Twenty-four adult Wistar mice of both sexes were divided into four groups, consisting of six mice each. The control group was injected with only venom (lethal dose, 8.5 mg/kg) intramuscularly (i.m.) while the remaining three groups were treated separately with *E. ocellatus* venom (8.5 mg/kg, i.m.), after 30 min of oral administration of *C. jagus* extract (500, 1000 and 1500 mg/kg), respectively. The mice were observed over the duration of the experiment for the number of mice which were dead and the time of death. The mean death time was analysed using ANOVA and Student's *t* test (Steel and Torrie, 1960).

2.5.2. Administration of both *E. ocellatus* venom (8.5 mg/kg) and *C. jagus* extract i.p. after pre-incubation of the mixture for 30 min

Four groups of mice consisting of six mice per group were used in the experiment. The control group was injected with *E. ocellatus* venom (8.5 mg/kg, i.p.) only. The other groups were each given a mixture of *E. ocellatus* venom (8.5 mg/kg) and one dose (500 or 1000 or 1500 mg/kg) of *C. jagus* extract i.p. The mixture of venom and extract was pre-incubated for 30 min at 37 °C before administration. The times taken by the mice to die and the mean death times were recorded and analysed as described previously.

2.5.3. Intraperitoneal administration of the extract, 30 min before injection of *E. ocellatus* venom (10 mg/kg, i.m.)

Four groups of mice with six mice per group were used for the experiment. Group A was administered a lethal dose (10 mg/kg, i.m.) of *E. ocellatus* venom only and this served as the control. Group B was given *C. jagus* extract at 250 mg/kg i.p., 30 min before the administration of *E. ocellatus* venom (10 mg/kg, i.m.). Group C was given *C. jagus* extract at 500 mg/kg i.p. 30 min prior to the injection of *E. ocellatus* venom (10 mg/kg, i.m.). Group D was similarly treated with the extract at 1000 mg/kg i.p. followed after 30 min with *E. ocellatus* venom (10 mg/kg, i.p.). All the mice were observed for the number that died over the period of the experiment and the mean time of death was analysed using ANOVA and Student's *t* test.

2.6. Evaluation of *C. jagus* extract for protective activity against *B. arietans* venom

Each experiment was performed with 24 Wistar mice divided into 4 groups of 6 mice each.

2.6.1. Oral administration of the extract, 30 min before the injection of *B. arietans* venom (9.5 mg/kg, i.m.)

The group that served as the control was injected with a lethal dose (9.5 mg/kg, i.m.) of *B. arietans* venom only. The remaining three groups were treated orally with the extract of *C. jagus* at 500, 1000 and 1500 mg/kg, respectively, and then allowed 30 min before *B. arietans* venom (9.5 mg/kg) was injected i.m. All the mice were observed for 24 h for the number that died and their times of death were recorded and subjected to ANOVA and Student's *t* test.

2.6.2. The i.p. administration of a mixture of the extract and *B. arietans* venom after 30 min pre-incubation

The control group was given a lethal dose (9.5 mg/kg) of *B. arietans* venom by i.p. injection. Each of the remaining three groups was treated with a mixture of *B. arietans* venom (9.5 mg/kg) and one dose of the extract (500 or 1000 or 1500 mg/kg, i.p.) after the mixture was pre-incubated for 30 min at 37 °C. The mice were observed for 24 h. The difference in the mean death time between mice groups was considered significant at $p < 0.05$.

2.7. Investigation of the extract for protective activity against *N. nigricollis* venom

Each experiment was performed with 24 mice, which were divided into 4 groups (A-E) of 6 mice each.

2.7.1. Administration of *C. jagus* extract orally, 30 min prior to the injection of *N. nigricollis* venom (6 mg/kg)

Group A (control) was injected with a lethal dose (6 mg/kg, i.m.) of *N. nigricollis* venom only. Group B was given 500 mg/kg of *C. jagus* extract orally,

followed after 30 min by the injection (i.m.) of 6 mg/kg of *N. nigricollis* venom. Group C was given 1000 mg/kg of the same extract by oral intubation, 30 min prior to the injection of the same dose (6 mg/kg) of *N. nigricollis* venom, i.m. Group D received 1500 mg/kg of *C. jagus* extract orally and after 30 min, 6 mg/kg of *N. nigricollis* venom was injected, i.m. The mice were observed over the duration of the experiment for the number that died.

2.7.2. Administration of a mixture of N. nigricollis venom (6 mg/kg) and C. jagus extract after both venom and extract were pre-incubated

The control group was injected with a lethal dose of *N. nigricollis* venom (6 mg/kg, i.m.). The rest of the three groups were separately given a mixture of *N. nigricollis* venom (6 mg/kg) and one dose (500 or 1000 or 1500 mg/kg) of *C. jagus* extract i.m. after the mixture was pre-incubated for 30 min. The mice were observed over 24 h and the number alive or dead were noted. The difference in the mean death time between mice groups was regarded as significant at $p < 0.5$.

2.8. Creatine kinase assay

2.8.1. Creatine kinase assay after injection of E. ocellatus venom (5 mg/kg, i.m.)

The evaluation of myonecrosis by quantifying mice plasma creatine kinase levels was conducted following a standard procedure (Grant et al., 2000). Twenty-four Wistar mice of both sexes (14–18 g) were divided into three groups, consisting of eight mice per group. Group A (control) was injected with a non-lethal dose (5 mg/kg, i.m.) of *E. ocellatus* venom only. Group B was given *C. jagus* extract (1000 mg/kg) orally, 30 min prior to injection of *E. ocellatus* venom (5 mg/kg, i.m.). Group C was injected (i.p.) with a pre-incubated mixture of *C. jagus* extract (1000 mg/kg) and *E. ocellatus* venom (5 mg/kg). At the end of 4 h, blood samples were collected from the mice by occular puncture, using capillary tubes, into eppendorf tubes with anticoagulant (EDTA). The samples were centrifuged and the plasma was collected. A specified volume (500 μl) of the working reagent from the creatine kinase kit and 20 μl of each plasma sample were mixed. Each mixture was transferred into a measuring cuvette. Absorbance was read after 4 min in a spectrophotometer at 365 nm. Further readings were taken during the next 4 min at 1 min intervals. The change in the concentration of creatine kinase per minute (ΔE/min) for every reading was recorded and the mean values were determined and analysed statistically using one-way ANOVA and Student's *t* test.

2.8.2. Creatine kinase assay with B. arietans venom (7 mg/kg)

A similar experiment using *B. arietans* venom (7 mg/kg) was performed with the same number of mice as previously described. Group A was injected with a non-lethal dose (7 mg/kg) of *B. arietans* venom, i.m., and it served as the control. Group B was given an oral intubation of *C. jagus* extract (1000 mg/kg), 30 min before injection of *B. arietans* venom (7 mg/kg, i/m). Group C was treated (i.p.) with a mixture of *C. jagus* extract (1000 mg/kg) and *B. arietans* venom (7 mg/kg) which was pre-incubated. After 4 h, blood collection, centrifugation, mixing of the specified volumes of the working reagent from creatine kinase kit and plasma as well as reading of the absorbance were done as previously described. The readings for each group were recorded and the mean creatine kinase values were analysed statistically using one-way ANOVA and Student's *t* test. Differences were considered significant at $p < 0.05$.

2.9. Histopathology.

A study of the effect of *C. jagus* extract on cellular necrosis induced by *E. ocellatus* venom (5 mg/kg) in mice was conducted in four groups of adult Wistar mice. Each group consisted of three mice weighing between 18 and 24 g. Group A (positive control) was injected with only *E. ocellatus* venom (5 mg/kg, i.m.) while Group B (negative control) was injected (i.m.) with an equivalent volume of distilled water. Group C was given *C. jagus* extract (1000 mg/kg) orally, 30 min before the injection of *E. ocellatus* venom (5 mg/kg, i.m.). Group D was injected (i.m.) with a mixture of *C. jagus* extract (1000 mg/kg) and *E. ocellatus* venom (5 mg/kg) after both were pre-incubated at 37 °C for 30 min before injection into mice. Tissue samples from the thigh muscles at the point of injections were cut with scalpel blade from each of the envenomed mice, 4 h post injection. The samples were fixed in 10% formol saline for a minimum of 24 h and then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned, stained with haematoxylin and eosin (H & E), and then mounted on glass slides with

Canada balsam. All sections were examined under light microscope ($\times 10$, $\times 20$, and $\times 40$). Photographs were taken with an Olympus photomicroscope (Tokyo, Japan) fitted with a camera.

3. Antihaemorrhagic test

The test was conducted following a standard method (Dunn and Boone, 1976; Sells et al., 1997). Day-old fertile eggs obtained from a local hatchery (John Gloree, Nsukka) were incubated till day 4 in a humid incubator at 38 °C. The eggs, which were disinfected, were cracked on day 4 into clingfilm hammocks and incubated further till day 6. Discs of 2 mm diameter cut with a handpunch from filter paper (Whatman no.1) were impregnated with a standard haemorrhagic dose (SHD) of *E. ocellatus* venom (2.8 mg/ml) alone or venom and various concentrations (1.7, 3.3 and 6.7 mg/ml) of *C. jagus* extract. Each of the discs was placed on the yolk sac membrane over a major bilateral vein and left for 3 h to form a haemorrhagic corona. The coronas were measured with a transparent metre rule. Control experiments were performed with the buffered saline solution used to prepare the extract and venom solutions. Readings were taken in duplicates. The minimum concentration of the extract required to completely abolish haemorrhage was recorded as the minimum effective neutralizing dose (MEND).

4. Results

4.1. Plant extract

The methanolic extract of the bulb of *C. jagus* was yellowish in colour with a sweet aromatic odour. The total solid recovered from the extract was 12.8% (w/w).

4.2. Acute toxicity study

No death was recorded in the mice treated orally with varying doses (250–2000 mg/kg) of the extract within 24 h. However, mice treated with 1500 and 2000 mg/kg of the extract showed transient dullness and inappetence, which disappeared 30 min after the administration of the extract.

4.3. Evaluation of *C. jagus* extract for protective activity against *E. ocellatus* venom

4.3.1. Oral administration of the extract, 30 min prior to injection of the venom

In the control group, which was treated with only *E. ocellatus* venom (8.5 mg/kg), 16.7% of the mice survived. Mice treated with *C. jagus* extract at 500 and 1000 mg/kg recorded 33.3% and 50% survival as presented in Table 1. The death time of mice treated with 1500 mg/kg of the extract was significantly ($p<0.01$) prolonged compared to the control.

4.3.2. Administration of a mixture of *E. ocellatus* venom and *C. jagus* extract after pre-incubation for 30 min

The results of this study are presented in Table 2. Pre-incubated mixtures of the venom (8.5 mg/kg) and various doses (500, 1000 and 1500 mg/kg) of *C. jagus* extract protected 50%, 100% and 33.3% of mice, respectively. Pre-incubation of the mixture of *E. ocellatus* venom (8.5 mg/kg) and 1500 mg/kg of *C. jagus* extract before injection into mice significantly ($p<0.05$) increased the mean death time.

4.3.3. Intraperitoneal administration of the extract, 30 min before injection of *E. ocellatus* venom (10 mg/kg, i.m.)

Two out of six died in the control mice that were treated with 10 mg/kg of *E. ocellatus* venom only.

Table 1
Oral administration of *C. jagus* extract, 30 min prior to injection of *E. ocellatus* venom (8.5 mg/kg, i.m.)

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract (control)	5/6	16.67	487.00 ± 51.48
B	500	4/6	33.33	333.75 ± 34.37[a]
C	1000	3/6	50	424.67 ± 51.19[a]
D	1500	5/6	16.67	678.00 ± 33.19[b]

[a]Significant (↓) at $p<0.05$ when compared with the control group.
[b]Significant (↑) at $p<0.01$ when compared with control and other treatment groups.

Table 2
Intraperitoneal (i.p.) administration of a mixture of *E. ocellatus* venom and *C. jagus* extract after pre-incubation for 30 min

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	5/6	16.67	487.00 ± 51.48
B	500	3/6	50	378.33 ± 54.99[a]
C	1000	0/6	100	–
D	1500	4/6	33.33	569.50 ± 71.43[b]

[a]Significant (↓) at $p<0.05$ when compared with the control group.
[b]Significant (↑) at $p<0.05$ when compared with control and *a*.

Table 3
Intraperitoneal administration of the extract, 30 min before the administration of *E. ocellatus* venom (10 mg/kg, i.m.)

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	2/6	66.67	738.50 ± 99.5
B	250	1/6	83.33	1320[a]
C	500	2/6	66.67	838.00 ± 51.00
D	1000	2/6	66.67	736.50 ± 59.5

[a]Significant (↑) at $p<0.01$ when compared with control and other treatment groups.

Table 4
Oral administration of *C. jagus* extract, 30 min before injection of *B. arietans* venom (9.5 mg/kg, i.m.)

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	5/6	16.67	113.80 ± 28.89
B	500	3/6	50	166.67 ± 26.87
C	1000	6/6	0	185.00 ± 25.20
D	1500	6/6	0	364.83 ± 57.31[a]

[a]Significant (↑) at $p<0.01$ compared to control and other treatment groups.

This represented 66.7% mice that survived while 250 mg/kg of the extract produced a survival of 83.3% (Table 3). There was no difference in the effect of 1000 or 1500 mg/kg of the extract when compared with the control. *C. jagus* extract at 250 mg/kg significantly ($p<0.01$) increased the time of death in the envenomed mice.

4.4. Evaluation of C. jagus extract for protective activity against B. arietans venom

4.4.1. Oral administration of the extract, 30 min before injection of B. arietans venom (9.5 mg/kg, i.m.)

The control group that was injected with *B. arietans* venom (9.5 mg/kg) only had one mouse alive, representing 16.7%. When 500 mg/kg of *C. jagus* extract was given orally, 30 min prior to injection of the venom, 50% of the mice survived. The administration of 1000 and 1500 mg/kg of the extract orally before injecting the venom, resulted in 100% mortality (Table 4). There was no significant difference in the mean death times between the mice treated with 500 mg/kg of the extract and control mice or between 500 and 1000 mg/kg of the extract. The extract at 1500 mg/kg significantly ($p<0.01$) prolonged the mean death time when compared with untreated mice.

4.4.2. Administration (i.p.) of a mixture of the extract and B. arietans venom after 30 min pre-incubation

In the control group which was injected with *B. arietans* venom (9.5 mg/kg), only 16.7% of the mice were alive. A pre-incubated mixture of *B. arietans* venom (9.5 mg/kg) and 500 mg/kg of *C. jagus*

extract offered 33.3% protection to the mice while no mouse survived in the groups treated with 1000 and 1500 mg/kg of the extract, respectively (Table 5). There were significant ($p<0.05$ and $p<0.01$) increases in the mean times of death at 500 and 1500 mg/kg of the extract, respectively, compared to the control mice.

4.5. Investigation of the extract for protective activity against N. nigricollis venom

4.5.1. Administration of C. jagus extract orally, 30 min prior to the injection of N. nigricollis venom (6 mg/kg, i.m.)

The control group which was injected with only *N. nigricollis* venom (6 mg/kg) had 16.7% of the mice alive. At 500 mg/kg of *C. jagus* extract in the presence of the venom, 16.7% of mice were alive as in the control. The extract at 1000 and 1500 mg/kg offered no protection (100% mortality) in each case (Table 6). There was no significant difference in the time of death between mice treated with 500 mg/kg of *C. jagus* extract and mice in the control group. The mean death times induced by the extract at 1000 and 1500 mg/kg were significantly ($p<0.05$) decreased compared to the effect of the extract at 500 mg/kg.

4.5.2. Administration of a mixture of N. nigricollis venom and C. jagus extract after pre-incubation

The administration of pre-incubated mixture of venom and 500 mg/kg of *C. jagus* extract protected 50% of the mice from death. Similarly, 33% of the mice treated with 1000 and 1500 mg/kg of the extract, pre-incubated with the venom for 30 min before administration of the mixture, survived (Table 7). There was a significant ($p<0.01$) increase in the mean death time when the pre-incubated mixture of venom and extract (500 mg/kg) was administered as compared to the control mice. However, the extract at 1000 mg/kg significantly ($p<0.01$) decreased the time of death when compared with the control mice. Similarly, the administration of the pre-incubated mixture of *N. nigricollis* venom and *C. jagus* extract (1500 mg/kg) significantly ($p<0.05$) decreased the mean time of death in mice compared to the control group.

4.6. Histopathology

Group 1 (negative control): The result showed a normal condition of the musculature devoid of necrotic lesion in the muscle fibres. The scattered arrangement of the fibres was due to bluntness of the knife used for sectioning (Plate 1).

Table 5
Administration of a mixture of *C. jagus* extract and *B. arietans* venom after 30 min pre-incubation

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	5/6	16.67	113.20 ± 29.03
B	500	4/6	33.33	191.75 ± 63.75[a]
C	1000	6/6	0	95.00 ± 26.02
D	1500	6/6	0	399.33 ± 71.18[b]

[a]Significant (↑) at $p<0.05$ when compared with control at $p<0.05$.
[b]Significant (↑) at $p<0.01$ compared to control and the other treatment groups.

Table 6
The oral administration of *C. jagus* extract, 30 min prior to injection of *N. nigricollis* venom (6 mg/kg, i.m.)

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	5/6	16.67	670.40 ± 73.86
B	500	5/6	16.67	643.20 ± 83.36
C	1000	6/6	0	217.17 ± 12.37[a]
D	1500	6/6	0	202.67 ± 8.83[a]

[a]Significant (↓) when compared with control at $p<0.01$.

Table 7
Administration of a mixture of *N. nigricollis* venom and *C. jagus* extract after pre-incubation

Groups	Dose of extract administered (mg/kg b.w.)	No. of dead mice out of the total in the group	Percentage (%) of mice alive	Mean death time (min ± s.e.)
A	No extract	5/6	16.67	670.40 ± 73.86
B	500	3/6	50	964.33 ± 49.98[a]
C	1000	4/6	33.33	295.50 ± 5.61[b]
D	1500	4/6	33.33	539.25 ± 87.58[c]

[a]Significant (↑) at $p<0.01$ when compared with control and the other treated groups.
[b]Significant (↓) at $p<0.01$ when compared with control.
[c]Significant (↓) at $p<0.05$ when compared with control but significant (↑) at 0.01 when compared with group treated with 1000 mg/kg of the extract.

Group 2 (positive control): *E. ocellatus* venom (5 mg/kg), alone was injected i.m. into the mice. There was moderate necrosis of the muscle fibres with slight lymphocytic infiltration (Plate 2).

Group 3: *C. jagus* extract (1000 mg/kg) was given orally, 30 min before the injection of *E. ocellatus* venom (5 mg/kg, i.m.). There was very severe necrosis and degeneration of the muscle fibres with massive infiltration of lymphocytes (Plate 3).

Group 4: Injected with a pre-incubated mixture of the extract (1000 mg/kg) and *E. ocellatus* venom (5 mg/kg, i.m.); it showed minimal lymphocytic infiltration even though evidence of cellular degeneration was present (Plate 4).

4.7. Creatine kinase assay

4.7.1. Creatine kinase assay after treatment with E. ocellatus venom (5 mg/kg) and C. jagus extract (1000 mg/kg)

The mean concentration of creatine kinase enzyme induced by venom alone in the control group was 322.63 ± 29.44 U/l. The creatine kinase concentration of 349.16 ± 27.80 U/l was recorded after the oral administration of *C. jagus* extract (1000 mg/kg), 30 min before injection of *E. ocellatus* venom. This enzyme concentration was not significantly different from that of the control group. The pre-incubated mixture of *E. ocellatus* venom (5 mg/kg) and *C. jagus* extract (1000 mg/kg) significantly ($p<0.01$) reduced the level of creatine kinase concentration to 184.66 ± 26.95 U/l (Fig. 1).

4.7.2. Creatine kinase assay in mice treated with B. arietans venom (7 mg/kg) and C. jagus extract (500 mg/kg)

B. arietans venom (7 mg/kg) as control produced 364.14 ± 11.68 U/l of creatine kinase in mice. Oral administration of *C. jagus* extract (500 mg/kg), 30 min before the injection of *B. arietans* venom (7 mg/kg), induced a creatine kinase concentration of 355.29 ± 18.14 U/l, which is not significantly different from the effect of the venom alone. Preincubation of *B. arietans* venom (7 mg/kg) and *C. jagus* extract (500 mg/kg) for 30 min, prior to administration, significantly ($p<0.05$) decreased the concentration of creatine kinase to 297.14 ± 11.32 U/l in poisoned mice (Fig. 2).

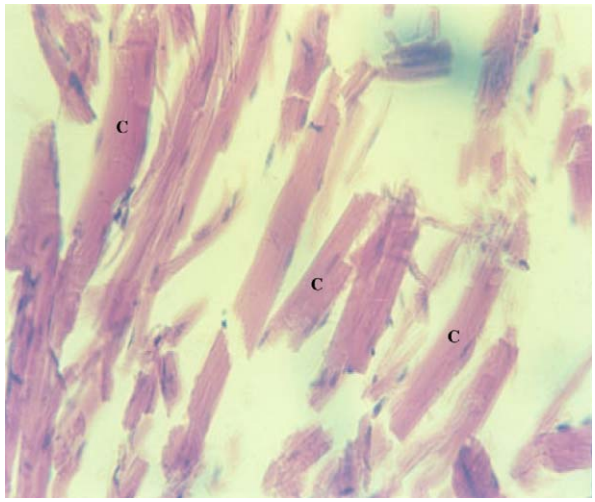

Plate 1. Longitudinal section of mouse muscle tissue from negative control, injected with only distilled water (×200 magnification). C is showing scattered normal muscle fibres.

4.8. Antihaemorrhagic test

An SHD of *E. ocellatus* venom (2.8 mg/ml) produced a distinct haemorrhagic lesion, 2 mm in diameter. A mixture of *E. ocellatus* venom (2.8 mg/ml) and various concentrations (6.7, 3.3 and 1.7 mg/ml)

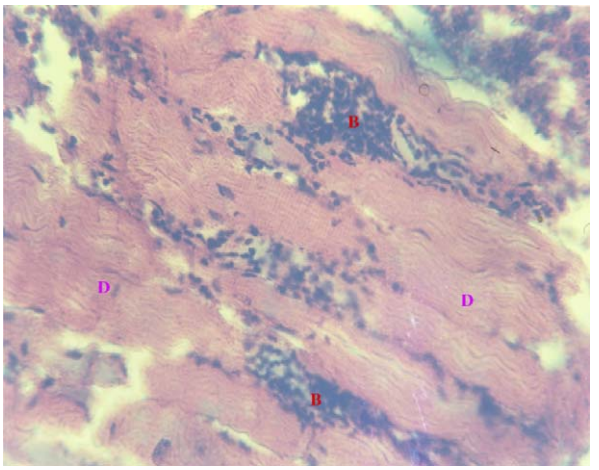

Plate 2. Longitudinal section of muscle tissue of mouse from positive control; only *E. ocellatus* venom (5 mg/kg) was injected i.m. (× 200 magnification). B shows inflammatory cells; D indicates degenerating muscle fibres.

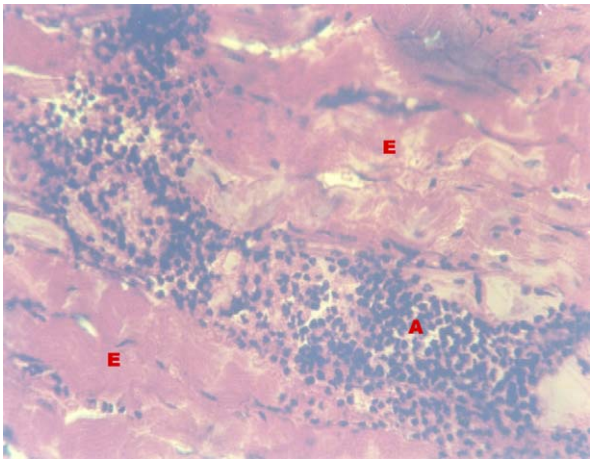

Plate 3. Longitudinal section of muscle tissue from mouse treated orally with *C. jagus* extract (1000 mg/kg) 30 min prior to injection of 5 mg/kg of *E. ocellatus* venom i.m. (× 200 magnification). A shows lymphocytic cells; E is necrotic muscle fibres.

of the methanolic extract of the bulb of *C. jagus* did not produce any haemorrhagic spot (Table 8).

5. Discussion

The methanolic extract of the bulb of *C. jagus* was safe in mice because the highest dose (2000 mg/kg) did not cause any death. The oral administration of the extract at 500 and 1000 mg/kg conferred 33.3% and 50% protection, respectively, against the lethal effect of *E. ocellatus* venom. This

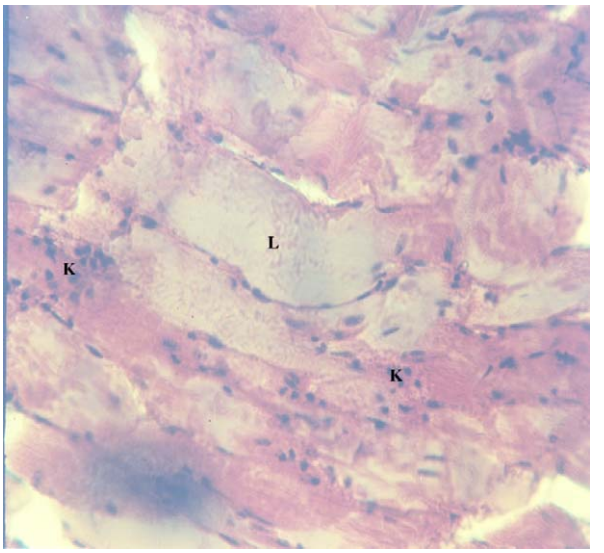

Plate 4. Longitudinal section of muscle tissue from mouse injected (i.m.) with pre-incubated mixture of *C. jagus* extract (1000 mg/kg) and 5 mg/kg of *E. ocellatus* venom (× 200 magnification). K indicates lymphocytes; L is muscle fibre.

effect was significant compared to 16.7% in the control mice. The most significant protective effect of the extract was obtained when it was pre-incubated for 30 min with *E. ocellatus* venom, prior to intraperitoneal administration of the mixture. Fifty and 100% protections were recorded in the mice treated with 500 and 1000 mg/kg, respectively. Although the highest dose (1500 mg/kg) of the extract gave only 33.3% protection, it significantly increased the mean death time in the mice (Table 2). Pre-incubation of venom and antidote is an approved method for preclinical assessment of antivenom efficacy (Theakston and Reid, 1983; Theakston et al,. 1995; World Health Organisation, 1981). *C. jagus* extract only showed significant protective effect (83%) when it was administered intraperitoneally at 250 mg/kg 30 min before the injection of *E. ocellatus* venom (Table 3). The oral administration of the extract at 500 mg/kg protected 50% of the mice poisoned with *B. arietans* venom but this effect reduced to 33% after pre-incubation of extract and venom. The ability of *C. jagus* extract to protect mice from the lethal effects of venoms (*E. ocellatus* and *B. arietans*) after oral administration is an indication that its action is pharmacological and not the result of physical interaction with the venom as is the case with most plant polyphenols. Other reasons to support the pharmacological action of the extract are the absence of a simple dose

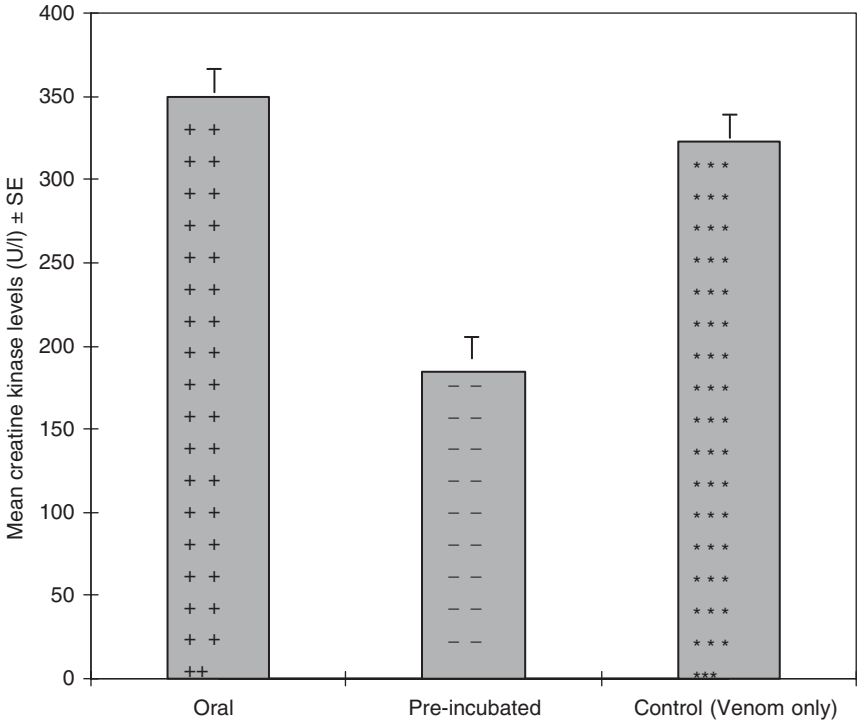

Fig. 1. Bar chart presenting the result of creatine kinase assay in mice treated with *Echis ocellatus* venom (5 mg/kg, i.m.): + + + / + + + *C. jagus* extract (1000 mg/kg) given orally, 30 min before injection of *Echis ocellatus* venom (5 mg/kg) i.m.; – – – / – – – mixture of 1000 mg/kg of *C. jagus* extract and *Echis ocellatus* venom (5 mg/kg), pre-incubated for 30 min before injection i.m.; * * * / * * * control, only *Echis ocellatus* venom (5 mg/kg), was injected i.m.

dependency with the extract and its unequal effectiveness against all three venoms. The effectiveness of the extract when administered orally is also an advantage since the oral route is the easiest for drug administration. *C. jagus* extract did not show protective effect against the lethal effect of *N. nigricollis* venom at the doses (500, 1000, 1500 mg/kg) tested. However, 30 min pre-incubation with *N. nigricollis* venom protected 50% of the mice.

The extract seemed to be an effective antidote against *E. ocellatus*, *B. arietans* and *N. nigricollis* venoms in that descending order. The differential protective effect of the extract in relation to the kind of venom may be related to the mechanism of venom toxicity. *E. ocellatus* and *B. arietans* are haemotoxic, while *N. nigricollis* is neurotoxic. It should be noted that the toxic dose of the venom was adjusted to suit the individual experiment. The purpose was to obtain a dose of the venom which will not be too toxic (70–80% lethality) to the mice and to allow the extract sufficient time to manifest its protective effect. Victims of snakebite are seldom inoculated with 5 LD_{50} or 4 LD_{50} (as used in most experiments) of the venom in a single attack.

The oral administration of *C. jagus* extract at 1000 mg/kg did not alter the level of creatine kinase induced by *E. ocellatus* venom but pre-incubation of the same dose of extract and venom significantly ($p<0.01$) reduced the plasma concentration of creatine kinase (Fig. 1). Similarly, the pre-incubated mixture of 500 mg/kg of the extract and *B. arietans* venom (7 mg/kg) significantly ($p<0.05$) decreased the concentration of creatine kinase compared to control mice (Fig. 2). The concentration of serum creatine kinase increases when there is necrosis in muscles (Baccus et al., 1972). Hence, the extract of *C. jagus* is able to reduce the degree of myonecrosis induced by *E. ocellatus* venom and *B. arietans* venoms. To support this is the ability of the pre-incubated mixture of the extract (1000 mg/kg) and *E. ocellatus* venom (5 mg/kg) to significantly inhibit infiltration by inflammatory cells into the site of assault (Plate 4). It means that the swelling accompanied by pain, which is normally experienced after a viperian bite, would be reduced by the

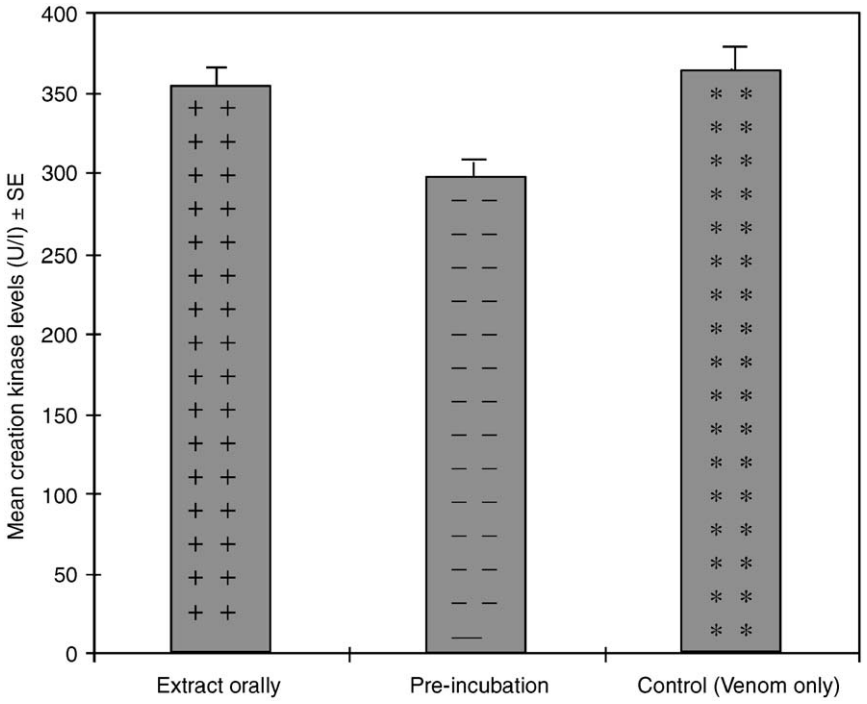

Fig. 2. Creatine kinase assay in mice treated with *B. arietans* venom (7 mg/kg) and *C. jagus* extract (500 mg/kg): + + + / + + + *C. jagus* extract (500 mg/kg): given orally, 30 min before injection of *Bitis arietans* venom (7 mg/kg) i.m.; : : : / : : : mixture of *C. jagus* extract (500 mg/kg) and *Bitis arietans* venom (7 mg/kg), pre-incubated for 30 min before injection i.m.; * * * / * * * control, only *Bitis arietans* venom (7 mg/kg), was injected i.m.

Table 8
Effect of *C. jagus* extract on haemorrhage induced by *E. ocellatus* venom (2.8 mg/ml) in the egg embryo

Extract/venom	Conc. of extract (mg/ml)	Haemorrhaghic zone (mm)	% reduction from control	State of the embryo after 4 h
Echis 2.8 mg/ml	–	2.0	–	All alive
Extract + *Echis* 2.8 mg/ml	1.7	0.0	100.0	All alive
Extract + *Echis* 2.8 mg/ml	3.3	0.0	100.0	All alive
Extract + *Echis* 2.8 mg/ml	6.7	0.0	100.0	All alive

extract. *C. jagus* extract (1.7, 3.3 and 6.7 mg/ml) completely blocked the haemorrhagic activity of *E. ocellatus* venom (Table 8) with a MEND of 1.7 mg/ml. The antihaemorrhagic activity of the extract is very important in dealing with bites from viperian snakes, which normally cause haemorrhage.

In conclusion, the methanol extract of *C. jagus* plant has shown significant antivenom activity. Its antivenom effect appears to be more significant against *E. ocellatus* venom. Pre-incubation of the extract and venom appears to be most effective compared to the other modes of treatment used in this study. The effect of the extract against *N. nigricollis* venom, which is neurotoxic, was very minimal. The present study has confirmed the ethnomedical use of the extract for treating snakebite victims among the rural Nigerian population. It is hoped that subsequent fractionation of the extract to obtain the pure active compound (s) will enhance its antivenom potential.

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