

Research Article

Anti-Venom Activity of *Cinnamomum zeylanicum* Extracts Against *Naja kaouthia* Snake Venom

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ABSTRACT

Cinnamomum zeylanicum also called as *Cinnamon bark* is the dried inner bark of the coppiced trees, belonging to the family Lauraceae. It is considered to be native of Sri Lanka and Malabar Coast of India. It contains tannins and flavonoids which have been reported to have anti-venom activity. The main aim is to evaluate the anti-venom activity of aqueous and alcoholic extracts of *Cinnamomum zeylanicum* extracts against various toxic effect of *Naja kaouthia* snake venom. Aqueous (AQCZ) and alcoholic (ALCZ) extracts of *C. zeylanicum* (both extracts at 500 and 1000mg/kg dose) were tested for their neutralization effect on lethality induced by *N. kaouthia* both *in vivo* and *in vitro*, neutralization of PLA₂ activity of *N. kaouthia* by indirect haemolysis method and also evaluated for their effect on *N. kaouthia* induced bleeding time in mice. AQCZ and ALCZ showed significantly protective effect against various complications induced by *N. kaouthia* venom like lethality both *in vivo* and *in vitro*, PLA₂ and bleeding time rise. AQCZ and ALCZ showed inhibitory effect on various physiological responses induced by *N. kaouthia* snake venom. Further study is required to isolate the active constituent and mechanism which is involved in anti-venom activity.

Keywords: *Cinnamomum zeylanicum*, antivenom, lethality, myotoxicity, paw edema, Tannins.

1. INTRODUCTION

Snake bite is frequent accident faced by villagers. In India there are about 216 species of snakes of which 52 are poisonous.¹ The four major venomous species responsible for fatality in India are Cobra (*Naja naja*), Krait (*Bungarus caeruleus*), Russell's viper (*Vipera russelli*) and Saw-scaled viper (*Echis carinatus*).² In north-eastern India *Naja naja* and *Naja kaouthia* are the two venomous snakes most often responsible for snakebite deaths.³ According to various estimates about more than 200000 cases are reported and an estimated 35000 to 50000 people die of snakebite every year in India.⁴ It has been estimated that 5 million people are bitten by venomous snakes annually around the world, thereby resulting in about 100,000 fatalities.⁵ Envenomation are associated with a variety of pathophysiological manifestations, frequently including a severe local tissue damage with myonecrosis, edema and hemorrhage, which may result in irreversible lesions and even amputation of the affected limb.⁶ Polyvalent antivenom therapy is the most common remedy available for treatment of snake bite. But, the polyvalent antivenom therapy is less effective and requires high doses

of antivenom that leads to adverse reactions that may be fatal.⁷ The adverse reactions observed are hypersensitivity reactions, serum sickness.⁸ Along with this, antiserum sometimes does not provide enough protection against hemorrhage, necrosis, nephrotoxicity and often produced hypersensitive reactions. Antiserum development in animal is time consuming, expensive and requires ideal storage condition.⁹ Traditional herbal medicine is readily available in rural areas for the treatment of snakebite. Plants are used either single or in combination, as antidotes for snake envenomation by rural populations in India and in many parts of the world. However, in most cases the efficacy of this traditional treatment regimen is unproven. Thus, the study of herbal antidotes against snake venom is of great importance in the management of snakebite.¹⁰ For example The methanolic root extracts of *Vitex negundo* and *Emblica officinalis* were tested for antisnake venom activity.¹¹ The root extracts of *Mimosa pudica* was tested for neutralization of lethality, myotoxicity and toxic enzymes of *Naja kaouthia*.¹² The aqueous extract of *Mikania glomerata* was tested for antiophidian properties.¹³

Cinnamomum zeylanicum also called as *Cinnamon* bark belonging to family Laureaceae. It is widely distributed in native of Sri Lanka and Malabar coast of India. The bark contains several volatile oils, aldehydes, tannins, phenols, hydrocarbons. It is being used widely as carminative, astringent, stimulant, aromatic, antiseptic, antihypertensive, antibacterial and antifungal, etc.^{14, 15, 16, 17} It has been mentioned in certain literature that *Cinnamomum zeylanicum* was used for snake bite in ancient Indian medicinal folkfare.¹⁸

Therefore an effort was made to study the Neutralizing activity of aqueous and alcoholic extracts of *Cinnamomum zeylanicum* against the *Naja kaouthia* venom induced lethality, Phospholipase A₂ activity and bleeding time activity.

2. MATERIALS AND METHODS

2.1 VENOM

Lyophilized *Naja kaouthia* venom was procured from the authenticated supplier from Hindustan Park, Kolkata, India and was preserved in a desiccator at 4°C for further use. It was dissolved in PBS of pH 7.2 at concentration of 100µg/ml for further use.

2.2 COLLECTION OF PLANT MATERIAL

About 3 kg of dried bark of *Cinnamomum zeylanicum* were procured from GKVK, Bangalore and Amruth kesari depot, Bangalore, and was identified and authenticated by Dr. P. Santhan, Plant Taxonomist, Natural Remedies Private Limited, Bangalore.

2.3 ANIMALS

Swiss albino mice weighing between 18-25g were procured. All the animals were acclimatized for seven days under standard laboratory conditions, i.e.; room temperature of 24 ± 10°C; relative humidity 45-55% and a 12:12h light/ dark cycle. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of P.E.S. College of Pharmacy Bangalore (Karnataka) was taken prior to the experiments. (Ref No. PESCP/IAEC/3/11)

2.4 EXTRACTION PROCEDURE

2.4.1 Aqueous Extraction

Collected *Cinnamomum zeylanicum* bark was finely powdered in a mechanical mixture. 10g of fine powder of *Cinnamomum zeylanicum* bark

was weighed and mixed with 100ml of water and kept on water bath at 60°C for 2h and filtered. Same procedure followed several time for obtaining required amount of extract.¹⁹

2.4.2 Alcoholic extraction

Collected *Cinnamomum zeylanicum* bark was finely powdered in a mechanical mixture. The powdered bark about 100g was extracted using ethanol 80 % about 700ml in Soxhlet apparatus for about 8 h. Then the obtained extract was evaporated to dryness using rotary flash evaporator under reduced pressure using vacuum. Same procedure followed several time for obtaining required amount of extract.²⁰

Then both the extracts were stored in desiccator until use. This extract was diluted with distilled water and administered orally to mice.

2.5 PHYTOCHEMICAL EVALUATION²¹

Both the extracts are evaluated for presence of flavonoids, tannins, saponins, alkaloids, terpenoids by chemical method.

2.6 ACUTE ORAL TOXICITY OF PLANT EXTRACTS²²

Acute oral toxicity of both the aqueous and alcoholic extracts was carried out according to OECD guidelines for acute oral toxicity.

2.7 DETERMINATION OF MEDIAN LETHAL DOSE OF VENOM (LD50 OR MLD)²³

The MLD was determined by administering varying dose of *N. Kaouthia* venom in Phosphate buffer of pH 7.2 in different groups of mice containing six in each using two different routes.

Initially a dose of 0.1µg/g, 0.3µg/g, and 0.5µg/g was administered by *i.p.* and a dose of 0.1µg/g, 0.3µg/g and 0.5µg/g was administered *i.v.* and observed for mortality and signs of neurotoxicity up to 72 hours.

2.8 NEUTRALIZATION OF LETHAL EFFECT

2.8.1 *In-vivo* Neutralization activity of AQCZ and ALCZ extracts against lethality induced by *N. kaouthia* venom^{3, 24}

The ability of the Aqueous (AQCZ) and Alcoholic (ALCZ) extract of *Cinnamomum zeylanicum* to neutralize lethal toxicity of venoms was assessed by *in-vivo* neutralization tests. *In-vivo* neutralization was performed in mice (n=6). The mice were administered with 500 & 1000 mg/kg of AQCZ and ALCZ through *p.o* route one hour prior to administration of 1 to 3.0 folds of MLD of venom by intraperitoneal route; all the animals

were observed for mortality for 24 h. Control animals were administered only with venom.

2.8.2 *In-vitro* Neutralization activity of ALCZ against lethality induced by *N. Kaouthia* venom^{25, 26}

For *In-vitro* neutralization, 1-3 folds of MLD of *N. kaouthia* venom were mixed with fixed doses of ALCZ (500 mg/kg and 1000 mg/kg) and incubated for 60 min at 37°C. Then it is centrifuged at 2000 rpm for 10 min. Then supernatant was collected and injected *i.p.* into mice. About 6 mice were used for each extract dose.

For comparison purpose control mice received the same amount of venom without plant extract. Here all the animals were observed for mortality for 24 h.

2.9: PHOSPHOLIPASE A₂ ACTIVITY^{27, 28}

2.9.1: Determination of MHD of *N. Kaouthia* venom

In order to evaluate the phospholipase A₂ activity, the indirect hemolytic activity was assayed. 300 µl of packed sheep erythrocytes (1.2%) washed four times with saline solution, 300 µl of 1:3 egg yolk solution in saline as a source of lecithin (1.2%) and 250 µl of 0.01 M CaCl₂ (10 mM) solution were added to 25 ml of 1% (w/v) of agar at 50°C dissolved in 0.8% PBS pH 7.2. The mixture was applied to Petri plates (135 x 80 mm) and allowed to gel. Then, 3 mm diameter wells were filled with 15 µl of samples. After 20 h of incubation at 37°C, the diameters of hemolytic halos were measured. To determine the minimum hemolytic dose (MHD) of isolated enzyme 15 µl of solutions containing different amounts of venom concentration (from 1 to 18 µg), were applied into the wells. The minimum hemolytic dose (MHD) was defined as the amount of enzyme that induced a hemolytic halo of 11-mm diameter.

2.9.2 Neutralization of Phospholipase activity (MHD) by AQCZ and ALCZ

Neutralization of phospholipase A₂ activity (indirect hemolytic activity) by AQCZ and ALCZ was done. Samples of constant amount of [12µg (1 MHD)] venom were incubated with different amount of AQCZ (1-300 µg) and ALCZ (1-300 µg) for 30 min at 37°C. Then aliquots of 15µl of the mixtures (venom + AQCZ or ALCZ) were added to wells in agar-egg yolk-sheep erythrocytes gels plates were incubated 37°C for 20 h. Control samples [12µg (1 MHD)] contained

venom without extract. Plates were incubated at 37°C for 20 h.

2.10 Effect on bleeding time of mice²⁹

Effect of AQCZ and ALCZ on *N. kaouthia* venom induced bleeding time in mice was performed under normal physiological condition. Bleeding time test was performed using male Swiss Albino mice. The animals in treatment groups received required doses of AQCZ and ALCZ by oral route one hour before the administration of *N. Kaouthia* venom (*i.v.* route). Control animals (group 2) received a similar injection of *N. Kaouthia* venom (LD 50 Dose) only. After one hour all the animals were anaesthetized with anesthetic ether. A wound was made on the tail vein of the mouse by surgical razor at 5 cm from the tip. The blood was absorbed on whatmann no.1 filter paper at every 15 sec interval till no blood stain was appeared on the filter paper then total bleeding time was recorded.

Statistical Analysis

Values will be expressed as Mean ± S.E.M. Statistical difference in mean will be analyzed using one way ANOVA followed by Dunnett's multiple comparison tests.

For all tests P<0.05 will be considered statistically significant.

3. RESULTS

3.1 EXTRACTION

Both aqueous and alcoholic extract were dark brown in colour and semisolid in nature. Results replicated in table no.1

3.2 PHYTOCHEMICAL EVALUATION

Phytochemical evaluation of both AQCZ and ALCZ was done and results replicated in table no.2.

3.3 ACUTE ORAL TOXICITY

Oral administration of aqueous and alcoholic extracts of *Cinnamomum zeylanicum* bark in mice for determination of toxicity studies were observed for up to 24 and 72 h. Animals did not showed any mortality or toxic symptoms but showed signs of slight sedation and perspiration at a dose up to 5000 mg/kg body weight. So it was concluded that both aqueous and alcoholic extracts were safe up to 5000mg/kg.

Based on this 1/10th of the acute oral toxicity of plant extract i.e. 500mg/kg and 1000mg/kg dose were selected for both aqueous and alcoholic extracts.

3.4 DETERMINATION OF MLD (LD 50) OF *N. KAOUTHIA* VENOM

The venom of *N. Kaouthia* is highly lethal to mice with an LD 50 (*i.p*) of 0.5 µg/g of mice with a mean survival time of 185 ± 34.26 min and LD 50 (*i.v*) of 0.3µg/g of mice with a mean survival time of 54 ± 22.16 min.

3.5 NEUTRALIZATION OF LETHALITY

3.5.1: *In- vivo* Neutralization activity of AQCZ and ALCZ against lethality induced by venom in mice

N. kaouthia venom induced lethality was partly antagonized by AQCZ. 50% neutralization of lethality up to 0.5 µg/g (1 fold) in 500 mg/kg and up to 100% neutralization of lethality up to 0.5 µg/g (1 fold) in 1000 mg/kg was observed. ALCZ at dose of 500 mg/kg showed 100 % neutralization of lethality up to 0.5 µg/g (1 fold) and 1000 mg/kg showed 100 % neutralization of lethality up to 0.5 µg/g (1 fold) and 50% neutralization of lethality up to 1 µg/g (2 folds). However animals in which extracts does not prevent death shows increase in survival time compare to venom control in which at 1 fold dose of venom survival time is 240 ± 17.32 min, at 2 fold dose of venom shows survival time of 36.83 ± 2.272 min and at 3 fold dose of venom shows survival time of 15.83 ± 3.135 min. Results are replicated in table: 3.

3.5.2: *In-vitro* Neutralization activity of ALCZ against lethality induced by *N. Kaouthia* venom

There was 100% neutralization of lethality up to 1 µg/g (2.0 folds) and 50% neutralization in 1.5µg/g (3.0 folds) of venom in both ALCZ 500mg/kg and 1000mg/kg. Mice which are not protected from lethality also show increase in survival time. In venom control group survival time for 3 folds dose of venom is 47 ± 5.66. Results are given in table no: 4

3.6 PHOSPHOLIPASE ACTIVITY

About 12µg of *N. kaouthia* venom produced 11 mm diameter haemolytic haloes which was taken as MHD. This shows that *N. kaouthia* venoms have the enzymes (phospholipase A₂) that has the ability to lyse sheep RBC's. Incubating different concentration of AQCZ from 25-300µg with 12µg of *N. kaouthia* venom, it was found that AQCZ prevented hemolysis by minimum of 4.5% to maximum of 63.6% ranging from 10.5 to 4 mm diameter. A dose of 250µg of AQCZ was able to prevent 50% hemolysis (5.5 ± 0.5mm). Whereas when incubated with different

concentration of ALCZ from 25-300µg with 12µg of *N. kaouthia* venom, it was found that ALCZ prevented hemolysis by minimum of 9.09% to maximum of 68.16% ranging from 10 to 3.5mm diameter. A dose of 225µg of ALCZ was able to prevent 54.54% hemolysis (5 ± 0mm). Figure no.1

3.7 Bleeding Time Test

The neutralization of bleeding time by AQCZ and ALCZ 500 and 1000 mg/kg in venom induced bleeding effect in mice was performed. *N. kaouthia* venom (LD50dose) showed increase in bleeding time at 1st h 304±8.860s, gradually decreased from 2nd to 4th h when compared to that of the normal bleeding time of the mice. AQCZ with lower dose (500mg/kg) showed significant (P<0.01) at 1st and 2nd h and 3rd and 4th h showed highly significant (P<0.001) decrease in bleeding time as compared to venom control group. AQCZ at dose of 1000mg/kg showed highly significant (p<0.001) at all 4 h decrease in bleeding time compare to venom control group. ALCZ at dose of 500mg/kg showed significant (p<0.01) at 1, 2 and 4th h and at 3rd h showed highly significant (p<0.001) decrease in bleeding time compare to venom control group. ALCZ at 1000mg/kg dose showed highly significant (p<0.001) at all 4 h decrease in bleeding time compare to venom control group. Results are replicated in table no 5 and figure 2.

4. DISCUSSION

Snakebite is a major health hazard that leads to high mortality rate especially in India. *Vipera russellii* and *Naja kaouthia* are the common snakes found throughout India and a large number of deaths occur due to envenomation by these snakes. Antisnake venom remains the specific antidote for snake venom poisoning. This antisnake venom is usually derived from horse sera. They contain horse immunoglobulins, which frequently caused complement mediated side effects, and other proteins that cause serum sickness and occasionally, anaphylactic shock. Although, the use of plants against the effects of snakes bite has been given since last 20 years.³⁰ Many Indian medical plants are recommended for the treatment of snakebite.¹¹

In this study we examined the preliminary phytochemical screening and antivenom potential *Cinnamomum zeylanicum* bark extract. This study was performed using *Cinnamomum zeylanicum*, one of the herbal plant traditionally used in phyto-therapeutic treatment for diabetes,

dyspepsia, intestinal colic and digestive atony³¹ in nausea and vomiting, as antimicrobial³², anti-inflammatory³³, osteostogenesis³⁴ infantile diarrhoea uterine stimulant. Besides, it has been notably used against snake bite in folklore medicine by traditional healers around the world. *Cinnamomum zeylanicum* was selected because of anti-venom property reported in ancient literature and it also contains some tannins and flavonoids which have been mentioned as antivenom constituent.¹⁸

In this study the aqueous and alcoholic extracts of *Cinnamomum zeylanicum* bark extracts of 500 and 1000 mg/kg were tested for its neutralization capacity of the *N. kaouthia* venom induced lethality, PLA₂ (indirect hemolysis) and bleeding time.

From the results of *in-vitro* animal studies, it was found that pre-incubating the ALCZ with NK venom prior to treatment yielded 100% survival in 1 and 2 folds, but with 3 folds only 50% survival in venom challenged mice (table 2). This finding is in compliance with previous studies done.^{13, 35, 36} Since the crude venom is composed of mostly proteinaceous components (approximately 90–95%) which possess many diverse enzymatic activities,³⁷ the most likely mechanism for antisnake venom activities by these plant extracts could undoubtedly be due to the binding of venom proteins with polyphenolic and tannin like substances of the extracts.³⁸ As a result, precipitation of the venom proteins occurred and the venom activities were inhibited. On the other hand, the *in vivo* test of AQCZ and ALCZ administration of venom first followed immediately by the plant extract using separate injection routes failed to rescue mice from the lethal effects of *N. kaouthia* venom. Nevertheless, in *in vivo* studies tannins may interact with plasma proteins from the blood circulation. Therefore, there is a decrease in binding to *N. kaouthia* venom proteins and, hence, extracts containing tannins become less efficient as anti-venom. This finding agree with the fact revealed by the previous work.³⁶

Indirect hemolysis exhibited by *N. kaouthia* venom PLA₂ in presence of egg-yolk phospholipids was due to formation of phospholipid hydrolysis products like lysophospholipids and free fatty acids which are lytic by themselves.³⁹ It is difficult to distinguish between *N. kaouthia* venom PLA₂ induced release of hemoglobin from the tissues due to haemolysis followed by RBC release. But rupturing of blood capillaries of tissues was

necessary for the release of hemoglobin and it may be contributed by phospholipid hydrolysis and protease activity present in NK-PLA₂.³⁹ PLA₂ activity induced by *N. kaouthia* snake venom (Figure 1) in sheep RBC's were inhibited by different doses of both AQCZ and ALCZ. The PLA₂ activity was maximum inhibited by ALCZ. The reason may be due to the extract rich in flavanoids. The several exogenous agents from medicinal plants such as flavanoids, aristocohlic acid and coumestan are also known to inhibit PLA₂.^{40, 41}

In *N. Kaouthia* snake envenomation, the presence of both local and systemic hemorrhage is common, mainly due to the presence of zinc-dependent metalloproteases which act by damaging the endothelium of blood vessels causing serious injuries to the skeletal muscle, such as myonecrosis followed by tissue ischemia. The inhibition of the hemorrhagic effect of *N. Kaouthia* venom by extracts is shown in Table No.5 and Figure 2. Results suggest that possible presence of compounds, in both the extracts, able to inhibit the activity of hemorrhagic proteins present in the venoms. These proteins are mostly Zn²⁺-dependent, and the active compounds of this plant may be interfering in the binding of the proteins to the cofactor, or may be binding to other active sites essential for this activity.⁴²

For the development of plant-derived therapeutic antagonist against snakebite for the community in need further investigation of plant; the isolation and investigation of active chemical constituent needed.

5. CONCLUSION

Present investigation revealed the *Naja kaouthia* venom neutralization by aqueous and alcoholic bark extract of 500 and 1000mg/kg of *Cinnamomum zeylanicum*. The neutralization capacity of the aqueous and alcoholic extract of *Cinnamomum zeylanicum* was checked for *N. kaouthia* venom. Various *In vivo* and *In vitro* models were used to determine the anti-venom capacity of the plant extract by using two dose levels and it showed a significant neutralization of lethality, PLA₂ activity and bleeding time. Hence aqueous and alcoholic extracts of *Cinnamomum zeylanicum* at low dose ranges showed a good Anti-venom activity. Further study is needed to determine the mechanism of action and to find out the active component responsible for its activity.

Table 1: Percentage yield of aqueous and alcoholic extracts of the *Cinnamomum zeylanicum*

Particulars	Aqueous Extract	Alcoholic extract
% Yield	6.4%	7.2%

Table 2: Phytochemical results

Chemical constituents	Aqueous extract	Alcoholic Extract
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Terpenes	+	+

Table 3: In vivo Neutralization studies of *Naja kaouthia* venom by AQCZ and ALCZ in mice

Treatment	Venom dose ($\mu\text{g/g}$)	MLD	No. Of Survival / No. of mice used	Survival time (min)	% Neutralized
Aqueous Extract 500 mg/kg	0.5	1	03/06	280 \pm 23.09	50 %
	1	2	00/06	65.83 \pm 2.71	0 %
	1.5	3	00/06	24 \pm 1.932	0 %
Aqueous Extract 1000 mg/kg	0.5	1	06/06	All survived	100 %
	1	2	00/06	76.17 \pm 2.58	0 %
	1.5	3	00/06	45.67 \pm 2.459	0 %
Alcoholic Extract 500 mg/kg	0.5	1	06/06	All survived	100%
	1	2	00/06	165 \pm 4.91	0 %
	1.5	3	00/06	70.17 \pm 5.576	0 %
Alcoholic Extract 1000 mg/kg	0.5	1	06/06	All survived	100%
	1	2	03/06	246.3 \pm 5.19	50 %
	1.5	3	00/06	96.83 \pm 4.64	0 %

Table 4: In vitro Neutralization studies of *N. kaouthia* venom by ALCZ in mice

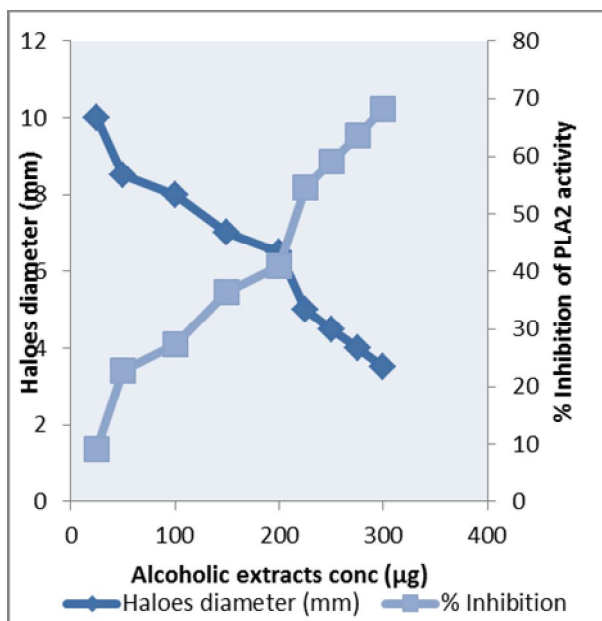
Treatment	Dose of venom	MLD	No. Of Survival/ No. of Mice used	Survival time of dead mice	% Neutralized
Alcoholic Extract 500 mg/kg	0.5	1	06/06	All survived	100 %
	1	2	06/06	All survived	100 %
	1.5	3	03/06	70 \pm 8.66	50 %
Alcoholic Extract 1000 mg/kg	0.5	1	06/06	All survived	100 %
	1	2	06/06	All survived	100 %
	1.5	3	03/06	96.67 \pm 7.26	50 %

Table 5: The effect of AQCZ and ALCZ on bleeding time

Time (h)	Vehicle	Venom	Aq 500mg/kg	Aq 1000mg/kg	Al 500mg/kg	Al 1000mg/kg
1	135 \pm 9.220	304 \pm 8.860	250 \pm 13.69 ^a (17.76%)	219 \pm 7.141 ^b (27.96%)	250 \pm 9.487 ^a (17.76%)	220 \pm 7.071 ^b (27.63%)
2	135 \pm 7.583	288 \pm 5.831	237 \pm 12.51 ^a (17.7%)	201 \pm 9.798 ^b (30.2%)	236 \pm 9.670 ^a (18.05%)	205 \pm 8.944 ^b (28.81%)
3	133 \pm 8.602	265 \pm 8.062	204 \pm 8.276 ^d (23.01%)	160 \pm 3.536 ^b (39.62%)	200 \pm 7.416 ^d (24.52%)	170 \pm 5.701 ^b (35.84%)
4	131 \pm 8.573	215 \pm 8.660	159 \pm 13.55 ^b (26.04%)	125 \pm 2.236 ^b (41.86%)	170 \pm 7.906 ^a (20.93%)	140 \pm 3.536 ^b (34.88%)

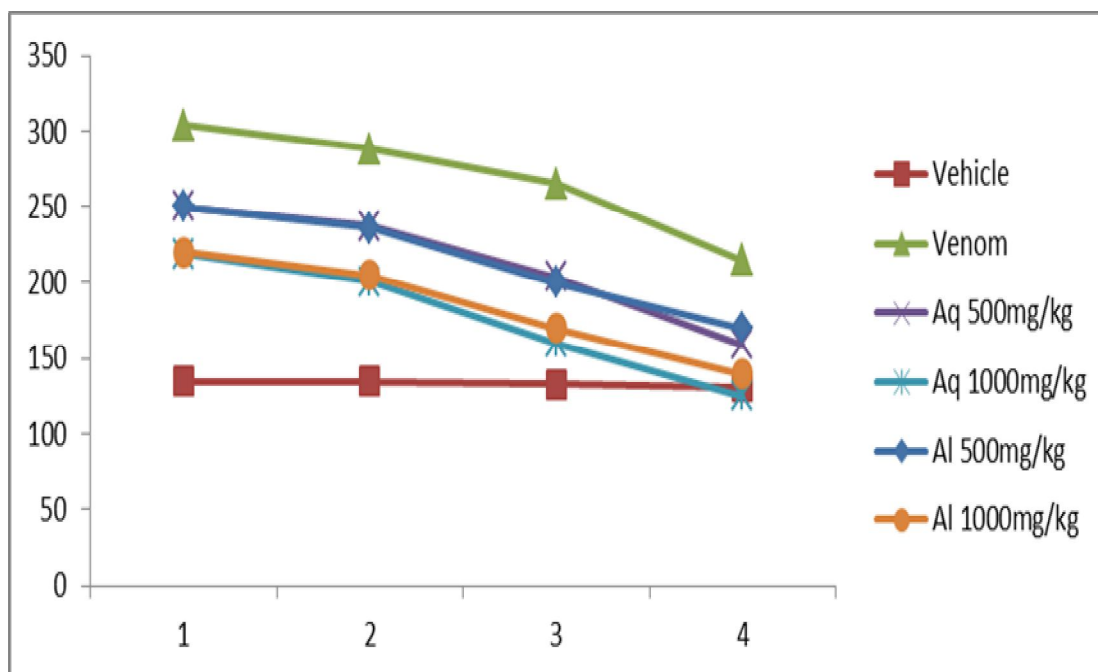
Values are expressed as mean \pm SEM; n = 6, a = **P < 0.01,

b = ***P < 0.001. compared with venom treated group using one way ANOVA followed by Dunnett's test.



Results are expressed as mean of 2 observation as Mean ± SEM.

Fig. 1: The effect of AQCZ and ALCZ on the *N. kaouthia* induced haemolysis of sheep RBC's gel plate



Values are expressed as mean± SEM; n = 6, **P<0.01, ***P<0.001.compared with venom treated group using one way ANOVA followed by Dunnett's test.

Fig. 2: Effect of AQCZ and ALCZ on Bleeding Time

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