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Prophylactic *In vivo* Neutralization Efficacy of Chitosan-Encapsulated Seed Extract of *Tamarindus indica* Against *Naja nigricollis* Venom-Induced Lethality and Coagulopathy

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ABSTRACT

Naja nigricollis is a medically significant venomous snake in Nigeria, particularly in the Northern regions, where it causes hundreds of annual deaths and permanent disabilities in rural communities. This study evaluated the prophylactic neutralization efficacy of chitosan-encapsulated extract of *Tamarindus indica* seed against *N. nigricollis* envenomation in mice. *In vivo* neutralization of venom-induced lethality and coagulopathy were determined using standard methods. Lethality neutralization study revealed that encapsulated and unencapsulated extracts dose-dependently neutralized the venom lethality. At 400 mg/kg, encapsulated extract conferred 100% survival in the envenomed mice at venom doses of 3 LD_{50} and 5 LD_{50}, respectively while unencapsulated extract achieved 75% and 50% at 3 LD_{50} and 5 LD_{50}, respectively. Furthermore, encapsulated extract normalized bleeding time and clotting time (1.00 min and 1.50 min, respectively). Remarkably, the efficacy of the encapsulated extract was comparable ($p > 0.05$) to Echitab plus at 100 mg/kg body weight and normal control. In contrast, unencapsulated extract showed higher levels of bleeding time and clotting time (1.42 min and 2.25 min, respectively) compared to these groups. Similarly, encapsulated extract exhibited higher protective efficacy against venom-induced haemorrhage (82% inhibition) which was significantly higher ($p < 0.05$) than unencapsulated extract (73% inhibition). The efficacy of encapsulated extract was comparable to that of Echitab plus with 83% inhibition. To our knowledge this is the first study evaluating the neutralization efficacy of chitosan-encapsulated extract of *T. indica* seed against *N. nigricollis*. The chitosan-encapsulated extract shows promise as a phytomedicine candidate for adjunctive snakebite therapy.

Keywords: *Naja nigricollis*, *Tamarindus indica*, antivenom, chitosan nanoparticles, lethality, coagulopathy

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INTRODUCTION

Snakebites represent a public health hazard that leads to high morbidity and mortality especially in the African continent and Indian subcontinent. This tropical disease is classified under class one of abandoned global health issues the World Health Organization (WH (Sofyantoro *et al.*, 2022). Malik *et* (2021) reported an annual incidence snakebite of 497/100,000 and a morta of 12.2% in Savannah Region of North Nigeria. Although, the data available

epidemiology statistics for occurrence and death as a result of snakebite incidence are said to be underestimated, this is due to inadequate computer-based and instant research software and tools needed in Asia and Sub-Saharan Africa where snakebite incident is endemic (Farooq *et al.*, 2022). Another reason for the perceived low data estimation record of snakebite injuries, especially in Africa, is limited attendance to health facilities by most of the snakebite victims and this practice is against the WHO guidelines on first aid treatment of snakebite for Africa (Chippaux, 2021).

The cobras originated from Africa and belonged to the family Elapidae, this snake species is the most regular snake associated with snakebite cases in hospitals (Deikumah *et al.*, 2023). Generally, these spitting cobras have active venom that causes ulceration and necrosis within the bite site, accompanied by systemic neurotoxic effects. Hospital records revealed that the black-necked spitting cobra (*Naja nigricollis*) is the most urbanized and clinically essential snake in Northern Nigeria (Musah *et al.*, 2022). Administration of serum-based antivenoms remain the cornerstone for the treatment of snakebites. However, challenges such as limited available, cold transport chain, allergic reactions, and exorbitant cost limited the use of these antivenoms (Gamulin *et al.*, 2023). Thus, it is imperative to search for generally available, effective, and cost-friendly alternatives such as medicinal plants extracts.

Tamarindus indica of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. Asian countries such as India and Myanmar have used *Tamarindus indica* for the treatment of snakebite (Ghaly *et al.*, 2023). Although this plant extracts have been explored for the treatment of snakebites, effectiveness is believed to be reduced poor solubility and stability resulting lower concentration of bioac principles at the target site (Faria et 2024).

Nanotechnology has enabled signific improvements in the pharmacok profiles, pharmacodynamic prope and therapeutic efficacy of plant-der bioactive compounds through contro and targeted delivery syste Nanocarrier encapsulation of extracts has demonstrated enha efficacy against diverse pathol including snakebite envenom Chitosan nanoparticles exhibit toxicity, high biocompatibility, tuna degradability, positioning it among most promising nanocarriers for pl bioactive compounds (Kulabhusan et 2020). This study aimed to evaluate neutralization efficacy of chitos encapsulated extract of *T. indica* se against *N. nigricollis* venom-induc lethality and coagulopathy.

MATERIALS AND METHODS

Collection and identification of pla material

The whole parts *Tamarindus indica* wa collected from Bosso, Minna, Niger sta Nigeria (Latitude 13° 4' 48.84" Longitude 13° 18' 37.08" E) on the 17th September 2024. The plant material w identified at the Department of Pla Biology, Federal University of Technolo Minna, Nigeria with voucher numb FUT.MIN/SLS/PB-020-024 and t specimen was deposited at the herbariu unit of the University.

Chemicals and reagents

Chemicals and reagents

The chemicals and reagents used in this study were of analytical grade, and products of Central Drug House (CDH)-India. The chemicals and reagents employed in this study include distilled water, phosphate buffer, chitosan, acetic acid, phosphate-buffered saline, sodium tripolyphosphate, potassium hexacyanoferrate (III), among others.

Preparation of chitosan nanoparticles

Chitosan solution concentration of 1% w/v was prepared by dissolving 1 g of chitosan with deacetylation degree of 90 in 100 mL of 1% acetic acid. The mixture was stirred on a magnetic stirrer at a temperature of 37 °C until the chitosan had completely dissolved (indicated by appearance of clear solution). Then, 5 mL of tripolyphosphate (TPP) solution (0.5% w/v) was slowly added to the chitosan solution at the rate 0.2 mL/minute with continuous stirring. Afterwards, the solution was further stirred for 1 hour at room temperature (37 °C) to ensure homogeneity. Finally, the chitosan nanoparticles were separated by centrifugation at a speed of 20,000 g and temperature of 4 °C for 30 minutes (Farid *et al.*, 2024).

Encapsulation of *T. indica* seed extract in chitosan nanoparticles

To encapsulate the *T. indica* seed extract in chitosan nanoparticles, 3 g of the synthesized chitosan nanoparticles were dissolved in 30 mL of 1% acetic acid and stirred on a magnetic stirrer until complete dissolution. Thereafter, 15 mL of *T. indica* seed extract (10 mg/mL) was added to the solution of chitosan nanoparticles. The mixture was stirred for 1 hour to ensure the extract was maximally entrapped (Farid *et al.*, 2024).

Experimental animals

A total of one hundred and fifty (150) mice weighing 31 ± 2.00 g were purchased from Murine Top Farms, Minna, Niger State, Nigeria. The animals were kept in polypropylene cages under environmental conditions of temperature 27 ± 2 °C and relative humidity 46-53%. The animals were given free access to water and fed with pelletized commercial grower feed (Vital Feeds, Jos Nigeria) *ad libitum*. The experiment was performed following the review protocol (1997) of Canadian Council on Animal care and use guidelines. Ethical clearance number 00021 was issued by FUT, MINNA/Nigeria Ethical Review Committee.

Animal grouping in anti-antivenin studies

There was execution of a fully randomized design experiment in which animals of equal number (n= 4), body weight, and gender were randomly allocated to different experimental groups as shown below:

Group 1 (Naive control): administered mL/kg body weight normal saline

Group 2(Positive control): envenomed and administered 100 mg/kg body wei of echitab plus

Group 3(Negative control): intoxicate and administered 2 mL/kg body weig normal saline

Group 4: envenomed and administere 100 mg/kg body weight encapsulate extract

Group 5: envenomed and administer 200 mg/kg body weight encapsulate extract

Group 6: envenomed and administered 400 mg/kg body weight encapsulated extract

Group 7: envenomed and administered 100 mg/kg body weight unencapsulated extract

Group 8: envenomed and administered 200 mg/kg body weight unencapsulated extract

Group 9: envenomed and administered 400 mg/kg body weight unencapsulated extract

Group 10: envenomed and administered 400 mg/kg body weight chitosan nanoparticles

Neutralization of Lethality

The prophylactic neutralization of *N. nigricollis* venom lethality by the encapsulated and unencapsulated extracts of *T. indica* seed was carried out following the method described by Muhammad *et al.* (2022) with slight modifications. The *in vivo* neutralization potencies of the extracts were assessed by intraperitoneal (i.p) administration of 3 LD_{50} and 5 LD_{50} doses of the venom into different groups of mice. The 3 LD_{50} and 5 LD_{50} doses of the venom were respectively administered one hour (1 h) after the oral administration of various doses of the encapsulated and unencapsulated extracts.

Determination of bleeding time

The bleeding time of the mice envenomed with 5LD_{50} of the venom was measured using the method of Deshpande *et al.* (2022) with slight modifications. Thirty minutes after respectively administering the extracts (i.e encapsulated and unencapsulated extracts), the venom (at dose of 5LD_{50}) was intraperitoneally injected into the mice. Pressure was built at the tail of the animal by stroking it ... fingers after thirty minutes (30 min) ... venom administration. Then a ... needle was used to puncture one of ... blood vessels lying alongside the tail. ... blood that oozed out of the site was bl... gently but completely with filter p... every 20 sec until bleeding ceases. ... time of the first appearance of blood ... stopping of the blood flow was taken ... bleeding time.

Determination of clotting time

The clotting time of the mice envenomed with 5LD_{50} of the venom was ev... using capillary coagulation me... described by Deshpande *et al.* ... Mice were respectively adm... different doses of encapsula... unencapsulated extracts thirty ... prior to venom injection. Non-h... capillary tube was used to co... from the retro-orbital plexus of ... through capillary action until w... of the tube was filled with bloo... intervals, one half of the capilla... carefully broken off at one en... pulled apart to look for the ... (fibrin clot) which is an i... clotting. The breaking of the t... at 20 sec intervals until fibri... seen. The time it took the thr... taken as the clotting time.

Determination of anti... activity

The minimum haemorrhag... of *N. nigricollis* venom was ... the method described by M... (2022). The minimum hae... is defined as the least an... which when injected intra... to mice results in a haemo... 10 mm diameter in ... prophylactic study, the M...

was intradermally injected in to the shaved dorsal skin of the mice in their respective groups 30 min following oral administration of different doses of the encapsulated and unencapsulated extracts.

RESULTS

Figure 1 shows the prophylactic venom-induced lethality neutralization efficacy of T. indica seed extract in both encapsulated (EE) and unencapsulated (UE) forms against *N. nigricollis* at venom at 3 LD_{50}. All envenomed mice and in the untreated group (negative control) exhibited 100% mortality (0% survival). Treatment with either EE or UE increased survival rates, though EE demonstrated higher neutralization capacity compared to UE exhibited higher neutralization potential compared to the unencapsulated extract. Both extracts showed dose-dependent effects. At 400 mg/kg, UE achieved 75% survival against 3 LD_{50} venom, while EE at the same dose conferred 100% survival. The positive control (Echitab plus, 100 mg/kg) also showed 100% survival. In contrast, chitosan nanoparticles (CNPs) alone (400 mg/kg) conferred no protection (0% survival), confirming that neutralization requires bioactive compounds rather than nanocarrier materials.

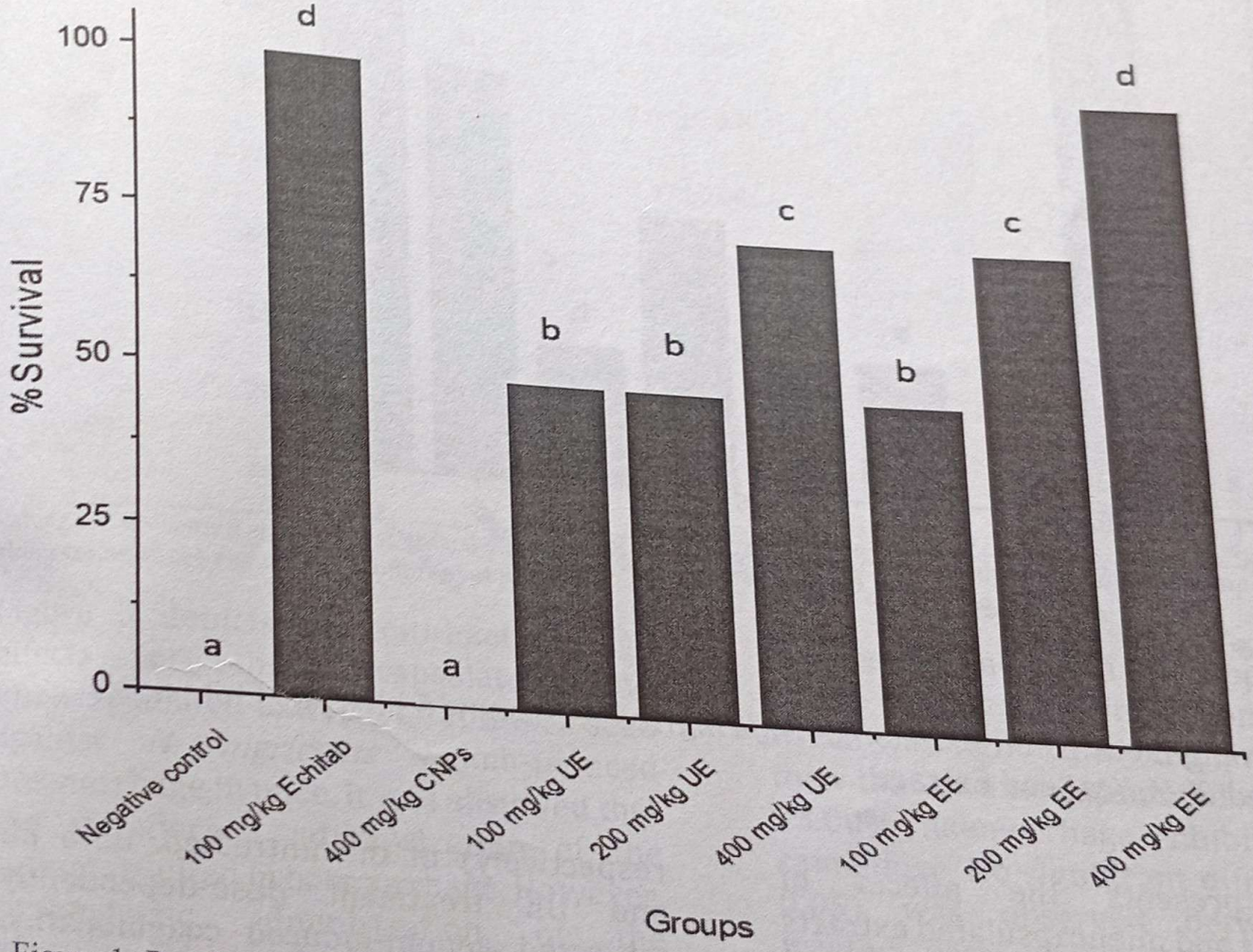

Figure 1: Prophylactic Neutralization of *Naja nigricollis* venom (3 LD_{50}) by Chitosan-Encapsulated *T. indica* Seed Extract

Figure 2 illustrates the dose-dependent neutralization efficacy of encapsulated and unencapsulated extract of T. indica seed against *N. nigricollis* venom at 5LD50. All untreated envelopes experienced 100% mortality (0% survival). However, the administration of either EE or UE significantly reduced percentage mortality (increased % survival rate), though EE exhibited higher efficacy. Consistent with results at 3 LD_{50}, survival increased with extract dosage, with the highest survival being obtained at 400 mg/kg. EE achieved 100% survival at 400 mg/kg, while only 50% survival was obtained for UE at the same dose. In addition, mice treated with 100 mg/kg body weight Echitab Plus recorded 100% survival while 0% survival was obtained for CNPs.

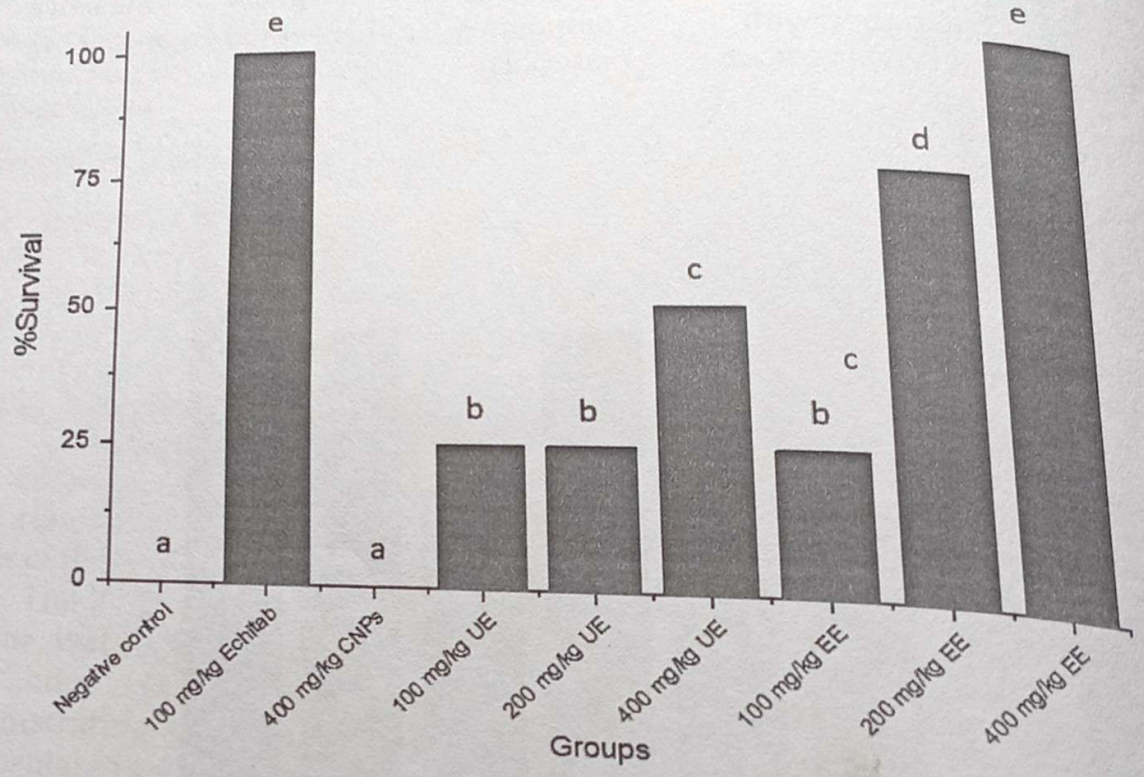

Figure 2: Prophylactic Neutralization of *Naja nigricollis* venom (5 LD_{50}) by Ch Encapsulated *T. indica* Seed Extract

Table 1 presents the effects of encapsulated and unencapsulated extracts of *T. indica* seed on bleeding time (BT) and clotting time (CT) in mice envenomed with N. nigricollis venom (5 LD_{50}). Intraperitoneal venom injection significantly prolonged ($p < 0.05$) in BT and CT (4.08 min and 4.58 min, respectively) in the untre and UE treatment do mitigated venom-induced with maximal protection a normalized BT and CT (1. min, respectively). Nota mg/kg showed compar Echitab plus (1.00 mir

respectively) and normal control (1.00 min and 1.33 min, respectively) ($p > 0.05$). Conversely, UE (1.42 min and 2.25 min, respectively) remain significantly elevated compared to these groups ($p < 0.05$). Chitosan nanoparticles alone reduced BT (3.42 min) and CT (4.08 min), which were significantly lower than the untreated ($p < 0.05$) but significantly higher than the treated groups ($p < 0.05$).

Table 1: Prophylactic Efficacy of Chitosan-Encapsulated Extract of *T. indica* Seed on Naja nigricollis-induced coagulopathy in Mice

Treatment group	Bleeding time (minutes)	Clotting time (minutes)
Normal control	1.00 ± 0.00^{a}	1.33 ± 0.08^{a}
Negative control	4.08 ± 0.08^{e}	4.58 ± 0.08^{f}
Echitab plus	1.00 ± 0.00^{a}	1.42 ± 0.08^{a}
400 CNPs	3.42 ± 0.08^{d}	4.08 ± 0.08^{e}
100 UE	2.83 ± 0.08^{c}	3.58 ± 0.08^{d}
200 UE	2.67 ± 0.08^{c}	3.08 ± 0.08^{c}
400 UE	1.42 ± 0.08^{b}	2.25 ± 0.14^{b}
100 EE	1.50 ± 0.00^{b}	3.08 ± 0.08^{c}
200 EE	1.17 ± 0.17^{a}	1.58 ± 0.08^{a}
400 EE	1.00 ± 0.00^{a}	1.50 ± 0.14^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscri along columns are significantly different at $p < 0.05$.

Figure 3 depicts the anti-haemorrhage effects of the encapsulated and unencapsulated extracts of *T. indica* seed against *N. nigricollis* venom-induced haemorrhage in mice. It was observed that the intradermal administration of the venom resulted in haemorrhage. However, prophylactic administration of the extracts mitigated the venom-induced haemorrhage. The effects of the extracts were observed to be dose dependent. Groups treated with 400 mg/kg body weight of the encapsulated extract showed the highest haemorrhage-suppress with a haemorrhage inhibition of 8 which was significantly higher (p than that of unencapsulated extra 73.00% haemorrhage inhibition same dose. No significant differer 0.05) was observed betweer treated with encapsulated ext group treated with 100 mg/kg plus. Groups treated with 400 mg inhibited haemorrhage by only which was the least haemorrhag among treated groups.

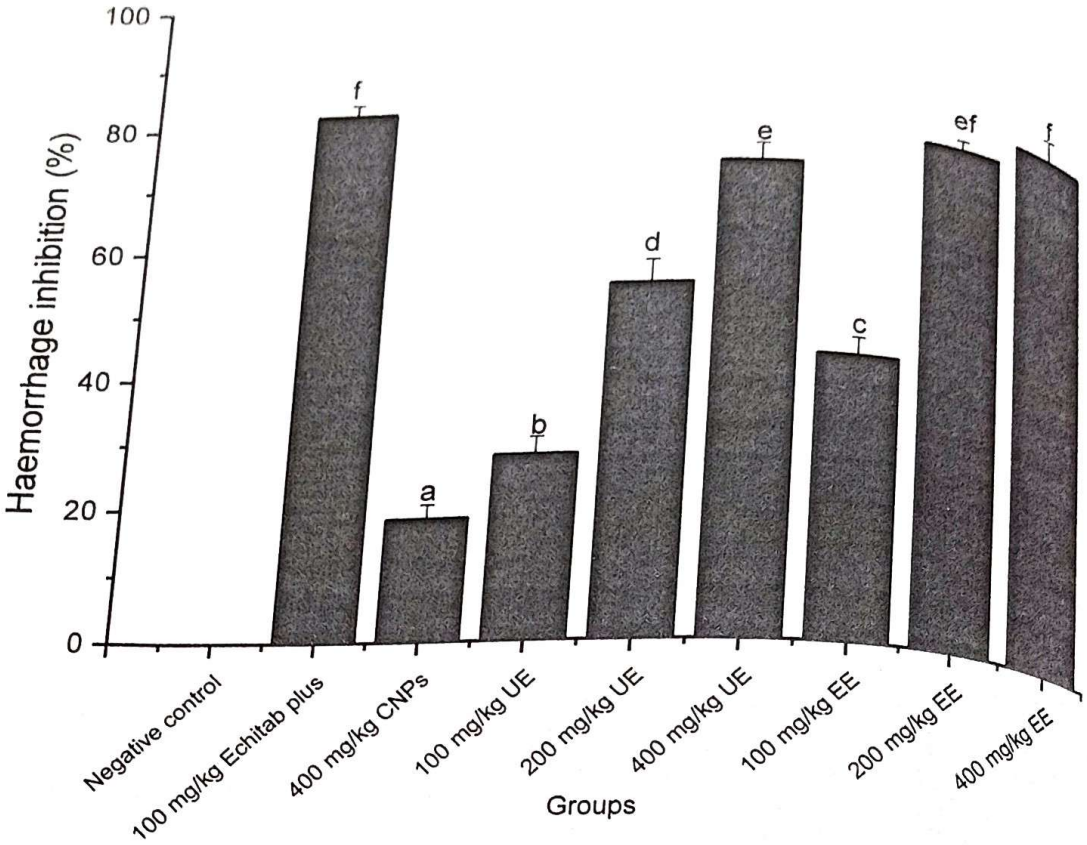

Figure 3: Prophylactic Efficacy of Chitosan-Encapsulated Extract of *T. indica* Seed o
nigricollis Venom-induced haemorrhage.

DISCUSSION

This study revealed that both encapsulated and unencapsulated extract significant neutralized the *N. nigricollis* venom-induced lethality, with encapsulated achieving 100% protection against the venom at 400 mg/kg body weight. To our knowledge, this is the study valuating the neutralization potentials of ncapsulated and unencapsulated extracts *T. indica* seed against N. nigricollis nom-induced lethality. N. nigricollis nom like other snake venoms contains diverse array of toxins contributi lethality through enzymatic hy (metalloproteinases, serine protea phospholipase A_2) or three-finge and other non-enzymatic toxins cause haemorrhage, coagulopati tissue damage ultimately resul death (Oliveira *et al.*, 2022). mechanisms through which plant- antivenoms elicited their effects ha postulated including protein precip enzyme inactivation/inhibition, ch adjuvant action, and antioxidant (Ravi *et al.*, 2024; Melo *et al.*, 20

such, the neutralization potential of the extracts against this snake venom could be attributed to one or combinations of these mechanisms.

Treatment of the envenomed mice with encapsulated and unencapsulated extracts of T. indica seed respectively mitigated the prolonged bleeding time and clotting time induced by the venom of N. nigricollis. Bleeding determines platelet function and vascular integrity by measuring primary haemostasis, which is the time for bleeding to stop after skin incision (Metkar& Saraf, 2023). Clotting time on the other hand measures secondary haemostasis (coagulation cascade) which is an essential tool for detecting life-threatening coagulopathy in snakebites. Venomous Snakes from the family 'Elapidae' have been shown to alter primary haemostasis by inhibiting platelet aggregation or destroying platelets using metalloproteinases, blocking platelets receptors (e.g GPIb, αIIbβ3 integrin), or vascular damage by metalloproteinases (Alvitigala *et al.*, 2025). Furthermore, the secondary haemostasis is disturbed by direct conversion of fibrinogen to fibrin by the venom components resulting in rapid depletion of clotting factors, inhibition of blood clotting factors such as X, IX V, or thrombin, degradation of fibrin clots by metalloproteinases, and destruction of fibrinogen (Metkar & Saraf, 2023). Hence, the protective effects of the extracts could stem from their abilities to potently inhibit venom toxins, especially the activity of metalloproteinases resulting in restored primary and secondary haemostasis. Furthermore, the encapsulated and unencapsulated extracts of *T. indica* seed significantly mitigated venom-induced haemorrhage. However, the encapsulated extract was found to exert higher protective effect. Hemorrhage is said to be an exceptional characteristic of haematotoxic snake venom brought about by the activity of proteases on the vascular endothelium (Tan *et al.*, 2021). Thus, the abilities of the extracts to significantly subdued the haemorrhagic effect of the venom could be attributed to their inhibitory effects on the activity of protease contained in the venom. In addition, the higher neutralization potential elicited by the encapsulated compared to unencapsulated extract is not a surprise since nanoencapsulation has been reported to shield plant extracts from oxidative degradation, improve pharmacokinetics and stability of plant extracts (Soltanzadeh *et al.*, 2021).

Conclusion

The encapsulated and unencapsulated extracts of *T. indica* seed exhibited significant in vivo antivenom effects, with encapsulated extract exhibiting higher neutralization effects. By implication, the encapsulated extract can be employed in the treatment or management of *N. nigricollis* envenomation or used as a drug lead for the development of efficacious antivenom against *N. nigricollis* envenomation. Further studies should be focused on isolation of bioactive compounds and in vivo antivenom evaluation of such compounds.

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