Antidote Potential of Ethanolic Leaf Extract of *Portulaca oleracea* on Mice Challenged with *Naja nigricollis* Snake Venom

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ABSTRACT

Portulaca oleracea is a well-known medicinal plant in Nigerian ethnomedicine for the management of many diseases. Investigations concerning its phytochemicals and pharmacological characteristics have been carried out but its potency against snake envenomation has not been evaluated. In this study, we evaluated its venom neutralizing (antidote) properties against *Naja nigricollis* venom in mice. Freshly collected leaves of *Portulaca oleracea* were room dried, powdered and extracted in ethanol. LD_{50} of the plant extract and N. nigrocollis venom were carried out using Lorke's method. Blood clotting time was measured using the modified method of Igboechi and Anuforo and bleeding time was measured using the modified procedure of Mohammed et al. To study the antivenom potentials of the ethanolic leaf extract of *Portulaca oleracea*, fifty-six (56) male and female albino mice weighing between 17-20g were randomly divided into six (6) groups of five (5) mice each. Group 1 received normal saline + venom, groups 2-5 received venom + *P. oleracea* extract at 0, 5, 10 and 15 mins delay respectively, while group 6 received venom + a combined extract of *Portulaca oleracea* and *Euphorbia hirta*. *N. nigricollis* venom was administered intraperitoneally at a dose of 1414 µg/kg body weight of mice and *Portulaca oleracea* extract was orally administered at a dose of 250 mg/kg body weight at different time interval in the in vivo assay. The result for the LD_{50} of the plant extract showed no mortality. It was found to be > 5000 mg/Kg body weight and that of *N. nigricollis* venom showed mortality in the second phase of mice that received a higher dose range of 2000-5000 µg/ml of the venom. The result for blood clotting time showed that *N. nigricollis* venom is an anticoagulant and the plant extracts brought the increased values back to normal. The result for bleeding time showed that *N. nigricollis* venom cause severe external hemorrhage but the plant extract stoped the excess bleeding. The result of the effects of time-lag after the

administration of *N. nigricollis* venom and *Portulaca oleracea* extract showed a 40% death in the group 1 (control), 80% survival in group 4 and the other groups had a 100% survival. This study showed that the ethanolic leaf extract of *Portulaca oleracea* showed anti venom neutralizing potentials in animal models. The above results 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: Ethnomedicine; envenomation; mortality; antidote; neutralize.

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INTRODUCTION

Snake bites are considered a neglected tropical trauma that affects thousands of people worldwide.

The number of snakebites that occur each year may be as high as five million. They result in about 2.5 million poisonings and 20,000 to 125,000 deaths (World Health Organization, 2015). The frequency of bites varies greatly in different parts of the world. They occur most commonly in Africa, Asia and Latin America with rural areas more greatly affected.

Although anti-venom immunotherapy is the only treatment available against snake envenomation, it is associated with many side effects which include; anaphylactic shock, pyrogen reaction and serum sickness. These are possible outcomes of the action of antigenic proteins present in higher concentrations in anti-venom (*Assafim et al.*, 2006). In addition, they do not neutralize the local tissue damage (*Gutierrez et al.*, 2009). Also, anti-venoms (antisera) are not available in remote areas and they are quite expensive.

Snake Venom is a complex mixture of many substances such as; toxins, enzymes (hydrolytic growth factors, activators and inhibitors) with a wide spectrum of biological activities (*Rhamy and Hemmard, 2000*). Snake Venoms are also known to cause various physiological changes in organs of different animals. The composition of snake venom determines the

physiological effects on their target and it varies with snake type, age and environment where there are found. The components of snake venom are mostly enzymes which include; L-amino acid oxidase, Alanine amino transferase, Phospholipase A_2, 5^1 Nucleotidase, Phosphodiesterase, Deoxyribonuclease, Ribonuclease I, Adenosine triphosphatase, Amylase, Hyaluronidase, NAD-Nucleotidase, Glusamine ammonium lyase and Kininogenase which are found in all snake types and Lactate dehydrogenase, Lysophospholipase, Acetylcholinesterase, Alkaline Phosphatase, Acid phosphatase, Factor-X activator, Heparinase, α-fibrinogenase, β-fibrinogenase, α-β-fibrinogenase, Fibrinolytic enzyme, Prothrombin activator, Collagenase and Elastase which are found in some species (*Bauchot,* 1994).

The World Health Organization (WHO) has estimated that up to 80 percent of people in the developing world are dependent on traditional system of medicines primarily because of their easy accessibility, wide affordability and cultural familiarity.

Over the years, many attempts have been made for the development of snake venom antagonists from plant sources. Some works have been done in other to find out the phytochemicals in some plants that have ethno botanical usage for snake envenomation (*Ukachukwu,* 2015). Some plants have shown to possess anti-snake venom potentials. Plants like *Annona senegalensis, Moringa oleifera, Alluim cepa, Allium sativum* have been used by the Fulani Herdsmen in Taraba State for the management of snake bite (Ameen *et al.*, 2015). Extracts of *Uvaria chamae* has also shown to neutralize some biological effects of *Naja nigricollis* snake venom in rats (Omale *et al.,* 2013).

Recently, work has been carried out in the department of Biochemistry, ANSU, Uli on phytochemical composition of *Portulaca oleracea.* In silico work has been carried out which revealed that some of the phytochemicals obtained from this plant have strong affinity for snake venom PLA_2 compared with the control ligand used (Ukachukwu, 2015). The phytochemical constituents of *Portulaca oleracea* that have been isolated include; flavonoids (Xu and Chen, 2006), alkaloids (Xiang *et al*., 2005), fatty acids, terpenoids, polysaccharides, vitamins, sterols, proteins, and minerals. Research has shown that this plant is a good source of omega-3 fatty acid. Its pharmacological effects include; antibacterial (Zhang *et al*., 2002), anti-ulcerogenic (Karimi *et al.,* 2004), anti-inflammatory (Chan *et al.,* 2000), antioxidant and wound-healing (Rashed *et al*., 2003) properties. Aside the insilico work done on this plant, few work have been done to ascertain its snake venom

neutralizing potentials. The aim of this study therefore is to carry out an *in vivo* assessment of the anti-snake venom neutralizing potentials of *Portulaca oleracea* ethanolic extract in mice.

MATERIALS AND METHODS

Chemicals, solutions and equipment: All chemicals used in the present study were of analytical grade and purchased from QULIKEM, India. Centrifuge (Heraeus Christ GMBH Estrode), Analytical balance, measuring cylinder, micropipette, mortar, pestle, beakers, retord stand, burette, syringes and deep freezer.

Collection, Identification and Extraction Procedure of the Plant Sample

The leaves of *Portulaca oleracea* were harvested from Girls High School, Agulu, Anaocha Local Government Area in Anambra State and was identified by Mrs. Emezie, A. U., a pharmacologist in the department of pharmacology, school of pharmacy, Nnamdi Azikiwe University, Agulu campus. Its voucher number is PCG 474/A/028. These leaves were dried at room temperature (25^0C) for a couple of days and later blended into fine powder using dry blender. 250g of the powder was macerated in 1litre of ethanol in an air tight plastic container and allowed to stand for 48hrs. The extract was filtered successively using muslin cloth and Wattman filter paper No. 42. The extract was then concentrated to dryness over a water bath at 50^oc.

Laboratory Animal

Albino mice were purchased from the animal house of the Department of Pharmacology, School of Pharmacy, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria. This study was approved by the Department of Biochemistry, Anambra State University, Uli, according to the institutional ethics. These animals were used as approved in the study of snake venom toxicity. The mice were maintained under normal laboratory condition of humidity, temperature (25 ± 1^0C) and light (12 hours night / day cycle) with access to clean water. An ethical clearance for animal use was obtained for this research work.

Snake Venom

Lyophilized *Naja nigricollis* venom was purchased from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria Nigeria.

EXPERIMENTAL DESIGN

Determination of LD_{50} of plant extract:

The LD_{50} of the ethanolic leaf extract of *Portulaca oleracae* was carried out according to the method described by Dietrich Lorke (1983).

Procedure:

This method has two phases.

Phase 1

This phase required nine mice. The nine animals were divided into three groups of three animals each. Each group of animals were orally administered different doses (10, 100 and 1000 mg/kg) of test substance (leaf extract). The animals were placed under observation for 24 hours to monitor their behavior as well as any mortality.

Phase 2

This phase involved the use of four animals, which were distributed into four groups of one animal each. The animals were administered higher doses (2000, 3000 4000 and 5000 mg/kg) of test substance (leaf extract) and then observed for 24 hours for behavior as well as mortality.

Then the LD_{50} was calculated with the formula:

$$LD_{50}\sqrt{D_0 \; x \; D_{100}}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

The formula below was used to determine the dose volume of extract and snake venom administered to each mouse.

$$\text{Dose volume} = \frac{\text{dose (mg/kg) x weight (kg)}}{\text{Stock (mg/ml)}}$$

Determination of LD_{50} of snake venom:

The LD_{50} of *Naja nigricollis* venom was carried out according to the method described by Dietrich Lorke (1983).

Bleeding Time:

For the determination of the bleeding time, modified procedure of Mohammed et al. (1969) was used. Four hours after envenoming the mice and treating them with plant extract, the tail of each rat was gently pieced with lancet. A piece of white filter paper was used to blot the blood gently from the punctured surface of the body. The readings were taken every 15 sec. The end result occurs when the paper was no longer stained with blood.

Clotting Time:

For the determination of the clotting time, the modified method of Igboechi and Anuforo (1986) was used. Clotting time is the time required for a firm clot to be formed in fresh blood on glass slides. The blood sample was collected from, the rats via tail bleeding and a drop was placed on a clean plain glass slide and every 15 sec, a tip of office pin was passed through the blood until a thread-like structure was observed between the drop of blood and tip of the pin. The thread-like structure was an indication of a fibrin clot. The time was recorded.

In vivo snake venom toxicity neutralizing potential of *Portulaca oleracea* plant extract on envenomed mice:

Thirty albino mice were randomly divided into six groups of five mice each.

Group 1: Control group that received snake venom and normal saline.

Group 2: Envenomed mice that received plant extract at zero minute delay.

Group 3: Envenomed mice that received plant extract at five minutes delay.

Group 4: Envenomed mice that received plant extract at ten minutes delay.

Group 5: Envenomed mice that received plant extract at fifteen minutes delay.

Group 6: Envenomed mice that received combined extracts of *Euphorbia hirta* and *Portulaca oleracea* at ten minute delay.

The venom was administered intraperitoneally at a dose of 1414 µg/kg body weight of mice and the extract was administered orally at a dose of 250 mg/kg body weight of mice at different time intervals.

RESULTS

Twenty-four hour acute toxicity (LD_{50}) result of the ethanolic leaf extract of *Portulaca oleracae*.

The different groups for the LD_{50} of plant extract were given the different doses (ml) of extract above and the result presented in table 1.

Table 1: Result for the LD_{50} experiment for plant extract

	Dose (mg/kg)	No of death	Behavior
Phase I	10	0/3	Normal
	100	0/3	Normal
	1000	0/3	Normal
Phase II	2000	0/1	Normal
	3000	0/1	Normal
	4000	0/1	Normal
	5000	0/1	Normal

In the first phase of the experiment, the first three groups of mice that received a dose range of 10-1000 mg/kg of the extract had no mortality. The four groups of mice in the second phase of the test that received a higher dose range of 2000-5000 mg/kg of the extract had no mortality as well.

LD_{50} value is > 5000 mg/kg since there was no mortality.

Table 2: Result for the LD_{50} experiment for *Naja nigricollis* venom

	Dose (µg/ml)	No of death	Behavior
Phase I	10	0/3	Normal
	100	0/3	Normal
	1000	0/3	Normal
Phase II	2000	1/1	Palpitating
	3000	1/1	Palpitating
	4000	1/1	Palpitating
	5000	1/1	Palpitating

In the first phase of the experiment, the first three groups of mice that received a dose range of 10-1000 mg/kg of the venom had no mortality. The four groups of mice in the second phase of the test that received a higher dose range of 2000-5000 mg/kg of the venom all died within 24hours.

This result shows that the venom of *Naja nigricollis* administered is very toxic and harmful in animal model.

According to Lorke's method, the LD_{50} is calculated as follows

$LD_{50} = \sqrt{1000 \ x \ 2000}$

LD_{50} = 1414.21 µg/ml.

Clotting time: The result of the effects of *Portulaca Oleracea* against *Naja nigricollis* venom on blood clotting time is as presented in Table 3:

Table 3: Effect of *Portulaca Oleracea* extract on clotting time after envenomation

Treatment groups	Clotting time (sec)
Group 1: Control	1.2±0.10
Group 2: Administered snake venom only	2.2±0.10
Group 3: Administered venom and *Portulaca Oleracea*	1.4±0.29
Group 4: Administered venom, *Portulaca Oleracea* and *Euphorbia hirta*	1.1±0.10

Values are Mean ± S.E.M (n=4)

The result of the effects of *Portulaca Oleracea* plant extracts against *Naja nigricollis* envenomation on blood clotting time shows that *Naja nigricollis* venom increased the clotting time in mice and the plant extract decreased the clotting time.

Bleeding time: The result of the effects of *Portulaca Oleracea* against *Naja nigricollis* venom on blood bleeding time is as presented in Table 4.

Table 4: Effect of *Portulaca Oleracea* extract on bleeding time after envenomation

Treatment groups	Bleeding time(sec)
Group 1: Control	0.9±0.33
Group 2: Administered snake venom only	1.9±0.43
Group 3: Administered venom and *Portulaca Oleracea*	0.8±0.18
Group 4: Administered venom, *Portulaca Oleracea* and *Euphorbia hirta*	0.5±0.23

Values are Mean ±S.E.M (n=4)

The result of the bleeding time of *Portulaca Oleracea* plant extracts against *Naja nigricollis* envenomation shows that group 2 that was administered snake venom has a higher value compared to the control group indicating a deleterious effect of the snake venom. The result of groups 3 and 4 treated with plant extracts had a reduced bleeding time when compared with group 2.

Effects of Time-lag After the Administration of 1414.21 µg/ml of Snake Venom and 250 mg/ml of Plant Extract on the Survival Rate of Mice

Table 5: Effect of the administration of snake venom and normal saline (Control)

Control (sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of normal saline administered (ml)	Result after 24hrs.
1 (m)	21.0	0.021	0.3	0.6	Died
2 (m)	27.4	0.027	0.4	0.7	Survived
3 (f)	24.3	0.024	0.4	0.6	Died
4 (f)	21.1	0.021	0.3	0.6	Survived
5 (m)	19.1	0.019	0.3	0.5	Survived

This group of mice had a 60% survival and a 40% death after the administration of *N. nigrocollis* venom and normal saline.

Table 6: Effect of the administration of snake venom and plant extract at zero minute delay.

(sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of extract administered (ml)	Result after 24hrs.
1 (m)	31.9	0.032	0.5	0.8	Survived
2 (m)	29.6	0.030	0.4	0.8	Survived
3 (f)	18.7	0.019	0.3	0.5	Survived
4 (f)	23.3	0.023	0.3	0.6	Survived
5 (f)	30.2	0.030	0.4	0.8	Survived

The plant extract was effective after the zero minute delay. There was 0% death and 100% survival.

Table 7: Effect of the administration of snake venom and plant extract at five minutes delay

(sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of extract administered (ml)	Result after 24hrs.
1 (m)	32.8	0.033	0.5	0.9	Survived
2 (m)	32.2	0.032	0.4	0.8	Survived
3 (f)	28.8	0.029	0.4	0.8	Survived
4 (f)	19.1	0.019	0.3	0.5	Survived
5 (f)	25.9	0.026	0.4	0.7	Survived

The plant extract was still effective after the five minutes delay because there was 0% death and 100% survival.

Table 8: Effect of the administration of snake venom and plant extract at ten minutes delay

(sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of extract administered (ml)	Result after 24hrs.
1 (m)	28.3	0.028	0.4	0.7	Survived
2 (m)	32.5	0.033	0.5	0.9	Survived
3 (f)	27.9	0.028	0.4	0.7	Survived
4 (f)	27.4	0.027	0.4	0.7	Survived
5 (f)	24.3	0.024	0.4	0.6	Died

The plant extract was still effective after the ten minutes delay. There was 20% death and 80% survival.

Table 9: Effect of the administration of snake venom and plant extract at fifteen minutes delay

(sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of extract administered (ml)	Result after 24hrs.
1 (m)	32.2	0.032	0.5	0.8	Survived
2 (m)	31.5	0.032	0.5	0.8	Survived
3 (f)	31.0	0.031	0.4	0.8	Survived
4 (f)	29.7	0.030	0.4	0.8	Survived
5 (f)	20.6	0.021	0.3	0.6	Survived

The plant extract was still effective after the fifteen minutes delay. There was 0% death and 100% survival

Table 10: Effect of the administration of snake venom and combined extracts at 10 minutes delay (*Portulaca oleracea and Euphorbia hirta*)

(sex)	Weight of animal (in g)	Weight of animal (in kg)	Volume of venom administered (ml)	Volume of extract administered (ml)	Result after 24hrs.
1 (m)	16.8	0.017	0.3	0.5	Survived
2 (m)	16.6	0.017	0.3	0.5	Survived
3 (f)	28.2	0.028	0.4	0.7	Survived
4 (f)	28.2	0.028	0.4	0.7	Survived
5 (f)	27.3	0.027	0.4	0.7	Survived

The mice were envenomated and were treated with the combined extract of *Portulaca oleracea and Euphorbia hirta*. There was 0% death and 100% survival.

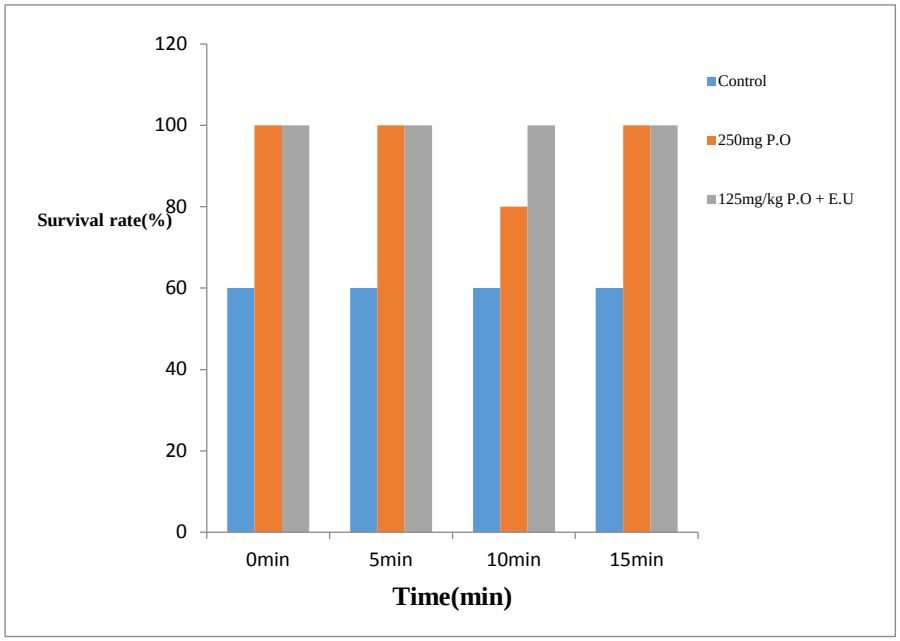

Fig 1: A Bar Chart of % survival rate against the dose or treatment in time.

The bar chat shows a 60% survival rate in the control of all the groups and 80% survival rate in the 10min group. All other groups show a 100% survival rate.

Discussion

Snake bites are considered a neglected tropical trauma that affects thousands of people worldwide. Although anti-venom (which is prepared from animal sera) immunotherapy is the only treatment available against snake envenomation, it is associated with many side effects which include; anaphylactic shock, pyrogen reaction and serum sickness. These are possible outcomes of the action of antigenic proteins present in higher concentrations in anti-venom (*Assafim et al.*, 2006). Also, antivenom (antisera) do not neutralize the local tissue damage (*Gutierrez et al.*, 2009), are not available in remote areas and are quite expensive. Although, the use of plants against the effects of snake bites has been recognized, more scientific attention has been given to it since last 2 decades (Alam and Gomes, 2003).

Several plants have been used in folk medicine throughout the world as treatment against snakebites (Houghton and Osibogun, 1993; Melo et al., 1994; Maiorano *et al.*, 2005; Oliveira et al., 2005; Cavalcante *et al.*,2007; Lomonte *et al.*,2009; De Paula *et al.*,2010; Omale *et al.*, 2013). Till date few plants materials have been evaluated in well controlled assays and only a few of them have been found to be effective against Snake envenomation. Snake bite is an important cause of morbidity and mortality and is one of the major health problems in rural areas in Nigeria.

The search for bioactive molecules in plants used in folk medicine has been growing in the past few years. This study shows that *Naja nigricollis* venom can inhibit or induce metabolism in mice and has also shown that *Portulaca oleracea*, a medicinal plant, neutralized some biological effects induced by *Naja nigricollis* venom.

The phytochemical constituents of *P. oleracea* has shown the presence of saponin, alkaloid, tannin, flavonoid, cardiac glycoside, terpenoids, protein and starch as its active phytoconstituents with saponin as the major constituent. The presence of these constituents in most plants has been reported to have proven its medicinal usage in health issues (Okafor and Ezejindu, 2014).The phytochemical analysis of *Portulaca oleracea* has also shown that it is a good source of omega-3 fatty acid and vitamins which means that it is good for consumption. Pharmacologically, it is a good antibiotic, antioxidant, anticancer, antimicrobial, anti-inflammatory, antiulcerogenic, and hepatoprotective agent.

The LD_{50} test for the the ethanolic leaf extract of *Portulaca oleracea* plant showed no mortality or abnormal behavior on the groups of mice that received both the lower and higher dose after 24hrs (table 1), hence, its phytochemical studies that showed that it is good for consumption. Its LD_{50} value is > 5000 mg/kg.

The LD_{50} of *Naja nigricollis* venom showed no mortality on the groups of mice that were envenomed with a lower dose of 10-1000 mg/ml but the experimental animals in the groups that received a higher dose range of 2000-5000 mg/ml of *Naja nigricollis* venom all died within 24 hrs (table 2) which tells us how toxic this venom can be when it gets in contact with one's biological system.

Regardless of the precise mechanism, *Portulaca oleracea* appear to be a promising chemical agent for use as first and treatment, or in combination with antiserum. Many snake venoms are known to cause pathological properties associated with haematological disturbances leading to in coagulability of blood. Some local tissue necrosis always accompany envenomation from this snake species. Spontaneous bleeding and coagulation disturbances are some of the haematological effects of Naja nigricollis in patients (Warrell et al., 1976).

A prolonged blood clotting time is a function of clotting factors deficiency. The result of clotting time shows *that Naja nigricollis* venom is an anticoagulant. In the envenomated animals (group 2) that were not treated with extract there was significant increase in clotting time due to the presence of venom. In groups 3 and 4 treated with *Portulaca oleracea* and *Portulaca oleracea* + *Euphorbia hirta* extract respectively, the extract neutralized this effect of the venom and the clotting time was maintained at the normal level when compared with the control group 1. The decrease in clotting time level observed in Table 3 establishes the fact that treatment of animals with venom/extract mixture abolished the blood incoagulability. The capacity of plasma to form thrombin is also relevant in the blood coagulation system. These entire blood characteristic are affected by the toxic components of *Naja nigricollis* venom (Denson et al., 1992).

Bleeding time is associated with integrity of blood vessels and is known to cause pathological disturbances leading to incoagulability of blood.

The result for the bleeding time of this study shows that *Naja nigricollis* venom caused severe external hemorrhage. The level of bleeding time increased significantly ($p<0.05$) in the envenomated animals in group 2 that were not treated with extract. In groups 3 and 4 treated with *Portulaca oleracea* and *Portulaca oleracea* + *Euphorbia hirta* extract respectively, the extract neutralized this effect of the venom and the bleeding time returned to the normal level when compared with the control group 1. The decrease in bleeding time level observed in Table 4 establishes the fact that treatment of animals with venom/extract mixture proved effective.

LD_{50} is meant to kill 50% of the experimental animals and that of *Naja nigricollis* venom (1414.21 µg/ml) killed 40% of the experimental animals in the control group within 24 hrs (table 5). This shows how effective the used *Naja nigricollis* venom is.

The result of the zero minutes delay shows the effectiveness of the plant extract used because there was 100% survival (table 6).

Portulaca oleracea plant extract neutralized the toxic effects of the venom at the five minutes delay. They was a 100% survival (table 7).

Though one death was encountered in the ten minutes delay, the plant extract still showed its effectiveness because they was an 80% survival (table 8).

The result of the fifteen minutes delay also showed a 100% survival. This shows that the neutralizing potentials of *Portulaca oleracea* plant extract was still intact fifteen minutes after envenomation (table 9).

Portulaca oleracea + *Euphorbia hirta* plant extracts neutralized the toxic effects of the venom at the fifteen minutes delay as shown in table 10.

Conclusion

From this study, it can be concluded that the extract of *Portulaca oleracea* is effective in neutralizing the toxic effects of *Naja nigricollis* venom. Time of treatment is of essence because the longer the time taken after the envenomation, the more disastrous the toxins in the venom damages the body cells.

Further experiment could address the fractioning of the *Euphorbia hirta* 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 accidents-victims.

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References

Abdel Moneim, A. E. (2013). "The neuroprotective effects of purslane (*Portulaca oleracea*) on rotenone-induced biochemical changes and apoptosis in brain of rat". *CNS & Neurological Disorders—Drug Targets*. 12 (6): 830–841.

Ahmed, A., Rajendaran, K. and Jaiswaletal, D. (2010). "Anti-snake venom activity of different xtracts of Pouzolzia indica against Russel viper venom". *International Journal of Chem Tech Research.* 2 (1): 744–751.

Akhila, J. S., Deepa, S. and Alwar, M. C. (2007). "Acute toxicity studies and determination of median lethal dose". *Curr Sci.* 93: 917–20.

Alam, M.I. and Gomes, A. (2003) "Snake Venom Neutralization by Indian Medicinal Plant (Vitex negundo and Emblica officinalis) Root Extracts". *Journal of Ethnopharmacology*, 86: 75-80.

Ameen, S.A., Salihu, T., Mbaoji, C.O., Anoruo Dibia, C.A. and Adedokin, R.A.M. (2015). Medicinal plants used to treat snake bite by Fulani Herdsmen in Taraba State, Nigeria. *International Journal of Applied Agriculture and Apicultural Research.* 11(1and 2) 10 – 21.

Assafim, M., Ferreira M.S. and Frattani, F.S. (2006). Counteracting effect of glycyrrhizin on the hemostatic abnormalities induced by bothrops jararaca snake venom. *Braz. J. Pharmacol.*, 148: 807–813.

Awad, N. (1994). "Lipid content and antimicrobial activity of phenolic constituents of cultivated *Portulaca oleracea* L". *Bull Fac Pharm*. 32: 137–142.

Bauchot, R. (1994). "Snakes: A Natural History." New York City, NY, USA: *Sterling Publishing Co., Inc*. 194–209.

Behravan, J., Mosafa, F., Soudmand, N. Taghiabadi, E., Razavi, B. M. and Karimi, G. (2011)."Protective effects of aqueous and ethanolic extracts of *Portulaca oleracea* L. aerial parts on H_2O_2-induced DNA damage in lymphocytes by comet assay". *Journal of Acupuncture and Meridian Studies.* 4 (3): 193–197.

Bottrall, J. L., Frank, M., Christopher, D. B., Michael, G. V. and Peter, J. M. (2010)."Proteolytic activity of Elapid and Viperid Snake venoms and its implication to digestion". *Journal of Venom Research* 1 (3): 18–28.

Bruce, R. D. (1985). "An up-and-down procedure for acute toxicity testing." *Fundam Appl Toxicol.* 5: 151–7.

Cavalcante, W.L.G., Campos T.O. and Pai-Silva, M.D. (2007). "Neutralization of snake venom phospholipase A2 toxins by aqueous extract of casearia sylvestris (Flacourtiaceae) in mouse neuro-muscular preparation". *J. Ethnopharmacol.* 112: 490-497.

Chan, K., Islam, M. W. and Kamil, M. (2000). “The analgesic and anti-inflammatory effects of Portulaca oleracea L. subsp. Sativa (Haw.) Celak.” *Journal of Ethnopharmacology*. 73 (3): 445–451.

De Paula, R.C., Sanchez, E.F. and Costa, T.R. (2010). “Antiophidian properties of plant extracts against Lachesis muta venom”. *J. Venom. Anim. Toxins Trop. Dis*. 16: 311-323.

Elkhayat, E. S., Ibrahim, S. R. M. and Aziz, M. A. (2008). “Portulene, a new diterpene from *Portulaca oleracea* L.” *Journal of Asian Natural Products Research*. 10 (11-12): 1039 1043.

El-Sayed, M.-I. K. (2011). “Effects of *Portulaca oleracea* L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy,” *Journal of Ethnopharmacology*. 137 (1): 643–651.

Engelmann, Wolf-Eberhard (1981). “Snakes: Biology, Behavior, and Relationship to Man.” *Leipzig; English version NY, USA: Leipzig Publishing; English version published by Exeter Books* (1982) 71.

Gold, B. S., Richard C. D. and Robert A. B. (2002). "Bites of venomous snakes". *The New England Journal of Medicine* 347 (5): 347–56.

Gong, F., Li, F., Zhang, L., Li, J., Zhang, Z. and Wang, G. (2009). “Hypoglycemic effects of crude polysaccharide from Purslane”. *International Journal of Molecular Sciences.* 10(3): 880–888.

Gutiérrez, J. M., Bruno L., Guillermo L., Alexandra R., Fernando C. and Yamileth A. (2007). "Trends in Snakebite Envenomation Therapy: Scientific, Technological and Public Health Considerations". *Current Pharmaceutical Design* 13 (28): 2935–50.

Houghton, P.J. and Osibogun, I.M. (1993) Flowering Plant Used against Snakiebite. *Journal of Ethnopharmacology*, 39: 1-29.

Igboechi, A.C. and Anuforo, D.C. (1986). “Anticoagulant Activities of Extracts of Eupatorium Odoratum and Vernonia Amygdalina. In: Sofowora, A.” (Ed.), the State of Medicinal Plants Research in Nigeria. University of Ile-Ife Press, Nigeria.

Ihejiofor F.C. (2015). “Phytochemical Analysis of the Leaves of Euphorbia hirta Plant”.

Karimi, G., Hosseinzadeh, H. and Ettehad, N. (2004). “Evaluation of the gastric antiulcerogenic effects of *Portulaca oleracea* L. extracts in mice,” *Phytotherapy Research*. 18 (6): 484–487.

Kasturiratne, A., Wickremasinghe, A.R., Silva, N., Gunawardena, N.K. and Pathmeswaran, A. (2008). The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Med* 5: 1591–1604.

Lee, A. S., Lee, Y. J. and Lee, S. M. (2012). "*Portulaca oleracea* ameliorates diabetic vascular inflammation and endothelial dysfunction in db/db mice". Evidence-Based Complementary and Alternative Medicine. 2012, Article ID 741824, 9.

Lomonte, B., Leon, G., Angulo, Y., Rucavado, A. and Nunz, V. (2009). "Neutrilization of bothrops asper venom by antibodies, natural products and synthetic drugs: Contributions to understanding snake bite envenomings and their treatment." *Toxicon,* 54: 1012-1028.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch Toxicol*. 54: 275–287.

Maiorano, V.A., Marcussi, S. and Daher, M.A.F. (2005). "Antiophidian properties of the aqueous extracts of mikania glomerata." *J. Ethnopharmacol.*, 102: 364-370.

Marais, Johan (2004). "A Complete Guide to the Snakes of Southern Africa. Cape Town, South Africa." *Struik Nature*.

Martin, C. J. and Lamb, G. (1907). "Snake-poison and Snake-bite". In Allbutt, T.C., Rolleston N.D. *A System of Medicine. London: MacMillan*. pp. 783–821.

Mastenbroek, Richard. (2011). "Black-neck Spitting Cobra" (PDF). *DEVENOMIZED.* www.devenomized.com.

Mattison Chris (2007). "The New Encyclopedia of Snakes." *New Jersey, USA: Princeton University Press*. 117.

Mehrtens, J. M. (1987). "Living Snakes of the World in Color." New York: Sterling Publishers. 480.

Melo, P.A., Do Nascinento, M.C. Mors W.B. and Suarez-Kurtz, G. (1994). "Inhibition of the myotoxic and hemorrhagic activities of crotalid venoms by eclipta prostrate (Asteraceae) extracts and constituents." *Toxicon*, 32: 395-603.

Mohammed, A. I. and Hussein, A. S. (1994). "Chemical composition of purslane (*Portulaca oleracea)*". *Plant Foods for Human Nutrition*. 45 (1): 1–9.

Mohammed, A. H., Serougi, M. S. and Hanna, M. M. (1969). "Observations on the effects of *echis carinatus* venom on blood clotting." *Toxicon*, 6: 215–219.

"Naja melanoleuca". Encyclopedia of Life. Retrieved 2014-03-22.

"Naja melanoleuca". Integrated Taxonomic Information System. Retrieved 11 January 2014.

Okafor, I. A. and Ezejindu D. N. (2004). "Phytochemical studies on *Portulaca oleracea* (purslane) plant." *Global 1Journal of Biology, Agriculture & Health Sciences*. 3 (1): 132–136.

Oliveira, C.Z., Maiorano, V.A. and Marcussi, S. (2005). "Anticoagulant and antifibrinogenolytic properties of the aqueous extracts from bauhinia forficata against snake venoms." *J. Ethnopharmacol.*, 98: 213-216.

Omale, J., Ebiloma U. and Idoko G.O. (2013). *Uvaria chamae* (Annonaceae) plant extract neutralizes some biological effects of *Naja nigricollis* snake venom in rats. *British Journal of Phamacology and Toxicology*. 4(2): 41-50.

Rahmy, T. R. and Hemmaid, K. Z. (2000). "Histological and histochemical alterations in the liver following intramuscular injection with sublethal close of the Egyptian cobra venom." *J. Nat. Toxins*. 9: 21–32.

Rashed, A. N., Afifi, F. U. and Disi, A. M. (2003). "Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in Musmusculus JVI-1," *Journal of Ethnopharmacology*. 88 (2-3): 131–136.

Simopoulos, A. P., Tan, D. X., Manchester, L. C. and Reiter, R. J. (2005). "Purslane: a plant source of omega-3 fatty acids and melatonin". *J Pineal Res*. 39 (3): 331–332.

Uddin, M. K., Juraimi, A. S., Hossain, M. S., Nahar, M. A. U., Ali, M. E. and Rahman, M. M. (2014). "Purslane weed (*Portulaca oleracea*): a prospective plant source of nutrition, omega-3 fatty acid, and antioxidant attributes," The Scientific World Journal. 6.

Ukachukwu, C.I. (2015). "Insilico determination of binding affinity of an anti- snake venom component of ethno medicinal origin to phospholipase A2 by molecular docking simulating". (Not published)

World Health Organization. (2015). "Neglected tropical diseases Snakebite".

Xiang, L., Xing, D., Wang, W., Wang, R., Ding, Y. and Du, L. (2005). "Alkaloids from *Portulaca oleracea* L." *Phytochemistry*. 66 (21): 2595–2601.

Xin, H. L., Xu, Y. F. and Hou, Y. H. (2008). "Two novel triterpenoids from *Portulaca oleracea* L." *Helvetica Chimica Acta.* 91 (11): 2075–2080.

Xu, X., Yu, L. and Chen, G. (2006). "Determination of flavonoids in *Portulaca oleracea* L. by capillary electrophoresis with electrochemical detection," *Journal of Pharmaceutical and Biomedical Analysis*. 41 (2): 493–499.

Yan, J., Sun, L.R., Zhou, Z.Y., Chen, Y.C., Zhang, W.M., Dai. H.F. and Tan, J.W. (2012). Homoisoflavonoids from the medicinal plant *Portulaca oleracea."Phytochemistry*. 80: 37–41.

Yang, Z., Liu, C., Xiang, L., and Zheng, Y. (2009). "Phenolic alkaloids as a new class of antioxidants in *Portulaca oleracea*". *Phytother Res*. 23 (7): 1032–1035.

Zhang, X. J., Ji, Y. B., Qu, Z. Y., Xia, J. C. and Wang, L. (2002). "Experimental studies on antibiotic functions of *Portulaca oleracea* L. in vitro," *Chinese Journal of Microecololgy*. 14 (6): 277–280.