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EVALUATION OF IPOMOEA SETOSA CRUDE TITER EXTRACT EFFECT ON NAJA PHILIPPINENSIS MICE INDUCED VENOM TOXICITY

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ABSTRACT

The study determined the weakest concentration in serial dilution of 1:60, 1:100 and 1:120 titers of Ipomoea setosa crude seed extract that exerted a counter toxic effect against cobra venom induced toxicity test in 30 ICR laboratory mice. The titer looked for is watered down repeatedly to see how long it remained in the blood of mice and kept mice alive 24 hours post treatment. It also evaluated the independent effect of routes and titers, interaction of titers and routes in terms of onset and duration of toxic signs, and onset of mice recovery. Descriptive statistics and 2-way ANOVA yielded slow to fast onset and duration of toxic signs when administered by oral and intravenous routes. Titer and Routes did not interact in terms of onset of toxic signs. However, titers can either slow down or prolong the duration of toxic signs. Result concludes 1:120 titer per oral route as the endpoint or weakest concentration by 10% (1)mice survival after 18hrs 20% (2) survival for 1:100 after 12 hours and 0%(10) deaths for 1:60. Research Institute for Tropical Medicine suggested isolation and purification of active substances to standardize dosing and utilize appropriate route of administration.

Keywords: *cobra venom, ipomoea setosa, titers, routes, Naja philippinensis*

Introduction

Naja philippinensis also known as Philippine cobra is one of the deadliest venomous snakes in the world. The neurotoxin impairs heart and lung function causing heart and respiratory failure. Other signs and symptoms include feeling of doom, fright headache, nausea, vomiting, abdominal pain, diarrhea, dizziness, collapse and convulsions. The death time of the victims is usually about several minutes in average but the fastest recorded time is at 30 seconds after the envenomation when a specific anti venom is not administered or delayed (Ortega, 2011).

According to Alirol E,etal,(2015) of the 24 neglected tropical diseases and conditions listed by the World Health Organization, snakebite is among the top killers. About 5 million people are bitten by snakes every year, including 100,000 deaths and several hundred thousand others who suffer amputations or other disabilities. Tens of thousands of people die each year as a result of snakebite envenoming, with close to 50,000 deaths in India alone (Mohapatra et al., 2011) and up to 32,000 in sub-Saharan Africa (Kasturiratne A, et.al 2008). Unfortunately, Médecins Sans Frontières (MSF) has been informed that the production of Fav-Afrique by Sanofi Aventis will be permanently discontinued. The last batch was released in January 2014, with an expiry date of June 2016. All the vials produced have already been sold by Sanofi Pasteur. Apart from being scarcely available, it is costly that most people in the villages turn to herbal alternatives.

Herbal medicines are a common practice in the developing countries, where venomous snakes are prevalent. *Ipomoea setosa* also known as Brazilian Morning Glory Ker Gawl was used as an emergency treatment by local villagers in the Philippines. It is thus the proponents with participating agency, the University of the Philippines, Institute of Plant Breeding Los Banos conducted the Qualitative and Quantitative analysis of the plant per 100 grams sample and found it to contain alkaloids (+++ highly positive), flavonoids (1.42 mg), saponin (12.36 mg), phenols (2.86 mg) and tannins (1.86 mg). Because claims are not yet proven scientifically, it has to demonstrate efficacy without severe toxicity or damaging properties (Bergensen, 1976). Thus, an Oral Lethal dose50 was subsequently assessed in cooperation with (DOST ITDI Standard and Testing Division, 2006) and found the sample to be practically non toxic in mice (13.8573 ± 0.3113 g per kilogram). Bioassay both *in vitro* and *in vivo* studies are performed to determine the required amount of the substance to produce a definite effect on a suitable laboratory animal (Karch, 2008). Therefore an evaluation of its venom counter effect was conducted considering routes of administration and dosage similar to human use. It is based on the assumption that a high degree of correlation exists between the effects of drugs in animals and man though variations occur in terms of size, concentration, and activity in individual systems of different species. Moreover, the amount of drug available at the actual site of action depends on whether it was given orally, rectally, or by injection (Mendjiwa, 2010). The core institutions of Philippines National Health Research System composed of PCHRD-DOST, DOH, CHED consolidated the regional and institutional research priorities through consultation process with the involvement of stakeholders and experts from public and private sectors to come up with the National Unified Health Research Agenda 2011-2016 one of which is the topic drug discovery and development. The National Unified Health Research Agenda (NUHRA) specifies the areas and topics that need to be addressed in line with global and national initiatives influencing the health sector. It serves as a guide for the health research community on research studies that should be undertaken to address the health needs of the Filipinos and other countries.

2. Material and Methods

2.1 General Methods and procedures

The study conducted was in the pre-clinical stage wherein the subjects were mice and grouped into four (4). Three groups (3) were treated with Ipomoea seed extract with different titer of 1:60, 1:100, 1:120 using 1 gram herb concentration and a control group treated with cobra venom.

2.2 Extraction and Filtration

The researchers initially performed the standard water based extraction and filtration of crude extract to obtain a clear solution by using sterile technique

2.2 Dosage and Solution

The test initially prepared sterile water and powdered seeds according to desired concentration making 1 gram/2 ml of 1:2 dilutions. Collected clear solution in sterile vials and refrigerated until use. Next was preparation of
2 sterile vials for 1:10 dilutions of venom and herb clear extract with each corresponding vials for titers 1:60, 1:100 and 1:120 titer.

2.3 Treatment

Ten (30) mice were used as subjects Baseline measurement of vital signs was observed and recorded pretest to obtain normal values. .05 mg/5ml lethal dose of cobra venom and titer of 1:60 1:100, and 1:120 ipomoea seed
extract was mixed and allowed to settle for 30 minutes before administering it orally, intraperitoneally, intramuscularly and intravenously to each mouse.

3. Results and Discussion

Table 1.1 shows a slow toxic sign onset in both control and experimental groups through oral 52-59 secs and intraperitoneal 47-59 secs, while fast onset following intramuscular 35-50 secs and intravenous route: 31-45 secs. According to Kee, Hays, Mc Cuistion (2006), onset of action is the time it takes to reach the minimum effective concentration after a drug is administered. When an immediate effect is needed to achieve most reliable therapeutic levels, solutions may be given directly into a vein (Karch, 2008) as in intravenous injection which results to rapid absorption as shown by fast toxicity onset. Following oral administration, drug action has slower onset due to variations in absorption as a result of drug composition, gastric and intestinal pH motility, and food content.

Moreover, alteration of the drug resulting from its retention, inactivation or partial destruction b y the liver before entering the general circulation is another factor to consider (Bergensen,1976). However, there are exceptional variations after intramuscular application showing slow to fast onset in case titer is increased from 1:100 to 1:120 a condition when the test solution are becoming more diluted. Result implies that routes and increasing titer affects treatment speed of absorption. According to Karch (2008), intramuscular administration offer varying rates of absorption. The suspension form a depot of drug in a tissue, and slow gradual absorption usually result (Bergensen,1976).

Table 1.1 Effect of routes and titer in terms of Onset of Toxic Signs after cobra venom was administered to control group and treatment groups through different routes and titers of Ipomoea setosa

Sources and variable	Oral			Intraperitoneal				Intramuscular				Intravenous			
	Mean sec	SD	VI	N	Mean sec	SD	VI	N	Mean	SD	VI	N	Mean	SD	VI
Control	59.00	1.1414	slow	2	59.00	1.414	slow	2	50.0	7.071	slow	2	41.50	16.263	fast
1:60	54.00	8.485	slow	2	47.50	7.778	slow	2	49.0	12.728	slow	4	36.50	12.503	fast
1:100	52.50	9.899	slow	2	49.00	1.414	slow	2	56.5	4.950	slow	4	45.75	10.436	fast
1:120	54.13	10.607	slow	2	51.50	4.950	slow	2	35.0	7.071	fast	4	31.25	6.292	fast

Note : VI = verbal interpretation , O, IP, IM,IV, Average onset of toxic signs for control and titer groups: oral: 52-59 secs = slow, intraperitoneal:
47-59= fast to slow, intramuscular: 35-50 secs = fast to slow, Intravenous: 31-45 secs = fast

Simple main effects analysis in table 1.2 shows that the type of routes influences the onset of toxic sign whether the concentration is weak or strong as shown by p value of .043. If the drug is administered through oral route, it undergoes the first pass effect. It is a process in which the drug passes to the liver first where it may be metabolized in a more active or inactive form. (Kee, Hays, McCuistion,2006). On the other hand titer only implies the length of time the chemical substance remain in the blood and vital organs as presented by severe or mild toxic effects. It goes to say that routes and titers have independent effects. Titer is a way of expressing concentration. It employs serial dilution to obtain activity approximate quantitative information from an analytical procedure that inherently only evaluates as positive or negative. The substance looked for is diluted (watered down) repeatedly to see how long the substance remains.

Table 1.2 The mean difference in the subjects' reaction in terms of onset of toxic signs for the independent effects and interaction of titers and routes as a result of ANOVA test

Sources	df	Mean S	f	p	VI
Routes	3	362.044	5.100	.043	sig
Titers	2	104.250	1.445	.300	Not sig
Routes*Titers	6	70.994	.743	.623	Not sig
Errors	18	95.583			Not sig

Result in table 2.1 shows the average short duration of toxic signs for Control and titer 1:60 within 20-30 minutes as death finally occur. But a long duration for Titer 1:100 in all routes was shown as life is prolonged until toxicity signs gradually disappeared to full mice recovery. Duration of action is the length of time the drug has pharmacologic effect Kee, Hays, McCuistion (2006). Result implies that mice were able to manage the toxic effect of cobra venom when added to Titer 1:100 ipomoea setosa extract administered per oral route as shown by increased number of mice recovery in table 3. Whereas a more watered down concentration of 1:120 cause a long duration and

reduction rate of survival. According to Karch (2008) and Bergensen (1976), oral route have slow, prolong but less potent effect. Parenteral drug action is more rapid but of shorter duration. However, different rates of absorption can be manifested in varying degrees of effect when an aqueous solution or suspension is administered in intramuscular and subcutaneous routes due to slow gradual absorption. A prolonged score of 25 minutes for intramuscular route is related to the deposition and slow release of injected substance in the muscles.

Table 2.1. Effects of routes and titers in terms of Duration of Toxic Signs after cobra venom was administered to control group and treatment groups through different routes and titers of Ipomoea setosa

	Or				Intraperitoneal				Intramuscular				Intravenous			
	N	Mea	SD	VI	N	Mean	SD	VI	N	Mean	SD	VI	N	Mea	SD	VI
Contr	2	30mi	848.	shor	2	.20	.006	shor	2	25	1272	shor	2	.0.	.006	short
1:60	2	53mi	565.	long	2	47.	466.	long	2	49	763.	long	4	36.	750.2	short
1:100	2	52mi	593.	long	2	49.	84.8	long	2	56.	296.	long	4	43.	576.2	long
1:120	1	54mi	339.	long	2	13.	127.	shor	2	12.	212.	shor	4	17.	172.3	short

Note: VI= verbal interpretation. Average duration of toxic signs for control and experimental group per routes and titer. Control and Titer 1:120 per O, P, IM, IV: short duration 20-35 minutes, Titer 1:60 and Titer 1:100 per O,IP, IM, long duration 36-63, IV short 36 min and long duration 43 min

Table 2.2 Result shows that Titer has an effect on the duration of toxic signs with p value of .000. It is implied in the result that duration of toxic sign is associated with titers which is the amount or concentration of absorbed substances remaining in the body of mice. Dancel quoted (2006), "Titer is the endpoint concentration of a substance capable of producing an effect".

Table 2 2 The mean difference in duration of toxic signs for the independent effects and interaction of titers and routes as result of 2-way ANOVA

Sources	df	Mean Score	f	p	VI
Routes	3	865244.444	2.923	.122	Not sig
Titers	2	9606776.190	32.689	.000	sig
Routes*Titers	6	296024.444	1.179	.361	Not sig
Errors	18				

Table 3.1 revealed that all mice of control and 1:60 titer died following treatment per routes. Mice Onset of recovery for Titer 1:100 occurred after a maximum of 12 hours which produced two (2) mice recoveries. 1:120 yield
1 mice recovery after 18 hours. As observed 1:100 is the standard dilution capable of keeping mice alive and 1:120 titer is the endpoint that yields 1 mice recovery through oral route. The principle of titration in this study shows that as titer increases or (watered down) the concentration becomes weaker as shown by death of mice. Titer as defined by Kapllit and Leowy (1995), is a way of expressing concentration. It employs serial dilution to obtain approximate
quantitative information from an analytical procedure that inherently only evaluates as positive or negative. The titer corresponds to the highest dilution factor that still yields a positive reading (Timbury 1994).

Table 3.1 Effect of routes and titer in terms of onset of recovery in terms of Onset of Recovery after cobra venom was administered to control group and treatment groups through different routes and titers of Ipomoea setosa

Treatment	Oral				Intraperitoneal				Intramuscular				Intravenous			
	N	Mean	SD	VI	N	Mean	SD	VI	N	Mean	SD	VI	N	Mean	SD	VI
Control	2	.00	.00	2 deaths	2	.00	.000	death	2	.00	.000	death	2	.00	.000	death
1:60	2	.00	.00	2 deaths	2	.00	.000	death	2	.00	.000	death	4	.00	.000	death
1:100	2	780.00 min (12) hrs	.00	2recovery	2	.00	.000	death	2	.00	.000	death	4	.00	.000	death
1:120	1	1110.00 min (18) hrs	1569.	1recovery	2	.00	.000	death	2	.00	.000	death	4	.00	.000	death

Control average onset of recovery for oral: .00 sec = death, intraperitoneal:.00= death, intramuscular: .00 = death, Intravenous: .00= death Experimental 1:60 oral: .00 sec =death, intraperitoneal:.00 = death, intramuscular:.00 =.death, intravenous: .00 =death .Experimental 1:100 oral: 780.00 = two recovered, intraperitoneal: .00=death , intramuscular: .00=death , intravenous: .00=death Experimental 1:120 oral: = one recovered, intraperitoneal:.00= death ,intramuscular: .00=death, intravenous: .00 =death

Table 3.1 below shows that Routes revealed a significant result in terms of onset of recovery which suggests that appropriate route must be considered in administering the treatment aside from fact that the active substance is still in the crude state and needs to be purified and isolated to determine whether there is an interaction between routes and titer.

Table 3.1.The mean difference in the subjects' reaction (onset of recovery) for the different titers, routes, and interaction of titers and routes as a result of ANOVA test after cobra venom was administered to control group and treatment groups through different routes and titers of Ipomoea setosa

Sources	df	Mean Square	f	p	VI
Routes	3	362.044	5.100	.043	significant
Titers	2	104.250	1.445	.393	Not significant
Routes * Titers	6	70.994	1.266	.321	Not significant
Error	18				

Two -way anova:routes p =.082: not significant, titers p =..393:significant, Routes and Titers interaction p =.321: not significant

Conclusion

1.Routes have independent effect in the onset of toxic sign whether the titer is weak or strong
2.Titers emerged to have independent effect in terms of duration of toxic signs
3.Routes have independent effect in terms of onset of recovery
4.Evaluation result: The standard and endpoint serial dilution that counteract venom toxicity in mice is 1:100 and
1:120 titer per oral route

Recommendation

Isolate and purify the active substance for standard dosing and route of administration.

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