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Screening of Anti Snake Venom Potential of the Seed Oil of *Balanites aegyptiaca* L. Del

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Abstract

The Seed Oil of *Balanites aegyptiaca* L. (Del) Zygophyllaceae was tested for its inhibitory activity against lethal and inflammatory effect of *Vipera russellii* (Russell's viper) venom. The seed oil of the plant displayed significant inhibitory action against the lethal toxic and inflammatory effects of the viper venom. The present findings suggest that the seed oil of *Balanites aegyptiaca* possess compound(s) which inhibit the lethal and inflammatory effects of viper venom.

Keywords: *Balanites aegyptiaca*, inflammation, Russell's viper

1. Introduction

Snakebite is a serious occupational hazard in India. Every year India reports more than 2 lakhs diagnosed cases of snakebite and near about 35000 to 50000 deaths [1]. Snake bite occurrences among the farmers and the tribals of Satpura range forests of the Khandesh region of the Maharashtra State are always of growing concern. In this region cases of snake bites especially comprising the Cobras (*Naja* species), Kraits and Vipers (e.g. Russell's viper) are prevalent. Russell's viper (*Vipera russelli)* has been reported to be a very dangerous snake and its victims are mainly the farmers and fieldsmen. This snake is a common snake of Khandesh region of Maharashtra state and viper bite is one of the worst snakebite hazards. Deaths are reported within an average time of 3 hours after viper bites. This time span is too short since most bites occur in areas situated at a long distance away from hospitals, and even when the victim is right on time to a point of medication, sometimes the lack of availability of the Anti venom serum in the rural hospitals may pose a grave concern to the victim's life. Also the anti venom serum may have some lethal adverse effects. Envenoming by Russell's viper is common in this region which causes frequent systemic poisoning, local swelling, tissue necrosis, nephrotoxicity, cardiac effects and Disseminated Intravascular Coagulation (DIC) leading to the death of the victim within a few hours if not treated promptly. Tribal people of this area mostly treat snakebite victims by local herbs and medicinal plants. Our ethno medical survey has revealed many such plants used by the tribes of the Satpura range forests for this purpose. We have observed that several plants are used by the tribals of the northern hilly part of this Khandesh region for treating snake bite victims. Use of different plants and their parts for various medicinal purposes by the tribal community of the Satpura range forests of Jalgaon district has already been reported [2]. During intensive study of the medico-ethno biology of the tribes Bhils, Pawara, Paradhi and Vasave inhabiting the northern hilly regions of Jalgaon district *Balanites aegyptiaca* was found to be used for treating various ailments. In this work, an effort has been made to evaluate the anti snake venom activity of this plant against *Vipera russellii* venom with reference to its anti inflammatory and lethal effect.

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2. Materials and Methods

2.1 Snake Venom

A commercial sample of lyophilized venom of Russell's viper (*Vipera russelii*) (Batch No. 787J Haffkine Institute Mumbai) was supplied by Principal; K.E.S's College of Pharmacy, Amalner (M.S.) and preserved at 4^0C until further use. Venom was dissolved in normal saline solution immediately before the administration. The venom concentration was expressed in terms of dry weight (mg/ml stock venom.)

2.2 Plant Material

Fresh samples of small leafy branches and fruits of *Balanites aegyptiaca* L. (Del) were collected from the Northern Hilly Regions of Jalgaon district in the month of August. The plant was identified and authenticated by Botanical Survey of India, Pune vide Herbarium sheet No.RHM1.

2.3 Animals

Throughout the study healthy adult Albino mice weighing 18–20 g of either sex were used. The animals were obtained from National Toxicology Center, Pune. Animals were housed by in groups of five per cage at a temperature of 25° ± 1°C and relative humidity of 45–55% and acclimatized for one week. A 12:12 hr dark: light cycle was followed during the experiments. Animals had free access to standard pellet diet and water except 18 hrs before and during the period of experiment. The Institutional Animals Ethics Committee approved the protocol of the study vide number NIB/ IAEC/ 12-13/114.

2.4 Extraction of Seed oil From Fruits

Collected fruits samples were made free from aerial parts and dirt thoroughly washed with running water to remove adherent soil and dried in sun for about 7 days. The dried nuts were cracked to remove the seed kernels. The kernels were further dried on a polythene sheet followed by pounding and roasting of the dried kernels. The roasted kernels were allowed to cool and ground to a coarse powder on a mortar pestle. Oil was immediately extracted from kernel powder in boiling water by hot water floatation process. The oil was decanted into a clean container.

2.5 Preliminary Phytochemical Screening of the Seed Oil

Phytochemical screening was done using colour forming and precipitating chemical reagents on the seed oil of *Balanites aegyptiaca* to generate preliminary data on the constituents of the extracted seed oil. Small quantity of the extracted seed oil and fraction were dissolved in respective solvents and were subjected to preliminary phytochemical analysis for the detection of individual components [3]. Preliminary phytochemical screening revealed the presence of phytosterols, glycosides, flavonoids, proteins, carbohydrates, steroids in the seed oil of *Balanites aegyptiaca*. The seed oil was analyzed by HPLC to develop a finger print chromatogram. The oil methanolic extract was injected against available flavonoid reference compound (rutin). Rutin was identified and used for standardization of the oil extract (Fig. 1, 2). Presence of rutin in the *B. aegyptiaca* seed oil is already mentioned in the classical literature [4].

2.6 Inhibition of Lethality

This acute toxicity study was carried out to determine the median Lethal Dose (LD_{50}) and Minimum Lethal Dose (MLD) of the *Russell's viper* venom. The venom was reconstituted with the normal saline and concentration of 1mg/ml was obtained. The methods of Theakston and Reid [5] and Abubakar [6] were adopted for the experiment. The median Lethal Dose (LD_{50}) of *Russell's viper v*enom was assayed by injecting different concentrations of venom 0.2 ml normal saline into the peritoneum of the mice. (Table 1, Fig. 3).

The median Lethal Dose (LD_{50}) and Minimum Lethal Dose (MLD) of the venom were calculated using the probit analysis [7]. The LD_{50} and MLD of the viper venom were established 0.3 mg/kg and 0.2 mg/kg respectively. For venom inhibition study, the animals were divided into five groups (8 mice per group) and they were administered with the median lethal dose (0.3 mg/kg) of the venom intraperitoneally. Immediately one minute after the administration of the venom, different doses of (0.1–0.25ml/mouse) the oil of *B. aegyptiaca* were administered to the mice by the same route. The treatment groups (2–5) received the oil. The control group (1) received the LD_{50} i.e. test dose of the venom alone in normal saline (0.9% w/v). In oil pretreated mice group the animals

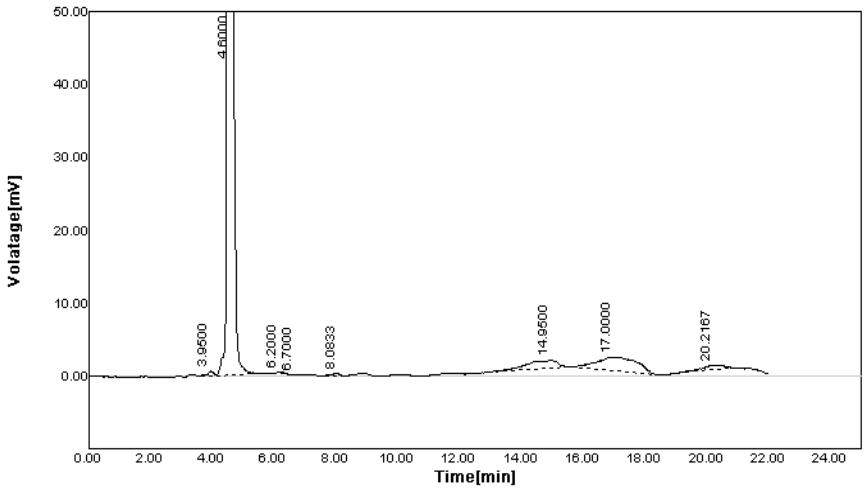

Fig. 1. HPLC Finger Printing of *Balanites aegyptiaca* Seed Oil Methanolic Extract: (100% Methanol-0.7 ML-254).

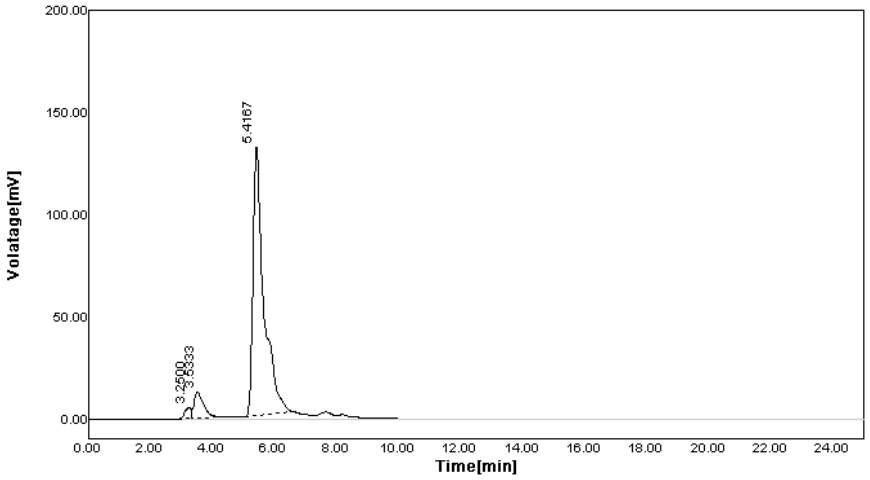

Fig. 2. HPLC Finger Printing of Standard Rutin.

Table 1: Acute toxicity test (LD_{50} determination) of Russell's viper (*Vipera russellii*) Venom

Group	Venom Mg/kg	No. of Deaths	% Death	Probit
1	-	0/6	0	-
2	0.1	0/6	0	-
3	0.2	1/6	16.67	4.17
4	0.3	3/6	50	5.00
5	0.4	5/6	83.33	5.97
6	0.5	6/6	100	8.09

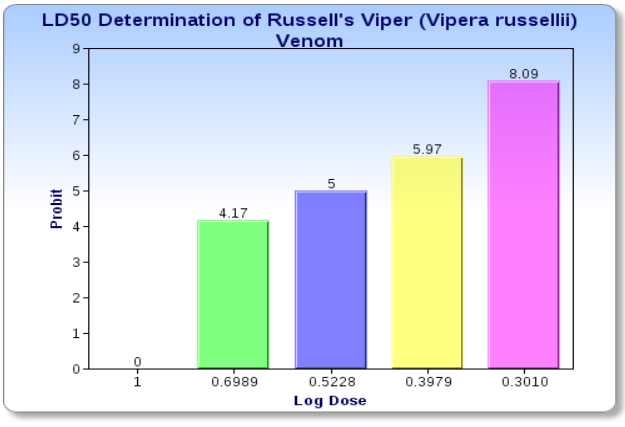

Fig. 3. Acute toxicity test (LD_{50} determination) of Russell's viper (*Vipera russellii*) Venom.

were pretreated with different doses of oil (0.1- 0.25 ml) using intraperitoneal route and were challenged with the venom (Test dose 0.3 mg/kg) one hour after the administration of the oil. The animals in the experiment were observed for toxicity signs and mortality up to 24 hours. The Effective Doses (ED_{50}) of the oil were estimated using probit analysis.

(Table 2. and 3. Fig. 4 and 5)

2.7 Evaluation of Anti Inflammatory Effect

The anti inflammatory activity of the seed oil of *Balanites aegyptiaca* L. Del was assessed by inducing paw edema in albino mice by Russell's viper venom. All the experiments were carried out as per the methods described by Mishal H, Al-Asmari and Alam [8–10]. The Russell's viper (*Vipera russellii*) venom (5 µg/paw) was injected in the sub plantar region of the right hind paw followed by different doses of the *Balanites* oil intra peritoneally. 0.1 ml solution of viper venom was administered each time to produce time and dose dependent swelling leading to paw edema.

Table 2: Effect of *B. aegyptiaca* oil in venom pre-treated mice group

Group	Dose of oil (ML)	Mortality after 24hrs. No. of deaths	% Survival	Probit of % survival
1	0	8/8	0	--
2	0.1	7/8	13	3.87
3	0.15	5/8	37.5	4.69
4	0.2	4/8	50	5.00
5	0.25	2/8	75	5.67

Table 3: Effect of *B. aegyptiaca* oil in oil pre-treated mice group

Group	Dose of oil (ML)	Mortality after 24hrs. No. of deaths	% Survival	Probit of % survival
1	0	8/8	0	–
2	0.1	8/8	0	–
3	0.15	5/8	37.5	4.69
4	0.2	4/8	50	5.00
5	0.25	2/8	75	5.67

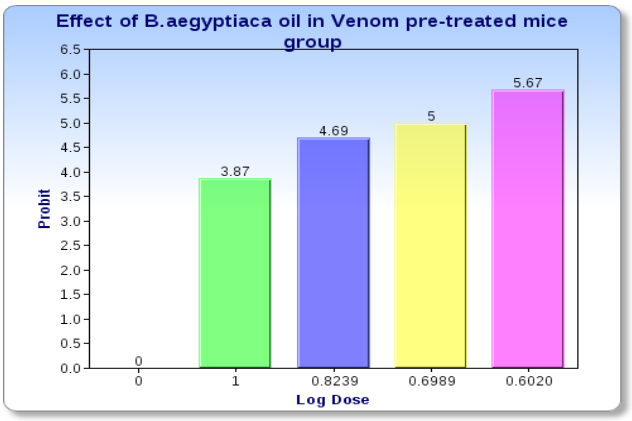

Fig. 4. Effect of B. aegyptiaca oil in venom pre-treated mice group.

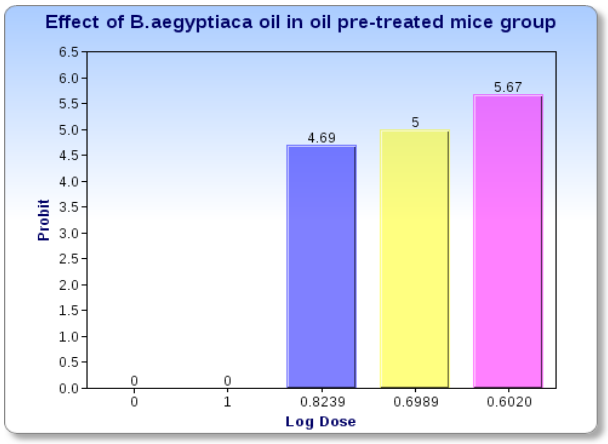

Fig. 5. Effect of *B. aegyptiaca* oil in oil pre-treated mice group.

The paw volume was measured after 0.25, 0.5, 1 and 2 hours respectively, by using an arm plethysmograph (make DOLPHIN Mercurial Treatment). Appropriate controls were performed by injecting the normal saline solution into the sub plantar region of the left foot of mice (Table 4.).

2.8 Statistical Analysis

The median lethal dose (LD_{50}), Minimum Lethal Dose (MLD) of the venom and the Effective Doses (ED_{50}) of the oil were calculated using the probit analysis. All values are expressed as mean ± SEM. Statistical significance was calculated using Student's t-test with $p< 0.01$ being considered significant.

Table 4: Effect of different doses of *B. aegyptiaca* oil on the viper venom induced edema in mice

Treatment With oil ML	Venom µg/0.1 ml	Paw volume ml ± SEM				Edema %
		0.25 hr	0.50 hr	1.00 hr	2.00 hr	
--	5	0.35 ± 0.204	0.43 ± 0.020	0.52 ± 0.020	0.81 ± 0.020*	46.64
0.1	5	0.32 ± 0.020	0.41 ± 0.019	0.54 ± 0.020	0.93 ± 0.020	31.93
0.15	5	0.34 ± 0.204	0.43 ± 0.020	0.54 ± 0.020	0.92 ± 0.020*	36.55
0.2	5	0.32 ± 0.016	0.40 ± 0.027	0.48 ± 0.027	0.90 ± 0.017*	34.39
0.25	5	0.30 ± 0.015	0.38 ± 0.018	0.47 ± 0.026	0.89 ± 0.016*	32.36
Control	–	–	–	–	–	–

N=4, P*<0.001, Values are expressed as ± SEM

3. Results

Phytosterols, glycosides, flavonoids, proteins, carbohydrates, steroids, were found to be present in seed oil of *Balanites aegyptiaca* as observed by qualitative tests. The LD_{50} and MLD of viper venom was calculated to be 0.3 mg/kg and 0.2 mg/kg respectively. The Effective Dose 50% (ED_{50}) of *B. aegyptiaca* oil in venom pre-treated mice group after intraperitoneal injection was estimated to be 0.2 ml in mouse, while the Therapeutic Index (TI) was calculated to be 1.75. The Effective Dose 50% (ED_{50}) of *B. aegyptiaca* oil in oil pre-treated mice group after intraperitoneal injection was estimated to be 0.2 ml in mouse, while the Therapeutic Index (TI) was calculated to be 1.75. This shows the seed oil of *Balanites aegyptiaca* offers equal protection before and after venom envenomation. While assessing the anti inflammatory activity of the crude *B. aegyptiaca* seed oil after inducing paw edema in Albino mice, significant reduction of the paw edema was observed at 0.25 ml dose of the seed oil.

4. Discussion

Russell's viper or *Daboia* is a monotypic genus of old world viper, found all over the Asian as well as in Indian subcontinent. Russell's viper bite is a serious medical problem in these areas. Russell's viper venom is the rich source of different enzymes including Phospholipase A_2, ATPase, Hyaluronidase and certain other components which act on the different parts of the human blood and induce severe haemostatic disturbances in the human beings. This procoagulant activity of the Russell's viper venom is because of the presence of specific peptide components of the venom which activate different clotting factors in the blood including factor IX (Christmas factor), factor X (Stuart Prower factor) factor V as well as platelets. This activation of many factors simultaneously produces a very contradictory effect in the systemic circulation of the humans, enhancing blood clotting as well as severe haemorrhage, bleeding and haemolysis called as Disseminated Intravascular Coagulation (DIC) of the blood. Because of edema of muscle and bleeding there is development of compartment syndrome characterized by swelling, pain full passive movement and loss of sensation over the nerve areas passing through the compartment. Subsequently there is development of wet gangrene or non-healing ulcers. If untreated the bitten part usually toe or finger results in auto amputations. On the other hand in smaller animals, Russell's viper venom promptly produces blood coagulation leading to the death of the animal because of the blocking of blood vessels of heart, kidneys and lungs. Many cases subsequently develop anuria, oliguria and acute renal failure. As such Anti Snake Venom (ASV) serum is the only anti snake bite regimen, available till date. This conventional therapy though successful, always have many short comings like severe side effects like allergic reactions to horse serum, difficulty in judging the exact quantity of the ASV to be administered, as well as inability of the ASV to resolve the local manifestations of snake bite. Successful treatment much more depends on the skill and experience of the physician in treating the snake bite victims. Failure of the ASV in curing the patients bitten by the same snake species in different geographical areas is one more limitation apart from its cost. On the other hand, herbal extracts are expected

to have similar efficiency without side effects as that of conventional anti snake venom serum therapy. Hence, in the present study, we have tried to evaluate the anti snake venom potential of *Balanites aegyptiaca* seed oil with various parameters in animal models. Studies were done to assess the safety and therapeutic profile of the seed oil. Administration of single dose of the seed oil of *Balanites aegyptiaca* at the limit dose of 0.3 ml i.p. did not have any toxic effects. Thus, the seed oil of *Balanites aegyptiaca*, emerged safe for administration and further studies. In the experimental modes the seed oil was able to neutralize the viper venom (LD_{50} dose 0.3 mg/kg) and this was expressed by the efficacy of the mice to survive the lethal and toxic effects of venom. This very effectiveness of *Balanites aegyptiaca* seed oil may be due to the presence of the flavonoid glycoside rutin which was identified in the HPLC fingerprint chromatogram. The significant protection and anti-inflammatory activity observed with the different doses of the seed oil may be due to the presence of rutin which exerts anti inflammatory effect [11]. It is also reported that rutin efficiently inhibits PLA_2s present in *Vipera russelli* venom [12].

5. Conclusion:

This justifies the use of *Balanites aegyptiaca* in the traditional system of medicine, especially by the tribes of Satpura range of forests and the seed oil of this plant can also be recommended for further studies as an antidote to the snake bite poisoning.

References:

1. Bawaskar H. Snake venoms and anti venoms issues. J Asso Phy Ind. 2004; 52:11–13.
2. Kamble SY, Patil SR, Pawar SG, Sawant PS, Sawant S, Singh EA. Studies on plants used in traditional medicine by bhilla tribe of maharashtra. Ind J Trad Knowl. 2010; 9(3): 591–8.
3. Kokate CK, Purohit A, Gokhale S. Textbook of pharmacognosy. 7th Ed. Nirali Prakashan, India: 2001.
4. Motaal AA, Shaker S, Haddad PS. Anti diabetic activity of standardized extracts of *Balanites aegyptiaca* fruits using cell based bio assays. Phcog J. 2012. Available from PhcogJ.com/105530pj2012304.
5. Reid HA, Theakston. RDG Development of simple standard assay procedures for the characterization of snake venoms. Bull WHO. 1983; 61(6):949–56.
6. Abubakar MS, Sule MI, Pateh UU, Abdurahman, Haruna AK, Jahun BM. In vitro snake venom detoxifying action of *Guiera senegalensis*, J Ethnopharmacol. 2000; 69: 253–7.
7. Akah PA, Odita IO Ex, Odita IO. Experimental methods in physiology and pharmacology (for medical and pharmacy students). Enugu, Nigeria: AIBC Publishers; 2001.
8. Mishal H. Anti snake venom medicinal plants among certain adivasis in the satpura region of maharashtra. Hamdard Medicus. 2006; XLIX (2):13–15.
9. Al-Asmari AK. Pharmacological characterization of rat paw edema induced by *Naje haje Arabica* venom. J Venom Anim Toxins incl trop Dis. 2005; 11(1):51–67.
10. Alam MI, Gomes A. Snake venom neutralization by indian medicinal plant *Vitex negundo* and *Emblica officinalis* Linn. Root Extracts. J Ethnopharmacol. 2003; 86(1):75–80.
11. Laid S, Hamama B, Chafia T, Chahra B. Anti-inflammatory effect of rutin on rat paw oedema, and on neutrophils chemotaxis and degranulation. Exp Toxicol Pathol. 2003; 54(4):313–8.
12. Samy RP, Gopalakrishnakone P, Chow VTK. Therapeutic application of natural inhibitors against snake venom phospholipase A. Biotransform (open access). 2012; 8(1):48–57.