

## Detoxified *Vespa orientalis* venom improves memory and learning activities in rats

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**Abstract:** The work was aimed to study the effect of photooxidized *Vespa orientalis* venom (PVOV) on memory and learning activity on rats in presence of scopolamine and ondansetron using T maze, elevated plus maze and passive avoidance. UV radiation of *Vespa orientalis* venom for 15 to 60 min intervals in presence to methylene blue to detoxify venom and studied by change in UV spectrum. Antigenicity study, in-vivo and in-vitro neutralization study of PVOV against immunoglobulin from hyperimmunized rabbit was performed. 45 min exposed venom showed shift in  $\lambda_{max}$  and increase absorbance indicated alternations in venom protein concentration, this is supported when PVOV showed loss of toxicity and decrease in mortality time in mice. Administration of PVOV for 28 days produced a notable improvement in spatial and long memory in rats when subjected in several tasks. When PVOV administered with scopolamine and ondansetron, all the parameters of spatial and long term memory tasks were significantly reduced inferred that PVOV acted by modulating either muscarinic or serotonergic receptors. There is a possibility of employing PVOV in the treatment of depressive and chronic degenerative illnesses as a non-herbal and non-synthetic alternative for patients not responding to the available therapy.

**Keywords:** Elevated plus maze, Ondansetron, Photooxidized *Vespa orientalis* venom (PVOV); Scopolamine; *Vespa orientalis* venom (VOV)

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### I. Introduction

Hymenoptera venoms are complex mixtures of biochemically and pharmacologically active components such as biogenic amines, peptides and proteins. (1) *Vespa orientalis* venom contain many biological components acetylcholine, serotonin, adrenaline, nor-adrenaline, dopamine, kinins, and high molecular weight compounds such as phospholipase-A; hyaluronidase; histidine decarboxylase; acid, alkaline, and neutral DNase; poly and disaccharidase; and several polycationic peptide and protein that act together to produce biological effects. (2) Photooxidation is one of the method carried out to detoxify venom exposing gamma, visible and ultraviolet radiations (UVR) to generate antigenically-active detoxified venom products. (3) Detoxified snake venom has been used treat dengue fever, atherosclerosis, cancer, and diabetes. Photooxidized *Echiscarinatus* venom demonstrated antidepressant and central nervous system stimulant activities, and enhancement of memory and learning in rats. (4, 5) Envenomation with hornets caused specific central nervous system effects in laboratory animals due to cholinesterase like compounds present in the wasp venom. (6) Numerous studies have been carried out to test the properties of wasp venom as a pharmacological agent. However, no attempts have been made to investigate the effects of Photooxidized *Vespa orientalis* venom (PVOV) on cognition and dementia and effect in presence of various antagonist like scopolamine and ondansetron in animal models as an alternative mechanism that may create lead compounds for the treatment of neurodegenerative diseases. Thus, the present work aimed to evaluate the psychopharmacological effects of PVOV using suitable animal models for memory and learning.

### II. Material And Methods

Animals: Swiss albino mice (20-25g) for toxicity study, Wistar rats (180-220g) for psychopharmacological studies and albino rabbits (2-2.5kg) were used in hyper immunization studies. The Institutional Animal Ethics Committee of PES College of Pharmacy, Bangalore, India, approved all animal experiments. All procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India, under recommended temperature and relative humidity.

Drugs and chemicals : Piracetam was received as a gift sample from UCB India Private Ltd. (India), Freund's complete and incomplete adjuvants were purchased from Genie Bioscience, Bangalore, (India), Diethylaminoethyl cellulose, Methylene blue (MB – Qualigens Fine Chem Ltd, Mumbai, India) and all other chemicals used in the study were of analytical grade.

### **2.1 Collection of *Vespa orientalis* venom**

Total of 3000 wasps were collected in the month of November to March from a village Belavanaki, India in the whole period of work (Seasonal collection) and were authenticated by the entomologist Dr. Chandrashekhar, University of Agricultural Sciences, Bangalore, India. A specimen wasp is maintained in the Department of Entomology. The method reported by Duvdevani *et al*, with suitable modification used for milking of venom was followed.(7) The lyophilized venom subsequently referred to as *Vespa orientalis* venom (VOV). The venom concentration was expressed in terms of mg/ml.

### **2.2 Determination of minimum lethal dose**

Groups of three mice each were injected (via intraperitoneal and intravenous routes) with VOV dose range from 12-20 mg/kg and 10-15 mg/ kg. Control group animals were treated with phosphate buffer only. Following with administration of VOV, the signs of toxicity and behavioral changes were observed for 1, 2, 4, 24 and 72 h.(8-10)

### **2.3 Photooxidation of *Vespa orientalis* venom**

Standard photochemical method with suitable modification was followed to generate photooxidized venom products.(11-13) Briefly, the reaction mixture containing 2ml of VOV (25mg in 2 ml of 0.05M phosphate buffer at pH 6.8) and 2ml of methylene blue (0.003% w/v in phosphate buffer) solution was kept on a magnetic stirrer in a Photooxidation chamber exposed to UV light (tubular ultraviolet 15 W lamp, 615, T8, Philips, Holland, UV output 4.8 W, 49  $\mu$ W/ cm<sup>2</sup>) at about 10-cm distance and gently stirred in different time intervals (15, 30, 45, and 60 minutes) at 37°C. Then, 200  $\mu$ l of activated charcoal (1% w/v in phosphate buffer at pH 6.8) was added, the mixture was stirred five more minutes without light exposure. The photooxidized *Vespaorientalis* venom (PVOV) exposed different interval were filtered through a 0.2- $\mu$ m filter using a syringe filtering unit (Minisart®, Sartorius, India). The absorbance of the control mixture (2 ml of VOV and MB without UV exposure maintained in similar conditions) was measured separately at 200-400nm using UV-spectrophotometer.

### **2.4 Hyper immunization of rabbit for antigenicity test**

The purpose of the hyper immunization of rabbit and preparation of IgG was to test the detoxified PVOV for its antigenicity. Male rabbits were immunized with 1 mg of VOV in 5ml of phosphate buffer mixed with Freund's adjuvant were inoculated intradermally at 16 sites along the animals' spine. Two weeks later, animals were boosted with 1.5 mg VOV *i.p* route. The same procedure was repeated twice, three and four weeks later; then animals were bled and their sera were pooled. During the immunization schedule, serum was continuously applied on to the immunodiffusion gel for appearance of precipitin lines.(14)

### **2.5 Purification of immunoglobulin**

The isolation of antisera from rabbit was carried out by ion-exchange method using diethyl amino ethyl cellulose (DEAE). The purified immunoglobulins were then tested for antigenicity against PVOV.(15)

### **2.6 Antigenicity Test**

The antigenicity test of PVOV submitted to different UV time exposure was carried out using immunogel diffusion method, in which antigens and antibodies migrate through an agarose gel and react resulted precipitin lines.(14)

### **2.7 In vitro neutralization Studies with Immunoglobulin:**

A constant amount of VOV (1 MLD) was allowed to react with 1% and 0.1% dilutions of isolated immunoglobulin and the mixtures were incubated at 37°C for 30 minutes. The protection or death (if any), was determined after intraperitoneal injection 0.2 ml into mice (n = 3) and observed for seven days.(16)

### **2.8 In-vivo detoxification Test in Mice**

The detoxification of PVOV exposed to UV light for 45 minutes (which preserved antigenicity as tested out by immunogel diffusion method) was intraperitoneal injected into a group of mice (n = 3) at a dose based on body weight (0.1 ml/25 g or 5 mg/ml) and observed up to seven days.(4)

### **2.9 Total protein content**

Total protein concentration of VOV and PVOV was determined quantitatively by Bradford method using Bovine serum Albumin as a standard.(17)

## **2.10 Memory and learning activity using T-maze**

Eight groups of six Wistar rats of either sex weighing between 180-200g were used for the experiment. Group-1 normal control animals received vehicle for 28 days; Group-2 animals received 1.5mg/kg VOV alone for 28 days; Group-3 animals received 1.5mg/kg PVOV alone for 28 days; Group-4 received first 21 day 1.5mg/kg PVOV following by scopolamine for (0.5mg/kg) up to 28days; Group-5 received first 21 day 1.5mg/kg PVOV following by Scopolamine for (1mg/kg) up to 28days; Group-6 received first 21 day 1.5mg/kg PVOV following by Ondansetron (50mg/kg) up to 28days; Group-7 received first 21 day 1.5mg/kg PVOV following by Ondansetron (100mg/kg) up to 28days and Group-8 Piracetam (150 mg/kg) up to 28days. Training of the animals was carried out for three consecutive days. On the first day, each rat placed on the maze completed 15 trials to become familiarized with the maze and access food. Then, animals were deprived of food for 24 hours. On the second day, 60 minutes before experiment, each group was treated as describe above. Rats were trained until they attained nine correct arm choices out of 10 consecutive trials. The number of correct responses and time needed to reach the food (TRF) in each trial were recorded. The percentage of correct responses (%CR) was calculated. (18)

### **2.10.1 Transfer latency in elevated plus maze**

In elevated plus maze (EPM) rats were placed individually at end of the open arm facing away from the central platform. The time taken to enter any closed arm with all four paws was taken as transfer latency (TL). On the first day, rats were allowed to explore the maze for five minutes and TL was recorded (acquisition) and on the second day, 60 minutes prior to experiment, animals were treated as described above and the retention of the learned task was examined. TL was recorded on completion of the fourth week of treatment.(19)

### **2.10.2 Step down passive avoidance**

The method suggested by Camacho *et.al.* 1996 was followed. Briefly, on the first day, each rat was placed on the shock free zone, when it stepped down and placed all four paws on the grid floor, an electric shock was delivered (1 mA for 5 seconds). Rats were given 3 to 5 trials until the time spent in the shock free zone was 120 seconds, the step down latency (SDL) and time spent in the shock zone (TSS) were recorded (familiarization). Twenty four hours later, acquisition phase was recorded again. Animals were treated as described above. All Animals were given three trials and parameters such as SDL, step down error (SDE) and time spent in shock zone were recorded. Retention was tested on the third day and all the parameters were recorded again. The drugs were administered next day of familiarization and one hour before the acquisition phase on 28th day and recorded.(20)

## **III. Results**

### **3.1 Minimum lethal dose of venom**

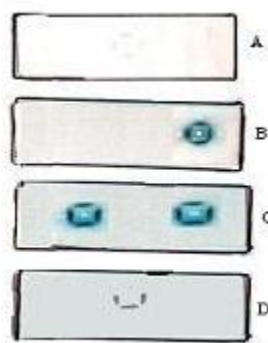
About 5.212 g of lyophilized VOV was obtained from 3000 *Vespa orientalis* with an average of 1.5 mg VOV/sac. Toxicity by intraperitoneal and intravenous routes in mice showed characteristics symptoms such as, dyspnoea, akinesia, diarrhoea, haematuria, stupor, loss righting reflex, hyper excitation to external stimuli and death at 15mg/kg and 12 mg/kg by *i.p* and *i.v* route respectively. The average time of death were  $1.57 \pm 0.2$  &  $1.25 \pm 0.1$ h by *i.p* and *i.v* route, hence the dose of 15mg/kg *i.p* was considered as 1 MLD of VOV and chosen for Photooxidation studies to generate antigenically active detoxified PVOV.

### **3.2 Photooxidation of venom**

Photooxidation of VOV in presence methylene blue caused changes in UV absorbance value and  $\lambda_{max}$  shift at various exposure time. The UV spectrum of VOV was biphasic with maximum optical density of 1.034 AU at 265 nm and 3.432 AU at 214 nm. In the UV spectrum of PVOV, the  $\lambda_{max}$  shifted from 265 nm to 255.5 nm. The absorbance increased from 1.034 to 1.424 at 265.5nm and venom products obtained after UV exposure were abbreviated as PVOV (Photooxidized *Vespaorientalis* venom).

### **3.3 Antigenicity Test**

Immunogel diffusion was performed using antisera to each four photooxidized venoms to test retention of antigenicity after Photooxidation at different time interval. The Figure.1 shows distinct precipitin lines visible with 30 and 45min PVOV, when incubated with Immunoglobulins from in central well, however the number of precipitin lines are less with 30 min PVOV (Fig .1 B) than 45 min (Fig 1 C), whereas, with PVOV 60 min exposure showing reduced intensity and number of precipitin lines (Fig.1 D).



**Fig. 1** Immunogel diffusion slide. The photooxidized *Vespa orientalis* venom at different time interval were incubated with immunoglobulin isolated from rabbits sera (central well). (A) 15min exposure, (B) 30min (right well), (C) 45min, (right and left well) and (D) 60min.

### 3.4 Total Protein Estimation

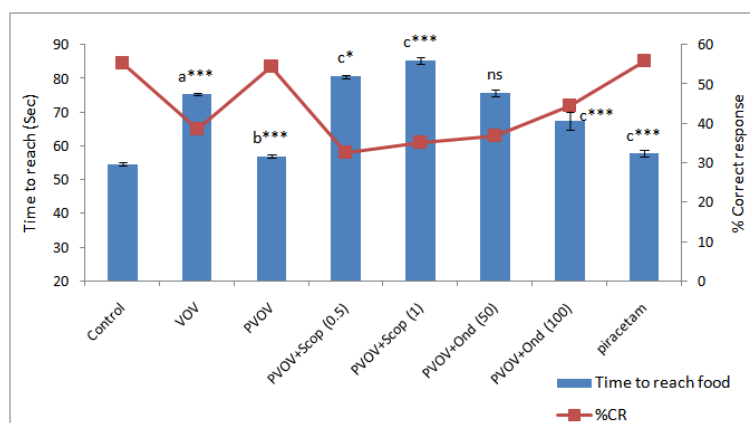
The results of protein content of crude venom VOV was 68.5% (w/v) upon UV photo-oxidation at various time interval, a reduction in protein content was observed. There was a 8.61%, 11.8%, 17.8 and 22.3% protein content was loss when VOV was exposed at 15, 30, 45 and 60 min respectively indicated that alteration in protein concentration.

### 3.5 Neutralization and detoxification Studies of Purified Immunoglobulins

It was observed that 1% of immunoglobulins neutralized the 1 MLD of VOV in mice indicated that the 1% antivenin was sufficient to protect mice toxicity. During in-vivo detoxification studies, mice exhibited continuous restlessness, respiratory abnormality, immobility, urination, defecation, dyspnoea, and all animals died within  $2.45 \pm 0.3$  hour. Based on antigenicity and toxicity tests, 45-minute UV-exposed venom product was considered Photooxidized *Vespa orientalis* venom (PVOV), and it was used to study psychopharmacological activities.

### 3.6 Memory and learning using T-maze

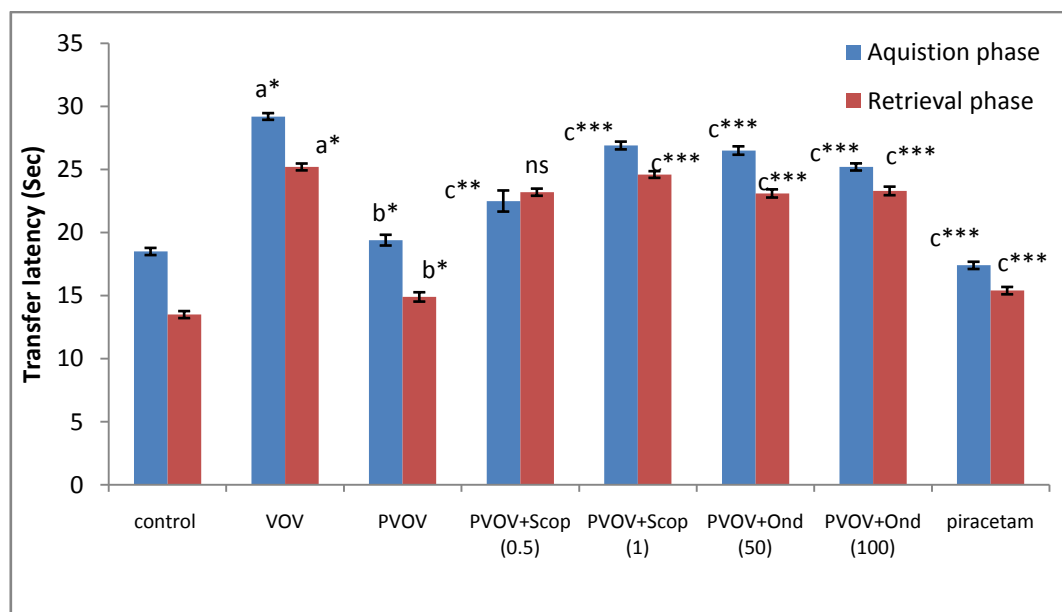
Administration of VOV to group of animals for 28 days had significantly increased ( $P < 0.001$ ) by 59.5% for reach food (transfer latency) and decreased the % Correct responses by 23 % compared to control animals during retrieval phase. These effects were countered significantly ( $P < 0.001$ ) by 1.5mg/kg PVOV (45 min) administration for 28 days with decrease in transfer latency by 66.8% and increase in correct response by 28.4% compared VOV administered group animals. When PVOV was administered with scopolamine a dose dependant change in transfer latency & % CR was observed with significant ( $P < 0.05$ ) increase in latency by 64 and 76.6% and correct of correct responses were reduced by 69.4 and 73% respectively compared to PVOV. Whereas PVOV with ondansetron 50mg/kg and 100mg/kg, there was significant ( $P < 0.001$ ) increase by 50.7 and 28% in time latency compared to control and number of correct choices were increase by 75.4 and 86.2% compared to PVOV alone. Piracetam was used as reference standard. The details of results are shown in Fig. 2.



**Fig 2.** Effect of Photooxidized *Vespa orientalis* venom (PVOV) Time to reach food during retrieval phase in T-maze; % CR= Correct response; a = Control Vs VOV & b = VOV Vs PVOV by Paired t- test; c= PVOV Vs all groups by one way ANOVA and Dunnet's multiple comparison test, \*\*\*  $P < 0.001$ ; \*  $P < 0.5$ . ns=non-significant.

### 3.7 Transfer latency in Elevated plus maze

During acquisition study, VOV administration to rats for 28 days caused significant ( $P < 0.5$ ) increase in transfer latency by 57.8 % compared to normal animals, whereas, this effect was significantly ( $P < 0.5$ ) reversed by treatment with 1.5 mg/kg PVOV for 28 days by 64.4% compared to VOV. PVOV administration along with scopolamine at 0.5 and 1 mg/kg for 28 days significantly ( $P < 0.001$ ) increase time latency by 15 and 38.6% and with PVOV with Ondansetron at 50 and 100 mg/Kg increased significantly ( $P < 0.001$ ) by 36.5 and 29.8 % compared to PVOV alone. In Retrieval phase, the transfer latency of VOV was significantly ( $P < 0.5$ ) increased by 86.6 % compared to normal animals whereas, this effect was significantly ( $P < 0.5$ ) increased when administered with PVOV by 59.1% when compared VOV group animals. When PVOV was combined with two doses of scopolamine and Ondansetron, there was a significant ( $P < 0.001$ ) increase in latency time by 55.7 & 65.1% and 55& 56.3 % when compared with PVOV group. Piracetam was used as reference standard produced a significant decrease of 3.3 % change in transfer latency compared to PVOV. The details are depicted in Fig. 3



**Fig 3.** Effect of Photooxidized *Vespa orientalis* venom (PVOV) on transfer latency in elevate plus maze; a = Control Vs VOV & b= VOV Vs PVOV by Paired t- test; c= PVOV Vs all groups by one way ANOVA and Dunnet's multiple comparison test, \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.5$

### 3.8 Step down passive avoidance test

During acquisition phase of study, VOV treated animals showed significant ( $p < 0.05$ ) increase in SDL by 81.9% compared to control animals, in turn the PVOV group animals showed significant decrease ( $P < 0.001$ ) in SDL by 41.2 % compared to VOV treated animals. When PVOV was combined with two doses of scopolamine and Ondansetron, the step down latency was significantly ( $P < 0.001$ ) increased by 4.5 & 32.2% and 30.8 & 30.1 % when compared with PVOV group, whereas Piracetam treated animals showed significant ( $p < 0.05$ ) decrease by 3.2% compared to PVOV. Similarly, there was an increase in step down errors and significant ( $P < 0.001$ ) increase in time spent in shock zone by animals when PVOV was administered with scopolamine and ondansetron compared PVOV alone.

During retrieval phase, the step down latency of was significantly ( $P < 0.001$ ) increased by 15.3% with VOV compared to control, whereas, the PVOV group animals showed significant decrease ( $P < 0.001$ ) in SDL by 61.5 % compared to VOV treated animals, similarly Piracetam decrease significantly ( $P < 0.001$ ) by 3.2% when compared PVOV group. When PVOV was administered with scopolamine and ondansetron, there was a dose dependant significant ( $P < 0.001$ ) increase in SDE and TSS in comparison with PVOV alone group. The details of results shown in the table 1.

Treatment	Acquisition			Retrieval		
	SDL	SDE	TSS	SDL	SDE	TSS
Normal control	140.5±0.1	4.4	40.5±0.8	120.7±0.8	3.8	32.5±0.5
VOV	115.1±0.5*	3.5	55.2±0.1 <sup>a***</sup>	102.2±0.4 <sup>c***</sup>	3.4	41.2±0.6 <sup>a***</sup>
PVOV	162.6±0.3* 1.6	25.2±0.7 <sup>b***</sup>	165.1±0.8 <sup>b**</sup>	1.4	23.4±0.6 <sup>b**</sup>	
PVOV + Scop(0.5)	155.2± 0.2 <sup>c***</sup>	1.7	60.3±0.5 <sup>c***</sup>	163.1±0.3 <sup>c***</sup>	1.5	24.4±0.5 <sup>c***</sup>
PVOV + Scop(1)	110.2±0.3 <sup>c***</sup>	4.2	61.2±0.5 <sup>c***</sup>	122.6± 0.5 <sup>c***</sup>	3.5	53.7±0.7 <sup>c***</sup>
PVOV +Ond(50)	112.4±0.2 <sup>c***</sup>	3.9	59.2±0.5 <sup>c***</sup>	119.1± 0.5 <sup>c***</sup>	3.6	51.8±0.4 <sup>c***</sup>
PVOV +Ond(100)	113.6± 0.2 <sup>c*</sup>	5.1	62.2±0.6 <sup>c***</sup>	122.8± 0.6 <sup>c***</sup>	3.4	49.8±0.8 <sup>c***</sup>
Piracetam	167.9±0.4 <sup>c***</sup>	4.9	22.5±0.4 <sup>c***</sup>	170.5±1.2 <sup>c***</sup>	1.4	21.7±0.6 <sup>c*</sup>

Table 1. Effect of Photooxidized *Vespa orientalis* venom (PVOV) step down latency in rats using passive avoidance paradigm. Statistical analysis: a = Control Vs VOV & b= VOV Vs PVOV by Paired t- test; c= PVOV Vs all groups by one way ANOVA and Dunnet's multiple comparison test, \*\*\**P*<0.001; \*\**P*<0.01; \**P*<0.05; ns= non-significant.

#### IV. Discussion

*Vespa* venom are still widely studied for their component activity and applications in toxicology related fields. These venom contain high molecular mass proteins, biological active amines (acetylcholine, histamine, serotonin and Catecholamine) and small peptides. Many bioactive peptides (kinins, mastoparans and chemotactic peptides) have been isolated and characterized from various species of vespids venoms.(21) Due these components, most of venom cause complications.(22) In the present study, the minimum lethal dose of VOV was found to be 15mg/kg and 12 mg/kg by *i.p* and *i.v* route in mice, which is higher than the reported dose of 2.5mg/kg (22), probably due presence non-protein material might have increased the MLD. We have selected 1/10th of MLD of VOV for Photooxidation studies to generate antigenically active detoxified *Vespa orientalis* venom products (PVOV) and to study effect on memory and learning activity in presence and absence of scopolamine and ondansetron. The UV-Photooxidation of venom proteins in presence methylene blue have been widely used to detoxify and to study the biological activity.(3, 23, 24) UV exposure of VOV caused shift in the ODmax in PVOV absorption profile compared to VOV. An increased absorbance was reciprocated with loss of venom protein in photooxidized venom. Comparative absorbance changes in reaction mixture of VOV and PVOV revealed the loss of protein in PVOV probably due to unfolding of proteins in the presence of sensitizer dye. Similar alterations were also observed in photooxidized venom product of *Naja siamensis* and *Echis carinatus*, which support our hypothesis.(4, 25) In immunogel diffusion test, Innumoglobulins isolated from rabbitsera showed prominent precipitin lines with 45min exposure of venom indicated the retention of antigenicity, where as other photooxidized venom products produced either reduced intensity or number of precipitin line, hence we have selected 45 min exposure PVOV for in-vivo and in-vitro neutralization study of venom. When isolated immunoglobulins were tested against 1 MLD VOV, it was found that 1% diluted antivenom was sufficient to neutralize 1 MLD and protected mice from death and even when PVOV when injected in mice the mortality time was prolonged compared to VOV indicated that decreased toxicity with retention of antigenicity. From the results of above study, we have chosen 1.5mg/kg (1/10<sup>th</sup> of 1MLD) of PVOV as therapeutic dose to rats in memory and learning model. *Vespa orientalis* venom is complex mixture of many enzymatic and non-enzymatic proteins. Nearly 4% of soluble proteins of *Vespa orientalis* are known to contain acetylcholine-like substances.(26) The pure venom and venom sac extract of *Vespa orientalis* provoked CNS anticholinesterase-like activity in various animals, and may open the blood-brain barrier and cause central mediated changes in auditory and somatosensory activities in cats(27,28) and pharmacological and toxicological effects of toxins of snake and wasps thought to mediated through central acetylcholine receptor and ion channels. (29-31) These facts encouraged us explore study of effect of Photo detoxified *Vespa orientalis* venom along administered with Scopolamine and Ondansetron to check mechanism of memory enhancement. PVOV generated at 45 min UVR exposure showed improvement both spatial and long term memory in rats when subjected in T maze, EPM and conditioned avoidance paradigm.

Administration of PVOV shortened the time required to reach the food in T-maze and transfer latency in EPM, while a significant improvement was observed in step down passive avoidance test, which indicates a clear cut improvement in learning and memory function in all the models. These experimental results indicated that PVOV components might have acted directly or indirectly upon repeated administration and some water soluble components of venom responsible for boosting of blood flow in central cortex.(32) Scopolamine, an anticholinergic agent acts as a competitive inhibitor at post ganglionic muscarinic M1 receptors site of parasympathetic system. The improvement was significantly suppressed when scopolamine was administered indicated that activity of PVOV could be mediated through cholinergic mechanism which was antagonized by

scopolamine and ondansetron. However, we cannot rule out the role of other low-molecular weight protein and peptides or enzymes, which may act by serotonergic / cholinergic system that affect CNS action.

## V. Conclusion

We provide first reported evidence that photooxidized *Vespa orientalis* venom with UV radiation for 45 min undergo detoxification having improvement in both spatial and long term memory. There is a possibility of using PVOV in the treatment of degenerative illnesses such as Dementia, Alzheimer's disease as a non-herbal and non-synthetic alternative for patients not responding to the available therapy; The results support that possible mode of action could be through M1 receptor or 5HT-3 modulation or anticholinesterase action.

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## 7. Conflicts Of Interest

There is no conflict.

## References

- [1]. Piek T. Venoms of the Hymenoptera : biochemical, pharmacological and behavioral aspects Academic Press; London, Orlando; San Diego; 1986.
- [2]. Nakajima T. Biochemistry of vespid venoms AT T, editor. New York: Marcel Dekker; 1984.
- [3]. Bennacef-Heffar N, Laraba-Djebari F. Evaluation of the effect of gamma rays on the venom of *Vipera lebetina* by biochemical study. Canadian journal of physiology and pharmacology. 2003;81(12):1110-7.
- [4]. Gawade SP, S P. Pharmacodynamics of photooxidized *Echis carinatus* venom product using UV sensitized methylene blue Indian J Pharma Edu. 2004;38(2):81-5.
- [5]. Reddy CM, SP G. Evaluation of the effects of photooxidized *Echis carinatus* venom on learning, memory and stress. J Venom Anim Toxins incl Trop Dis. 2006;12(4):632-52.
- [6]. Ishay JS. Anticholinesterase-like activity by oriental hornet (*Vespa orientalis*) venom and venom sac extract. Experientia. 1979 May 15;35(5):636-9.
- [7]. Duvdevani P, Ishay JS, Schejter A. Mastoparan induces hypothermia in mice. Experientia. 1991 May 15;47(5):460-2.
- [8]. Reed LJ, Muench H. A Simple method of estimating Fifty percent Endpoint American Journal of Epidemiology. 1938;27(3):493-7.
- [9]. Turner R. . Screening methods in pharmacology. New York: Academic Press; 1965.
- [10]. Pizzi M. Sampling variation of the fifty percent end point, determined by Reed Muench Method Human Biology. 1950;22(3):151-90.
- [11]. Gawade SP, Sankar A. Pharmaco-photodynamics of photooxidised Russell's viper venom product using UV radiation in the presence of methylene blue Ind J Pharm Education Res 2007;41:21-8.
- [12]. Flowers HH. The Effects of X-Irradiation on the Biological Activity of Cottonmouth Moccasin (*Agkistrodon Piscivorus*) Enom. Rep 588. Rep US Army Med Res Lab. 1963;16:1-14.
- [13]. Bellin JS, Yankus CA. Influence of dye binding on the sensitized photooxidation of amino acids. Arch Biochem Biophys. 1968;123(1):18-28.
- [14]. Ouchterlony O. Antigen-antibody reactions in gels. Acta Pathol Microbiol Scand. 1949;26(4):507-15.
- [15]. Hudson L, FC H. Isolation and structure of immunoglobulins. In: Hudson L, Hay FC, editors. Practical Immunology. . 3 ed: Boston: Blackwell Scientific Publication; 1989.
- [16]. Akbari A, H R, A H, N M, H Z, SH T. Production of effective antivenin to treat cobra snake (*Naja naja oxiana*) envenoming Arch Razi Inst. 2010;65(1):33-7.
- [17]. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
- [18]. De-Mello N, AP C. Elevated T-maze as an animal model of memory: effects of scopolamine. Behav Pharmacol. 2012;13(1):139-48.
- [19]. Kulkarni S, A V. Evidence for nootropic effect of BR-16A (Mentat). A herbal psychotropic preparation, in mice Ind J Physiol Pharmacol 1992;36(1):29.
- [20]. Camacho F, CP S, HM V, JT W. Alpha 2 adrenoceptor antagonists, potentiate acetylcholinesterase inhibitor effects on passive avoidance learning in rat. Psychopharmacology (Berl). 1996;124(4):347-54.
- [21]. Ho CL, Chen WC, Lin YL. Structures and biological activities of new wasp venom peptides isolated from the black-bellied hornet (*Vespa basalis*) venom. Toxicon. 1998 Apr;36(4):609-17.
- [22]. Ishay J. Hyperglycemia produced by *Vespa orientalis* venom sac extract. Toxicon. 1975;13(4):221-6.
- [23]. Andriao-Escarso SH, Soares AM, Rodrigues VM, Angulo Y, Diaz C, Lomonte B, et al. Myotoxic phospholipases A(2) in bothrops snake venoms: effect of chemical modifications on the enzymatic and pharmacological properties of bothrotoxins from *Bothrops jararacussu*. Biochimie. 2000 Aug;82(8):755-63.
- [24]. Gawade SP, Tamboli MA, Thorat SS. Anti-Tumor Activity of Viper Snake Venom Photo-products SV1 and SV2 against Ehrlich Ascites Carcinoma in Mice. Indian Journal of Pharmaceutical Education and Research 2016;50(1):123-9.
- [25]. GAWADE SP. The photodynamic action of UV sensitized methylene blue on the venom of Thailand cobra *Naja siamensis*. Journal of Venomous Animals and Toxins. 2000;6(2):271-80.
- [26]. Friedman J, Ishay JS. Inhibition of protein synthesis by an extract of the venom sac of the oriental hornet (*Vespa orientalis*). Toxicon. 1987;25(6):673-6.
- [27]. Ishaya J. Anticholinesterase-like activity by oriental hornet (*Vespa orientalis*) venom and venom sac extract. Cell Mol Life Sci. 1979;35(5):636-9.
- [28]. Barenholz-Paniry V, Ishay JS, Freeman S, Sohmer H. Evoked potential changes in cats following injection of an extract from the venom sac of the oriental hornet (*Vespa orientalis*). Toxicon. 1990;28(11):1317-24.
- [29]. Osipov A, Utkin Y. Effects of snake venom polypeptides on central nervous system. Cent Nerv Syst Agents Med Chem. 2012 Dec;12(4):315-28.

- [30]. Lee SM, Yang EJ, Choi SM, Kim SH, Baek MG, Jiang JH. Effects of bee venom on glutamate-induced toxicity in neuronal and glial cells. *Evid Based Complement Alternat Med.* 2012;2012:368196.
- [31]. Piek T, Hue B, Mantel P, Nakajima T, Pelhate M, Yasuhara T. Threonine6-bradykinin in the venom of the wasp *Colpa interrupta* (F.) presynaptically blocks nicotinic synaptic transmission in the insect CNS. *Comp Biochem Physiol C.* 1990;96(1):157-62.
- [32]. Kaspi T, Ishay JS. Cortical cerebral blood flow is enhanced by hornet venom. *Pharmacol Toxicol.* 1990 Sep;67(3):205-8.

Mound H "Detoxified *Vespa orientalis* venom improves memory and learning activities in rats." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 13.1 (2018): PP 16-22.