ANTI-INFLAMMATORY ROLE OF PEPTIDES ISOLATED FROM VENOM OF *ANDROCTONUS FINITIMUS* (POCOCK, 1897) (SCORPIONES: BUTHIDAE)

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ABSTRACT

Scorpion venom has great medicinal value as it contains biologically active compounds. Some of these compounds enhance production of anti-inflammatory cytokines; the mediators of hyperactive immune system. Present study is based on the fact that scorpion venom or its components have anti-inflammatory potentials by boosting anti-inflammatory mediators. Venom of *Androctonus finitimus* was extracted and its toxicity was determined by calculating LD_{50} using Probit analysis. Remedial activity of venom and its selected peptide fractions on carrageenan induced paw edema in *Mus musculus* (Swiss Webster albino mice) was estimated. Total leukocyte count (TLC), differential leukocyte count (DLC), and anti-inflammatory cytokines i.e., interleukin 4 (IL-4), IL-6, IL-10 and IL-13 were measured in blood of venom treated animals. Results of study illustrated that protein fractions of venom were more potent anti-inflammatory agents as compared to whole venom because animals treated with different protein fractions showed rapid recovery in paw edema as well as elevated TLC, DLC and concentrations of anti-inflammatory cytokines. As venom and protein fractions under study were found effective in healing the inflammation, further study on isolation and biochemical characterization of these fractions is recommended to use them for synthesis of new and cheap drugs for curing edema/inflammation related diseases.

Keywords: inflammation, peptides, venom, scorpions, *Androctonus finitimus,* cytokines.

INTRODUCTION

Scorpion's venom has drawn much attraction of medical researchers because of its toxic properties. During the 400 million years of evolution, scorpion has developed a complex mixture of mucoproteins, various biochemically active water soluble enzymes, enzyme inhibitors, amines, histamine, lipids, glucose-aminoglycanes, small peptides and various salts in their venom (Zlotkin *et al*., 1978; Gwee *et al*., 2002; Nisani *et al*., 2007; Adiguzel, 2010; Gomes *et al*., 2010). Though venoms are inducers of many patho-physiological effects but their modified use turn them into healer of several pathologies. Researchers are trying to intricate pathophysiological effects of venom to discover some logical application of venom toxin for developing therapeutically potent agents. Scorpion venom contain bioactive peptides with variety of therapeutic applications (Chen and Ji, 2002; Garcia *et al*., 2003; Fiske *et al*., 2006; Gomez-Varela *et al*., 2007; Zhao *et al*., 2008; Pillozzi *et al*., 2009; Cheong *et al*., 2010). Many scientists have reported cytotoxic and anti-inflammatory potentials of scorpion venom and its components (Salarian *et al*., 2012), which may be helpful in the treatment of cancers of numerous types (Incesu *et al*., 2005; Zhang *et al*., 2009; Di-Lorenzo *et al*., 2012; Joseph and George, 2012). Some peptides from scorpion venom are immunosuppressive agents (Chen and Chung, 2012). Anti-inflammatory effects of venom from *Heterometrus laoticus* and *Androctonus bicolor* have also been reported (Ayed *et al*., 2013; Hoang *et al*., 2014).

After scorpion envenomation a cascade of inflammatory responses occurs immediately (Fabiano *et al*., 2008), including initiation of antibodies production against venom antigens and induction of a wide variety of cytokines and other mediators of inflammation by T lymphocytes (Mosmann *et al*., 2005). Hence, cytokines are the direct mediators of inflammatory process (Romgnani, 1994). The cytokines are of two types i.e., pro-inflammatory and anti-inflammatory. Production of both type of cytokines have been reported in severe scorpion envenomation cases (Petricevich, 2004). However, this process is slightly biased towards production of anti-inflammatory cytokines (Petricevich, 2006). Anti-inflammatory cytokines such as IL-4, IL-10, IL-5 and IL-6 down regulates hyperactive inflammatory process (Bone, 1996; Fisher & Zheng, 1996). Secretion of IL-4 initiate differentiation of Th cells into Th2 sub-populations which are capable of secreting more IL-4 and IL-10 (Mosmann *et al*., 2005).

Interleukin-6 plays dual role in an inflammatory response, as it has both pro- and anti-inflammatory effects (Barton, 1997). IL-6, down regulate the production of IL-1 and tumor nacrosis factor-α (TNF-α),

the pro-inflammatory cytokines, inhibits production of granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon γ (IFN-γ) (Gay *et al*., 2006) and also has little role in IL-10 production (Xing *et al*., 1998). IL-10 is produced by several immune cells (Asadullah *et al*., 2003), and probably the most important anti-inflammatory cytokine of human immune response. It is the compelling inhibitor of Th1 derived cytokines, including IFN-*γ*, TNF-*α*, IL-1*β*, IL-2, IL-6, IL-8, and IL-12 (Asadullah *et al*., 2003; Mosmann *et al*., 2005). It also down regulates other mediators of inflammation such as, prostaglandin as well as macrophage originate production of free oxygen radicals and nitric oxide (Goldman *et al*., 1996), platelet activating factor and leukotrienes (Bogdan *et al*., 1991; Malefyt *et al*., 1991). Increased production of IL-10 is believed to counteract the inflammation process so that hemostasis can be reestablished. Keeping in view of the ample therapeutic applications of scorpion venom present study has been designed. Aim of the study was to evaluate anti-inflammatory potentials of selected low molecular weight fractions of scorpion venom.

MATERIALS AND METHODS

Venom and treatments: Venom was extracted from *Androctonus finitimus* (Pocock, 1897) by electric stimulation of telson (Ozkan and Filazi, 2004). Crude venom as well as following bioactive protein fractions eluted from crude venom by native-Polyacrylamide gel electrophoresis (Native-PAGE) (Arndt *et al*., 2012) were used in experiment.
AF1: fraction containing proteins of ~54 kilo Dalton (KDa)
AF2: fraction containing proteins of ~36KDa
AF3: fraction containing proteins of ~8.5KDa

Model animals: *Mus musculus* (Swiss Webster albino mice) with an average weight of 30-35 grams were used as a model organism. Animals were purchased from National Institute of Health (NIH), Islamabad, Pakistan. Five to seven animals were kept in one cage (24″×12″). Each cage was lined with saw dust as bedding material. Animals were maintained at controlled environmental conditions i.e., light (325 lux), humidity (40-50%), temperature (26-30°C) and 12 hours alternating light-dark cycle. All animals were provided with standard food and water.

Determination of median lethal dose (LD$_{50}$) for mice: Lethal potency of venom was determined by probit analysis method as recommended by World Health Organization (WHO, 1981). Dried crude venom was dissolved in double distilled water to prepare three venom doses i.e., 0.1mg/ml, 0.05mg/ml and 0.02mg/ml. Intravenous injections of treatments were given to mice in tail. Volume of injection was kept constant i.e., 100µl/ mice. An equal volume of 5% normal saline was injected

to control group. Percent mortality was recorded for 24 hours post injection.

Evaluation of Anti-inflammatory potential of venom: Albino mice were divided into 6 groups of 3 animals each. Paw edema was induced in animals of all groups. To induce edema 50µl of 1% carrageenan in saline solution was injected to left hind tibio-tarsal joint. After 1 hour of injecting cargeenan, Group 2 received Betamethasone (50µl) as standard treatment after induction of paw edema and considered as positive control group. Animals of group 1 were considered as negative control, thus received 50 µl of normal saline only. Different venom fractions and sub lethal dilutions of whole venom were injected to remaining groups as a treatment. Treatment given to each group were as follow:

- **Group 1 (Negative control):** 50µl of 1% carrageenan + 50µl of normal saline
- **Group 2 (Positive control):** 50µl of 1% carrageenan + 50µl of Betamethasone
- **Group 3**: 50 µl of 1% carrageenan + 50 µl of AF1
- **Group 4**: 50 µl of 1% carrageenan + 50 µl of AF2
- **Group 5**: 50 µl of 1% carrageenan + 50 µl of AF3
- **Group 6**: 50 µl of 1% carrageenan + 50 µl of *A. finitimus* whole venom

       Diameter of tibio-tarsal joint of each animal was recorded before the treatment. The swelling of ankle joints (tibio-tarsal) was measured at 1, 2, 4, 6, 12 hour intervals post treatment. After 48 hours, animals were sacrificed and blood was drawn via heart puncture. The blood withdrawn was used for total leukocyte count (TLC) and differential leukocyte count (DLC). Experiment was replicated thrice and results were compared through one way ANOVA by using SPSS 13. Normality of the data was determined by using Kolmogorov–Smirnov test.

Effect of venom on cytokine levels: Blood serum from above described groups was collected and stored at -20°C for determination of anti-inflammatory cytokine level. Levels of IL-4, IL-6, IL-10 and IL-13 in mice serum were determined by sandwich-enzyme-linked immuno sorbent assay (ELISA) (Schumacher *et al*., 1988). Qiagen kit (Mouse Inflammatory Cytokines Multi-Analyte ELISArray Kit: MEM-004A) was used for this purpose. Solutions were prepared according to manufacturer's instruction and after preparation of plates the absorbance was recorded at 450nm. One way analysis of variance (ANOVA) followed by Tukey's test was used to compare the level of cytokines in the treated and control groups.

RESULTS

       Results of Probit analysis showed that median lethal dose of *A. finitimus* venom was 0.019mg of venom/g of body weight and a dose of 0.031mg/g was

sufficient to kill 95% of treated animals. Injection of carrageenan resulted in remarked swelling of tibio-tarsal joints within one hour of injection (Fig. 1). Paw swelling in negative control animals increased progressively up to 3 hours post treatment and then decreased gradually but do not return to normal size even after 12 hours. When effect of venom and its protein fractions on carrageenan induced paw edema was recorded, decline in paw swelling was observed in all treated groups as compared to negative control. Increase in paw diameter was observed in ACV and AF1 treated animal during first hour after treatment which settle down within 4 hour post treatment. Although increase in paw size was more obvious in ACV injected group as compared to AF1 treated group. Animals treated with AF2 and AF3 protein fractions there is reduced paw swelling during first hour of treatment and recovery in paw size was more apparent in AF2 treated group.

Results of the study showed that injection of *A. finitimus* venom (ACV) and its protein fractions AF1 significantly affected the total leukocyte count (TLC) in relation to negative control ($F_{5,12} = 131.89$; *P*: <0.05) (Table 1). Highest TLC was recorded in animals which

receive protein fraction AF3. On the other hand neutrophil and lymphocyte levels elevated significantly in all venom treated groups except ACV ($F_{5,12} = 14.42$; *P* <0.05 for neutrophils; $F_{5,12} = 7.37$; *P* = 0.002 for lymphocytes). There was no significant difference in monocyte number ($F_{5,12}$ 1.52; *P* = 0.256) however, eosinophil levels significantly decreased in venom treated animals ($F_{5,12} = 3.77$; *P* = 0.028).

Levels of cytokines in blood serum of mice treated with venom of *A. finitimus* are depicted in Table (2). Increased concentrations of IL-4 were recorded in all venom treated animals except ACV treated group, as compared to negative control. IL-4 was highest in AF2 treated animals ($F_{5,12}$=607.43; *P*<0.05). IL-6 levels were elevated in ACV and AF3 injected groups ($F_{5,12}$=269.460; *P*<0.05). Animals in negative control group had lowest IL-10 and IL-13 levels while positive control groups had comparatively high concentrations. IL-10 and IL-13 levels were lower in ACV treated animals while, high in AF3 treated animals amongst all venom fraction treatments ($F_{5,12}$=1420.459; *P*<0.05 for IL-10; $F_{5,12}$=214.460; *P*<0.05 for IL-13).
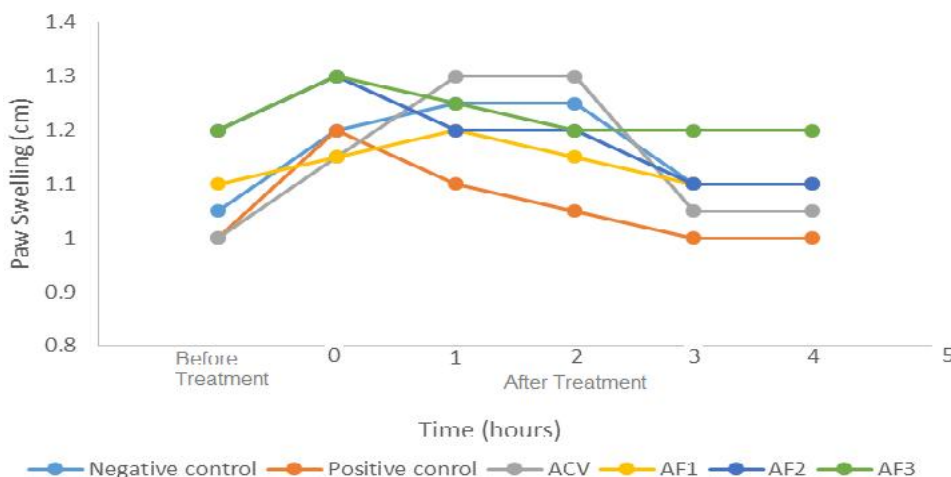

Fig. 1: Extent of paw edema after treatment of crude venom and protein fractions from venom of *A. finitimus*.

Table 1. *Effect of A. finitimus venom and its protein fractions on different blood parameters of Mice.*

Treatments	TLC (10⁻³/ul)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Negative control	3.33[c]±0.17	60[a]±5.77	23.33[ab]±2.02	2.67[a]±0.67	3[b]±0.57
Positive control	4.75[cd]±0.18	70[a]±2.88	31.33[b]±3.75	2[a]±0.57	1.33[ab]±0.33
ACV	1.53[a]±0.17	54.66[a]±5.17	14.66[a]±1.45	1.33[a]±0.33	1.67[ab]±0.33
AF1	5.1[d]±0.17	63[a]±1.73	26.33[ab]±2.33	3[a]±0.57	1.67[ab]±0.33
AF2	2.4[b]±0.21	66.66[a]±1.45	34[b]±3.21	3[a]±0.57	1[a]±0
AF3	7.1[e]±0.17	94[b]±2.3	27[b]±1.15	3[a]±0.57	1.33[ab]±0.33
F 5,12	131.89	14.42	7.34	1.52	3.77
P	<0.05	<0.05	0.002	0.256	0.028

Note: Data represented as Mean ± SEM
Values in a column having different superscripts indicate significant difference i.e., P≤0.05

Table 2. *Dynamics of Anti-inflammatory cytokines in serum of mice injected with whole venom and various venom fractions of A. finitimus.*

Treatments	IL-4	IL-6	IL-10	IL-13
Negative control	0.216[b]±0.011	0.219[c]±0.002	0.245[a]±0.002	0.216[a]±0.028
Positive control	0.276[e]±0.009	0.24[e]±0.000	0.293[c]±0.002	0.25[e]±0.016
ACV	0.181[a]±0.006	0.231[d]±0.006	0.246[a]±0.020	0.218[a]±0.002
AF1	0.246[d]±0.014	0.212[b]±0.004	0.291[c]±0.004	0.227[b]±0.001
AF2	0.278[e]±0.006	0.201[a]±0.004	0.284[b]±0.003	0.233[c]±0.006
AF3	0.23[c]±0.010	0.25[f]±0.008	0.336[d]±0.008	0.24[d]±0.004
$F_{5,12}$	607.43	264.46	1420.459	214.46
P	<0.05	<0.05	<0.05	<0.05

Note: Data represented as Mean ± SEM

Values in a column having different superscripts indicate significant difference i.e., P≤0.05

Values in table indicating absorbance at wavelength of 450nm

DISCUSSION

Inflammation is the body's typical response for the removal of unnecessary material from infested tissue (Mahat and Patil, 2007). Sometimes body encounters extended periods of inflammation that are considered as sign of chronic diseases. To deal such indications use of steroids and non-steroidal anti-inflammatory drugs (NSAID) is a common practice. Use of these chemicals is often associated with many hormonal and gastric complications (Suleyman *et al*., 2007) as well as liver and kidney malfunctioning (Wellehan and Gunkel 2004). Development of unique anti-inflammatory treatments and preparations is immediately required to resolve the complication associated with these drugs. In the proposed study, potential anti-inflammatory effect of scorpion venom and its protein fractions was investigated.

We observed a profound suppressive effect of protein fractions of venom on carrageenan induced paw edema. Previously, venom of *Heterometrus laoticus* and *Mesobuthus eupeus* has been reported to be involved in reducing carrageenan and adjuvant induced paw edema (Ahmadi *et al*., 2009; Hoang *et al*., 2014). During inflammation remarkable changes take place including edema, accumulation of white blood cells i.e., neutrophils, macrophages and monocytes and sensation of pain and fever (Gallin, 1989). Carrageenan induced paw edema is thought to be most suitable test for various investigations regarding evaluation of anti-inflammatory products because carrageenan is a strong inducer of various inflammatory and pro inflammatory mediators such as interleukins, leukotriene, histamine, bradykinins, etc. (Wills, 1960; D-Armour *et al*., 1965).

In the present study, decrease in the TLC was observed in crude venom treated animals compared to negative control. In case of inflammation, leukocytes migrate towards inflamed tissue. This may cause low levels of leukocytes in peripheral blood which in turn trigger the production of more WBCs under the influence of IL-1β. IL-1β induces the production of neutrophils

(Eric and Lawrance, 1996; Kalpana *et al*., 2007). Decrease in leukocytes levels was reported by Jhon and Shobana, (2012) while evaluating potential anti-inflammatory role of some organic products by Rached *et al*. (2010) working on anti-inflammatory activity of bee venom and Al-Sadoon *et al*. (2012) who observed decline in WBCs during 24 post injection hours of snake venom (*Walterennesia aegyptia).*

A transient increase in TLC was recorded in positive control group. Similar results were observed by Ahmadi *et al*. (2009) while working on venom of *M. eupeus*. In our experiment, increase in leukocyte level was observed in animals injected with F1 and F2 protein fractions. This increased production of WBC may be due to the enhanced release of leukocyte from bone marrow and reduced clearance from peripheral circulation (Veiseh *et al*., 2009).

The extent of inflammation is determined by subtle balance in pro-inflammatory and anti-inflammatory cytokines. Anti-inflammatory cytokines down regulate pro-inflammatory cytokines to limit the inflammatory response (Petricevich, 2006). Scorpion venom contains peptides which are found to be associated with elevated levels of such anti-inflammatory cytokines. In our experiments we observed prominent increase in IL-4 levels in response to various venom protein fractions. According to another study an immediate increase in serum cytokine concentrations of rats treated with *Androctonus australis* venom was observed during one hour of exposure (Adi-Bessalem *et al*., 2008). Elevated levels IL-4 were also observed previously in serum of humans, envenomed by *Tityus serrulatus* (Magalheaes *et al*., 1999) and in the mice which were experimentally injected with venom and Ts2 fraction from venom of *T. serrulatu* (Petricevich *et al*., 2007; Zoccal *et al*., 2013). IL-4 is secreted by T cells and found to suppress various pro-inflammatory cytokines produced by monocytes which may help to quell hyperactive inflammatory process (Heller *et al*., 2008; Barros *et al*., 2009). Some studies also reported immunosuppressive

effect of IL4 via activation of B and T lymphocytes (Rogler and Andus, 1998). IL-4 imposes an inhibitory effect on neutrophil and monocyte derived IL-8 and TNF (Lee *et al.*, 2002) by inhibiting gene expression and mRNA degradation (Standiford *et al.*, 1990).

IL-6 down regulate production of many pro-inflammatory mediators i.e., IL-1, TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon γ (IFN-γ) as well as induce production of other anti-inflammatory cytokine i.e., IL-10 (Xing *et al.*, 1998; Gay *et al.*, 2006). IL-10 regulates damage caused by hyperactive immune response (Moore *et al.*, 2001; Bazzoni *et al.*, 2010). IL-10 is also a powerful inhibitor of various pro-inflammatory cytokines (Yokoyama *et al.*, 2004) and known as "human cytokine synthesis inhibitor factor". Many of its target molecules are Th1 lymphocyte derived cytokines i.e., IFN-*γ*, TNF-*α*, IL-1*β*, IL-2, IL-6, IL-8, and IL-12 (Asadullah *et al.*, 2003; Mosmann *et al.*, 2005). IL-6 and IL-10 were observed to be over expressed in venom fraction F3 treatment as compared to other fractions. An increase in IL-6 was also observed in rats exposed to *M. eupeus* venom (Jalali *et al.*, 2015). In patients envenomed by scorpion venom, a rapid increase in IL-6 was observed (Magalheaes *et al.*, 1999; Petricevich and Pena, 2002; Pessini *et al.*, 2003; Petricevich, 2006; Cruz *et al.*, 2008) i.e., peaks at 15 minute of exposure, followed by return towards normal levels (Fukuhara *et al.*, 2003).

Induced production of IL-10 in mice has also been documented by group of researchers while evaluating activity of *A. australis* (Adi-Bessalem *et al.*, 2008). Another study suggested that venom of *T. serrulatus* and its different fractions (Ts2) enhance IL-6 and IL-10 production which may impose anti-inflammatory effect by acting on macrophages in a dose dependent manner. According to them, same fraction was responsible for inhibition of pro-inflammatory cytokines in presence of inflammation inducer (Lipopolyscharide) (Zoccal *et al.*, 2011 & 2013). The increase in IL-10 in response to TsV is much rapid that within 6 hours of venom treatment considerable elevation could be observed (Fialho *et al.*, 2011). Reduced sepsis and lung inflammation was found to be associated with augmented IL-10 that could be helpful in reducing lethality due to sepsis and lung inflammation (Gerard *et al.*, 1993; Van-Der-Poll *et al.*, 1995; Maciel *et al.*, 2014).

IL-13 is another Th2 cell derived anti-inflammatory cytokine. It has the ability to inhibit production of multiple pro-inflammatory cytokines i.e., TNF, IL-1 and IL-8 (Zurawski and De-Vries, 1994) and enhance IL-1 receptor antagonist which is an anti-inflammatory cytokine. IL-13 shares many activities with IL-4 and receptors as well (Malefyt *et al.*, 1993). In present study, we found little increase in IL-13 concentrations in positive control animals. This may be associated with reduction of inflammation. Previously involvement of IL-13 in reduction of NF-kB activation was found in lipopolysaccharide induced inflammation which was associated with decline in lethality (Di-Santo *et al.*, 1997; Mijatovic *et al.*, 1997; Muchamuel *et al.*, 1997). IL-13 was also reported to play anti-inflammatory role in lung inflammation (Mulligan *et al.*, 1997; Lentsch *et al.*, 1999).

Conclusion: In current study, protein fractions extracted from scorpion venom are found to be effective in healing inflammation so, further study to modify them for synthesis of new and cheap drugs for curing edema/inflammation related diseases is recommended. Different fractions act in different manner so use of venom peptides in different combinations yet to be evaluated to get maximum curative effects. Furthermore, sequencing of these separated peptides is required to get better insight of mode of action of those peptides.

REFERENCES

Adi-Bessalem, S., D. Hammoudi-Triki, and F. Laraba-Djebari (2008). Pathophysiological effects of *Androctonus australis hector* scorpion venom: tissue damages and inflammatory response. Exp. Toxicol. Pathol. 60: 373-380.

Adiguzel, S. (2010). In vivo and in vitro effects of scorpion venoms in Turkey: a mini-review. J. Venom. Anim. Toxins. Inc. Trop. Dis. 16: 198-211.

Ahmadi, M., M. Zare-Mirakabadi, M. Hashemlou, and M. Hejazi (2009). Study on anti-inflammatory effect of scorpion (*Mesobuthus eupeus*) venom in adjuvant-induced arthritis in rats. Arch. Razi Inst. 64: 51-56.

Al-Sadoon, M.K., A. Fahim, S.F. Salama, and G. Badr (2012). The effects of LD50 of *Walterinnesia aegyptia* crude venom on blood parameters of male rats. Afr. J. Microbiol. Res. 6: 653-659.

Arndt, C., S. Koristka, H. Bartsch, and M. Bachmann (2012). Protein Electrophoresis: Methods and Protocols, Methods in Molecular Biology. Kurien, B. T. and Scofield, H. R. (eds.). Springer Science Business Media, LLC. pp 49-53.

Asadullah, K., W. Sterry, and H.D. Volk (2003). Interleukin- 10 therapy—review of a new approach. Pharmacol. Rev. 55: 241-269.

Ayed, A.S., M.M.A. Daim, Z.I. Nabil, and M.A. Abdel-Rahman (2013). Anti-inflammatory, antipyretic and antinociceptive effects of the Egyptian scorpion venom *Androctonus bicolor*. Abstract

presented in 1st Young researchers conference of Suez canal University. Retrieved from http://scuegypt.edu.eg/ar/wp-content/uploads/2013/02/pdf.

Barros, V.E.D., B.R. Ferreira, M. Livonesi, and L.T.M. Figueiredo (2009). Cytokine and nitric oxide production by mouse macrophages infected with Brazilian flaviviruses. Rev. Inst. Med. Trop. *Sao Paulo.* 51: 141-147.

Barton, B.E (1997). IL-6: insights into novel biological activities. Cinic. Immunol. Immunopathol. 85: 16-20.

Bazzoni, F., N. Tamassia, M. Rossato, and M.A. Cassatella (2010). Understanding the molecular mechanisms of the multifaceted IL-10-mediated anti-inflammatory response: lessons from neutrophils. Eur. J. Immunol. 40: 2360-2368.

Bogdan, C., Y. Vodovotz, and C. Nathan (1991). Macrophage deactivation by interleukin 10. J. Exp. Med. 174: 1549-1555.

Bone, R.C. (1996). Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndromes (MODS). Ann. Intern. Med. 125: 680-687.

Chen, R., and S.H. Chung (2012). Engineering a potent and specific blocker of voltage-gated potassium channel Kv1.3, a target for autoimmune diseases. Biochemistry, 51: 1976-1982.

Chen, B., and Y. Ji (2002). Anti hyperalgesia effect of BmK AS, a scorpion toxin, in rat by plantar injection. Brain Res. 952: 322-326.

Cheong, A., J. Li, P. Sukumar, B. Kumar, F. Zeng, K. Riches, C. Munsch, and I.C. Wood (2010). Potent suppression of vascular smooth muscle cell migration and human neointimal hyperplasia by KV1.3 channel blockers. Cardiovasc. Res. 89: 282-289.

Cruz, H.A., S. Garcia-Jimenez, M.R. Zucatelli, and V.L. Petricevich (2008). Pro- and anti-inflammatory cytokines release in mice injected with *Crotalus durissusterrificus* venom. Hindawi Publishing Corporation Mediators of Inflammation. 2008: 1-10.

D-Armour, F.E., F.R. Blad, and J.D.A. Belden (Ed.) (1965). Manual for Laboratory Work in Physiology. 3rd Edition. The University of Chicago Press, Chicago, Illinois, 4-6p.

Di-Lorenzo, L., P. Chiara, C. Antonio, and F. Calogero (2012). Cancer pain management with a venom of blue scorpion endemic in Cuba, called rhopalurusjunceus "escozul". Open Cancer J. 5: 1-2.

Di-Santo, E., C. Meazza, M. Sironi, P. Fruscells, A. Mantovani, J.D. Sipe, and P. Ghezzi (1997). IL-13 Inhibits TNF production but potentiates that of IL-6 in vivo and ex vivo in mice. J. Immonol. 159: 379-382.

Eric, G.B., and J.L. Lawrence (1996). Rheumatoid Arthritis and its Therapy. The Text Book of Therapeutics Drug and Disease Management. 16th Ed, Williams & Wilkins Company, Blatimore. 579-595p.

Fabiano, G., A. Pezzolla, M.A. Filograna, and F. Ferrarese (2008). Traumatic shockphysiopathologic aspects. G. Chir. 29: 51-57.

Fialho, E.M.S., M.C.G. Maciel, A.C.B. Silva, A.S. Reis, A.K.M. Assunçao, T.S. Fortes, L.A. Silva, R.N.M. Guerra, F.H. Kwasniewski, and F.R.F. Nascimento (2011). Immune cells recruitment and activation by *Tityus serrulatus* scorpion venom. Toxicon. 58: 480-485.

Fisher, J.C.J. and Y. Zheng (1996). Potential strategies for inflammatory mediator manipulation: retrospect and prospect. World J. Surg. 20: 447-53.

Fiske, J.L., V.P. Fomin, M.L. Brown, R.L. Duncan, and R.A. Sikes (2006). Voltage sensitive ion channels in cancer. Cancer Metastasis Rev. 25: 439-500.

Fukuhara, Y.D.M., M.L. Reis, R. Dellalibera-Joviliano, F.Q.C. Cunha, and E.A. Donadi (2003). Increased plasma levels of IL-1β, IL-6, IL-8, IL-10 and TNF-α in patients moderately or severely envenomed by *Tityus serrulatus* scorpion sting. Toxicon. 41: 49-55.

Gallin, J.I. (1989). Fundamental Immunology. 2nd Ed. Raven press, New York. 721-33 p.

Garcia, C., E.S. Calderon-Aranda, G.A. Anguiano, B. Becerril, and L.D. Possani (2003). Analysis of the immune response induced by a scorpion venom sub-fraction, a pure peptide and a recombinant peptide, against toxin Cn2 of *Centruroides noxius* Hoffmann. Toxicon 41: 417-427.

Gay, J., E. Kokkotou, M. O-Brien, C. Pothoulakis, and K.P. Karalis (2006). Interleukin-6 genetic ablation protects from trinitrobenzene sulfonic acid-induced colitis in mice: putative effect of anti-inflammatory cytokines. Neuroimmuno. Modulation 13: 114-121.

Gerard, C., C. Bruyns, A. Marchant, D. Abramowicz, P. Vandenabeele, A. Delvaux, W. Fiers, M. Goldman, and T. Velu (1993). Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J. Exp. Med. 177: 547-550.

Goldman, D., Y. Cho, M.L. Zhao, A. Casadevall, and S.C. Lee (1996). Expression of inducible nitric oxide synthase in rat pulmonary Cryptococcus

neoformansgranulomas. Am. J. Pathol. 148: 1275-1282.

Gomes, A., P. Bhattacharjee, R. Mishra, A.K. Biswas, S.C. Dasgupta, and B. Giri (2010). Anticancer potential of animal venoms and toxins. Indian, J. Exp. Biol. 48: 93-103.

Gomez-Varela, D., E. Zwick-Wallasch, H. Knotgen, A. Sanchez, T. Hettmann, D. Ossipov, R. Weseloh, C. Contreras-Jurado, M. Rothe, W. Stuhmer, and L.A. Pardo (2007). Monoclonal antibody blockade of the human Eag1 potassium channel function exerts antitumor activity. Cancer Res. 67: 7343-7349.

Gwee., M.C.E., S. Nirthanan, H.E. Khoo, P. Gopalakrishnakone, R.M. Kini, and L.S. Cheah (2002). Autonomic effects of some scorpion venoms and toxins. Clin. Exp. Pharmacol. Physiol. 29: 795-801.

Heller, N.M., X. Qi, I.S. Junttila, K.A. Shirey, S.N. Vogel, W.E. Paul, and A.D. Keegan (2008). Type I IL-4Rs selectively activate IRS-2 to induce target gene expression in macrophages. Sci. Signal. 1: 1-7.

Hoang, A.N., D.M.V. Hoang, P.V. Nguyen, S.K. Kseniya, V.N. Oksana, V.F. Alexey, P.K. Mikhail, V.A. Tatyana, V.S. Mariana, I.T. Victor, and N.U. Yuri (2014). Vietnamese *Heterometrus laoticus* scorpion venom: evidence for analgesic and anti-inflammatory activity and isolation of new polypeptide toxin acting on Kv1.3 potassium channel. Toxicon. 77: 40-48.

Incesu, Z., F. Caliskan, and Zeytinoglu (2005). Cytotoxic and geltinolytic activities of *Mesobuthus gibbosus* (Brulle, 1832) venom. *Rev.* CENIC, Cienc. Biol. 36: 1-7.

Jalali, M.R., M.T. Jalali, and Z. Mapar (2015). Evaluation of plasma cytokine levels in *Mesobuthus eupeus* (Scorpionida: Buthidae) scorpion envenomation in rats treated with polyvalent antivenom. Jundishapur J. Health Sci. 7: 2715.

Jhon, N.A.A., and G. Shobana (2012). Anti-inflammatory activity of *Talinum fruticosum* L. on formalin induced paw edema in albino rats. J. App. Pharm. Sci. 2: 123-127.

Joseph, B. and J. Jeorge (2012). Scorpion toxins and its applications. Int. J. Toxicol. Pharmacol. Res. 4: 557-61.

Kalpana, S., J. Patil, and M.A.G. Phcog (2007). Effect of celastruspaniculatus wild seed on adjuvant induced arthritis in rats. Pharmacognosy Magazine. 3: 177-181.

Lee, D.U., S. Agarwal, and A. Rio (2002). Th2 lineage commitment and efficient IL-4 production involves extended demethylation of IL-4 gene. Immunity 16: 649-660.

Lentsch, A.B., B.J. Czermak, J.A. Jordan, and P.A. Ward (1999). Regulation of acute lung inflammatory injury by endogenous IL-13. J. Immunol.162: 1071-1076.

Maciel, M.C.G., E.M.S. Failho, R.N.M. Guerra, V.M. Borges, F.H. Kwasniewski, and F.R.V. Nascimento (2014). *Tityus serrulatus* scorpion venom improves survival and lung inflammation in lethal sepsis induced by CLP in mice. Toxicon 89: 1-8.

Magalheaes, M.M., M.E.S. Pereira, C.F.S. Amaral, N.A. Rezende, D. Campolina, F. Bucaretchi, R.T. Gazzinelli, and Jr. Cunha-Melo (1999). Serum levels of cytokines in patients envenomed by *Tityus serrulatus* scorpion sting. Toxicon 37: 1155-1164.

Mahat, M.A. and B.M. Patil (2007). Evaluation of anti-inflammatory activity of methanol extract of *Phyllanthus amarus*in experimental animal models. Ind. J. Pharmaceut. Sci. 69: 33-36.

Malefyt, D., J. Abrams, B. Bennett, C.G. Figdor, and J.E. De-Vries (1991). Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174: 1209-1220.

Malefyt, D., C.G. Figdor, R. Huijbens, S. Mohan-Peterson, B. Bennett, J. Culpepper, W. Dang, G. Zurawski, and J.E. De-Vries (1993). Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes: comparison with IL-4 and modulation by IFN-gamma or IL-10. J. Immunol. 151: 6370-6381.

Mijatovic, T., V. Kruys, D. Caput, P. Defrance, and G. Huez (1997). Interleukin-4 and -13 inhibit tumor necrosis factor-alpha mRNA translational activation in lipopolysaccharide-induced mouse macrophages. J. Biol. Chem. 272: 14394-14398.

Moore, K.W., R. De-Waal-Malefyt, R.L. Coffman, and A. O'Garra (2001). Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19: 683-765.

Mosmann, TR., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman (2005). Two types of murine helper T cell clone. I. definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 175: 5-14.

Muchamuel, T., S. Menon, P. Pisacane, M.C. Howard, and D.A. Cockayne (1997). IL-13 protects mice from lipopolysaccharide-induced lethal endotoxemia: correlation with down-modulation of TNF-alpha, IFN-gamma, and IL-12 production. J. Immunol. 158: 2898-903.

Mulligan, M.S., M.L. Jones, M.A. Bolanowski, M.P. Baganoff, C.L. Deppeler, D.M. Meyers, U.S. Ryan, and P.A. Ward (1997). Inhibition of lung

inflammatory reactions in rats by an anti-human IL-8 antibody. J. Immunol. 150: 5585-5595.

Nisani, Z., S.G. Dunbar, and W.K. Hayes (2007). Cost of venom regeneration in *Parabuthus transvaalicus* (Arachnida: Buthidae). Compara. Biochem. Physiol. 147: 509-513.

Ozkan, O. and A. Filazi (2004). The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different methods from scorpions, *Androctonus crassicauda* (Olivier 1807). Acta Parasitol. Turcica 28: 50-53.

Pessini, A. C., A. M. Souzam, L. H. Faccioli, O. Z. M. Gregorio, and E.C. Arantes (2003). Time course of acute-phase response induced by *Tityus serrulatus* venom and TsTX-I in mice. Int. Immunopharmacol. 3: 765-774.

Petricevich, V. L. (2004). Cytokine and nitric oxide production following severe envenomation. Curr. Drug Targets Inflamm. Allergy. 3: 325-332.

Petricevich, V. L. (2006). Balance between pro- and anti-inflammatory cytokines in mice treated with *Centruroides noxius*scorpion venom. Mediat. Inflamm. 6: 54273.

Petricevich, V. L., and C.F. Pena (2002). The dynamics of cytokine d nitric oxide secretion in mice injected with *Tityus serrulatus* scorpion venom. Mediat. Inflam. 11: 173-180.

Petricevich V.L., H.A. Cruz, F.I.V. Coronas, L.D. Possani (2007). Toxin gamma from *Tityus serrulatus* scorpion venom plays an essential role in immunomodulation of macrophages. Toxicon 50: 666–675.

Pillozzi, S., M. Masselli, E. De Lorenzo, E. Cilia, O. Crociani, A. Amedei, B. Accordi, M. Veltroni, G. Basso, D. Campana, A. Becchetti, and A. Arcangeli (2009). Overcoming chemotherapy resistance in childhood acute lymphoblastic leukemia by targeting ion channels. Blood, 114: 3085.

Rached, I.C., F.M. Castro, L.M. Guzzo, and S.B.V. de-Mello (2010). Anti-inflammatory effect of bee venom on antigen-induced arthritis in rabbits: Influence of endogenous glucocorticoids. J. Ethno. Pharmacol. 130: 175-178.

Rogler, G. and T. Andus (1998) Cytokines in inflammatory bowel disease. World J. Surg. 22: 382-389.

Romagnani, S. (1994). Lymphokine production by human T cells in disease states. Ann. Rev. Immunol. 12: 227-257.

Salarian, A.A., A. Jalali, A.Z. Mirakabadi, H. Vatanpour, and F.H. Shairazi (2012). Cytotoxic effects of two Iranian scorpions *Odontobuthus doriae* and *Buthus saulcyi* on five human cell lines and

fractions of toxic venom. J. Iranian Pharmachol. Res. 11: 357-367.

Schumacher, J.H., A. O-Garra, B. Shrader, A. Van-Kimmenade, M.W. Bond, T.R. Mosmann, R.L. Coffman (1988). The characterization of four monoclonal antibodies specific for mouse IL-5 and development ofmouse and human IL-5 enzyme-linked immunosorbent. J. Immunol. 141: 1576-1581.

Standiford, T.J., R.M. Strieter, S.W. Chensue, J. Westwick, K. Kasahara, and S.L. Kunkel (1990). IL-4 inhibits the expression of IL-8 from stimulated human monocytes. J. Immunol. 145**:** 1435-1439.

Suleyman, H., B. Demircan, Y. Karagoz (2007). Anti-inflammatory and side effects of cyclo-oxygenase inhibitors. Pharmacol. Rep. 59: 247-258.

Van-Der-Poll, T., A. Marchant, W.A. Buurman, L. Berman, C.V. Keogh, D.D. Lazarus, L. Nguyen, M. Goldman, L.L. Moldawer, and S.F. Lowry (1995). Endogenous IL-10 protects mice from death during septic peritonitis. J. Immunol. 155: 5397-5401.

Veiseh, O., C. Sun, C. Fang, N. Bhattarai, J. Gunn, F. Kievit, K. Du, B. Pullar, D. Lee, R.G. Ellenbogen, J. Olson, and M. Zhang (2009). Specific targeting of brain tumors with an optical/magnetic resonance imaging nanoprobe across the blood-brain barrier. Cancer Res. 69**:** 6200-6207.

Wellehan, J.F.X. and C.L. Gunkel (2004). Emergent diseases in reptiles. Seminars in Avian and Exotic Pet Medicine 13: 160-174.

Wills, A.L. (1960). Release of histamin, kinin and prostaglandins during carrageenin induced inflammation of the rats. In: Prostaglandins, Peptides and Amins, P. Montagazza and E. W. Horton, (Eds.). Academic Press, London, UK. pp. 31–48.

World Health Organization (1981). Progress in the characterization of venoms and standardization of antivenoms. WHO offset Publication, Geneva. 58: 21-28.

Xing, Z., J. Gauldie, G. Cox, H. Baumann, H. Jordana, X.F. Lei, and M.K. Achong (1998). IL-6 is an anti-inflammatory cytokine required for controlling local or systemic acute inflammatory responses. J. Clin. Invest. 101: 311-320.

Yokoyama, Y., W.C. Kitchens, B. Toth, M.G. Schwacha, L.W. Rue, K.L. Bland, and I.H. Chaudry (2004). Role of IL-10 in regulating proinflammatory cytokine release by Kupffer cells following trauma-hemorrhage. Am. J. Physiol. Gastrointest. Liver Physiol. 286: 942-946.

Zhang, Y.Y., L.C. Wu, Z.P. Wang, Z.X. Wang, Q. Jia, G.S. Jiang, and W.D. Zhang WD (2009). Anti-proliferation effect of polypeptide extracted from scorpion venom on human prostate cancer cells in vitro. Clin. Med. Res. 1: 24-31.

Zhao, R., X.Y. Zhang, J. Yang, C.C. Weng, L.L. Jiang, and J.W. Zhang (2008). Anticonvulsant effect of BmK IT2, a sodium channel-specific neurotoxin, in rat models of epilepsy. Br. J. Pharmacol. 154: 1116-1124.

Zlotkin, E., F. Miranda, and H. Rochat (1978). Chemistry and pharmacology of Buthinae scorpion venoms. In: Handbook of Experimental Physiology Bettini, S. (eds.). Berlin: Spinger-Verlag. pp 317-329.

Zoccal, K.F., C.S. Bitencourt, A. Secatto, C.A. Sorgi, K.C.F. Bordon, S.V. Sampaio, E.C. Arantes, and L.H. Faccioli (2011). *Tityus serrulatus* venom and toxins Ts1, Ts2 and Ts6 induce macrophage activation and production of immune mediators. Toxicon 57: 1101-1108.

Zoccal, K., C.S. Bitencourt, C.A. Sorgi, K.C.F. Bordon, S.V. Sampaio, E.C. Arantes, and L.H. Faccioli (2013). Ts6 and Ts2 from *Tityus serrulatus* venom induce inflammation by mechanisms dependent on lipid mediators and cytokine production. Toxicon 61: 1-10.

Zurawski, G. and J.E. De-Vries (1994). Interlukin 13 and interleukin 4-like cytokine that act on monocytes and B cells, but not on T cells. Immunol. Today 15: 19-26.