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The protective effects and ameliorative potency of the haemolymph from the Saudi scorpion *Androctonus crassicauda* against the oxidative stress induced by its crude venom: A pharmacological study

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Abstract:

Background: Envenomation from dangerous scorpions remains a horrible threat in many parts of the globe, especially the developed countries, reflecting a reliable cause of a lot of mortalities and morbidities for both children and adolescents; as the annual number of scorpion stings exceeds 1,200,000 resulting in approximately 3250 deaths. In Saudi Arabia, scorpions constitute an acute major medical problem with about 15, 000 average stung, yearly. The Saudi government has given high priority to the development of health care services to monitor scorpionism carefully, to manage its treatment protocols successfully, and to explain the pathophysiological effects of the venom. A wide variety to treat scorpionism was used, either singly or in combination. Mostly, treatment modalities include antivenom immunoglobulin (SAV) and chemical antidotes, with varying degrees of effectiveness and side effects, though the cons and the so expensive wholesale cost associated with SAV treatment. We are in bad need and warranty challenged to obtain safer, more effective, and not economically burdensome bioactive antivenins. Fourteen medically important scorpion species belonging to *Buthidae* have been identified in KSA. Although *Androctonus crassicauda* (*A. c.*) is an endemic and highly venomous scorpion in KSA, few studies were dealing with it. **Objective:** To evaluate the possible involvement of cellular oxidative reactions of the crude venom of the Saudi scorpion *A. c.*, as the main cause of multiple organ dysfunctions, and how far scorpion haemolymph (SH) could be effective to protect and/or treat the envenomation-associated metabolic disorders. **Materials and methods:** Twenty-four adult male albino mice (25-32 g) were randomly divided into four groups; six in each. The controls; injected subcutaneously (SC) with 0.01 ml NaCl 0.9%/ kg BW, envenomed animals; receiving a single dose of crude venom of the Saudi scorpion *A. c.* (0.12 mg/kg BW; SC) and tested two hours post-injection, envenomed animals treated within 5 min. with an SC dose of 0.12 mg of SH/kg BW, and envenomed animals treated with the same dose and route with SH, two hours before scorpionism. Animals were dissected and different biochemical parameters and oxidative stress biomarkers were measured in serum, liver, and brain tissues. **Results:** Scorpion envenomation was accompanied by oxidative damage and hyperglycemia; which is causative for a generation of additional

reactive oxygen species, and its subsequent metabolic disorders. Also, SH was predicted to significantly protect from and reverse all the cytotoxic manifestations following envenomation. **Conclusion**: The cytotoxic effects of crude venom of the Saudi scorpion *A. crassicauda* could be attributed to the generation of reactive oxygen species causing oxidative damage. The scorpion haemolymph can be used as a bioactive therapeutic agent to protect and treat the dysfunctions after envenomation, as it may contain novel molecule (s) to do this.

Key Words: Scorpionism, *Androctonus crassicauda*, Scorpion venom, Scorpion haemolymph, antivenins, Oxidative stress, antioxidants

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1. Introduction:

The scorpion (Order: *Scorpiones*) is one of the oldest known creatures that existed as the most ancient and species-rich terrestrial animals on the earth (Ding et al., 2014 & Al Asmari and Khan, 2016). Order Scorpiones is a distinctive group of class *Arachnida*; representing only 1.5 % of its members and including its oldest fossils, within the phylum *Arthropoda*, and is dating back to the Silurian period (almost 433–438 mya) (Dunlop and Selden, 2013; Waddington et al., 2015; Santibáñez-López et al., 2016 & Hosseini et al., 2017). Scorpions are a largely nocturnal secretive group of animals widely distributed worldwide; in tropical, subtropical, and desert habitats; generally south of 45˚ N latitude with increased diversity in tropical regions at altitudes between 23 and 38 degrees (Polis, 1990 & Bowles and Swaby, 2006). Although most scorpions are not aggressive and stinging incidences usually occur accidentally (Bowles and Swaby, 2006), they have developed a negative reputation due to their stings and envenomation, where they pose a serious health hazard due to their venoms (Uawonggul et al., 2006; Al Asmari et al., 2012; 2015; 2016 & 2017) in many underdeveloped regions (Kshirsagar et al., 2012 & Khatony et al., 2015).

One undeniable fact is that all scorpions are venomous and scorpion venom is the cause of widespread fear in the globe (Frembgen, 2004 & Morrow, 2017), especially in developing countries. Fortunately, only very few are dangerous to humans (Mansour, 2001 & Alkahlout, et al., 2015), where out of 1500 scorpion species about 50 are medically important. *Buthidae* is not only the largest family among scorpion lineages, but is the one that includes all the medically significant and the most potentially lethal species primarily distributed in Africa and Southeast Asia, as well (Goyffon and El Ayeb, 2002; Soleglad et al., 2003; Prendini and Wheeler, 2005; Bosmans and Tytgat, 2007; Chippaux and Goyffon, 2008; Caliskan et al., 2013; Quintero-Hernándeza et al., 2013; Sunagar et al., 2013; Hmed et al., 2013 & Al Asmari et al., 2016). Members of this family show extreme potency of their venom and the relatively smaller pincers; particularly pronounced in the fat-tailed scorpions' genera; *Androctonus* and *Parabuthus*, so they heavily rely on their venom arsenal for predation (Sunagar et al., 2013).

Scorpionism is a medical, social, and economic problem (Telmisany and Othman, 1993) that represents a real threat in many parts of the world (Jarrar and Al-Rowaily, 2008; Mohamad et al., 2014 & Selmane et al., 2017), including Saudi Arabia (Fatani et al., 2006; 2010 & Al Asmari et al., 2017). Bawaskar and Bawaskar (2012) and Jalalia and Rahim (2014) reported that stings by venomous

animals; especially scorpions are a concern to health authorities in many Middle East countries, because of the severity, extent, and wide range of clinical effects. Due to its scattered geographical distribution, the at-risk population exceeds 2.5 billion people (Al Asmari et al., 2008). Scorpion stings (Ss) are reported to occur by an average of approximately 1.2 million/year, leading to more than 3250 deaths with an annual mortality rate of about 3‰. Scorpions exceed snakes in their toxicity. For every person killed by a poisonous snake, 10 are killed by a poisonous scorpion (Chippaux and Goyffon, 2008; Bawaskar and Bawaskar, 2012; Quintero-Hernández et al., 2013; Yılmaz et al., 2013; Adi-Bessalem et al., 2014; Ding et al., 2014 & Ebrahimi et al., 2017). Thus scorpion sting, and the resulting envenomation, constitutes a real predicament for healthcare professionals (Freire-Maia et al., 1994 & Daoudi et al., 2017).

In Saudi Arabia, scorpions are widely scattered. The Saudi fauna comprises at least 28 species and subspecies belonging to *Buthidae*, *Scorpionidae*, and *Hemiscorpiidae* families (Desouky and Al-Shammari, 2011 & Al Asmari et al., 2013). Different fatal species; as *Androctonus*, are well identified (Al Asmari et al., 2007, 2009a & 2009b). Scorpion stings are common in this country due to their climate, socioeconomic structure, and social habits. An average exceeding 18,500 annual stings is recorded in the different regions of Saudi Arabia (Desouky and Al-Shammari, 2011). Ss cases are a frequent presentation to emergency departments (EDs) in many areas and are encountered daily, in certain regions (Alkahlout et al., 2015). Young children and elderly people are particularly at high risk (Telmisany and Othman, 1993). The most vulnerable people include the shepherds who tend the livestock in the desert, farmers, and the people who spend their weekends in the desert for recreation. Scorpion stings cause a wide range of conditions, from severe local skin reactions, like pain, swelling, and hypertension; that can be treated with analgesics and/or antihistamines, to neurologic, respiratory, and cardiovascular collapse and other systemic manifestations/ supportive care, and coma (Al Sadoon and Jarrar, 2003; Chippaux and Goyffon, 2010; Jalali et al., 2011; Azzam, 2012 & Yılmaz et al., 2013). The synergistic impact of cardiac, respiratory, autonomic, and metabolic abnormalities in scorpion sting patients may lead to multisystem failure and death (Murthy et al., 1991; Yugandhar et al., 1999; Uawonggul et al., 2006; Bawaskar and Bawaskar, 2007 & Al–Asmari et al., 2017). The clinical picture and response to scorpion envenomation vary depending on many factors; as the scorpion species, scorpion size, number of stings, the bulk of the venom glands, the status of the venom canals of the telson, the amount of venom injected, the age of the offender, the site and depth of sting penetration, a quantity of the injected venom, and the proportion of the venom reaching the circulatory system, victim's age, weight, general health physiology status, genetics, and emotional conditions, the interval between envenomation and hospitalization, the anatomical location of the sting and the season (time of sting) (Krishnan et al., 2007; Boşnak et al., 2009; Karnad, 2009; Uluğ et al., 2012; Azzam; 2012; Kassiri et al., 2014; Khatony et al., 2015 & Ebrahimi et al., 2017). The symptoms of a sting start immediately within few minutes and usually progress to a maximum severity (death of the victim) within 5-7 hours, depending on the scorpion species (Mebs, 2002; Zargan et al., 2003; Petricevich, 2010 & Azzam, 2012).

The crude venom of a scorpion, produced by the columnar cells of the venom gland (Al–Asmari et al., 2016), is a water-soluble antigenic and heterogeneous complex multi-component mixture (Bosnak et al., 2009) of mostly small molecular weight peptides and small proteins that have evolved for prey capture and/or defense (Dutertre and Lewis, 2010) and can impair the cell function by interfering with ion channels permeability of the excitable cell membranes (Allen, 1985). Scorpion venoms consist of hundreds of different pharmacologically active components including variable combinations of proteins; usually called toxins, of 1,000–9,000 Da in size (Omran, 2003; Cao et al., 2006; Dutertre and Lewis, 2010 & Zhu et al., 2011). Toxins of a scorpion species are almost similar in structural and functional characteristics (Cao et al., 2014). It includes insoluble, generally non-toxic, fraction and a soluble fraction containing toxic peptides that exhibit activity on ion channels, in addition to mucopolysaccharides, nucleotides, vasoactive amines (serotonin or histamine), protease inhibitors, and enzymes (Gazarian et al., 2005; de la Vega et al., 2010). Additionally, scorpion venom constituents include water, lipids, mucosa, ions, low molecular weight peptides, metals, mucoproteins, hyaluronidase, phospholipase, biogenic amines, glycosaminoglycans, phosphodiesterases, cytokine-releasers, and relatively low molecular mass molecules, like serotonin, histamines, tryptophans, polynucleotides, protease inhibitors, and histamine-releasers, small organic molecules; as polyamines, amino acids, and neurotransmitters, salts, non-toxic proteins, bioactive amines are and many unidentified substances (Mahadevan, 2000; Gwee et al., 2002; Dittrich et al., 2002; Inceoglu et al., 2003; Sollod et al., 2005; Gazarian et al., 2005; Olivera and Teichert, 2007; Fox and Serrano, 2008 & Fry et al. 2009), that exert acute toxicological effects in humans (Bawaskar and Bawaskar, 2012; Venancio et al., 2013; Ding et al, 2014 & Al Asmari et al., 2016). In general, the toxic activity of scorpion venom is predominantly attributed to the presence of peptides that disrupt Na^+, K^+, Ca^{2+}, and Cl^- channels in neuronal cells (Possani et al., 2000 & Venancio et al., 2013). Apart from these peptides, several molecules that play a role in scorpion poisoning or that exhibit properties of biotechnological interest are also present in scorpion venoms (Wu et al., 2010; Zeng et al., 2012; Zhao et al., 2011 & Venancio et al., 2013). The main constituents of the venom are neurotoxins; chemicals affecting the nervous system, ultimately killing or paralyzing their prey (Tan et al., 2006 & Francielle et al., 2015).

Androctonus crassicauda; one of the five most dangerous scorpions in the world (Al-Ramahi and Al-Hasnawi, 2002), inhabits KSA especially Tehamah, Al-Baha, Ha' il and Al-Riyadh regions (Jarrar and Al-Rowaily, 2008 & Al Asmari et al., 2013). The venom of this species is a neurotoxin; which is fast-acting and can be absorbed very quickly, because of its small molecular weight proteins, resulting in fatal or paralytic effects (Al-Ramahi and Al-Hasnawi, 2002; Ozkan and Filazi, 2004 & Wikipedia, 2018). They are especially acting on the nerves responsible for respiration, leading ultimately to death by respiratory failure (Wikipedia, 2018). The symptoms of the sting start immediately within few minutes after stinging and usually progress to maximum severity within 5 hours (Mebs, 2002).

A wide variety of treatments to scorpion envenomation were used either singly or in combination. No one treatment or combination of treatments showed any clear superiority (Curry et

al., 1984 & Azzam, 2012). Historically, the main modes of therapy for scorpion stings were supportive treatment and immunotherapy in the form of antivenom (Rodrigo and Gnanathasan, 2017). Scorpion sting treatment employs various strategies including the use of specific medicines such as antiserum, especially for patients with severe symptoms (Carmo et al., 2015). All of the used antivenoms are animal-derived antisera of equine origin. Although effective in neutralizing scorpion venoms, these animal-derived antisera are draw backed due to the heterologous nature of their protein contents, which may elicit both early and late adverse reactions in human recipients (Chippaux, 2012 &.Rodríguez, 2015). Additionally, only a subset of the antibody/antibody fragments present in these antivenoms have a therapeutic value since the presence of non-toxic immunogens in the venoms used for immunization may elicit therapeutically irrelevant antibodies in the immunized animal (Pucca et al., 2011 & Laustsen et al., 2016). Also, as these antivinines are derived from animal serum, individual differences in the immune responses of the production animals may give rise to batch-to-batch variation (Rodríguez-Rodríguez et al., 2016). Finally, due to the very minute amounts of venom that can be extracted from scorpions, the production of antisera against Ss is dependent on a highly laborious venom collection process, where large numbers of scorpions are needed to be milked to procure enough venom for immunization (Meadows and Russell, 1970 & Laustsen et al., 2016). Thus making antivenom (AV) is a complicated and so prohibitively expensive process (Danny, 2015). Also, AV is generally in short supply in most parts of Africa and many parts of Asia (William, 2015 & Shrestha, 2017). These challenges warrant technological innovation, not only to obtain safer, cheaper, and more effective AVs with therapeutic impact (Azzam, 2012) but also to establish more sustainable productions processes that are independent of both venoms and animals (Rodríguez, 2015 & Laustsen et al., 2016) with evaluation on their cost-competitiveness (Laustsen et al., 2016).

The present work was to investigate the possible involvement of cellular oxidative reactions in envenomation-induced changes by the crude venom of the Saudi scorpion *Androctonus crassicauda*; which were estimated to be the main concerned metabolic disorders in the brain and liver tissues of the victim and to evaluate how far the scorpion haemolymph could be effective in protecting and/or ameliorating the cytotoxic changes due to scorpionism in the examined tissues and thus in restoring the envenomation associated metabolic abnormalities to be more or less within the normal values.

2. Materials and Methods:

2.1. The Experimental Animals:

Approval of this study was obtained from the animal use and care committee at the (65cm × 25cm × 15 cm) home cages, with sawdust-covered floors, the standard conditions of controlled humidity (70-80 %), temperature (25 ± 5°C) and light (12 hrs. darkness/ light cycle) and the conventional animal facilities of the lab. Healthy male Swiss Webster albino mice, weighing 20-25g, and, were used. Each cage contains six animals. They had access to stable food (mice chow) and water ad-libitum.

2.2. Scorpions and Venom collection and preparation:

Forty of *the Androctonus crassicauda* (*A.c.*) scorpions were collected by using ultraviolet (UV) lamps during the no-moon nights from the wild in

Tehamah (Assir region; KSA) by professional hunters, specifically recruited for this job. Scorpions were brought to the laboratory in tightly closed plastic bottles. The scorpion house was designed so that each scorpion batch was housed in larger plastic containers, which were placed in rows on shelves (Al Asmari et al., 2007; 2009a & 2009b). Avoiding scorpion cannibalism, scorpions were singly kept in separate plastic boxes (6×6×10 inch) (L×W×H) on top of a soil bed of about 2 inches in depth, above which proper size stones, mimicking their natural habitat. Lids of boxes were designed to provide proper aeration and water-sprayed periodically to maintain humidity. Scorpions were fed on mealworms (Al Asmari et al., 2009a), once weekly (de Roodt et al., 2010); as they do not require food frequently due to their low metabolic rate (Neubauer, 2013 & Yaqoob et al., 2016). Scorpions in the laboratory were stored for one month and water was given to them through small humid cotton rolls, soaked in water, and supplied for each animal regularly (Moghadam et al., 2013 & Yaqoob et al., 2016).

Venom was extracted two weeks after the SH collection, not on the day immediately after feeding, and also no food is added until three days after the venom extraction (Whittemore et al., 1963 & Candido and Lucas, 2004), by the electrical stimulation method explained by Abdel-Rahman (Abdel-Rahman, 2013); as the major components of venom collected by this method are toxins (Bücherl and Buckley, 1971). The venom was dissolved with sterile double-distilled water and centrifuged at 15,000 rpm for 15 min at 4°C (Özkan and Filazi 2004). The precipitate was discarded and the supernatant was stored at −20 °C until use. When required, the venom was reconstituted by adding 0.9% sodium chloride solution (physiological saline solution; PSS). It was used for cytotoxic assays at a dose equals 1/5 LD_{50}, subcutaneously (SC); to simulate the natural route of envenomation, and all envenomed mice were sacrificed two hours post-injection to ensure absorption and common cement of venom's deleterious actions (Fatani et al., 2010; Azzam, 2012 & Azzam et al., 2013).

2.3. Determination of Median Lethal Dose (LD_{50}):

The median lethal dose; MLD, of *A. crassicauda* crude venom, was determined according to the method of Meier and Theakston (Meier and Theakston, 1986). Different known concentrations of the venom solution dissolved in a PSS were prepared. Ten weighted mice were treated subcutaneously (SC) with different doses (D) of scorpion venom; as the SC route provides the highest LD_{50} value and is the most frequent route of accidental scorpion envenomation (Krifi et al., 1999 & Özkan et al., 2007). Accordingly, the approximate SC LD_{50} of *A. crassicauda* crude venom was calculated to be 0.60 ± 0.05 mg/kg.

2.4. Haemolymph collection and preparation:

To collect the haemolymph, a scorpion was starved five days prior to the collection of haemolymph (Geethabali and Rajashekhar, 1988) and treated according to Azzam (2012). After resting and feeding, the animal can be used again (Geethabal and Rajashekhar, 1988), either for more haemolymph or for venom production. The haemolymph was centrifuged at 3000 r.p.m. for 20 min to avoid coagulation. The supernatant was lyophilized and stored at −20°C until use. The used dose equals 0.05 mg/kg dissolved in a PSS. Immediately before usage 1/5 LD_{50} of *A. crassicauda crude* venom solution dissolved in saline were incubated 10 min. at 37˚C with the SH solution at a ratio of 2:1 (SH: SV, v:v). In treated

animals in all groups, an injection was applied 5 min. after envenomation and half an hour before dissection (Azzam, 2012 & Azzam et al., 2013).

2.5. Cytotoxic Assays:

2.5.1. Study Design:

The experimental animals were adapted to the lab. environment for one week, then they were randomly divided into the experimental groups (n=6). 24 male Swiss Webster albino mice, weighing 20-25g, were allocated into four main groups:

1- The control (C) group: given a SC injection of the PSS (0.9% NaCl); 0.01 ml / kg B.W.
2- Envenomed (E) animals; received a single SC dose of 1/5 LD_{50} of the *A. crassicauda* crude venom (0.12 mg/kg B. W.) and sacrificed for cytotoxic assays two hours post-injection of crude venom.
3- Envenomed animals protected with SH (EHP); treated with SH two one week earlier before injection with a single SC dose of 0.05 mg of the *A. crassicauda* crude venom/ kg B. W. of the experimental animal, and the examined tissues were used for biochemical measurements two hours after envenomation, as discussed earlier.
4- Envenomed animals treated with SH (EHT); injected with SH after 5 min. of envenomation and then dissected after two hours for bioassays, as mentioned before.

At the end of each experimental period, animals were sacrificed by decapitation, using a sharp razor blade. Blood and tissue samples were treated and prepared for the biochemical assays as explained by Azzam (2012).

The following biochemical parameters were measured in the control and treated groups, in serum, liver, and brain tissues:

2.5.2. Serum Glucose (SG) Assay:

SG was estimated by using the kit obtained from Biodiagnostic Company for Laboratory Services, referenced to the method of Trinder (1969).

2.5.3. Oxidative Stress Biomarkers:

Protein carbonyl content (PCOC) was determined according to the method of Reznick and Packer (1994) and Levine et al. (1994) while nitric oxide (NO) concentration was investigated according to Green et al. (1982) method. The Biodiagnostic Company for Laboratory Services kit; applying the techniques of Satoh (1978) and Ohkawa et al. (1979) was used to determine the lipid peroxidation end product; malondialdehyde (MDA).

2.5.4. Enzymatic and Non-Enzymatic Antioxidants Assays:

Assayment of Cu/Zn superoxide dismutase (SOD) activity was as per the procedures of Winterbourne et al. (1974) and Maral et al. (1977). The reduced glutathione (GSH) was estimated using the method adopted by Beutler et al. (1963). According to the techniques used in the kits of Biodiagnostic Company for Laboratory Services all the following parameters were estimated; glutathione peroxidase (GPx) (Paglia and Valentine,1967), glutathione reductase (GSH-R) (Goldberg and Spooner,1983), glutathione-s-transferase (GST) (Habig et al.,1974), Liver catalase (CAT) (Aebi,1974 & 1984) activities, hydrogen peroxide (H_2O_2) content (Fossati et al.,1980 & Aebi, 1984) and finally the total antioxidant capacity (TAC) (Koracevic et al., 2001).

2.6. Statistical Analysis:

Raw data were statistically analyzed by using SPSS software (Statistical Package for Social Science, version 17.01, Illinois, USA) (Dancey and

Reidy, 2002). Tabulation and graphics of data were done using Microsoft Excel 2007. According to the mathematical principles described by Field, all of the data of control and treated groups were expresses as mean value ± standard error (Field, 2000). One-way analysis of variance (ANOVA) was carried out followed by *post hoc* Duncan's test (Bewick et al., 2004); for comparison between control and treated groups. The probability criterion for significance for each statistical test was $P \leq 0.05$.

3. Results:

The results of this work; presented in tables "1, 2, and 3", indicated that a single SC injection of 0.12 mg of the crude venom of *A.c.*/kg B.W. in the experimental animals causes marked significant changes in the biochemical parameters in the serum and tested organs of the envenomed group, in comparison with the control values, at $P \leq 0.05$. Serum glucose (SG) concentration, malondialdehyde content in brain and liver (bMDA & lMDA), brain and liver protein carbonyl contents (bPCOC & lPCOC), nitric oxide in serum and the examined tissues (sNO, bNO & lNO), glutathione-s-transferase activity in brain and liver tissues (bGST & lGST) were significantly elevated post venom treatment in comparison with the control values. Otherwise, significantly declined brain and liver reduced glutathione (bGSH & lGSH) level, activities of liver catalase (lCAT) and superoxide dismutase activity in serum, brain and liver (sSOD, bSOD & lSOD), brain and liver glutathione peroxidase (bGPx & lGPx) and tissues glutathione reductase (bGSH-R & lGSH-R) hydrogen peroxide content in serum, brain and liver (sH_2O_2, bH_2O_2 & lH_2O_2) were noticed with consequently sharp and significant decremented total antioxidant capacity (TAC) (tables "1"-"3" & figures "1"-"12").

Pre and post-treatment of the scorpion-envenomed animals with the desired dose of the SH as mentioned earlier; has been predicted to prevent and restore all the metabolic disturbances and significantly ameliorate them to be more or less within the normal values; as cleared from the investigated data.

Applying one-way ANOVA revealed that the difference between treated and control groups for the increased SG to be very highly significant ($F_{6,6}$ = 119.682, $P \leq 0.05$). This was the same for differences in brain and liver MDA contents ($F_{6,6}$ = 685.314 and 270.131 respectively, $P \leq 0.05$), brain and liver PCOC ($F_{6,6}$ = 71.081 and 95.126, respectively, $P \leq 0.05$), serum, brain and liver NO contents($F_{6,6}$ = 211.423, 424.192 and 327.090, respectively, $P \leq 0.05$), the activity of GST in brain and liver tissues ($F_{6,6}$ = 281.312 and 39.011, respectively, $P \leq 0.05$) and H_2O_2 content in serum, brain and liver of the examined animals ($F_{6,6}$ = 96.050, 182.015 and 212.075, respectively, $P \leq 0.05$). Also, one-way ANOVA test between groups certified very highly significant differences in the decreases for the mean values for the GSH content in sera, brain, and liver tissues of the experimental animals ($F_{6,6}$= 139.411, 143.228 and 143.016, respectively, $P \leq 0.05$), lCAT ($F_{6,6}$ = 61.24, $P \leq 0.05$), Cu/Zn SOD ($F_{6,6}$ = 82.124, 127.039 and 114.615 for serum, brain and liver brain and liver, respectively, $P \leq 0.05$), GPx activities ($F_{6,6}$= 45.307, 20.276, respectively, $P \leq 0.05$), GSH-R activity in brain and liver tissues ($F_{6,6}$= 37.003 and 31.954, respectively, $P \leq 0.05$), and finally gave insurance for very highly significant differences between groups in their venom-evoked decreases in sera, brain and liver TAC concentrations ($F_{6,6}$=75.448, 187.123 and 182.213, respectively, $P \leq 0.05$).

Table (1): Effects of the Experimental Conditions on Serum Glucose and Oxidative Stress Biomarkers

Group	SG Mg / 100 ml	MDA mg / g wet tissue		PCOC mM / g wet tissue		NO mM / g wet tissue		
		bMDA	lMDA	bPCC	lPCC	sNO	bNO	lNO
C	91.37 ± 6.97	12.40 ± 1.30	67.65 ± 6.68	5.35 ± 0.76	4.56 ± 1.02	8.72 ± 0.98	12.48 ± 0.91	12.40 ± 1.30
E	393.79 ± 15.31*	31.23 ± 2.29*	326.17 ± 9.23*	32.39 ± 0.94*	33.85 ± 0.79*	39.22 ± 1.39*	33.53 ± 1.00*	31.23 ± 2.29*
SHP	92.92 ± 5.99¥	12.69 ± 1.02¥	67.75 ± 5.07¥	5.39 ± 1.01¥	4.60 ± 1.12¥	8.85 ± 1.04¥	12.49 ± 1. 22¥	12.69 ± 1.02¥
SHT	95.86 ± 6.66¥	12.64 ± 1.99¥	68.91 ± 6.17¥	6.02 ± 1.17¥	4.99 ± 0.97¥	8.12 ± 1.12¥	12.79 ± 1.65¥	12.64 ± 1.99¥

Table (2): Effects of the Experimental Conditions on the Glutathione Circuits

Group	GSH mg/100 ml & mg/g wet tissue			GPx U/g wet tissue		GSH-R U/g wet tissue		GST U/g wet tissue	
	sGSH	bGSH	lGSH	bGPx	lGPx	bGSH-R	lGSH-R	bGST	lGST
C	2.87 ± 0.13	4.47 ± 0.11	3.15 ± 0.06	48.88 ± 1.14	41.47 ± 2.08	140.23 ± 5.95	52.01 ± 2.99	49.89 ± 6.06	88.93 ± 4.92
E	0.39 ± 0.09*	0.61 ± 0.12*	0.49 ± 0.06*	14.06 ± 2.51*	11.87 ± 1.90*	43.02 ± 1.97*	15.98 ± 3.36	231.19 ± 9.97*	300.02 ± 7.34*
SHP	2.88 ± 0.23¥	4.46 ± 0.19¥	3.19 ± 0.25¥	48.84 ± 1.18¥	41.05 ± 1.46¥	140. 63 ± 4.80¥	52.29 ± 3. 96¥	49.66 ± 8.46¥	89.01 ± 6.07¥
SHT	2.77 ± 0.31¥	4.11 ± 0.07¥	2.99 ± 0.29¥	48.03 ± 1.16¥	41.01 ± 0.97¥	139.63 ± 4.02¥	51.79 ± 3.08¥	50.71 ± 4.97¥	91.98 ± 4.98¥

(*) Represents a significant difference between the control and envenomed groups at $P \leq 0.05$.
(¥) Represents a significant difference between the envenomed and treated groups at $P \leq 0.05$.

Table (3): Effects of the Experimental Conditions on the Enzymatic and Non-Enzymatic Antioxidants

Group	lCAT U/g wet tissue	H_2O_2 mg / 100 ml & mg / g wet tissue			SOD mg / g wet tissue			TAC mM / L & mM / g tissue		
		sH_2O_2	bH_2O_2	lH_2O_2	sSOD	bSOD	lSOD	sTAC	bTAC	lTAC
C	40.01 ± 0.31	124.68 ± 2.97	171.44 ± 3.90	162.16 ± 6.40	28.24 ± 1.096	6.89 ± 0.87	34.25 ± 0.94	3.95 ± 0.52	19.14 ± 1.05	19.83 ± 0.99
E	16.89 ± 0.16*	250.10 ± 3.864*	403.11 ± 12.44*	385.22 ± 9.87*	10.94 ± 0.57*	3.03 ± 0.28*	19.05 ± 1.14*	1.01 ± 0.75*	6.96 ± 0.81*	5.04 ± 0.99*
SHP	39.95 ± 1.55¥	124.89 ± 6.16¥	171.64 ± 6.13¥	161.95 ± 8.08¥	28.43 ± 0.71¥	6.28 ± 1.09¥	34.00 ± 0.79¥	3.94 ± 0.41¥	19.04 ± 1.88¥	19.86 ± 0.63¥
SHT	38.97 ± 1.92¥	126.58 ± 8.88¥	174.80 ± 5.33¥	163.61 ± 5.89¥	27.01 ± 0.77¥	6.00 ± 0.90¥	33.15 ± 0.95¥	3,19 ± 0.33¥	18.05 ± 0.71¥	18.98 ± 0.96¥

(*) Represents a significant difference between the control and envenomed groups at $P \leq 0.05$.
(¥) Represents a significant difference between the envenomed and treated groups at $P \leq 0.05$.

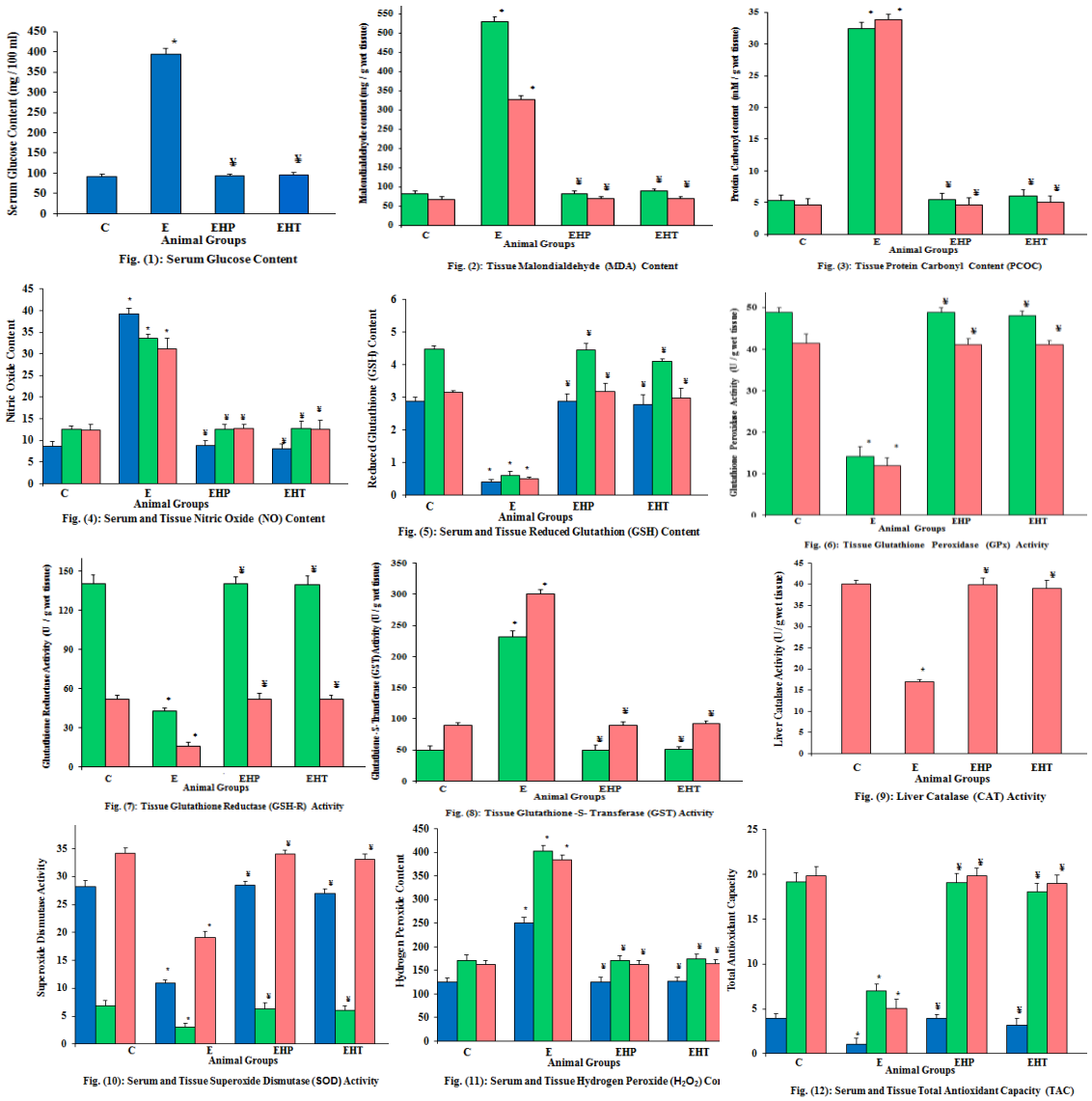

Fig. (1): Serum Glucose Content

Fig. (2): Tissue Malondialdehyde (MDA) Content

Fig. (3): Tissue Protein Carbonyl Content (PCOC)

Fig. (4): Serum and Tissue Nitric Oxide (NO) Content

Fig. (5): Serum and Tissue Reduced Glutathion (GSH) Content

Fig. (6): Tissue Glutathione Peroxidase (GPx) Activity

Fig. (7): Tissue Glutathione Reductase (GSH-R) Activity

Fig. (8): Tissue Glutathione -S- Transferase (GST) Activity

Fig. (9): Liver Catalase (CAT) Activity

Fig. (10): Serum and Tissue Superoxide Dismutase (SOD) Activity

Fig. (11): Serum and Tissue Hydrogen Peroxide (H_2O_2) Co

Fig. (12): Serum and Tissue Total Antioxidant Capacity (TAC)

Discussion:

Although the mechanism of the hyperglycemia-induced by scorpions' venom; indicated as one of the pathophysiological effects of scorpion envenomation in this work (table "1" & fig."1"), and are in line with other investigators (Abd Rabo, 2006; Choudhry et al., 2011; Azzam, 2012; Azzam et al., 2013; de Oliveira et al., 2016 & Chaubey, 2017), is not clearly understood, there are some suggestions. This can occur through the reduced insulin secretion, excessive release of catecholamines, decreased thyroid hormone levels, and increased cortisol and glucagon levels (Murthy and Zare, 1998; Goncalves et al., 2003; Oliveira et al., 2016 & Chaubey, 2017). This appears to be secondary to cause an autonomic storm that activates both the sympathetic and parasympathetic autonomic nervous system (Mirakabadi et al., 2006), where envenomation is a powerful stimulant of the autonomic nervous system, especially the peripheral and central adrenergic systems, causing intense autonomic discharge and leading to a massive release of neurotransmitters (Murthy and Haghnazari, 1999; Dittrich et al., 2002; Vasconcelos

et al., 2004; Azzam, 2012 & Azzam et al., 2013) and activation of b-receptors with catecholamine and serotonin secretion (Adam, 1959), which give rise to several clinical manifestations including hyperglycemia (Murthy and Haghnazari, 1999; Dittrich et al., 2002; Vasconcelos et al., 2004; Mirakabadi et al., 2006; Azzam, 2012; Azzam et al., 2013 & Murthy et al., 2015). The released catecholamines result in a variety of metabolic effects and mobilize substrates from fuel depots; as mobilization and break down of liver glycogen causing an increase in blood sugar levels and activation, via cyclic AMP, of hepatic glycogen phosphorylase, the enzyme responsible for converting glycogen to glucose-1-phosphate, the rate-limiting step in glycogenolysis (Ganong, 1987; Keele et al., 2000; Jackson, 2006 & Murthy et al., 2015). Also, the epinephrine-like effects, mediated by the serotonin, present in the scorpion venom, was said to be responsible for the predicted hyperglycemia and liver and muscle glycogenolysis after envenomation (Mohamed et al., 1972) where it acts as a stimulant of gluconeogenesis (Murthy, 2000) and inhibits glycolysis (Murthy et al., 2003). Otherwise, envenomation was estimated to enhance glucose production by combined epinephrine, glucagon, and cortisol infusion that occurs as a result of an additive effect on hepatic glucose production, without any additional change in glucose clearance (Goldstein et al., 1995). Additionally, the suppressed insulin secretion, block of insulin secretion (El-Asmar et al., 1974; Mirakabadi et al., 2006 & Murthy et al., 2015), and insulin resistance (Zare, 1993) by scorpion envenomation may be causative. Furthermore, the actions of the scorpion neurotoxins on voltage-gated sodium (Nav) or potassium (Kv) channels have demonstrated a gamut of physiological responses, including the increase of plasma glucose levels (Vasconcelos et al., 2004; Ribeiro et al., 2010; Pucca et al., 2012; 2015 & Oliveira, et al., 2016). Goncalves and his team work clarified a direct effect of a scorpion toxin on isolated islets of Langerhans (not provided by catecholamine's action), enhancing the β-cells membrane depolarization and significantly potentiating glucose-induced insulin secretion (Goncalves et al., 2003 & Oliveira et al., 2016). This is inconsistent with imitating OS to be the major causative factor of venom-induced toxicity and has been associated with renal failure, hepatic impairment, and acute pancreatitis in viper and other envenomed experimental animals and humans.

Of great importance, the significant increases of the oxidative stress (OS) biomarkers in the envenomed animals, revealed by our results (table "1" & figures "2" -"4") and previously by others (De Roodt et al., 2003; Meki et al., 2003; Hammoudi-Trik et al., 2004; Fatani et al., 2006; 2010; Azzam, 2012; Azzam et al., 2013; Al Asmari et al., 2016 & Al-Sheikh et al., 2017), indicating that scorpion venom could induce oxidative damage in the envenomed animals. Besides causing many human pathologies (Valko et al., 2007) (Yamasaki et al., 2008; Valenta et al., 2010; Sagheb et al., 2011; Al Asmari et al., 2014; 2016 & Al-Sheikh et al., 2017).

Oxidative stress is a cellular phenomenon or condition simply defined as a case of physiological imbalance between the levels of antioxidants and oxidants (free radicals; FRs or reactive species; RS) (Boveris and Navarro, 2008; Kalam et al., 2012 & Moustafa, 2015) in favor of oxidants (Ighodaro and Akinloye, 2017). OS occurs as a deleterious process implicated in the development of many diverse diseases including hypertension, cardiac dysrhythmia, and myocardial damage (Satoh and

Nishida, 2004; Fatani et al., 2006 & Liang and Juan, 2011), all of which are present in scorpion envenomation (Ismail, 1995; Amitai, 1998; de Roodt et al., 2003; Hammoudi-Triki et al., 2004; Dousset et al., 2005; Fatani et al., 2006; Liang and Juan, 2011 & Aksoy et al., 2012). An oxidant/free radical is defined as any chemical species containing one or more unpaired electrons in the outer atomic or molecular orbital/s (Kohen and Nyska, 2002; Pryor et al., 2006; Aksoy et al., 2012 & Aprioku, 2013). Because of these unpaired electrons, free radicals are highly reactive and readily take part in chemical reactions with virtually all cell components (lipids, proteins, complex carbohydrates, and nucleic acids) in the body. These reactions occur through a chain of oxidative reactions to cause tissue injury (Kohen and Nyska, 2002 & Aprioku, 2013). FRs can be formed from two groups of sources; endogens, mostly mitochondrial electron transport chains, and exogens; as cigarettes, pesticides, solvents, petrochemical products, drugs, alcohol, solar rays, stress, x-rays, and heavy physical activity (Valko et al., 2007 & Aksoy et al., 2012). Although they have a very short life cycle, FRs may be harmful to organisms due to their activity levels (Braunersreuther and Jaquet, 2012). The FRs of interest, in most biological systems, are referred to as reactive oxygen species (ROS); where the most significant free radicals are oxygen-centered (Aprioku, 2013). ROS in the cells includes singlet oxygen ($^{\bullet}O$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{-\bullet}OH$), hypochlorous acid (HOCl), and hydrogen peroxide (H_2O_2) (Slater, 1984; Halliwell, 2006; Pryor et al., 2006 & Aprioku, 2013). Although H_2O_2 contains no unpaired electrons, it is considered a reactive oxygen species because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive transition metals like iron and copper (Slater, 1984; Aruoma et al., 1991 & Valko et al., 2005). Reactive nitrogen species (RNS) is another group of free radicals referring collectively to nitrogen-centered radicals that alter cellular functions. RNS includes nitric acid (NO), peroxynitrate radical ($ONOO^-$), nitrogen dioxide radical ($^{\bullet}NO_2$) and other oxides of nitrogen and products arising when NO reacts with O^{2-}, peroxyl radical ($RO^{\bullet}$) and alkoxyl radical ($ROO^{\bullet}$) (Kohen and Nyska, 2002; Victor et al., 2004; Valko et al., 2005 & Aprioku, 2013). ROS and RNS are generated as a consequence of normal cellular metabolism (Lobo et al., 2010), but increased under conditions of both physiological and psychological stress (Jackson, 2000) as in scorpionism (Azzam, 2012 & Azzam et al., 2013). They are well recognized to play a dual role; both deleterious and beneficial species (Valko et al., 2007). The human body has antioxidative mechanisms of two groups; enzymatic (SOD, CAT, GPx, GST, GSH-R, etc.) and nonenzymatic (GSH, ascorbic acid; Vitamin C, α-tocopherol; Vitamin E, carotenoids, flavonoids, melatonin, albumin, uric acid, etc) to overwhelm oxidants (Al-Gayyar et al., 2007; Valko et al., 2007; Gill et al., 2011; Zengin et al., 2014; Thatoi et al., 2014 & Moustafa, 2015). Total antioxidant capacity (TAC) of an organism represents the sum of all antioxidant activities in the organism, including both enzymatic and non-enzymatic activities of antioxidants, which can be regarded as the ability of an organism to suppress ROS (Lesser, 2006 & Dann, 2017).

OS can occur due to an overproduction of oxidants, over-exposure to exogenous oxidants, a decrease in antioxidant defenses, or a combination of these factors (Zengin et al., 2014). In this context, the scorpion–induced cytotoxic oxidative damage

was shown to cause lipid and protein oxidation to the experimental animals as compared with the controls, indicated by significant increases in MAD, NO, and PCOC levels in brain and liver tissues (table "1"). It is well known that scorpion venoms, by their action mainly on sodium channels, enhance the release of various neurotransmitters, such as adrenaline and noradrenaline, (Ismail, 1995; Meki et al., 2003 & Azzam, 2012) that can induce the generation of FRs leading to OS and apoptosis (Khorchid et al., 2002).

The mechanisms by which FRs and lipid peroxidation (LPO) are generated in scorpion envenomation are complex and may depend on a multitude of interacting factors (Fatani et al., 2006 & Azzam, 2012); 1) Scorpion venoms contain neurotoxins that act on many ionic channels resulting in an alteration of ionic transport and cytosolic calcium overload (Harvey et al., 1992; Fatani et al., 2006 & Azzam, 2012), which 2) elevates adenosine monophosphate (AMP) concentration and increases its catabolism, leading subsequently to a generation of FRs. 3) These FR's attack membrane phospholipids causing their peroxidation, that 4) eventually causes impairment of biological membrane functioning, decreased fluidity, and inactivation of membrane-bound receptors, all of which may culminate in multiple organ dysfunction (MOD) (Bhaumik et al., 1995; Love, 1999; Al-Omar et al., 2004; Fatani et al., 2006 & Azzam, 2012); a feature encountered in scorpion envenomation (Meki et al., 2003 & Azzam, 2012).

LPO is a natural metabolic process under normal aerobic conditions and it is one of the most investigated consequences of ROS action on membrane structure and function (Blokhina et al., 2003). Generally, LPO can be described as a process under which oxidants such as FRs or non-radical species attack lipids containing C = C bond/s; especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from carbon, with oxygen insertion, resulting in lipid peroxyl radicals and hydroperoxides (Yin et al., 2011) causing its deterioration (Devasagayam et al., 2003). Also, the presence of phospholipases (PLA), as a constituent in the toxins produced by venomous animals (De Maria et al., 2007), including scorpions (Zamudio et al., 1997; Conde et al., 1999; Valdez-Cruz et al., 2004; Hariprasad et al., 2007; Hmed et al., 2013 & Incamnoi et al., 2013) may be causative. PLA is a group of enzymes that hydrolyze one of the ester bonds on phospholipids to release a variety of products (Shimizu et al., 2006), as arachidonic acid whose metabolism results in the formation of potentially toxic ROS and lipid peroxides (Adibhatla and Hatcher, 2008 & Abou-Elezz et al., 2017). Currently, LPO is considered as the main molecular mechanism involved in the oxidative damage to cell structures and in the toxicity process that leads to cell death (Repetto et al., 2012). The peroxidation process of membrane lipids include loss of polyunsaturated fatty acids (PUFAs), decreased lipid fluidity, altered membrane permeability, effects on membrane-associated enzymes, altered ion transport, the release of material from subcellular compartments, and the generation of cytotoxic metabolites or lipid hydro-peroxides (Santamaria et al., 2002 & Abou-Elezz et al., 2017). LPO produces a wide variety of oxidation products (Ayala et al., 2014). MDA which is one of the aldehydes formed as a secondary end-product product during LPO as is a final metabolite of LPO formed from a variety of unsaturated fatty acids in biological membranes stimulated by ROS and RNS overproduction (Valko et al., 2007), and generated by decomposition of unsaturated fatty acids

(arachidonic acid and larger PUFAs) (Esterbauer et al., 1991; Valko et al., 2007; Yin et al., 2011 & Ayala et al., 2014), through enzymatic or non-enzymatic processes, appears to be the most mutagenic product of LPO (Esterbauer et al., 1990), widely used as a convenient biomarker for LPO (Ayala et al., 2014).

Additionally, as predicted from our results, the single SC dose of 1/5 LD_{50} (0.12 mg/kg BW) of the crude venom of Saudi scorpion *Androctonus crassicauda*, induced evidenced protein oxidation, as indicated by the accumulation of protein carbonyl (PCO) in the examined tissues (table "1" & fig. "3"). Previous studies agreed with these data (Krishnan and Kodrik, 2006; Abdel-Rahman et al., 2009; Azzam, 2012; Azzam et al., 2013 & Béchohra et al., 2016). Proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells because they are often used as catalysts (Pandey and Rizvi, 2010). Exposure of proteins to reactive oxygen species (ROS) alters the physical and chemical structure of the proteins causing oxidation of side-chain groups, protein scission, backbone fragmentation, cross-linking, and unfolding. These alterations are consequent to the formation of new reactive groups, generation of protein carbonyls (PCOs), oxidation of -SH groups, the formation of dityrosine containing cross-linked protein products known as protein products (AOPPs), and many others (Levine et al., 1990; Witko-Sarsatet al., 1996; Cakatay et al., 2008 & Pandey and Rizvi, 2010). Protein carbonyl content (PCOC) is the most general indicator and the most commonly used marker of protein oxidation (Berlett and Stadtman, 1997; Shacter, 2000; Chevion et al., 2000; Beal, 2002 & Deori et al., 2016), particularly associated with OS derived from free radicals and other activated oxygen species (Levine et al., 1990 & Halliwell and Whiteman, 2004). Protein carbonyls are formed early during OS conditions and are not a result of one specific oxidant, thus they can be called a marker of overall protein oxidation (Weber et al., 2015). PCO is a product of irreversible non-enzymatic oxidation or carbonylation of proteins (Dalle-Donne et al., 2006) that represents an irreversible form of protein modification, demonstrated to be relatively stable (degradation/clearance in hours/days) in contrast to LPO products that are removed within minutes (Grune et al., 1995 & 1996). The usage of the PCO group as a biomarker of OS has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of the carbonylated protein (Berlett and Stadtman, 1997 & Dalle-Donne et al., 2003).

Carbonyl (C=O; CO) is a functional group consisting of a carbon atom that has a double-bound to an oxygen atom, thus aldehydes, ketones, carboxylic acids, esters, and amides are all carbonyl groups (Danielsen, 2015). The carbonyl groups can be introduced on protein side chains, when they are oxidized, by a variety of oxidative pathways (Dalle-Donne et al., 2003; Fedorova et al., 2014 & Abou-Elezz et al., 2017). Oxidation of certain amino acids within proteins can result in the formation of PCO which are disturbers of protein function (Dalle-Donne et al., 2003 & Danielsen, 2015), either by modifying their structural function or by causing loss of catalytic activity. Oxidation can arise from direct oxidation of most protein residues or with secondary oxidation products (Beal, 2002). Oxidative cleavage of proteins; by either the α-amidation pathway or by oxidation of glutamyl side chains, leading to the formation of a peptide in which the N-terminal amino acid is blocked by an α

-ketoacyl derivative, is a way (Berlett and Stadtman, 1997 & Dalle-Donne et al., 2003). Also, CO groups may be introduced into proteins by a secondary reaction of the nucleophilic side chains of Cys, His, and Lys residues, with the aldehydes produced during LPO, or with reactive carbonyl derivatives generated as a consequence of the reaction of reducing sugars, or their oxidation products with lysine residues of proteins with the eventual formation of the advanced glycation / lipoxidation end products (AGEs/ALEs) (Berlett and Stadtman, 1997; Stadtman and Berlett, 1997; 1998; Uchida, 2000; Dalle-Donne et al., 2003 & Halliwell and Gutteridge, 2015). The venom-induced oxidative stress which elevates the steady level of ROS causes protein carbonyls to accumulate on the side chains of proteins, decreases the TAC, also indicated in this work; table "3" and fig. "12", (Chaudhuri et al., 2006; Krishnan and Kodrik, 2006; Abdel-Rahman et al., 2009; 2010, Azzam, 2012 & Azzam et al., 2013) and degrades the ability to oxidized proteins by either decreased proteases concentration or increased levels of proteases inhibitors (Stadtman, 2004).

On contrary, there is a relation between the observed increase in nitric oxide (NO) concentration after envenomation and the resulted elevations in PCOC; where the increase in PCOC might be in part due to the increase in the NO concentration. NO; a free radical generated as a byproduct of five-electron oxidation of the amino acid L-arginine mediated by one of three isoforms of nitric oxide synthase (Moncada et al., 1991; Annane et al., 2000 & Abdoon, 2004), readily reacts with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($ONOO^-$), a potent oxidant and nitrating agent capable of attacking and modifying proteins and depleting antioxidant defenses (Mc Cord, 2000 & Abdel-Rahman et al., 2010).

In this respect, our work results (table "1" & fig. "4") were in harmony with the previous works (Dousset et al., 2005; Petricevich, 2010; Abdel-Rahman et al., 2010; Azzam, 2012; Sahan-Firat et al., 2012; Azzam et al., 2013; & Ahmed et al., 2015) denoting that scorpion envenomation causes elevated NO content. Within a cell, the effects of NO are concentration-dependent and its actions are dependent on its levels, the redox status of the cell, and the number of metals, proteins, and thiols, amongst other factors (Rosselli et al., 1998 & Agarwal et al., 2012). NO is known to have a dual role; as a ubiquitous intracellular messenger in many mammalian systems and a contributor to lethal processes (Ignarro, 2000), and the cardio-vascular autonomic nervous system has proven to be no exception (Chowdhary and Townend, 1999). NO as one of the smallest and most known diffusible signal molecules, can freely cross membrane barriers to exert its effects through direct binding and/or reaction with target proteins (Stamler et al., 1992 & Stanley, 2000). It is known to be involved in multiple reactions (Stamler et al., 1992) of biological importance; such as being an endogenous regulatory mediator in the control of vascular tone (Moncada et al., 1991) and vasomotor function (Petricevich, 2004; 2010; Ahmed et al., 2015 & Abou-Elezz et al., 2017), its implication in the genesis of various pathological conditions such as inflammation, arteriosclerosis, hypertensive vascular lesions in which the infiltration of inflammatory cells occurs (Olsen, 1972; B¨usse and M¨ulsch, 1990; Libby and Hansson, 1991) and insulin resistance, as which resulted from envenomation (Petricevich, 2002; Petricevich and Pe˜Na, 2002 & Azzam, 2012). The high levels of

NO in the venom-treated animals may abolish the effect of catecholamines, as a reflex control of sympathetic nerve activity (Chowdhary and Townend, 1999; Abdel-Rahman, 2009; Azzam, 2012 & Azzam et al., 2013). Several inducers of insulin resistance; including pro-inflammatory cytokines and oxidative stress that can modify the levels of nitrites, and nitrate production have been associated with several conditions as envomation. Envenomation by scorpions, bees, snakes, spiders, and wasps involves the release and activation of pro-inflammatory cytokines that may be at least partially responsible for NO synthesis (Petricevich, 2004; 2010 & Azzam, 2012). NO could be released by tissues after scorpionism via induction of the inducible nitric oxide synthase (iNOS) (Stamler et al., 1992) and production of ROS (Meki and Mohey El-Deen, 1988). Also, the significantly high serum level of NO supports the evidence for the role of these potent vasodilators in the terminal hypotension that is usually observed in humans and animals after envenomation (Abdoon and Fatani, 2009). In our findings, NO secretion may be increased for its prominent protective role against the neurotoxic effects of the scorpion toxins, either by direct activation of Ca^{2+}-activated-K^{+}-channel (K^{+}-Ca) in cell-free membrane patches (Bolotina et al., 1994 & Azzam, 2012) or by inhibiting the mitochondrial Ca^{2+} uptake and increasing the probability for opening the mitochondrial ATP-sensitive K^{+}-channel by modulating affinity to ATP (Rakhit et al., 2001 & Azzam, 2012). On the other hand, NO can give rise to toxic secondary radical species (Stamler et al., 1992).

Naturally, cells have developed an extensive defense system (antioxidants) to neutralize the harmful oxidative effects of excessive ROS generation (Volko et al., 2007). Antioxidant enzyme activity and oxidant generation rates have been postulated to be useful in estimating the level of oxidative damage (Volko et al., 2007 & Aboul-Soud et al., 2011). Antioxidants are important inhibitors of LPO, not only for protection but also as a defense mechanism of living cells against oxidative damage (Dauqan et al., 2011 & Padmanabhan et al., 2012). Apart from being involved in damaging cellular components, ROS seem to play a major role in venom-induced toxicity (Dousset et al., 2005). This is evident from the present results; as the fine balance, normally maintained between antioxidant activity and oxidant generation, has shifted in favor of oxidative reactions, causing OS in the E group (tables "1" - "3" & figures "5" - "12"). Cellular antioxidant defense systems including SOD, CAT, and glutathione redox cycle; which reduce ROS to water (Volko et al., 2007; Lee et al., 2015 & Patlevič et al., 2016; Ighodaro and Akinloye, 2017) are the most important organic mechanisms against tissue damage induced by FRs (Kochar and Umathe, 2009 & Azzam, 2012), that may prevent disturbances in ROS homeostasis, or reduce the effect of oxidative stress in cells.

GSH is the first essential defense mechanism for the maintenance of homeostasis and redox balance and the prevention of LPO and depletion of cellular thiols (Halliwell and Gulteridge, 1989 & Couto et al., 2013). It is considered the most abundant intracellular ubiquitous thiol-based antioxidant involved in numerous processes essential for normal biological functions, as DNA and protein synthesis (Peña et al., 2003 & Azzam. 2012). In the GSH redox cycle, GPx catalyzes the decomposition of H_2O_2 and lipid peroxides to water. The present results (table "2" & fig."5") indicated very significant decreases in GSH, thus producing a pronounced OS. Furthermore, there was a very

significant reduction in GSH-R activity (Table "2" & fig. "7"). The drop in GSH levels of the venom-treated animals may be caused by the pronounced decrease in GSH-R activity or as a result of GSH reacting directly with excessively generated H_2O_2. Valko and his co-workers reported that GSH levels can be regulated by its denovo synthesis catalyzed by γglutamate-cysteine ligase and glutathione synthase, hence, the noted drop in GSH levels of examined tissues of the E group may be a result of the crude venom acting to lower the gene expression levels and consequently the activities of these enzymes (Valko et al., 2007 & Ghneim, 2017).

A remarkable decline in liver CAT activity was noticed in parallel with the decrements in GSH in the tested organs of envenomed animals (table "3" and fig. "9"). These changes were properly due to the dexterous effects of reactive oxygen species as $O_2^{\bullet-}$, H_2O_2 and NO. The depletion of liver GSH, as a nucleophilic scavenger of reactive metabolites, maybe the main cause of the fallen serum GSH levels (Ballatori and Rebbeor, 1998). On the other hand, this may be likely to reduce the ability of the liver and brain tissues to eliminate OFR (Yu, 1999; Basu et al., 2001 & Azzam, 2012). Also, GPx and GSH-R were decreased while GST was elevated in brain and liver tissues of envenomed animals (table "2" & figures "6", "7" & "8"). GSTs; a family of enzymes catalyzing the addition of GSH to endogenous and xenobiotic substrates with electrophilic functional groups, play important role in detoxification and metabolism of many xenobiotic and endobiotic compounds (Ji et al., 1992). The increment in GST activity (table "2" & fig. "8") is inconsistent with the generation of FRs; as one of the defense mechanisms to detoxify or neutralize the toxic metabolites generated in the liver (El-Demerdash et al., 2005). GPx plays a primary role in minimizing oxidative damage. GPx and GST work together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of GSH. On contrary, significant decreases in SOD activity and TAC (table "3" & figures "10" &"12") were reported. The role of SOD is to protect cells against oxidative damage caused by superoxide (SO) anion radicals. SODs cause rapid dismutation of SOD to a less reactive H_2O_2 molecule which is further degraded by CAT to water and oxygen (Wetscher et al., 1995). Thus the decreased SOD activity, in our study, may be related to an adaptive response to the generated FRs to $O_2^{\bullet-}$ in the treated animals (Giller and Winge, 1984; Kale et al., 1999; Azzam, 2012 & Azzam et al., 2013). An association between GSH depletion and reduced SOD activity was suggested (Loven et al., 1986). Also, the decrements in SOD and CAT activities may be due to the inactivation of mitochondrial and cytosolic enzymes, leading to a decrease in the metabolic activity (Kochar and Umathe, 2009). Alternatively, SO anion radicals are dismutated and catalyzed by SOD to H_2O_2 (Schild et al., 2003). Both pathways of SO anion radical reactions; the formation of H_2O_2 and the formation of $ONOO^-$, lead to a decrease in the activity of both SOD and CAT in the current study.

Concerning recovery trials of scorpion venom toxicity in animal models with safer, more effective, cheaper, and more sustainable products with therapeutic impact, this work is an attempt and a developing effort pointing towards an upcoming transition from the wholesale coastisity of the serum-based antivenom to discover therapeutic natural solutions based on modern biotechnology. Thus scorpion haemolymph (SH) was used in this work, as discussed earlier, to ensure it's protective

and/or treating ability of the scorpion-evoked OS. Scorpions are known to be quite resistant to their venom. Some kill the scorpion and apply the scorpion's fluid into the site of the sting thinking that it will neutralize the venom. SH was insured to have beneficial effects when applied to envenomed individuals in a controlled environment (Tripod.com, 2011; Azzam, 2012; Azzam et al., 2013 & Abdou et al., 2015). Our results; presented in tables "1", "2" & "3", investigated that the venom-induced hyperglycemia was returned to euglycemia in the pre and post-envenomed groups treated with SH. Thus, the noticed cellular dysfunctions after hyperglycemic oxidative stress after envenomation conditions in this study were normalized to the control values. The haemolymph has reported having antihyperglycemic effects due to the presence of insulin-like peptides; structurally related to that of insulin of mammals (Padmanabhan et al., 2012), so can reverse the metabolic disturbances by changes in the hormonal environment, especially insulin (Kankonkar et al., 1992; Murthy et al., 2002; 2003; Natu et al., 2006 & Azzam. 2012). Otherwise, the neutralization effect may be attributed to the non–enzymatic antioxidant activity predicted on the oxygenated haemocyanin (Quéinnec et al., 1999; Bolton et al., 2009 & Dann, 2017); a large multisubunit extracellular pigmented metalloprotein component in the SH that performs the important function of oxygen transport (Ali et al., 1995 & Azzam, 2012). It is worth mentioning that the haemolymph is known for its ability to inhibit ROS by its bioactive peptides (Moselhy, 2012 & Padmanabhan et al., 2012). It was found to contain several effective antitoxins that may reverse the venom-toxins-metabolic disturbances (Harpaz, 1973 & Balasubramanian et al., 2017). Also, the haemolymph was reported to have potent peptides showing a diverse array of activity (Arul Prakash et al., 2011 & Padmanabhan et al., 2012) and plays a prominent role in host defense response including superoxide anion activity, (Kramer et al. 1977; Sahoo et al., 2005 & Moselhy, 2012). On other hand, many studies have reported that scorpion haemolymph has cross-species toxin inhibitory effects (Smith et al., 2000 & Abdou et al., 2015) but its mechanism of action remains unclear (Azzam, 2012 & Azzam et al., 2013). This may explain its activity to reverse the venom-toxins-metabolic disturbances. The attainment of normal blood glucose levels following the SH indicated that SH may act as the scorpion antivenom (SAV) to neutralize its toxicity. Also, the amelioration efficacy of SH, under our experimental conditions, ensured that the time of treatment, the IV injection, and the desired dose effectively and fastly neutralize the circulating venom and durably clears of free toxins from the circulation as well as their rapid redistribution from the peripheric compartment to the vascular one (Azzam, 2012 & Azzam et al., 2013).

Recommendations:

The management of diseases without any side effects remains a challenge in medicine. More trials to elucidate the therapeutic value of other scorpions' haemolymph against the cytotoxicity of venom, and to separate its novel active molecule (s) is very important. More efforts to discover natural antivenins to prevent and treat scorpion sting symptoms are recommended. Also, a well-planned health education program might be useful to overcome the problems of sting bites especially scorpionism, predominately in developing countries**.**

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