THE EFFECT OF VITAMIN C ON HISTOPATHOLOGICAL ALTERATIONS-INDUCED BY *HEMISCORPIUS LEPTURUS* SCORPION VENOM IN RAT

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ABSTRACT – *Hemiscorpius lepturus* (HL) is one of the most dangerous scorpion in khuzestan. HL envenomation causes various pathological changes in the affected organs, including lung, liver and kidney. To our knowledge there is no data available regarding the effect of the antioxidants on HL-induced organ cell damage. The study of the effect of vitamin C on HL venom on experimental animals may be useful for better understanding of the clinical pictures following HL scorpion sting. To evaluate the effect of vitamin C on HL venom induced histopathological alterations in rat liver, kidney and lung tissues. Adult male rats were pretreated with 0 or 300mg/kg vitamin C prior to receive HL venom at doses of 1, 2 or 3 mg/kg (ip). Control rats were given vehicle only. The animals were killed after 4,8,12 or 24h. The liver, kidney and lung tissues were removed, fixed for 24 h in 10% formalin, then processed for light microscopy. Using Haeotoxylin- Eosin (H&E) staining methods. Ten animals were used for each treatment group.

The main toxic effect of the venom was primarily on the liver, kidney and lung tissues in 3ug/g treted rats in various time. Various doses of HL venom induced cell damage in liver, kidney and lung. Vit c had no effect on rat tissues .but it cause considerable depletion of HL venom-induced cell damage. We concluded that structural alterations caused by HL venom reflected to the toxic effect of the venom on vital organs. It appears that accumulation of the venom in vital organs induced toxicity, alternatively, translocation of toxic reactive metabolite(s) from target organ(s) may be at least in part is responsible for organ histopathological damage. The finding that the effectiveness of vitamin C in ameliorating venom-evoked histopathological changes indicative of involvement of oxidative stress in venom-evoked cellular damages. Thus free radical generation may be at least in part is responsible for HL venom induce cell damage in rat tissues.

Key words : Vitamin C, Hemiscorpius lepturus, histopathology, liver, kidney, lung rat

INTRODUCTION

*Hemiscorpius lepturus* (HL) is one of the most dangerous scorpion in khuzestan. Severe and fatal hemolysis, secondary renal failure, deep and necrotic ulcers, psychological problems and death are reported in the scorpion sting victims(Radmanesh 1998,1990).

The HL venom is known to produce adverse effect on different organs in human and experimental animals (Saghab et al, 2012; Hedian pur et al, 2012; Ahmadizadeh and Razi Jalaly, 2010). Saghab et al, 2012 found that renal toxicity of scorpion envenomation is mostly related to HL stings and this complication obvious in younger children. Histopathological changes in various organs of mice were reported following subcutaneous HL administration (Hedian pur et al, 2012). Interaperitoneal HL

Venom administration produced damage in various tissues of rat (Ahmadizadeh and Razi Jalaly, 2006)

Large body of evidence showed that scorpion venoms have potential to develop cytotoxicity. El Nasr et al(1992) observed that *Buthus quinquestriatus* induced cell injury in mice liver and kidney. Ultrastructural study indicated that *Heterometrus fulvipes* scorpion venom caused rat liver mitochondrial swelling (Venkaiah and Parthasarathy, 1983). The histopathological changes were observed in the liver, lung and kidney of rat after treated with *Buthus quinquestriatus* venom (Nawar et al, 1979; ).

Lung edema induced in rat following administration of tityustoxin a toxic fraction of *Tityus serrulatus* scorpion venom (Mesquita et al, 2002). Regardless of the venom species, histopathological alterations in both human and animals were reported (Ahmadizadeh et al, 2006; Meki et al, 2002 ; Meki et al, 2006; More et al, 2004; Omram et al, 2004; Sahnoun et al, 2007; Yugandhar et al, 1999; Zare et al, 1994; Zare et al, 1994; ).

Oxidative stress has been implicated to various diseases including hypertension, myocardial damage renal injury and respiratory failure, all of which noted after scorpion envenomation Saghab et al, 2012; Hedian pur et al, 2012; Ahmadizadeh and Razi Jalaly, 2010). The mechanism by which scorpion venom produced toxicity is not clear. Dousset et al(2005) found lipid peroxidation
in different organs following envenomation of rats by toxic fractions of *Androctonus australis Hector* venom. These authors found that considerable lipid peroxidation in various vital organs. They also reported that pretreatment of rat with N-acetylcysteine (NAC) protected animals against toxicity. Meki et al. (2002) showed that the level of nitric oxide and lipid peroxidation significantly higher in serum of envenomed children stung with *Buthus occitanus* or *Leurus quinquestrctus* when compared to healthy children (control subjects).

Vitamin C play an important role as an antioxidant to prevent cellular damage from free radical. This agent acts directly to scavenge free radicals and also protecting cell membrane by regenerating the antioxidant (Beyer, 1994).

Previously we found that vitamin C protected rats against HL venom induced biochemical alterations (Ahmadizadeh and Razy Jalaly, 2010) As clinical symptoms were noted following HL envenomation in human and experimental animals, thus the antioxidant chemicals may have the potential to diminish HL toxicity.

The study of the effect of vitamin C on HL venom induced alteration in various tissues of rat may be useful for better understanding of the clinical pictures following HL envenomation in humans. This experimental in vivo study was conducted to investigate the effect of vitamin C on HL-induced hisopathological changes in rat lung, liver and kidney tissues.

**MATERIALS AND METHODS**

Freeze-dried fresh venom from scorpion *Hemiscorpius lepturus* (HL) was obtained from the Ahwaz Razi Institute. Adult male Sarague-Dowley rats (150-200g) were purchased from animal house of Ahvaz Jundishapur medical sciences university and were housed in a groups of 3 in clear polypropylene cages in a light cycle (12 h light and 12 h dark) and temperature-controlled room. The animals were allowed food and tap water ad libitum. The rats were pretreated with 300mg/kg vitamin C. Control animals were received vehicle only. 30 min later, the animals were given HL venom (ip) at doses of 1, 2or 3µg/g. Control rats received vehicle (normal saline) only. Animals were killed (with over dose of sodium pentobarbital) 4h, 8h, 12 or 24 h following administration of the venom. The liver, kidney and lung tissues were removed, fixed for 24 h in %10 formalin , then processed for light microscopy. Using Hемatoxylin- Eosin (H&E) staining methods . Five histological sections each at least 15µm apart were taken from each tissue block and stained with H&E. Ten animals were used for each treatment group.

**RESULTS**

Administration of vehicle alone did not produce detectable injury in rat liver (Figure. 1). In control rats (vehicle treated animals) liver cells were intact . There was no detectable injury in hepatocytes (Figure 1). However, dose and time related injury in HL venom -treated rats were noted.

Liver: LH scorpion venom induced injury in the liver. The extent of injury appeared to be a dose and time related manner. HL venom induced liver injury as early as 4 hr after administration of various doses of HL venom. However, the most remarkable histopathological alterations were noted in rats 24 h treated with 3ug/g HL venom . The liver cells were intensively swollen. The vessels of portal space and centrolobular vein being distended and congested with hemolyzed material. The hepatocytes were enlarged and granular with many vacules. The nuclei were appeared to be larger. Hydropic degeneration was obvious (Figure 2).

Vitamin C had no effect on liver tissue and there was no obvious injury . However, this agent protected hepatocytes against HL venom produced cytotoxicity (Figure 3).

Kidney : In control, kidney was intact and no detectable injury was seen (Figure 4). Kidney damage were noted in animls treated with various doses of LH scorpion venom. However, dose and time related injury in HL venom -treated rats were noted.

Congestion and hemorrhagic area and sign of disorganization were seen. The extent of injury was dose and time related manner. The tubular cells were swollen with increased granularity and attenuation of tubular lumen. ; hydropic degeneration were noted.

However, the most remarkable kidney histopathological alterations were noted in rats 24 h treated with 3ug/g HL venom (Figure 4).

Vit C had no effect on kidney tissues when compared to control animals. However, pretreated animals with this agent markedly reduced the level of cell damage when compared to HL venom-treated rats (figure5). Similarly, Vit C had no effect on kidney cells. However, the extent of HL-induced injury markedly decreased in vitamin C pretreated rats when compared to the HL treated animals (figure6).

Lung : Lung tissue was intact in vehicle treated rats (Figure 7). Histopathological alterations were noted in the rat lung as early as 4 hr treated with HL venom. However, the extent of injury was noted in a dose and time related manner. the most remarkable
Fig. 1: Light micrograph of rat liver treated with vehicle (normal saline). There was no obvious injury in the liver cells. Note hepatocytes (arrow) are intact. Centrolobular vein (CV) H&E X200.

Fig. 2: Light micrograph of rat liver treated with 3µg/g LH scorpion venom and was killed 24 hrs later. Enlarged hepatocytes and hydropic degeneration (arrow). The centrolobular vein is distended and congested with hemolysed material H&E x200.

Fig. 3: Light micrograph of rat liver pretreated with 300 mg/kg vitamin C and received 3 3µg/g HL venom. Showing HL venom-induced liver injury markedly diminished in compare with non-pretreated animals which received the same dose of this venom (figure 2). Note hepatocytes (arrow) are intact. H&E x200.

Fig. 4: Light micrograph of rat kidney treated with vehicle (normal saline). There was no obvious injury in the kidney cells. H&E X200.

Fig. 5: Light micrograph of rat kidney treated with 3µg/g HL scorpion venom and was killed 24 hrs later. Disorganization and congestion are apparent (arrow) H&E x200.

Fig. 6: Light micrograph of rat kidney pretreated with 300 mg/kg vitamin C and received 3 3µg/g HL venom. Showing HL venom-induced kidney damage markedly diminished in compare with non-pretreated animals which received the same dose of this venom (figure 5). Note renal tubular cells (arrow) are intact. H&E x200.
Histopathological changes were noted in rats 24 h treated with 3μg/g HL venom. Dilated thrombosed vessels were observed. Severe congestion of alveolar capillaries, pulmonary edema and hemorrhagic areas were seen in HL venom treated rat lung. (Figure 8).

**DISCUSSION**

We found that HL scorpion venom induced injury in rat lung, liver and kidney in a dose and time dependant manner. Histopathological changes in mice lung, liver and kidney were reported following SC injection of HL venom in Balb/c mice (Heidarpour et al., 2012). El Nasr et al. (1992) showed that sublethal doses of Buthus quinquestriatus scorpion venom produced injury in rat liver. Similarly, liver lesion was noted in rats envenomed with Leiurus quinquestriatus scorpion (Mansour et al., 2007). Scorpion Heterometrus fulvipes venom induced liver mitochondrial swelling in rat (Venkaiah and Parhasarathy, 1983). Our histopathological finding revealed that similar to Tityus serrulatus (Correa et al., 1997), Buthus Quinquestriatus (Nawar et al., 1979), Heterometrus fulvipes (Venkaiah and Parhasarathy, 1983) Leiurus quinquestriatus (Mansour et al., 2007). We observed that HL scorpion venom produced damage in rat liver as early as 3h after intraperitoneal injection. Histological alteration was consistent with liver biochemical changes (Ahmadizadeh and Razi Jalali, 2010). We observed that HL scorpion venom produced damage in rat kidney as early as 3h after intraperitoneal injection. Histological alterations was consistent with kidney biochemical changes (Ahmadizadeh and Razi Jalali, 2010). Administration of Tityus trivitattus to mice produced renal injury (de roodt et al., 2001). Kidney damage was noted in rats envenomed with Leiurus quinquestriatus scorpion (Mansour et al., 2007).
Similar to the liver and kidney, we observed that HL venom induced pulmonary damage following intraperitoneal administration. Mesquita (2002) found that injection of tityus toxin (TSTX), a toxic fraction of the tityus serrulatus venom caused lung edema. Pathology studies of lung showed severe congestion of alveolar capillaries, pulmonary edema and hemorrhagic areas in mice lung treated with tityus trivittatus scorpion venom (de Roodt et al., 2001). Pulmonary edema was reported in patients victims of scorpion stings by Tityus serrulatus (Cupo et al., 1994; Benvenuti et al., 2002). Tityus discrepans venom produced lung lesion and respiratory distress in rabbit (D’Suze et al., 1999).

The mechanism by which scorpion venom induced cell damage is not completely understood. Dousset et al. (2005) observed lipid peroxidation in different organs following envenomation of rats by toxic fractions of Androctonus australis Hector venom. These authors showed that pretreatment of rat with N-acetylcysteine (NAC) protected animals against toxicity. Mekí et al. (2002) showed that the level of nitric oxide and lipid peroxidation significantly higher in serum of envenomed children stung with Buthus occitanus or Leirus quinquestratus when compared to healthy children (control subjects). We observed vitamin C considerably depleted lung, liver and kidney tissues against HL venom induced cell damage. Previously we found that vitamin C protected rats against HL venom induced biochemical alterations (Ahmadizadeh and Razy Jalali, 2010) As clinical symptoms were noted following HL envenomation in human and experimental animals, thus the antioxidant chemicals may have the potential to diminish HL toxicity.

El-Alfy et al. (2008) reported lipid peroxidation in cardiac tissues of rats injected Leirus quinquestratus. These authors showed that pretreatment of rats with red grape seeds proanthocyanidins (GSP) markedly decreased the cardiovascular manifestation induced by yellow Leirus quinquestratus scorpion envenomation. These authors concluded that GSP had a significant role in protecting cells against lipid peroxidation probably due to enhancement of the antioxidant defense system. Sahnoun et al. (2007) concluded that the level of oxidative stress markedly enhanced in rats myocardial tissues injected with Buthus Occitanus tunetansus. Fatani et al. (2006) reported Protective effects of the antioxidant Ginkgo biloba extract against Leirus quinquestratus venom-induced tissue damage in rats.

We observed that vitamin C protected lung, liver and kidney tissues of rat against HL venom induced toxicity. On the basis of these results, we conclude that vitamin C may prevent the occurrence of HL induced adverse effect in humans. The mechanism by which vitamin C protected cells against HL venom toxicity may be related to vitamin C is able to reduce reactive metabolites.

In summary, HL venom produced dose- and time- dependant in jury in various tissues of rat. This finding support the view that these cells may have the potential to bioactivate HL venom. The observation that vitamin C had potential to ameliorating HL venom toxicity further support this hypothesis. The effectiveness of the antioxidant, vitamin C in ameliorating venom-evoked changes indicate the involvement of oxidative stress in venom-induced cellular damages seen in the rat lung, liver and kidney tissues. However, further studies will be needed to clarify this hypostheses.

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