Pain, hyperglycemia, cytokines and insulin resistance in scorpion (Buthidae family) envenoming syndrome -Insulin-Glucose administration reverses all clinical manifestations

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Abstract
Scorpion sting causes excruciating pain at the site of sting, autonomic storm, release of catecholamines, renin, angiotensin – II, aldosterone, glucagon, glucocorticoids, either suppressed insulin secretion or hyper-insulinemia, hyperglycemia, sudden increase in free fatty acids, secretion of pro-inflammatory cytokines IL-1α, IL-1β, IL- 4, IL- 6, IL- 10, TNF-α, IFN-γ, NO; acute myocarditis, initial hypertension, cardiovascular disturbances, peripheral circulatory failure, cardiac pulmonary edema, ARDS, hyper-aggregation of platelets; failure of Na+ - K+ ATPase activity, stimulation of Ca2+ ATPase activity, pulmonary edema and many other clinical manifestations causing insulin resistance in scorpion envenoming syndrome. Scorpion envenoming is a syndrome of fuel – energy deficits, MSOF and death. Administration of insulin has a primary metabolic role in preventing, reversing ARDS, MSOF, metabolic, cardiovascular, haemodynamic, ECG changes; all the clinical manifestations, suppresses secretion and actions of pro-inflammatory cytokines and insulin resistance developed for the endogenously secreted insulin when that has become a determinant to survival in scorpion envenoming syndrome. Treatment: Continuous infusion of regular crystalline insulin should be given at the rate of 0.3 U/g glucose and glucose at the rate of 0.1 g/kg body weight/hour, for 48 - 72 hours, with supplementation of potassium as needed and maintenance of fluid, electrolytes and acid-base balance.
1. Introduction

Death due to scorpion envenoming syndrome is a common event all over the world

Scorpion stings are responsible for a number of deaths in infants, children, and adults in the tropical and sub-tropical countries all over the world (Radha Krishna Murthy, 2014 a; b; c; d; e; 2013; 2002; 2000; Ahmed et al., 2015; Elatrous et al., 2015; Sahin et al., 2015; Bahliou 2005; Cordeiro et al., 2006; Benvenuti et al., 2002; Bouaziz et al., 2008; Deshpande and Aparna, 2012; Deshpande 1998; Deshpande 1993; Deshpande et al., 2005; Deshpande and Alex, 2000; Gajanan and Dammas, 1999; Mirakabadi et al., 2006; Meki et al., 2002; Abdel-Haleem 2005; Praveen Kumar and Vasudeva Murthy, 2013; Freire-Maia and Campos, 1991; 1994; Freire-Maia and De Matos, 1993; Gueron and Weizman, 1968; Gueron and Ovsyshcher, 1987; Gueron et al., 1992; 1993; Ismail, 1993; Ismail, et al., 1992; Cologna et al., 2009; Ravi Babu and Radha Krishna Murthy, 2014; Radha Krishna Murthy and Prabhakara Rao, 2014; Amucheazi and Umeh, 2012; Tarasiuk et al., 1994; 1997; Yugandhar et al., 1999; Sofer, 1995).

The annual number of scorpion stings is more than five hundred thousand and hundreds of thousands of these victims die every year. For every person killed by a poisonous snake, 10 persons are killed by poisonous scorpions. The treatment of scorpion envenoming syndrome is a difficult poison treatment requires extensive knowledge of the patho-physiological mechanisms behind the clinical manifestations and an understanding of the clinical symptomatology.

1.1 Distribution of killer scorpions of Buthidae family

All the different species and genera of poisonous scorpions of the world belong to Buthidae family. The lethal members of the Buthidae family include the genera of Buthus, Parabuthus, Mesobuthus, Centruroides.
1.3 (b) Insulin plays an important role in thermogenesis
Insulin plays an important role in thermogenesis and insulin resistance may therefore be implicated in the defective thermogenesis and severe hyper-hydrosis in scorpion envenoming syndrome.

1.3 © Insulin lack – high blood pressure
Insulin spares sodium and uric acid from excretion, and in hyperinsulinemia – insulin resistance, these effects may contribute to transient high blood pressure in scorpion envenoming syndrome.

1.3 (d) Insulin hyperpolarizes the plasma membranes
Insulin hyperpolarizes the plasma membranes of both excitatory and non-excitatory tissues, with consequences ranging from "Baro-receptor desensitization" to "Cardiac refractoriness"—"Prolongation of QT interval", arrhythmias and various conduction defects in scorpion envenoming syndrome (Ferrannini et al., 1999).

1.3 (e) Insulin is vaso-dilatory agent
Insulin is a vaso-dilatory agent by regulating the Sodium – Potassium pump (Na+ - K+ ATPase activity) and intra-cellular calcium transients (Ca2+ ATPase activity) (Radha Krishna Murthy, 1982).

1.3 (f) Insulin exerts a host of central effects
(a) All the above mentioned clinical manifestations are reversed by administration of insulin in the experimental scorpion envenoming syndrome (Radha Krishna Murthy et al., 1988 b; 1990) and in the scorpion sting victims (Yugandhar et al., 1999, Radha Krishna Murthy et al., 1991) demonstrated by us in the years 1988-1999!

(b) Insulin is the physiological antagonist to the actions of catecholamines and all the other counter-regulatory hormones.

(c) The catecholamines bring out the cardiovascular, respiratory & ECG changes, metabolic disturbances through their action on stimulation of Alpha receptors and suppressed insulin secretion. Alpha blockers are essentially acting indirectly through insulin secretion (Radha Krishna Murthy and Anita, 1986 a; Radha Krishna Murthy et al., 2003; 1988 a; b; c; Radha Krishna Murthy and Haghnazari, 1999).

(d) Scorpion envenoming is a serious medical emergency due to over-stimulated secretions and stimulation of counter-regulatory hormonal actions.

(e) Therefore, it is logical to use insulin as insulin infusion in the critically ill scorpion sting victims with focus on the cytokines, Reactive Oxygen Species (ROS), myocardial damage, and many other...
clinical manifestations.

f) Ahmed and his co-investigators from Egypt (Ahmed et al., 2015) demonstrated insulin deficiency in their scorpion sting children which confirmed our experimental work carried out in 1986 (Radha Krishna Murthy et al., 1986 a; Radha Krishna Murthy et al., 1988 a; b; 1989; 1990 a; 1992; 1999; 2003)!

g) We also advocate the use of either insulin alone or combination of insulin and anti-scorpion serum to treat the scorpion sting victims (Radha Krishna Murthy et al., 2002; Natu et al., 2006).

h) We recommend continuous infusion of regular crystalline insulin should be given at the rate of 0.3 U/g glucose and glucose at the rate of 0.1 g/kg body weight/hour, for 48 - 72 hours, with supplementation of potassium as needed and maintenance of fluid, electrolytes and acid-base balance in scorpion sting victims.

The experimental basis of the various pathophysiological mechanisms involved in the genesis of glycogenolysis - hyperglycemia in scorpion envenoming syndrome and its reversal (in the experimental animals and scorpion sting victims) by administration of insulin, role of hyperglycemia as a toxic agent, role of pro-inflammatory interleukins – insulin resistance, insulin – an anti-inflammatory agent are reviewed.

Insulin crosses the blood brain barrier, and exerts a host of central effects such as sympahto-excitation, vagal withdrawl, stimulation of corticotrophin releasing factor, collectively resembling a stress reaction in scorpion envenoming syndrome (Ferrammini et al., 1999).

1.4 Administration of Insulin as treatment for scorpion sting victim

2.0 Pain in Scorpion Envenoming Syndrome
Poisonous scorpion stings result in excruciating pain at the site of sting and the pain reappears despite repeated local infiltration of xylocaine and other drugs.

Therefore an account of Physiology of pain, pain pathways – role of Hypothalamus – activation of neuro-endocrine axis, adverse physiological effects of acute pain and the injury response, metabolic and endocrine responses to injury, is given to understand various mechanisms in acute pain in scorpion envenoming syndrome. (Fig. 1, 2)

2.1 Apical skin - high basal sympathetic tone - cortico-hypothalamic control
Apical skin is found in those areas of the body that are exposed but are poorly insulated by subcutaneous fat, like hands and feet, web spaces of the fingers and toes. The arterioles in apical skin have a high basal sympathetic tone, and also contain arteriovenous (A-V) anastomoses, which are under cortico-hypothalamic control (Sabyasachi Sircar, 2014).

2.2 Scorpion stings at apical skin
Majority of the scorpion stings take place on the finger tips and web spaces of the fingers with apical skin (Yugandhar et al., 1999; Radha Krishna Murthy et al., 1991; Radha Krishna Murthy, 2014 a; b; c; d; e; 2013; 2002; Ahmed et al., 2015; Elatrous et al., 2015; Sahin et al., 2015; Bouaziz et al., 2008; Gajanana and Damm, 1999; Meki et al., 2002; Praveen Kumar and Vasudeva Murthy, 2013; Freire-Maia et al., 1994). This could be the reason for the rapid disappearance/ absence of scorpion venom from the site of sting (Murugesan et al., 1999).

2.3 Acute pain
Acute pain is defined as “pain of recent onset” related to injury such as scorpion sting. The ability of the somatosensory system to detect noxious and potentially tissue-damaging stimuli involves multiple interacting peripheral and central mechanisms.

2.4 Nociceptors
Nociceptors have membrane proteins that convert chemical energy into depolarizing potential. Secondary hyperalgesia occurs due to the sensitization of the Spinothalamic neurons. The pain producing substances are called algogenic substances.

2.5 Algogenic substances
Potassium ions, histamine, serotonin, bradykinin (formed from dying cells) and prostaglandin E₂ are few of the algogenic substances released from injured tissues.

2.6 Signalling molecules in pain pathways
Tissue damage associated with infection, inflammation or ischaemia; produces disruption of cells, degranulation of mast cells, secretion by inflammatory cells, and induction of enzymes such as cyclo-oxygenase-2 (COX-2). Ranges of chemical mediators act either directly via ligand-gated ion channels or via metabotropic receptors to activate and/or sensitise nociceptors. Endogenous modulators of nociception, including proteinases, pro-inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, TNF-α, IFN-γ and NO), anti-inflammatory cytokines (IL-10) and chemokines (CCL3, CCL2, CCL3CL1) can also act as signalling molecules in pain pathways (McGillicuddy et al., 2011; Macintyre, 2010; Nalini et al., 2009; Greisen et al., 2015; Elatrous et al., 2015; Bouaziz et al., 2008; Gajanan and Damm, 1999; Meki et al., 2002; Praveen Kumar and Vasudeva Murthy, 2013; Freire-Maia et al., 1994). This could be the reason for the rapid disappearance/ absence of scorpion venom from the site of sting (Murugesan et al., 1999).
Many of these signalling molecules (IL-1α, IL-1β; IL-4, IL-6, TNF-α, IFN-γ, NO, IL-10, CCL3, CCL2, CX3CL1) participating in pathways are increased either in experimental scorpion envenoming or in scorpion sting victims.

**2.7 Nociception- tissue damage**

“Nociception” is processing of noxious stimuli. Noxious stimuli activate “Nociceptors” (peripheral sensory organs), generate action potentials and transmit the information through “pain pathways” to central nervous system.

**2.8 Noxious soup of chemicals**

Pain is detected by nociceptors– the unmyelinated nerve endings. Nociceptor activation leads to generator potential. Generator potentials depolarize the distal axonal segment and initiate self propagating action potential.

Tissue injury causes release of cellular mediators like potassium ions (K⁺), hydrogen ions (H⁺), prostaglandins, bradykinin, substance P (sP), cholecystokinin (CCK), calcitonin gene-related peptide (CGRP) and activate the terminal nerve endings of sensitized nociceptor sensory afferent fibers. Substance P (sP) is responsible for further release of bradykinin and histamine from mast cells and 5HT from platelets. These cellular mediators increase vascular permeability, neurogenic edema and nociceptor irritability. The noxious soup of chemicals exacerbates the inflammatory response, and recruits adjacent nociceptors.

**2.9 Pain sensations**

The pain sensations are carried to spinal cord in spinal nerve. The dorsal root ganglion contains the soma of sensory neuron. The Primary sensory neuron carries sensory information from the sensory receptor to the spinal cord or brain stem. The lateral division of the dorsal root consists of thinly myelinated group – III (A delta) fibers and unmyelinated group-IV fibers (McGillicuddy et al., 2011; Macintyre, 2010; Nalini et al., 2009; Greisen et al., 2001).

**2.10 Secondary sensory neurons**

The secondary sensory neuron carries the sensations from the spinal cord or brain stem to the specific relay nuclei of the Thalamus. The nociceptive – specific neurons respond only to nociceptive stimuli, and the wide dynamic range (WDR) neurons respond to noxious and non-noxious stimuli. After crossing, the fibers for pain ascend in the lateral Spinothalamic tract (spinal lemniscus).

**2.11 Spinothalamic tract**

The Spinothalamic tract conveys pain sensation to the intra-laminar nuclei of the thalamus after passing and relaying in the medullary and pontine reticular formation. This pathway provides information on the sensory- discriminative aspects of pain.

**2.12 Spino-mesencephalic tract**

The spino-mesencephalic tract conveys pain sensation to the reticular formation and periaqueductal gray matter to the amygdala through parabrachial nucleus.

**2.13 Spino-parabrachial pathway**

The spino-parabrachial pathway originates from superficial dorsal horn lamina I neurons and projects to the ventromedial hypothalamus and central nucleus of the amygdala. Multiple further connections include those with cortical areas involved in the affective and motivational components of pain, projections back to the periaqueductual grey (PAG) region of the midbrain and rostroventromedial medulla (RVM), which is crucial for fight or flight responses and stress-induced analgesia, and projections to the reticular formation.

**2.14 Hypothalamus - activation of neuro-endocrine axis**

The Spinothalamic tract conveys pain fibers to the hypothalamus, which activates neuro-endocrine axis (McGillicuddy et al., 2011; Macintyre et al., 2010; Nalini et al., 2009; Greisen et al., 2001).

**2.15 Reflex sympathetic efferent response**

Reflex sympathetic efferent responses sensitize nociceptors by releasing norepinephrine which produces peripheral vasoconstriction at the site of injury. Norepinephrine stimulates the release of bradykinin (BK) and substance P (sP) leading to peripheral vasoconstriction and trophic changes.

**2.16 Tertiary sensory neuron**

The tertiary sensory neuron carries the sensations from spinal & medial lemniscus and terminates in the specific thalamic nuclei to the sensory cortex.

**2.17 Increased synthesis and extravasation of humoral proinflammatory cytokines**

Acute tissue injury results in an increased synthesis and extravasation of humoral proinflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, TNF-α, IFN-γ and NO). These cytokines are responsible for exacerbating edematous and irritative components of inflammatory pain.

**2.18 Persistent pain**

Elevated levels of IL-1β result in the development of persistent pain. The inflammatory mediators and proinflammatory cytokines activate transducer molecules such as transient receptor potential (TRP) ion channel.
2.19 Central projections of pain pathways
Different qualities of the overall pain experience are subserved by projections of multiple parallel ascending pathways from the spinal cord to the midbrain, forebrain and cortex.

The spinoreticular (spinoparabrachial) and spinomesencephalic tracts project to the medulla and brainstem and are important for integrating nociceptive information with arousal, homeostatic and autonomic responses as well as projecting to central areas mediating the emotional or affective component of pain.

The spinoparabrachial pathway originates from superficial dorsal horn lamina I neurons that express the NK1 receptor and projects to hypothalamus and central nucleus of the amygdala (McGillicuddy et al., 2011; MacIntyre et al., 2010; Nalini et al., 2009; Greisen et al., 2001).

2.20 Adverse physiological effects of acute pain and the injury response
Acute pain is one of the activators of the complex neurohumoral and immune response to injury. Thus acute pain and injury of various types are inevitably interrelated and if severe, the injury response becomes counterproductive and can have adverse effects on outcome. Combination of surgery or trauma and the associated acute pain result in a painful experience and an associated hormonal/metabolic response. This includes increased cortisol, catecholamines and glucagon, and a decrease in insulin sensitivity. (Fig. 1, 2)

2.21 Adverse physiological effects of painful experience
Clinically significant injury responses can be broadly classified as
Inflammation,
Hyperalgesia,
Hyperglycemia,
Protein catabolism,
Increased free fatty acid levels (lipolysis) and Changes in water and electrolyte flux.
In addition, there are cardiovascular effects of increased sympathetic activity and diverse effects on respiration, coagulation and immune function.

2.22 Metabolic and endocrine responses to injury in scorpion envenoming syndrome
(McGillicuddy et al., 2011; MacIntyre et al., 2010; Nalini et al., 2009; Greisen 2001)

2.23 Endocrine Secretions - responses to injury (Fig. 1, 2, 3)
Increased Catecholamines – Adrenaline & Nor-adrenaline
Increased secretions of Glucocorticoids

2.24 Metabolic responses to injury
2.24 [I] Carbohydrate Metabolism
Hyperglycemia,
Glucose intolerance,
Increased Glycogenolysis,
Increased gluconeogenesis,
Increased insulin secretions or increased insulin secretions (Hyperinsulinemia) – Insulin resistance

Increased IL-1, TNF-α, IL-6

2.25 [II] Protein catabolism
Muscle protein catabolism,
Increased synthesis of acute phase proteins
Increased secretions of Glucocorticoids
Increased Catecholamines – Adrenaline & Nor-adrenaline
Increased Glucagon secretions

Increased IL-1, TNF-α, IL-6

2.23 [III] Lipid Metabolism
Increased lipolysis and oxidation due to
Increased Catecholamines – Adrenaline & Nor-adrenaline
Increased secretions of Glucocorticoids
Increased Glucagon secretions
Increased growth hormone

2.24 [IV] Water and electrolyte flux
Retention of water and sodium
Increased excretion of potassium
Reduced functional ECF with shifts to ICF
Increased Catecholamines – Adrenaline & Nor-adrenaline
Increased aldosterone,
Increased ADH,
Increased secretions of Glucocorticoids
Increased angiotensin II,
Increased prostaglandins

2.25 Hyperglycemia due to pain
Hyperglycemia is proportional to the extent of the injury response. Injury response mediators stimulate insulin-independent membrane glucose transporters glut-1, 2 and 3, which are located diversely in brain, vascular endothelium, liver and some blood cells.

Circulating glucose enters cells that do not require insulin for uptake, resulting in cellular glucose overload and diverse toxic effects. Excess
intracellular glucose non-enzymatically glycosylates proteins such as immunoglobulins, and render them dysfunctional.

Excess glucose enters glycolysis and oxidative phosphorylation pathways, leading to excess superoxide molecules that bind to nitric oxide (NO), with formation of peroxynitrate, result in mitochondrial dysfunction and death of cells served by glut-1, 2 and 3. (Macintyre et al., 2010; Nalini et al., 2009; Greisen et al., 2001) (Fig. 3, 4, 5, 6, 7, 8)

2.26 Increased Free Fatty Acid levels associated with pain
Free fatty acid (FFA) levels are increased due to several factors associated with the injury response and can have detrimental effects on cardiac function. High levels of FFA can depress myocardial contractility, increase myocardial oxygen consumption (without increased work), and impair calcium homeostasis and increase free radical production leading to electrical instability and ventricular arrhythmias (Macintyre et al., 2010; Nalini et al., 2009; Greisen et al., 2001). We have demonstrated elevated levels of free fatty acid levels in our experimental animals injected with scorpion venom (Radha Krishna Murthy and Medh, 1986 (d); Radha Krishna Murthy et al., 1988 (d); 1988 (a); 1990; 1992 (b); 1999; 2003; Radha Krishna Murthy and Zare, 2002) (Fig. 3, 4, 5, 6, 7, 8, 9).

2.27 Protein catabolism
The injury response is associated with an accelerated protein breakdown and amino acid oxidation, in the face of insufficient increase in protein synthesis (Greisen et al., 2001).

2.28 Painful trauma - disturbed metabolic state with impaired insulin sensitivity
Painful trauma results in a disturbed metabolic state with impaired insulin sensitivity, which is related to the magnitude of the trauma. Pain reduced whole-body insulin-stimulated glucose uptake because of a decrease in non-oxidative glucose disposal. The suppression of endogenous glucose output during hyperinsulinemia tended to be decreased after pain.

Pain elicited a twofold to threefold increase in serum cortisol, plasma epinephrine, and serum free fatty acids. Similarly, circulating concentrations of glucagon and growth hormone tended to increase during pain.

2.29 Acute severe pain decreases insulin sensitivity
Acute severe pain decreases insulin sensitivity, primarily by affecting non-oxidative glucose metabolism. Counter regulatory hormonal response plays an important role. This may indicate that pain relief in stress states is important for maintenance of normal glucose metabolism (Greisen et al., 2001).

2.30 Impaired insulin action resides in skeletal muscle
The impaired insulin action after pain probably resides in skeletal muscle. Defects in both skeletal muscle GLUT-4 translocation and glycogen synthase activity could be the intracellular mechanisms of the impairment of insulin action immediately after surgery.

2.31 Noncardiogenic pulmonary edema after scorpion envenoming
Pulmonary edema, an important finding and commonly the cause of death, has two components: a cardiogenic (left ventricular dysfunction) and a non-cardiogenic component. The latter appears to be related to an increase of pulmonary vascular permeability that accompanies activation of the inflammatory cascade. Complement system activation, release of substance P, activation of mast cells and subsequent release of other mediators (PAF, leukotrienes and prostaglandins) can contribute to triggering of the inflammatory cascade (Cologna et al., 2009; Amaral and Rezende, 1997; Aparna et al., 2015; 2013; Deshpande and Aparna, 2012; Deshpande, 1998; 1993; Deshpande et al., 2005).

2.32 Scorpion venom causes release of mediators that affect inflammatory processes
Scorpion stings result in a massive release of neurotransmitters, mainly from the autonomic nervous system; however, the central nervous system is also affected. The injection of scorpion venom also causes damages to the tissue, which induces a systemic release of mediators that affect inflammatory processes, including kinins, eicosanoids, and platelet activating factor, nitric oxide, and cytokines (Ana Dorce et al., 2015).

2.33 Pregnant women are among the possible scorpion sting victims
Due to the high incidence of scorpion stings, pregnant women are among the possible victims. During pregnancy, the immune system plays an important role ensuring the normal development of the pregnancy, also preventing the development of complications. Cytokines also play a fundamental role in the development of neurons, including the proliferation, survival, differentiation and axodendritic growth, and regulation of neuronal synapses (Ana Dorce et al., 2015).

2.34 Cytokines - key role for a successful pregnancy
Cytokines are important mediators playing a key role for a successful pregnancy, where they are
involved in the implantation of the blastocyst and formation and development of the embryo, particularly in the development of the central nervous system. The uterus promotes immunological adaptations during pregnancy to prevent rejection of the fetus by the mother. IL-10 appears to be the key cytokine for protective effect on the fetal-placental unit for the maintenance of pregnancy since it inhibits the secretion of Th1 and cytokines such as IL-6, TNF-α, and IFN-g.

**Decreased levels of IL-10 - implantation loss**

Decreased levels of IL-10 in the fetuses were reported after scorpion venom injection. This alteration would explain the implantation loss. (Fig. 2). Elevated levels of proinflammatory cytokines generated by the maternal or fetal immune system have been associated with abnormal fetal brain development and increased risk for neurodevelopmental disorders (Ana Dorce et al., 2015).

### 2.35 Behavioral alterations in scorpion envenoming

We have reported behavioral alterations in our experimental animals. Changes in the levels of TNF-α, and IL-1α after the venom injection were reported. These alterations could be related to behavioral alterations, in which adult males had a decrease in the locomotor activity and females had enhanced anxiety and depression. Furthermore, TNF-α, can play an integral part in modulating anxiety-like behavior as well as in fear conditioning (Ana Dorce et al., 2015).

**2.36 IL-1 system associated with an increased risk of placental and perinatal brain damage**

The IL-1 system is associated with an increased risk of placental and perinatal brain damage. It is possible that the alterations on IL-1 levels are responsible for the increase in weight of the placenta. IFN-g along with other components of the immune system is involved in the process of neurogenesis. Under normal conditions, the microglia is activated by IFN-g, a cytokine produced by T cells, which induces cell differentiation. Scorpion venom decreased the level of IFN-g which would explain the reduction in the number of hippocampal cells in the offspring of dams envenomed during pregnancy (Ana Dorce et al., 2015). (Fig. 2)

**2.37 Scorpion envenoming causes release of proinflammatory cytokines**

The signs and symptoms of Tityus serrulatus (Ts) envenoming involve intense activation of the immune system with the release of mainly proinflammatory cytokines such as TNF-α, IL-6, INF-alpha and other mediators such as leukotriene B4 and prostaglandin E2. The uncontrolled release of pro-inflammatory mediators by macrophages can induce a generalized inflammation that can lead to multi-organ failure. *Tityus serrulatus* alpha-like toxin, (Ts5) is a proinflammatory toxin inducing the cytokine production of TNF-alpha and IL-6 (Pucca et al., 2015).

### 3. Scorpion venom increase the membrane permeability to Na+

Scorpion venom increase the membrane permeability to Na⁺ by opening the voltage sensitive Na⁺ channels, and this effect is associated with Ca²⁺ entry blockade of Ca²⁺ - activated K⁺ channels. This will result in a disturbance of transmembrane K⁺ gradient resulting in either absolute or relative hyperkalemia (Radha Krishna Murthy, 2014 a; b; c; Ismail, 1993).

### 3.1 Factors contributing and aggravating hyperkalemia

a) The deficiency of some of the normal body mechanisms causing K⁺ influx arising primarily from the pronounced prolonged hyperglycemia (Radha Krishna Murthy, 2014 a; b; c; e; Ismail, 1993; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy et al., 2003; 1999; 1992; 1990; 1988 a; b; d).

b) Enhanced glycogenolysis (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy, 2014 a; b; c; e; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy et al., 2003; 1999; 1992; 1990; 1988 a; b; d) and

c) Inhibited glycogenesis from decreased insulin secretion (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy, 2014 a; b; c; e; Ismail, 1993; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy et al., 2003; 1999; 1992; 1990; 1988 a; b; c; d) and

d) Venom induced release of catecholamines which in turn cause K⁺ efflux from the liver (Ismail, 1993; Ismail et al., 1991)

**e) Decreased serum Ca²⁺ secondary to disseminated intravascular coagulation (DIC)** (Radha Krishna Murthy et al., 1988 d), increased deposition in the heart and /or decreased intestinal absorption and increased urinary excretion (Ismail, 1993; Ismail et al., 1991)

**f) Decreased serum magnesium** (Ismail, 1993; Ismail et al., 1991)

**g) Changes in [K⁺] may shift the balance between glycogen synthesis and glycogen breakdown** (Ganong, 1987; Keele, Neil, and Joels 2000).
We have demonstrated “hyperkalemia (Radha Krishna Murthy, 1988 a); hyperglycemia, “hypocalcaemia - decreased serum Ca$^{2+}$ levels” (Radha Krishna Murthy, 1988 a); “suppression of insulin secretion” (Radha Krishna Murthy and Anita, 1986; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy et al., 2003; 1999; 1992; 1990; 1988 a; b; c; d ) and “depletion of glycogen content” (enhanced glycogenolysis) and inhibited glycogenesis in the liver, atria, ventricle and skeletal muscles in the experimental animals in acute myocarditis produced by scorpion (Mesobuthus tamingus Concanensis, Pocock) venom (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Ismail, 1993; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy et al., 2003; 1999; 1990; 1988 a; b; d )

There is sufficient experimental and clinical evidence that venoms from different scorpion species release catecholamines from the sympathetic nervous system and stimulate the cardiac adrenergic nerve endings causing sinus tachycardia, arrhythmias, conduction defects and arterial hypertension. Sinus tachycardia is due to an effect of catecholamines released by toxin on beta adrenergic receptors. Arterial hypertension is due to an effect of catecholamines released by toxin from adrenal glands and postganglionic nerve endings on alpha adrenergic receptors. Arterial hypertension is due to an effect of catecholamines released by toxin on beta adrenergic receptors. Arterial hypertension is due to an effect of catecholamines released by toxin on beta adrenergic receptors.

3.2 Scorpion envenoming - Autonomic storm - Angiotensin II

Scorpion envenoming results in an autonomic storm which leads to a massive release of catecholamines, suppressed insulin secretions (Mirakabadi et al., 2006; Gueron and Weizman, 1968; Gueron et al., 1992; Ismail, 1993; Ismail et al., 1992; Radha Krishna Murthy and Prabhakara Rao, 2014; Radha Krishna Murthy and Haghnazari, 1999) and an increase in angiotensin II levels (Radha Krishna Murthy and Vikal, 1988). Angiotensin II stimulates the release of catecholamines, thereby synergizing and amplifying each other’s actions, and these may act at least in part, at similar sites (Gueron et al., 1992; Edwin Jackson, 2006; Ganong, 1987; Keele, Neil and Joels 2000) (Fig. 1).

3.3 Severe Scorpion Envenoming causes an increase in Blood Sugar, Insulin, Glucagon, and Cortisol

Severe scorpion envenoming causes an increase in the circulating levels of blood sugar (Radha Krishna Murthy, 2014; Ahmed, 2015; Aparna et al., 2014; Elatrous et al., 2015; Cordeiro et al., 2006; Bouaziz et al., 2008; Gajanan and Dammas, 1999; Meki, 2002; Praveen Kumar and Vasudeva Murthy, 2013; Freire-Maia et al., 1994; Gueron et al., 1992; Ismail, 1993; Radha Krishna Murthy, 2015; 2014 b; 1992; 1990; Radha Krishna Murthy and Prabhakara Rao, 2014 a; Radha Krishna Murthy et al., 1999; 1988 a; b; c; d; 1986 b;) insulin, glucagon (Radha Krishna Murthy and Anita, 1986; Johnson and Ensinck, 1976; Johnson et al., 1976; Radha Krishna Murthy et al., 2003; 1988 a; d; 1992; Radha Krishna Murthy and Zare, 2002) and Cortisol (Radha Krishna Murthy et al., 2003; Radha Krishna Murthy and Zare, 2002). Subcutaneous or intravenous injection of scorpion venom (Mesobuthus Concanensis, Pocock) in the dogs caused hypo-insulin secretion 30 min after venom injection, and elevated insulin levels 60 min after venom injection. Insulin and blood glucose were higher at 60 and 120 min after venom injection in our experimental animals.

3.4 Mechanism of increased glucagon secretion

Nor-epinephrine released from the adrenergic nerve terminals of the pancreas may be more effective stimulus to glucagon secretion.

3.5 Mechanism of release of glucocorticoid and insulin secretion

Glucocorticoids could be released following stress or injury. The sympatho-adrenal axis primarily serves to maintain the pressure necessary for organ perfusion. Thus, during the “ebb phase”, the insulin levels are reduced and With the onset of hyper-metabolism, characteristic of the “flow phase”, the hormonal environment is changed and the insulin levels are increased (Douglas, 1986).

3.6 Effects of the hormonal actions are synergistic in the presence of increased circulating levels of all the catabolic counter-regulatory hormones

In the presence of increased circulating levels of all the catabolic counter-regulatory hormones, the effects of these hormonal actions are synergistic and produce hyperglycemia (Douglas, 1986). Thus, the counter-regulatory hormones and a simultaneous suppressed insulin secretion or insulin resistance is responsible for glycogenolysis in the atria, ventricles, liver; and skeletal muscles (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy et al., 2003; 1988 a; b; c; d; Radha Krishna Murthy and Haghnazari, 1999); hyperglycemia (Radha Krishna Murthy et al., 1988 a; b; c; d); lipolysis and elevated free fatty acid levels (Radha Krishna Murthy et al., 1986 a; b; c; d; 1988 a; b; c; d) and increased protein breakdown products (Ismail, 1993).

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3.7 Hormonal disturbances cause metabolic changes
Suppressed insulin secretion or hyper-secretion of insulin (insulin resistance) would result in glycogenolysis – hyperglycemia, sudden increase in Free Fatty Acids and increased protein breakdown products (Fig. 1, 2).

3.8 Hyperinsulinemia - insulin resistance
Hyperinsulinemia observed in our studies could be equated with insulin resistance. Insulin resistance could be caused by a change in the receptor membrane, a change in hormone-receptor binding characteristics, or a change in the post receptor events (Izzo, 1991) (Fig. 3).

3.9 Severe scorpion envenoming is a syndrome of fuel-energy deficits & Result in Multi-System-Organ-Failure (MSOF)
Severe scorpion envenoming is a syndrome of fuel-energy deficits and an inability of the vital organs to utilize the existing metabolic substrates. This ultimately may result in Multi-System-Organ-Failure (MSOF) and death. These changes are brought about by a massive release of catecholamines, angiotensin II, glucagon, glucocorticoids, and either insulin deficiency or insulin resistance (Fig. 3, 4, 5, 6, 7, 8, 9).

4.0 Mechanisms of production of Hyperglycemia due to scorpion venom (Fig. 3, 4, 5, 6, 7, 8)
a) Epinephrine elevates blood glucose and lactate concentration by a series of enzyme activities. In addition, insulin secretion is predominantly inhibited via alpha receptors (Edwin Jackson 2006).

b) Epinephrine also can cause glycogenolysis in muscle (Edwin Jackson 2006) thus providing substrate in the form of lactate for hepatic gluconeogenesis.

c) In addition to circulating catecholamines, Nor-epinephrine released from nerve endings in the liver might influence glucose production (Defronzo, 1987; Naomi Karau-Friedmann, 1984).

d) The liver is richly innervated by Sympathetic nerves; Stimulation of Sympathetic system can lead to increased glucose production, an effect mediated mostly through activation of alpha adrenergic receptors. This indicates a possible role of Nor-epinephrine released by the nerve terminals in the glucose production (Naomi Karau-Friedmann, 1984).

4.1 Physiological Basis of the Glycogenolysis -- Hyperglycemia in scorpion envenoming

4.2 Role of Glucagon (Fig. 3, 4, 5, 6, 7, 8)
a) Glucagon acts mostly on the liver and adipose tissue where it antagonizes the action of insulin. Glucagon raises blood glucose concentration by enhancing the breakdown of liver glycogen to glucose (glycogenolysis) and by promoting gluconeogenesis from lactate, pyruvate, glycerol and amino acids.

b) Glycogenolysis produces a rapid rise in blood glucose within a few minutes; gluconeogenesis produces a slower, more sustained rise in blood glucose lasting for hours or days (Keele, Neil and Joels 2000).

c) Glycogenolysis is mediated by activation of adenyl cyclase in the hepatic cell membrane and subsequent increase in intracellular cAMP and activation of protein kinases which in turn activate the Phosphorylase responsible for converting glycogen to glucose-6-phosphate, and inactivates glycogen synthatase. Phosphatase in the liver then acts on glucose 6 phosphate to release glucose into the hepatic venous blood (Ganong 1987, Keele, Neil and Joels 2000).

4.3 Glucagon is potent stimulant of glucose output from the liver
Glucagon as a stimulant of glucose output from the liver is more potent than insulin as a promoter of glucose retention (Edwin Jackson, 2006; Ganong, 1987; Keele, Neil and Joels 2000).

Role of catecholamines (Fig. 3, 4, 5, 6, 7, 8)
a) Catecholamines act similarly (like glucagon) to enhance glycogenolysis but on molar basis, Adrenaline and Nor-adrenaline, are weaker than glucagon; however, Nor-adrenaline released locally at sympathetic nerve terminals might have powerful effects.

b) Catecholamines antagonize insulin by increasing cAMP formation in the liver, fat and muscle; in the liver this activates Phosphorylase, promotes glycogenolysis, and leads to hyperglycemia.

c) Catecholamines promote glycogenolysis in muscle and enhance lactate formation.

4.4 Adrenalin inhibits glucose-induced secretion of insulin

4.5 The acute hyperglycemia seen in acute
myocarditis induced by injection of scorpion venom (Mesobuthus tiburtinus, Pocock) could be because of suppression of insulin release from beta cells of the pancreas as well as the capacity for adrenal catecholamines to provoke glycogen breakdown and peripheral inhibition of glucose uptake.

4.6 Reduction in glycogen content (Fig. 3, 4, 5, 6, 7)
Epinephrine stimulates inhibition of insulin secretion which in turn stimulates glycogenolysis in the muscle, thus providing a substrate in the form of lactate for hepatic gluconeogenesis (Keele, Neil and Joels, 2000). This might explain the reduction in glycogen content of atria, ventricle, and liver and skeletal muscle in rabbits, hyperglycemia in the dogs within 30 min after venom injection (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy, 2003; 1986 a; b; c; 1988 a; b; c).

4.7 Effects of acute ischemia on myocardial metabolism (Fig.4)
a) The immediate metabolic changes in the myocardium during acute ischemia are largely determined by the rates of glycolysis and glycogenolysis and to a lesser extent, of fatty acid availability in relation to the demand for phosphorylation (Oliver, 1975; Oliver and Opie, 1994).

b) Glycolysis increases with mild hypoxia, and in areas of profound hypoxia, decreased glycogenolysis occurs. Hydrolysis of stored triglycerides results with increases in Free Fatty Acids (FFA). Greater glycogenolysis was observed in atria, ventricle, and liver and skeletal muscle in rabbits, hyperglycemia in the dogs within 30 min after venom injection (Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy, 2003; 1986 a; b; c; 1988 a; b; c).

c) Important early systemic changes have been recorded in man in the first few hours of the onset of acute myocardial ischemia.

d) Feeling of impending death: This is due to an increase in catecholamines. These changes probably result from the anxiety and pain associated with ischemia, including a sustained rise in plasma catecholamines, FFA, cortisol concentrations, a transient elevation in blood glucose, and decreased plasma insulin levels (Bondy and Rosenberg, 1980; Goldstein et al., 1995; Serrano, 1992; Susan, 1996).

e) Plasma FFA is absorbed by tissues in an exponential relationship to their molar binding with plasma albumin, while glucose uptake depends on adequate concentrations of plasma insulin, which is reduced in acute myocardial infarction. In many patients, the increase in plasma FFA concentrations is such that the two main binding sites of albumin are saturated, and the ischemic myocardium extracts proportionately more FFA than at lower plasma concentrations. The ischemic myocardium is presented, therefore, with a considerable excess of FFA relative to glucose and, in a severely ischemic zone, the available oxygen may be insufficient for oxidation (Oliver, 1975; Oliver and Opie, 1994).

4.8 Renin-angiotensin system
Increased sympathetic activity causes elevated renin release by direct stimulation of juxtaglomerular cells. A subsequent increase in angiotensin II secretion enhances the ongoing sympathetic nerve output by a direct action on the brainstem and by a blunting of Baroreceptor mechanisms. Thus, the renin-angiotensin system is an important facilitator of ongoing Sympathoadrenal traffic (Radha Krishna Murthy and Vakil, 1988; Edwin Jackson 2006; Ganong, 1987; Keele, Neil and Joels 2000; La Grange, 1977; Bondy and Rosenberg, 1980).

4.9 Hyperglycemia in scorpion envenoming
Hyperglycemia (Cordeiro et al., 2006; Bouazziz et al., 2008; Gajanan and Dammas, 1999; Radha Krishna Murthy, 2002; Mirakabadi et al., 2006; Meki et al., 2002; Praveen Kumar and Vasudeva Murthy, 2013; Cologna et al., 2009; Radha Krishna Murthy, 2014 a; b; c; d; e; Radha Krishna Murthy, 2013; 2000; Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy et al., 2003; 1986 a; b; c; 1988 a; b; c; d) was found after envenoming. This could be due to a massive release of catecholamines (Ahmed et al., 2015, Aparna et al., 2015, Elatrous et al., 2015, Gueron and Weizman, 1968; Gueron et al., 1992; Ismail, 1993), increased glucagon, Cortisol (Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy et al., 2003) and changes in insulin secretion (Radha Krishna Murthy and Anita, 1986; Johnson and Ensinck, 1976; Johnson et al., 1976; Radha Krishna Murthy et al., 1988 a; b; c; d). (Fig. 3, 4, 5, 6, 7, 8)

4.10 Effects of an acute increase in epinephrine and Cortisol on carbohydrate metabolism during insulin deficiency
Elevations in plasma epinephrine (Ahmed et al., 2015; Elatrous et al., 2015; Gueron and Weizman, 1968; Gueron et al., 1992; Ismail, 1993) and Cortisol levels (Johnson and Ensinck, 1976; Johnson et al., 1976; Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy et al., 2003) are associated with scorpion envenoming. In diabetic patients, plasma epinephrine and Cortisol
levels increase during diabetic keto-acidosis. Goldstein et al. demonstrated an acute physiological rise in the plasma epinephrine level was associated with a transient increase in hepatic glucose production and a sustained fall in glucose clearance and persistent hyperglycemia (Goldstein et al., 1995). The initial increase in glucose production was primarily due to an increase in hepatic glycogenolysis, whereas the later elevation in glucose production was due to a stimulation of gluconeogenesis (Izzo, 1991).

4.11 Cortisol may be synergistic with other stress hormones
Cortisol may be synergistic with other stress hormones. Glucose production was reported to be synergistically enhanced by a combined epinephrine, glucagon, and Cortisol infusion, while glucose production was increased in an additive manner by epinephrine and glucagon infusions. Glucagon and Cortisol modify lactate gluconeogenesis in an additive rather than a synergistic manner (Goldstein et al., 1995).

4.12 Small increases in epinephrine level during insulin deficiency worsen hyperglycemia
Small increases in the plasma epinephrine level during insulin deficiency can significantly worsen the resulting hyperglycemia. This occurs as a result of what is probably an additive effect on hepatic glucose production, without any additional change in glucose clearance. The small increase in epinephrine significantly increases the importance of gluconeogenesis, as the period of insulin deficiency becomes prolonged.

5.0 Glucose toxicity
In virtually all tissues except the brain, glucose, at a fixed insulin concentration, promotes its own utilization in a concentration-dependent manner. The superiority of insulin in stimulating glucose oxidation seems to be explained by its anti-lipolytic effect. Even a small increment in serum insulin concentration promptly suppresses lipolysis, and consequently, the use of FFA for energy production, which in turn, enhances glucose oxidation. In contrast, glucose per se is unable to suppress lipolysis in man. Glucose per se (i.e. hyperglycemia) is a cellular toxin. Hyperglycemia may cause a generalized desensitization of all cells in the body through the down-regulation of the glucose receptors in the glucose transport system (Hannele, 1992).

5.1 Hyperglycemia is toxic
Hyperglycemia per se is toxic. Insulin is an anti-inflammatory agent, and is cardio-protective. Insulin can improve endothelial function, cardiac function, lipid profile and can lower blood pressure. Hyperglycemia increases the risk of myocardial infarction, peripheral vascular disease, stroke etc. Hyperglycemia is harmful.

All the above mentioned clinical manifestations are reversed after administration of insulin in our scorpion envenomed experimental animals (Radha Krishna Murthy et al., 1988 (a); Radha Krishna Murthy et al., 1990) and in scorpion sting victims (Radha Krishna Murthy et al., 1991; Radha Krishna Murthy et al., 1992 (a); Yugandhar et al., 1999).

5.2 Hyperglycemia - Reactive Oxygen Species (ROS) (Fig. 3, 4, 5, 6, 7, 8)

a) Hyperglycemia is known to increase the production of O$_2$ and other reactive oxygen species (ROS). Prostacyclin (PGI$_2$) and nitric oxide (NO) are secreted by endothelial cells, are potent vasodilators and platelet anti-aggregators. ROS inactivate prostacyclin (PGI$_2$) and nitric oxide (NO) (Das, 2007).

b) Hyperglycemia increase leukocyte rolling, leukocyte adherence and leukocyte transmigration through mesenteric venules (Booth et al., 2001). It is due to decreased release of endothelial NO (eNO), and result in inflammation. Local application of insulin completely attenuated the pro-inflammatory events, inhibited free radical generation, and NF-kappa B activation in mononuclear cells and reduced soluble intercellular adhesion molecule-1, monocytes chemo attractant protein-1 and plasminogen activator-1 production by enhancing NO synthesis (Aljada et al., 2000).

5.3 Hyperglycemia - suppressed insulin secretion – IL-6, TNF-α, IL-18 (Fig.9)
Hyperglycemia and suppressed insulin secretion cause an increase in plasma IL-6, TNF-α and IL-18 levels within 2 hours (Das, 2007).

5.4 Impaired glucose tolerance (IGT) - Increased pro-inflammatory cytokines
Hyperglycemia acutely increases pro-inflammatory cytokine concentrations by an oxidative mechanism and this effect is more pronounced in subjects with impaired glucose tolerance (IGT) (Das, 2007).

5.5 What is hyperglycemia?
Presence of hyperglycemia (hyperglycemia is defined as plasma glucose >110 mg %) is an indication that insufficient insulin (either qualitative or quantitative) is present in the circulation or more precisely there is peripheral insulin resistance.

5.6 Counter-regulatory hormones-Insulin resistance-pro-inflammatory cytokines (Fig. 4)
Peripheral insulin resistance is due to increased production of stress hormones such as
corticosteroids, adrenaline, nor-adrenaline, enhanced production of pro-inflammatory cytokines such as IL-6, IL-8, IL-18, TNF-α, and or decreased production of anti-inflammatory molecules IL-4, IL-10 and transforming growth factor – β (TGF-β) (Das, 2007) (Fig. 4).

5.7 Insufficient insulin - Increased Free Fatty Acids
Insufficient insulin causes a decrease in glycolytic substrate and an increase in free fatty acids that reduces myocardial contractility and promotes cardiac failure and arrhythmias (Oliver and Opie, 1994).

6.0 Increased TNF-α, IL-1β, IL-6 and IL-8 levels in moderate and severe cases of Tityus serrulatus scorpion sting victims
TNF-α, IL-1β, IL-6, IL-8 and IL-10 levels were significantly increased in moderate and severe cases of scorpion sting victims and the levels of these cytokines were positively correlated with the severity of envenomation, as evaluated by clinical profile and plasma venom concentration (Fukuhara et al., 2003).

6.1 Source of cytokines
a) The source of cytokines could be from the myocardium and/or from leukocytes, lymphocytes, monocytes, and macrophages.

b) Ischemia stimulates the production of pro-inflammatory cytokines from the myocardium.

c) Increased mesenteric venous pressure due to myocardial dysfunction causes increased endotoxin absorption from the gut.

d) Endotoxin is a potent stimulant of synthesis and release of pro-inflammatory cytokines from various cells/tissues especially macrophages (Das, 2007).

6.2 TNF-α (Fig. 9)
TNF-α is secreted by a variety of cells/tissues including adipose tissue, macrophages and heart.

6.3 TNF-α - insulin resistance, hypertension and inflammation
TNF-α plays a major role in insulin resistance, hypertension and inflammation. TNF-α is released by myocardium, and directly decreases myocardial contractility. TNF-α causes dysfunction and apoptosis of endothelial cells, decrease the production of eNO and enhance procoagulant activity and fibrin deposition. TNF-α stimulates endothelial cells and leukocytes to produce increased amounts of ROS that in turn inactivate eNO (Aljada et al., 2002; Meldrum and Donnahoo, 1999). (Fig. 9)

6.4 TNF-α and other pro-inflammatory cytokines depress myocardial function
Ischemic myocardium produces TNF-α. TNF-α in turn suppresses myocardial contractility, which results in increased mesenteric venous pressure and absorption of endotoxin from the gut. The absorbed endotoxin stimulates the production of TNF-α. TNF-α induces further myocardial depression and exacerbates cardiac failure. Thus, a vicious cycle is initiated that may prove fatal (Das, 2007).

6.5 Insulin – suppressed pro-inflammatory cytokines
Insulin suppresses circulating concentrations of FFA and production of pro-inflammatory cytokines TNF-α, IL-6, and ROS and enhances the production of IL-4, IL-10, eNO and PG12 (Das, 2007).

6.6 TNF-α - Apoptosis
TNF-α is secreted by a variety of cells/tissues – adipose tissue, macrophages and cardiac tissue. TNF-α plays a major role in insulin resistance, hypertension, inflammation and septic shock. TNF-α causes dysfunction and apoptosis of endothelial cells, decreases the production of eNO and enhances procoagulant activity and fibrin deposition (Levine et al., 1990).

6.7 Pregnant women are among the possible scorpion sting victims
Due to the high incidence of scorpion stings, pregnant women are among the possible victims. During pregnancy, the immune system plays an important role ensuring the normal development of the pregnancy, also preventing the development of complications. Cytokines also play a fundamental role in the development of neurons, including the proliferation, survival, differentiation and axodendritic growth, and regulation of neuronal synapses (Ana Dorce et al., 2015).

6.8 Cytokines - key role for a successful pregnancy
Cytokines are important mediators playing a key role for a successful pregnancy, where they are involved in the implantation of the blastocyst and formation and development of the embryo, particularly in the development of the central nervous system. The uterus promotes immunological adaptations during pregnancy to prevent rejection of the fetus by the mother. IL-10 appears to be the key cytokine for the maintenance of pregnancy due to its protective effect on the fetal-placental unit, since it inhibits the secretion of Th1 inflammatory cytokines such as IL-6, TNF-α and IFN-γ.

Decreased levels of IL-10 in the fetuses were reported after scorpion venom injection. This alteration would explain the implantation loss (Fig.
Elevated levels of proinflammatory cytokines generated by the maternal or fetal immune system have been associated with abnormal fetal brain development and increased risk for neurodevelopmental disorders (Ana Dorce et al. 2015).

6.9 Behavioral alterations in scorpion envenoming

We have reported behavioral alterations in our experimental animals (Radha Krishna Murthy et al., 1984 a; b; c; 1986 a; b; c; d; Radha Krishna Murthy, 1990). Changes in the levels of TNF-α and IL-1 α after the venom injection were reported. These alterations could be related to behaviour alterations, in which adult males had a decrease in the locomotor activity and females had enhanced anxiety and depression. Furthermore, TNF-α can play an integral part in modulating anxiety-like behavior as well as in fear conditioning (Ana Dorce et al., 2015).

6.10 IL-1 system associated with an increased risk of placental and perinatal brain damage

The IL-1 system is associated with an increased risk of placental and perinatal brain damage. It is possible that the alterations on IL-1 levels are responsible for the increase in weight of the placenta. IFN-γ along with other components of the immune system, it is involved in the process of neurogenesis. Under normal conditions, the microglia is activated by IFN-γ, a cytokine produced by T cells, which induces cell differentiation. Scorpion venom decreased IFN-γ levels which would explain the reduction in the number of hippocampal cells in the offspring of dams envenomed during pregnancy (Ana Dorce et al., 2015) (Fig. 8).

6.11 Scorpion envenoming causes release of proinflammatory cytokines & multi-organ failure

The signs and symptoms of Tityus serrulatus (Ts) envenoming involve intense activation of the immune system with the release of mainly proinflammatory cytokines such as TNF-α, IL-6, INF-α and other mediators such as leukotriene B4 and prostaglandin E2. The uncontrolled release of pro-inflammatory mediators by macrophages can induce a generalized inflammation that can lead to multi-organ failure. Tityus serrulatus alpha-like toxin, (Ts5) is a proinflammatory toxin inducing the cytokine production of TNF-α and IL-6 (Pucca et al., 2015) (Fig. 8).

6.12 Insulin administration decreased serum IL-1β, IL-6, Macrophage Migration Inhibitory Factor and TNF-α concentration

Insulin administration acts via the Insulin-like Growth Factor (IGF), enhances tissue perfusion and glucose uptake, suppresses lactate, FFA, glycerol production and lipolysis; improves survival. Insulin also suppresses excess production of IL-1β, IL-6, TNF-α and Macrophage Migration Inhibitory Factor (MIF), enhances the synthesis of eNO and anti-inflammatory cytokines IL-4 and IL-10, facilitates improvement in myocardial function and recovery (Das, 2001).

7.0 Free Fatty Acids (FFA) – Insulin - myocardial carbohydrate metabolism

At physiologic concentrations, plasma insulin is a major regulator of myocardial substrate exchange. Insulin promotes myocardial carbohydrate metabolism directly by stimulating carbohydrate uptake and indirectly through inhibition of lipolysis. The lowered plasma concentration of FFA mediates the indirect action of insulin, and infusion of FFA significantly blunts insulin stimulation of myocardial carbohydrate removal (Barrent et al., 1984).

Apart from controlling hyperglycemia, insulin also intervenes with many other metabolic and inflammatory pathways. Plasma free fatty acids, which are increased due to enhanced catabolism, would induce inflammation and also worsen the clinical outcome. Normalizing free fatty acids by exogenous insulin could yield significant results (Dandona et al., 2003).

7.1 Insulin – clot dissolution

Suppression of Plasminogen Activator Inhibitor-1 (PAI-1) production by insulin is of benefit as this would increase clot dissolution. There are many other potential benefits of insulin, mainly inhibition of pro-inflammatory Early Growth Response Gene-1(Egr-1) and tissue factor indicating its anti-inflammatory properties. The basic mechanism by which insulin acts as an anti-inflammatory factor is by enhancing nitric oxide production.

7.2 Insulin – Vasodilatory effect

The vasodilatory effect of insulin could also be one of the reasons for its favorable effects (Aljada et al., 2002).

7.3 Triggering of apoptosis

Apoptosis could be induced by extracellular inducers like toxins, hormones, growth factors, cytokines and nitric oxide. The process of direct initiation of apoptosis could be induced by tumor necrosis factor α (TNF α), elevated levels of cytokine IL-1α, IL-1β, IL-4, IL-6, IL-10, TNF-α IFN-γ and NO (Omran, 2003; Robbins and Cotran, 2010).
7.4 Increased production of pro-inflammatory cytokines in experimental scorpion envenoming

Scorpion envenoming causes increased production of pro-inflammatory cytokines. IL-1α, IL-1β, IL-4, IL-6, IL-10, TNF-α, IFN-γ and NO are produced and released in the experimental animals after injection of either crude scorpion venom or purified fractions of the Buthidae scorpion venoms (Omran, 2003) (Fig. 4).

7.4 Increased production of pro-inflammatory cytokines in scorpion sting victims (Fig. 4)

1. Scorpion envenoming by Leiurus quinquestriatus and B. judaicus resulted in increments of IL-6 in the serum of 8 of 10 children one to three hours after the sting. Increased plasma levels of IL-1β, IL-6, IL-8, IL-10, TNF-α, IFN-γ and NO were reported in scorpion sting patients (Omran, 2003; Robbins and Cotran 2010; Magalhaes et al., 1999; Petricevich, 2010; Ait-Lounis et al., 2012).

2. Cytokine IL-1β is produced by the venom from the scorpions Androctonus australis hector (Aah), Centruroides noxius and Tityus serrulatus.

3. Cytokine IL-4 is produced by the venom from the scorpion Aah.

4. Cytokine IL-6 is produced by the venom from the scorpions Androctonus australis hector, Centruroides noxius, Tityus serrulatus and Leiurus quinquestriatus (Magalhaes et al., 1999; Petricevich, 2010; Ait-Lounis et al., 2012).

Cytokine IL-10 is produced by the venom from the scorpions Androctonus australis hector, Centruroides noxius and Tityus serrulatus (Sofer, 1995).

Cytokine TNF-α is produced by the venom from the scorpions, Centruroides noxius and Tityus serrulatus.

Cytokine IFN-γ is produced by the venom from the scorpions Androctonus australis hector, Centruroides noxius and Tityus serrulatus (Magalhaes et al., 1999; Petricevich, 2010; Ait-Lounis et al., 2012).

Nitric Oxide (NO) is a free radical and is produced by the venom from the scorpion Buthus martensi Karch. Nitric Oxide (NO) is a free radical and the key endothelium derived releasing factor in the regulation of vascular tone and vasomotor function (Magalhaes et al., 1999).

NO is also produced by Macrophages and some neurons in the brain.

Nitric Oxide is also considered as a second messenger for a number of physiological mechanisms such as neurotransmission, vascular tone and arterial blood pressure.

NO is also responsible for penile erection (Boyd’s Textbook of Pathology 2013). This could be the reason for “Priapism” observed in scorpion sting victims.

There are three different types of NOS: endothelial (e NOS), neuronal (n NOS), and inducible (i NOS). i NOS is induced when macrophages and other cells are activated by cytokines - TNF-α, IFN-γ. NO relaxes vascular smooth muscle and promotes vasodilatation. NO reduces platelet aggregation and adhesion, inhibits mast-cell induced inflammation and inhibits leukocyte recruitment. High levels of i NOS – induced NO are produced by leukocytes, mainly neutrophils and macrophages (Magalhaes et al., 1999; Petricevich, 2010; Ait-Lounis et al., 2012).

7.5 Nitric Oxide (NO) - insulin resistance

Nitric Oxide (NO) decomposes to nitrite and nitrate. Several pro-inflammatory cytokines are capable to cause modifications in the levels of nitrite and nitrate production has been associated with severe scorpion envenoming, hypertension, septic shock and insulin resistance (Magalhaes et al., 1999; Petricevich, 2010; Ait-Lounis et al., 2012).

7.6 Action of scorpion venom - in vitro - Apoptosis

Omran carried out in vitro experiments on 293T and C2C12 cell lines to find out the mechanism of the action of the scorpion Leiurus quinquestriatus venom. The damage induced to 293T and C2C12 cell lines by scorpion Leiurus quinquestriatus venom is direct and is not secondary to disruption of the microcirculation. 50 ug/ml of the venom highly reduced the cell survival. The toxins in the Leiurus quinquestriatus venom act at the membrane level. The toxins may cross either sarcolemma or translocated by a carrier or move through pores or channels in the membranes. This allows the passage of ions down their concentration gradient resulting in osmotic changes followed by several mechanisms leading to cell death (Omran, 2003).

7.7 Cytotoxic effects due to dose of the venom

We have demonstrated that the hormonal and metabolic effects of the venom from scorpions of Buthidae family are dependent upon the dose of the venom (Radha Krishna Murthy, 1982; Balasubramaniam and Radha Krishna Murthy, 1981; 1984; Radha Krishna Murthy et al 1984 b; c; 1988 a; b; c; d; 1989; 1990; 1991; 1992; Radha Krishna Murthy, 2013; 2014 a; b; c; d; e). The
apoptotic effects of the scorpion venom are more prominent in the early stages of toxicity (Omran, 2003). Less glycogenolysis was observed in atria, ventricle, skeletal muscle and liver tissue in our experimental animals injected with higher dose of the scorpion (Mesobuthus tamulus concanesis Pocock) venom given compared to severe glycogenolysis in the animals injected with smaller dose of the venom given (Balasubramaniam and Radha Krishna Murthy, 1981; 1984).

8. Defect in insulin action - impairment in insulin secretion
In muscles and adipocytes, this would be reflected by a defect in insulin action, whereas at the level of beta cells of the islets of Langerhans, this would be manifested by impairment in insulin secretion (Defronzo, 1987; Goldstein et al., 1995).

8.1 Haemodynamic abnormalities in short-term insulin deficiency - Magnified lipolysis and beta oxidation of FFA
In diabetic keto-acidosis, the simultaneous relative insulin deficiency and excessive secretion of counter-regulatory hormones lead to magnified lipolysis and beta oxidation of FFA with a parallel hepatic overproduction and peripheral underutilization of ketone bodies. The clinical characteristics of patients are drowsiness and over-breathing. In addition, signs of circulatory collapse, such as tachycardia, weak pulse, and low blood pressure are normally present (Avogaro et al., 1996). Similar clinical manifestations are usually observed in scorpion sting victims (Ahmed et al., 2015; Aparna et al., 2015; Elatrous et al., 2015; Sahin et al., 2015; Bouaziz et al., 2008; Gajanan and Dammas, 1999; Meki et al., 2002; Praveen Kumar and Vasudeva Murthy, 2013; Freire-Maia et al., 1994; Gueron and Weizman, 1968; Gueron et al., 1992; Ismail, 1993; Choudhry et al., 2011).

8.2 Insulin levels in scorpion envenoming
Insulin levels, as measured by radioimmunoassay, were significantly either suppressed or elevated after Mesobuthus tamulus concanesis, Pocock scorpion venom injection (Radha Krishna Murthy and Anita, 1986; Choudhry et al., 2011; Radha Krishna Murthy et al., 2003; Radha Krishna Murthy and Zare, 2002; Radha Krishna Murthy, 2002; 2000; Radha Krishna Murthy and Hase, 1994; Radha Krishna Murthy et al., 1992 a; b; 1990; 1988 a; b; c; d).

8.3 Catabolic state with low Insulin / glucagon (I/G) ratio
The insulin/glucagon ratio (I/G ratio) may be more important than the levels of individual hormones. A high I/G ratio produces an anabolic state with more nutrient incorporation into peripheral tissues. A high ratio is associated with low levels of cAMP and a respiratory quotient close to 1, indicating that carbohydrates are the predominant energy source. When I/G ratio are low, a catabolic state is produced in which nutrients are mobilized. Scorpion envenoming causes a low I/G ratio (Susan 1996).

8.4 What is "Hyperinsulinemia"?
Hyperinsulinemia is said to exist when plasma insulin levels are inappropriate for the blood glucose estimated simultaneously.

8.5 Insulin resistance
When insulin levels are elevated with a normal glucose level, "true hyperinsulinemia" is the most appropriate term, while high insulin levels with elevated blood glucose levels may be referred to as "insulin resistance". Elevated insulin levels were observed 30 min following venom injection (Radha Krishna Murthy and Anita 1986; Choudhry et al 2011; Radha Krishna Murthy and Zare 2002; Radha Krishna Murthy 2002; 2000; Radha Krishna Murthy and Hase, 1994; Radha Krishna Murthy et al., 1992 a; b; 1990; 1988 a; b; c; d; 2003).

8.6 Hyper-insulinemia and hyperglycemia in scorpion envenoming syndrome
We have observed hyperglycemia along with suppressed/ reduced insulin secretion (hypo-insulin secretion) and hyperglycemia along with hyper-insulinemia in all our experimental animals. This was confirmed by Deshpande and his co-workers in their experimental animals (Choudhry et al., 2011).

8.7 Insulin receptor and the signaling pathways not defective
Exogenous insulin administration reversed haemodynamic, cardiovascular, metabolic, electrocardio graphic (ECG) changes, pulmonary edema and many other clinical manifestations in the experimental animals (Radha Krishna Murthy et al., 1988 a; 1990) and in the scorpion sting victims (Radha Krishna Murthy et al., 1991; Yugandhar et al., 1999) suggesting that the insulin receptor and the signaling pathways are not defective.

8.8 Counter-regulatory hormones and hyperglycemia
Hyper-insulinemia and hyperglycemia are observed in scorpion envenoming syndrome. The increased circulating insulin levels and failure to counter the hyperglycemia may indicate the action of counter-regulatory hormones (Radha Krishna Murthy and Haghnazari, 1999; Radha Krishna Murthy et al., 2003). We have reported an elevation of glucagon and Cortisol level in experimental animals along with changes in insulin secretion. Various investigators have reported increased Renin (Gueron and Weizman, 1968; Gueron et al., 1992), angiotensin II levels, (Radha Krishna Murthy and Hase, 1994; Gueron et al., 1992; Ismail, 1993; Choudhry et al., 2011).
Vakil, 1988) in the experimental animals and scorpion sting victims. Epinephrine and nor-epinephrine levels were also elevated in scorpion envenoming syndrome.

8.9 Hyperglycemia and hyper – insulinemia - insulin resistance
Hyperglycemia and hyper-insulinemia - insulin resistance is observed in all our experimental animals (Radha Krishna Murthy and Haghazari, 1999; Radha Krishna Murthy et al., 2003; Radha Krishna Murthy and Yeolekar, 1986; Radha Krishna Murthy et al., 1986 a; b; c; 1988 a; b; c; d) and in the studies reported by Choudhry et al 2011. Thus, development of insulin resistance (for the endogenously secreted insulin) is a possibility in scorpion envenoming syndrome.

8.10 Insulin resistant state
The relationship between insulin resistance, plasma insulin level, and glucose intolerance is mediated to a significant degree by changes in ambient plasma FFA concentration [57, 70]. Plasma FFA levels can be suppressed by relatively small increments in insulin concentration. Consequently, an elevation of circulating FFA concentration can be prevented if large amounts of insulin are secreted. If hyperinsulinemia cannot be maintained, plasma FFA concentration will not be suppressed normally, and the increase in plasma FFA concentration will result in increased hepatic glucose production. Short-term hyperglycemia can induce insulin resistance (Hannele, 1992).

8.11 Causes of insulin resistance
In any insulin-resistant state, the cause of insulin resistance can be due to an abnormal beta cell secretion, circulating insulin antagonists, or target tissue defect in insulin action (Defronzo, 1987; Douglas, 1986; Edwin Jackson2006; Oliver, 1975; Serrano et al., 1992).

8.12 Counter-regulatory hormones down-regulate insulin receptors
Insulin resistance could be caused by a change in the receptor number, hormone-receptor binding characteristics, or post-receptor events. Insulin receptors are probably down-regulated by high concentrations of agonist hormone/s (Izzo, 1991).

8.13 Insulin resistance - pre-receptor, receptor, and post-receptor abnormalities
Insulin resistance may occur because of pre-receptor, receptor, and post-receptor abnormalities (Serrano et al., 1992).

8.14 Excess endogenous or exogenous glucocorticoids are often associated with carbohydrate intolerance
Glucagon and glucocorticoids stimulate hepatic glucose production through increased activity of hepatic gluconeogenic enzymes (Serrano et al., 1992).

8.15 Post-receptor resistance - Hormonal antagonists
Post-receptor resistance can be caused by other hormones. Hormonal antagonists consist of all counter-regulatory hormones, such as growth hormone, Cortisol, glucagon, and epinephrine. Increases in circulating levels of glucagon, Cortisol and catecholamines have been demonstrated in scorpion envenoming (Izzo, 1991).

8.16 Corticosteroids decrease peripheral glucose utilization
A rise in corticosteroids decreases peripheral glucose utilization by diminishing the activity of glucose transporters and inhibiting insulin-mediated translocation of these facilitative transporters. Additionally, glucocorticoids affect insulin receptor affinity and number, decreasing insulin binding to its receptor.

8.17 Catecholaminergic hyperactivity antagonize insulin effects
States of Catecholaminergic hyperactivity antagonize insulin effects through several mechanisms. Catecholamines stimulate hepatic glucose production by direct stimulation of glycogenolysis and gluconeogenesis and indirectly by increasing glucagon secretion. Additionally, catecholamines decrease peripheral glucose disposal both in vitro and in vivo.

8.18 Effect of acidic pH to accelerate insulin dissociation from the receptor
The accelerated receptor degradation has been found to be responsible for a decreased number of receptors. The effect of acidic pH to accelerate insulin dissociation from the receptor is markedly reduced, leading to an inhibition of receptor recycling and acceleration of receptor degradation (Serrano et al., 1992).

We have demonstrated an increase in pH (acidosis) in the experimental scorpion envenoming (Radha Krishna Murthy et al., 1988 a).

8.19 Catecholamines induce inhibition of tyrosine Kinase activity
In addition to animal studies, several in vitro models of insulin resistance suggest defects in the
receptor Kinase activity. Catecholamines induce a 90% inhibition of the tyrosine Kinase activity (Serrano et al., 1992).

8.20 Tissue insensitivity to insulin
Tissue insensitivity to insulin is an important pathogenic disturbance that contributes to glucose intolerance (Bondy and Rosenberg, 1980; Serrano et al., 1992).

8.21 Tissue insensitivity to insulin in the basal state
In the basal state, the liver represents a major state of insulin resistance. This is reflected by an overproduction of glucose despite the presence of fasting hyperglycemia and hyperinsulinemia (Defronzo, 1987).

8.22 Tissue insensitivity to insulin in the insulin-resistance state
In the insulin-resistance state, muscle is the primary tissue responsible for insulin resistance (Defronzo, 1987). Severe scorpion envenoming syndrome is thus a syndrome of fuel-energy deficits and an inability of the vital organs to utilize the existing metabolic substrates. This ultimately may result in Multi-System-Organ-Failure (MSOF) and death. These changes are brought about by a massive release of catecholamines, Angiotensin II, glucagon, Glucagon, Glucocorticoids, and either insulin deficiency, suppressed insulin secretion, or insulin resistance.

8.23 Counter-regulatory hormones in scorpion envenoming syndrome – antagonistic to insulin
Knowledge of hormonal interactions have shown that the disease “Diabetes mellitus” is not due solely to insulin deficiency but also to the actions of hormones, such as glucagon, Growth hormone and glucocorticoids, which are many ways antagonistic to insulin (Keele, Neil and Joels 2000).

9.0 Central role of the adipocyte in insulin resistance
Insulin acts slowly in vivo, but rapidly in vitro, suggesting that the pathway insulin traverses from beta cell of the endocrine pancreas to insulin sensitive tissue may be altered. Insulin resistance may result from reduced capillary permeability to insulin. The transport of insulin across the endothelial barrier not only limits the rate of insulin to stimulate glucose uptake by skeletal muscle, but appears to determine the rate at which insulin suppresses liver glucose output. Because the liver circulation is fenestrated, it is not possible that insulin transport into the liver is the rate determining step for suppression of liver glucose output. Insulin is transported into an extra-hepatic tissue (Ramnanan et al., 2012).

9.1 "Second signal" is generated by the extra-hepatic tissue
A "second signal" is generated by the extra-hepatic tissue, the signal is released into the blood, and the signal in turn controls hepatic glucose output.

9.2 Second signal is FFA
The second signal is free fatty acids (FFA). Insulin, by regulating adipocyte lipolysis, controls liver glucose production. Thus, the adipocyte is a critical mediator between insulin and liver glucose output. Evidence that FFA also suppress skeletal muscle glucose uptake and insulin secretion from the B-cell supports the overall central role of the adipocyte in the regulation of glycemia.

9.3 Insulin resistance at the fat cell
Insulin resistance at the fat cell may be an important component of the overall regulation of glycemia because of the relationships between FFA and glucose production, glucose uptake, and insulin release. It is possible that insulin resistance at the adipocyte itself can be a major cause of the dysregulation of carbohydrate metabolism (Bergman and Mittelman, 1998).

9.4 Hepatic insulin resistance
Insulin is a primary regulator of hepatic glucose metabolism in healthy individuals. Failure of the liver to appropriately respond to insulin (hepatic insulin resistance) is an underlying cause of the increased hepatic glucose production (HGP).

Insulin is known to rapidly suppress hepatic glucose production (HGP) through its direct action at the liver, as well as through indirect effects thought to be mediated primarily at adipose tissue and the α-cell.

Insulin signaling in the central nervous system (CNS) has the ability to modify neural input to the liver, and as a result, to suppress hepatic glucose production (HGP). CNS insulin action has been suggested to suppress adipose tissue lipolysis and α-cell glucagon secretion, thereby providing additional potential mechanisms for central control of HGP.

9.5 Activation of the insulin-brain-liver signaling axis
The activation of the insulin-brain-liver signaling axis is required for the rapid action of insulin on glucose production. CNS insulin action plays a role in the suppression of HGP.

The insulin-driven suppression of lipolysis (and not CNS insulin-driven input to the liver) is responsible for the acute inhibition of hepatic glucose production in response to modest non-hepatic hyperinsulinemia (Ramnanan et al., 2012).
9.6 Acute brain insulin action in rodents

In rodents, acute brain insulin action reduces blood glucose levels by suppressing the expression of enzymes in the hepatic gluconeogenic pathway, thereby reducing gluconeogenesis and endogenous glucose production (EGP).

9.7 Acute brain insulin action in canine

The canine brain senses physiologic elevations in plasma insulin, and that this in turn regulates genetic events in the liver. In the context of basal insulin and glucagon levels at the liver, this input augments hepatic glucose uptake and glycogen synthesis, reducing net hepatic glucose output (NHGO) without altering EGP (Ramnanan et al., 2011).

9.8 Insulin’s central effects are redundant in acute regulation of hepatic glucose metabolism

Although the canine brain can sense insulin and, thereby, regulate hepatic gluco-regulatory enzyme expression, CNS insulin action is not essential for the rapid suppression of glucose production caused by the hormone. Insulin's direct hepatic effects are dominant, thus it appears that insulin's central effects are redundant in the acute regulation of hepatic glucose metabolism (Ramnanan et al., 2012).

9.9 Sympathetic nervous system regulates adipocyte cytokines

Scorpion venoms induce systemic inflammation associated with an increase in cytokine release and chemokine production. *Androctonus australis hector* (Aah) venom induces high plasma concentrations of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, and increased sympathetic tone. Sympathetic nervous system regulates the expression of several adipocyte cytokines through adipocyte beta-adrenergic receptor (Ait-Lounis and Laraba-Djeban, 2012).

9.10 Adipose tissue secretes various cytokines

Adipose tissue secretes various cytokines TNF-α, IL-6 and adipokines such as leptin and adiponectin. These are involved in glucose metabolism and insulin resistance. Overproduction of TNF-α in both adipose tissue and skeletal muscle contributes to insulin resistance.

9.11 TNF-α can induce insulin resistance

TNF-α can stimulate the production of other cytokines and chemokines, such as IL-6 and Monocyte Chemoattractant Protein 1 (MCP1), which can induce insulin resistance. TNF-α selectively stimulates the expression of a key component of its own signaling pathway, Mitogen-activated protein 4 kinase isoform 4 (Map4k4), through a TNFR1-dependent mechanism to induce insulin resistance in adipose tissue (Ait-Lounis and Laraba-Djeban, 2012).

9.12 Hyperglycemia and hyperinsulinemia

We have reported hyperglycemia and hyperinsulinemia in our experimental animals after scorpion envenoming (Radha Krishna Murthy, 2014 a; b; c; d; e; 2002; 2000; Radha Krishna Murthy and Haghmazari, 1999; Radha Krishna Murthy et al., 2003; 1992 a; b; 1991; 1990; 1988 a; b; c; 1986 a; b; c).

9.13 Glucose intolerance

We have reported hyperinsulinemia in our experimental animals (Radha Krishna Murthy, 2014 a; b; c; d; e; 2002; 2000; Radha Krishna Murthy and Haghmazari, 1999; Radha Krishna Murthy et al., 2003; 1992 a; b; 1991; 1990; 1988 a; b; c; 1986 a; b; c). The plasma insulin concentration increased (hyperinsulinemia) in mice injected with Aah venom. Aah venom blocks the action of insulin, resulting in glucose intolerance (Ait-Lounis and Laraba-Djeban, 2012).

9.14 Adipose tissue inflammation in scorpion venom

IL-1β, IL-6 and TNF-α concentration in adipose tissue were increased 24 hours after envenomation. These findings demonstrate that there is a local inflammatory profile in adipose tissue 24 hours following scorpion envenoming (Ait-Lounis and Laraba-Djeban, 2012).

9.15 Scorpion venom increase the TNF-α in Quadriceps skeletal muscle

TNF-α concentration was higher in animals injected with venom or its toxic fraction 24 hours post-envenomation (Ait-Lounis and Laraba-Djeban, 2012).

9.16 TNF-α and IL-1β work synergistically to cause insulin resistance

It is likely that several cytokines act collectively to amplify the inflammatory response of adipose tissue. The concentrations of TNF-α, IL-6 and IL-1β in adipose tissue were increased by venom. IL-1β and TNF-α act synergistically in adipose tissue to enhance secretion of IL-6. TNF-α induced IL-6 is in part mediated by IL-1. TNF-α induces IL-6 secretion in adipose tissue and skeletal muscle. Increased IL-6 expression in adipose tissue after envenomation may be due to TNF-α production. TNF-α and IL-1β work synergistically to cause insulin resistance.

9.17 Mechanisms of activation of pro-inflammatory cytokines

Sympathetic nervous system regulates lipolysis, fat
2015; Freire-Maia et al., 1994; Gueron and Weizman, 1968; Gueron et al., 1992; Ismail, 1993). In addition, the activity of sympathetic nervous system can contribute to insulin resistance through effects of catecholamines on adipocytes. The expression of adipocytokines is regulated through β-adrenergic receptors. The lipolytic action of venom mainly involves the β2/β1 subtype of adrenergic receptors (Ait-Lounis and Laraba-Djeban, 2012).

9.18 Adipocyte is a critical mediator between insulin and liver glucose output
Adipocyte is a critical mediator between insulin and liver glucose output. FFA also suppresses skeletal muscle glucose uptake and insulin secretion from the B-cell of the pancreas. This indicates the overall central role of the adipocyte in the regulation of glycemia.

9.19 Insulin resistance at the adipocyte itself can be a major cause of the dysregulation of carbohydrate metabolism
Insulin resistance at the fat cell may be an important component of the overall regulation of glycemia because of the relationships between FFA and glucose production, glucose uptake, and insulin release. It is possible that insulin resistance at the adipocyte itself can be a major cause of the dysregulation of carbohydrate metabolism (Bergman and Mittelman, 1998).

9.20 Insulin resistance syndrome
Insulin resistance causes many metabolic abnormalities. These are collectively known as the insulin resistance syndrome.

9.21 Insulin-sensitising mechanism
Reduced FFA availability from adipose tissues to liver and skeletal muscle is a pivotal component of the insulin-sensitising mechanism in liver and skeletal muscle. Adipocytes secrete multiple proteins which regulate insulin signalling and impact on abnormalities of the insulin resistance syndrome.

9.22 Low plasma adiponectin is associated with insulin resistance
Low plasma adiponectin is associated with insulin resistance. Like FFA, adiponectin is probably an important signalling molecule regulating insulin sensitivity in muscle and liver. Adipocyte production of Plasminogen Activator Inhibitor-1 (PAI-1), an inhibitor of fibrinolysis, and angiotensin II secretion are partially corrected by PPARγ activation (Greisen et al., 2001).

9.23 Uncontrolled release of pro-inflammatory mediators - generalized inflammation & MSOF
The signs and symptoms of T. serrulatus envenoming involve intense activation of the immune system with the release of mainly proinflammatory cytokines such as TNF-α, IL-6, INF-α and other mediators such as leukotriene B4 and prostaglandin E2. The uncontrolled release of pro-inflammatory mediators by macrophages can induce a generalized inflammation that can lead to multi organ failure.

Tityus serrulatus alpha-like toxin, Ts5 causes an increase in vitro of IL-1-α, IL-6, and TNF-α production in vitro and in vivo (Pucca et al., 2015).

9.24 Painful trauma - disturbed metabolic state with impaired insulin sensitivity
Painful trauma results in a disturbed metabolic state with impaired insulin sensitivity, which is related to the magnitude of the trauma. Pain reduced whole-body insulin-stimulated glucose uptake because of a decrease in non-oxidative glucose disposal. The suppression of isotopically determined endogenous glucose output during hyperinsulinemia tended to be decreased after pain.

Pain elicited a twofold to threefold increase in serum cortisol, plasma epinephrine, and serum free fatty acids. Similarly, circulating concentrations of glucagon and growth hormone tended to increase during pain (Greisen et al., 2001).

9.25 Acute severe pain decreases insulin sensitivity
Acute severe pain decreases insulin sensitivity, primarily by affecting non-oxidative glucose metabolism. Counter regulatory hormonal response plays an important role. This may indicate that pain relief in stress states is important for maintenance of normal glucose metabolism.

9.26 Impaired insulin action resides in skeletal muscle
The impaired insulin action after pain probably resides in skeletal muscle. Defects in both skeletal muscle GLUT-4 translocation and glycogen synthase activity could be the intracellular mechanisms of the impairment of insulin action.
immediately after surgery (Greisen et al., 2001).

9.27 Non-glucose actions of insulin
Insulin has many non-glucose actions. It acts anabolically on protein and fat metabolism and has many other actions, such as on water and salt, the autonomic and central nervous systems, and vascular and thermogenesis homeostasis (Ferrannini et al., 1999). It is tempting to suggest that pain may induce alterations in sensitivity to many of these insulin actions as well as to the insulin-stimulated glucose disposal.

The release of hormones and free fatty acids may in part explain the decrease in insulin sensitivity, suggested also by the notion of the hormones working in an additive manner on glucose metabolism.

Another possible mediator of the response to pain is neural signaling with nociceptive afferent impulses to the central nervous system and sympathetic efferent impulses to the muscles and the liver.

Sympathetic nerve activity in the liver causes increased glucose release from the hepatocytes. Cytokines could also be candidates for such mediation, as they have been shown to increase after surgery and to induce insulin resistance. Normally, the development of insulin resistance is taken to be an unfavorable sign. It invariably coexists with stressful situations.

10.0 Administration of insulin in scorpion envenoming syndrome
We were the first to report and demonstrated that the administration of insulin alone (Radha Krishna Murthy et al., 1990) or insulin and alpha-blocker (Radha Krishna Murthy et al., 1988 a) successfully reversed metabolic and ECG changes in experimental scorpion envenoming. Insulin administration along with alpha-blocker (Radha Krishna Murthy et al., 1988 a) produced more glycogenesis and lipogenesis and reversed the rise in plasma angiotensin II levels (Radha Krishna Murthy et al., 1988 b; c; d) than insulin administration alone. However, alpha-blockers are known to stimulate the gastric acid secretion; this may in turn, aggravate the existing sub-clinical or clinical acute pancreatitis known to occur in some patients, in to a fully blown-up fulminating acute pancreatitis in some scorpion sting victims.

We have demonstrated experimental acute pancreatitis and acute myocarditis due to scorpion envenoming. Sofer and his co-workers (Sofer et al., 1997; Sofer, 1995; Sofer et al., 1996; Sofer and Gueron, 1992; Sofer et al., 1991; Sofer and Gueron, 1988; Tarasiuk et al., 1994, Tarasiuk et al., 1997) observed pancreatitis in their scorpion sting children. Abdominal and mild epi-gastric pain was the complaint of 36% patients, and a mild elevation of serum amylase levels was found in 20% of these victims.

10.1 Actions of Insulin
Insulin acts on several enzymes. Insulin activates hexokinase to convert glucose to intracellular glucose 6-phosphate. Insulin activates glycogen synthetase and simultaneously inhibits enzymatic breakdown of glycogen. Insulin enhances the activity of some glycolytic enzymes and inhibits enzymes favoring glucose synthesis (Keele, Neil and Joels 2000).

10.2 Myocardial protective action
Insulin has myocardial protective action, which can be attributed in part, to its ability to control hyperglycemia. Insulin has anti-inflammatory actions and prevents apoptosis of myocardial tissue that facilitates myocardial recovery from ischemic insults (Das, 2007; 2001).

10.3 Importance of glycogen in the liver
When liver glycogen is high the rate of deamination of amino acids in the liver is depressed and the amino acids remain available for protein synthesis.

A high level of liver glycogen depresses the rate of ketone formation. A glycogen rich liver is protected against the harmful effects of many poisons (Keele, Neil and Joels 2000).

10.4 I.V. inj. of glucose + Insulin - quickest way of building up liver glycogen
The quickest way of building up liver glycogen, is by raising the blood glucose level + insulin; this obviously best done by the intravenous injection (Keele, Neil and Joels 2000).

10.5 About alpha blockers and scorpion envenoming syndrome
(a) If stimulation of nerves to the pancreas inhibits insulin secretion via the release of catecholamines (via alpha adreno-receptor stimulation to the pancreas), then it is logical to convert the inhibitory response to an excitatory response by using alpha blocking drugs.

(b) Scorpion envenoming causes massive release of catecholamines and the toxicity is mediated by alpha and beta adrenergic receptor stimulation. The Alpha adrenergic blocking action of the released catecholamines due to scorpion envenoming was demonstrated by us using Tolazoline Hydrochloride given intravenously (Radha Krishna Murthy et al., 1988 a).
Figure 1: Pain in scorpion stings – Neuro-endocrine – Metabolic Changes

Scorpion Stings

Severe excruciating pain at the site of sting
Noxious soup of chemicals, algogenic substances

Signalling molecules in pain pathways
Pro-inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, TNF-α, IFN-γ and NO),
Anti-inflammatory cytokines (IL-10)

IL-1β results in persistent pain

Massive release of catecholamines, glucocorticoids, glucagon, thyroid hormone secretions,
Angiotensin II
IL-1, TNF, IL-6 - Insulin resistance

Suppressed insulin secretions or hyperinsulinemia
Insulin resistance

Hyper-salivation, hyper-sweating, nausea, vomiting
Loss of water and electrolytes

Urine nitrogen

Intra-cellular dehydration
Loss of K⁺

Dehydration

RBC count, Hb, hematocrit,
Osmotic fragility changes, erythrocyte Na⁺-K⁺ ATPase
Cardiac sarcolemmal defects- Na⁺-K⁺ ATPase
Cardiac sarcolemmal Ca²⁺ ATPase
Initial transient hypertension, peripheral circulatory failure
Hypotension
Death

Ketogenesis in liver
Metabolic acidosis
Loss of Na⁺
Figure 2: Mechanism of effects of severe pain – cytokine production – insulin resistance - implantation loss and increased risk for neuro developmental disorders in scorpion envenoming syndrome

Scorpion Stings

Severe excruciating Pain at the site of sting; noxious soup of chemicals, algogenic substances

Pro-inflammatory cytokines *IL-1α, IL-1β, IL-4, IL-6, TNF-α, IFN-γ* and NO
Decreased anti-inflammatory cytokines - *IL-10*
Elevated levels of *IL-1β* result persistent pain

Massive Release of Catecholamines, Glucocorticoids, Glucagon, thyroid hormone secretions, ↑Angiotensin II
↑IL-1, TNF-α, IL-6 - Insulin resistance

Suppressed Insulin secretions/ Hyperinsulinemia - Insulin Resistance

**IL-10** Important cytokine for maintenance of pregnancy
Protective effect on the fetal-placental unit
**IL-10** secretion of Th1 inflammatory cytokines - *IL-6, TNF-α* and IFN-γ

**IL-1** levels - responsible for increased weight of placenta
*IL-1* system - risk of placental damage and perinatal brain damage
Levels of IL-10 in fetuses

Abnormal fetal brain development and increased risk for neuro developmental disorders

**Implantation loss**
Figure 3: Physiological Basis of the Glycogenolysis resulting in Hyperglycemia in scorpion envenoming

SCORPION ENVENOMING

AUTONOMIC STORM

MASSIVE RELEASE OF CATECHOLAMINES

ANGIOTENSIN II

LEVELS OF

GLUCAGON, CORTISOL, THYROID (T3, T4) HORMONES

SUPPRESSED INSULIN SECRETION

GLYCOGEN

GLUCOSE – 1 – PHOSPHATE

GLUCOSE – 6 – PHOSPHATE

GLUCOSE

HYPERGLYCEMIA
Figure 4: Mechanism of glycogenolysis and production of Hyperglycemia. Adrenaline and glucagon promote hepatic glycogenolysis by stimulating adenyl cyclase. Adrenaline also activates adenyl cyclase in skeletal muscle. Glucose-6-phosphatase is absent in muscle. ATP = Adenosine Triphosphate, CAMP = Cyclic AMP, ACTH = Adrenocorticotropic Hormone.
Figure 5: Hyperglycemia – Cytokines-Insulin Resistance in Scorpion Envenoming Syndrome
Hyperglycemia

Impaired Glucose Tolerance (IGT)

Pro-inflammatory events

↑ Glucose levels

↑ IL-6, Tumor necrosis factor-α (TNF-α)

↑ NADPH Oxidase activity

↑ Free radical generation

Dysfunctional endothelial eNOS synthase activity

↑ NO production

IL-6, TNF-α, IL-8, NO

Endothelial damage and dysfunction

Insulin Resistance

Organ Damage

Multi System Organ Failure

Death

Fig 6 - Hyperglycemia-Cytokines-Impaired glucose tolerance in Scorpion Envenoming Syndrome
Figure 8: Hyperglycemia - myocardial dysfunction

Scorpion Envenoming

Myocardial ischemia

Production of pro-inflammatory cytokines from myocardium

$TNF-\alpha$ & other pro-inflammatory cytokines

Depress myocardial function

Myocardial dysfunction

Absorption of endotoxin from gut

Endotoxin

Suppress myocardial contractility

Cardiac cachexia

DEATH
Figure 9: Actions of TNF-α

Scorpion Envenoming

TNF-α

- Released by myocardium, directly decreases myocardial contractility
- Insulin resistance, hypertension and inflammation
- Dysfunction and apoptosis of endothelial cells,
- Production of eNO and
- Enhances procoagulant activity and fibrin deposition
- Stimulates endothelial cells and leukocytes –
- Produce increased amounts of ROS that in tum inactivate eNO
(c) We chose Insulin (given parenterally) + Tolazoline Hydrochloride (alpha blocker) (given parenterally) + Sodium bicarbonate (given parenterally) to demonstrate the reversal of metabolic & electrocardiographic changes (Radha Krishna Murthy et al., 1988 a) and plasma angiotensin II levels (Radha Krishna Murthy et al., 1988 b; c,) induced by Indian red scorpion (Buthus tamulus) venom.

10.6 **Insulin administration reversed metabolic changes and other abnormalities due to envenoming**

10.7 **Insulin stimulates activation of glycogen synthatase system**
Insulin administration reversed metabolic changes and other abnormalities due to envenoming (Radha Krishna Murthy et al., 1988 a; 1990). Insulin stimulates activation of glycogen synthatase system. This could be the reason for an increase in glycogen content of cardiac, skeletal muscles and liver of the insulin, alpha blocker + sodium bicarbonate treated animals after envenomation. Moreover, glycogen availability may be an important independent determinant of cardiac function. Elevated glycogen in heart partially protects against mechanical deterioration in anoxia (Radha Krishna Murthy, 2014 a; b; c; d; e; 2003; 2002; 2000).

10.8 **Insulin stimulates glycogen synthesis**
Insulin stimulates glycogen synthesis. Insulin may reduce cAMP formation in adipose cells. This will reduce the lipolytic response to adrenaline or glucagon and favor anabolic processes leading to synthesis of glycogen. Thus insulin counter-acts the effects of catecholamines favoring glucose uptake and inhibition of glucose release from liver. This could be the reason for an increased glycogen content of atria, ventricle, liver and skeletal muscle after insulin administration (Ganong, 1987; Keele, Neil and Joels 2000).

**Recommendations**

**Treatment using Continuous infusion of regular crystalline insulin**

Use of xylocaine: Avoid using local xylocaine, as patient complains of more pain after half an hour once the action of xylocaine wears off. Patients with systemic manifestations do not complain of pain. Patients without systemic manifestations and patients improving from systemic manifestations complain of pain at the site. Use injection Diclofenac and injection Tramadol for pain and reassurance. Sometimes Injection Pentazocine and Injection Mezolam are required. Continuous infusion of regular crystalline insulin should be given at the rate of 0.3 U/g glucose and glucose at the rate of 0.1 g/kg body weight/hour; for 48 - 72 hours, with supplementation of potassium as needed and maintenance of fluid, electrolytes and acid-base balance.

**Special Finding**

This Review article will revolutionize the treatment of the “scorpion envenoming syndrome” caused by the poisonous scorpion stings of “Buthidae family” distributed in more than 45 developing countries.

This review article will revolutionize the perception about the new roles of life saving actions of insulin in different diseases.

**Research Highlights**

Poisonous scorpion stings result in
- Excruciating pain at the site of site of sting
- Cause “Autonomic storm”
- Produce an increase in Catecholamines, Angiotensin II, Aldosterone, Glucagon, Glucocorticoids, Thyroid hormones,
- Suppressed insulin secretion initially followed by Hyperinsulinemia
- Hyperglycemia
- Sudden increase in Free Fatty Acids
- Cardiac arrhythmias, Conduction defects, ischemia & infarction changes
- Cardiogenic & Non-cardiogenic pulmonary oedema
- Sudden increase in Pro-inflammatory cytokines
- Insulin Resistance for endogenously secreted insulin and result in
- Death
- Insulin is “Anti-inflammatory”, “Cardio-protective”, Reverses “Metabolic changes”

**Insulin administration saves “Life”**

**Conclusion**

Scorpion envenoming results in an autonomic storm, massive release of catecholamines, Angiotensin II, Glucagon, Glucocorticoids; either suppressed insulin secretion or hyperinsulinemia causing hyperglycemia, pro-inflammatory cytokines IL-1a, IL-1β, IL-4, IL-6, IL-10, TNF-α, IFN-γ and NO, insulin resistance, and many metabolic changes. Under these conditions, scorpion envenoming syndrome results in fuel-energy deficits, Multi-System-Organ-Failure (MSOF) and death. Administration of insulin-glucose infusion to scorpion sting victims appears to be the physiological basis for the control of the metabolic response when that has become a determinant to survival.
Author’s contribution and competing interests

Dr. K. Radha Krishna Murthy (Corresponding author) and all the authors declare that they have no conflict of interests. All the authors contributed equally in preparation of the manuscript. All the authors declare that they comply with the Principles of Ethical Publishing in the Journal for Endocrinology and Metabolism.

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Most important

This Review article will revolutionize the treatment of the “Scorpion Envenoming Syndrome” caused by the poisonous scorpion stings of “Buthidae family” distributed in more than 45 developing countries. This review article will revolutionize the perception about the new roles of life saving actions of insulin in different diseases.

Poisonous scorpion stings result in Excruciating pain at the site of sting Cause “Autonomic storm” Produce an increase in Catecholamines, Angiotensin II, Aldosterone, Glucagon, Glucocorticoids, Thyroid hormones, initially suppressed insulin secretion followed by Hyperinsulinemia Hyperglycemia Sudden increase in Free Fatty Acids

• Cardiac arrhythmias, Conduction defects, ischemia & infarction changes
• Cardiogenic & Non-cardiogenic pulmonary oedema
• Sudden increase in Pro-inflammatory cytokines
Insulin Resistance for endogenously secreted insulin and result in

Death

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