Characterization of biochemical pathways

Disscussion:

Scorpion envenoming resulted in alteration in pyramidin, histidin, tyrosine, glycerophospholipid and steroid hormone biosynthesis pathways after 48 hours. These effects are an outcome of acute seizures and early myocardial injuries disorder in the group of experimental rates. Metabolite set enrichment analysis results also showed that pancreas, nerve cells and mitochondria are the most affected organs.

The toxicity of a given compound refers to its ability to disrupt some biological functions at a certain level of biological organization (i. e., cell, tissue, or organ) It is also related to the amplitude and the duration of the exposure and also to the degree of absorption of the substance by the organism, its distribution, biotransformation and elimination or accumulation (Topol, 2004). Understanding the mechanism of a toxic event is a challenging task, especially in the field of drug research and development. The symptoms caused by scorpion stings in animal models are similar to those observed in humans (Padilla et al., 2005).

Glycerophospholipids are known to display a high degree of molecular heterogeneity in mammalian tissues. In addition to their function as structural components of membranes, some molecular species may participate in specific biophysical and biochemical functions (Mason and Dobbs, 1980). Krshina Murthy (2000) suggested that the probable mechanism of death due to scorpion envenoming syndrome is alteration in phospholipid fractions concentrations mainly phosphatidylinositol and phospatidylglycerol (Murthy, 2000).

He postulated that disruption of phosphatidylglycerol choline and phosphatidyl inositol are the main causes of death. Glycerophosphocholine metabolite are involved in cell signaling and membrane integrity of the cell and serine is the precursor for choline and acetylcholine biosynthesis which is required in the brain and therefore any damage of the cell membrane can affect glycerophospholipids metabolites pathway and its concentration. In our study, Glycerophospholipid metabolism was one of the main pathways that have been altered by envenomation and this confirms the Krishna Murthy hypothesis.

Scorpion envenoming causes fuel-energy deficits and results in inability to utilize the existing metabolic substrates by different organs, which ultimately may lead to death. This is caused by a massive release of catecholamine, angiotensin II, an increase in glucagon and cortisol (Amaral et al., 1994; Avogaro et al., 1996; Balasubramaniam and Murthy, 1984; Basu et al., 1990; el-Amin, 1992; Gajalakshmi, 1982; Gueron and Ilia, 1996; Mirakabadi, 2013). Steroid hormones mediate a wide variety of vital physiological functions such as anti-inflammatory agents. They are also principally, involved in cell signaling, integrity and stability of the cell membrane as well as fuel or energy storage and energy source in cells. In our study cholesterol, dehydroepiandrosterone, 2-methoxyesterone and aldosterone profiles have shown changes. Disruption of cell membrane and liver function failure resulted due to cytotoxic effect of the *H. lepturus* venom (Heidarpour et al., 2012; R . Dehghani 2012). So the alterations in steroid hormones metabolites pathway are due to cytotoxic effects of venom.

In the present investigation, envenomation by *H. lepturus* causes alteration in carnosine and 1-methylhistidine metabolite concentration of histidine metabolism pathway. Carnosine which is an endogenous cytoplasmic dipeptide(b-alanyl-l-histidine) and have numerous physiological activities in normal muscles activities (Nagasawa et al., 2001) Histidine and carnosine at low concentration could improve hyperglycemia complications with reduction of proinflammatory cytokine levels, increase insulin secretion, and enhance glutathione peroxidase activity. It has been showed that serum glucose elevated and hyperglycemia resulted by envenomation (Lee et al., 2005). Therefore, alteration in carnosine might be due to hyperglycemia state produced by scorpion venom.

GENNIP (1999) showed that pyrimidine metabolism which is the building blocks of DNA and RNA is responsible for the cell programming machinery. Pyrimidine metabolism along with purine pathway fulfills a variety of functions in the metabolism of the cell of which the most important are regulation of energy conservation and transport, formation of coenzymes and of active intermediates of phospholipid and carbohydrate metabolism (GENNIP, 1999). Nyhan(2005) postulated that the catabolic pathways for pyrimidine have yielded a number of patients with specific enzymatic deficiencies, most of them with mental retardation, seizures, or both (Nyhan, 2005; Nyhan, 2005). Degradation disorders of pyrimidine can be presented as anemia, neurological deficits or devastating multisystem mitochondrial disorder (H. Anne Simmonds and Gennip, 2003). Our results also showed that the concentration of four metabolites of pyrimidine pathway i. e. Dihydrothymine, Deoxyuridine, Deoxycytidine, and Beta-alanine were

changed which might be due to neurological deficits and acute seizures, which are caused by scorpion venom and change in cell metabolism.

Venom of many scorpions species cause the activation and delay of inactivation of neural sodium channel which induce a massive release of catecholamines and acetylcholines by the postganglionic nerve both in the circulation and in specific organs, such as increase in rate and force of contraction of the heart tissues.(Gueron and Yaron, 1970; Sofer et al., 1997) Tyrosine is the main precursor for catecholamine, dopamine, epinephrine, and norepinephrine. Our results demonstrated that some of intermediate metabolites in this pathway including iodotyrosine, p-hydroxyphenylacetic acid and acetoacetic acid metabolite show changes which might be due to increase in catecholamine secretion in the stung victim.

Similar clinical manifestations of diabetes are usually observed in scorpion sting victims(Zare and Tanikawa, 2002). Scorpion envenoming causes the release of counter-regulatory hormones (glucagon and cortisol) with suppressed insulin secretion. Under these conditions, the metabolism of carbohydrate, protein, and fat is directed towards catabolism. This provokes gluconeogenesis with glucose elevation in serum.

During catabolic states such as starvation and diabetes free fatty acids and ketone bodies, compete as fuels for muscle metabolism. Whereas, after prolonged starvation in man free fatty acids displaced ketone bodies as preferred fuels for oxidation in striated muscle (Hagenfeldt et al., 1971; Owen and Reichard, 1971) intracellular ketone-body concentrations in striated muscle increased plasma concentrations rose during starvation and

diabetic states and causes ketoacidosis and increase of H^+ ions concentration of blood . In our study, synthesis and degradation of ketone bodies specially acetoacetic acid was affected in this pathway which is in accordance with Krishna Murthy study (Murthy, 2000).

To summarize, our findings show that the application of metabolomics approaches could play an important role in the characterization of biochemical pathways in the animal model, which envenomed with *Hemiscorpius lepturus* venom. Our finding indicates the involvement of multiple system and organs in scorpion envenoming syndrome in which the most affected organs are pancreas, nerve cells, mitochondria, and spleen with similarities to acute seizures, the early marker of myocardial injuries and different seizures disorder, which confirm the earlier acute scorpion pancreatitis and myocardial injury reports (Bartholomew, 1970; Novikov et al., 2000).