HORMONAL ALTERATIONS ELICITED BY THE SCORPION VENOM OF SCORPIO MARUSPALMATUS, IN VIVO STUDY

BY

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ABSTRACT

Background: Scorpio mauruspalmatus belongs to the family scorpionidae and is common in the Middle East, and Mediterranean and regions. **Aim of the work**: is to study the hormonal alteration elicited by Scorpion mauruspalmatus. In vivo estimation of the dose according to the LD_{50}.The estimated LD_{50} of S. m. palmatus pooled venom was 10 mg/Kg and ¼ LD_{50} was used for in in vivo toxicological studies, which are hormonal alteration of this scorpion venom. **Material and Methods:** Male albino mice 4-6 months (20–25 g) were housed in polyethylene cages (5/cage), under controlled conditions of humidity (22 ±2 °C) and on a 12 h-light/dark cycle, with free access to standard laboratory food and water. All procedures relating to care and maintenance of the animals were in accordance with International Guiding Principles for Animal Research and were overseen and approved by the Suez Canal University Bioethics and Animal Ethics Committee. Adult mice were injected intraperitoneally with crude S. m. palmatus venoms (¼ LD_{50} 2.5mg/kg, 5 animals/group) from the four different locations (Wadi Sahab, Al-Agramia, Rahaba Plains and WMCD) and the levels of various hormones (testosterone, T3Triodothyronine, T4 Thyroxine, cortisol and insulin) have been determined in serum samples after 2, 6 and 24 (the time points) hours using ASD (USA) ELISA kits. A control group of animals was injected with 0.9% NaCl. **Results:** Testosterone increased at all timepoints except in 6 hour (time point). The T3&T4 level was increased at all different timepoints. Regarding the blood cortisol level was increased at three locations. However, in Sahab location the cortisol level was increased at 2 and 6 hours. However, at 24 h the concentration of cortisol was equal between treated and control groups. In Rehab location the blood insulin level in treated group was less than the control one. In Rehab location the blood insulin level in treated group was less than the control one. Regarding Sahab location the insulin concentration level was equal in treated and control groups in both 2, and 6 h time points, and increased at 24 h. In both Alagramia and WMCD locations, the insulin level concentration was increased in both 2, and 24h time point. **Conclusion:** Our study showed the S. m. palmatus venom has been altered the hormones of the study, statistically significant $p<0.05$ and remarkably increased at different time points.

Keywords: *Hormones alteration, Scorpion maurus palmatus, in vivo study.*

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INTRODUCTION

The venoms of scorpion have been used and applied as a remedy since thousands of years worldwide specially in Asia. The scorpion venom is a complicated mixture of proteins and peptides, enzymes, mucoprotiens, salts and nucleotides. Numerous studies spotlighted their robust effects against contagious agents and exposed their different biological mechanisms that are related to immunological system ***(Attrade and Pandit, 2016).***

Furthermore, a variety of elements are available in scorpion venoms. With the advent of both molecular biology and biotechnology, some of the venom components have been applied as therapeutic agents. It has been postulated that the pharmacologic action and mechanisms of those agents are performed with a completely different or at least unidentical with the mechanisms which usually performed by ordinary therapeutic agents ***(Zhijian et al., 2006; Attrade and Pandit, 2016).***

It is well known that the first introduction of scorpion antivenom treatment was in 1909, continues to be the only technique applied for the emergency medicine against scorpion sting. The primary usage of scorpion venom is the planning and elaboration of antibodies having the efficacy to be applied as anti-venom ***(Theakston, et al., 2003).***

Several investigations highlighted their potent effects against microbes and showed their potential to modulate varied biological mechanisms that are concerned in immune, nervous, cardiovascular and neoplastic diseases. As a result of their necessary

structural and purposeful diversity, it's projected that scorpion-derived peptides might be accustomed to develop new specific medicine. The therapeutic potential of scorpion venoms and toxins and also the doable mechanisms for their anticancer, anti-inflammatory, antimicrobial activities and for the treatment of autoimmune, cardiac, haematological, infectious, osteoporosis, homeostasis and neurological diseases ***(Erasto et al., 2020).***

Scorpionism is common and worldwide condition, especially in subtropical and tropical areas. In Upper Egypt, scorpions' poisoning so far is considered a life threatening condition, especially to children ***(Mohamed et al., 2014).*** Releasing the catecholamines and the interaction between both sympathetic and parasympathetic stimulation are behind the clinical manifestations of scorpionism ***(Bahloul et al., 2010; Bahloul, et al., 2018).*** Scorpion venom is an excellent and promising candidates for drug design that might be separated and isolated from various components of the venom. However, a variety of components are present in these venoms, some of which have shown potential applications as therapeutic agents. The advancements in biotechnology have made it feasible to synthesize new natural products like parts of venom refined with therapeutic properties. The therapeutic effects of those agents are typically achieved by mechanisms that are completely different from that of typical therapeutic agents. Scorpion and its organs are known to cure brain disorder, rheumatism and male impotency since medieval times ***(Shao et al., 2007).***

Scorpionism is considered a major problem in both emergency and clinical toxicology in numerous countries. About 1.2 million scorpion stings are recorded worldwide every single year ***(Chippaux, 2012).*** On the other hand, scorpions have been used and applied in conventional medicine, mainly in Africa and Asia ***(Goudet et al., 2002).*** It is well known that, the hormones formed and released by the glands in our bodies for control nearly all the processes in our body. Hormones have many physiological functional processes, which are extremely important in maintaining homeostasis in the body. Those hormones are in charge of our body's functions, such as metabolism, growth and development. In addition to, sexual function and even sleep. Testosterone has its impact on growth, development and also on both sexual and social life of the couples ***(Nouira et al., 2005).***

Scorpion venom is a wealthy supply of active molecules, like proteins, peptides, and Necessary therapeutic activities, especially in both diabetes and erectile dysfunction. Moreover, scorpions' venoms are wide utilized in Chinese ancient remedy ***(Zeng et al., 2005; Pajovici et al., 2012).***

Our study will address the gap of knowledge via studying the effect of the scorpion venom on the testosterone, T3, T4, cortisol, and. insulin.

AIM OF THE WORK

The aim of this work was to study the hormonal alteration elicited by Scorpion maurus palmatus in vivo.

MATERIAL AND METHODS

1. Extraction and preparation of scorpion venom

Adult specimens of the Egyptian scorpion S. m. palmatus were collected from two different geographically isolated locations in Egypt (Sinai Peninsula, and the North coast). Scorpions were captured from different locations (Wadi Sahab, El-Agramia and Rahaba Plains) in the southern region of the Sinai Peninsula (910–1676 m above sea level), an area geographically separated from the Western Mediterranean Coastal Desert (WMCD; 30.5 m above sea level) by the Suez Canal and Gulf of Suez from where a second group of scorpions were collected ***(Abdel-Rahman et al., 2009).*** Captive scorpions from the four locations were kept separately in individual containers. Scorpions were milked using the squeezing method according to ***Abdel-Rahman et al. (2009)*** and individual venom samples collected and lyophilized. The freeze-dried pooled venom was stored at -20 °C prior to use.

2. Determination of the median lethal dose (LD_{50}) value of S. m. palmatus venom.

The LD_{50} of S. m. palmatus venom was calculated according to the method described by ***Meier and Theakston (1986).*** According to this method, eight albino mice received graded doses of S. M. Palmatus pooled venom, ranging from 1.0 to 15 mg/kg (intraperitoneally injection), and monitored for 24 h. The toxicological symptoms as well as

the mortality were closely observed and recorded. The estimated LD_{50} of S. m. palmatus pooled venom was 10 mg/Kg and ¼ LD_{50} was used for toxicological studies (hormonal alteration) of this scorpion venom.

3. Experimental animals and Study design

Male albino mice (20–25 g), 4-6 months age, were housed in polyethylene cages (5/cage), under controlled conditions of humidity (22 ±2 °C) and on a 12 h-light/dark cycle, with free access to standard laboratory food and water. All procedures relating to care and maintenance of the animals were in accordance with International Guiding Principles for Animal Research and were overseen and approved by the Suez Canal University Bioethics and Animal Ethics Committee (REC94/2022). Adult mice were injected intraperitoneally with crude S. m. palmatus venoms (¼ LD_{50} 2.5mg/kg, 5 animals/group) from the four different locations (WadiSahab, El-Agramia, Rahaba Plains and WMCD) and the levels of various hormones (testosterone, T3Triodothyronine, T4 Thyroxine, cortisol and insulin) have been determined in serum samples after 2, 6 and 24 (time points) hrs using ASD (USA) ELISA kits. A control group of animals was injected with 0.9% NaCl.

Pooled venom: To prepare pooled venom, 40 adult scorpions from each location have been electrically milked in one Eppendorf tube and then the venom extracted, freeze, dried and stored at -20C.

Mortality had been taken place in determining e the lethal dose. We used 10 mice with different doses and the found that 10 mg/kg was the LD_{50}.Based on the equation we used the 1/4 LD_{50} 2.5mg/kg, as a toxic dose for hormonal alterations. Our findings showed no mortality during the experiments of hormone changes or alterations.

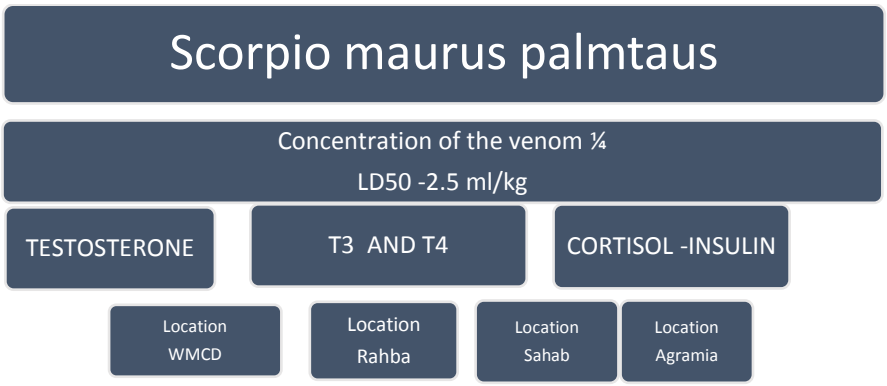

Fig. (1): Flow diagram shows the scorpion venom concentration, Hormones, and locations

STATISTICAL ANALYSIS:

The data will be collected, checked, revised, and organized in tables using Microsoft Excel 2016. Data will be subjected to outliers' detections and normality statistical test to detect whether the data are parametric or nonparametric. Data will be analysed for descriptive statistically numerical description. Inferential statistics for evaluating and comparing between different time points represents four sites (WMDC, Rahaba, Agramia, Sahab) was presented as mean and standard deviation. The difference between timepoints with repeated measures ANOVA. Difference between Control and Treated groups will be performed by independent t-test at 0.05 level. Data analyses will be carried out using computer software. Statistical Package for Social Science SPSS (IBM-SPSS ver. 28.0 for Mac OS) ***(Knapp, 2017)***.

RESULTS

Various blood biochemical parameters were presented in tables (1-5). The blood biochemical parameters include testosterone, T3, T4, cortisol, and insulin. A significant difference was observed between timepoints, Treatments, and sites. In addition to the interaction between variables as revealed by repeated measures two-way ANOVA.

Table (1) showed that the concentration of testosterone level at different time points for WMCD (Western Mediterranean costal desert) location. The treated group is increased at all different time points as compared to control group; the p value is statistically significant $p<0.001$. Regarding the Rahaba location, the concentration level increased in all time points except in 6 hour. In Algramia location the testosterone concentration was remarkably increased at 24

hour. In addition to Sahab location the testosterone concentration was increased at all the time points. **Table (2)** showed that T3 level at different timepoints in four different locations. The T3 concentration level was increased at all different time points and locations. In addition to Sahab location, the T3 concentration was remarkably increased as compared to control group.

Table (3) showed that T4 level was increased at all time points and locations. **Table (4)** showed that blood cortisol level at different timepoints in four locations. The concentrations of blood cortisol level were increased at all different time points at WMDC, Rahba, and Alagramia locations. However, in Sahab location the cortisol blood level was increased at 2 and 6 hours. However, at 24 hours concentration the treated group was equal to control group 0.4. The $p<0.001$ is significant in both 2, and 6 hours. **Table (5)** showed that the blood insulin level at different time points for four locations. In WMCD location the level of insulin concentration was increased in both time points 2, 24 hours In Rahaba location the concentration of insulin level in treated group was less than the control one. The Alagramia group showed that the level of insulin concentration at both time points 2, and 24 hours was increased. However, the control group was greater than treated group at 6 hours' time point. Regarding Sahab location the insulin concentration level was equal in treated and control group at both 2, and 6 hours timepoints. However, the insulin level was increased at 24 hours timepoints.

Table (1): Testosterone level at different timepoints represents four sites (WMCD, Rahaba, Agramia, Sahab) at both control and treatment group.

Site		**testosterone/ time points (hrs)**						**ANOVA**
		2		**6**		**24**		
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
WMCD	**C**	**3.0**	0.1	**3.0**	0.1	**2.8**	0.1	0.206ns
	T	**4.6**	0.2	**7.1**	0.0	**5.7**	0.2	<0.001***
	p	<0.001***		<0.001***		<0.001***		
RAHABA	**C**	**3.0**	0.1	**7.9**	1.7	**2.8**	0.1	0.021*
	T	**4.9**	0.1	**7.9**	1.7	**3.5**	0.2	0.04*
	p	<0.001***		1.00ns		0.005**		
AGRAMIA	**C**	**3.2**	0.1	**3.0**	0.1	**3.3**	0.2	0.223ns
	T	**2.9**	0.1	**3.7**	0.2	**16.2**	2.5	0.002**
	p	0.032*		0.002**		<0.001***		
SAHAB	**C**	**3.2**	0.1	**3.0**	0.1	**3.3**	0.2	0.223ns
	T	**4.5**	0.2	**7.0**	1.4	**5.4**	0.3	0.144ns
	p	<0.001***		0.011*		<0.001***		
Corr.model		<0.001***		**Groups x Site**		<0.001***		
Groups (G)		<0.001***		**Group x time**		<0.001***		
Site (S)		0.037*		**Site x time**		<0.001***		
Time (T)		<0.001***		**Group x Time x Site**		<0.001***		

Table (2): Blood T3 level at different timepoints represents four sites (WMCD, Rahaba, Agramia, Sahab) at both control and treatment group.

Site		T3/ time points (hrs)						ANOVA
		2		6		24		
		Mean	SD	Mean	SD	Mean	SD	p-value
WMCD	C	**155.3**	3.9	**166.0**	8.1	**161.0**	7.6	0.352ns
	T	**202.7**	1.3	**219.3**	3.7	**220.2**	5.5	0.047*
	p	<0.001***		<0.001***		<0.001***		
RAHABA	C	**155.3**	3.9	**166.0**	8.1	**161.0**	7.6	0.352ns
	T	**195.4**	9.2	**207.9**	8.4	**199.1**	4.8	0.254ns
	p	0.001***		0.003**		<0.001***		
AGRAMIA	C	**159.8**	5.0	**155.2**	4.6	**156.0**	5.9	0.635ns
	T	**170.4**	6.0	**199.8**	9.2	**183.2**	9.6	0.056ns
	p	0.169ns		0.001***		0.025*		
SAHAB	C	**159.8**	5.0	**155.2**	4.6	**156.0**	5.9	0.635ns
	T	**207.9**	3.8	**205.0**	6.1	**244.1**	13.5	0.025*
	p	<0.001***		<0.001***		<0.001***		
Two-Way repeated measures ANOVA								
Corr.model		<0.001***		**Groups x Site**		<0.001***		
Groups (G)		<0.001***		**Group x time**		<0.027*		
Site (S)		<0.001***		**Site x time**		<0.036*		
Time (T)		<0.005**		**Group x Time x Site**		0.032*		

Table (3): Blood T4 at different timepoints represents four sites (WMCD, Rahaba, Agramia, Sahab) at both control and treatment group.

Site		T4/ time points (hrs)						ANOVA
		2		6		24		
		Mean	SD	Mean	SD	Mean	SD	p-value
WMCD	C	**6.7**	0.2	**6.6**	0.5	**6.1**	0.4	0.34ns
	T	**10.1**	0.2	**11.4**	0.3	**13.0**	0.1	<0.001***
	p	<0.001***		<0.001***		<0.001***		
RAHABA	C	**6.7**	0.2	**6.6**	0.5	**6.1**	0.4	0.34ns
	T	**11.2**	0.5	**9.2**	0.1	**10.7**	0.8	0.032*
	p	<0.001***		<0.001***		<0.001***		
AGRAMIA	C	**6.6**	0.1	**6.4**	0.5	**6.2**	0.4	0.572ns
	T	**7.6**	0.3	**9.2**	0.7	**8.9**	0.1	0.121ns
	p	0.011*		0.006**		<0.001***		
SAHAB	C	**6.6**	0.1	**6.4**	0.5	**12.2**	1.0	0.004**
	T	**10.3**	0.1	**12.2**	1.0	**12.7**	0.6	0.11ns
	p	<0.001***		<0.001***		0.696ns		

Two-Way repeated measures ANOVA				
Corr.model	<0.001***	**Groups x Site**	<0.001***	
Groups (G)	<0.001***	**Group x time**	0.138 ns	
Site (S)	<0.001***	**Site x time**	<0.001***	
Time (T)	<0.001***	**Group x Time x Site**	<0.001***	

Table (4): Blood cortisol at different timepoints represents four sites (WMCD, Rahaba, Agramia, Sahab) at both control and treatment group.

Site		**cortisol/ time points (hrs)**						**ANOVA**
		2		**6**		**24**		
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value**
WMCD	**C**	**0.4**	0.0	**0.3**	0.0	**0.4**	0.0	0.002**
	T	**0.7**	0.0	**0.6**	0.0	**0.6**	0.0	0.002**
	p	<0.001***		<0.001***		<0.001***		
RAHABA	**C**	**0.4**	0.0	**0.3**	0.0	**0.4**	0.0	0.052ns
	T	**0.9**	0.0	**0.6**	0.0	**0.5**	0.0	<0.001***
	p	<0.001***		<0.001***		0.003**		
AGRAMIA	**C**	**0.5**	0.0	**0.4**	0.0	**0.4**	0.0	0.003**
	T	**0.6**	0.0	**0.6**	0.0	**0.6**	0.0	0.279ns
	p	0.006**		<0.001***		<0.001***		
SAHAB	**C**	**0.5**	0.0	**0.4**	0.0	**0.4**	0.0	0.003**
	T	**0.9**	0.0	**0.9**	0.0	**0.4**	0.0	<0.001***
	p	<0.001***		<0.001***		1.00ns		
Two-Way repeated measures ANOVA								
Corr.model		<0.001***		**Groups x Site**		<0.001***		
Groups (G)		<0.001***		**Group x time**		<0.001***		
Site (S)		<0.001***		**Site x time**		<0.001***		
Time (T)		<0.001***		**Group x Time x Site**		<0.001***		

Table (5): Blood insulin level at different timepoints represents four sites (WMCD, Rahaba, Agramia, Sahab) at both control and treatment group.

Site		**insulin/ time points (hrs)**						**ANOVA**
		2		6		24		
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	p-value
WMCD	**C**	**15.9**	0.6	**16.4**	1.0	**14.8**	0.3	0.129ns
	T	**18.0**	1.1	**14.2**	0.6	**15.8**	1.0	0.094ns
	p	0.095ns		0.053ns		0.345ns		
RAHABA	**C**	**17.0**	1.5	**17.0**	1.7	**15.1**	0.8	0.118ns
	T	**16.9**	1.1	**14.9**	0.8	**12.5**	0.1	0.007**
	p	0.955ns		0.242ns		0.004**		
AGRAMIA	**C**	**14.7**	0.6	**15.8**	1.3	**14.5**	0.6	0.472ns
	T	**19.1**	1.9	**13.4**	0.5	**19.0**	1.9	0.093ns
	p	0.04*		0.095ns		0.033*		

Site		insulin/ time points (hrs)						ANOVA
		2		6		24		
		Mean		**SD**		**Mean**		p-value
SAHAB	**C**	**14.4**	0.7	**15.8**	1.3	**14.5**	0.6	0.408ns
	T	**14.6**	0.9	**15.8**	1.3	**16.4**	1.0	0.383ns
	p	0.84ns		1.00ns		0.096ns		
Two-Way repeated measures ANOVA								
Corr.model		<0.001***		**Groups x Site**		0.013*		
Groups (G)		0.336 ns		**Group x time**		0.002**		
Site (S)		0.502 ns		**Site x time**		0.009**		
Time (T)		0.088 ns		**Group x Time x Site**		0.105 ns		

DISCUSSION

Our study revealed that the scorpio maurus palmtaus has altered the hormones of the study. And these changes disclosed by increase in the concentrations of the hormones at different time points 2, 6, 24 hours. The blood biochemical parameters include testosterone, T3, T4, cortisol, and insulin. A significant difference was observed between timepoints, Treatments, and sites. In addition to the interaction between variables as revealed by repeated measures two-way ANOVA.

Our findings showed that the concentration of Testosterone level was increased at different time points. In Rahaba location, Testosterone increased in all timepoints except in 6 hour (timepoints), and remarkably increased at 24 hour. In addition to Sahab location the level was increased at all-time points. The T3 and T4 level was increased at all different timepoints, in all locations and Sahab location as well regarding the blood cortisol level was increased at three locations at all the different time points. However, in Sahab location the cortisol level was increased at 2 and 6 hours. In Rehab location the blood insulin level in treated group was less than the control one. In both Alagramia and WMDC locations, the insulin level concentration was increased in both 2, and 24h time point.

Our findings could be supported and explained by ***Radha and Murthy, (2014)*** who found that hyperglycemia is often observed in severe scorpion-envenomed patients. It is due to a severe autonomic storm with a massive release of catecholamines, increased, cortisol levels, and either decreased of insulin levels or insulin resistance.

As evident from the results the scorpion venom increase the cortisol level which was in agreement of previous studies that shown that severe scorpion envenomation cause an automatic storm resulting in a massive release of counter-regulatory hormones (cortisol) ***(Radha et al., 2016).*** In agreement with Ahmed and colleagues who found that children with severe envenomation had significantly higher levels of cortisol compared with our findings showed that the cortisol level was increased in different locations, at different time points. Furthermore, our findings are partly consistent with theirs, in some location (Rahaba) the concentrations of treated group was less than the control one. And in other location the insulin concentration was equal to the control. Insulin levels decreased significantly in severe cases of scorpion compared with mild ones, leading to hyperglycemia. The same results were published in some experimental studies ***(Ahmed et al., 2015).***

Aldosterone diminishes glucose–stimulated insulin secretion in vivo and in vitro from isolated pancreatic islets and cultured B cells though a mineralocorticoid-receptor independent mechanism **(*Luther and Brown 2011).*** Insulin levels are either inhibited or elevated after envenoming ***(Radha et al., 2016),*** which is consistent with our findings.

Collectively, our results were similar to experiments with different animals, such as dogs. In previous studies, intravenous

injection of scorpion venom (Meso buthustambulus) 4mg/kg in experimental dogs resulted in a suppressed insulin secretion and subcutaneous injection of scorpion venom (3mg/kg)in dogs resulted in suppression of insulin secretion 30min after injection , and elevated insulin levels 60 min after venom injection ***(Murthy and Zera, 1998*).** In other experimental studies, both insulin and blood glucose were found to be higher after 60 and after 120 min of venom injection ***(Rahda et al., 2016).***

Our study was partly consistent with ***Razi et al., (2020)*** who revealed that there was a significant decrease in insulin levels in the treated rats compared to control group, with highest reduction at 8 and 24h after venom injection.

On the contrary, our results revealed the concentration of (T3 & T4) hormones was increase in all locations of the study due to injection of the scorpio mauruspalmatus venom. On the other hand, injection of M. eupeus venom resulted in a significant reduction in the thyroid hormones (T3&T4). These changes might be attributable to an autonomic storm through the release of catecholamines following envenomation, which led to a decrease in insulin secretion and subsequent reduction of T3 and T4 concentration in the blood. These findings are consistent with other previous studies in this regard ***(Mendil et al., 2016).***

In contrast to study performed by ***Murthy and Zare (1998)***, our data have shown an increase in both hormonesT3 and T4. Bu their findings were a significant decrease in T3 and T4 levels following the injection of Mesobuthus venom in dogs.

Furthermore, Andhector scorpion toxin was associated with severe inflammatory response, liver tissue damage and hyperglycemia accompanied by hyperinsulinemia in a previous study in rats. It is also possible that the insulin measured after venom injection was not functionally ***(Razi et al., 2020).***

Moreover, our findings were partly consistent with the M. eupeus scorpion venom which can affect the endocrine system by suppressing the secretions of essential metabolic hormones decrease T3 and T4, ours indicated the reverse (increased in both T3, T4) and excessive release of, cortisol, in which our findings agree with these alterations might be responsible for the pathogenesis of a variety of clinical symptoms following envenomation ***(Taibi, and Laraba, 2015).***

Arthropods and their products are present in impotence treatment even nowadays, due to century's long tradition, easy availability and high diversity ***(Razi et al., 2020).*** Therefore, our testosterone findings showed significant high concentration levels as compared to control group, in the entire locations as well as at different time points. And this is a salient feature in our findings that explains why scorpio mauruspalmatus venom is an excellent candidate for erectile dysfunction in general. The diversity in the results might be due to the difference in the type of studied scorpion species and venom as well as the route of administration.

Limitations of the study:

The findings of this study have to be seen in the light of some limitations. Such as the following points.

1- Chronotoxicology: The influence of biological rhythms on the toxicity of substance. Biorhythmicity of living organisms is well known for many dozens of years in numerous studies in the science of cell biology, physiological lab work and more recently in pharmacology and in different branches of Toxicology. Reality of temporal alterations in functions and structures of biological systems is clear whatever their level of complexity. Such temporal variations can explain that the same toxic does not induce the same efficacy in living organisms, if it is given at different hours in the day or at different seasons in the year. This considers the classical of the temporal dimension of the toxicology, so called chronotoxicity, describing the influence of time administration of drugs (toxic venom), may induce some variation on the experiments.

2-Methods of Milking or extraction of venom:

a. Electrical method of extraction is an efficient method to get good quality and higher quantity of venom as compared to manual venom. However, we used both the

manual method andelectrical method of extraction as well.

b. Manual extraction of venom: The telson were later separated from each scorpion and the venom was squeezed out and collected in a fine tube. A prevenom which is usually a watery secretion was collected and obtained followed via milky droplets (venom).One type produces the toxins, while the other method produces just mucus. And that is why we have to milk the scorpion personally and not let the students milking the scorpion alone.

3-Collection of scorpions and their maintenance: The collected scorpions might be from subspecies that is why we have to check after the hunter, to be certain that this is the species of the study. Effect of milking method, scorpion nutrition and temperature might have diversity on the venom production. However, the mentioned limitations did not affect the reliability of our findings.

CONCLUSION

Our study showed the S. M. Palmatus venom has been altered the hormones of the study, statistically significant $p<0.05$ and remarkably increased at different time points.

RECOMMENDATIONS

1- Other experimental studies on the hormones alteration of scorpio maurus palmatus with different materials and methods to give reliability for previous similar study findings.

2- Further studies should be conducted on endocrine hormones and investigate its effect with different assays.

3- Based on our findings the toxic effect of scorpio maurus palmatus is mild toxicity, and that is why the scorpion is a good candidate for drug design. Furthermore, studies to link the hormones alterations as novel therapeutic agents against cancer and chronic or refractory disorders.

4- The findings of our study open the field of drug design and vaccination.

5- Experimental medical research in clinical and basic sciences often related to medical application. Our hormones alterations findings could be further investigated, and applied as selective toxicity in certain harmful cells such as lethal common disorders.

6- The use of scorpion venom in both cytotoxicity and hormones alterations could be applied, not only for its immediate impact but also for the long term application and implications.

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التغيرات الهرمونية الناتجة عن سم العقرب سكوربيو مورس بالموتس، في دراسة في الجسم الحي

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الملخــص العربـــى

المقدمة: ينتمى عقرب سكوربيو مورس بالموتسالى عائلة سكوربيونأداسكوربواندا وهو شائع فى الشرق الاوسط ومناطق البحر الابيض المتوسط.
هدف الدراسة: هو دراسة التغيرات السمية لعقرب ا لدراسة على الهرمونات .الهرمونية
تحضير الجرعة المميتة:(LD_{50}). تجمع السم بالماتوس كان ١٠ ملغ / كغ . وربع الجرعة القاتلة ٢.٥ ملغ استخدمت للدراسات السمية لمعرفة (التغيير الهرموني) لهذا العقرب.
تصميم الدراسة: تم إيواء ذكور الفئران البيضاء فى سن ٤-٦ أشهر ، وزنهم من ٢٠-٢٥ جم، في أقفاص بولي إيثيلين (٥ / قفص) ، تحت ظروف رطوبة خاضعة للرقابة (٢٢ ± ٢ درجة مئوية) وفي دورة مظلمة / ضوء ١٢ ساعة ، مع توفر الغذاء والماء في المختبر. كانت جميع الإجراءات المتعلقة برعاية الحيوانات وصيانتها متوافقة مع المبادئ التوجيهية الدولية لأبحاث الحيوان وتم الإشراف عليها والموافقة عليها من قبل لجنة الأخلاقيات الحيوية وأخلاقيات الحيوان بجامعة قناة السويس. تم حقن فئران بالغة داخل الصفاق بسموم سكوربو مورس بلاتوس. تم تحديدالاهرمونات تستوستيرون T4 ، T٣ وكورتيزول وانسولين) في عينات المصل بعد ٢ و ٦ و ٢٤ ساعة (النقاط الزمنية) باستخدام ELISA ASD (الولايات المتحدة الأمريكية). تم حقن مجموعة مراقبة من الحيوانات بنسبة ٠.٩٪ كلوريد الصوديوم.
النتائج: زاد هورمون التستوستيرون فى جميع النقاط الزمنية باستثناء ٦ ساعات (نقطة زمنية). كان المستوى T3 وT4 فزيادة في جميع النقاط الزمنية المختلفة .. فيما يتعلق بمستوى الكورتيزول في الدم تم زيادة في ثلاثة مواضع. ومع ذلك ، في موقع سحاب ارتفع مستوى الكورتيزول في ٢ و ٦ ساعات. ولكن ، في توقيت ٢٤ ساعة كان تركيز الكورتيزول متساوياً بين المجموعات المعالجة ومجموعة المراقبة. في موقع رحاب ، كان مستوى الأنسولين في الدم في المعالجة أقل من المجموعة الضابطة. فيما يتعلق بموقع سحاب، كان مستوى تركيز الأنسولين متساويًا بينالمجموعات المعالجة والضابطة في كلتا النقطتين الزمنيتين ٢ و ٦ ساعات، وزادت عند ٢٤ ساعة. في كل من Alagramia وموقع WMCD ، تمت زيادة تركيز مستوى الأنسولين في كل من ٢ و٢٤ ساعة.
الخلاصة: أظهرت الدراسة أن سم العقرب.قد زاد من نسبة هورمونات الدراسة، التستيرون ،الكورتيزول والانسولين، ت ٣و ت٤ . زادت في معظم النقاط الزمنية المختلفة.