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Effect of Fractionated Doses of *Cerastes Cerastes* Crude Venom on Tissues of Irradiated Mice

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THIS WORK aims to study the effect of fractionated doses of *Cerastes cerastes* (*C. cerastes*) crude venom (CCV) on physiological alterations in different tissues of 5.5 Gy γ-irradiated mice. Male mice were grouped into: Control group. CCV group; mice received via inter peritoneum (i.p.) ⅓LD50 CCV in fractionated doses over a period of 2 weeks. Irradiated group; mice whole body exposed to 5.5 Gy γ-rays. Irradiated+ CCV; mice received via i.p ⅓LD50 CCV in fractionated doses over a period of 2 weeks starting 1 h post irradiation. Exposure to 5.5 Gy γ-rays elevated advanced oxidation protein products (AOPP) and malondialdehyde (MDA) levels and decreased glutathione (GSH) content of liver, spleen and kidney. Moreover, γ-irradiation significantly decreased calcium (Ca) and elevated zinc (Zn), and cupper (Cu) in liver, spleen and kidney tissues compared to the control, whereas, iron (Fe) was significantly elevated in liver and spleen and decreased in kidney. In addition, serum urea and creatinine and their ratio were significantly increased. Irradiated mice treated with fractionated CCV showed significant amelioration of oxidative stress and element alterations in the different tissues. It could be concluded that the fractionated doses of CCV (⅓LD50) might have favourable potential against irradiation induced-biochemical injuries.

Keywords: Cerastes crude venom, Oxidative stress, Trace Elements, γ-rays.

Venoms are excellent sources of molecules for drug discovery, and once purified, characterized, and cloned, could have potential applications in medicine. Snake procoagulant molecules, especially from the Viperidae family, have been used in medical applications and as diagnostic tools (Suntravat *et al*., 2010). *Cerastes* is a small genus of vipers found in North Africa and the Middle East (Phelps, 2010).

Indian Monocellate Cobra venom treatment increased glutathione (GSH) level and thereby it may prevent peroxidation in arthritis. Cobra venom not only modulates inflammatory activity, but it also possesses anti complementary activity (Gomes *et al.,* 2010). A cytotoxic and antioxidant protein (NN-32) from the Indian spectacled cobra *Naja naja* venom increased the antioxidant markers GSH, glutathione peroxidase, glutathione transferase, superoxide dismutase and catalase activity. NN-32 increased serum IL-10 level (Das *et al.,* 2011). A bradykinin potentiating factor extracted from the Egyptian viper *Androctonus amoreuxi* venom offered a radio protective effect to irradiated rats (Ashry *et al.,* 2012).

The growing application of radiation science in different settings (e.g., radiotherapy, biomedical research, military and space research) necessitates protecting humans against the harmful effects of radiation. During radiotherapy, ionizing radiation interacts with biological systems to produce free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, leading to serious cellular damage (Shirazi *et al.,* 2013).

The antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and enzymes of the hexose monophosphate shunt, require cofactors such as trace elements and other relevant ones: selenium (Se), manganese (Mn), Cu, Zn, and also Mg to maintain their function (Jozanov-Stankov *et al*., 1998). Deposition of these elements may have some additional role on the toxicity caused by direct radiation of the kidney (Cengiz *et al*., 2003). A marked increase was noted in serum calcium content with concomitant decrease in the bone calcium which suggests that irradiation of rats resulted in disturbances of calcium metabolism (Edrees *et al.,* 2008).

Our work aims to study the effects of fractionated CCV (⅓LD50) on γ-rays induced oxidative stress biomarkers, biochemical changes and trace elements in tissues of mice.

Materials and Methods

Animals

Twenty four adult male mice, weighing (24± 2 g) were obtained from the Nuclear Research Centre (NRC), Anchas, Atomic Energy Authority-Egypt.

Animals were allowed to acclimate in metal cages inside a well-ventilated room for 2 weeks prior to the commencement of the experiment. They were maintained under normal conditions, temperature 18-23°C, fed on standard commercial pellet diet and had free access to tap water.

Venom

Snakes; *C. cerastes* venom was kindly provided by Dr Ahmad Abd El Baset, PhD, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Snakes were collected from the Western Desert in Upper Egypt. The snakes were kept in large tanks, heat was provided from a 100 watt lamp for a daily period of 9 h. Water was always available. Venom was milked from adult snakes, lyophilized and Venom was dissolved in saline solution (0.9% Na Cl) at a concentration of 1 mg/ ml prior to use.

Radiation facility

Irradiation was performed at NCRRT, Cairo, Egypt, using a Gamma Cell-40 (^{137}Cesium) biological irradiator manufactured by the Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada. Mice were irradiated at a single dose of 5.5 Gy given at a dose rate of 0.49 Gy/ min.

The study protocol

Animals matched in age and body size were randomly assigned into four groups; control group; animals were injected i.p. with saline solution only. CCV group; animals injected via i.p. ⅓LD50 (Al-Sadoon *et al.*, 2013) CCV in a diluted form over a period of 2 weeks. Irradiated group; animals were exposed to 5.5 Gy of γ-radiation. Irradiated and CCV; animals were irradiated at 5.5 Gy and injected i.p., 1 h post irradiation, with ⅓LD50 of diluted crude CCV daily for 2 weeks. After 2 weeks, mice were anesthetized with ether. Blood was collected by heart puncture, centrifuged and serum was stored at -20°C until analyzed.

The liver, kidney and spleen were dissected out, washed in deionized water. For oxidative stress biomarkers a known wt of the different organs was used to obtain a 10 % tissue homogenate in 0.15M KCl. Reduced GSH content was measured by spectrophotometry at 412nm in blood, based on the determination of yellow colour that develops (Beutler *et al*., 1963). Lipid

peroxidation was based on the determination of MDA which reacts with thiobarbituric acid in acidic medium to yield a pink colour trimethine complex at 532 nm (Yoshioka *et al*., 1979). AOPP were measured by spectrophotometry on a microplate reader (990 win6 software for DV990BV4.GIO.DE VITA. Roma, Italy) and were calibrated with chloramine-T solutions that is measured in the presence of potassium iodide at 340 nm (Witko-Sarsat *et al*. 1998). AOPP concentrations were expressed as μmol/L of chloramine-T equivalents. To determine possible kidney injury by venom utilization, urea and creatinine were determined using Stanbio kits according to the method of Young (2001) and Spierto *et al*. (1979), respectively.

Liver, spleen and kidney were isolated and tissue samples were digested in pure nitric acid and H_2O_2 (4:1) by Microwave Sample Preparation Labstation, MLS-1200 MEGA model, Italy (Kingston and Jassie, 1988). Trace elements (Cu, Zn, Fe and Ca) were estimated using Atomic Absorption Unicam 939 Solar Spectrometer, England.

Statistical analysis

The results are presented as percentage and mean± S.E. Statistical analysis was performed using one-way analysis of variance (ANOVA), statistical package of social science (SPSS) version 15.0 for windows. Individual difference among groups was analysed by Duncan's test. Significance was indicated at $P< 0.05$

Results

Results showed that CCV treated animals revealed no significant differences ($P< 0.05$) compared to control group after 2 weeks for all investigated parameters. Exposure of animals to 5.5 Gy γ-rays induced oxidative stress demonstrated by a significant ($P< 0.05$) decrease of GSH content associated with significant increase of AOPP and MDA levels in liver tissue compared to the control group (Table 1). Results also revealed that irradiation induced significant elevation of liver Zn, Cu and Fe, whereas Ca was significantly depressed compared to the control group (Table 2). Irradiated group that received CCV showed significant decrease of liver AOPP, MDA, Zn, Cu and Fe, while GSH content and Ca were significantly increased compared to the irradiated group. (Tables1 and 2).

TABLE 1. Effect of CCV on liver AOPP, MDA, and GSH contents (nmol/g tissue) of 5.5 Gy irradiated mice.

Groups	AOPP	MDA	GSH
Control	201.5± 5.3	200.2± 5.9	21.8± 1.3
CCV	193.5± 7.2	191.2± 7.2	23.1± 1.5
Irradiated (5.5Gy)	233.2± 5.2a	262.3± 5.6a	16.5± 1.8a
Irradiated+ CCV	214.8± 3.6b	220.5± 10.8b	19.0± 1.1b

a: significantly different from control. b: significantly different from irradiated group.

TABLE 2: Effect of CCV on liver Zn, Cu, Ca nd Fe contents (µg/g tissue) in 5.5 Gy irradiated mice.

Groups	Zn	Cu	Ca	Fe
Control	29.2± 1.1	3.4± 0.02	69.2± 3.4	83.2± 3.5
CCV	28.4± 1.1	3.6± 0.3	70.5± 4.8	78.6±2.6
Irradiated (5.5Gy)	35.2± 1.5a	4.5± 0.2a	54.1± 2.8a	104.5± 4.6a
Irradiated+ CCV	27.3± 1.6b	3.8± 0.1b	68.9± 4.9b	87.8± 3.1b

Legends as in Table 1.

Exposure of mice to 5.5 Gy provoked oxidative stress in the spleen demonstrated by significant ($P< 0.05$) decrease of GSH content and Ca associated with significant elevations of AOPP and MDA in addition to Zn, Cu and Fe levels compared to the control group (Tables 3 and 4). Irradiated group that received CCV showed significant ($P< 0.05$) decrease of spleen AOPP, MDA, as well as Zn, Cu and Fe levels, while GSH content and Ca level were increased significantly compared to the irradiated group.

TABLE 3. Effect of CCV on spleen AOPP, MDA and GSH contents (nmol/g tissue) of 5.5 Gy irradiated mice.

Groups	AOPP	MDA	GSH
Control	198.8± 5.7	194.8± 5.6	20.0± 1.2
CCV	196.3± 6.7	195.9± 8.9	22.9± 0.8
Irradiated (5.5Gy)	243.3± 4.4a	259.9± 4.5a	14.8± 0.7a
Irradiated+ CCV	218.3± 5.0ab	220.9± 6.2ab	17.9± 1.0b

Legends as in Table 1.

TABLE 4. Effect of CCV on spleen Zn, Cu, Ca and Fe contents (µg/g tissue) of 5.5 Gy irradiated mice.

Groups	Zn	Cu	Ca	Fe
Control	29.4± 1.5	1.5± 0.1	76.1± 3.7	166.8± 6.7
CCV	30.0± 1.3	1.6± 0.2	74.3± 3.7	161.5± 5.3
Irradiated (5.5Gy)	35.6±1.1a	2.5±0.2a	61.7± 3.9a	210± 9.4a
Irradiated+ CCV	30.1± 1.0b	1.8± 0.1b	73.3± 3.6ab	180.8± 7.4b

Legends as in Table 1.

Irradiation induced significant increase in AOPP, MDA, Zn and Cu accompanied with a significant decline in GSH content, Ca and Fe in the kidneys as compared to the control, (Table 5 & 6). Irradiated group treated with CCV exhibited significant ($P< 0.05$) decline in AOPP, MDA, Zn and Cu whereas kidney GSH content, Ca and Fe were elevated significantly compared to the irradiated group.

TABLE 5. Effect of CCV on kidney AOPP, MDA and GSH contents (nmol/g tissue) of 5.5 Gy irradiated mice.

Groups	AOPP	MDA	GSH
Control	212.7± 6.3	101.4± 3.4	20.0± 0.9
CCV	205.8± 5.4	83.6± 4.5	18.4± 1.2
Irradiated (5.5Gy)	243.0± 5.6a	124.4± 4.5a	14.5± 0.9a
Irradiated+ CCV	214.8± 5.6b	107.2± 4.5b	18.9± 0.9b

Legends as in Table 1.

TABLE 6. Effect of CCV on kidney Zn,Cu , Mg, Ca and Fe contents (µg/g tissue) of 5.5 Gy irradiated animals.

Groups	Zn	Cu	Ca	Fe
Control	29.3± 1.8	5.4± 0.1	82.6± 4.1	76.4± 3.2
CCV	29.5± 1.5	5.6± 0.3	80.0± 4.7	78.6±3.6
Irradiated (5.5Gy)	35.1± 1.6a	6.5± 0.2a	63.7± 3.2a	69.4± 2.3a
Irradiated+ CCV	29.9± 1.5b	5.1± 0.3b	77.0± 4.6b	78.5± 2.6b

Legends as in Table 1.

Also, serum urea, creatinine levels and their ratio showed a significant increase in irradiated animals (Table 7). Treatment of irradiated animals with CCV induced significant decline of serum urea, creatinine levels and their ratio.

TABLE 7. Effect of CCV on serum creatinine, urea and urea/creatinine ratio of 5.5 Gy irradiated animals.

Groups	Creatinine mg/dl	Urea mg/dl	Urea/creatinine
Control	5.1± 0.17	53.5± 2.1	9.4± 0.3
CCV	4.4± 0.2	50.6± 2.1	11.9± 0.9
Irradiated (5.5Gy)	5.8± 0.32a	65.3± 1.7a	12.3± 0.3a
Irradiated+ CCV	4.4± 0.36ab	50.5± 1.9b	10.4± 0.6b

Legends as in Table 1.

Discussion

Ionizing irradiation exerts its biological effects by initiating ROS generation through the radiolysis of water. ROS include O_2-derived free radicals including superoxide anion radical (O_2^-) and the hydroxyl radical (^-OH), as well

as non radical derivatives of O_2 such as hydrogen peroxide (H_2O_2). It is a well established fact that ionizing radiation causes damage to DNA, proteins, lipids and carbohydrates in the various organs (Chen *et al.,* 2013). In some organs, damage is expressed early while in others, it may be expressed over a period of time depending upon the cell kinetics and the radiation tolerance of the tissues (Jagetia and Baliga, 2003).

In the present study γ-irradiation of mice has induced imbalance between oxidant and antioxidant species. Significant elevation in the level of MDA and AOPP levels was accompanied by significant depletion of GSH level in liver, spleen and kidney after 15 days. This may be attributed to the utilization of antioxidants by the enhanced production of ROS (Prasad *et al.,* 2005) or due to the decreased transport activity of the oxidized GSH through membranes with a decrease in the activity of glutathione reductase which is augmented by riboflavin deficiency (Abou-Bedair *et al*., 2002). Parihar *et al.* (2006) attributed the increase in tissue MDA to the susceptibility of lipids to free radical attack. Witko-Sarat *et al.* (1996) suggested that AOPP accumulation that coexisted with decreased GSH and elevated MDA support the occurrence of oxidative stress. An interaction between oxygen and nitrogen species has drawn extensive attention because of the deleterious effects of peroxynitrite on antioxidant systems (Yousefipour *et al.,* 2010). Plasma membrane fluidity and permeability are directly affected by radiation-induced lipid damage (Corre *et al.,* 2010). Free radicals attack biomolecules such as fatty acid component of membrane lipids, proteins and DNA, leading to lipid peroxidation, AOPP, strand breaks and ultimately cell death (Morita *et al.,* 2011). Spleen damage was confirmed by DNA fragmentation and necrosis of DNA of spleenocytes as well as elevation of MDA and AOPP (Salama and Montaser, 2013).

Intra-peritoneal treatment with fractionated $⅓LD_{50}$ of crude CCV over 2 weeks, significantly improved oxidative stress in irradiated mice implicating possible antioxidant effect of low doses of *C. cerastes* crude venom. Also Hou *et al.* (2004) described that the fraction F and H isolated from *Naja naja atra* venom has anti-lipid peroxidation effect, may scavenge active oxygen free radical and increase the activity of SOD. A dose of $1/10^{th}$ LD_{50} of *Naja kaouthia* venom and *Vipera russelli* venom strengthened antioxidant system of Ehrlich ascites carcinoma treated mice (Debnath *et al.,* 2007).

Although trace elements constitute a minor part of living tissues, they are important for vital processes. Some metals, usually present in proteins, enzymes and cellular membranes, are essential for the normal physiological function (Silva, 2009). The present elevation of Cu and Zn are in agreement with Cengiz *et al.* (2003) who noted that deposition of trace elements may have some additional role on the toxicity caused by direct radiation. The increase of Zn may be due to its accumulation from the damaged lymphoid organs, bone marrow and spermatogonia (Okada, 1970). El-Nimr and Abdel-Rahim (1998) attributed the significant change in the concentrations of essential trace elements in lung, liver, spleen and kidney to the long term disturbances of enzymatic functions and possible retardation of cellular activity by irradiation. The significant decrease in spleen, kidney and liver Ca in the present study might be attributed to the inhibitory effect of ionizing radiation on Ca ion channels (Nunia *et al.*, 2007). Kotb *et al.* (1990) attributed the disturbances in calcium metabolism to the insufficient renal function after irradiation. It has previously been observed that calcium homeostasis is essential for the maintenance of cellular mitosis. Induced hypocalcaemia would be expected to depress mitotic activity and may be partially responsible for activity of pathological alterations produced by irradiation. While in the kidney, the iron content was significantly decreased. This was attributed by Hampton and Mayerson (1950) to that the kidney is capable of forming ferritin from iron released from haemoglobin. It is probable that iron present in the kidney is used for excretion and conversion to ferritin.

The venom of *C. cerastes* is a low-complexity proteome composed of 25–30 toxins belonging to 6 protein families, mainly targeting the haemostatic system. Cysteine-rich secretory proteins and C-type lectin-like molecules, each accounted for less than 4% of the total venom toxins (Fahmi *et al.*, 2012).

A protective effect of animal venom was attributed to the cysteine-rich secretory proteins found in animal venoms (CRISP-Vs), which are members of a large family of cysteine-rich secretory proteins (CRISPs) that act on different ion channels as proved in Elapidae, Colubridae and Viperidae families (Ramazanova *et al.*, 2009).

Radiation-induced oxidative stress in the kidney was associated with a significant increase in the level of serum urea and creatinine. Reduction-

oxidation imbalances lead to kidney injury and proteinuria (Nistala *et al.*, 2008). It could be referred to increased ammonia formed by deamination of amino acids in the liver converted to urea (Ganong, 1999). The impaired detoxification function of the liver by irradiation could also contribute in the increase of urea in the blood (Robbins et al., 2001). Serum creatinine elevation might be attributed to the interaction of ionizing radiation with the sites of biosynthesis (El-Kashef and Saada, 1988). Dai *et al.* (2012) described renal protective effects of *Naja naja atra* venom (NNAV) treatment, at the dose of 90 μg/ (kg/day), in mice with streptozotocin (STZ)-induced diabetes manifested by improved creatinine, blood urea nitrogen, urinary protein excretion, MDA, SOD in serum and kidney tissue in diabetic nephropathy. Egyptian viper *Androctonus amoreuxi* venom offered radio protective effect to irradiated rat kidneys (Ashry *et al.*, 2012).

In conclusion: *C. cerastes* crude venom at fractionated doses ($1/3LD_{50}$) reduces oxidative stress induced by γ-rays exposure and prevents trace elements disturbances. The present study provides details about the effect of *C. cerastes* crude venom in mice exposed to whole body γ-irradiation, suggesting that it could be a natural potential radio protective agent for radiotherapy, though the mechanism is not yet clear.

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تـأثير الجرعـات المجزئــة مـن السـم الخـام للافعـى المقرنــة سراستس سراستس على أنسجة الفئران المشعة

اميمة محمد عشري و صفوت فريد سلامة و هشام فاروق حسن

قسم بيولوجيا الإشعاع ، المركز القومي لبحوث وتكنولوجيا الإشعاع ، ص. ب. ٢٩ مدينة نصر ، مصر.

سم الافعى المقرنة هو مخلوط من البروتينات و العديد من الإنزيمات و بعض العناصرالطبيعية والنادرة. يهدف البحث دراسة آثار الجرعات المنخفضة من السم الخام للافعى المقرنة (٣/١ الجرعة المميتة للنصف) المجزأة على الاجهاد االتاكسدى و العناصر النادرة الرئيسية في الكبد و الكلى و الطحال و كذلك و ظائف الكلى للفئران المعرضة لاشعة جاما بجرعة ٥ر٥ جراى .تم تقسيم الفئران الذكور إلى اربع مجموعات مجموعة الضابطة ، و مجموعة تحقن بالسم الخام للافعى المقرنة (٣/١ الجرعة المميتة للنصف) مجزأة على فترة اسبوعين. ثم مجموعة مشعة و فيها يتعرض الفئران ٥ر٥ جراى من أشعة جاما، ومجموعة مشعة :تتعرض ٥ر٥ جراى من أشعة جاما ثم تحقن بجرعة من السم الخام المجزا يوميا على مدى أسبوعين بعد ساعة من تشعيع الفئران.

التعرض لأشعة جاما ٥ر٥ جراى تسبب في زيادة مستوى ناتج أكسدة البروتين المتقدم و مالون ثنائى الالدهيد في الكبد و الطحال و الكلى في حين أنه ادي الي انخفاض مستوى الجلوتاثيون في كل من الكبد و الكلى وعلاوة على ذلك ، اثر التشعيع الجامي سلبيا علي مستويات العناصر النادرة (الزنك ، النحاس ، الكالسيوم و الحديد) في الكبد ، و الكلى و الطحال. و بالإضافة إلى ذلك ، زادت مستويات اليوريا و الكرياتينين ونسبتهم في مصل الدم في المجموعة المعرضة للإشعاع.

أظهرت النتائج في المجموعة المشعة و المعاملة بالسم الخام المقسم على مدى اسبوعين أن مستويات ناتج أكسدة البروتين المتقدم و مالون ثنائى الالدهيد و الزنك و النحاس و الكالسيوم والحديد في الطحال والكبد و الكلى و اليوريا و الكرياتينين و نسبتهم في مصل الدم قد انخفضت انخفاض معنوى بينما ارتفع كل من الجلوتاثيون في الكبد و الجلوتاثيون في الكلى بالمقارنة مع مجموعة المشعة الضابطة.

و من هذا يتضح أن السم الخام للحية المقرنة (٣/١ الجرعة نصف المميتة) بجرعات صغيرة قد تكون له فعالية عندما يتم استخدامه كمادة طبيعية للتخفيف من حدة الإصابات البيوكيميائية التي يسببها الاشعاع في الأنسجة.