Pakistan J. Zool., vol. 48(6), pp. 1971-1978, 2016.

Radioprotective Effects of Adenosine in Gamma Irradiated Rats

N.A. Abd El-Rahman,[1,]* E.M. Kamal El-Dein,[1] A.M. Abd El-Hady[2] and Saeed M. Soliman[1]
[1]Radiation Biology Dept., National Centre for Radiation Research and Technology, P.O. Box 29, Nasr City, Egypt
[2]Faculty of Applied Medical Sciences, Misr University for Sciences and Technology 6th October Giza, Egypt

ABSTRACT

Adenosine is a non –toxic purine abundant in meat and sugar beets, was reported to provide health benefits including antioxidant, anti-inflammatory effects as well as vascular protective properties. This study was dedicated to determine the cardio-protective efficacy of adenosine against oxidative stress due to radiation exposure in male albino rats. Rats were divided into four groups *viz.* Control group: rats not subject to any treatment; Rats receiving adenosine (150ml/Kg body weight) by gavages for 14 days. Radiation groups in which rats were whole body gamma irradiated at 7Gy. Rats receiving adenosine for 14 days before whole body gamma irradiation at 7Gy. Animals were sacrificed 24 h post irradiation. Irradiated rats revealed a significant increase of heart thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD) and catalase (CAT) activities as well xanthine oxidase activity (XO) in parallel to a significant decrease of reduced glutathione (GSH) content, xanthine dehydrogenase (XDH) and uric acid. Blood showed elevation of TBAR, XO and uric acid accompanied by decrease of antioxidants and XDH. Radiation exposure induced a significant rise in the activities of creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and aspartate aminotransferase (AST), markers of heart damage, both in the heart and blood indicating acute cardiac toxicity. The results revealed that administration of adenosine before irradiation induced significant protection to the parameters of oxidative stress, amelioration of xanthine oxidoreductase (XOR) system and uric acid, besides significant improvement of markers of heart damage. In addition, radiation induced myocardial degenerative changes, interstitial oedema between muscle fibres, necrosis and inflammatory cells infiltration, fibrotic and cellular damage to the heart. Administration of adenosine ameliorates the histological changes induced by gamma irradiation in the heart. It is concluded that the use of adenosine as an antioxidant is safe and may provide some beneficial effects, and could exhibit modulatory effects on γ-radiation-induced oxidative damage in rats.

Article Information
Received 1 February 2015
Revised 21 April 2016
Accepted 21 May 2016
Available online 20 October 2016

Authors' Contribution
NAAR conceived and designed the study. NAAR, EMKD and AMAH performed the experimental work and analyzed the data. SMS wrote the article.

Key words
γ-radiation, Adenosine, oxidative stress, reactive oxygen species, antioxidant, superoxide dismutase, glutathione, CPK, LDH, Xanthine oxidase, TBARS.

INTRODUCTION

Exposure to ionizing radiation initiates a cascade of events including oxidative damage that leads to alteration of tissue physiological function **(**Zhao *et al*., 2007). Lipid peroxidation is considered to be a critical event of ionizing radiation effect (Agrawal and Kale, 2001). Most of the toxic effects of ionizing radiation are due to generation of reactive oxygen species (ROS) by radiolysis of water which triggers formation of several reactive intermediates (Adaramoye *et al*., 2011). Therefore, to overcome this oxidant stress, the body is equipped with defense system including enzymatic and non-enzymatic radical scavengers that can either directly detoxify ROS or indirectly regulate their levels (Sandeep and Nair, 2012). Hence, an over production of ROS leads to uncontrolled chain reactions and lipid peroxidation, resulting in various pathological conditions that may include liver injury (Kotzampassi *et al*. 2009) testicular tissues injury (Adaramoye *et al*., 2012) in addition to lung and kidney damage (Sener *et al*., 2006)**.**

Ionizing radiation is known to generate ROS in irradiated tissue. Because most tissues contain 80% water, the major radiation damage is due to the aqueous free radicals, generated by the action of radiation on water. Hydroxyl radicals (OH), are considered the most damaging of all free radicals generated in organisms (Spitz and Gius, 2004). These free radicals react with cellular macromolecules, such as DNA, RNA, proteins and cause cells dysfunction and mortality (Tominaga *et al*., 2004). Attention has been given to the roles of free radicals generated through the oxidative stress, especially induced by ionizing radiation (Riabchenko *et al*., 2011). Radiation induces an inflammatory response in target and surrounding tissues which is characterized by accumulation of plasma proteins and leukocytes (Johnson *et al*., 2004). The inflammatory reaction is a classical feature of radiation exposure and appears to be a key event in the development of the acute radiation syndrome (Van der Meeren *et al*., 2005). Oxidative stress occurs due to excessive free radical production and/or low

* Corresponding author: nehadaliibrahim@yahoomail.com
0030-9923/2016/0006-1971 $ 8.00/0

antioxidant defence, and results in chemical alterations of bio-molecules causing structural and functional modifications (Robbins *et al*., 2002). ROS in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Ozkan and Fiskin, 2004). One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissues. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Superoxide dismutase (SOD) catalyzes the reduction of $O_2^{\bullet -}$ to H_2O_2, the majority of which is broken down to oxygen and water by catalase (CAT). In addition to CAT, glutathione peroxidase in presence of adequate amount of reduced glutathione (GSH) can also break down H_2O_2 (Sun *et al*., 1998).

Adenosine, an endogenous nucleoside, has potent effects on the immune, neural and cardiovascular systems (Guinzberg *et al*., 2006). It has been shown to be an immunomodulator and anti-inflammatory agents (Scneider and Klein, 2005).

Adenosineis classified as a non–toxic purine. The adenosine extract contains high amounts of purine (Gudkov *et al*., 2006). The extract protects mouse bone marrow cells against physical and chemical mutagenic agents. Antioxidants in natural products, meat and sugar beets, prevent oxidation reactions associated with cancer and heart diseases with minimum side effects (Gudkov *et al*., 2006a). Adenosine may have antioxidant, anti-inflammatory, antiallergic, antiviral, hypolipidimic and vasoprotective effects (Guinzberg *et al*., 2006), as well as powerful protective effects on the radiation-induced DNA damage (Hou *et al*., 2007) and was proven to be highly effective for protection (Schneider and Klein, 2005). The possible health benefits of adenosine is partly attributed to their potent antioxidant and free–radical scavenging activities (Schneider and Klein, 2005).

This study has been conducted to investigate the possible protective role of adenosine against the toxic effects of exposure to whole body gamma-irradiation in albino rats.

MATERIALS AND METHODS

Experimental animals

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland and according to approval from the Ethics Committee for Animals Care at the National Research Centre, (ethic No.10-230).

Adult male Sprague-Dawley rats (180±20 g, body weight), were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). The animals were housed under standard laboratory conditions (constant temperature 25-27°C, with 12 h light /dark cycle) during the experimental period. The rats were provided with tap water and commercial diets. The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment.

Radiation facility

Irradiation of rats was carried out using a Canadian Gamma Cell-40 (137Cs), manufactured by Atomic Energy of Canada Ltd., located at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The animal's whole body was exposed to gamma rays at a dose of 7Gy administered at a dose rate of 1Gy/2.10 min.

Adenosine treatment

Adenosine was purchased from Sigma chemical Co., St. Louis, MO, USA. The product is provided as concentrated exudates, dissolved in distilled water. Adenosine was administered to rats by gavages at a dose of 150 mg/kg body weight, according to Mabley *et al*. (2003) for 14 days, prior to irradiation. Animals were sacrificed 24 h post irradiation.

Animal groups

Animals were divided into 4 groups, each of 6 rats.

1. Control: received no treatment.
2. Adenosine: received adenosine via gavages (150mg/kg body weight/day) for 14 days.
3. Radiation: were whole body gamma irradiated at 7Gy.
4. Adenosine+Radiation: received adenosine via gavages (150mg/kg body weight/day) before whole body gamma irradiation at 7Gy.

Biochemical analysis

The animals were sacrificed twenty four hours post irradiation and blood was collected and plasma samples were obtained by centrifugation at 3000 rpm for 10 min. Immediately after sacrifice, the heart was rapidly excised from the body of each animal, accurately weighted and tissues were homogenized in normal saline. The freshly prepared homogenate were then used for determination of thiobarbituric acid reactive substances (TBARS), CAT, GSH, SOD, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), xanthine oxidase (XO), xanthine dehydrogenase (XDH), and uric acid contents.

Lipid peroxide content was determined by quantifying thiobarbituric acid TBARS content in blood and tissue homogenates according to the method

described by Yoshioka *et al.* (1979). The activity of SOD was determined in blood erythrocytes according to the method of Minami and Yoshikawa (1979). The CAT activity was determined following the procedure described by Johansson and Hakan Borg (1988). Blood glutathione was determined according to Beutler *et al.* (1963). XO and XDH were assayed in serum following the procedure described by Kaminski and Jezewska (1979). LDH, CPK and AST activities were determined according to the methods of Henderson and Moss (2001), Rec (1977) and Reitman and Frankel (1957), respectively. Uric acid was determined according to Haismann and Muller (1977).

Histological methods

Hearts were removed and fixed in Bouin's solution for 24 h according to Hartz (1972). Samples were serially sectioned at a thickness of 4-5 µm and stained applying the technique of Conn and Darrow (1960) using hematoxylin and eosin. Tissue sections were examined under a research light microscope.

Statistical analysis

ANOVA (one-way classification F-test) followed by Duncan (Multiple Range-test) were carried out for the statistical analysis as described by Lind and Masson (1996) and data were represented in Tables as mean ± standard error.

RESULTS

The oxidative stress parameters *viz.* SOD and CAT activities and GSH content as well as TBARS concentration in heart and blood are presented in Table I. The results pointed to a significant increase in the activity of SOD and CAT both in the heart of rats subjected to 7Gy gamma radiation whereas their activities decreased in the blood. However, 24 h post irradiation, there was a significant reduction of GSH accompanied by a significant increase of TBARS both in the heart and blood as compared to that of control group. The results showed significant protection to oxidative stress parameters in animals treated with adenosine as compared to irradiated group.

Table also revealed a significant increase in XO associated with significant decrease in XDH activities in the heart and blood of animals exposed to 7 Gy gamma radiation as compared to control group. Uric acid was significantly decreased in the heart but was elevated in the blood. Administration of adenosine before irradiation ameliorated xanthine oxidoreductase system and protected uric acid values.

CPK, LDH and AST increase significantly after exposure to gamma rays compared to control both in the heart and blood. Administration of adenosine reduced the increase of the enzymes activities compared to irradiated groups.

Each value represents the mean ± SE (n=6) $P<0.05$: significant, a: Significantly different compared to control. b: Significantly different compared to radiation.

Histopathological observation

Heart section of the control group and group treated with adenosine (150 mg/kg body wt) showed normal architecture. The heart cells have normal amount of cytoplasm with one or two nuclei and defined cell boundaries. Cardiac tissues, cardiac muscle fibers, appeared as short branching and anastomosing cylinders with moderately stained eosinophilic sacroplasm and centrally located oval nuclei (Fig. 1 A,B). Exposure to gamma rays induced changes manifested as slight disruption of the striated appearances and disorganization of the myofilamentous arrangement in many cardiomyocytes, discontinued, fragmented and lysis. Structural changes in the cardiac muscle fibers, deformation of the striated appearance and areas of vacuolation (Fig. 1C), were also detected, in addition to patches, necrosis of muscle fibres, pyknotic myocardial cells and myocardial damage. In the groups treated with adenosine (150 mg/kg body wt) prior to whole body gamma irradiation, amelioration of many of the radiation induced changes in the histological structure was observed. The pyknotic cells were not observed, the degree of myocardial damage was less than that of irradiated groups, the interstitial odemas as well as inflammation were less than irradiated group, myonecrosis was also not remarkable in this group (Fig. 1D).

DISCUSSION

Several evidences had indicated that accumulation of ROS led to the alteration in wide range of gene expression such as antioxidant enzymes, stress response genes and cytokines (Zhang *et al.*, 2002). During oxidative stress, the endogenous antioxidant defenses are likely to be weakened because of overproduction of oxygen radicals, consumption of antioxidant and failure to adequately replenish these antioxidant enzymes in tissues (Droge, 2002). Exposure of mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs, which cause changes in the structure and function of cellular components. Oxidative stress with the subsequent production of ROS was postulated as one of the mechanisms of radiation toxicity.

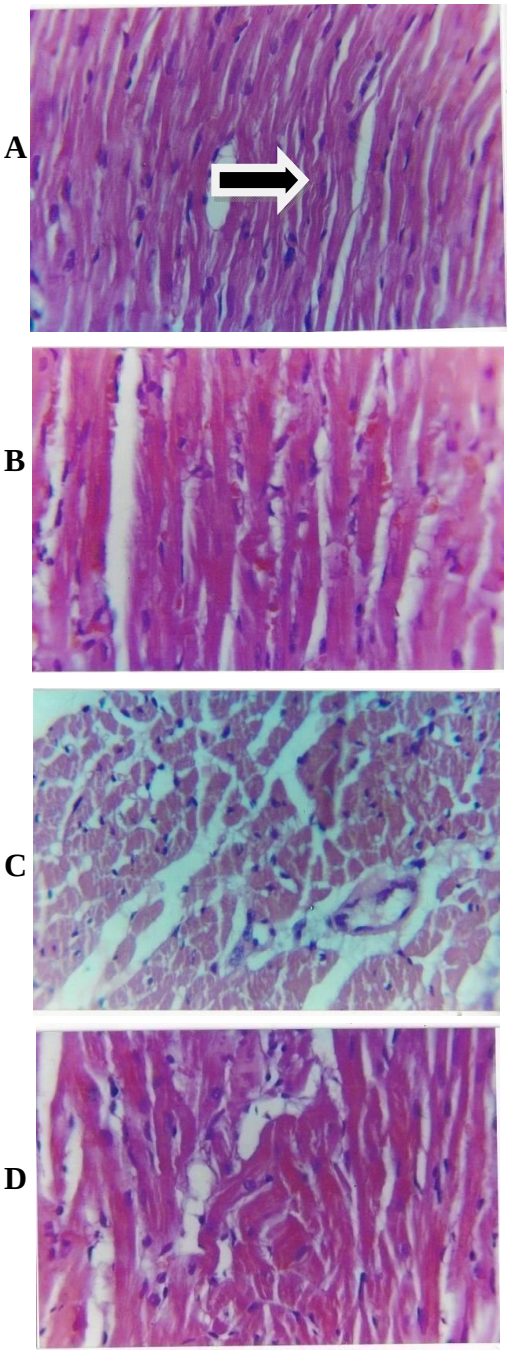

Fig. 1. Histological structure of a section in the heart of rats after different treatments (H&E-400x); **A,** Control rats showing normal structure (arrows Showed cardiac muscle fiber). **B,** Adenosine treated rat showing no deviation from the normal structure. **C,** Irradiated rats showing patches of necrosis of muscle fibers. **D,** Irradiated rats given adenosine showing amelioration of the radiation induced changes.

In the present study, whole body exposure of male albino rats to gamma radiation (7Gy) has provoked an imbalance between oxidant and antioxidant species. Significant increase in the level of TBARS, accompanied by significant decreases of SOD and CAT activities were recorded in the blood. The increase of TBARS level is probably due to the interaction of OH resulting as a bi-product of water radiolysis, upon exposure to ionizing radiation, with the polyunsaturated fatty acids present in the phopholipids portion of cellular membranes (Spitz *et al*., 2004 Oktem *et al*., 2004; CAT directly neutralizes the H_2O_2 produced from the superoxide dismutation reaction into water and molecular oxygen. The significant decrease in the activity of SOD and CAT might also be attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation (Kregel and Zhang. 2007). The ROS as chemically reactive molecules can modify most cell components such as lipids, nucleic acid, carbohydrates and proteins (Stadtman and Levine, 2003).

GSH is the most abundant non-protein sulfhydryl-containing compound and constitutes the largest component of the endogenous thiol buffer (Holmgren *et al*., 2005). Assessment of GSH in biological samples is essential for evaluation of the redox homeostasis and detoxification status of cells in relation to its protective role against oxidative and free radical-mediated cell injury (Rossi *et al*., 2005)**.** Depletion of GSH may be partly attributed to inflammation (Meister, 1991). Excessive lipid per oxidation can increase GSH consumption (Gudkov *et al*., 2006 a). Elevation of MDA by irradiation which could be attributed to enhanced utilization of the antioxidant system in an attempt to detoxify radiation generated free radicals (Krishna and Kumar, 2005) which probably also accounts for the decrease of SOD and CAT activities.

Adenosine treatment improved CAT, SOD and TBARS that may be due to adenosine free radical scavenging ability by redox active sulphydryl group directly reacting with oxidants (Guinzberg *et al*., 2006)**.** In the past few years, innovations in the management of poisoning have also been directed toward the use of antioxidants, since gamma rays induces its toxic effect via oxidative stress-mediated mechanisms (Hou *et al*., 2007)**.**

CPK, LDH and AST are important enzymes used to confirm a myocardial infarction or heart injury. In the present study, the elevation in these enzyme activities after whole body gamma irradiation was attributed by Fahim (2008) to the alterations in dynamic permeability of membranes induced by ionizing radiation, allowing leaking of biologically active materials out of the injured cells. The amount of these markers in plasma is directly

Table I.- Effect of adenosine on thiobarbituric acid reactive substance (TBARS), glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activity xanthine oxidase (XO), xanthine dehydrogenase (XDH), uric acid, creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and aspartate aminotransferase (AST) in blood and heart of rat groups.

	Control		Adenosine		Radiation		Adenosine+Radiation	
	Heart (mIU/mg protein	Blood (U/g Hb)	Heart (mIU/mg protein	Blood (U/g Hb)	Heart (mIU/mg protein	Blood (U/g Hb)	Heart (mIU/mg protein	Blood (U/g Hb)
SOD	13.69 ±0.31	448.12± 11.9	15.53±0.39	422.00±9.3	27.33±1.69[a]	329.00±8.43[a]	18.35±0.63[b]	376.00±8.61[ab]
CAT	5.19±0.10	16.64±045	5.04±0.07	15.98±0.36	7.20±3.61[a]	11.47±0.28[a]	6.43±0.18[ab]	13.89±0.39[ab]
GSH	31.09±0.67	58.63±186	34.89±0.87	60.18±1.28	23.56±1.09[a]	42.71±0.93[a]	25.28±1.42[ab]	48.80±1.08[ab]
TBARS	188.00±4.93	17.38±0.49	182.70±4.09	15.64±0.36	298.13±5.06[a]	8.19±0.90[a]	310.18±5.42[ab]	20.58±0.85[ab]
XO	5.06.±0.23	1.28±0.09	4.63±0.28	1.14±0.07	6.64±0.30[a]	2.48±0.13[a]	6.08±0.36[a]	2.07±0.11[a]
XDH	13.73±0.39	1.18±0.06	13.59±0.08	1.09±0.08	9.34±0.59[a]	0.81±0.05[a]	8.79±0.46[a]	0.97±0.10[a]
Uric acid	50.46±1.36	2.43±0.09	41.65±1.24	2.39±0.05	32.44±1.0[a]	4.38±0.21a	58.13 ±1.1[ab]	3.37±0.14a
CPK (U/ml)	28.6±0.18	148.2±6.40	27.8±0.21	145.40.±6.00	38.54±0.50[a]	231.51.±8.9[a]	31.49±0.46[b]	173.80.±7.0[ab]
LDH (U/ml)	14.5±0.21	89.00±3.30	12.7±0.24	82.71.±2.8	25.60±0.29[a]	108.20.±3.69[a]	21.11±0.32[ab]	93.00.±2.90[b]
AST (U/L)	21.4±1.46	60.0±2.9	23.8±1.22	55.70.±2.08	31.95±1.6a	88.9.±3.1a	26.15±1.58[ab]	62.0.±2.2[b]

proportional to the number of necrotic cells present in cardiac tissue (Farvin *et al*., 2004) The recorded protection to the heart enzymes is attributed to that adenosine inhibits superoxide radical production by activated human neutrophils (Marton *et al*., 2001). The exogenous administration of larger doses of adenosine is reported to prevent ischemia-reperfusion injury in the heart and brain (Hasko *et al*., 2004).

The present study revealed significant aleration in XOR system of irradiated rats. The changes in XOR was manifested by significant increase in the activity of XO associated with significant decrease in XDH activity as compared with control rats. XOR is a member of the molybdoenzyme family that catalyze purine degradation, hypoxanthine and xanthine metabolism to uric acid with concomitant generation of ROS (Berry and Hare, 2004). Uric acid, the end products of DNA catabolism was elevated significantly in serum of rats exposed to gamma rays in the present study, indicating the increased rate of purine bases degradation and DNA damages. It was suggested that serum uric acid correlates with circulating markers of inflammatory process (Struthers *et al*., 2002). This implies a relation between increase in XO activity and endothelial injury secondary to increase of oxidative damages (Farquharson *et al*., 2002). Ionizing radiation has been shown to convert XDH into XO and contribute to increase ROS release which leads to cell damage (Srivastava *et al*., 2002). XO and XDH are two inter convertible forms of XOR. Administration of adenosine after exposure to gamma radiation induced significant decrease in XO and significant increase in XDH activities associated with significant decrease in serum uric acid ,postulating the inhibitory effect of adenosine on ROS generation systems (Kudina *et al*., 2003). Adenosine is an endogenous purine nucleoside that modulates many physiological processes. Modulation of XOR system by adenosine is due to that cellular signaling by adenosine occurs through four known adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3In regard to stress or injury, the function of adenosine is primarily that of cytoprotection preventing tissue damage. Activation of A_{2A} receptors produces a constellation of responses that in general can be classified as anti-inflammatory (Mustafa *et al*., 2009).

Regarding the histological changes in the heart recorded in the present study, it was found that exposure of rats to whole body gamma irradiation caused patchy necrosis of muscle fibers with infiltration of acute and chronic inflammation cells. Marked interstitial edema was also noted. These results coincide to some extent with the results of (Said *et al*., 2002**;** Mansour and Abuo El-Nour, 2009; Gorg, 2014) who concluded that this damage may be due to generation of oxidized, reactive lipoproteins and through direct attacks on the DNA of the arterial wall cells. In this study, irradiation of rats induced the formation of structural changes in their aortas, degeneration of the endothelial cell layer of the tunica intima, changes in the endothelium of the intima that was the cause for the development of edema, fibrosis and increase of vascular permeability, as well as degeneration and decrease of the number of smooth muscle cells of the tunic media of the aorta: the results agreed with (Soliman, 1997).

The significant protection observed for the heart tissue support the role of adenosine in minimizing radiation-induced damage. The treatment with adenosine before radiation exposure might lead to reduction of the damaging effect of oxygen free radicals acting as oxygen scavengers, which by its turn preserved the normal like

appearance of the heart tissue. This was observed upon the use of different free radical scavenging agents (Soliman, 2007; Gaur *et al*., 2011). Adenosine as a natural antioxidant prevents oxidative damage to DNA, decreases the generation of radical oxygen species and protects the animal against gamma-radiation induced death, and could especially enhance the survival of animals when administrated shortly after irradiation (Gudkov *et al*., 2005b). It could be concluded that adenosine could protect against radiation-induced oxidative stress in experimental rats via affecting ROS generation system and salvage of antioxidant status thus it can be conveniently incorporated in the diet as a nutritional supplement.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Abd El-Rahman, N.A. , Kamal El-Dein, E.M., Abd El-Hady A.M. and Soliman S.M., 2016. Effect of hesperidin on γ-radiation- and/or paraquat herbicide-induced biochemical, hematological and histopathological changes in rats. *Pakistan J. Zool.*, **48**: 1407-1415.

Adaramoye, O.A., Adedara, I.A. and Farombi, E.O., 2012. Possible ameliorative effects of kolaviron against reproductive toxicity in sub-lethally whole body gamma-irradiated rats. *Exp. Toxicol. Pathol*., **64:** 379-385.

Adaramoye, O.A., Okiti, O. and Farombi, E.O., 2011. Dried fruit extract from *Xylopia aethiopica* (Annonaceae) protects Wistar albino rats from adverse effects of whole body radiation. *Exp. Toxicol. Pathol*., **63:** 635-643.

Agrawal, A. and Kale, R.K., 2001. Radiation induced per oxidativedamage: mechanism and significance. *Indian J. exp. Biol*. **39:** 291-309.

Berry, C.E. and Hare, J.M., 2004. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J. Physiol*., **555:** 589-606.

Beutler, E., Duron, O. and Kefly, B.M., 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med*., **61**: 882-888.

Conn, H.J. and Darrow, M.A.A., 1960. *Staining procedure used by the biological stain commission.*, N.Y. Biotech. Publication, Geneva..

Droge, W., 2002. Free radicals in the physiological control of cell function. *Physiol. Rev*. **82:** 47-95.

Fahim, T.H.M., 2008. Possible radio-protective efficiency of bee-pollen against radiation induced cardiotoxicity in male rats. *Egypt. J. Rad. Sci. Applic*., **21:** 547-563.

Farvin, K.H.S., Anandan, R., Kumar, S., Sankar, T.V. and Thankappan, T.K., 2004. Effect of squalene on tissue defense system in isoproterenol – induced myocardial infarction in rats. *Pharmacol. Res.*, **50:** 231-236.

Fraquharson, C.A., Butler, R., Hill, A., Belch J.J. and Struthers, A.D., 2002. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation*, **106:** 221-226.

Gaur, V., Aggarwal, A. and Kumar, A., 2011. Possible nitric oxide mechanism in the protective effect of hespiridin against ischemic perfusion cerebral injury in rats. *Indian J. exp. Biol*., **49**: 609-618.

Gudkov, S., Gudkova, O., Shtarkman, I., Gapeev, A., Chemeris, N. and Bruskov, V., 2006b. Guanosine and inosine as natural geneprotectors for mice blood cells exposed to X-rays. *Rad. Biol. Radioecol*., **46**: 713-718.

Gudkov, S.V., Shtarkman, I.N., Smirnova, V.S., Chernikov, A.V. and Bruskov, V.I. 2006a. Guanosine and inosine display antioxidant activity, protect DNA in vitro from oxidative damage induced by reactive oxygen species, and serve as radioprotectors in mice. *Radiat. Res*., **165**: 538-545**.**

Guinzberg, R., Cortes, D., Diaz-Cruz, A., Riveros-Rosas, H., Villalobos-Molina, R. and Pina, E. 2006. Inosine released after hypoxia activates hepatic glucose liberation through A3 adenosine receptors. *Am. J. Physiol. Endocrinol. Metab*., **290:** E940-951.

Haismann, P. and Muller, B.R., 1977. *Glossy of clinical chemistry terms.* Butterworth, London, pp.617.

Hamza, R.G. and El-Shennawy, H.M. 2016. Study of the influence of Egyptian apple against oxidative stress in gamma-irradiated rats. *Pakistan J. Zool.*, **48**: 547-556.

Hartz, 1972. Tissues were fixed in bouins solution for 24h according to hartz technique *Am. J. clin. Pathol*., **17**: 750.

Hasko, G., Sitkovsky, M.V. and Szabo, C., 2004. Immuomodulatory and neuroprotective effects of inosine. *Trends Pharmacol. Sci*., **25:** 152-157.

Henderson, A.R. and Moss, D.W., 2001.: *Enzymes tietz fundamentals of clinical chemistry*, 5th Ed. (eds. C.A. Burtis, C.A. and E.R. Ashwood) Philadelphia, USA, pp. 352.

Holmgren, A., Johansson, C., Berndt, C., Lonn, M.E., Hudemann, C. and Lillig, C.H., 2005. Thiol redox control via thioredoxin and glutaredoxin systems. *Biochem. Soc. Trans*., **33:** 1375-1377.

Hou, B., Xu, Z.W., Yang, C.W., Gao, Y., Zhao, S.F. and Zhang, C.G., 2007. Protective effects of inosine on mice subjected to lethal total-body ionizing irradiation. *J. Radiat. Res.*, **48:** 57-62.

Johansson, L.H. and Borg, L.A., 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal. Biochem*., **174:** 331-336.

Johnson, C.R., Chun, J., Bittman, R. and Jarvis, W.D., 2004. Intrinsic cytotoxicity and chemomodulatory actions of novel phenethylisothiocyanate sphingoid base derivatives in HL-60 human promyelocytic leukemia cells. *J. Pharmacol. exp. Ther*., **309**: 452-461.

Kaminski, W. and Jewezska, M.M., 1979. Intermediate

dehydrogenase-oxidase form of xanthine oxidoreductase in rat liver. *Biochem. J*., **181:** 177-182.

Kotzampassi, K., Tzitzikas, Y., Papavramidis, T.S., Kolettas, A., Vrettou, E., Spiliadi, C.H., Paramtthiotis, D., Metaxas, G. and Eleftheriadis, E., 2009. N-3 fatty acids ameliorate radiation-induced liver injury in the rat. *Annls. Gastroenterol*., **22:** 106-111.

Kregel, K.C., and Zhang, H.J., 2007. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **292**: R18–36.

Krishna, A. and Kumar, A., 2005. Evaluation of radioprotective effect of Rajira (*Amaranthus paniculatus*) extract in Swiss albino mice. *J. Rad. Res*., **46:** 233-239.

Kudina, N.H., Andriichuk, T.R. and Tsudzevych, B.O., 2003. Activity of purine enzyme metabolism in rat thymocytes after irradiation and after administration of riboxine. *Ukr. Biokhim. Zh*., **75**: 109-112.

Mabley, J.G, Rabinovitch, A., Suaez-pinzon, W., Gyorgy, H., Pacher, P., Power, R., Southan, G., Salzman, A. and Szabo, C., 2003. Inosine protects against the development of diabetes in multiple-low-dose streptozotocin and nonobese diabetic mouse models of type 1 diabetes. *Mol. Med*., **9:** 96-104.

Mansour, H.H. and Abuo El-Nour, S.M., 2009. Biochemical and histopathological studies on the protective effect of propionyl-L-carnitine against cardiotoxicity in rats. *Egypt. J. Rad. Sci. Applic*., **22**: 99-128.

Marton, A, Pacher, P., Murthy, K.G., Németh, Z.H., Haskó, G. and Szabó, C., 2001. Anti-inflammatory effects of inosine in human monocytes, neutrophils and epithelial cells *in vitro*. *Int. J. mol. Med.*, **8**: 617–621.

Meister, A., 1991. Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol. Ther*., **51:** 155-194.

Minami, M.X. and Yoshikawa, H., 1979. A simplified assay method of superoxide dismutase. *Clin. Chim. Acta*, **92**: 337–342.

Mustafa, S.J., Morrison, R.R., Tengm, B. and Pelleg, A., 2009. Adenosine receptors and the heart: role in regulation of coronary blood flow and cardiac electrophysiology. *Handb. Pharmacol*., **193**: 161-188.

Oktem, F., Arslan, M.K. and Dundar, B., 2004. Renal effects and erythrocyte oxidative stress in long-term low-level lead-exposed adolescent workers in auto repair workshops. *Arch. Toxicol*., **78:** 681-687.

Ozkan, A. and Fiskin, K., 2004. Free radicals, carcinogenesis and anti-oxidant enzymes. *Turkish J. Hematol. Oncol*., **14:** 52-60.

Rec, 1977 German Clinical Chemistry Society. Kinetic determination of creatine phosphokinase activity. *J. clin. Biochem.*, **15:** 255.

Reitman, S. and Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. clin. Pathol*., **28:** 56-63.

Riabchenko, N.I., Ivannik, B.P., Riabchenko, V.I. and Dzikovskaia, L.A., 2011. Influence of ionizing radiation, application of iron ions and their chelate complexes on the oxidative status of blood serum of rats. *Rad. Biol. Radioecol*., **51:** 229-232.

Robbins, M.E., Zhao, W., Davis, C.S., Toyokuni, S. and Bonsib, S.M., 2002. Radiation induced kidney injury: a role for chronic oxidative stress? *Micron*, **33:** 133-141.

Rossi, R., Dalle-Donne, I., Milzani, A., and Giustarini, D., 2006. Oxidized forms of glutathione in peripheral blood as biomarkers of oxidative stress. *Clin. Chem*., **52:** 1406-1414.

Said, U.Z., Soliman, S.M. and El-Tahawy, N.A., 2002. Possible protective and curative role of thiamine pyrophosphate against radiation induced biochemical and histological changes in male albino rats. *Egypt. J. Rad. Sci. Applic*., **15**: 17.

Sandeep, D. and Nair, C.K., 2012. Protection from lethal and sub-lethal whole body exposures of mice to γ- radiation by *Acoruscalamus* L: studies on tissue antioxidant status and cellular DNA damage. *Exp. Toxicol. Pathol*., **64:** 57-64.

Schneider, S. and Klein, H.H., 2005. Inosine improves islet xenograft survival in immunocompetent diabetic mice. *Eur. J. med. Res*., **10**: 283-286.

Sener, G., Kabasakal, L., Atasoy, B.M., Erzik, C., Velioglu-Ogunc, A., Cetinel, S, Contuk, G., Gedik, N. and Yegen, B.C., 2006. Propylthiouracil-induced hypothyroidism protects ionizing radiation-induced multiple organ damage in rats. *J. Endocrinol*., **189:** 257-269.

Soliman, S.M., 1997**.** *Biochemical and histological studies on lipid pattern in blood and cardiovascular system as affected by dietary fat intake and/or whole body radiation exposures*. Ph.D. thesis, Faculty of Science, Cairo University, Egypt..

Soliman, S.M., 2007**.** Protective role of oregano oil against histological changes in whole body gamma irradiated albino rats. *J. Egypt. Ger. Soc. Zool*., **52C:** 46-56.

Spitz, D.R., Azzam, E.I., Li., J.J. and Gius, D., 2004. Metabolic oxidation reduction reaction and cellular responses to ionizing radiation: a unifying concept in stress response biology. *Cancer Metast. Rev*., **23:** 311-322.

Srivastava, M., Chandra, D. and Kale, R.K., 2002. Modulation of radiation-induced changes in the xanthine oxidoreductase system in the liver of mice by its inhibitors. *Rad. Res*. **157:** 290-297.

Stadtman, E.R. and Levine, R.L., 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*, **25:** 207-218.

Struthers, A.D., Donnan, P.T., Lindsay, P., McNaughton, D., Broomhall, J., and MacDonald, T.M., 2002. Effect of allopurinol on mortality and hospitalizations in chronic heart failure: a retrospective cohort study. *Heart*, **87:** 229-234.

Sun, J., Chen, Y., Li, M. and Ge, Z., 1998. Role of antioxidant enzymes on ionizing radiation resistance. *Free Radic. Biol. Med.*, **24**: 586-593.

Tominaga, H., Kodama, S., Matsuda, N., Suzuki, K. and Watanabe, M., 2004. Involvement of reactive oxygen species (ROS) in the induction of genetic instability by radiation. *J. Rad. Res*., **45**: 181.

Van der Meeren, A., Monti, P., Vandamme, M., Squiban, C. Wysock, J. and Griffiths, N., 2005. Abdominal radiation exposure elicits inflammatory responses and obscopal effects in the lungs of mice. *Rad. Res.*, **163**: 144.

Yoshioka, T., Kawada, K., Shimada, T., Mori, M., 1979. Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in the blood. *Am. J. Obstet. Gynecol*. **135**: 372–376.

Zhang, H.J., Zhao, W., Venkataraman, S., Robbins, M.E.C., Buettner, G.R., Kregel, K.C. and Oberley, L.W., 2002. Activation of matrix metalloproteinase-2 by overexpression of manganese superoxide dismutase in human breast cancer MCF-7 cells involves reactive oxygen species. *J. biol. Chem*., **277:** 20919-20926.

Zhao, W., Diz, D.I. and Robbins, M.E., 2007. Oxidative damage pathways in relation to normal tissue injury. *Br. J. Radiol*., **80:** S23-31.