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Possible Role of *Withania somnifera* Against Gamma Radiation Induced Cardiotoxicity in Male Albino Rats

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ABSTRACT

The study aims to investigate the possible role of *Withania somnifera* (WS) against γ-rays induced cardiac lesions in male albino rats. Forty albino rats were divided into four equal groups as follows: control group, rats were administered vehicle by gastric tube for 7 consecutive days. Irradiated group (animals subjected to a single dose of whole body γ-rays (10 Gy), treated group (each rat received 100 mg/kg body weight WS once daily, orally by gastric tube for one week), and treated irradiated group (each rat received 100 mg/kg body weight WS once daily, orally by gastric tube for one week, then one hour later after the last treatment, rats were exposed to a single dose of whole body γ-rays (10 Gy). The results revealed that administration of WS to rats pre-irradiation significantly abolish the radiation-induced elevation of serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and asparatate amino transferase (AST) activities, total cholesterol and low-density lipoprotein-cholesterol (LDL-C) levels, and restored high-density lipoprotein-cholesterol (HDL-C), creatine kinase-MB (CK-MB) and cardiac troponin 1 (cTnI) to normal levels. Moreover, WS treatment elevates cardiac catalase (CAT) and superoxide dismutase (SOD) activities while reduced malondialdehyde (MDA) level compared to irradiated group. The histopathological results showed distinctive pattern of myocardial injuries in irradiated group, while in treated-irradiated group the myocardial tissues showed minimum injury with or without congested blood vessels or edema. In conclusion, WS acts as a potent scavenger of free radicals to prevent or ameliorates the toxic effects of γ-rays.

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AAE and ETM designed the study. AAE performed histopathological analysis. ETM performed biochemical studies. AAE wrote the article.

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Antioxidant, *Withania somnifera*, γ-rays, cardotoxicity.

INTRODUCTION

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). ROS also negatively impact the antioxidant defense mechanisms, reduce the intracellular concentration of glutathione (GSH) and decrease the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) (Gracy *et al.*, 1999). Azab *et al.* (2011) found that radiation may cause the generation of ROS that interacts with biological molecules and producing toxic free radicals. The final product of peroxidation is malondialdehyde (MDA), major aldehyde product that is mutagenic in cells and could be assessed to evaluate tissue injury (Dalle-Donne *et al.*, 2006). Plasma lipids increased by day 6 post irradiation; plasma cholesterol, triglycerides (TG) were increased. Also low-density lipoprotein (LDL) was increased and accumulated in plasma while high-density lipoprotein (HDL) level was decreased (Feurgard *et al*., 1999). The exposure of the human body to ionizing radiation leads to depletion of endogenous antioxidants (Koc *et al.*, 2003) and ultimately to the development of systemic disease. Recently, research has focused on finding effective and reliable antioxidants that can protect tissues against radiation-induced damage.

Withania somnifera Dunal (ashwagandha, Indian ginseng,WS) belonging to the family solanaceae, is widely used in Ayurvedic medicine (Sangwan *et al.*, 2007). The pharmacological effects of the WS roots are attributed to the presence of withanolides, a group of steroidal lactones (Udayakumar *et al.*, 2009). It restores physical and mental health of the body in debilitated conditions, and increase longevity (Kulkarni and Dhir, 2008). WS is known to have anti-inflammatory (Alhindawi *et al.*, 1992), anti-tumor (Widodo *et al.*, 2010), anti-diabetic (Prasad *et al.*, 2010), anti-oxidant (Das *et al.*, 2010), cardio-protective (Deocaris *et al.*, 2008) and anti-stress effect (Udayakumar *et al.*, 2010). WS also increases tumor sensitization to radiation and chemotherapy while reducing some of the most common side effects of these conventional therapies (Sharada *et al.*, 1996). Therefore, the present study was aimed to determine the cardio-protective effects of WS root

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extracts on ionizing radiation-induced oxidative stress.

MATERIALS AND METHODS

Animals

Adult male Swiss Albino rats (100-110g) were obtained from the Egyptian organization for biological product and vaccines Giza, Egypt. Animals were kept under good ventilation and illumination condition and received standard diet and water. The animals' treatment protocol was approved by the animal care committee of the National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Radiation processing

It was performed by using gamma cell-40 (Cesium - 137) located at NCRRT, Cairo, Egypt. Animals were irradiated with a single dose level of (10 Gy) delivered at a dose rate of 0.46 Gy/min. at the time of experimentation. Animals were not anesthetized before irradiation.

Chemicals

Withania somnifera (WS) roots extract manufactured by Idea Sphere Inc., American Fork, USA. The alcoholic extract was filtered through a Whatman filter paper #4 and evaporated in a rotary evaporator under reduced pressure at 60°C, which was stored in refrigerator for further use (Senthilnathan *et al.,* 2006). The required amount was suspended in 0.5 ml of double distilled water (DDW) (Balgia *et al.,* 2004).

Experimental design

Forty rats were divided into four groups (n = 10). Group I (control group), rats received orally by gastric tube an equivalent volume of distilled water (vehicle of WS) during one week. Group II (irradiated group) received orally by gastric tube an equivalent volume of distilled water during one week, then whole body exposed to an acute single dose level of 10 Gy. Group III (treated group), rats received WS orally by gastric tube 100 mg/kg body weight once daily, for one week according to Rajasankar *et al.* (2009). Group IV (treated irradiated group) rats received WS orally by gastric tube (100 mg/kg body weight) once daily, for one week, then one hour post the last dose of WS, rats were whole body gamma-irradiated with an acute single dose of 10 Gy. Rats were sacrificed on the 6^{th} day post radiation exposure or 13^{rd} days from WS administration.

Samples collection

After an overnight fast, rats were anesthetized with ether and then sacrificed. Blood samples from each rat were collected by retro-orbital puncture using blood capillary tubes. Serum was obtained immediately by centrifugation of blood samples at 3000 rpm for 10 min. Heart was directly separated after sacrifice, washed in ice-cold saline then the heart samples were homogenized in distilled water (10% W/V) using homogenizer then the cell debris was removed by centrifugation at 3000 rpm for 10 min. The homogenates supernatant were subjected to biochemical analysis. Tissue specimens from heart were collected and fixed in 10% buffered formalin solution followed by dehydration, clearing and embedding in paraffin. Paraffin sections of 5µ thickness were prepared and stained routinely with haematoxylin and eosin according to Bancroft and Stevens (1996) and examined microscopically.

Estimation of biochemical parameters

Creatine phosphokinase (CPK) level was estimated according to the method of Okinaka *et al.* (1964) and LDH was assayed depending on the method of Henery (1974). Moreover, serum total cholesterol (TC) concentration was estimated as described by Allain *et al.* (1974). HDL-C and LDL-C were determined according to the methods described by Demacker *et al.* (1980) and Marchal, (1992) respectively. The activity of AST was determined according to the method of Reitman and Frankel (1957). CK-MB and cTnI were performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. CAT activity was assayed using the method of Sinha (1972). SOD was determined according to the method described by Kakkar *et al.* (1984) and MDA level was estimated following the method reported by Buege and Aust (1978).

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) followed by LSD as is post hoc test. The results obtained were expressed by mean ± standard deviation. Differences were considered significant at $P \leq 0.05$ (George and William, 1980).

RESULTS

As presented in Table I, whole body gamma-irradiation induced a significant increase in the activity of serum CPK, LDH, cholesterol and LDL-C while a significant decrease in HDL-C concentration was noticed compared to control group. Pretreatment with WS prior to gamma irradiation was found to significantly abolish these radiation-induced elevations in the levels of serum CPK, LDH, cholesterol and LDL-C and also maintained the level of HDL-C near the normal level. CK-MB and

cTnI were a significant increase in irradiated group. Animal group treated with WS showed non-significant changes in the concentration of serum CPK, LDH, cholesterol, LDL-C, HDL-C, cTnI and CK-MB compared to those of control group.

The effects of γ-rays on endogenous antioxidant status are shown in Table II. Gamma-irradiation induced a significant decrease in the activity of heart CAT and SOD as well as a significant increase in the level of MDA compared to control group. Administration of WS prior to gamma irradiation of rats restored the reduced CAT and SOD activity, while it decreased MDA level compared to irradiated group. Animal group treated with WS showed insignificant changes in the activity of heart CAT, SOD and MDA compared to those of control group.

Histopathological finding

Light microscopic examination of cardiac muscle of group I (controls) showed normal myocytes with one or two large oval nuclei occupying a central position. The perinuclear sarcoplasm region is distinct. The intercalated disks are irregular and wider than the normal cross-striations, and represent specialized junctions between cardiac muscle fibers (Fig. 1). In group II (irradiated group), the myocardial muscles were necrotic with effacement of their structural details of their and replaced by spindle cells (fibroblasts) in some cases. In other cases the necrotic muscles fibers were middle infiltrated with leucocytes, edema, associated with loss of striation and disappearance of nuclei and intercalated disks (Fig. 2). Moreover, in few cases, fibrinoid necrosis in coronary vessel represented by circumferential bright pink area of necrosis with protein deposition (Fig. 3), other cases showed removal of necrotic myocytes by phagocytosis (Fig. 4). In group III (WS treated rats), the cardiac muscle showed normal structure. On the other hand, most cases of group IV (treated irradiated group) myocardial muscle showed slightly preserved architecture without necrosis, degenerative changes. In few cases preserved myocardial muscle were seen with dilatation of coronary blood vessels and edema (Fig. 5).

DISCUSSION

It has been proposed that radiation-induced effects are caused by oxidative stress and inflammation. Increased production of reactive oxygen species (ROS), which leads to lipid peroxidation, oxidation of DNA and proteins, as well as activation of pro-inflammatory factors has been observed *in vitro* and *in vivo* (Zhao and Robbins, 2009).

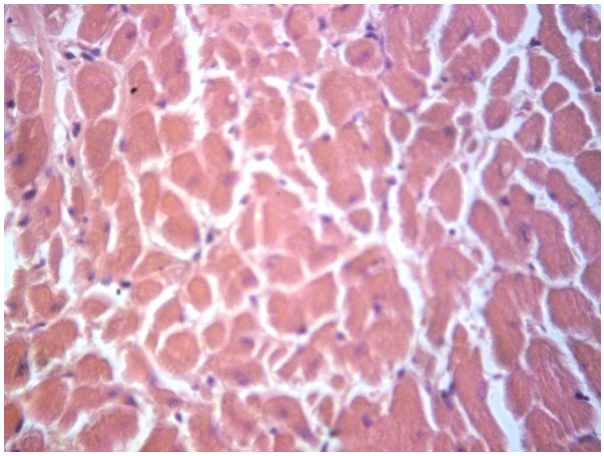

Fig. 1. Heart of control rat (group 1) showing normal structure (H& E × 400).

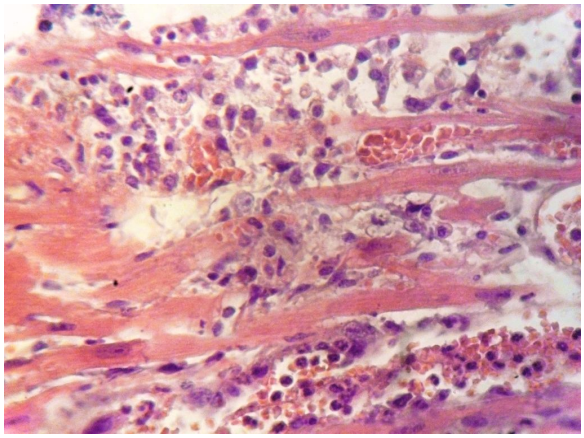

Fig. 2. Heart of irradiated rat (group II) showing necrotic myocytes, edema, associated with inflammatory cell infiltrated (H& E × 400).

A single dose (10 Gy) of whole body gamma irradiation (Table I) induced a marked increase in the activity of cardiac serum enzymes (CPK and LDH activity), in addition to the increase in total cholesterol, LDL-C, HDL-C, CK-MB and cardiac troponin I (cTnI). It is well known that the magnitude of CPK and LDH activities in blood after myocardial injury reflects the extent of damage in its musculature (Preus *et al.*, 1988). The result are in accordance with previous findings reported by Elkady and Ibrahim (2014) and Khaled *et al.* (2011). The mechanism of radiation-induced cardiotoxicity has been reported to be through formation of superoxide anions and their derivatives, particularly highly reactive and damaging hydroxyl radicals, which induces peroxidation of cell membrane lipid (Hemnani and Parihar, 1998).

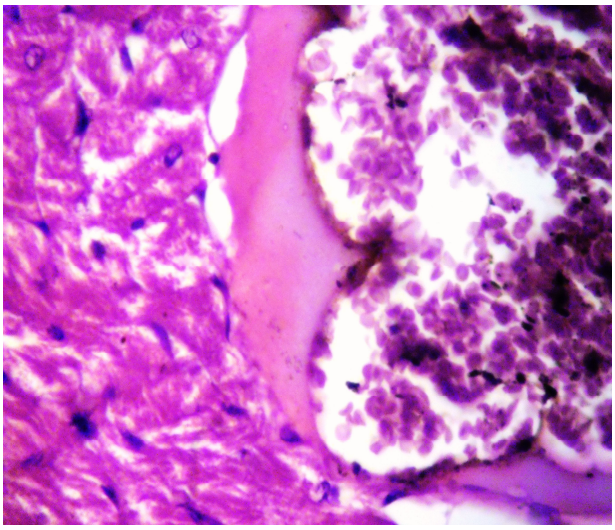

Fig. 3. Heart of irradiated rat (group II) showing fibrinoid necrosis in coronary vessel (H& E × 400).

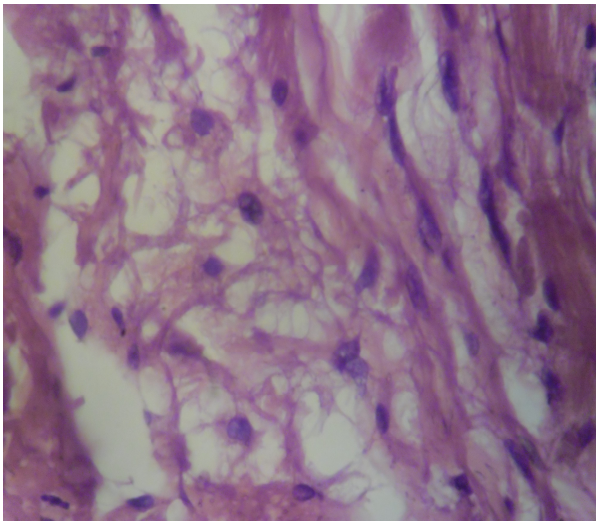

Fig. 4. Heart of irradiated rat (group II) showing removal of necrotic myocytes by phagocytosis (H& E × 400).

In the present study gamma-irradiation induced a significant decrease in the activity of heart CAT and SOD in parallel to a significant increase in the level of MDA compared to control group (Table II). Such results explain that the exposure to ionizing radiation increases the production of ROS and directs the irradiated cells into a state of oxidative stress that has been implicated in a variety of natural and pathological processes (Hong *et al.*, 1999). Some authors reported that injury to the heart from irradiation appears to be indirect, supporting the notion that injury to abdominal organs, principally the kidneys, is responsible for the increased risk factors for and the occurrence of cardiac disease after total body irradiation (Lenarczyk *et al.*, 2013). Renal dysfunction may be a part of the mechanism responsible for the increased risk for heart disease following total body irradiation in adults (Adams *et al.*, 2012).

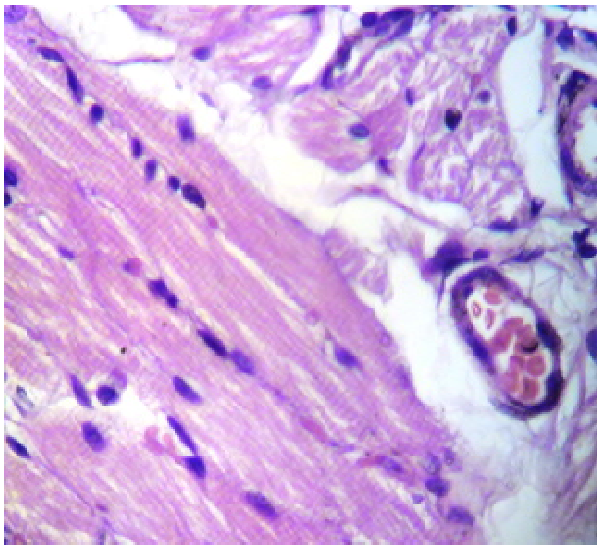

Fig. 5. Heart of treated irradiated rat (group IV) showed minimal architecture distrcture of normal myocytes with dilated blood vessel and edema (H& E × 400).

In the present study, treatment of rats with WS prior to gamma irradiation restored the activity of serum CPK, LDH, as well as the level of total cholesterol, LDL-C, HDL-C, cTnI and (CK-MB) to their normal control levels. Such results indicate that WS pretreatment protects against radiation damage. The results corroborates the findings of Mansour and Hafez (2012) that WS exhibits a radio-protective effect against gamma radiation by preventing oxidative stress. In the same line, Khaled *et al.* (2011) reported that WS enhanced superoxide dismutase and catalase activities in heart tissue in ischemic reperfusion injury. Also WS treatment before irradiation protected against oxidative stress, evidenced by increased SOD and CAT activities and decreased MDA levels of heart tissues compared to the irradiated non treated rats (Table II), thus indicating protective role of WS against radiation toxicity, which is substantiated by the previous findings of Bharavi *et al.* (2010), Rajasankar *et al.* (2009), Udayakumar *et al.* (2010), Sharma *et al.* (2011a,b). These findings suggest that the protective actions of WS are mediated via its

Table I.- Effect of *Withania somnifera* (WS) root extracts on creatine phosphokinase (CPK), creatine kinase (CK-MB), cardiac troponin I (cTnI), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein (HDL-C) of γ radiated male albino rats.

Parameters	Control	WS	γ-rays	WS- γ-rays
CPK (U/L)	265.53±6.23	260.22±8.75	482.25±14.54[a]	326.31±12.32[b]
CK-MB (ng/mL)	3.33± 0.72	2.8± 0.66	7.97± 0.98 [a]	5.8± 0.85 [b]
cTnI (ng/mL)	24.74±0.96	23.51±1.02	77.31±2.87 [a]	47.77± 1.65 [b]
AST (U/L)	34.22±1.14	32.47±1.30	75.76±2.34 [a]	45.26± 1.24 [b]
LDH (U/ml)	220.3±11.76	221.3± 12.76	448.4± 23.79[a]	330.4± 17.88 [b]
Cholesterol (mg/dl)	136.26±3.11	130.71± 3.01	269.86± 5.37 [a]	185.27±3.24 [b]
LDL-C (mg/dl)	40.25±1.23	39.12± 1.56	150.67±4.77 [a]	74.92±3.24 [b]
HDL-C (mg/dl)	47.61± 1.01	44.92± 1.54	35.41±2.87 [a]	37.01±2.21 [b]

All values are expressed as mean± SD. [a] Significant *(P<0.05)* when compared to the control group. [b] Significant *(P<0.05)* when compared to the irradiated group.

Table II.- Effect of *Withrania somnifera* (WS) root extract on catalase (CAT), superoxide dismutase (SoD) and malondialdhyde (MDA) of γ irradiated male albino rats

Parameters	Control	WS	γ-rays	WS- γ-rays
CAT (U/mg protein)	2.77±0.72	2.95±0.83	1.22±0.91[a]	2.20±0.98[b]
SOD (U/mg protein)	3.21±0.97	3.32±1.15	1.40±0.94 [a]	2.41±0.88 [b]
MDA (nmol/g protein)	8.70±1.98	9.14±1.77	16.80±2.54 [a]	12.91±2.35 [b]

All values are expressed as mean± SD [a] Significant *(P < 0.05)* when compared to the control group. [b] Significant *(P < 0.05)* when compared to the irradiated group.

antioxidant activity. In the same context, Priyandoko *et al.* (2011) suggested that the cells treated with WS could be protected against toxicity by multiple mechanisms including reduction in the production of ROS, subsequent damage at DNA and mitochondrial level, and induction of cellular defense machinery.

In the current study oxidative stress in cardiac tissues were accompanied by severe histopathological changes. The results revealed that in the irradiated group (Group II), the myocardial muscles were necrotic with effacement of their details and replaced by spindle cells (fibroblasts), highly infiltrated with leucocytes infiltration, fibrinoid necrosis in coronary vessel, edema and hyalinization of myocardial muscles were remarked which is in accordance with the previous results observed by Baker *et al.* (2009), Khaled *et al.* (2011) and Elkady and Ibrahim (2014). While in treated irradiated group (Group IV) the structure of myocytes showed slightly preserved architecture without necrosis, degenerative changes or hyalinization. In few cases preserved myocardial muscle were seen with dilatation of coronary blood vessels and edema. Such results indicated the cardio-protective role of WS (Deocaris *et al.*, 2008) as antioxidant agent which prevent the action of highly acute dose (10 Gy whole body radiation) of gamma rays which is in agreement with previous results (Mansour and Hafez, 2012; Priyandoko *et al.*, 2011). In the current study the amelioration of the histopathological changes observed in cardiac tissues was associated with a significant improvement in oxidative stress and MDA suggesting that WS protect cardiac tissues from radiation-induced lipid peroxidation and damage of cell membrane. The protective action of WS could be attributed also to its anti-inflammatory (Alhindawi *et al.*, 1992), antioxidant (Das *et al.*, 2010).

In conclusion, the present work documents that treatment with WS offers protection from radiation-induced cardotoxicity. The deterioration of biochemical parameters and histological damage in heart caused by gamma irradiation are markedly improved by WS treatment. These observations may be attributed partly to the considerable antioxidant effect of WS and suggest that it may be a valuable prophylactic agent against a variety of conditions and diseases.

Conflict of interests statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Adams, M.J., Grant, E.J., Kodama, K., Shimizu, Y., Kasagi, F., Suyama, A., Sakata, R. and Akahoshi, M., 2012. Radiation dose associated with renal failure mortality: a potential pathway to partially explain increased cardiovascular disease mortality observed after whole-body irradiation. *Radiat. Res.*, **177**:220-228.

Alhindawi, M.K., Alkhafaji, S.H. and Abdulnabi, M.H., 1992. Antigranuloma activity of Iraqi *Withania somnifera*. *J. Ethnopharmacol.*, **37**: 113-11.

Allain, C., Poon, L., Chan, C. and Fu, P., 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.*, **20**: 470.

Azab, K.S., Bashandy, M., Salem, M., Ahmed, O. Tawfik, Z. and Helal, H., 2011. Royal jelly modulates oxidative stress and tissue injury in gamma irradiated male Wister Albino rats. *N. Am. J. med. Sci.*, **3**): 268-76.

Baker, J.E., Fish, B.L., Su, J., Haworth, S.T., Strande, J.L., Komorowski, R.A., Migrino, R.Q., Doppalapudi, A, M., Harmann, L., Li, X.A., Hopewell, J.W. and Moulder, J.E., 2009. 10 Gy total body irradiation increases risk of coronary sclerosis, degeneration of heart structure and function in a rat model. *Int. J. Radiat. Biol.*, **85**:1089-1100.

Balgia, M., Jagetia, S., Venkatesh, G.C., Reddy, P. and Ulloor, J.N.R., 2004. Radiprotective effect of abana, a polyherbal drug following total body irradiation. *Br. J. Radiol.*, **77**: 1027-1103.

Bancroft, J.D. and Stevens, A.E., 1996. *Theory and practice of histological techniques*. 4th ed. Edinburgh; Churchill Livingstone. P.766.

Bharavi, K., Gopala reddy, A., Rao, G.S., Rajasekhara, A. and Rama, S.V., 2010. Reversal of cadmium-induced oxidative stress in chicken by herbal adaptogens *Withania somnifera* and *Ocimum sanctum*. *Toxicol. Int.*, **17**: 59-63.

Buege, J.A. and Aust, S.D., 1978. Microsomal lipid peroxidation. *Meth. Enzymol.*, **52**: 302–310.

Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A., 2006. Biomarkers of oxidative damage in human disease. *Clin. Chem.*, **52**: 601-623.

Das, K., Samanta, T.T., Samanta, P. and Nandi, D.K., 2010. Effect of extract of *Withania somnifera* on dehydration-induced oxidative stress-related uremia in male rats. *Saudi J. Kidney Dis. Transpl.*, **21**: 75-80.

Demacker, P., Vos-Janssen, H., Hijmans, A., Vant Laar, A. and Jansen, A., 1980. Measurement of high-density lipoprotein cholesterol in serum: comparison of six isolation methods combined with enzymic cholesterol analysis. *Clin. Chem.*, **26**: 1780.

Deocaris, C.C., Widodo, N., Wadhwa, R. and Kaul, S.C., 2008. Merger of ayurveda and tissue culture-based functional genomics: inspirations from systems biology. *J. Transl. Med.*, **6**: 14–19.

Elkady, A.A. and Ibrahim, R.M., 2014. Royal jelly attenuates ionizing radiation induced cardiac toxicity in male albino rats. *Med. J. Cairo Univ.*, **82**: 13-18.

Feurgard, C., Boehler, N. and Ferezou, J., 1999. Ionizing radiation alters hepatic cholesterol metabolism and plasma lipoproteins in Syrian hamster. *Int. J. Radiat. Biol.*, **75**:757–766.

George, W. and William, G., 1980. *Statistical methods*, seventh edition, pp. 217.

Gracy, R.W., Talent, J.M., Kong, Y., and Conrad C.C., 1999. Reactive oxygen species: the unavoidable environmental insult? *Mutat. Res.*, **428**:17–22.

Hemnani, T. and Parihar, M.S., 1998. Reactive oxygen species and oxidative DNA damage. *Ind. J. Physiol. Pharmacol.*, **42**: 440.

Henery, R.J., 1974. Colorimetric determination of lactic dehydrogenase. In: *Clinical chemistry: Principles and techniques* (ed. R.J. Henery). Second ed. Harper & Row. Hagerstown MD, pp. 819-831.

Hong, J.H., Chiang, C.S., Tsao, C.Y., Lin, P.Y., McBride, W.H. and Wu, C.J., 1999. Rapid induction of cytokine gene expression in the lung after single and fractionated doses of radiation. *Int. J. Rad. Biol.*, **75**:1421.

Kakkar, P., Das, B. and Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. *Ind. J. Biochem. Biophys.*, **21**:130-132.

Khaled, S.A., Mohamed, B., Mahmoud, S., Osama, A., Zaki, T. and Hamed, H., 2011. Royal jelly modulates oxidative stress and tissue injury in gamma irradiated male wister albino rats. *N. Am. J. med. Sci.*, **3**: 268-2776.

Koc, M., Taysi, S., Buyukokuroglu, M.E. and Bakan, N., 2003. Melatonin protects rat liver against irradiation-induced oxidative injury. *J. Radiat. Res.*, **44**:211–15.

Kulkarni, S.K. and Dhir, A., 2008. *Withania somnifera*: An Indian ginseng. *Prog. Neuropsychopharmacol. Biol. Psychiat.*, **32**: 1093-1105.

Lenarczyk, M.I., Lam, V., Jensen, E., Fish, B.L., Su, J., Koprowski, S., Komorowski, R.A., Harmann, L., Migrino, R.Q., Li, X.A., Hopewell, J.W., Moulder, J.E. and Baker, J.E., 2013. Cardiac injury after 10 Gy total body irradiation: indirect role of effects on abdominal organs. *Radiat. Res.*, **180**:247-258.

Mansour, H.H. and Hafez, H.F., 2012. Protective effect of *Withania somnifera* against radiation-induced hepatotoxicity in rats. *Ecotoxicol. Environ. Safety*, **80**:14-19.

Marchal, W., 1992, *Clinical chemistry*, 2nd. Gower Medical Publishing, U.K. pp. 222.

Okinaka, S., Sugita, H., Momoi, H., Toyokura, Y., Watanabe, T. and Ebashi, F., 1964. Cysteine-stimulated serum creatine kinase in health and disease. *J. Lab. Clin. Med.*, **64**: 299.

Prasad, S.K., Kumar, R., Patel, D.K. and Hemalatha, S., 2010. Wound healing activity of *Withania coagulans* in streptozotocin-induced diabetic rats. *Pharm. Biol.*, **48**:

1397-1404.

Preus, M., Bhargava, A.S., Khater, A.E. and Günzel, P., 1988. Diagnostic value of serum creatine kinase and lactate dehydrogenase isoenzyme determinations for monitoring early cardiac damage in rats. *Toxicol. Lett.*, **42**: 225.

Priyandoko, D., Ishii, T., Kaul, S.C. and Wadhwa, R., 2011. Ashwagandha leaf derived withanone protects normal human cells against the toxicity of methoxyacetic acid, a major industrial metabolite. *PloS One*, **6**: 1-10.

Rajasankar, S., Manivasagam, T. and Surendran, S., 2009. Ashwagandha leaf extract: a potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease**.** *Neurosci. Lett.*, **454**: 11-15.

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

Sangwan, R.S., Chaurasiya, N.D., Lal, P., Misra, L., Uniyal, G.C., Tuli, R. and Sangwan, N.S., 2007. Withanolide A biogeneration in *in vitro* shoot cultures of ashwagandha (*Withania somnifera* Dunal), a main medicinal plant in ayurveda. *Chem. Pharm. Bull.*, **55**: 1371-1375.

Senthilnathan, P., Padmavathi, R., Banu, S.M. and Sakthisekaran, D., 2006. Enhancement of antitumor effect of paclitaxelin combination with immunomodulatory *Withania somnifera* on benzo(a) pyrene induced experimental lung cancer. *Chem. Biol. Interact.*, **159**: 180-185.

Sharada, A.C., Solomon, F.E., Devi, P.U., Udupa, N. and Srinivasan, K.K., 1996. Antitumor and radiosensitizing effects of withaferin on mouse Ehrlich ascites carcinoma *in vivo*. *Acta Oncol.*, **35**: 95-100.

Sharma, K.K., Swarts, S.G. and Bernhard, W.A., 2011a. Mechanisms of direct radiation damage to DNA: the effect of base sequence on base end products. *J. Phys. Chem. B*, **115**: 4843-4855.

Sharma, V., Sharma, S., Pracheta, S., Paliwal, R. and Sharma, S., 2011b. Therapeutic efficacy of *Withania somnifera* root extract in the regulation of lead nitrate induced nephrotoxicity in Swiss albino mice. *J. Pharm. Res*., **4**:755: 775.

Shirazi, A., Mihandoost, E., Mohseni, M., Ghazi-Khansari, M. and Rabie Mahdavi, S., 2013. Radio-protective effects of melatonin against irradiation-induced oxidative damage in rat peripheral blood. *Phys. Med.*, **29**: 65.

Sinha, K.A., 1972. Colorimetric assay of catalase. *Anal. Biochem.*, **47**: 389.

Udayakumar, R., Kasthurirengan, S., Mariashibu, T.S., Rajesh, M., Anbazhagan, V.R., Kim, S.C., Ganapathi, A. and Cho, C.W., 2009. Hypoglycaemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan-induced diabetic rats. *Int. J. mol. Sci*., **10**: 2367-2382.

Udayakumar, R., Kasthurirengan, S., Vasudevan, A., Mariashibu, T.S., Rayan, J.J., Choi, C.W., Ganapathi, A. and Kim, S.C., 2010. Antioxidant effect of dietary supplement *Withania somnifera* L. reduce blood glucose levels in alloxan- induced diabetic rats. *Pl. Fds. Hum. Nutr.*, **65**: 91-98.

Widodo, N., Priyandoko, D., Shah, N., Wadhwa, R. and Kaul, S.C., 2010. Selective killing of cancer cells by ashwagandha leaf extract and its component withanone involves ROS signaling. *PloS One*, **5**: 3536-e13537.

Zhao, W. and Robbins, M.E., 2009. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr. med. Chem.*, **16**: 130-143.