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Amelioration of oxidative stress induced by gamma radiation in rats using some medicinal plants

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Radiation exposure of living organisms is known to produce many harmful effects in biological systems due to generation of free radicals. Many antioxidants have been investigated as hepato-protectors against ionizing radiation induced injury since they reduce the oxidative effect of the reactive oxygen species (ROS) on normal cells. Whole body exposure to γ-irradiation provokes oxidative damage, organ dysfunction and metabolic disturbances. Fenugreek (*Trigonella foenum*-graccum L.) and Jerusalem artichoke (*Helianthus tuberosus* L.), two important medicinal plants rich in polyphenolic compounds possess antioxidant properties. This study was designed to evaluate the hazardous effects of γ-irradiation induced oxidative stress and to investigate the role of fenugreek seeds and Jerusalem artichoke tubers treatments as radio protectors in rats followed exposure to a single dose whole body gamma irradiation of 5.0 Gy. Rats irradiated at 5 Gy delivered as single dose produced a significant decrease in albumin, total protein and globulin levels as compared to normal control rats. However, γ-irradiated rats pretreated with fenugreek and Jerusalem treatments increased significantly these parameters as compared to gamma irradiated rats (5 Gy). Rats irradiated at 5 Gy produced a significant increase in the activity of the liver enzymes (ALT, AST, ALP and GGT), kidney function tests (creatinine and urea) and lipid profile (cholesterol, triglyceride, HDL and LDL). Meanwhile, gamma irradiated rats pretreated with fenugreek and Jerusalem treatments decreased significantly these parameters as compared to gamma irradiated rats. It could be concluded that fenugreek seeds and Jerusalem artichoke tubers treatments would protect from oxidative damage and tissue injury induced by γ-irradiation. Thus, supplementation with fenugreek seeds and Jerusalem artichoke tubers may have a benefit for safe application of γ-irradiation technology in medicine and industry.

Keywords: Gamma radiation, Oxidative stress, Fenugreek seeds, Jerusalem artichoke tubers, Enzymes.

INTRODUCTION

Exposure to ionizing radiation causes many health hazardous effects. Such exposure produces biochemical lesions that initiate a series of physiological symptoms (Halliwell and Gutteridge, 1989). Ionizing radiation passing through living tissues generates reactive free radicals which interact with critical macromolecules, such as DNA, proteins or membranes, and can induce cell damage and potentially, cell dysfunction and death (Pellmar and Rockwell, 2005). ROS are the cause of certain disease such as cancer tumors, cardiovascular diseases, and liver dysfunction (Florence, 1995). The damaging effects of ionizing radiation on DNA lead to cell death and are associated with an increased risk of cancer (Azab and El-Dawi, 2005). After a high dose of γ-irradiation exposure, patients usually suffer from fatal damage to multiple organs, which have been referred to collectively as acute radiation syndrome (Pellmar and Rockwell, 2005). Exposure to high amounts of ionizing radiation results in damage to the haematopoietic, gastrointestinal and central nervous system, depending on radiation dose (Halliwell and Gutteridge, 1989). Plants products appear to have an advantage over synthetic products in terms of low/no toxicity at effective dose radiation–induced free radicals in turn impair the antioxidative defense mechanism, leading to an increased membrane lipid peroxidation that results in damage of the membrane bound enzyme (Halliwell and Gutteridge, 1989).

Fenugreek (*Trigonella foenum-graecumL.)* is one of the oldest medicinal plants belonging to the family Leguminosae. Fenugreek was found to treat glucose and lipid homeostasis in several metabolic disorders (Raju and Bird, 2006).The chemical constituents of fenugreek seeds include volatile oils, alkaloids, saponins, sapogenins, polyphenolic flavanoids, and mucilage (Billaud, 2001). Furthermore, fenugreek seed extract was found to reduce triglycerides accumulation in the liver (Raju and Bird, 2006). It was reported, also, that fenugreek seed polyphenols had a positive influence on both lipid profile and on quantitative and qualitative properties of collagen in hepatotoxicity (Kavirasan et al, 2007). Furthermore, fenugreek was shown to possess antioxidant activity, and to afford protection against cancer of the breast (Amin et al, 2005) and colon (Raju et al, 2004).

Jerusalem artichoke (*Helianthus tuberosus*), also called the sunroot, sun choke, earth apple or topinambour, which is used as a root vegetable (Kays and Nottingham, 2007). The tuber contains small amounts of anti-oxidant vitamins such as vitamin C, vitamin A and vitamin E. These vitamins together with flavonoid compound like carotenes helps scavenge harmful free radicals, and thereby offers protection from cancers and inflammation (Waters et al, 1981). Further, Jerusalem artichokes are very good source of minerals and electrolytes especially potassium, iron, and copper. It also contains small levels of some of valuable B-complex group of vitamins such as folates, pyridoxine, pantothenic acid, riboflavin, and thiamin (Kays and Nottingham, 2008). Recent studies improved that, the diets containing Jerusalem artichoke tubers reduced glucose levels, triglycerides, total cholesterol and LDL-cholesterol in the hyperglycemic rats (Eid, 2009).

This study was designed to evaluate the hazardous effects of γ-irradiation induced oxidative stress and to investigate the role of fenugreek seeds and Jerusalem artichoke tubers treatments as radioprotectors in rats irradiated with a single dose whole body gamma irradiation of 5.0 Gy.

MATERIALS AND METHODS

Experimental animals:

A total of 60 male Swiss Albino rats weighing (110-120g) were obtained from the Animal Farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Animals were housed in plastic cages and maintained under standard conditions of temperature, humidity and 12 h light/dark cycle along the experimental period. Rats were provided with a pellet concentrated diet containing all the necessary nutritive elements. Food and water were available throughout the experiment adlibitum. Rats were left to acclimatize for one week before starting the experiment. Animal procedures were consistent with the guide lines of Ethics by Public Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) in accordance with the recommendations for the proper care and use of laboratory animals approved by animal care committee of the National Center for Radiation Research and Technology, Cairo, Egypt.

Radiation facility:

γ-irradiation was performed at the National Center for Radiation Research and Technology, Nasr city, Cairo, Egypt. The source of radiation was (^{137}Cesium) gamma cell-40 which ensured a homogenous dose distribution all over the irradiation tray. Rats were placed in a specially designed well-ventilated acrylic container where their bodies were exposed to a single dose of 5 Gy. The dosage rate of the gamma radiation is 0.84 Gy/min and the radiation time for that dose was 4.2 minutes.

Fenugreek seeds and Jerusalem artichoke tubers:

Fenugreek seeds and Jerusalem artichoke tubers were purchased from Swanson Health Products. PO 2803. Fargo, ND 58108 (USA). The products were supplied as tablets of 610 mg and

400 mg respectively which were dissolved in distilled water. Rats received 1g/kg body weight, by gavages, according to (Annida et al, 2005).

Experimental design:

Sixty rats were divided equally into six groups (each group contains 10 rats) as following:

Group I: Control group: Animals were daily received basal diet only for 14 consecutive days. Animals of this group were neither treated with medicinal plants nor exposed to gamma irradiation.

Group II: Control + γ-irradiation group: Animals were daily received basal diet only for 14 consecutive days, then whole body exposed to a dose of 5 Gy gamma irradiation applied as one shot does.

Group III: Fenugreek group:

Animals were daily received basal diet plus fenugreek seed powder in distilled water (1g/kg body weight/day) by oral gavages for 14 consecutive days.

Group IV: Fenugreek + γ- irradiation group: Animals were daily received basal diet plus fenugreek seed powder in distilled water (1g/kg body weight/day) by oral gavages for 14 consecutive days then, the whole body exposed to a dose of 5 Gy gamma irradiation applied as one shot does.

Group V: Jerusalem group:

Animals were daily received basal diet plus Jerusalem tuber powder in distilled water (1g/kg body weight/day) by oral gavages for 14 consecutive days.

Group VI: Jerusalem + γ-irradiation group: Animals were daily received basal diet plus Jerusalem tuber powder in distilled water (1g/kg body weight/day) by oral gavages for 14 consecutive days then, the whole body exposed to a dose of 5 Gy gamma irradiation applied as one shot does.

Preparation of biological samples:

48 hours post γ-irradiation and after an overnight fast, the animals were anesthetized with ether. Blood samples from each rat were collected from the eyes by retro-orbital puncture using blood capillary tubes. Sera were obtained immediately by centrifugation of the blood samples at 4000 rpm for10 minutes at 4 ^{o}C then the sera were frozen at -20 ^{o}C for future measurements. Cholesterol was measured by the method of (Ellefson and Caraway, 1976), Triglyceride was measured by the method of (McGowan et al., 1983). High density lipoprotein (HDL) was determined by the method of (Burstein et al., 1970), Total protein was measured by the method of (Burtis and Ashwood, 1999). Albumin was measured by the method of (Doumas et al., 1971). Urea was measured by the method of (Burtis and Ashwood, 1999), Creatinine was measured by the method of (Bowers and Wong, 1980). The activity of Alanine Aminotransferase (ALT) and Aspartate Amino Transferase (AST) was measured by the kinetic method of (Sherwin, 1984). Alkaline Phosphatase (ALP) was measured according to the kinetic method of (Tietz et al, 1983), and Gamma-Glutamyltransferase (GGT) was measured according to the method of (Tietz, 1995).

Statistical analysis.

All obtained data were subjected to the statistical analysis and means were compared according to LSD at 5% level test described by Gomez and Gomez (1992).

RESULTS AND DISCUSSION

Rats irradiated at a dose of 5 Gy, delivered as single dose, produced a significant decrease ($p \leq 0.05$) in albumin, total protein and globulin levels as compared to normal control rats (Table1). However, γ-irradiated rats pre-treated with fenugreek or Jerusalem was increased significantly the above parameters as compared to normal control rats. Increased levels of these diagnostic markers of hepatic function in irradiated rats are implicative of the degree of hepatocellular dysfunction caused by the radiation (El-Missiry et al, 2007). Results of the current study go in parallelism with previous studies that revealed a potential hepatoprotective effect of fenugreek seeds (Abdelgawad et al, 2012) or Jerusalem artichoke tubers (Samal et el, 2012) in rats. Rats irradiated at 5 Gy, delivered as single dose, produced a significant increase ($p \leq 0.05$) in urea and creatinine as compared to normal control rats (Table 2). However, γ-irradiated rats pretreated with fenugreek or Jerusalem decreased significantly the above parameters as compared to normal control rats. In the present study, urea and creatinine levels, which are considered as markers of kidneys function were significantly elevated after exposure the animals to γ-irradiation indicating renal impairment (Moussa et al, 2015). Elevation of urea and creatinine levels by γ-irradiation might result from reduced activities of several enzymes, that play a role in renal function as well as reduced adenosine triphosphate production due to uncoupling of the mitochondrial oxidative phosphorylation (Ezzat, 1996) or may be due to impairment of glomerular selective properties caused by γ-irradiation (Berry et al, 2001).

Table (1): Influence of fenugreek seeds (1g/kg B.W), jerusalem artichoke tubers (1g/kg B.W) and/or γ-irradiation (5 Gy) application on albumin (g/dl), total protein (g/dl), globulin (g/dl) and A/G ratio in different rat groups.

Treatments	Albumin	Total protein	Globulin	A/G ratio
Group I	2.81±0.24^{a}	5.53±0.20^{a}	2.72±0.15^{a}	1.03±0.05^{d}
Group II	1.71±0.13^{c}	2.90±0.12^{d}	1.19±0.11^{f}	1.44±0.07^{a}
Group III	2.93±0.17^{a}	5.31±0.16^{a}	2.38±0.10^{c}	1.23±0.04^{b}
Group IV	2.05±0.08^{b}	4.52±0.11^{b}	2.47±0.23^{d}	0.83±0.06^{f}
Group V	2.86±0.12^{a}	5.25±0.2^{a}	2.39±0.08^{b}	1.2±0.03^{c}
Group VI	1.83±0.06^{c}	3.72±0.13^{c}	1.89±0.13^{e}	0.97±0.05^{e}

Data are means ± standard error of at least 6 replicates. Means with different letters were significantly different (p≤0.05).

Treatment of γ-irradiation rats with Jerusalem artichoker resulted in a significant improvement in urea and creatinine levels towards the normal values of the control (Samal et al, 2012).

Table (2): Influence of fenugreek seeds, jerusalem artichoke tubers (1g/kg BW) and/or γ-irradiation (5Gy) application on urea level (mg/dl) and creatinine concentration (mg/dl) in different rat groups.

Treatments	Urea	Creatinine
Group I	19.2±1.7^{d}	0.82±0.03^{d}
Group II	45.6±1.8^{a}	1.7±0.06^{a}
Group III	20.1 ±1.4^{d}	0.79±0.03^{d}
Group IV	27.18±1.2^{c}	1.02±0.04^{c}
Group V	18.6±1.8^{d}	0.85±0.05^{d}
Group VI	32.8±1.3^{b}	1.4±0.06^{b}

Data are means ± standard error of at least 6 replicates. Means with different letters were significantly different (p≤0.05).

Influence of fenugreek seeds, jerusalem artichoke tuber (1g/kg B.W) and/or γ-irradiation (5 Gy) application on enzyme activities of alanine aminotransferase (ALT), aspartate Aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) in different rat groups.

Rats irradiated at a dose of 5 Gy produced a significant increase in the activities of ALT, AST, ALP and GGT, respectively as compared to normal control rats (Table 3). Meanwhile, γ-irradiated rats pretreated with fenugreek or Jerusalem showed a significant improvement in the activities of these enzymes towards the normal values of the control. In the present study, gamma irradiation caused a marked increase in the levels of AST, ALT, ALP and GGT indicating liver injury (Pradeep et al, 2008). The significant increase in these enzyme activities result from γ-irradiation induced destruction of cellular membranes of hepatocytes, which in turn causes an increase in the membranes permeability followed by the release of intracellular enzymes to the blood stream (Khamis and Roushdy, 1991). In addition, γ-irradiation caused peroxidation for the lipids of hepatocytes membranes which contains large amount of fatty acids and excessive generation of free radicals leading to an increase in the permeability of the cytoplasmic membrane to organic substances and leads to leakage of cytosolic enzymes such as ALT, AST, ALP and GGT (Weiss and Lander, 2003).

Gamma irradiated rats pre-treated with fenugreek seeds showed a significant improvement in the activities of ALT, AST, ALP and GGT towards the normal values of the control (Kaviarasan et al. 2007).

Influence of fenugreek seeds (1g/kg B.W), jerusalem artichoke tubers (1g/kg B.W) and/or γ-radiation (5Gy) application on cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol concentrations in different groups:

The concentrations of cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol increased significantly (p ≤0.05) in irradiated rats at 5 Gy as compared to normal control rats (Table 4). On the other hand, pre-treated with fenugreek or Jerusalem, the concentration of these parameters decreased significantly (p ≤ 0.05) as compared to γ-irradiated rats.

Table (3): Influence of fenugreek seeds, Jerusalem artichoke tuber (1g/kg B.W) and/or γ-irradiation (5Gy) application on enzyme activities of ALT (U/L), AST (U/L), ALP (U/L) and GGT (U/L) in different rat groups

Treatments	ALT	AST	ALP	GT
Group I	16.6±0.52[d]	19.3±0.56[d]	65.1±1.4[d]	42.2±1.5[d]
Group II	42.4±1.28[a]	50.7±0.22[a]	180.1±1.6[a]	93.5±0.71[a]
Group III	16.1±0.96[d]	17.8±0.54[d]	66.2±0.99[d]	41.8±1.06[d]
Group IV	20.6±1.24[c]	30.1±0.16[c]	101.2±0.76[c]	55.2±1.4[c]
Group V	15.9±0.96[d]	18.5±0.77[e]	65.4±1.47[d]	43.05±1.6[d]
Group VI	30.7±1.07[b]	39.4±0.18[b]	126.97±1.8[b]	67.6±1.3[b]

Data are means ± standard error of at least 6 replicates. Means with different letters were significantly different (p≤0.05).

Table (4): Influence of fenugreek seeds (1g/kg B.W), Jerusalem artichoke tubers (1g/kg B.W) and/or γ-irradiation (5 Gy) application on cholesterol (mg/dl), triglycerides (mg/dl), HDL-cholesterol (mg/dl) and LDL-cholesterol (mg/dl) concentrations in different rat groups.

Treatments	Cholesterol	Triglycerides	HDL	LDL
Group I	123.7±2.5[e]	102±0.94[d]	47.4±1.3[a]	55.9±1.1[e]
Group II	241.3±1.8[a]	230.1±1.3[a]	29.6±1.1[d]	165.7±2.26[a]
Group III	117.4±2.1[e]	98.5±1.4[d]	45.7±1.6[a]	53.1±1.3[e]
Group IV	142±1.4[c]	123.5±1.6[c]	40.1±1.1[b]	77.2±0.96[c]
Group V	131.2±1.7[d]	96.8±1.1[d]	46.8±1.05[a]	65.04±1.4[d]
Group VI	165±1.8[b]	151.2±1.4[b]	37.02±1.4[c]	97.7±1.1[b]

Data are means ± standard error of at least 6 replicates. Means with different letters were significantly different (p≤0.05).

The results are in accordance with (Nada, 2008) who showed that; whole body exposure of rats to γ-irradiation (6.5 Gy) produced biochemical alteration in lipid profile fractions as triglyceride, total cholesterol, low density lipoprotein cholesterol and high density lipoprotein cholesterol. The increased level of serum cholesterol fractions were probably due to their release from tissues and destruction of the cell membranes caused by γ-irradiation (Moussa et al, 2015). Also, γ-irradiation could modify low and high density lipoproteins metabolism indirectly through the action of various inflammatory products (Jedidi et al, 2003).

Safwat et al. (2012) reported that, there is an improvement in the hypolipidemic effect of Jerusalem artichoke tubers in alloxan-induced diabetic rats. Also, Al-Sultan et al. (2015) reported that; a hypolipidemic effect was obtained when aqueous extract of fenugreek seeds was given for rats intoxicated with CCL4 as compared to their corresponding values of CCL4-intoxicated rats.

CONCLUSION

Supplementation with fenugreek seeds and Jerusalem artichoke tubers may have a benefit for safe application of γ-irradiation technology in medicine and industry.

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CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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