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Ameliorative Effect of Graviola Fruit Juice on the Damaged Tissues of Gamma-Irradiated Male Rats

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ABSTRACT

Exposure to ionizing radiation is characterized by production of reactive oxygen species (ROS) associated with increase in lipid peroxidation. Graviola fruits have been reported to possess antioxidant and anti-inflammatory activities. The present study was designed to determine the possible protective effects of graviola fruit juice (GFJ) against radiation induced oxidative stress and biochemical alterations in the liver and kidney of male albino rats. In the present study, gamma- irradiation (2 Gy every 3 days up to 8 Gy total doses) induced biochemical and oxidative damage in rats when compared to control group. In GFJ (10ml/Kg B Wt. /day) treated irradiated group, there were noticeable decreases recorded in lipid contents, concentration of serum urea, creatinine and uric acid, glucose level, activity of some liver enzymes and liver and kidney TBARS levels with remarkable increases in level of HDL-C, insulin and showing elevation in GSH level and SOD and CAT activities of the liver and kidney of gamma-irradiated treated rats compared to irradiated group. It was suggested that the biochemical and antioxidant amelioration observed in GFJ treated irradiated rats might be due to the antioxidant potential effects of graviola active constituents.

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ANS and AMM carried out the experiment with support from RGH. RGH, AMM and MNS wrote the manuscript.

Key words
Graviola fruits, gamma-radiation, antioxidant, oxidative damage, anti-inflammatory

INTRODUCTION

Humans have become more exposed to gamma radiation than before due to the over using of gamma rays in many therapeutic and research fields (Fouad *et al*., 2019). Many studies have shown that over exposure to gamma rays can cause liver and renal tissue damage directly by affecting the DNA molecule and cause cell destructions or indirectly through water radiolysis causing generation of reactive oxygen species (ROS) (Fouad *et al*., 2020). Moreover, it was found that oxidative stress is associated with abnormal changes that can disrupt the permeability of hepatocytes cell membranes accompanied by over release of liver enzymes into the blood stream causing liver dysfunction (Fouad *et al*., 2019). Also, γ-rays affecting the kidney function causing glomerulosclerosis and/or tubulointerstitial fibrosis (Fouad *et al*., 2019). Furthermore, exposure to gamma rays leads to an imbalance between antioxidant enzymes and the overproduction of free radicals, thereby reducing the total antioxidant capacity associated with oxidative stress (Rahmouni *et al*., 2019). Therefore, the body must be fortified with external antioxidants from natural products to be able to resist the damage caused by gamma rays. This natural antioxidant can be found in different types of vegetables, fruits and plants that might help to elucidate the mechanisms of interaction of radiation and molecules of biological importance (Prasad *et al*., 2005).

Natural compounds are much preferred for use in radioprotection rather than synthetic chemical compounds due to their safety and their effect on preventing or destroying free radicals, activating enzymes involved in the repair of DNA breaks, stimulating hematopoiesis and the immune system, or interacting with proteins in signaling and apoptotic execution pathways (Mun *et al*., 2018; Moghadamtousi *et al*., 2015).

Antioxidants are free radical scavengers because they seek out free radicals, reduce their energy, stabilize them, and end the damaging oxidative chain reaction (El-Seifi *et al*., 2015). *Annona muricata* is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. *A. muricata*, also known as soursop, graviola and guanabana, is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world. The fruits of *A. muricata* are extensively used to prepare syrups, candies, beverages, ice creams and shakes (Moghadamtousi *et al*., 2015). The *A. muricata* has essential oils (β-caryophyllene, δ-cadinene and cadinol), chemical components such as alkaloids (reticulin, coreximine, coclarine and anomurine) and antineoplastic substances

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such as acetogenins, which exert a selective cytotoxicity on tumor cells without affecting normal cells (Holanda *et al*., 2014). Numerous investigations have proven that graviola has the ability to protect against oxidative stress and has many activities such as anti-cancer, anti-convulsive, anti-arthritic, anti-parasitic, anti-malarial, hepatoprotective and anti-diabetic activities (Moghadamtousi *et al*., 2013). Additionally, graviola can increase the level of hepatic antioxidant enzymes (catalase, SOD, and glutathione peroxidase) and levels of glutathione to reduce oxidative stress in this tissue. However, other positive effects of this treatment included improvements in blood lipid levels, specifically a decline in diabetes-induced levels of LDL, total cholesterol, and triglycerides and an increase in HDL (Rady *et al*., 2018). Thus, the purpose of this study was to investigate the possible effect of graviola fruit juice on liver enzymes, kidney function and lipid profile of male rats exposed to gamma radiation.

MATERIALS AND METHODS

Materials

The fruits of graviola (*Annonamuricata L.*) were obtained from local market, Cairo, Egypt. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of fruit extract

The fruits of graviola were washed, peeled and cut into tiny pieces and then liquidized by blending with distilled water by using juice blender and then graviola fruit juice (GFJ) was stored in glass bottle and refrigerator at 2-4°C until used for the experiment (Ekaluo *et al*., 2013).

Radiation facility

Whole body gamma irradiation of rats at a fractionated dose (2 Gy every 3 days up to total dose 8 Gy) was performed using a Canadian gamma cell-40, (^{137}Cs) housed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate was 0.43 Gy/min at the time of the experiment.

Animal groups

Male albino rats Sprague Dawley (10 ± 2 weeks old; 120 ± 20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on rodent diet and tap water ad libitum. All animals procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, 1996).

Experimental design

Animals (24 rats) were randomly divided into 4 groups each of 6 animals as follows: (i) Control rats fed on a balanced diet for 35 days. (ii) GFJ group rats were administrated orally with GFJ (10ml/Kg B Wt. /day) (Ekaluo *et al*., 2013) for 35 days. (iii) Gamma-irradiated group rats were exposed at the 15th day of the experimental period to fractionated γ-radiation dose of 8 Gy (2 Gy every 3 days up to total dose 8 Gy), (iv) GFJ+γ-irradiated rats were administered orally with GFJ (10ml/Kg B Wt. /day) for 35 days and exposed to fractionated γ-radiation dose of 8 Gy (2 Gy every 3 days up to total dose 8 Gy) at the 15th day of the experimental period (35 days). At the end of the experiment, rats were fasted for 24 h and anaesthetized with diethyl ether. Blood sample were collected though heart puncture and allowed to coagulate and centrifuged to obtain serum for biochemical analysis.

Biochemical analyses

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain *et al*. (1974), Fossati and Prencipe (1982) and Demacker *et al*. (1980), respectively. Low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (vLDL-C) and risk ratio were evaluated according to Friedwald's formula (Friedwald *et al*., 1972) by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5. Serum samples were analyzed for glucose according to Trinder (1969) and insulin hormone was determined by radioimmunoassay kit supplied by Diasari, Italy. Urea, creatinine and uric acid levels were determined in serum according to Henery *et al*. (1974). The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957) and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954).

Liver was dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate TBARS (Yoshioka *et al*., 1979), GSH (Beutler *et al*., 1963), superoxide dismutase activity (SOD) (Minami and Yoshikawa, 1979) and catalase activity (CAT) (Johansson and Borg, 1988).

Statistical analysis

Results were presented as mean±SE (n=6). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS, 1988). Differences between means were considered significant at $P < 0.05$.

RESULTS

Data represented in this study showed that exposure of rats to fractionated dose (2 Gy every 3 days up to total dose 8 Gy) γ-radiation induced significant increases in lipid contents, concentration of serum urea, creatinine and uric acid, glucose level and activity of some liver enzymes with significant reduction in the level of HDL-C, and insulin compared to the corresponding control values. Whereas, co-administration of GFJ resulted in a significant reduction in the level of TC, TG, LDL-C and vLDL-C, concentration of serum urea, creatinine and uric acid, glucose level and activity of ALT, AST and ALP and remarkable increases in level of HDL-C, and insulin compared to irradiated group (Figs. 1, 2 and 3).

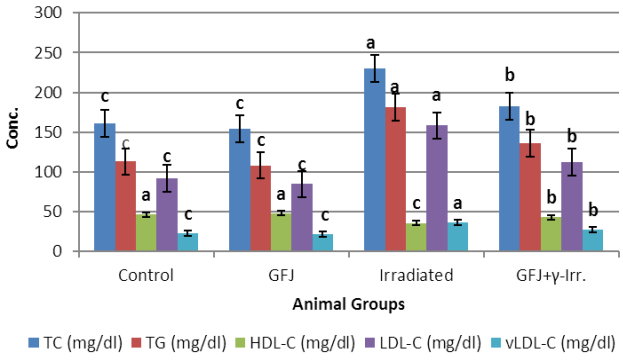

Fig. 1. Ameliorative Effect of GFJ on serum lipid profile of gamma-irradiated rats. GFJ, graviola fruit juice; Values are expressed as mean ± S.E. (n=6), (P<0.05).

Figure 4 shows that liver TBARS levels were significantly elevated and GSH level and *SOD and CAT* activities were significantly reduced by 8Gy (2 Gy every 3 days) gamma-irradiation compared to control. GFJ administration has significantly diminished the elevation of liver TBARS levels and showing elevation GSH level and *SOD and CAT* activities in compared to irradiated-group.

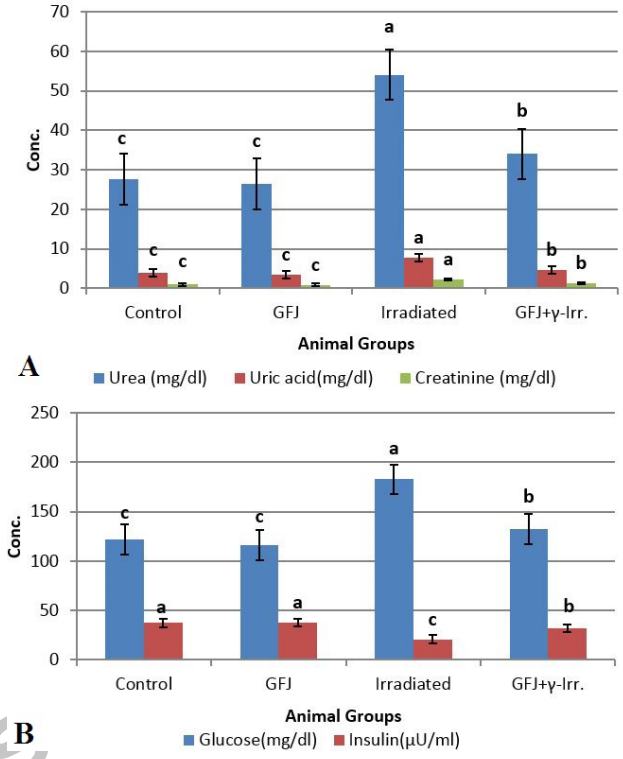

Fig. 2. Ameliorative effect of GFJ on (A) serum urea, creatinine and uric acid, and (B) serum insulin and glucose of gamma-irradiated rats. GFJ graviola fruit juice; Values are expressed as mean ± S.E. (n=6), (P<0.05).

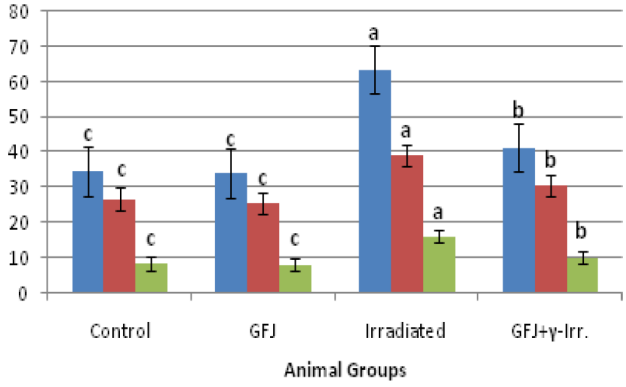

Fig. 3. Ameliorative effect of GFJ on liver enzymes of gamma-irradiated rats. For details see Fig. 1.

DISCUSSION

In the present study, significant changes in serum lipid profile post- irradiation were observed when compared to control values. This result came in accordance with El-Desouky *et al*. (2017) and Azab *et al*. (2011). The

hyperlipidaemic state observed in the serum of γ-irradiated rats could be explained on the basis that γ-radiation can act directly on the metabolic capacity of the liver and intestine which synthesize lipoproteins. Cholesterol is derived almost equally from exogenous diet and endogenously from acetyl-CoA in series of biosynthesis reactions (Garcia *et al*., 1996). In the present study, a marked significant elevation was observed in the total cholesterol content in plasma of irradiated animals. This was in accordance with the observation of El-Desouky *et al*. (2017) and Bok *et al*. (1999) who reported an increase in its plasma level in rats post-irradiation. Bok *et al*. (1999) attributed the observed hypercholesterolemia to the stimulation of cholesterol synthesis in the liver after gamma rays exposure. Moreover, Bok *et al*. (1999) contributed the irradiation-induced hypercholesterolemia to the increase of activation of HMG CoA-reductase enzyme; the key regulatory enzyme in the reaction of the overall process of cholesterol synthesis. The induced hyper triacylglycerolemia following gamma rays exposure may attribute to lipoprotein lipase activity inhibition effects (Fungwe *et al*., 1993). Lipoprotein lipase activity has a significant correlation with the ability of tissue to incorporate the fatty acids of triacylglycerols lipoproteins.

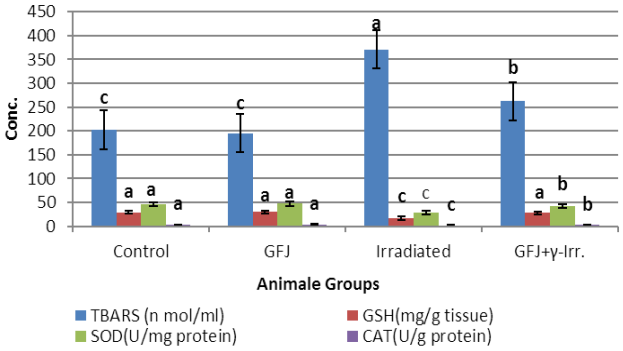

Fig. 4. Effect of GFJ on the level of hepatic TBARS, GSH content and SOD and CAT activities of gamma-irradiated rats. For details see Fig. 1.

The level of HDL-C was significantly increased and the levels of TC, TG, LDL-C and vLDL-C were significantly reduced in the group of γ-irradiated rats treated with GFJ when compared to γ-irradiated. Agbai *et al*. (2015) reported that the hypolipidemic effect of GFJ could be attributed to its phytochemical constituents. The study of Adeyemi *et al*. (2009) showed a significant reduction in the serum TC, TG, LDL-C and vLDL-C and a significant increase in the serum HDL-C and a significant antiatherogenic index of GF-treated group when compared to untreated diabetic group of rats.

The result of this study showed that whole body γ-irradiation of rats has induced significant increases in the concentration of serum urea, creatinine and uric acid. The increase of urea might be attributed to radiation-induced amino acids catabolism. The increased proteins catabolic rate in irradiated rats is accompanied by a decrease in liver total proteins and an increase in the content of non-protein nitrogen of both liver and serum as well as increased levels of serum amino acids and ammonia which depends mainly on the protein destruction after irradiation (El-Kashef and Saada,1985). The impaired detoxification function of the liver by irradiation could also contribute in the increase of urea in the blood (Robbins *et al*., 2011). Serum creatinine elevations might be attributed to the interaction of ionizing radiation with the sites of biosynthesis (El-Kashef and Saada, 1988). Significant elevations in serum uric acid of irradiated rats might be related to the breakdown of nucleotides into uric acid (Ganong, 1999).

However, co-administration of GFJ to γ- irradiated rats remarkably reduced the level of serum urea, creatinine and uric acid relative to γ- irradiated group. The reduction in the level of serum urea and creatinine levels reveals the potency of graviola to abate the effects of the radiation on the kidney. This could be attributed to the antioxidants present in *Annonamuricata* (Adewole and Caxton-Martins, 2006). Usunomena and Ngozi (2016) found that pre-treatment prior to dimethylnitrosamine administration significantly lowered the serum urea and creatinine levels, thus enhancing renal function.

In this work, the glucose level of γ- irradiated rats significantly increased with reduction in the level of insulin and that may be correlated with hepatic gluconeogensis and glycogenolysis (Verspohl *et al*., 2003). Radiation induced hyperglycemia could be attributed to the diminished utilization of glucose by irradiated tissues. Irradiation could induce the transport of certain amino acids and thus increased glucose formation through the processes of deamination and transamination (Alhersova *et al*., 1981) as well as acceleration of gluconeogenesis, which resulted as an indirect effect of radiation exposure (Sedlakova *et al*., 1998) On the other side, treatment with GFJ showed a significant antihyperglycaemic activity in γ- irradiatedrats received GFJ evidenced by elevation of glucose level and increase in insulin when compared to γ- irradiated rats. It has been suggested that bioactive compounds from plant sources having antihyperglycaemic activities might act by several mechanisms such as stimulating insulin secretion, increasing repair, or proliferation of β -cells and enhancing the effects of insulin and adrenalin (Fayed *et al*.,1998; Shanmugasundaram *et al*., 1990). The results of the study of Adeyemi *et al*. (2010) indicated that the decrease in the blood glucose concentration of diabetic rats by *A.muricata*

treatment is due to regeneration/proliferation in the pancreatic β-cells.

The rise in the serum transaminases activities and alkaline phosphatase in γ- irradiated animals in this work may be due to the drastic physiological effect caused by irradiation, either directly by interaction of cellular membranes with γ- ray or through the action of free radicals produced by radiation. These findings are supported by previous finding reported by Ramadan *et al*. (2001) and Nada (2008) who explained that changes in the enzymatic activities after irradiation may be due either to the release of enzymes from radiosensitive tissues or to changes in its synthesis and may be related to the extensive breakdown of liver parenchyma and renal tubules.

Khamis and Roushdy (1991) explained that the increase in serum aminotransferase activities by radiation may be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in blood serum. Also, ionizing radiation enhanced lipid peroxidation in cell membrane which contains fatty acids and excessive production of free radicals; this in turn increases the cytoplasmic membrane permeability to organic substances and causes leakage of cystosolic enzymes such as AST, ALT (Weiss and Lander, 2003).

GFJ administration to γ- irradiated rats significantly reduced the activities of ALT, AST and ALP comparing to non-treated γ- irradiated group indicating stabilization of plasma membrane as well as repair of hepatic tissue damage induced by γ-radiation (Owolabi *et al*., 2013). Nwogu *et al*. (2010) reported that Graviola leaf-extracts at 250 and 500mg/kg body weight significantly reduced the elevated serum levels of ALP and AST in acute liver damage induced by different hepato-toxins. Owolabi et al. (2013) recorded that treatment of rats with Graviola aqueous leaf-extracts significantly reduced the elevated activities of the ALT. The hepato-protective effect of Graviola leaves may be due to presence of flavonoids, saponins, alkaloids, tannins and ascorbic acid (Usunobun and Okolie, 2015; Usunobun *et al*., 2015). Gamma radiation resulted in decrease in the GSH level and the antioxidant enzymes activity with significant increase in the TBARS level of the liver of γ- irradiated rats comparing to control and GFJ group. The increase in MDA level may be due to the attack of free radicals on the fatty acid component of membrane lipids. The observed decreased in the activities of SOD and CAT could be due to a feedback inhibition or oxidative inactivation of the enzyme protein caused by ROS generation, which in turn can impair the antioxidant defense mechanism, leading to an increased membrane lipid oxidation (LPO) (Srinivasan *et al*., 2008). Thus, it seems that the activities of antioxidant enzymes are in close relationship with the induction of LPO, where the activities of SOD and CAT declined with the increase in LPO (Jagetia *et al*., 2003).

A significant reduction in the level of hepatic TBARS and remarkable elevation in GSH content and the activity of SOD and CAT were observed in the group of irradiated rats treated with GFJ compared to γ-irradiated rats. Graviola has been shown to possess antioxidant properties, due to the presence of acetogenins, which probably play the role of effective free radical scavengers (Baskar *et al*., 2007). The antioxidant effect of graviola leaf extract has been demonstrated by its ability to significantly reduce serum ROS, GSSG and MDA, while significantly raising hepatic levels of CAT, GTPx, SOD and GSH in streptozotocin diabetic rats (Adewole and Ojewole, 2009). Usunobun *et al*. (2015a) reported that graviola extract caused a significant reduction in MDA levels of in the liver tissues of rats by reduction in lipid peroxidation and increasing of tissue antioxidant enzyme activities.

CONCLUSION

The finding of this study obtained that the ability of graviola to modulate the damage effects induced by γ-irradiation may be probably due to the plant richness in chemical compounds including phenols and flavonoids as well as its ability to enhance antioxidants and reduce oxidative stress induced by gamma-rays.

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Statement conflict of interest

the authors declare that there is no conflict of interest.

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