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A Study on The Preventive Effect of Mulberry (*Morus alba l.)* Fruits in Rats Exposed to Gamma Radiation

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ABSTRACT

Background: Ionizing radiation is known to generate reactive oxygen species. This study was carried out to investigate the efficacy of mulberry fruit against γ-irradiation induced oxidative stress in rats. **Materials and methods:** Male albino rats were divided into four groups, Group (I): (control group) rats fed on balanced diet for 4 weeks, Group (II): rats fed on balanced diet contained 5% mulberry fruits powder (MFP), Group (III): rats were exposed at the 1st week of the experiment to fractionated γ-irradiation dose of 8 Gy administered as 2 Gy, every other day and fed on balanced diet for 4 weeks, and Group (IV): irradiated rats fed on balanced diet contained 5% mulberry fruits powder, at the end of the experiment, animals from each group were sacrificed, 24 hrs post the last dose of treatment, six rats from each group were sacrificed. Blood samples were taken and analyzed for Lipid peroxides as malondialdehyde (MDA) level, Xanthine Oxidoreductase system (XO and XDH), Glutathione (GSH) content, Superoxide dismutase (SOD), catalase (CAT), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP) activities and total bilirubin in rats. **Results:** The results showed that, irradiation induced high significant decrease in hepatic Glutathione contents (GSH), Xanthine Dehydrogenase (XDH), Superoxides Dismutase (SOD) and Catalase (CAT) activity. Moreover, a remarkable increase in the Malondialdehyde (MDA) concentration, xanthine oxidase activity (XO), the activity of some liver enzymes was observed in γ-irradiated rats. In contrast, administration of Mulberry Fruit Powder (MFP) to γ-irradiated rats was found to offer protection against γ-irradiation induced oxidative stress, by elevating the activity of antioxidant enzymes, enhancing liver function. All results in this study suggested that mulberry fruit had high potential to be developed as radio protective agent.

Conclusion: Therefore, MFP could have a beneficial role in modulating oxidative stress by improving the natural antioxidant mechanism.

Keywords: Gamma-irradiation; Mulberry fruits; Antioxidants.

INTRODUCTION

Radiation exposure produce highly reactive and dangerous molecular species called free radicals in cells and tissues, which have high energies and can break chemical bonds. Free radicals may be formed within cells as well as in the extra cellular medium and can interact with membrane lipids, nucleic acids, carbohydrates and proteins. These reactions disturb membrane structure and transport processes which leads to histological and biochemical disorders and ultimately resulted in acute and chronic disease.[1]

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs.[3,4]

All living organisms are exposed to some amount of radiation coming from outer space or emitted from the radioisotopes present in the environment.[5] Radiations are commonly used in a number of medical and industrial situations; however, their prooxidative effects limit their applications.[6]

The scavenging of free radicals and inhibition of lipid peroxidation has been suggested to be the key target activities for developing successful radioprotection strategies.[7,8]

Natural antioxidants play a major role by continuously inactivating ROS, to keep only a small amount necessary to maintain normal cell function.[9] Considerable epidemiological evidence has been gathered to suggest an association between consumption of fruits

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containing antioxidants, and a reduced risk of certain chronic diseases.[10,11]

Mulberry (*Morus alba L.*) belongs to the family *Moraceae*. Mulberry fruit is widely regarded as a nutritious food, and it can be eaten freshly or widely used in the production of wine, fruit juice, jam and canned food.[12,13] Mulberry fruit is not only used as fruit, but also it has been used effectively in natural medicine for the treatment of sore throat, fever, hypertension and anemia.[14,15] Moreover, mulberry fruit is used to protect against liver and kidney damage, strengthen the joints, improves eyesight, and have anti-aging effects.[13,17]

Aim of the work

The present study was undertaken to investigate the possible ameliorative effects of mulberry fruits on oxidative damage, resulting from exposure of normal male rats to γ-radiation.

Materials and Methods

Materials and plant preparation

Standard commercial rodent diet and fresh purple-colored mulberry fruits were purchased from the local market (Cairo, Egypt). All berries were dried at 70ºC for 4 days and grounded to powder.[18]

Radiation facility

Irradiation was performed by gamma cell 40 source (Cesium-137) belonging to the National Centre for Radiation Research and Technology (NCRRT), Egypt. This Cesium source offers a dose rate 1.3 rad/sec at the time of experiment. Rats were exposed to fractionated dose of 8.0 Gy γ-irradiation administered as 2 Gy, every other day.

Experimental design

Fifty eight male Swiss albino rats (150±10 g) were used. Animals were housed in stainless steal cages. They were kept under the same controlled laboratory conditions of temperature, lighting and ventilation. All rats were fed on standard casein diet and water *ad libitum*. Rats were categorized into 4 groups each of 12 rats as follows: Group (I): (control group) rats fed on balanced diet for 4 weeks, Group (II): rats fed on balanced diet contained 5% mulberry fruits powder (MFP), Group (III): rats were exposed at the 1st week of the experiment to fractionated γ-irradiation dose of 8 Gy administered as 2 Gy, every other day and fed on balanced diet for 4 weeks, and Group (IV): irradiated rats fed on balanced diet contained 5% mulberry fruits powder.

At the end of the experiment, animals from each group were sacrificed, 24 hrs post the last dose of treatment. Blood samples were collected though heart puncture, after light anesthesia and centrifuged to obtain serum for biochemical analysis. Also, liver tissue was removed for biochemical investigation.

Biochemical analysis

The lipid peroxidation was determined colorimetrically as Malondialdehyde (MDA).[19] Hepatic Xanthine Oxidase (XO) and Xanthine Dehydrogenase (XDH) were determined.[20] Whereas, the value of hepatic Glutathione content (GSH) and the activity of Superoxides Dismutase (SOD) and Catalase (CAT) were measured by the method of [21,22,23], respectively. In addition, the activity of serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), serum Gamma Glutamyl Transferase (GGT), serum Alkaline Phosphatase Activity (ALP) and serum total bilirubin was estimated by biome kits.

Statistical analysis

Results were expressed as the mean ± SE. Data were statistically analyzed for variance and the least significant difference (LSD) using one way analysis of variance (ANOVA) according to [24]. SPSS version 13 was used for analysis.

RESULTS

The data presented in table 1 revealed a significant decrease in the value of hepatic GSH contents and the activity of XDH, SOD, and CAT activity, associated with a significant increase in MDA level and XO activity of rats exposed to γ-radiation, as compared to the corresponding values of control and all treated groups; while rats receiving MFP after γ-irradiation exposure had a lower concentration of MDA and XO activity, and higher level of GSH, as well as SOD and CAT activity, than the γ-radiated group.

Also, the results presented in table 2 revealed a significant elevation in the concentration of total bilirubin and the activities of AST, ALT, ALP and GGT in γ-irradiated group, compared to control; whereas, the level of total bilirubin in addition to the activity of liver enzymes, were

decreased in the group of γ-irradiated rats supplemented with MFP.

DISCUSSION

It is well documented that dietary antioxidants play an important role in mitigating the damaging effects of oxidative stress on cells. [25] indicated that mulberry fruit is a natural health food with antioxidant effects and these beneficial effects may be because of phytochemical constituents, which might include fiber, fatty acids, phenolics, flavonoids, anthocyanins, vitamins and trace elements.

According to the data obtained, it appears that the detrimental damage of radiation is associated with the alteration of XOR system, and conversion of XDH into XO activity. The significant increase in XO activity might be attributed to radiation-induced hypoxia, where insufficient oxygen availability elevates calcium concentration, which activates a protease capable of converting the dehydrogenase to oxidase form [26,27]. Also, the level of MDA in the present study was elevated in the serum of untreated irradiated animals. The observed increased Thiobarbituric Acid Reactive Substances (TBARS) level in irradiated rats could be attributed to the peroxidation of membranes lipid, resulted in cellular structure modifications and oxygen radicals-mediated tissue damage. [28,29]

In the present study, the activities of Superoxide Dismutase (SOD) and Catalase (CAT) were significantly decreased in irradiated rats. The existence of a mutually supportive relationship between enzymatic antioxidants; SOD and CAT against accumulation of ROS inactivates the superoxide anion and peroxide radicals, by converting them into water and oxygen. In this study, the observed decrease in SOD activity suggests inactivation of the enzyme, possibly due to increased superoxide radical production or an inhibition by the H2O2, as a result of corresponding decrease in the activity of CAT, which selectively degrades H2O2. In previous studies, activities of SOD, CAT and GPx have been reported to decrease in the liver of irradiated rats [30,31]. The significant decrease in GSH levels observed in untreated irradiated animals may lead to decreased protection against oxidants.

This decrease could be due to an enhanced utilization in large amount, to combat the radiation-induced free radical damage, as glutathione is a major non-enzymatic antioxidant [32]. Similar decrease in hepatic GSH [29], and testicular GSH has been reported, following gamma irradiation in rats [33].

In this study, irradiated rats treated with Mulberry Fruits Powder (MFP) showed a significant decrease in the level of MDA content and XO activity, with concomitant significant increase in the activity of XDH, SOD and CAT, and in the content of GSH. Thus, MFP has potential as an anti-peroxidative agent, and as an antioxidant. [25] reported that MFP contained vitamin C and low levels of vitamin E, in addition to anthocyanins and flavonoids; all of which are powerful natural antioxidants that increase SOD and GSH-Px activities, and decrease TBARS concentration and improve lipid profiles in rats [34,35]. In addition, mulberry fruit contains many trace elements include ing Cu, Mn, Zn and Fe, which are necessary components of SOD [26]. Among these, Cu, Mn and Fe are prosthetic groups of SOD and play a decisive role in its enzyme activity. Zn stabilizes the structure of SOD [36]. Se is an important element of GSH-Px, which regulates lipid metabolism, prevents fatty liver formation, and improves antioxidant ability in rats [37].

The activities of ALT, AST, ALP and GGT, as well as the level of total bilirubin in serum, showed a significant rise, following γ-irradiation exposure. The increase in 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 liver and blood serum [38,39]. Also, it is proposed that oxidative stress is linked to the organ damage, following exposure to ionizing radiation [40,41].

However, the activity of liver enzymes was decreased, as a result of MFP administration to γ-irradiated rats. Several studies revealed that mulberry fruit, leaves, bark and branches have been used in Chinese medicine to treat fever, facilitate discharge of liver, protect the liver damage and lower blood pressure [42,43].

CONCLUSION

In conclusion, the present study revealed that the mulberry fruit is the potential functional food that can protect against oxidative damage induced by gamma-irradiation in male rats, through its positive effects on the activity of some antioxidant enzymes, liver enzymes and inhibition of lipid peroxidation. Moreover, the ameliorating effects of MF attributed to its phenolic and flavonoid contents that possess antioxidant activity.

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Table (1): Lipid peroxides as malondialdehyde (MDA) level, Xanthine Oxidoreductase system (XO and XDH), glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activities in rats after whole body gamma irradiation and/or mulberry fruits powder (MFP) administration.

Parameters / **Groups**	**MDA (nmol/ml)**	**XO (mU/mg protein)**	**XDH (mU/mg protein)**	**GSH (mg/g tissue**	**SOD (U/mg protei**	**CAT (U/g protein)**
G. I	a 193.27± 3.2	a 2.44 ± 0.07	a 3.15 ± 0.16	a 27.31± 0.92	a 46.08 ± 1.06	a 3.21 ± 0.02
G. II	a 181.76 ± 2.8	a 2.30 ± 0.06	a 3.19 ± 0.14	a 27.73± 0.86	a 47.10 ± 0.88	a 3.34 ± 0.02
G. III	c 388.51 ± 4.7	c 3.72 ± 0.07	c 1.56 ± 0.11	b 15.68± 0.64	c 30.11 ± 0.81	c 1.79 ± 0.02
G. IV	b 242.53 ± 4.6	b 2.56 ± 0.05	b 2.83 ± 0.13	a 25.86 ±0.75	b 41.63 ± 0.74	b 2.83 ± 0.03

Table (2): Activities of serum Aspartate Transaminase (AST), Alanine Transaminase (ALT), Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase Activity (ALP) and total bilirubin in rats after whole body gamma irradiation and/or mulberry fruits powder (MFP) administration.

Parameters / **Groups**	**AST (U/ml)**	**ALT (U/ml)**	**ALP (U/100ml)**	**γGT (U/ml)**	**Bilirubin (mg/dl)**
G. I	a 30.15 ± 0.45	a 23.54 ± 0.71	a 8.92 ± 0.31	a 3.96 ± 0.29	a 0.58 ± 0.02
G. II	a 29.71 ± 0.48	a 22.92 ± 0.59	a 8.83 ± 0.45	a 3.88 ± 0.36	a 0.57 ± 0.02
G. III	c 53.27 ± 0.73	c 41.32 ± 0.62	c 15.87 ± 0.61	c 6.48 ± 0.47	c 1.12 ± 0.03
G. IV	b 36.57 ± 0.83	b 28.41 ± 0.83	b 11.06 ± 0.52	b 4.91 ± 0.52	b 0.70 ± 0.02