



## Efficiency of Kumquat Fruit (*Fortunella margarita*) Extract against Hepatotoxicity and Infertility Induced by Gamma Irradiation in Male Albino Rats

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IONIZING radiation (IR) generate reactive oxygen species (ROS). Imbalance between ROS and antioxidants level leads to oxidative stress. *Fortunella margarita* (*F. margarita*) fruit contains a lot of bioactive compounds, such as polysaccharides, flavonoids, phenolic acids, ..etc. Therefore, it has high radical scavenging capacities and strong antioxidant activity. This study was designed to determine the probable efficiency of *F. margarita* extract against liver damage and sperm abnormalities induced by  $\gamma$ -radiation in male rats. Thirty six rats were divided into 6 groups (6 rats each): control group, treated groups: 14 days *F. margarita* group and 28 days *F. margarita* group, irradiated group (Irr): rats were exposed to 6Gy of whole body  $\gamma$ -radiation, pre-treated (14 days *F. margarita* extract + Irr) group and pre- and post- treated (28 days *F. margarita* extract + Irr) group. Biochemical investigations included lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C). Liver function enzymes involved analyzing serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) level, hepatic tissue malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione reduced (GSH) levels. Fertility competence (sperm head, tail and head and tail abnormalities) were recorded. Results obtained revealed the strong efficiency role of *F. margarita* fruit extract as an antioxidant against hepatotoxicity through enhancement of liver functions (ALT, AST and GGt), reduction of oxidative stress (MDA, SOD and GSH), and powerful recovering of sperm abnormalities induced by  $\gamma$ -irradiation. Also, adjusting and normalization of TC, HDL-C, LDL-C, VLDL-C and TG have been shown.

**Keywords:** *Fortunella margarita*,  $\gamma$ -radiation, Lipid profile, Hepatotoxicity, Infertility.

### Introduction

IR is one of the environmental toxicants used in modern medicine for diagnostic and therapeutic purposes, including cancer treatment (De & Devasagayam, 2011; Lertworapreecha et al., 2013) and in a large number of industrial applications such as developing new diversities of high-yield crops and enhancing storage of food materials (Maurya & Devasagayam, 2013). Exposure to IR causes different changes in the structure and function of cellular components resulting in tissue

damage, and in some cases may lead to death (Abdelhalim & Moussa, 2013) depending on absorbed dose, duration, interval after exposure and its ability to initiate a series of reactions that increase the levels of free radicals in cells and tissues (Sankaranarayanan, 2006).

Thus, scientific researches and studies focus on scavenging and capturing these hazardous circulating free radicals. The most effective and less toxic radioprotective agents are derived from plant sources (Yamini & Gopal, 2010; Arora et al., 2005).

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Consequently, phenolic and flavonoid compounds in vegetables and fruits were discovered as potent antioxidants in the human diet and protect normal cells from free radicals and other ROS that are produced during exposure to ionizing radiation (Arora, 2008).

Citrus is one of the important fruits of high medicinal value and have long been the basis of commonly used traditional medicines in several Asian countries (Kubo et al., 2005). The genus *Fortunella* Swingle is a close relative of the genus *Citrus* L, and both belong to the true Citrus group of the family Rutaceae. *Fortunella* is believed to originate in China and then introduced to other countries in the late of 18th century (Huang et al., 2011).

The liver is an active metabolic organ that is easily influenced by many environmental conditions. IR is one of such environmental factors, and living organisms exposed to relatively high-dose radiation can sustain severe damage or die within a short period due to acute effects (Nakajima et al., 2018).

In addition, radiation can induce fatty livers (Akahoshi et al., 2003). Acute irradiation induces fat metabolic pathways (Martius et al., 2014). Changes in fatty metabolism may be involved in radiation-induced liver carcinogenesis. In modern life, relationships between radiation effects and the three lifestyle factors of obesity, alcohol intake and intake of food-derived supplements are discussed at the molecular level in liver diseases (Akiba & Mizuno, 2012).

Oxidative stress may be an important determinant of altered lipid metabolism due to  $\gamma$ -radiation exposure (Makhlouf & Makhlouf, 2012). Earlier reports demonstrate hyperlipidemia as a consequence of whole body irradiation (El-Khafif et al., 2003). Moreover, radiation-induced alterations in hepatic function include changes in carbohydrate, lipid, heme, fatty acid and cholesterol, and protein metabolism, as well as detoxification reaction. In addition, excessive production of ROS increased MDA, TC, TG, LDL-C and VLDL-C level and decreased the level of GSH, activities of SOD and HDL-C level (Abou-Zeid et al., 2018; Baker et al., 2009).

In accordance with observations of many researches, whole body irradiation showed a

significant increase of serum lipid profile after irradiation whether this irradiation is applied as a single or fractionated doses (Feurgard et al., 1998; Hassan et al., 2015).

The consumption of *F. margarita* fruits may be of health-promotion effect due to its highly nourishing and its contents of bioactive compounds, such as polysaccharides, limonoids, essential oils, flavonoids, phenolic acids, vitamins, dietary fiber, amino acids and carotenoids including,  $\beta$ -Carotene,  $\beta$ -cryptoxanthin, lutein, violaxanthin and zeaxanthin etc. (Wu, 2013). Hence, *F. margarita* has effective role in amelioration of the drastic effects of IR especially for liver function, lipid profile and free radicals scavengers (Zeng et al., 2016; Wu et al., 2010).

A decline in fertility rates is becoming an increasingly prevalent issue worldwide, with current estimates indicating that 1 in every 6 couples experience issues with conception. It is clear that, the leading cause of male infertility stems from a loss of sperm function, ultimately resulting in a loss of fertilization potential. This loss of function is causatively linked to oxidative stress within the cell, driven by the presence and/or overproduction of ROS. ROS are oxygen-containing molecules that can contain unpaired electrons (radicals) or be non-radical oxidizing agents (Jessica et al., 2018).

*F. margarita* can protect the morphology of sperm through scavenging of free radicals and DNA repair (Hazra et al., 2011; Rosen et al., 2014). Additionally, it can be responsible for the regulation of spermatogenesis (Soghra et al., 2012).

Therefore, the present study is attempted to evaluate the protective and treatment capacities of *F. margarita* against the deleterious effects of IR exposure.

## **Materials and Methods**

### *Plant material*

Fresh mature fruits of *F. margarita* was collected during January month from the seedlings production unit belonging to Citrus Research Department, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

There are five *Fortunella* species: *F. margarita*, *F. crassifolia*, *F. obovata*, *F. hindsii*, and *F. japonica* (Güney et al., 2015).

### Experimental animals

Adult male albino rats (Sprague Dawley strain), weighting about 140-170 gm were obtained from Biological Products and Vaccines of the Egyptian Holding Company (VACSERA); Cairo, Egypt. All animals were allowed one week to acclimatize in animal housing conditions before being used for the study. The rats were housed in plastic cages (6 rats in each) under standard laboratory conditions that include all hygienic measures with constant illumination and ventilation, temperature and humidity. The experiment was performed at the NCRRT, Atomic Energy Authority, Egypt. Animals were fed standard diet and provided with water *ad libitum*, the standard diet was formulated according to the American Institute of Nutrition 93 (AIN-93) (Reeves, 1997) containing salt mixture which was prepared according to Viviani et al. (1964) in addition to vitamin mixture prepared according to A.O.A.C. (1975).

### Radiation source

Whole-body gamma-irradiation process was performed with a Canadian Gamma Cell, Caesium-137 ( $^{137}\text{Cs}$ ) unit at the NCRRT, Egyptian Atomic Energy Authority, Nasr City, Cairo, Egypt, with a dose rate of 0.4 Gy/min at the time of experiment.

### Extract Preparation of *F. margarita*

Fruits of *F. margarita* were washed and dried by hybrid solar convective drying system, belonging to the solar energy dept., National Research Center, Dokki, Egypt. The dried fruits were ground into fine dried powder (900g) and this powder was crushed in 3L 70% aqueous ethanolic solution and kept overnight. The extract was filtered through Whatman filter paper. The step was repeated several times till exhaustion and the extracts were combined. The combined extracts were then condensed in a rotatory evaporator at a temperature not exceeding 50°C under vacuum according to Premkumar et al. (2001). The condensed extract lyophilization was conducted using freeze dryer (Lab Conco- Free Zone 12 Liter Console Freeze Dry System with Stoppering Tray Dryer, collector Temp. -50°C, electrical standard: International, ice holding capacity: 12L, pressure: 0.1mp).

### Experimental design

Adult male albino rats were randomly categorized into six equal groups (n= 6). These

groups were classified as: control group: rats were neither irradiated nor treated. 14 days *F. margarita* group: group of rats received *F. margarita* extract (150 mg/kg of body weight per day) dissolved in 0.5 ml of distilled water for the first fourteen days of the experiment. This dose was chosen according to the study of Elgizawy (2014). 28 days *F. margarita* group: group of rats received *F. margarita* extract for continuous twenty eight days. Irradiated group (Irr): rats were exposed to a single dose (6Gy) of whole body  $\gamma$ -radiation with a dose rate of 0.4Gy/min at day fourteen of the experiment. This dose was chosen according to according to Ashry et al. (2017) and Cihan et al. (2013). 14 days *F. margarita* extract + Irr group: rats received *F. margarita* extract for the first fourteen days before irradiation. 28 days *F. margarita* extract + Irr group: rats received *F. margarita* extract for fourteen days before irradiation and continued for fourteen days after irradiation (the treatment pre- and post-irradiation).

### Samples collection and biochemical analysis

After treatment periods, the animals were sacrificed under anesthesia and blood samples were collected by cardiac puncture immediately after sacrificing and serum was separated by centrifugation of blood sample at 3000rpm for 15min to separate the serum and stored frozen (-20) until being assayed. The liver was removed and homogenized in two ways for determination of the various biochemical analyses according to Ohkawa et al. (1979), Satoh (1978), Beutler et al. (1963) for determination of MDA, GSH and according to Nishikimi et al. (1972) for determination of SOD.

Determination of serum TC, TG, LDL-C and HDL-C was performed by an enzymatic colorimetric method using kits purchased from Bio-diagnostic Company (Giza, Egypt) according to Allain et al. (1974), Fossati & Prencipe (1982), Fossati & Seidel (1983), Lopes-Virella et al. (1977), respectively.

For assay of liver function, ALT and AST activities in serum were determined using the Bio-diagnostic kit (Giza, Egypt) according to Reitman & Frankel (1957) while GGT was determined using the Egyptian Company for Biotechnology kits according to Heersink et al. (1980), Persjn & Van der Slike (1976), Saw et al. (1983).

### Sperm abnormalities

Epididymides of each rat were minced finely with small scissors in 3ml phosphate buffers pH (7.8). Evaluation of sperm head, tail and head and tail abnormalities and amorphism were conducted according to the criteria of Wyrobek et al. (1984). Smears were prepared on clean dry slides which stained in 1% Eosin-Y (1gm Eosin Y dissolved in 100ml distilled water). Sperm smears were examined at 40X magnification by light microscopy. For each dose, about 1000 sperms were assessed for morphological abnormalities of the sperm shape.

Mutation factor and mutation index were calculated as follows: Mutation factor= frequency of abnormal sperm head, tail and head and tail (treated)/ frequency of abnormal sperm head, tail and head and tail (control). Mutation index= frequency of abnormal sperm head, tail and head and tail (treated- control)/ frequency of abnormal sperm head, tail and head and tail (control) according to Ekaluo et al. (2008).

### Statistical analysis

Results were expressed as mean S.E. Data were subjected to one-way analysis of variance (ANOVA- test) to determine the statistical significance of the differences according to Snedecor & Cochran (1989). Differences were considered statistically significant at  $P < 0.05$ .

The statistical package for social science (SPSS) version 20 was used for these calculations.

### Results:

Results in Table 1 show that the irradiated group demonstrated significant increments in TC, LDL-C, VLDL-C and TG accompanied by decrease in HDL-C values when compared with the control and both *F. margarita* treated groups. While groups of animals received *F. margarita* pre & pre and post irradiation recorded significant reduction in lipid profile data and enhancement of HDL-C values, in comparison with the irradiated group only.

Table 2 shows that the concentrations of ALT, AST and GGT in serum samples recorded significant increments ( $\uparrow$  4.9, 3.5 & 3.6 folds) respectively in the irradiated rats. Whereas, animals treated with *F. margarita*. pre & pre and post irradiation recorded a significant amelioration in liver enzymes levels.

Table 3 demonstrates that radiation exposure induced a significant increase in the levels of MDA ( $\uparrow$ 1.33 folds) and significant decrease in both SOD & GSH contents ( $\downarrow$ 0.74 & 0.71 folds) respectively compared with the control group. Levels of antioxidant profile enhanced significantly at both *F. margarita* groups.

**TABLE 1. Effect of *F. margarita* fruit extract on serum lipid profile levels (mg/dl) in irradiated rats.**

Experimental groups	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	TG (mg/dl)
Control	163.34±1.48	49.33±1.17	74.83±1.33	13.03± 0.55	77.4±4.02
14 days <i>F. margarita</i>	163.17±1.06	a 39.03±1.64	71.67±0.97	13.63± 0.63	a 71.28±1.11
28 days <i>F. margarita</i>	164.37±1.40	ab 35.78±1.38	70.33±0.94	13 ± 0.52	a 70.63±0.82
Irradiation (Irr)	abc 388.83±2.16	ab 29.53±5.61	abc 246.17±2.35	abc 23.67± 0.41	abc 296±0.82
14 days <i>F. margarita</i> +Irr	abcd 259.67±1.31	a 38.54±2.56	abcd 180.5±1.44	abcd 19.51± 0.43	abcd 256.83±2.52
28 days <i>F. margarita</i> +Irr	abcde 243.83±1.22	ab 39.11±2.46	abcde 165. ±1.37	abcd 18.69± 0.36	abcde 244.33±2.32

a: significant when compared with control group

b: significant when compared with 14 day *F. margarita* group

c: significant when compared with 28 day *F. margarita* group

d : significant when compared with Irradiation group

e : significant when compared with 14 day *F. margarita* + Irr group

**TABLE 2. Effect of *F. margarita* fruit extract on serum liver enzymes, ALT, AST and GGT levels in irradiated rats.**

Experimental groups	ALT U/ml	AST U/ml	GGT U/ml
Control	27.17 ± 1.66	33.33±1.56	9.04 ± 0.09
14 days <i>F. margarita</i>	29.50 ± 1.18	35±2.15	8.80 ± 0.14
28 days <i>F. margarita</i>	29.17± 1.05	32.83±1.9	8.15 ± 0.12
Irradiation (Irr)	abc 133±5.66	abc 115±8.12	abc 32.87 ± 1.46
14 days <i>F. margarita</i> + Irr	abcd 71.33±1.15	abcd 65.5±2.06	abcd 13.5± 0.99
28 days <i>F. margarita</i> + Irr	abcde 48.0±3.44	acde 41.67±2.51	abcde 10.87± 2.8

Legends as in Table 1.

**TABLE 3. Effect of *F. margarita* fruit extract on MDA, SOD and GSH levels in liver tissues of irradiated rats.**

Experimental groups	MDA (nmol/g. tissue)	SOD (U/g. tissue)	GSH (mg/g. tissue)
Control	23.60 ± 1.23	62.73±9.1	46.44 ± 2.74
14 days <i>F. margarita</i>	23.52 ± 1.29	62.28±8.8	45.78 ± 2.28
28 days <i>F. margarita</i>	23.20 ± 1.10	62.66±9.0	ab 61.78 ± 1.47
Irradiation (Irr)	abc 31.49 ± 2.16	abc 46.46±6.6	abc 32.99 ± 0.61
14 days <i>F. margarita</i> + Irr	abc 31.29 ± 1.74	abcd 52.1±7.47	abcd 35.02 ± 1.21
28 days <i>F. margarita</i> + Irr	de 25.55 ± 1.51	abcde 56.55±8.17	abcde 38.11 ± 1.88

Legends as in Table 1.

Table 4 illustrates that the data of *F. margarita* 150mg/kg doses before and/or after radiation exposure led to reduction in the values of mutation factor and mutation indices. Moreover, a significant decrease in the percentage of abnormalities was recorded in the two treated groups, but its average number did not reach to the control values.

As shown in Tables 1, 2, 3 and 4 all data of protection and/or treatment periods with *F. margarita* administration 14 days (pre) and 28 days (pre and post) *F. margarita* groups revealed that no significant differences were observed between them and the control except for HDL-C, TG and GSH which were significantly different, but within normal ranges.

It is apparent that (14 days *F. margarita* + Irr) group represents protective group while (28 days *F. margarita* + Irr) group represents protective and treatment group and the last mention verified more improvement in all investigated parameters than protection only.

### Discussion

Exposure to the IR is common to certain people including professionals handling radioactive materials or to the patients undergoing radio-diagnostics and radiotherapy or as millions of people who travel by air are exposed by X-rays scanning every day. Though it is indirect cause, IR may trigger a mutation in healthy cells which further induces molecular alterations. It is

well known that ionizing radiation generates free radicals from cytoplasmic water and ultimately induces biomolecules lesions especially DNA damage. These damages may lead to neoplasm in normal and healthy cells, however IR is not by itself recognized and certain carcinogens present in the environment. In order to develop some type of cancer, they have to interact within the organisms and cells with other multiple factors of high complexity from physiological to environmental components (genetics of the living being, cellular microenvironment, epigenetic factors, environmental conditions, and others, perhaps still unknown) (Marignac et al., 2019).

Plant parts such as fruits, roots, stem/bark, leaves, and medicinal herbs have been found to have antioxidant capacity due to the presence of phenolic compounds, vitamins, nitrogen compounds, terpenoids, and other metabolites. These compounds have been shown to possess antioxidant, immunostimulatory, and antimicrobial activity and to impart radioprotective effects

(Samarth et al., 2015). Therefore, it has been found that *F. margarita* is a rich resource of bioactive compounds enriched with antioxidants more effective than those of citrus species (Tan et al., 2014).

In the current study,  $\gamma$ -irradiation induced significant elevation in the activities of ALT, AST and GGT in serum. This elevation is in agreement with the previous findings of Mansour et al. (2014) and Rezk et al. (2018). This elevation is might be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes, facilitates the passage of cytoplasmic enzymes outside the cells leading to the elevation in aminotransferase activities in the serum (Gaur & Bhatia, 2009).

The data demonstrated that irradiated rats treated with *F. margarita* fruit extract showed a significant decrease the level of liver enzymes (ALT, AST and GGT) of irradiated rats, which is in agreement with the findings of Elabd (2015).

**TABLE 4. Mutation factors and mutation indices of sperm morphology abnormalities in all rats groups (1000sperm/rat).**

Groups		Control group	14 day <i>F. margarita</i>	28 day <i>F. margarita</i>	Irradiation (Irr)	14 day <i>F. margarita</i> + Irr	28 day <i>F. margarita</i> + Irr
	%	0.72	0.80	0.78	13.81	13.07	11.45
Head abnormalities	Mutation factor	1.00	1.12	1.09	19.29	18.23	15.98
	Mutation index	0.00	0.12	0.09	18.28	17.23	14.98
	%	0.85	0.87	0.87	15.17	14.00	13.11
Tail abnormalities	Mutation factor	1.00	1.02	1.02	17.84	15.47	15.43
	Mutation index	0.00	0.02	0.02	16.84	14.43	14.43
	%	0.20	0.22	0.20	2.52	2.48	2.22
H & T abnormalities	Mutation factor	1.00	1.08	1.00	12.58	12.42	11.08
	Mutation index	0.00	0.08	0.00	11.58	11.42	10.08
	%	1.77	1.89	1.85	31.50	29.55	26.78
Total abnormalities	Mutation factor	1.00	1.07	1.05	17.80	16.69	15.13
	Mutation index	0.00	0.07	0.05	16.80	15.69	14.13

Legends as in Table 1

This indicates that *F. margarita* fruit extract tends to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through membranes, exhibiting hepatoprotective activity. The protective action of *F. margarita* fruit extract on hepatocyte membranes may be related to the antioxidant specially carotenoids and anti-inflammatory potential effects of the active constituents of these fruits which were previously proved by several authors (Wu et al., 2010).

Lipid profile is affected hugely by exposing to IR, since cholesterol is derived almost equally from exogenous diet and endogenously from acetyl-Co A in a series of biosynthesis reactions. In addition, radiation exposure induced hypercholesterolemia which may be attributed to activation of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase enzyme, the key regulatory enzyme in the reaction of the overall process of cholesterol synthesis (Bok et al., 1999).

Other opinions attributed the cause of hypercholesterolemia to the stimulation of cholesterol synthesis in the liver after gamma irradiation (Ladan et al., 2008). Also, free radicals destruct cell membranes and enhanced cholesterol release and increase lipid peroxidation in addition to decreasing fatty acid oxidation (Karbownik & Reiter, 2000).

In the present results, whole body exposure of male albino rats to 6Gy  $\gamma$ -irradiation caused in significant increases in serum total lipid; TC, TG, LDL and VLDL as well as significant decrease in the level of serum HDL. These results are in agreement with the findings of Ahmed et al. (2017).

Table 1 reveals that administration of *F. margarita* fruit extract reduced significantly the hazard effect of  $\gamma$ -irradiation on the lipid fractions of the irradiated rats, indicating that *F. margarita* has hypolipidemic effect. This improvement may be attributed to the presence of polysaccharides, carotenoids and sterol in the extract. Polysaccharides, as the main bioactive compounds from *F. margarita*, approximately accounted for 12% of dried *F. margarita* (Zeng, 2015). The hypolipidemic mechanism of polysaccharides was achieved by increasing the lipase activity, enhancing the activity of antioxidant enzymes and the ability of polysaccharides to bind bile

acid (Zeng et al., 2016) which affects cholesterol solubility and then suppresses its absorption (Nagaoka et al., 2005).

In addition, plant sterol reduces the absorption of cholesterol and thus increases the fecal excretion of steroids that results in a decrease of body lipids. Plant secondary metabolites *i.e.*, flavonoids and polyphenolics from polar extracts may be responsible for the anti-hyperlipidemic effect (Guimarães et al., 2000).

Table 2 shows that irradiated rats provoked imbalance between oxidant and antioxidant species in the hepatic tissues. A significant elevation in MDA level accompanied by significant depletion of GSH content and SOD activities were recorded, which in are agreement with Ammar (2015) and El-Desouky et al. (2017).

The elevation of MDA level in the liver tissues of irradiated rats indicates the presence of radiation-induced oxidative damage. Also, the depletion of GSH content and SOD activities might result from the damage of cell membrane and alterations in dynamic permeability of membranes due to peroxidation after radiation exposure followed by the release of intracellular enzymes to the blood stream (Saada et al., 2003) and enhanced utilization of the antioxidant enzymes in an attempt to detoxify radiation-generated free radicals (Krishna & Kumar, 2005). Furthermore, the decrease of SOD activity suggests inactivation of the enzyme, possibly due to increased superoxide radical production or an inhibition by the  $H_2O_2$  (Ahmed et al., 2018).

Treatments with *F. margarita* fruit extract showed a significant decrease in the level of MDA content, with a parallel to significant enhancement in the activity of GSH which is in accordance with the finding that extracts of *F. margarita* fruits increase antioxidant capacity (Mohamed, 2011) as well as a significant increase in the level of SOD.

These previous effects may be attributed to the presence of carotenoids which reported to have antioxidant activities as they are capable of scavenging ROS, inhibiting lipid peroxidation and inducing detoxifying enzymes (Yeh et al., 2009). Many studies revealed that *F. margarita* contains carotenoids including,  $\beta$ -Carotene,  $\beta$ -cryptoxanthin, lutein, violaxanthin and zeaxanthin (Schirra et al., 2007; Wang et al., 2007).

Tan et al. (2016) reported that the antioxidant capacity of *F. margarita* is very high and this is attributed to the high content of flavonoids since flavonoids were usually regarded as the basis of antioxidant capacity. It was reported that flavonoids strongly inhibit production of lipid peroxidation and enhance the endogenous antioxidant defense mechanism (Pavanato et al., 2003).

It is well documented that IR disturb the reproductive functions and the over doses of it can lead to infertility. Infertility is one of the major health problems in life, and approximately 30% of infertilities are due to a male factor (Isidori et al., 2006).

As a sperm morphology is an excellent biomarker of sperm dysfunction(s), the extent of sperm abnormality is closely related to sperm function. Moreover, fertilization failures *in vitro* are strongly related to impaired sperm morphology (Evenson & Wixon, 2006).

In the present study, gamma irradiation induced a significant elevation of sperm head, tail and both together abnormalities, this is in agreement with the results of Fatehi et al. (2018). The effect of gamma irradiation and the abnormal forms of sperm were correlated to the disruption of spermatogenesis and sperm DNA. Also, mammals sperm membrane has a lot of non-saturated fatty acids which make it sensitive to lipid peroxidation of oxidative stress (Bonisoli-Alquati et al., 2011).

It has been reported that several incidences of reduction in the sperm count are due to Sertoli cell morphology and function (Allenby et al., 1990) or to the alterations in germ cell apoptosis by a disruption in contact mediated communication between the Sertoli cells and germ cells (Mishra et al., 2009).

The results of Table 4 illustrated that irradiated rats treated with *F. margarita* fruit extract ameliorated morphological abnormalities of sperms. These effects can be attributed to the presence of high amount of flavonoids in *F. margarita* fruit extract which are potent antioxidants. Flavonoids can protect the morphology of sperm through scavenging of free radicals and DNA repair mechanisms (Hazra et al., 2011; Rosen et al., 2014). Also, the reduction of the deformation of sperms (mutation factors and indices) might be attributed to the effect of

flavonoids through protection of Leydig cells against oxidative stress a factor affecting the fertility of sperm (Chang et al., 2008).

According to the presented data, it was observed that *F. margarita* administration pre- and post-irradiation (28 days) was more effective than pre-irradiation treatment only (14 days) for protection and treatment of hepatotoxicity, oxidative stress and infertility.

## Conclusion

Finally, the results obtained in the current study suggested that the high antioxidant properties of *F. margarita* fruit extract may be attributed to free radical scavenging activity. Furthermore, the results demonstrated the effectiveness of *F. margarita* fruit extract in the protection of liver damage and sperm mutation induced by  $\gamma$ -irradiation.

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## كفاءة مستخلص فاكهة الكمكوات (فورتينلا مارجرينا) ضد التسمم الكبدي وضعف الخصوبه المستحث بالتشعيع الجامى فى ذكور الجرذان البيضاء

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تنتج جميع الأشعة المؤينة شوارد حرة والتي بدورها تؤدي إلى إنتاج أنواع الأوكسجين النشط. يؤدي وجود شوارد الأوكسجين النشطة أو نقص مستويات مضادات الأوكسدة إلى إنتاج ما يعرف بالإجهاد التأكسدي. يحتوي نبات الفورتينلا مارجرينا على نسبة عالية من العناصر المغذية و الكثير من المواد النشطة البيولوجية مثل السكريات المتعددة والفلافونيدات والأحماض الفينولية ألخ، ولذلك فهو له قدرة عالية في مكافحة الشوارد الحرة ونشاط قوى مضاد للتأكسد.

تهدف هذه الدراسة لتحديد مدى كفاءة مستخلص نبات فاكهة الفورتينلا مارجرينا المحتملة ضد التسمم الكبدي وتشوهات الحيوانات المنوية الناجمه عن التعرض لإشعاع جاما فى ذكور الفئران. 36 فأرا تم تقسيمهم إلى 6 مجموعات (6 فئران لكل مجموعة): مجموعة ضابطة، ومجموعة 14 يوم تحقن بمستخلص الفورتينلا، ومجموعة تحقن بنفس المستخلص لمدة 28 يوم، ومجموعة تم تعريضها للإشعاع جاما لجرعة (6 جراى) للجسم كله، ثم مجموعة تعرض للإشعاع بعد 14 يوم من حقن مستخلص الفورتينلا وأخيرا مجموعة تعرضت للإشعاع بعد حقن المستخلص لمدة 14 يوم قبل التشعيع وتم اكمال الحقن لمدة 14 يوم بعد الإشعاع. تم اجراء القياسات البيوكيميائية التي تتضمن قياس الكوليستيرول الكلي والجلسريدات الثلاثيه والدهون عاليه الكثافة والدهون منخفضة الكثافة بالإضافة إلى تعيين مستوى إنزيمات الكبد والتي شملت: إنزيم الألانين أمينو ترانسفيريز وأسبرتات أمينو ترانسفيريز وجاما جلوتاميل ترانسفيريز، أيضا دهون الكبد فوق مؤكسدة وسوبر أكسيد ديسميوتيز والجلوتاثيون المختزل. بالإضافة إلى تشوهات الحيوانات المنويه (الرأس، الذيل، الرأس والذيل معا). وقد أظهرت النتائج تحسن ملحوظ في كل القياسات السابق ذكرها مع الحفاظ على الشكل الطبيعي للحيوانات المنويه بدون ظهور التشوهات المعتاد عليها بعد الإشعاع مما يدل على مدى الكفاءة العاليه لمستخلص الفورتينلا مارجرينا ضد التسمم الكبدي والإجهاد التأكسدي وتشوهات الحيوانات المنويه الناجمه عن التعرض لإشعاع جاما.