

Influence of *Tropaeolum majus* Leaves Extract in Ameliorating Damage Induced by Gamma Radiation in Rats

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Abstract

Background: Medicinal plants were commonly used in primary health care. They can ameliorate the action of oxidative stress and free radical-induced damage. The present study was proposed to investigate the possible hepatoprotective role of hydroethanolic extract of *Tropaeolum majus* leaves (HETM)₂ in modulating the toxicity and the oxidative stress induced by exposure to gamma radiation (IR) in the liver of male rats.

Material & methods: HETM (300 mg/kg body weight) was administered to male albino rats via gavages during 21 successive days before whole body exposure to gamma rays (6 Gy) and during 7 days after irradiation. The animals were sacrificed on the 7th day post-irradiation. Results: the exposure to gamma-radiation caused liver dysfunction manifested by significant ($p < 0.05$) increases in the plasma level of the alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), total cholesterol, low density lipoprotein (LDL-C) and triglycerides. Moreover, IR induced oxidative stress as indicated by a significant increase in the level of thiobarbituric acid reactive substance (TBARS) with a concomitant decrease in the reduced glutathione (GSH) content as well as in the activity of superoxide dismutase, (SOD) and catalase (CAT) in the liver tissue. Pretreatment with HETM in IR-treated rats alleviated the previously mentioned alterations in the biochemical and oxidative stress parameters and restored their values toward the normal value of the control group.

Conclusion: hydroethanolic extract of *T. majus leaves* by its bioactive components and essential trace elements content might attenuate the severity of radiation-induced biochemical disorders in liver tissues.

Key words: *Tropaeolum majus*, γ -radiation, antioxidant, Lipid profile, liver enzymes.

Introduction

Free radicals are produced from normal oxygen metabolism within the body, and from exposure to certain chemicals, environmental pollutants, sunlight, radiation, parasites, dietary fats, and more. During radiation exposure or as a consequence of the physiological metabolic reactions, molecular oxygen undergoes sequential reduction to form reactive oxygen species, including superoxide anion and hydroxyl radical, in addition to hydrogen peroxide. All are implicated in the pathogenesis of aging and diseases including cancer⁽¹⁾.

The body naturally protects itself against pollutants by forming antioxidant compounds e.g. superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase. Antioxidant plays an important role in scavenging oxidants and preventing cell damage. Unfortunately, the continual bombardment of stress, environmental pollution, and the

popular practice of food industrialization and food conversion processes destroy

antioxidants, allowing the body to be more susceptible to disease and ill health. Consequently, the body has an extremely difficult time producing enough antioxidants to combat the contaminants. Although antioxidant administration during radiation exposure has been shown to reduce the severity of the radiation injury, yet some of their properties such as cytotoxicity⁽²⁾, pro-oxidant activity⁽³⁾ limited their therapeutic application. Use of plant extracts, food supplement which augment major cellular endogenous antioxidants following chronic administration have been identified as a promising therapeutic approach to combat oxidative stress associated with heart disease⁽⁴⁾.

According to World Health Organization (WHO), 65-80% of the world populations rely on traditional medicine to treat various diseases⁽⁵⁾. The WHO recommends

research into the use of the local flora for therapeutic purposes, with the intention of reducing the number of people excluded from effective therapy in the government health systems, which could constitute an economically viable alternative treatment of several diseases, especially in developing countries⁽⁶⁾⁽⁷⁾.

Nasturtium herb and its extracts have been used for years in folk medicine in many countries as medicinal product⁽⁸⁾.

Tropaeolum majus (*T. majus*) commonly known as nasturtium is an herbal plant that rich in polyphenols, benzyl isothiocyanate, and flavonoids (isoquercitroside, quercetol 3-triglucoside, and kaempferolglucoside)⁽⁹⁾.

These polyphenolic compounds are exerting powerful biological benefits. *T. majus* has a very broad range of action. It has proven efficacy as an antihypertensive and diuretic and it has been used abundantly as food supplement rich in vitamin C and minerals⁽¹⁰⁻¹²⁾. Fresh leaves of *T. majus* were traditionally used for the treatment of infected wounds and the gall bladder, as diuretic, aphrodisiac, and as medicine against chronic diseases such as obstructive pulmonary disease and infections of kidneys and bladder⁽¹³⁾. It was shown that *T. majus* has also an anti-carcinogenic potential⁽¹⁴⁾. Moreover, it is used externally in dermatology and cosmetology for the treatment of diseases of the skin, nails and hair, sunburns⁽¹⁵⁾.

Therefore, the present study firstly aimed to investigate the possible radiation exposure-induced changes in enzyme activities, oxidative stress biomarkers. Since there is always need for a successful therapeutic approach that might inhibit the initiation and progression of diseases. The present study was aimed also to evaluate the potential radioprotective effect of HETM leaves extract in ameliorating these possible alterations.

Material and methods

Plant material

T. majus leaves have been kindly supplied from Medicinal and Aromatic Plants Department, National Research Centre, Cairo, Egypt.

Animals

32 Male albino rats, 120-150 g obtained from the Egyptian Holding

Company for Biological products and Vaccines, Helwan, Egypt were used. They were kept under standard housing and handling conditions. Food pellet diet and water intake were available *adlibitum*.

Radiation facility

Irradiation of rats was carried out with a ¹³⁷Cs source in a Gamma Cell 40 (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), located at the National Center for Radiation Research and Technology (NCRRT), in Nasr City, Cairo, Egypt. The animals' whole bodies were exposed to gamma rays and received a dose of 6Gy administered at a dose rate of 0.5 Gy/min calculated according to the Dosimeter department in the NCRRT.

Preparation of ethanolic extract of *T. majus* leaves

Leaves of the *T. majus* were air-dried in an oven at 40 °C for 4 days, the dried powdered plant material macerated using 70% ethanol as a solvent. The solvent was then eliminated by a rotary vacuum evaporator under reduced pressure and lyophilized. The hydroethanolic *Tropaeolum majus* (HETM) of leaves is dissolved in distilled water just before administration to animals at doses of 300mg/kg body weight⁽¹⁶⁾.

Experimental design

Animals were divided into four equal groups (n= 8).

(i) Control group: Rats received distilled water via gavages during 28 successive days.

(ii) *T. majus* group: Rats received *T. majus* (300 mg/kg/ day) via gavages during 28 successive days.

(iii) RAD group: Rats received distilled water via gavages during 21 successive days before whole body gamma irradiation with 6 Gy and continued during 7 successive days after irradiation.

(iv) *T. majus*+ RAD group: Rats received *T. majus* (300 mg/kg/day) during 21 successive days before whole body gamma irradiation with 6 Gy and during 7 successive days after gamma irradiation.

The animals were sacrificed by decapitation on the 7th day post-irradiation.

The liver was rapidly excised and blood samples were collected and serum obtained by centrifugation at 3000 rpm for 10 min and stored frozen for biochemical analysis.

Biochemical and metal analysis

All chemicals and reagents used were pure chemical materials from Sigma-Aldrich, St Louis, MO, USA. For the assessment of oxidative stress biomarkers a portion of the liver was weighed, then 10% weight/volume (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4) using Teflon homogenizer (Glas-Col, Terre Haute, Ind., USA). The homogenates were centrifuged at 10,000 g for 15 min. Aliquots of supernatants were separated for use.

Lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substance (TBARS) levels according to the method of **Yoshioka et al.**⁽¹⁷⁾. The activity of SOD was determined according to the method of **Nisikimiet al.**⁽¹⁸⁾. The activity of CAT was determined according to the method of **Sinha**⁽¹⁹⁾ in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. Reduced thiols content was estimated according to **Beutler et al**⁽²⁰⁾. The activity of serum aspartate and alanine transaminases (ASAT and ALAT) were assayed using available commercial kits (Spinreact, Spain) according to the method described by **Reitman and Frankel**⁽²¹⁾, while serum alkaline phosphatase (ALP) activity was assayed depending on the method of **King and Armstrong**⁽²²⁾ using Biodiagnostic kit. The content of serum total cholesterol was measured by the method of **Allianet al.**⁽²³⁾. Serum triglycerides (TG) and low density lipoprotein cholesterol LDL-C were measured according to **Fossati and Principe and Demackeret al.**⁽²⁴⁻²⁵⁾, respectively.

Fe, Cu, Zn, Ca and Mg were determined in plant material after digestion in concentrated pure nitric acid and hydrogen peroxide in 5: 1 ratio (**IAEA, 1980**)⁽²⁶⁾. Samples digestion is carried out with acids at elevated temperature and pressures by using Microwave Sample Preparation Lab Station, MLS-1200 MEGA. The selected

elements were then estimated by using SOLAR System Unicam 939 Atomic Absorption Spectrometer, England, equipped with deuterium background corrections.

Statistical analysis

All the values are expressed as mean \pm standard error (SE). Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test as a post hoc ANOVA test according to **Snedecore and Cochran**⁽²⁷⁾ and means were compared between groups by Duncan multiple range test⁽²⁸⁾. Differences between means were considered significant at $P < 0.05$.

Results

T. majus group rats showed insignificant changes in the oxidant/antioxidant status of liver tissue, compared to the control group rats. The levels of TBARS, SOD and CAT showed insignificant changes ($P > 0.05$), compared to their corresponding levels in the control group (Table 1). The activity of serum (ALAT), (ASAT), and (ALP) as well as lipid profile (cholesterol, TG and LDL-C) showed insignificant changes ($P > 0.05$), compared to their corresponding levels in the control group (Tables 2&3).

Tables 2 & 3 also show that the gamma radiation induced a significant increase ($P < 0.05$) in the activity of serum ALAT, ASAT and ALP as compared to control values, indicating that radiation induced significant liver injury. Whole body exposure of male albino rats to gamma radiations (6 Gy) provoked oxidative stress demonstrated by significant increases ($P < 0.05$) of TBARS levels associated with significant decreases ($P < 0.05$) of SOD and CAT activity compared to their respective values in the control group. The results also revealed significant increases ($P < 0.05$) of cholesterol, TG and LDL-C levels.

The same tables 2 & 3 also show prolonged administration of ethanolic extract of *Tropaeolum majus* leaves before whole body exposure to gamma rays (6 Gy) and after irradiation, significantly attenuated the severity of radiation-induced oxidative stress.

Table (1): Antioxidant status in liver fresh tissues of different animal groups

Groups	TBARS (nmol/g fresh tissue)	GSH (mg/g fresh tissue)	SOD (U/mg protein)	CAT (U/mg protein)
Control	198±2.23	16.84±0.85	41.39±2.10	18.7±0.91
IR	369±3.28*	11.02±0.54*	26.15±1.65*	11.6±0.64*
<i>T. majus</i>	194.5±2.92	16.88±0.93	40.98±3.11	18.2±0.80
<i>T. majus</i> + IR	210±3.60 [#]	13.99±0.65 ^{*#}	34.12±1.67 ^{*#}	15.2±0.76 [#]

Each value represents the mean ±SE of 8 observations.

* Significant difference compared to the value of control

Significant difference compared to irradiated rats

Table (2): The activity of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and alkaline phosphatase (ALP) in the serum of different animal groups

Groups	ASAT (U/L)	ALAT (U/L)	ALP (U/L)
Control	43.7±1.21	29.2±0.86	170±1.97
IR	63.2±2.1*	38.3±1.40*	243±2.67*
<i>T. majus</i>	44.7±1.08	27.9±1.03	172±1.82
<i>T. majus</i> + IR	52.1±14.9 ^{*#}	33.2±1.2 [#]	184±1.9 [#]

Legend as table 1

Table 3: Changes in the concentration of total cholesterol, triglycerides (TG), and Low-density lipoprotein (LDL-C) in the serum of different animal groups

Groups	Cholesterol (mg/dL)	TG (mg/dL)	LDL-C (mg/dL)
Control	54.1± 1.68	44.2±1.25	27.62±0.79
IR	82.67± 2.28*	73.13±1.78 *	36.84±0.78 *
<i>T. majus</i>	54.73±1.66	42.94±1.40	27.03±0.80
<i>T. majus</i> + IR	74.63± 1.27 ^{*#}	47.25±1.08 ^{*#}	31.4±0.89 [#]

Legend as table 1

Table (4): Concentration levels of metal in *T. majus* leaves (µg/g)

Elements	Concentration
Fe	150.515±24.303
Cu	7.302±0.209
Zn	149.8±1.301
Ca	13484.4±1222.76
Mg	8400.94±248.032

Each value represents the mean of 2 samples recorded±SD

Discussion

In the present study, whole body exposure of male albino rats to gamma radiation (6Gy) has provoked an imbalance between oxidant and antioxidant species in the rats liver tissues. Significant increases in the level of TBARS accompanied by significant decreases in SOD and CAT activities and reduced thiols content (GSH) were recorded. The increase of TBARS level is probably due to the interaction of hydroxyl radical ($\cdot\text{OH}$) resulting as a byproduct of water radiolysis, upon exposure to ionizing radiation, with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes⁽²⁹⁾.

Intracellular reduced glutathione (GSH) plays a role in protecting cells from toxicity by maintaining intracellular redox status, conjugating with electrophilic xenobiotics and free radicals and detoxifying reactive peroxides.⁽³⁰⁾ Glutathione plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is a key function in the protective process. SOD and CAT are involved in the clearance of superoxide and hydrogen peroxide⁽³¹⁾.

The changes in the total antioxidant status could be considered as one of the alterations concerned in the assessment of radiation and the determination of the efficiency of antioxidant compounds used to prevent the development of radiation syndromes. The decrease of SOD and CAT activity and reduced thiols contents might probably be the consequence of cellular membrane damages. In favor of this postulation, **Saada**⁽³²⁾ reported that radiation induced oxidative damage to cell membrane and alterations in dynamic permeability followed by the release of intracellular molecules to the blood stream. One must consider, also, that the decrease of antioxidants might result from their increased utilization to neutralize the excess of free radicals generated in the body after exposure to ionizing radiations. Cytotoxicity of ROS is possibly associated with depletion of antioxidant enzymes⁽³³⁾. The decrease in GSH has been related to an enhanced oxidation of GSH to oxidized glutathione (GSSG) as a consequence of increased generation of ROS⁽³⁴⁾.

Liver was considered earlier relatively resistant to gamma radiations but **Johnet al.**⁽³⁵⁾ found that it is moderately sensitive to radiations and to lower doses also. Liver is an organ which suffers from direct and indirect radiations damage. Also, ionizing radiation is an important environmental risk factor known to produce various types of reactive oxygen species in biological systems provoking oxidative damage, organ dysfunction and metabolic disturbances⁽³⁶⁾. The results obtained revealed that, γ -irradiation (6Gy) showed a significant increase of serum ASAT, ALAT and ALP when compared to their respective values in the control group reflecting liver damage. Excess ROS can damage hepatocytes and activate hepatic stellate cells⁽³⁷⁾, which play a central role in liver damage and fibrosis⁽³⁸⁾ or may be related to extensive breakdown of liver parenchyma with subsequent enzyme release, or to increase in permeability of the cell membrane that could enhance the movement of enzymes from their sites of production⁽³⁹⁾.

In the present study, whole body gamma irradiation produced significant increases of triglycerides (TG), total cholesterol (TC), and low density lipoprotein-cholesterol (LDL-C). Radiation exposure resulted an increased in lipid peroxidation and loss of membrane integrity which might be important determinants of altered lipid metabolism and are closely associated with hyperlipidemia and/or hypercholesterolemia in many animals and human studies⁽⁴⁰⁾. Free radicals impair liver function and can be a major cause of hormone imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of TG and LDL⁽⁴¹⁾.

The increase in serum cholesterol level due to irradiation may be originated from the migration of tissue cholesterol via the blood circulation or/and the decrease in utilization of cholesterol for synthesis of higher substances⁽⁴²⁾. Elevated serum LDL-C levels might result from radiation-induced damage to the receptors on the surface of many cells in the body that prevents the ingestion of LDL-C by endocytosis⁽⁴³⁾. The increase in serum TG levels occurring after irradiation might result from inhibition of lipoprotein lipase⁽⁴⁴⁾.

Medicinal herbs are widely used for treatment of several diseases, as well as for research and development of new drugs being used in traditional medicine and these are potential sources for discovery of new therapeutic compounds⁽⁴⁵⁾. In the present study, pretreatment with HETM leaves extract improved the altered level of most studied parameters in irradiated rats. This protective effect of *T.majus L* may be primarily attributed to its antioxidant activity and the protection of cellular membrane integrity from IR-induced oxidative damage due to presence of fatty acids, benzyl isothiocyanate, flavonoids particularly isoquercitrin, which are major components of the *T.majus* extract⁽⁴⁶⁾, in addition to its trace elements content. Several *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory potential of isoquercitrin⁽⁴⁷⁾⁽⁴⁸⁾. These studies, associated with the popular use of *Tropaeolum majus L.* leaves to treatment of acute inflammation.

T.majus is a herbal plant that rich in polyphenols, benzylisothiocyanate, and flavonoids (isoquercitroside, quercetol 3-triglucoside, and kaempferolglucoside)⁽⁹⁾. These polyphenolic compounds are exerting powerful biological benefits. *T.majus* has a very broad range of action. These phytochemical components have been found to be responsible for the various medicinal activities of *T.majus* and these activities include chemoprotective activity⁽⁴⁹⁾ by blocking the initiation of tumors. **Fahey and Stephenson**⁽⁵⁰⁾ have focused on glycosinolates as minor dietary constituents that elevate the activity of cellular detoxication enzymes (Phase 2 enzymes) based on evidence indicating that induction of these enzymes blocks the formation of tumors in experimental animals.

T.majus protects liver from cholestatic disease⁽⁵¹⁾, anticancer properties by photo-activated polyacetylenes⁽¹⁴⁾. It has proven its efficacy as antihypertensive, diuretic and it has been used abundantly as food supplement rich in vitamin C and minerals⁽¹²⁾.

In conclusion, administration of ethanol extract of *T.majus leaves* could minimized radiation harmful effects and protected the liver against its toxicity which may be due to its phytochemical components as well as its essential elements which could be stimulate the body defense mechanisms against oxidative stress. Additional studies should be performed with *T.majus*

supplementation to use as hepatoprotective therapy.

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