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Effect of Fenugreek (Trigonella Foenum-Graecum) Supplementation on Radiation-Induced Oxidative Stress in Liver and Kidney of Rats

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ABSTRACT

Whole body exposure to ionizing radiation provokes oxidative damage, organ dysfunction and metabolic disturbances. Fenugreek (*Trigonella foenum-graecum L. Leguminosae*), one of the oldest medicinal plants rich in polyphenolic compounds is known to possess antioxidant properties. The present study was designed to determine the possible protective effect of fenugreek, against γ-radiation-induced oxidative stress in liver and kidney tissues of rats. In parallel, the alteration in the activity of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as markers of liver function, creatinine and urea levels as markers of kidney function. In addition, serum glucose and insulin levels were determined as markers for carbohydrate metabolism. Irradiated rats were whole body exposed to 3.5Gy (Acute dose) γ-radiations. Fenugreek-treated irradiated rats received 1g fenugreek seed powder/kg body weight/day, by gavages, during 7 days before irradiation. Animals were sacrificed on the 1st day after irradiation. The results obtained demonstrated that exposure to ionizing radiation induced significant decreases in SOD and CAT activities and GSH content associated to significant increase of TBARS levels in liver and kidney. Fenugreek treatment has significantly attenuated radiation-induced oxidative stress in both tissues, which was substantiated by the significant amelioration of serum ALP, AST and ALT activities, creatinine, urea, glucose, and insulin levels. It could be concluded that fenugreek would protect from oxidative damage and metabolic disturbances induced by ionizing irradiation.

INTRODUCTION

Radiation damage, is to a large extent, caused by the overproduction of reactive oxygen species (ROS), including superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2), that overwhelm the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes, and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur[1].

Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Among the enzymes involved in antioxidative defense, particularly well documented are the antioxidative properties of the superoxide dismutases (SOD), glutathione peroxidases (GSH-Px), and catalase (CAT). SOD catalyzes the reduction of $O_2^{\bullet -}$ to hydrogen peroxide (H_2O_2). The majority of H_2O_2 is broken down to oxygen (O_2) and water (H_2O) by CAT. In addition to CAT, GSH-Px can also break down H_2O_2 and also any peroxides that form on lipids within the body[2]. The activity of GSH-Px depends on the presence of adequate amounts of reduced glutathione (GSH). Exposure to ionizing radiation induces the process of lipid peroxidation with the consequent damage of cellular biomembranes and organ dysfunction[3].

In order to overcome the potential harmful effect of free radicals several natural compounds have been proven to be efficient protectors against ionizing radiation such as water cress oil[3], clover oil[4], and parsley oil[5]. Interest in fenugreek is growing rapidly since it was found to be rich in polyphenolic compounds[6] and to possess many therapeutic applications[7]. Fenugreek *(Trigonella foenum-graecum* L. *Leguminosa*e) is one of the oldest medicinal plants, originating in India and Northern Africa. Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making [8]. Fenugreek is an annual plant that grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. Fenugreek was found to treat glucose and lipid homeostasis in several metabolic disorders[9]. The seeds have been shown to possess hypoglycemic action[10,11]. Furthermore, fenugreek seed extract was found to decrease plasma triglycerides[12] and to reduce

triglycerides accumulation in the liver[9]. 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[13,14]. Furthermore, fenugreek was shown to possess antioxidant activity, and to afford protection against cancer of the breast [15] and colon[16].

This study has been carried out to evaluate the possible advantage that could be gained by using fenugreek seed powder in the protection of liver and kidney against radiation-induced oxidative damage in parallel to some metabolic disorders.

MATERIALS AND METHODS

Male Swiss Albino rats (100 – 120g) obtained from the Egyptian organization for biological products and vaccines were used. Animals were kept under standard condition along the experiment period. The rats were fed on standard pellets. Liberal water intakes were available. All animal treatment procedures met the guidelines of National Institute of Health[17].

Whole body gamma irradiation was performed with a Canadian Gamma Cell-40 at the National Center for Radiation Research and Technology, Cairo, Egypt, at a dose rate 0.61Gy/min. Rats were exposed to 3.5Gy (one shot dose).

Fenugreek

GNC herbal plus, FINGERPRINTED, was purchased from General Nutrition Corp. Pittsburgh, PA 15222 (USA). The product is supplied as tablets of 500 mg that were dissolved in distilled water. Rats received 1g/Kg body weight, by gavages, according to Annida *et al.*[18].

Experimental design

Animals were divided into 4 groups of 6 rats each:

Control group: animals received distilled water, by gavages, for 7 consecutive days. Animals of this group were neither exposed to gamma irradiation nor treated with fenugreek powder.

Fenugreek group: animals received fenugreek seed powder in distilled water (1g/Kg body weight) for 7 consecutive days.

Irradiated group: animals were exposed to 3.5Gy gamma irradiation applied as one shot dose.

Fenugreek-irradiated group: animals treated with fenugreek seed powder for 7 consecutive days before exposure to 3.5Gy whole body gamma irradiation.

Animals were sacrificed on the 1st day post-irradiation. Blood samples were collected. Liver and kidney tissues were quickly removed.

Biochemical analysis

The activities of alkaline phosphatase and transaminases (AST and ALT) were assayed according to Kind & King[19] and Reitman & Frankel[20], respectively. Reduced glutathione (GSH) content was determined according to the method described by Beutler *et al.*[21]. Insulin content was determined by immunometric assay using immuolite kit technique and glucose content was determined after the method of Trinder[22]. Thiobarbituric acid reactive substances were determined according to the method of Yoshioka *et al.*[23]. Superoxide dismutase and catalase activity were determined according to the methods described by Minami and Yoshikawa[24] and Aebi[25] respectively. Blood urea and creatinine were determined according to the method described by Patton[26] and Houot [27].

Statistical analysis

Results were presented as mean ± S.E. (n=6). Experimental data were analyzed using analysis of variance (ANOVA). Duncan's multiple range tests was used to determine significant differences between means. The statical analysis systems (SAS) package was used for statistical analysis. Differences between means were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the present study, whole body exposure of rats to 3.5Gy gamma radiation resulted in oxidative stress in liver and kidney tissues obvious by significant increases ($P \leq 0.05$) in the level of TBARS associated to significant decreases ($P \leq 0.05$) in the activity of SOD and CAT and in the content of GSH on the 1st day post-irradiation, compared to control values (Tables 1 and 2). The increase of TBARS is probably the consequence of interaction of the excess of $^{\bullet}OH$ generated in the cells after exposure to ionizing radiation with the polyunsaturated fatty acids in the phospholipids portion of cell membranes initiating the lipid peroxidation chain reaction[1]. The significant decrease in the activity of antioxidant enzymes might result from radiation-induced cell

membrane damage and alterations in dynamic permeability of membranes due to peroxidation, which is followed by the release of intracellular enzymes to the blood stream. In addition, the excess of $^{\bullet}OH$ resulting from water radiolysis causes oxidative damage to enzymes that lead to the modification of their activities[28]. Glutathione is a major endogenous antioxidant involved in the protection of normal cells structure by quenching free radicals[29]. In the present study, the depletion in GSH content after exposure to gamma radiation may be due its interaction with free radicals.

Table 1. Liver superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) contents in different rats groups.

Biochemical Parameters	Rats groups			
	Control	Fenugreek	Irradiated	Fenugreek-Irrad.
SOD (U/ mg protein)	$6.30^{a} \pm 0.16$	$6.18^{a} \pm 0.10$	$5.20^{b} \pm 0.13$	$6.00^{c} \pm 0.16$
CAT (U/ mg protein)	$3.75^{a} \pm 0.10$	$3.20^{a} \pm 0.15$	$3.03^{b} \pm 0.17$	$3.37^{b} \pm 0.16$
GSH (µg/ mg protein)	$25.1^{a} \pm 0.96$	$24.0^{a} \pm 0.94$	$19.0^{b} \pm 0.79$	$23.6^{a} \pm 0.72$
TBARS (n mol/g protein)	$253^{a} \pm 1.20$	$257^{a} \pm 1.40$	$340^{b} \pm 0.95$	$269^{c} \pm 1.20$

Each value represents the mean of 6 records ± S.E.
Values with different superscript for each item are significantly different from each other at $P < 0.05$

Table 2. Kidney superoxide dismutase (SOD) and catalase (CAT) activities, reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) contents in different rats groups.

Biochemical Parameters	Rats groups			
	Control	Fenugreek	Irradiated	Fenugreek-Irrad.
SOD (U/ mg protein)	$6.45^{a} \pm 0.20$	$6.43^{a} \pm 0.11$	$5.13^{b} \pm 0.11$	$5.52^{c} \pm 0.18$
CAT (U/ mg protein)	$3.12^{a} \pm 0.21$	$3.16^{a} \pm 0.11$	$2.82^{a} \pm 0.10$	$2.95^{a} \pm 0.13$
GSH (µg/ mg protein)	$24.6^{a} \pm 0.75$	$23.4^{a} \pm 0.96$	$19.5^{b} \pm 1.30$	$22.6^{a} \pm 1.20$
TBARS (n mol/g protein)	$223^{a} \pm 0.88$	$220^{a} \pm 2.00$	$275^{b} \pm 1.63$	$252^{c} \pm 1.26$

Each value represents the mean of 6 records ± S.E.
Values with different superscript for each item are significantly different from each other at $P < 0.05$

Fenugreek-treated irradiated rats showed significant increase ($P<0.05$) in SOD and CAT activities as well as GSH content, in liver and kidney tissues, compared to their corresponding values in irradiated rats. Furthermore, the contents of TBARS were significantly lower than in irradiated rats (Tables 1

and 2). The results are in accordance with Anuradha and Ravikumar [30] who observed that fenugreek seed powder supplementation decreased susceptibility to oxidative stress and lipid peroxidation in the liver, kidney and pancreas of alloxan-diabetic rats. Thirunavukkarasu *et al.*[31] found, also, that in case of ethanol toxicity of rats the aqueous extract of fenugreek seeds prevented the enzymatic leakage and the rise in lipid peroxidation and enhanced the antioxidant potential. It was reported, also, that fenugreek modulates hepatic oxidative stress during colon cancer by decreasing lipid peroxidation and enhancing the activity of SOD and CAT in the liver [32].

It is well documented that interaction of free radicals with lipids disrupts membrane structure and transport processes, and interaction of free radicals with proteins may cause structural damage in many hormones and enzymes[33]. These structural changes result in biochemical disorders[34]. In the present study, whole body exposure of rats to 3.5Gy results in significant increase ($P<0.05$) in the level of serum glucose and insulin on the 1st day post-irradiation, compared to control values (Table 3). The hyperglycemic state observed after irradiation may result from enhanced gluconeogenesis, diminished utilization of glucose by irradiated tissues and insulin resistance [35]. Insulin resistance is characterized by a decline in skeletal muscle glucose utilization and/or an excessive hepatic glucose production. As consequences of insulin resistance the pancreas continues to produce insulin causing hyperinsulinemia[36].

Table 3. Serum urea, creatinine, glucose and insulin levels in different rat groups.

Biochemical Parameters	Rats groups			
	Control	Fenugreek	Irradiated	Fenugreek-Irrad.
Urea (mg/dl)	$51.61^{a} \pm 1.17$	$48.90^{a} \pm 1.70$	$60.10^{b} \pm 1.10$	$54.80^{c} \pm 1.10$
Creatinine (mg/dl)	$0.660^{a} \pm 0.011$	$0.665^{a} \pm 0.012$	$0.798^{b} \pm 0.014$	$0.705^{c} \pm 0.015$
Glucose (mg/dl)	$90.3^{a} \pm 0.95$	$88.5^{a} \pm 1.80$	$127.2^{b} \pm 0.97$	$110.2^{c} \pm 1.30$
Insulin (µIU/dl)	$24.1^{a} \pm 0.93$	$23.5^{a} \pm 1.20$	$43.1^{b} \pm 0.93$	$33.0^{c} \pm 1.30$

Each value represents the mean of 6 records ± S.E.
Values with different superscript for each item are significantly different from each other at $P < 0.05$

Fenugreek-treated irradiated rats showed significant decrease ($P<0.05$) in glucose and insulin levels, compared to their corresponding values in irradiated rats (Table 3). The hypoglycemic effect of fenugreek seed powder may be attributed to the insulin-mimetic effect, inhibition of intestinal α-amylase activity [11], activation of insulin signaling pathway in adipocytes and liver

cells[37], and enhancement of hepatic glucokinase and hexokinase enzymes activities[38]. In addition the hypoglycemic effects of fenugreek may be mediated through inhibition of carbohydrate digestion and absorption and enhancement of peripheral action[39]. It was demonstrated, also, that fenugreek seed powder treatment to diabetic animals resulted in a marked decrease in the plasma glucose level by restoring the altered expression of liver pyruvate kinase and phosphoenol-pyruvate carboxykinase the key enzymes of glycolysis and gluconeogenesis[40].

In the present study, whole body exposure of rats to 3.5 Gy induced alteration in liver and kidney functions, obvious by significant increases ($P \leq 0.05$) in serum creatinine and urea levels (Table 3) markers of kidney function, and significant increases ($P \leq 0.05$) in the activity of serum ALP, AST and ALT markers of liver function, on the 1st day post-irradiation, compared to control values (Table 4). Elevation in serum urea and creatinine levels may 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[41], or the interaction of irradiation with their sites of biosynthesis[42]. The significant increase in the activity of serum ALP, AST and ALT might result from radiation-induced liver cell membrane damage and alterations in dynamic permeability of membranes due to peroxidation, which is followed by the release of intracellular enzymes to the blood stream. Treatment with fenugreek seed powder restored the levels of serum urea and creatinine levels (Table 3), as well as ALP, AST and ALT activities (Table 4).

Table 4. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase activities in different rat groups.

Biochemical Parameters	Rats groups			
	Control	Fenugreek	Irradiated	Fenugreek-Irrad.
ALT U/ml	$30.47^{a} \pm 0.51$	$31.15^{a} \pm 0.96$	$40.8^{b} \pm 1.02$	$35.9^{c} \pm 0.36$
AST U/ml	$147.7^{a} \pm 0.67$	$147.2^{a} \pm 0.63$	$159.7^{b} \pm 0.82$	$153.9^{c} \pm 0.98$
ALP U/ml	$25.4^{a} \pm 0.54$	$42.2^{a} \pm 0.36$	$32.5^{b} \pm 1.6$	$26.02^{c} \pm 0.42$

Each value represents the mean of 6 records ± S.E.
Values with different superscript for each item are significantly different from each other at $P < 0.05$

The results are in accordance with Kaviarasan *et al.*[13] who reported that, treatment with fenugreek seed polyphenolic extract protects against ethanol-induced hepatic injury and apoptosis in rats. Fenugreek was also shown to restore membrane fluidity of liver and kidney[43]. According to the results obtained in the present study, it could be concluded that fenugreek seed powder could afford protection against radiation-induced oxidative stress in liver and kidney and organ dysfunction.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

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تأثير نبات الحلبة على التغيرات التى يحدثها التعرض للإشعاع الجامى فى الكبد والكلى للجرذان

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يتناول هذا البحث الدور الوقائى لنبات الحلبة ضد العبء التأكسدى الذى يحدثه التعرض للإشعاع الجامى فى أنسجة الكبد والكلى. تم حقن معلق مسحوق بذور الحلبة عن طريق الفم بجرعة مقدارها 1 جم لكل كيلو جرام من وزن الجسم لمدة 7 أيام متتابعة قبل التعرض للإشعاع بجرعة حادة مقدارها 3.5 جراى من الإشعاع الجامى.

ولدراسة مدى كفاءة مسحوق بذور الحلبة للوقاية من الإشعاع فقد تم قياس كل من مستوى الجلوتاثيون المختزل ، نشاط السوبر أكسيد ديسميوتيز والكتاليز فى أنسجة كل من الكبد والكلى ، كذلك تم قياس نشاط بعض الإنزيمات فى مصل الدم (ALP, ALT, AST) كدلالات لوظائف الكبد وكذلك اليوريا والكرياتينين كدلالات لوظائف الكلى. وأيضا تقدير نسبة السكر والأنسولين فى الدم وقياس عملية فوق الأكسدة للدهون (مستوى TBARs) لأنسجة الكبد والكلى.

وقد أظهرت النتائج أن الجرذان التى تجرعت معلق مسحوق بذور نبات الحلبة قبل التعرض للإشعاع الجامى حدث لها تحسن معنوى فى المعايير البيولوجية المقاسة لوظائف الكبد والكلى بالمقارنة بجرذان المجموعة المعرضة للإشعاع فقط، كما لوحظ إستعادة المستوى الطبيعى لمستوى السكر والأنسولين بالدم. هذا بالإضافة إلى النقص الواضح فى مستوى نواتج فوق الأكسدة للدهون فى أنسجة كل من الكبد والكلى.

ومن النتائج السابق ذكرها يتضح أن مسحوق بذور نبات الحلبة يلعب دورا فعالا فى الحد من الأضرار الإشعاعية نتيجة لما يحتويه من مواد مضادة للأكسدة مما يؤدى إلى إتزان المعايير البيولوجية المقاسة.