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EFFECT OF USING GAMMA-IRRADIATED MIXTURE EXTRACT OF CAROB AND ROSELLE IN DIABETIC RATS

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ABSTRACT

This study aimed to investigate the antidiabetic and antioxidant effects of gamma irradiated mixture of carob and roselle (10 kGy) in diabetic male rats. The results with test rats indicated that alloxan administration induced the elevation of glucose level, activity of some liver enzymes and malondialdehyde concentration, while the level of insulin, testosterone and glutathione content (GSH) and the activity of superoxide dismutase (SOD) and catalase (CAT) were decreased under the effect of alloxan. As a result of treatment of diabetic rats with either raw or γ-irradiated mixture extract of carob and roselle (10 ml/Kg b.wt., for 6 weeks), hyperglycemia, hepatic and endocrine abnormalities induced by alloxan were improved. The results support the conclusion that raw and γ-irradiated mixture extract of carob and roselle can effect a partial normalization of the biochemical changes associated with alloxan-induced diabetes mellitus in rats. Furthermore, gamma-irradiation (10 kGy) increases the total phenolic content of dried carob and roselle.

KEY WORDS: **Diabetes mellitus; Carob; Roselle; Alloxan; Gamma-irradiation.**

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INTRODUCTION

Diabetes mellitus is a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both [1]. The prevalence of diabetes is rapidly rising all over the world [2]. There are at least 150 million people living with diabetes worldwide of which two-thirds are in developing countries. The total number of people with diabetes is predicted to rise above 300 million by 2025 [3]. A wide variety of medicinal plants have been found to possess capacity to control metabolic problems and oxidative stress in diabetes, and their uses are considered safer than synthetic drugs [1]. Among them, *Hibiscus sabdariffa* Linn. (HS) (*Malvaceae*, local name Roselle) is a valuable source of traditional medicine [4]**.** It is a natural phenolic rich plant that has been reported to have a wide range of pharmacological properties, such as antioxidant activity and free radical scavenging capacity [5], hypoglycaemic [6] and hypolipidaemic effects [7]. Also *Ceratonia siliqua*.L., Fabacae (Carob) is considered one of medicinal plants that has been widely cultivated in Mediterranean area. The plant is grown locally in Egypt, and the pods are used mainly for preparing a popular beverage. Carob pods and leaves contain antioxidant agents and is rich in polyphenols and flavonoids [8]**.** Importantly, carob fruit presents an in vitro and in vivo antioxidant effects and might be proposed as a food additive to protect against oxidative stress damage [9]**.** Various conventional methods of sterilization and reduction of microbial loads are used. Many studies dealing with the use of irradiation technology for medicinal and aromatic herbs and spices have concluded that irradiation can be considered a radiologically hygienic and toxicologically safe technology and effective method to improve the quality of plant materials in terms of reduction of microbial contamination [10].Since carob and roselle are used for preparing a popular beverage in Egypt, and cause less complications and since they are used in traditional medicine to treat diabetes. This study aimed to investigate the antidiabetic and antioxidant effect of gamma irradiated mixture of carob and roselle in diabetic male rats.

MATERIALS AND METHODS

2.1. Plant material

The dried flowers of roselle plant and carob pods were purchased from a commercial herbal market in Cairo, Egypt.

2.2. Gamma Irradiation treatment

Both of dried flowers of roselle and carob pods samples were transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a ^{60}Co source at a dose rate of 2.50 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

2.3. Determination of total phenolic compounds

The water extract of raw and γ-irradiated carob and roselle was prepared by adding 10 g of dried samples into 100 mL of hot water for 15 min with constant stirring and was vacuumed-filter twice, centrifuged (3,000 rpm, 10 min).The total phenolic contents of raw and γ-irradiated carob and roselle water extract were determined by using the Folin-Ciocalteau calorimetric method [11]. Briefly, 1 mL of sample extract was added into a 25 mL volumetric flask followed by the addition of 1 mL Folin-Ciocalteau reagent (1N). The mixture was shaken slowly and left to react at room temperature for 5 min. After 5 min, 10 mL of sodium bicarbonate (7% w/v) was added into the mixture. The flask was filled with distilled water and left to stand at room temperature in the dark for 40 min. Distilled water was used as blank. Sample absorbance was recorded at 750 nm against the blank. The total polyphenol content was compared to that of gallic acid standard curve previously prepared covering the concentration of 20 to 100 µg/mL. Samples were measured in triplicate analysis.

2.4. Preparation of roselle and carob mixture extract

The raw and γ-irradiated carob pods were grinded to fine powder before extraction. In order to prepare mixture extract: 7.5 g of raw or γ-irradiated roselle were mixed with 7.5 g of raw and γ-irradiated carob pods powder. Then, either raw or γ-irradiated mixture (15 g) was slowly boiled in distilled water (100 ml) for 5 min [12]. The raw and γ-irradiated carob and roselle mixture extract (CRME) was then allowed to cool and filtered, then daily administered orally by gavage to the rats (10 ml/Kg b.wt.).

2.5. Experimental design

Adult male albino rats of Wistar strain (200-220g) were obtained and maintained in the Central Animal House. The rats were divided into four groups, seven animals each and housed in plastic cages under controlled conditions of 12 h light and dark cycle, 50% humidity and 22°C–25°C for 6 days to acclimate them to the experimental environment before the start of the experiment. The animals had access to food and water at all times. Hyperglycemia was induced by intraperitoneal administration of alloxan monohydrate dissolved in saline at a dose of 150 mg/kg body weight [13]. Alloxan can be induced fatal hypoglycemia as a result of massive pancreatic insulin release; therefore, rats were treated with 30% glucose solution orally at different time intervals after 6 h of alloxan induction, and 5% glucose solution was kept in bottles in their cages for the next 24 h. After one week, blood was extracted from the tail vein for glucose analysis by the method of Trinder [14]. Experimental animals exhibited fasting blood glucose levels in the range of 200 to 250 mg/dl. Group I: rats fed on balanced diet and served as control, group II: diabetic group and group III- IIII: diabetic rats received oral extract of raw or γ-irradiated M.E. (10 ml/Kg b.wt.) for 6 weeks. At the end of the experimental period, the rats in each group were fasted overnight, anaesthetized with diethyl ether and sacrificed. Blood samples were collected through heart puncture, allowed to coagulate and centrifuged to obtain serum and plasma for biochemical analysis. Also, liver was removed and washed in ice-cold saline.

2.6. Biochemical Analysis

Liver was minced and homogenized (10%w/v), in the ice-cold saline. The homogenate was centrifuged at 10,000 g for 20 min, and the resultant supernatant was used for measuring lipid peroxidation as TBARS following the method of Yoshioka et al. [15], superoxide dismutase (SOD) [16] and catalase (CAT) [17] activity, as well reduced glutathione content (GSH) [18]. Serum glucose was evaluated by the method of Trinder [14]. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) were assayed by the method of Reitman and Frankel [19], as well as serum alkaline phosphatase activity (ALP) was assessed according to Kind and King [20]. Finally, the serum testosterone concentration was measured by the enzyme linked immunosorbent assay (ELISA) according to the method of Engrall and Perlman [21] and also insulin hormone level was determined by radioimmunoassay kit supplied by Diasari, Italy.

2.7. Statistical analysis

Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range tests was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS) [22]. Differences between means were considered significant at $P < 0.05$.

RESULTS

The results in table (1) showed that the total phenolic compounds content of raw carob and roselle were 1.83 and 1.75 mg GAE/g, respectively. While, these values changed to 1.93 and 1.85 mg GAE/g, respectively after gamma-irradiation processing of carob and roselle (Table 1).

Table 1
Effect of gamma irradiation on total phenolic content of dried carob and roselle.

Parameters	Total phenolics (mg GAE/g)		% Change
	Raw	Irradiated	
Carob	1.83 ± 0.67	1.93 ± 0.56	5.5%
Roselle	1.75 ± 0.56	1.85 ± 0.53	5.7%

Each value represents the mean ± SE (n = 3)

As seen in table 2, the serum glucose level was significantly higher in diabetic groups than in the control group. Whereas, alloxan-induced diabetes caused a significant decline of insulin level compared to the control. Nevertheless, raw or γ-irradiated CRME (carob and roselle mixture extract) administration to diabetic rats led to a significant reduction of glucose level and a significant increase of insulin level compared to the diabetic group (Table 2).

Table 2
Effect of administration of raw and irradiated CRME on the level of glucose and insulin of diabetic rats.

Parameters	Control	Diabetic	Diab.+ M.E.	Diab.+ Irr. M.E.
Glucose(mg/dl)	91.70 ± 6.71^{a}	228.45 ± 8.23^{c}	133.42 ± 6.15^{b}	119.03 ± 5.91^{ab}
Insulin(µU/ml)	31.42 ± 3.93^{a}	17.93 ± 3.18^{c}	26.22 ± 3.57^{b}	29.12 ± 3.74^{ab}

Values are expressed as means ± S.E. (n=7).
Values in the same raw with different superscripts are differing significantly at P<0.05.

The concentrations of MDA, enzymatic antioxidants activity (SOD and CAT) and the level of GSH content in the liver of alloxan-induced diabetic rats were significantly higher, compared with control and other treated groups. Administration of raw and γ-irradiated CRME to diabetic rats showed a significant decrease in MDA and increase in the activity of enzymatic antioxidants (SOD, CAT) and GSH content in the liver (Table 3).

Table 3
Effect of administration of raw and irradiated CRME on hepatic MDA, GSH, activity of SOD and CAT of diabetic rats.

Parameters	Control group	Diabetic group	Diab.+ M.E. group	Diab.+ Irr. M.E group.
MDA (n mol/g tissue)	176.55 ± 6.37^{a}	366.23 ± 7.72^{c}	230.54 ± 6.62^{b}	204.57 ± 5.93^{ab}
GSH (mg/g tissue)	25.82 ± 2.11^{a}	16.37 ± 1.54^{c}	22.66 ± 1.81^{b}	23.95 ± 1.92^{ab}
SOD (U/mg protein)	43.88 ± 3.74^{a}	33.75 ± 3.13^{c}	40.07 ± 3.64^{b}	41.92 ± 3.60^{ab}
CAT (U/g protein)	3.71 ± 0.11^{a}	1.79 ± 0.09^{c}	2.91 ± 0.08^{b}	3.24 ± 0.10^{ab}

Values are expressed as means ± S.E. (n=7).
Values in the same raw with different superscripts are differing significantly at P<0.05.
ME: mixture extract of raw carob and roselle Irr. M.E : mixture extract of γ-irradiated carob and roselle

Alloxan administration significantly produced adverse effects on the liver function of the rats and increased the activity of ALT, AST and ALP enzymes as compared with normal (Table 4). Treatment of diabetic rats with either raw or irradiated mixture extract exhibited improvement in liver function compared to diabetic rats.

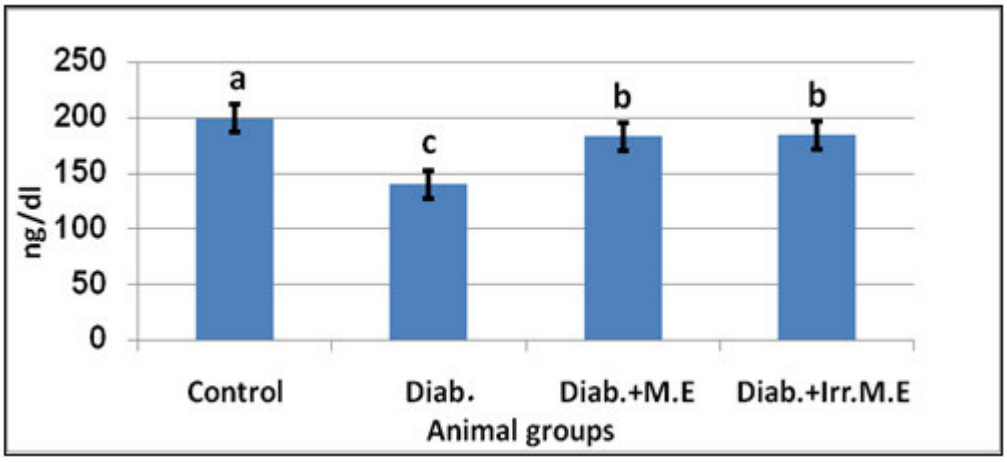
Table 4
Effect of administration of raw and irradiated CRME on the activity of AST, ALT and ALP of diabetic rats

Parameters	Control	Diabetic	Diab.+ M.E.	Diab.+ Irr. M.E.
AST (U/ml)	23.47±1.27[a]	50.27±3.15[c]	33.51±2.18[b]	29.77±1.82[ab]
ALT (U/ml)	19.72±1.63[a]	41.92±2.83[c]	25.18±2.12[b]	21.57±2.72[a]
ALP (U/100ml)	9.08±0.88[a]	14.87±0.98[c]	10.08±0.78[b]	9.58±0.85[ab]

Values are expressed as means ± S.E. (n=7).
Values in the same raw with different superscripts are differing significantly at P<0.05.

Hormonal measurement indicates a significant decrease in serum testosterone level in alloxan-induced diabetic group in comparison to control and other treated groups. In contrast, the testosterone level increased significantly only in diabetic groups that received raw or γ-irradiated mixture extract (Figure 1).

Figure 1
Effect of administration of raw and irradiated CRME on testosterone level of diabetic rats.



Values are expressed as means ± S.E. (n=7).
Values with different superscripts are differing significantly at P<0.05.

DISCUSSION

Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances, some may inhibit insulinase activity, some plants are involved in the stimulation of cells to produce more insulin and others may increase cells in the pancreas by activating regeneration of pancreatic cells [23]. Table 1 shows that the total phenolic content of raw dried carob pods and roselle flowers were increased by 5.5% and 5.7% respectively after gamma-irradiation (10 kGy). This increase in total phenolic content could be attributed to the degradation of tannins having higher molecular weight into the release of simple phenolic compounds like gallic acid, tannic acid, etc. Irradiation may break this complex to facilitate release of active ingredients, which were contributed to increase the total phenolic content [24]. Also, Villavicencio et al. [25] presented that γ-irradiation could be results in increasing in the total phenolic contents compared with raw samples and that might be due to the decomposition of some

insoluble phenolic compounds. Results in this study obtained a significant low level of insulin and high glucose level in untreated diabetic rats compared to the normal control rats. Verma et al. [26] showed that there was extensive damage of the langerhans in alloxan induces diabetic rats. This effect was previously explained on the basis of alloxan's ability to produce hydrogen peroxide and other free radicals, including $O_2^{\bullet}$ and $^{\bullet}OH$ that damage β-cells hence leading to their death [27]. The sensitivity of β-cells to oxidative stress has been attributed to their low levels of antioxidants compared with other tissues [28]. Beta cell dysfunction eventually culminates in a reduction in insulin release leading to hyperglycemia. The alloxan induced sustained hyperglycemia aggravates the oxidative stress status by auto-oxidation of glucose and its primary and secondary adducts [29]. Administration of raw or γ-irradiated mixture extract of carob and roselle to diabetic rats reduced the glucose level and increase insulin concentration. The antidiabetic effect of the mixture extract could be attributed to the ability of roselle to regenerate pancreatic β-cells [30]. Moreover, several study reported that *Ceratonia siliqua* contain phytosterols that can reduce glucose concentration by stimulating pancreatic beta cells to secret more insulin in blood circulation; in this way, blood glucose level is controlled better [31]. Hyperglycemia has been shown to generate free radicals from auto-oxidation of glucose, formation of advanced glycated end products with concomitant increase the cellular lipid peroxidation and damage of cellular membranes [32]. In the present study the formation of MDA, an indicator of lipid peroxidation, was significantly increased in diabetic liver tissues. The results are in agreement with the study of Mohamad et al. [33]. Whereas, treatment of alloxan-induced diabetic rats with either raw or γ-irradiated mixture extract lowered the level of hepatic MDA, suggesting that the extract possesses potent antioxidative properties. *H. sabdariffa* and its phenolic compounds, which are widely distributed in plant, have been considered to play an important role as dietary antioxidants in the prevention of oxidative damage in living system [34]. As well as, the reduction of the level of lipid peroxidation could be related to the flavonoid ingredient of *C. siliqua* such as catechin, naringenin, naringin, and quercetin [35]. In this study, a reduced activity of SOD and catalase in liver has been observed in diabetic group and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide [23]. *In vitro* and *in vivo* studies have reported that in a variety of tissues, hyperglycemia and possibly elevated free fatty acid levels result in the generation of oxygen free radicals and considerably increased oxidative stress [36]and reduced the activity of antioxidant enzymes. Furthermore administration of alloxan to rats in this study resulted in depletion in GSH content and that may be related to the apparent increased in lipid peroxidation in the liver of rats exposed to alloxan. Several studies have revealed lowered antioxidant enhanced peroxidative status diabetic condition particularly in the liver [37]. In this work, the activity of enzymatic antioxidants (SOD and CAT) and the level GSH content were significantly increased after treatment of diabetic rats with raw and γ-irradiated mixture extract. *H. sabdariffa* composed of flavonoids and phenolic compounds which have been reported to antioxidant activity and made the plant to be useful for pharmacological investigations as antioxidant agent. In agreement with this, anthocyanins and protocatechuic acid extracted from roselle have shown to have strong antioxidant activity [38]. Also, it is well known that many phenolic compounds, which are found in carob, exert powerful antioxidant effects and inhibit lipid peroxidation by scavenging reactive oxygen species (ROS), such as $^{\bullet}OH$ [39]. Liver cell destruction shows its effects mostly as impairment in the liver cell membrane permeability, which results in the leaking out of tissue contents into the blood stream. In diabetic rats, the activity of serum ALP, ALT and AST were significantly increased. Supporting these findings, it has been found that the liver was necrotized in diabetic rats. Therefore, the increase of the activity of liver enzymes in serum is mainly due to the leakage of these enzymes from the liver into the blood stream [40],

which gives an indication on the hepatotoxic effect of alloxan. According to the results of this study, administration of alloxan to rats induced a significant decrease in the level of testosterone. It has been reported that induction of diabetes by alloxan in male testes lead to reduction in testosterone production, suggesting a decrease in the function of both Leydig (testosterone producing cell) and Sertoli (supporting cell), which might be due to a reduction in insulin secretion [41]. On the other hand, diabetic rats daily consumed raw and γ-irradiated mixture extract of carob and roselle have higher testosterone concentration than the non treated diabetic group. It seems that the increase in testosterone levels by the mixture extract is due to its direct effect on leydig cells and on testosterone biosynthesis. Carob contain gammalinolenic acids and alpha linolenic acid that can be transformed into dihomogamma linolenic acid and then into arachidonic acid which is the precursor of all prostaglandins, like PGE2. In addition, carob contain own arachidonic acid [42], that plays a major role in testis steroidogenesis. Studies indicate that this organic acid and its metabolite (PGE2) can increase the production of cyclic adenosine monophosphate (cAMP), thereby elevating cholesterol side chain break-down, and stimulating testosterone production [43]. Another compound found in carob is aspartic acid [44] which can function as a second messenger in leydig cells through increasing synthesis of cAMP and testosterone release. Moreover, the effect of roselle on the testosterone concentration could be attributed to the antioxidant activity of its phenolic contents such as protocatechuic acid [45] and anthocyanins [46] that can protect against oxidative stress induce by alloxan.

CONCLUSION

The observed improvement in the hepatic antioxidants, liver enzymes, glucose concentration and the level of testosterone and insulin in alloxan-induced diabetic rats suggested that the hypoglycemic effect of the mixture of carob and roselle was attributed to their phenolic compounds which possess antioxidant potential can reduce the oxidative stress induced by alloxan administration.

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ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No.85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee.

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