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Using of Coffee and Cardamom Mixture to Ameliorate Oxidative Stress Induced in ?-irradiated Rats

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ABSTRACT

Human exposure to ionizing radiation induced overproduction of free radicals leading to oxidative stress. This study aimed to evaluate the possibility of using of coffee and cardamom mixture; as natural antioxidant compounds; to ameliorate oxidative stress in rats induced by exposure to ionizing radiation. Phenolic contents in coffee and essential oils in cardamom were identified by using HPLC chromatography and GC/MS analysis. Four groups of adult male rats were used; the control group (A), the second group (B) received orally the mixture extract of coffee and cardamom (60 mg/100g body weight) for 8weeks, the third group (C) ?-irradiated (6 Gy) and the fourth group (D) received orally the mixture extract for 8 weeks and exposed to ?-radiation at the 4th week. The results revealed that the administration of mixture extract of coffee and cardamom to rats significantly reduced the damage effect induced by ?-irradiation via the adjustment of the antioxidant status, decreasing of malondialdehyde content and the subsequent amending of different biochemical parameters as well as some hormones. Accordingly, it is possible to indicate that coffee-cardamom reduced the radiation exposure induced oxidative stress.

Key words: Ionizing Radiation / Coffee and Cardamom/ Phenolic contents/ Essential oil/ Antioxidants/ Hormones.

INTRODUCTION

Ionizing radiation being used in large number of therapeutic, industrial and other applications of nuclear power generation, developing new varieties of high-yielding of new crops and enhancing storage-period of food materials [1-3]. In addition, Gamma radiation associated with the generation of reactive oxygen species (ROS), causing oxidative damage particularly to various tissues and induce damages in DNA and cellular membrane [3-5].

A number of medicinal plants evaluated for their radioprotective efficacy have shown protective efficacy against the damaging effects of ionizing radiation. Plant extracts eliciting radioprotective efficacy contain a plethora of compounds including antioxidants, immunostimulants, cell proliferation stimulators, antiinflammatory and antimicrobial agents, some of which may act in isolation as well as in combination with other constituents from the same plant [6-10].

Coffee (*Coffea Arabica)* has scientific interests because it is a rich source of a number of phenol compounds with antioxidant effects *in vitro* and contains several species of xanthines such as caffeine, teobromine and theophylline [11&12]. Main polyphenols in coffee are chlorogenic acids such as caffeic, ferulic, and p-coumaric acid, caffeoylquinic acid, with 5-O-caffeoyl-quinic acid [13&14]. Meta-analyses have concluded that moderate to high coffee consumption (three to six cups/day) is not significantly

associated with an increased risk of coronary death or heart attack [15&16]. In addition, habitual coffee drinking has been associated with the prevention of diseases including cancer, cardiovascular disorders, obesity and diabetes as well as neurodegenerative disorders [15-20].

Cardamom (*Elettaria cardamomum* Maton), the Queen of all spices has a history as old as human race [21]. The major use of Cardamom on worldwide is for domestic culinary purpose and in medicine. The aroma and medicinal properties of cardamom are due to the volatile oil present in it and it is obtained from the seeds by steam distillation. The composition of cardamom oil has been studied by various workers and the major compounds found were 1, 8 cineole (20-60 %) and a-terpinyl acetate (20-55 %) [21]. Studies have implicated cardamom's potential therapeutic value as an inhibitor of human platelet aggregation [22] and it has antihypertensive, gastroprotective , anticancer and antidiabetic properties[23]. Cardamom has antioxidant properties and can increase levels of glutathione and antioxidant enzymes in the body [24&25]. Reports are available on the antioxidant activity of extract of cardamom from different countries [26-30].

Due to oxidative stress induced by ?-radiation exposure and the occurrence of different damage, the aim of the present study was oriented to evaluate the ameliorating effects of coffee-cardamom mixture against biochemical changes induced by ?-irradiation.

MATERIAL AND METHODS

Materials:

Coffee and cardamom powder as well as standard commercial rodent diet were purchased from local herbal market (Cairo, Egypt).

Preparation of coffee and cardamom mixture:

The mixture of coffee and cardamom were prepared by adding 5 g of cardamom powder for 100g of mixture (95g coffee + 5g cardamom).

Extraction and determination of phenolic compounds of coffee:

Grounded dry powder of coffee (10 g) was weighed into a test tube. A total of 100 ml of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonificated twice for 15 min and one left at room temperature (~ 20 °C) for 24 h. The extract was centrifuged for 10 min (10 min, 1500 x g), and supernatants were filtered through a 0.2 µm Millipore membrane filter then 1-3ml was collected in avail for the HPLC analysis of phenolic compounds.

According to the method of **Goupy et al.** [31], phenolic compounds of coffee were analyzed with high performance liquid chromatography (Hewllet Packared series 1050) equipped with outosampling injector, solvent degasser, ultraviolet (UV) detector set at 280nm and quarter HP pump (series 1050). The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. The phenolic standards from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for calculation of Phenolic compounds concentration by the data analysis of HEWLLET packared software.

Extraction, Separation and identification of the essential oils:

The essential oils of cardamom were obtained by water distillation in a glass apparatus for 3 hours. The separated volatile oils were dried over anhydrous sodium sulphate before hold glass bottles at -20ºC [32].

Separation and identification of essential oil components were performed by using Gas chromatography instrument, Model Hewlett-Packard- MS (5970) series II at the Agriculture Research Center, Giza, Egypt. Condition analysis as follow: Column: 30m hp Methyl silicon 0.1mm; Temperature: Initial 60 ºC; Rate: 3 ºC/ min up to 240 ºC; Carrier gas: Helium 1.0 ml/min; Injection port; Temperature: 250 ºC; Detector temperature: 270ºC; Integration: By using HP software Data; Injection volume: 0.3ml. The isolated peaks were identified by matching with data from the library of mass spectra and compared to those of authentic compounds and published data [33]. Quantitative determination was carried out based on peak area integration.

Radiation Facility:

Whole body gamma irradiation of rats at a dose level of 6Gy was performed using a Canadian gamma cell-40, (137Cs) housed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate was 0.44 Gy/min at the time of the experiment.

Experimental Animals:

Adult male albino rats, reared in NCRRT animal house, were used in the present experiments. Matched weight animals (150±10g) were selected and housed in plastic cages under controlled condition and fed on standard commercial rodent diet. Gamma irradiated rats were orally received extract of coffee and cardamom mixture at a dose level of 60 mg/100g body weight/day [20].

Experimental Design:

Animals (28 rats) were randomly divided into 4 groups each of six animals as follows:

Group A: rats fed on balanced diet for 8 weeks, served as control.
Group B: rats fed on balanced diet and received orally the extract of coffee and cardamom mixture at a dose level of 60 mg/100g body weight/day for 8weeks.
Group C: rats fed on balanced diet for 8 weeks and exposed to ?-radiation (6 Gy) at the 4th week.
Group D: rats fed on balanced diet and received orally the extract of coffee and cardamom mixture for 8 weeks and exposed to ?-radiation at the 4th week.

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 allowed to coagulate 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) [34]. Hepatic glutathione content (GSH) and activity of superoxides dismutase (SOD) were measured by the method of **Gross *et al.* [35] and Minami and Youshikawa [36],** respectively. Serum glucose was evaluated by the method of **Trinder [37].** The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to **Reitman and frankel [38],** as well as serum gamma glutamyl transferase (GGT) was assessed according to **Rosalk, [39]. In addition,** total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by **Allain *et al.* [40], Fossati and Prencipel, [41] and Demacker *et al.* [42],** respectively while low-density lipoprotein cholesterol and very Low-density lipoprotein-cholesterol were evaluated according to **Friedwald *et al.* [43] and Norbert [44]** formulas, respectively by the following equations: LDL-C (mg/dl) = TC - (TG/5+HDL-C), vLDL (mg/dl) = TG/5. **Finally,** the serum testosterone concentration was measured by the enzyme linked immunosorbent assay (ELISA)

according to the method of **Engrall and Perlman,** [45] and also insulin hormone level was determined by radioimmunoassay kit supplied by Diasari, Italy**.**

Statistical analysis:

Statistical analyses were performed using computer program Statistical Packages for Social Science [46] and values were compared with each other using one-way analysis of variance (ANOVA).

RESULTS

The phenolic compounds of coffee were identified by HPLC against standard compounds and the results were listed in table (1). The most abundant components of coffee were caffeine, pyrogallol and caffeic "820.59, 65.23 and 17.98 mg/100g", respectively.

The Essential oil contents found in cardamom were detected by GC/MS and the results were reported in table (2). The results showed that the main essential oil components were terpenyl acetate (60.65%) and 1,8 Cineol (13.63%).

Table (1): Phenolic compounds of coffee extract (mg/100g).

Phenolic compounds	mg/100g
Pyrogallol	65.23
Gallic	4.40
Protocatechouic	8.15
Catechin	8.67
Caffeic	17.98
Caffeine	820.59
Ferulic	2.40
P.coumaric	2.83
Ellagic	15.42
Cholchecien	7.32
Salicylic	16.69
Cinnamic	5.50

Table (2): Essential oil contents of cardamom.

Essential oil	%
a-Pinene	1.36
Sabimene	1.08
Limonene	7.12
1,8 Cineol	13.63
?-Terpinene	0.25
Terpinolene	0.31
Linaiool	0.92
Terpineol	5.10
Octyl acetate	0.05
Linalyl acetate	2.33
Germaniol	0.57
Terpenyl acetate	60.65
Neryl acetate	2.11
Total unknown	4.52

Results of irradiated rats revealed a significant increase in the level of MDA and a remarkable decrease in the content of GSH and SOD activity, while those rats that have received the oral extract of coffee and cardamom mixture showed a lower level of MDA accompanied by an increased in level of GSH and SOD activity **(Table 3).**

Table (3): Effect of administration of coffee and cardamom mixture extract to ?- irradiated rats on MDA, hepatic GSH and SOD activity.

Parameters	A	B	C	D
MDA (n mol/ml)	178.71 ±3.82^{c}	166.46 ±3.03^{d}	396.07 ±3.52^{a}	211.26 ±3.85^{b}
GSH (mg/g tissue)	24.62 ±1.35^{a}	25.58 ±1.46^{a}	15.48 ±1.22^{c}	22.18 ±1.41^{b}
SOD (U/mg protein)	43.60 ±1.65^{a}	43.86 ±1.71^{a}	36.65 ±2.47^{c}	40.96 ±1.47^{b}

Values are expressed as means ± S.E. (n=6).
Values in the same raw with different superscripts are differing significantly at P<0.05.

On the other hand, the data revealed a significant elevation in AST, ALT and GGT activity accompanied by high glucose level in irradiated group compared to control group but when the irradiated rats treated with the mixture extract a significant reduction was observed in the above mentioned liver enzymes as well as glucose concentration **(Table 4).**

Table (4): Effect of administration of coffee and cardamom mixture extract to ?- irradiated rats on the activity of some liver enzymes and glucose level.

Parameters	A	B	C	D
AST U/ml	27.57 ±1.49^{c}	26.09 ±1.43^{c}	49.66 ±2.90^{a}	32.35 ±2.29^{b}
ALT U/ml	15.92 ±1.05^{c}	15.16 ±1.16^{c}	47.49 ±1.54^{a}	29.31 ±1.55^{b}
GGT U/ml	3.24 ±0.22^{c}	3.20 ±0.37^{c}	6.13 ±0.53^{a}	4.46 ±0.41^{b}
Glucose (mg/dl)	112.49 ±4.34^{c}	109.46 ±3.68^{c}	195.95 ±4.69^{a}	126.00 ±3.96^{b}

Legend as table 3

Also, data in table 5 indicated that exposure of rats to ?-radiation resulted in a significant increase in TC, TG, LDL-C and vLDL-C concentration and decrease in the level of HDL-C. However, administration of coffee and cardamom mixture induced moderate improvement in the above mentioned levels.

Table (5): Effect of administration of coffee and cardamom mixture extract to ?- irradiated rats on serum lipid profile levels.

Parameters	A	B	C	D
TC mg/dl	152.48 ±3.83^{c}	149.45 ±3.09^{c}	208.13 ±4.06^{a}	186.42 ±3.17^{b}
TG mg/dl	112.47 ±2.91^{d}	108.41 ±2.90^{c}	181.45 ±2.06^{a}	156.50 ±2.86^{b}
HDL-C mg/dl	45.15 ±1.72^{a}	47.44 ±1.65^{b}	37.53 ±1.82^{d}	41.35 ±1.51^{c}
LDL-C mg/dl	84.84 ±2.22^{d}	80.32 ±1.95^{c}	134.31 ±2.44^{a}	113.77 ±2.76^{b}
vLDL-C mg/dl	22.49 ±1.18^{d}	21.68 ±1.11^{c}	36.29 ±1.21^{a}	31.30 ±1.17^{b}

Legend as table 3

The reducing effect of ?-irradiation on the level of testosterone and insulin is illustrated in **Figure 1&2**, while it was noticed that the treatment of irradiated rats with coffee and cardamom mixture reduced the adverse effects of ?-irradiation with an obvious enhancement in the level of the both hormones.

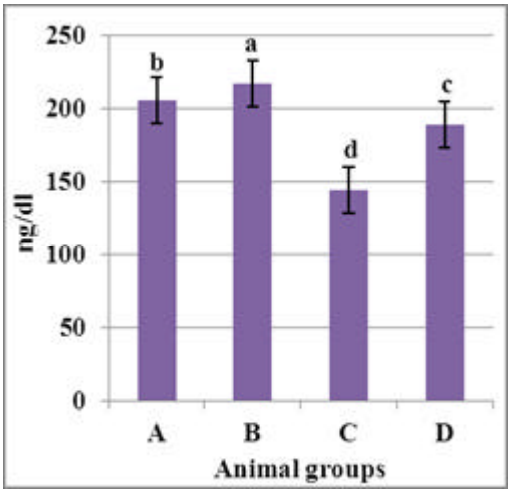

Figure (1): Effect of administration of mixture extract to ?-irradiated rats on testosterone level.

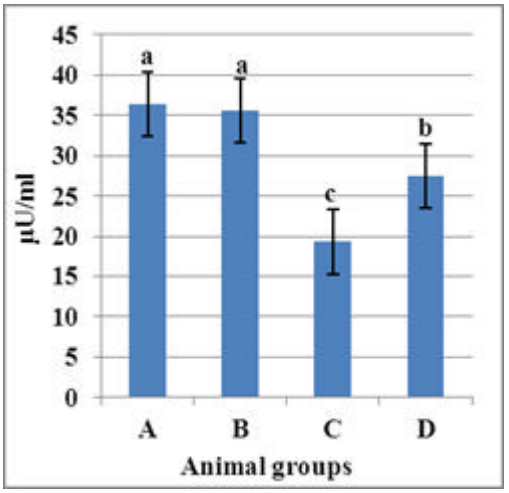

Figure (2): Effect of administration of mixture extract to ?-irradiated rats on insulin level.

DISCUSSION

Exposure to ionizing radiation might happen as a consequence of military or industrial accidents, or after therapeutic treatments [47, 48]. Also, it is well known that ionizing radiation generates reactive oxygen species (ROS) that interact with cellular molecules, including DNA, lipids, and proteins [49].

Coffee polyphenolic components (cafestol, kehweol, cholinergic acid and caffeine) [50], as well as essential oil of cardamom [51] function as an antioxidant by scavenging reactive oxygen species and

have chemoprotective and anitinflammatory property [26, 52] , reverse lipid peroxidation, enzymatic leakage and enhance cellular antioxidant defense mechanism [53].

Results of the present study demonstrated a significant increase in lipid peroxidation (MDA) and reduction in GSH concentration and SOD activity due to exposure of rats to ?-irradiation (6Gy). The results are in agreement with those revealed by several authors [54-56]. They recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after whole body gamma-irradiation. This could be due to an enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation exposure.

On the other hand, results of gamma irradiated rats received oral extract of coffee and cardamom mixture displayed a significant low level of liver MDA and high GSH concentration and SOD activity in comparison with those of irradiated group. Therefore, these results reflect the protective effects of both coffee and cardamom against the damaging effect of ?-irradiation. **Abreu et al.,** [57] found that the consumption of coffee decreased the lipid peroxidation of the membranes; increased the concentration of glutathione, a potent endogenous antioxidant; and increased the activity of two antioxidant enzymes, glutathione reductase and superoxide dismutase. Undoubtedly, these beneficial effects of coffee on antioxidant properties are partly due to caffeine, kahweol and cafestol [58-60]. In addition to the effect of coffee, cardamom also have a potential antioxidants such as 1,8-ceineoil and alphaterpineol, protocatechualdehyde and protocatechuic acid which have health benefits by inhibiting lipid peroxidation and activation of antioxidant enzymes, [24, 25].

The effect of ?-irradiation on liver function in this study was determined by detection of AST, ALT and GGT activity which were highly increased under the effect of ?-irradiation, while the increase in these enzymes significantly reduced by administration of the extract of coffee and cardamom mixture to irradiated rats. Also, the results in table (4) indicated the increasing effect of ?-irradiation exposure on the level of serum glucose which highly declined after treatment of irradiated rats with the mixture extract.

The significant increase of serum transaminases activity may be attributed to a radiation induced state of hypoxia in the parenchymal cells of contracting fibrous tissue as well as to extensive body tissue breakdown includes liver parenchyma and renal tubules and that can cause change in tissue permeability and could enhance the release of the transaminase enzymes from their subcellular sites of production to extracellular and consequently to the blood circulation, [61, 62]. On the other hand, the increased glucose level might be related to endocrine glands function abnormalities induced by irradiation that promote the secretion of biologically active peptide which has relation to carbohydrate metabolism by increasing gluconeogenesis in liver [63].

The effect of both coffee and cardamom resulted in a significant enhancement in liver function and serum glucose level by reducing the damage effect induced by ?-irradiation. The ameliorating effect of coffee on the serum AST, ALT and GGT activity might be due to some extent to caffeine, which is one of the major ingredients contained in coffee with various biological actions and possibly plays a crucial role in the observed associations of coffee intake [64]. Also, the coffee constituent chlorogenic acid may have the potential to influence glucose metabolism processes to prevent hyperglycemia by inhibition activity of glucose-6-phosphatase, an important regulator of blood glucose levels, [65, 66].

Moreover, **Abdel-Wahab and Aly** [67] observed that treatment of rats with clove and cardamom effectively decreased liver enzyme levels in the serum. This can be attributed to the presence of antioxidants in clove and cardamom which contain phenolic compounds that can act by scavenging free radicals. Also, the lowering effect of cardamom on glucose level was observed by **El-Yamani** [23],

who studied the hypoglycemic effect of cardamom as one of spices that possess antioxidant compounds.

The present study showed a significant increase in cholesterol, triglycerides and LDL-C levels associated with reduction in the concentration of HDL after exposure of rats to 6Gy irradiation compared to control group. In contrast, a moderate decrease in TC, TG and LDL-C contents and rise in HDL level were observed in group D. **El-Khafif et al., [68] and Mansour, [69]** reported that whole body exposure to gamma radiation induced high level of serum cholesterol fractions due to its release from tissues, destruction of cell membranes and increase rate of cholesterol biosynthesis in the liver and other tissues. Also, irradiation could modify low- and high density lipoproteins metabolism indirectly through the action of various inflammatory products. Gamma-irradiation might decrease the lipoprotein lipase activity in adipose tissue, leading to reduction in the uptake of triacylglycerols.

Choi et al. [20] reported that the cholesterol-raising effect of coffee has long been controversial, and observed significant increase in TC, decrease in HDL-C, and no effect on plasma TG levels after coffee intake (10 cups/day). They attributed this cholesterol-raising effect of coffee to its contents of diterpenes cafestol and kahweol. Therefore, the improving effect of coffee and cardamom mixture intake on lipid profile of the irradiated rats may be related to the effect of cardamom. The lowering effect of cardamom on cholesterol and triglyceride levels reflected its protective hepatocellular effects and the ability of the phenolic contents and essential oil to reduce the hyperlipidemia [70-72]. Furthermore, it has been reported that spices may inhibit hepatic 3-hydroxyl-3-methylglutaryl coenzyme A reductase activity, resulting in lowering hepatic and serum cholesterol levels [73].

The present results clearly demonstrate that gamma irradiation induced a significant decrease in testosterone and insulin hormones concentration. These results agree with those of **Michael and Amer[2]** and **Liu et al. [74]** who recorded that decreased testosterone level after whole-body irradiation dose of 4 and 5 Gy was due to alterations in DNA-single strand break, cell apoptosis and oxidative stress. Also, **Popoff and Kapich[75]** found a positive correlation between a decline in testosterone affinity and exposure to gamma irradiation. In addition, the lowering effect of ?-irradiation exposure on insulin level was observed due to production of free radicals that induced oxidative stress resulted in reduction in insulin secretion and DNA damage [3].

This study reported that coffee and cardamom ingestion to irradiated rats elevated testosterone secretion. When cardamom is combined with coffee it has especially strong effects on the genital reactions and sexual function and it was traditionally used in Arabic coffee for this purpose. **Sanovane et al. [76]** reported that cardamom intake stimulates the nervous system which in turn stimulates the pituitary gland to increase the secretion of hormones induced gonads to produce testosterone. In addition, previous animal experiments had found that coffee infusion elevated plasma concentrations of testosterone and that related to the effect of bioactive contents of coffee [77].

Also, coffee and cardamom administration to irradiated rats reduced the damaging effect of ?-irradiation exposure and enhanced serum insulin concentration. The effect of coffee and cardamom could be related to their antioxidant contents such as caffeine and terpenyl acetate which reduced oxidative stress by scavenging free radicals and stimulated pancreatic cells to secrete insulin [21, 78].

CONCLUSION

The observed improvement in the antioxidants, liver functions, lipid profile and level of testosterone and insulin in irradiated rats could justify that the radioprotective effect of the mixture of coffee and cardamom was attributed to the phenolic compounds and essential oil contents which possess potent antioxidant capable of ameliorating the oxidative stress induced by ?-radiation exposure.

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