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The role of Echinacea extract to protect the damage effects of gamma radiation on some biochemical constituents in male albino rats

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ABSTRACT

The present study was designed to determine the possible protective effects of *E. purpurea* extracts (EPE) against gamma (γ-) radiation exposure (6Gy) induced biochemical alterations and oxidative tissue damage (liver and testes) in male rats given EPE (100 mg/kg/day for 8 weeks) prior to γ-irradiation. It has been found that γ-irradiation led to hepatic and testicular oxidative stress with concomitant increase in liver function enzymes. Serum lipid profile and hormone level has also been found altered. Rats dosed with EPE before exposure to γ-rays showed significantly less severe damage and remarkable improvement in all of the measured parameters when compared to irradiated rats. It could be concluded that EPE attenuates the deleterious effects of radiation-induced biochemical disorders and tissue damage (liver and testes).

Key words: Antioxidants, *Echinacea purpurea*, Radioprotective agents, Tissue damage, γ- irradiation.

INTRODUCTION

Exposure of mammals to ionizing radiations leads to different changes in the structure and function of cellular components and resulting in tissue damage and death (Abdelhalim and Moussa, 2013). Thus, radiation-induced damage might result in adverse health effects within hours to weeks or delayed effects observable many months after exposure and these damages can restrict radiation applications in diagnostic, therapeutic, and industrial settings. Therefore, the concept of radiation consequences is important in medicine and other related occupations (Mihandoost *et al*., 2014).

Researches exert efforts to develop radio-protective and radio-recovery agents to protect from the damage effects of radiation by supplementation with phytochemicals, including polyphenols, flavonoids, sulfhydryl compounds and plant extracts (Tawfik *et al*., 2006).

Echinacea purpurea (L.) Moench is one of the most important and well-known medicinal plants in the world, belonging to the *Asteraceae* (Compositae) family. It contains a variety of medically important substances that play a role in its therapeutic ef-fects which include alkylamides, caffeic acid derivatives, glycoproteins, polysaccharides, polyacetylenes, phenolic compounds, cinnamic acids, essential oils and flavonoids. Moreover, *E. purpurea* has been reported to possess anti-tumour, anti-inflammatory, antibacterial, antiviral and antioxidant activities and immuno-stimulant properties (Manayi *et al*., 2015).

This study is therefore aimed at investigating the protective role of *Echinacea purpurea* extracts (EPE) against biochemical disturbances induced by γ-radiation exposure in male rats.

MATERIALS AND METHODS

Radiation facility: Whole body gamma irradiation of rats at a dose level of 6 Gy 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 0.43 Gy/min at the time of the experiment.

Administration of EPE: Pure sample of *Echinacea purpurea* extracts (EPE) was procured from Sigma Chemicals. EPE was given orally to rats at therapeutic dose 100 mg/kg/day for 8 weeks (Rezaie *et al*., 2013).

Experimental animals: Male albino rats *Sprague Dawley* (130 ± 10 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed *ad libitum*. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85 – 23, 1996).

Experimental design: Animals (28 rats) were randomly divided into 4 groups each of 7 animals as follows:

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Group (C): (control group) rats fed on balanced diet for 8 weeks, Group (EPE): (*E. purpurea*-treated group) rats received EPE orally at dose 100 mg/kg/day for 8 weeks, Group (Irr.): (irradiated group) rats fed on balanced diet and were exposed to g-irradiation dose (6 Gy) at the 6th week of the experiment period (8 weeks) and Group (EPE + Irr.): rats received EPE orally (100 mg/kg/day) and were exposed to ã-irradiation dose (6 Gy) at the 6th week of the experiment period (8 weeks).

At the end of the experiment, animals from each group were sacrificed 24 h post the last dose of treatment. Blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether and allowed to coagulate and centrifuged to get serum for biochemical analysis. Also, liver and testes were removed for biochemical investigation.

Biochemical analysis: Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain *et al*. (1974), Fossati and Prencipe (1982) and Demacker *et al*. (1980), respectively. Low-density lipoprotein-cholesterol, very Low-density lipoprotein-cholesterol and risk ratio were evaluated according to Friedwald's formula (Friedwald *et al*., 1972) by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5.

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957), serum g-glutamyl transferase (GGT) was assessed according to Rosalki (1975)and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954). Estimation of testosterone hormone was performed according to the method of Wilson and Foster (1992). Follicle stimulating hormone (FSH) and leutinizing hormone (LH) according to Garrett (1989)

Liver and testes were dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate TBARS(Yoshioka *et al*. 1979), GSH(Beutler *et al*. 1963), superoxide dismutase activity (SOD)(Minami and Yoshikawa 1979)and Catalase activity (CAT)(Johansson and Borg 1988).

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 test was used to determine significant differences between means. Statistical analyses were performed using computer program Statistical Packages for Social Science (SPSS, 1998). Differences between means were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the serum concentrations of TC, TG, LDL-C and vLDL-C showed significant increases with a significant decrease in HDL-C concentration in γ-irradiated group as compared to control. However, administration of EPE to rats prior to γ-irradiation significantly reduced the TC, TG, LDL-C and vLDL-C levels and increased the level of HDL-C comparing with irradiated group (Table 1).

Also, the results presented in Table 2 revealed a significant elevation in the activity of AST, ALT, ALP and γGT in the serum of γ-irradiated group compared to control; whereas, the activity of liver enzymes (AST, ALT, ALP and γGT) were decreased in the group of rats received EPE orally before exposure to γ-rays, compared to irradiated group.

The data in Table 3 revealed a significant increment in FSH and significant reduction in testosterone and LH

Table 1: Influence of EPE administration on lipid profile in γ-irradiated rats

Parameters	Control	EPE	Irradiated	EPE+ Irr.
TC (mg/dl)	154.78±3.75[a]	149.45±3.63[a]	223.17±4.82[c]	177.12±4.71[b]
TG (mg/dl)	116.33±2.51[a]	112.23±2.88[a]	185.66±2.58[c]	148.48±2.74[b]
HDL-C (mg/dl)	44.35±1.55[a]	46.28 ±1.86[a]	36.89±1.35[c]	41.72±1.92[b]
LDL-C (mg/dl)	87.16±2.37[a]	80.73±2.54[a]	149.15±3.48[c]	105.70±2.80[b]
vLDL-C (mg/dl)	23.27±1.52[a]	22.44±1.14[a]	37.13±1.47[c]	29.70±1.61[b]

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

Table 2: Influence of EPE administration on the activity of serum ALT, AST, ALP and γGT in γ-irradiated rats

Parameters	Control	EPE	Irr.	EPE+ Irr.
AST (U/ml)	35.23±1.66[a]	34.85±1.57[a]	61.25±2.56[c]	41.88±1.63[b]
ALT (U/ml)	24.92±1..22[a]	25.13±0.92[a]	39.86±0.87[c]	30.16±0.68[b]
ALP(U/100ml)	8.25±0.52[a]	8.10±0.34[a]	14.46±0.58[c]	10.85±0.62[b]
[3]GT (U/ml)	4.46±0.47[a]	4.30±0.52[a]	6.75±0.69 [c]	5.05±0.52 [b]

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

Table 3: Effect of EPE administration on the serum levels of follicle stimulating hormone (FSH), leutinizing hormone (LH) and testosterone hormone.

Parameters	Control	EPE	Irr.	EPE +Irr.
FSH (mIu/ml)	3.65±0.35 [a]	3.71±0.25 [a]	5.42±0.38[c]	4.11±0.36[b]
LH (mIu/ml)	1.26±0.22 [a]	1.32±0.19 [a]	0.57±0.12[c]	1.08±0.25[b]
Testosterone(ng/dl)	212.11±3.52[a]	216.75±3.45[a]	139.33±2.87[c]	188.97±3.23[b]

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

levels in γ-irradiated rats compared to normal rats. On the other hand, administration of EPE to rats before γ-irradiation resulted in remarkable decreased in the level of FSH with elevation in the level of testosterone and LH compared to γ-irradiated group.

Results in Table 4 and Table 5 showed that the whole body exposure to γ-radiation induced significant increase in the level of TBARs in parallel to significant decrease in GSH level and the activity of SOD and CAT in the liver and testes compared to their corresponding values in the control group. Administration of rats with EPE before γ-radiation exposure has significantly declined the level of hepatic and testicular TBARs in addition to a significant increase in the level of GSH of and the activity of SOD and CAT compared to γ-irradiated rats.

Ionizing radiation is commonly used in diagnostic, therapeutic, and industrial settings. However, the damaging effects of radiation restrict its applications. Therefore, various approaches have been developed for diminishing the effects of radiation on normal tissues or enhancing tumor cell killing by ionizing radiation (Yousri *et al*., 2011). Echinacea has numerous claimed medicinal properties including: anti-viral, antibacterial, antifungal, anti-oxidant, anti-carcinogenic, anti-inflammatory and wound healing properties (Nematalla *et al*., 2011).

The data in the present work revealed that exposure of animals to γ-radiation showed a significant elevation in serum TC, TG, LDL-C and vLDL-C and a decrease in HDL-C. Abou Safi *et al*. (2005) reported that the elevation in serum lipid fractions might result from ionizing radiation ability to accelerate other pathways of cholesterol formation like increasing its rate of biosynthesis in the liver and other tissues, or destruction of cell membrane by radiation and also to disturbance LDL cholesterol receptors. Also, the hyperlipidaemic state observed after irradiation of rats could be attributed to the mobilization of fats from the adipose tissue to the blood stream (Chajek-Shaul *et al*., 1989), in addition to mitochondrial dysfunction (Madamanchi and Runge, 2007).

In this study, it could be observed that administration EPE before whole body γ-irradiation to rats resulted in significant reduction in the lipid fraction when compared to γ-irradiated rats. Nematalla *et al*. (2011) concluded that administration of aged rats with Echinacea ethanolic and water extracts caused a significant improvement in the levels of lipid profiles (total cholesterol,

Table 4: Effect of EPE administration on TBARS and GSH levels in liver and testes of γ-irradiated rats

Group	TBARS (n mol/g tissue)		GSH(mg/g tissue)	
	Liver	Testes	Liver	Testes
Control	164.21 ± 3.12[a]	132.82± 4.25[a]	28.66± 2.17[a]	22.15± 1.68[a]
EPE	159.65 ± 3.72[a]	130.11± 3.63[a]	29.42± 1.66[a]	22.78± 1.35[a]
Irr.	229.27 ± 4.68[c]	187.35± 5.42[c]	17.22 ± 1.48[c]	13.64± 1.73 [b]
EPE +Irr.	180.24 ± 3.81[b]	155.56± 4.55[b]	24.63± 2.17[b]	20.85± 1.82[a]

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

Table 5: Effect of EPE administration on the activity of SOD and CAT in liver and testes of γ-irradiated rats

Group	SOD (U/mg protein)		CAT(U/mg protein)	
	Liver	Testes	Liver	Testes
Control	47.11 ± 3.55[a]	21.06± 1.25[a]	49.63 ± 1.47[a]	34.17± 1.21[a]
EPE	49.62 ± 2.61[a]	21.68± 1.36[a]	50.06± 1.33[a]	34.62± 1.83[a]
Irr.	29.23 ± 1.57[b]	12.89± 0.86[c]	33.57 ± 1.28[c]	18.52± 1.86[c]
EPE +Irr.	46.58± 3.06[a]	18.85± 1.37 [b]	41.36 ± 1.54[b]	28.37± 1.54[b]

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

LDL and VLDL) and significantly increased the level of HDL-C significantly compared with control aged rats.

The data from the present work revealed that the activity of liver enzymes (AST, ALT, ALP and gGT) significantly increased after whole body γ-irradiation when compared with the control. Some investigators have reported significant elevation in the activity of liver enzymes after γ-irradiation (Makhlouf and Makhlouf, 2012).The serum transaminases levels are common markers for hepatic toxicity; levels of these proteins were rapidly increased when the liver is damaged by any cause, including hepatitis or hepatic cirrhosis (Abdelhalim and Moussa, 2013). The increase in the serum aminotransferase activities could be due to liver damage induced by free radicals generated after radiation exposure (Jirtle *et al*., 1990 and Abdelhalim and Moussa, 2013). El-Khafif *et al*. (2003) ascribed the elevated serum liver enzymes to the cellular membrane-gamma ray interaction leading to increment hepatic cell membranes permeability. Furthermore, the increased serum enzymatic activities may also be referred to the damage of liver parenchymal cells and extrahepatic tissues caused by irradiation, followed by a release of intracellular enzymes into the circulation.

On the other hand, significant improvement in the liver functions were observed in group of rats received EPE prior to γ-irradiation relative to γ-irradiated group. This improvement might be reflected the ability of EPE to stopping the damage effect induced by γ-radiation in the cellular tissues of the liver. Bayramoglu *et al*. (2011) investigated the effect of echinacea on kidney and liver after experimental renal Ischemia / reperfusion injury in the rats. They showed that *E. Purpurea* can decrease the concentrations of dif-ferent liver enzymes and histopathologic changes such as inflammatory cell infiltration, necrosis, damage in hepatic cords and loss of intercellular border in liver. Ali (2008) reported that Echinacea extract has pro-tective effects on the liver against cyproterone acetate and mentioned antioxidant properties of *E. purpurea* induced these effects.

In the present work, whole body g-irradiation (6 Gy) induced a decrease in the serum levels of testosterone and LH with a significant increment in FSH level.

The elevation in FSH level is in agreement with that found by Hermann, *et al*.(2005) and Ahmed,(2006). This elevation in FSH might be attributed to inhibin: a peptide hormone produced by testicular tubules and acts by negative feedback mechanism to modulate the secretion of FSH by pituitary gland. The inhibin production mechanism appeared to have been disturbed by irradiation leading to increase in FSH level (Green and Harris, 1978).

Liu *et al*. (2009) 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. Furthermore, Ahmed and Abdel-Mageid (2011) recorded that rats exposed to 7 Gy of gamma irradiation showed a significant decrease in testosterone level, which could be attributed to hypothalamic and pituitary gland dysfunction, or to production of free radicals and increased lipid peroxidation in testicular tissue which attack the testicular parenchyma causing damage to seminiferous tubules and Leydig cells.

In the present study, group of rats given EPE before exposure to γ-rays exhibited higher levels of serum testosterone and LH and lower level of FSH than the their corresponding value in γ-irradiated group. The effect of EPE against damage induced by γ-radiation exposure in testes could be attributed to its phenolic contents that have antioxidant activity. Oi-Kano *et al*. (2013) proposed the mechanism of phenolic compounds supplementation enhances lipid and protein metabolism owing to hormonal regulation by the stimulation of noradrenalin secretion, thereby affecting the levels of steroid hormones, including testosterone and corticosterone, and other hormones in rats. The present study demonstrated that exposure to whole body γ- radiation induced oxidative stress in hepatic and testicular tissues of rats. The increased concentration of thiobarbituric acid reactive substances (TBARS) rat liver and testes, indicating high level of oxidative stress, markedly enhanced with increasing radiation dose (Makhlouf and Makhlouf, 2012); similar observations were reported on radiation-induced oxidative damage in several organs (Bhatia and Manda, 2004). The depletion in GSH in liver and testes after exposure of rats to γ-radiation may be due to reaction of GSH with free radicals resulting in the formation of thiol radicals that associate to produce GSSG. Further, normal synthesis/repair of GSH will be impaired due to damage to DNA and membranes (Navarro *et al*., 1999). In addition,the observed decrease in SOD activity following gamma radiation exposure suggests increased production of superoxide radicals. Farombi *et al*. (2010) suggested that superoxide radicals by themselves, or after their formation to H_2O_2, caused oxidation of CAT and GSH-Px enzymes and thus decrease SOD activity.Furthermore, the recorded reduction in the enzymatic activity of CAT in this study may be due to the increased utilization of this antioxidant to counteract lipid peroxidation production (Kalpana and Menon, 2004).

Administration of EPE to rats before exposure to g-rays obviously declined the level of TBARs with a significant elevation in GSH level and the activity of SOD and CAT in the liver and testes in comparison to γ-irradiated rats. This could be attributed to the presence of echinacoside and caffeic acid in *E. purpurea* which are potent scavengers of free radicals such as hydroxyl radicals produced by irradiation reducing cellular injury and preventing cellular

membrane destruction by oxidation (Mishima *et al*., 2004). Weiss and Landauer (2003) related the antioxidant protective effects of *E. purpurea* to the presence of polyphenols and a class of specific antioxidants known as caffeoyl derivatives.

CONCLUSION

On the basis of the data obtained in the present study it could be concluded that EPE exhibits a significant potential effects against γ-irradiation through modulation of disturbance induced in some biochemical constituents as well as protection against oxidative damage in hepatic and testicular tissues in male rats. Thus supplementation with EPE may have a benefit for safe application of radiation technology in medicine and industry.

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