Studying the antioxidant role of mushroom against hazards of gamma radiation exposure in male rats

R.G. Hamza*1, A.N. El Shahat1 and M.N. Al-seeni2

Food Irradiation Research Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.
Received: 21-10-2016 Accepted: 18-12-2017 DOI: 10.18805/ijar.B-387

ABSTRACT
This study aimed to investigate the antioxidant role of dried mushroom (DM) against hazards of gamma-irradiation in male rats. In this study, exposure of rats to whole body γ-radiation (6 Gy) resulted in hepatic oxidative stress (a significant increase in lipid peroxidation concomitant with a significant decrease in glutathione content and antioxidant enzyme activities); increase liver function enzymes and histopathological disorders. Treatment of irradiated rats with 10% DM significantly improved radiation-induced injury as indicated by the reduction of the indices of liver damage, lipid peroxidation product, the elevation of antioxidants and the attenuation of the tissues histological architecture. These results suggest that oyster mushroom can improve the antioxidant status and minimize the occurrence of oxidative stress-associated disorders.

Key words: Antioxidants, Gamma-radiation, Mushroom, Oxidative stress.

INTRODUCTION
Exposure to ionizing radiations can cause over production of reactive oxygen species (ROS) that interact with biological systems and induced oxidative stress (Acharya et al., 2011). Uncontrolled production of ROS results in tissue injury that cause change in the structure and permeability of cellular components leading to cellular damage and organ dysfunction (Abdelhalim and Moussa, 2013). Treatment of oxidative stress by using synthetic antioxidants can cause carcinogenicity and hepatotoxicity (Singh et al., 2014). Therefore, the modern medicine tends to use foods that contain phenolic and flavonoid compounds to protect against oxidative stress induced several diseases (Pal et al., 2010).

The biological functions of phenolic contents and flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Some flavonoids provide stress protection, for example, acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction (Williams et al., 2004).

Among different natural sources edible mushrooms are now becoming more attractive because of its strong nutritional value and therapeutic potentiality (Devi et al., 2014). Mushrooms are unlimited source of therapeutically useful and biologically active agents like phenolics, tocopherol, lycopene, β-carotene and are rich in proteins and certain essential amino acids. Compounds of mushrooms have been reported to have antifungal, anti-inflammatory, antibacterial, antiviral, antitumor, hepatoprotective, antidiabetic, antithrombotic, hypotensive, and antioxidant capacity (Punitha and Karan, 2014). The present study aimed to evaluate the biological effect of oyster mushroom (Pleurotus ostreatus) against radiation induced hazards in male rats.

MATERIALS AND METHODS

Oyster mushroom (Pleurotus ostreatus) was purchased from the Food Technology Research Institute, Agricultural Research Center.

Preparation of the dried mushroom (dm): The mushroom P. ostreatus was dried in the shade and then finely powdered. Dried mushroom were ground to pass through a 60 mesh sieve (Gaafar et al., 2010).

Radiation facility: Whole body gamma irradiation at 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 0.45 Gy/min at the time of the experiment.

Experimental animals: Matched weight of adult male albino rats (120-140 g) were reared in National Centre for Radiation Research and Technology animal house and kept under normal laboratory conditions (temperature remain 25 ± 2°C) for 48 hr before the initiation of experiment. During the period, animals were allowed free access of water and basal diet (A.O.A.C. 1971). The control diet is composed of as reported by Lane – Peter and Pearson (1971), 15% casein,
10% corn oil, 5% cellulose, 4% salt mixture (Schneeman et al., 1989), 1% vitamins mixture (Philip et al., 1993) and starch 65%.

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

- **Group C** (Control group) rats received basal diet for 6 weeks.
- **Group DM** (Mushroom group) rats received basal diet mixed with 10% dried mushroom (DM) (El-kholy et al., 2013) for 6 weeks.
- **Group Irr.** (Irradiated group) rats were exposed to γ-irradiation (6 Gy) and received basal diet for 6 weeks.
- **Group Irr. + DM** (Irradiated + Mushroom group) rats were exposed to γ-irradiation (6 Gy) and received basal diet mixed with 10% dried mushroom for 6 weeks.

At the end of the experiment, rats were fasted for 24 hours and anaesthetized with diethyl ether. Blood sample were collected though heart puncture and allowed to coagulate and centrifuged for to obtain serum for biochemical analysis.

**Biochemical analysis:** The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was assayed by the method of Reitman and Frankel (1957). Serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1959).

Moreover, the liver tissues were dissected and divided into two parts. One part for histopathological study and the other part was homogenate in saline solution. Liver homogenates were obtained using a tissue homogenizer. The homogenates (1:10 w/v) were prepared using a 100 mM KCl buffer (pH 7.0) containing EDTA 0.3 mM. All homogenates were centrifuged at 200× g for 20 minutes at 4°C, and the supernatants were used to estimate the level of thiobarbituric acid reactive substances (TBARS) (Yoshioka et al., 1979), glutathione (GSH) (Beutler et al., 1963), and for the assays of superoxide dismutase (SOD) (Minami and Johansson, 1979), xanthine oxidase (XO) and xanthine dehydrogenase (XDH) (Kaminski and Jewezska, 1979) activities. The activity of SOD was determined spectrophotometrically by the method of Lando and Borg (1988), respectively.

**Histopathological examination:** For histopathological study the tissue samples were taken rapidly from each rat, and fixed in 10% formalin. All the samples were dehydrated in ascending grades of ethanol, cleared in butanol and embedded in paraffin. Sections of 5-6 µm thick sections were obtained and stained with the following stains:

1. Haematoxylin and Eosin (H&E) staining for general histological studies.
2. Masson’s Trichrome stain for collagen fibres.

**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**

**Liver Biochemical studies:** The activity of liver enzymes (AST, ALT and ALP) was obviously increased in the serum of gamma-irradiated rats in comparison to control group (Table 1). However, the activity of the three enzymes was significantly reduced in the serum of irradiated rats treated with *P. ostreatus* compared to Group III rats.

In the liver of Group III (Irradiated rats), the level of TBARS and the activity of XO were markedly increased accompanied by significant decrease in the activity of XDH relative to normal rats (Table 2). Treatment of irradiated rats with *P. ostreatus* resulted in a significant lowering of the TBARS concentration XO activity with an obvious increase in XDH activity (Table 2).

Exposure to gamma-radiation in Group III rats resulted in significant decrease (relative to normal) in the level of reduced glutathione and CAT and SOD activity in liver (Table 3). Supplementation with *P. ostreatus* in Group III rats resulted in a significantly higher concentration of

### Table 1: Effect of feeding dried mushroom on the activity of serum AST, ALT and ALP of irradiated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>DM</th>
<th>Irr.</th>
<th>Irr.+dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/ml)</td>
<td>22.16±1.12a</td>
<td>22.16±1.33a</td>
<td>44.75±2.24c</td>
<td>31.82±1.78b</td>
</tr>
<tr>
<td>ALT (U/ml)</td>
<td>18.89±1.24a</td>
<td>18.27±1.62a</td>
<td>38.15±2.26c</td>
<td>23.66±1.85b</td>
</tr>
<tr>
<td>ALP (U/100ml)</td>
<td>8.55±0.91c</td>
<td>8.55±0.91c</td>
<td>14.93±0.82c</td>
<td>10.22±0.76b</td>
</tr>
</tbody>
</table>

*Values in the same row with different superscripts are differing significantly at P<0.05.*

### Table 2: Effect of feeding dried mushroom on TBARS level and xanthine oxidoreductase system (XO and XDH) in the liver of irradiated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>DM</th>
<th>Irr.</th>
<th>Irr.+dn</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (n mol/g tissue)</td>
<td>163.23±3.12a</td>
<td>158.11±5.32a</td>
<td>252.52±5.23c</td>
<td>175.62±6.17b</td>
</tr>
<tr>
<td>XO (mU/mg protein)</td>
<td>2.50±0.08b</td>
<td>2.47±0.07a</td>
<td>3.62±0.09c</td>
<td>2.82±0.07b</td>
</tr>
<tr>
<td>XDH (mU/mg protein)</td>
<td>3.27±0.12a</td>
<td>3.38±0.13a</td>
<td>1.73±0.16b</td>
<td>2.96±0.14a</td>
</tr>
</tbody>
</table>

*Values in the same row with different superscripts are differing significantly at P<0.05.*
Table 3: Effect of feeding dried mushroom on hepatic SOD and CAT activity of irradiated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GSH (mg/g fresh tissue)</td>
<td>53.27± 2.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD (U/ mg protein)</td>
<td>48.66 ± 3.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>16.32 ± 1.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same row with different superscripts are differing significantly at P<0.05.

GSH and activity of CAT and SOD than that in Group II (Table 3).

Liver Histopathological studies: In the histological study, the liver sections of control (Fig. 1A) and DM treated (Fig. 1B) rats display normal hepatic architecture. The central vein is appeared and the cellular architecture is formed of cords composed of hepatocytes, one to two cell layers thick, separated by blood sinusoids. In γ-Irr group (Fig. 1C), liver section showed discontinued the endothelial lining of the central vein and severely dilated blood sinusoids. Most hepatocytes in centrilobular area show focal necrosis and many hepatocytes are swollen and vacuolated (Fig. 1C). However, treatment of the irradiated rats with DM (Irr+DM group) showed sinusoidal dilatation and predicts great amelioration and preservation of the endothelial lining of central vein and ill-defined nuclei of the hepatocytes (Fig. 1 D).

Phenolic compounds are well-known, naturally occurring antioxidants and are believed to be beneficial to human health (Hu, 2011). In the present study, the harmful effect of γ—irradiation was noted in the increase in the activity of the serum enzymes AST, ALT and ALP in irradiated group in comparison to normal rats. Some investigators have reported significant elevation in the activity of liver enzymes (ALT, AST and ALP) after γ—irradiation that could indicate occurrence of liver injury (Makhlouf and Makhlouf, 2012). The damage of cellular membranes of hepatocytes following exposure to ionizing radiation leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in liver and blood serum (Ali et al., 2012).

However, the activity of AST, ALT and ALP was reduced by feeding irradiated rats on diet mixed with 10% dried mushroom (Group III). The results in consistent with the study of Jayakumar et al. (2006) who observed that administration of the extract of P. ostreatus reduces significantly the level of AST, ALT and ALP on carbon tetra chloride induced liver damage in male Wister rats. Also,

![Fig 1: Light micrographs of a rat hepatic tissue of rats (H&E X200).](image-url)

A): Microscopic appearance from the liver tissue of a rat belonged to healthy control group showing normal hepatic structure.
B): Microscopic view from the liver tissue of a rat belonged to rats received basal diet mixed with 10% DM, no deviation from the control.
C): Histological appearance from the liver tissue of γ-irradiated rats (6Gy) showing histopathological disorders.
D): Histological appearance from the liver tissue of γ-irradiated rats received basal diet mixed with 10% DM showing better-preserved appearance of tissues histological architecture comparing to γ-irradiated rats.
(V): Central vein ; (S): Blood sinusoid.
Choudhury et al. (2011) concluded that feeding of semi cooked fresh oyster mushroom decreased the serum levels of ALT and AST of male subjects indicating oyster mushroom is able to ameliorates liver functions. The antioxidant and hepatoprotective activities of oyster mushroom may be due to the presence of flavonoid and phenolic compounds (Punitha and Karan, 2014) that can inhibit nitrosation (Orhan et al., 2007).

Present study showed an obvious increase in TBARS level and in the conversion of XDH into XO activity in the liver of rats after irradiation relative to normal rats. Makhlouf and Makhlouf (2012) obtained that the high level of oxidative stresses resulted in increase in concentration of TBARS in the rat liver. The excess of •OH generated in the cells after exposure to ionizing radiation could interact with polyunsaturated fatty acids in the phospholipids portion of cell membranes initiating the lipid peroxidation chain reaction with obvious increase in the TBARS level (Azab et al., 2011). Study of Srivastava et al. (2002) indicated that ionizing radiation induces the conversion of XDH into XO. The enhanced specific XO activity induces oxidative stress whereby the excess of free radicals interact with various components of the cell and resulted in elevation of oxidative products.

On the other hand, addition of dried P. ostreatus to the diet of γ-irradiated rats showed significant ability to inhibit radiation-induced lipid peroxidation by reducing TBARS formation and the activity of XO with concomitant significant increase in the activity of XDH in rat liver. Study of Lakshmi et al. (2004) has shown that mushroom extracts possess significant radical scavenging properties of both primary and secondary radicals, in a concentration-dependent manner. Alam et al. (2011) concluded that mushroom might be beneficial to the antioxidant, xanthine oxidase, and tyrosinase protection system of the human body against oxidative damage and others complications. The presence of phenolic and flavonoid content in mushroom would have contributed towards xanthine oxidase inhibition. Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity (Costantino et al., 1992).

Radiation also in this work induced significant depletion in GSH concentration and remarkable reduction in the activity of SOD and CAT in the liver of irradiated rats (Group Irr.) compared to control group. Mansour and Hafez (2012) recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after whole body ė-radiation. The depletion in GSH may be attributed to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by irradiation or to the diminished activity of glutathione reductase due to the deficiency of NADPH, which is necessary to change oxidized glutathione to its reduced form (Pulpanova et al., 1982). The observed decrease in SOD activity suggests inactivation of the enzyme possibly due to increased superoxide radical production or an inhibition by the H2O2 as a result of corresponding decrease in the activity of CAT which selectively degrades H2O2 (El shahat, 2013). Moreover, Kregel and Zhang, (2007) attributed the significant decrease in the activity of SOD and CAT might be due to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation. Whereas, dietary supplementation with DM to γ-irradiated rats resulted in obvious increase in the level of hepatic GSH as well as the activity of hepatic SOD and CAT when compared to irradiated group only. The capability of dried mushroom to elevate glutathione content may be attributed to its phenolic contents that possess antioxidant effect.

An increase in the levels of GSH in aged rats treated with mushroom extract as a source of antioxidant has been reported by Jayakumar et al. (2006). Also, Mishra and Singh (2010) reported that supplementation with dried mushroom and their extract can improve the antioxidant status during aging and increase the level of glutathione. Study of Abdelazim and Afifi (2013) concluded that mushroom have the ability to reduce the oxidative stress and lower the level of MDA as an oxidative stress marker and increase the activity of antioxidant enzymes; SOD and CAT in diabetic mice livers.

Moreover, phenolic compounds found in mushroom are composed of one or more aromatic rings which bearing one or more hydroxyl groups and exhibit extensive free radical scavenging activities as hydrogen donors or electron donating agents, and metal ion-chelating properties (Alam et al., 2011). As well as, mushroom fruit are rich with different contents that have strong antioxidant, radical scavenging and other beneficial biological activities like vitamins (B1, B2, C and D2), polysaccharides and glycoproteins (such as chitin, hemicelluloses, β- and α-glucans, mannans, xylans and galactans) (Synytysa et al., 2009).

**CONCLUSION**

From the study it could be concluded that oyster mushroom (P. ostreatus) might be supplemented in basal diet as a natural food containing antioxidant active substances and have the ability to reduce the hazards effect and oxidative damage induced by γ- radiation exposure.

**REFERENCES**


