Ameliorative Effect of Salicin Against Gamma Irradiation Induced Electrophoretic Changes in Brain Tissue in Male Rats

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Abstract

The aim of this study was to investigate the radioprotective effect of salicin against irradiation effect on brain tissue of male rats. Lipid peroxidation level was measured as thiobarbituric acid reactive substance in brain tissue. The polyacrylamide gel electrophoresis for native protein, lipoprotein and zymogram were carried out in brain homogenate. As expected, salicin resisted the irradiation effect and declined the MDA level in brain homogenate of all treated groups (especially in the irradiated salicin post-treated group). Salicin minimized the qualitative mutagenic effect of irradiation on the electrophoretic protein pattern in all irradiated salicin treated groups and it showed the highest antagonistic effect against irradiation in irradiated salicin post-treated group (SI = 0.57). It could not prevent the abnormalities occurred qualitatively and quantitatively as a result of irradiation in lipoprotein pattern in all irradiated salicin treated groups. In the electrophoretic esterase pattern, salicin prevented the qualitative effect of irradiation in irradiated post-treated groups (SI = 1.00). Salicin minimized the qualitative irradiation effect on the catalase pattern in the irradiated salicin pre-treated group (SI = 0.73). While in the peroxidase pattern, salicin administration resisted the irradiation effect in the irradiated post-treated groups (SI = 0.67). The results suggested the radioprotective ability of salicin against gamma irradiation effect on various electrophoretic patterns in brain tissue of male rats.

Keywords: Gamma irradiation, Salicin, Brain, Protein electrophoresis, Isozymes

1 Introduction

The use of this radiation as a source to enhance the mutation frequency was recognized as early as 1930. These rays act either directly or by secondary reactions to produce biochemical lesions that initiate series of physiological symptoms. They are known to induce oxidative stress through the generation of reactive oxygen species (ROS) resulting in imbalance of the prooxidant and antioxidant activities, ultimately resulting in cell death. Studies showed that radiation can affect a wide variety of tissues, particularly those with greater levels of cellular turnover and divisions. Irradiation causes similar damage at a cellular level but gamma rays are more penetrating, causing diffuse damage throughout the body. This type of rays also used for diagnostic purposes in nuclear medicine in imaging techniques.

Irradiation causes damage to living tissue through a series of molecular events. The formation of ROS as a result of interaction of irradiation with cellular macromolecules is the cause of dysfunction and death, in both normal as well as tumor cells exposed to radiation. The energy exchange between the rays and the targeted molecules leads to changes produced in deoxyribonucleic acid (DNA), lipids, and proteins and then cell inactivation.

Gamma irradiation was found to interrupt energy supplies and blocking all key enzymes, which may stop normal metabolism of the exposed tissue. The radiation exposure whether occupational or during radiotherapy leads to serious systemic damage to various cellular and subcellular structures.

Irradiation causes induction of lipid peroxidation as evidenced by increased malondialdehyde (MDA). It causes damage of cells...
directly by ionizing DNA and other cellular targets and indirectly by effect through ROS. It produces oxygen-derived free radicals in tissue environment: these include hydroxyl radicals (the most damaging), superoxide anion radicals and other oxidants such as hydrogen peroxide.

Major radiation damage is due to the aqueous free radicals generated by the water radiolysis. These free radicals act as molecular marauders and in turn damage DNA which is considered to be the primary target. This gives rise to genomic instability leading to mutations, carcinogenesis and cell death.

The most important consequences of OS are lipid peroxidation, protein oxidation and depletion of antioxidants. The latter authors showed that the increase of MDA level is probably due to the interaction of •OH resulting as a by-product of water radiolysis with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes. The excessive free radicals can damage crucial macromolecules, including DNA, cell membranes and enzymes, and can cause cell death. DNA damage includes genotoxicity, chromosomal abnormalities, gene mutations and cell death if the damage is beyond repair.

Catalase (CAT) and glutathione peroxidase (GPx) play an important role in detoxification of hydrogen peroxide and ROS (Kula et al., 2000). The irradiation caused significant increase in markers of the oxidative stress as malondialdehyde (MDA) and alterations in activity of antioxidant enzymes such as CAT and GPx in a dose-dependent manner.

Salicin was discovered in 1831. It was isolated from the willow bark and leaves. It is a prodrug and thus a precursor of salicylic acid. Salicin is a phenolic glycoside. It exhibits analgesic effects, and it is used for the treatment of rheumatic pain. Its occurrence in willow (Salix) species is the major reason willow bark and its extracts are popular products. High-Performance Liquid Chromatography (HPLC) has been the standard method of analysis of quantifying salicin in the different Salix species.

The present experiment was concerned with studying the ability of salicin which represents the most abundant fraction of aqueous willow extract to impress radiation-induced electrophoretic alterations in brain tissue of male rats.

2 Materials and Methods

2.1 Salicin isolation

Fresh young leaves of the willow trees (Salix subserrata, Salix sahsaf) were collected from Orman garden, Giza, Egypt. This species was well authenticated by qualified specialists in plant taxonomy. Salicin was extracted and isolated from fresh leaves according to method described by Mabry et al. and purified according to method by Kamel et al. Ameliorative Effect of Salicin Against Gamma Irradiation suggested by Kur’yanov et al. then identified qualitatively by traditional and advanced chromatographic techniques.

2.2 Acute toxicity test

The lethal dose 50 (LD50) was evaluated on 6 groups of female albino mice (8 animals / group) receiving progressively increasing oral dose levels (500, 1000, 2000, 3000, 4000 and 5000 mg/kg body weight) of aqueous solution of salicin solution. Mortality was recorded 24 hrs post treatment. The LD50 was calculated according to the equation suggested by Paget and Barnes.

2.3 Animals

Seven groups of male rats weighing between 150-200 gm per one obtained from the animal house laboratory of the national research centre. Ten rats in each group. All the animals were kept under normal environmental and nutritional conditions. The animal groups were divided into Control group: Rats were non-irradiated and non-treated with salicin. Salicin treated group: Rats were non-irradiated but treated with the safe dose of salicin which was about 150 mg / Kg taking in the consideration weight of each rat. Irradiated group: Rats were irradiated at the dose 7 Gy and non-treated with salicin. Irradiated salicin pre-treated group: Rats were treated with salicin for 15 days followed by irradiation at the 15th day. Irradiated salicin pre-post-treated group: Rats were treated with salicin for 15 days followed by irradiation at the 15th day then the treatment was continued daily for another 15 days. Irradiated salicin simultaneous treated group: Rats were irradiated and treated with salicin at the same time of irradiation and continue daily for 15 days and Irradiated salicin post-treated group: Rats were irradiated at the same gamma dose then left without treatment for 15 days. At the 15th day, the rats were treated with salicin for another 15 days.

2.4 Irradiation

Whole body was gamma irradiated at Middle Eastern Regional Radioisotope Centre for the Arab Countries, Dokki, Egypt. Using Cobalt 60 (Co 60) as a suitable gamma source. Rats were irradiated at a single dose of 7 Gy delivered at the dose rate of 1.167 Rad / Sec.

2.5 Biochemical Assay

Lipid peroxidation level was measured as thiobarbituric acid reactive substance in brain homogenate according to method of Ohkawa et al.

2.6 Electrophoretic protein pattern

Total protein was determined in brain homogenate according to Bradford. The sample was mixed with the sample buffer. The protein concentration in each well must be about 70 μg protein. Proteins were separated through polyacrylamide gel electrophoresis.
(PAGE) with different concentrations. Electrode and gel buffer and polyacrylamide stock were prepared according to Laemmli\textsuperscript{31}. After electrophoretic separation, the gel was gently removed from the apparatus and put into a staining solution of coomassie brilliant blue for native protein pattern\textsuperscript{32} and staining solution of sudan black B (SBB) for lipoprotein pattern\textsuperscript{33}.

2.7 Electrophoretic isozyme

Native protein gel was stained for peroxidase pattern using certain stain prepared according to the method suggested by Rescigno et al\textsuperscript{34}. It was stained for catalase pattern according to the method described by Siciliano and Shaw\textsuperscript{35}. For esterase pattern, the native gel was stained according to the method suggested by Baker and Manwell\textsuperscript{36}.

2.8 Data analysis

The polyacrylamide gel plate was photographed, scanned and then analyzed using Phoretix 1D pro software (Version 12.3). The similarity index (S.I.) compares patterns within, as well as, between irradiated and non-irradiated samples. The similarity values were converted into genetic distance (GD) according the method suggested by Nei and Li\textsuperscript{37}.

2.9 Statistical analysis

All the grouped data were statistically evaluated with SPSS/16.00 software. The results were expressed as mean ± SE of studied groups using the analysis of variance test (one-way ANOVA) followed by student’s t-test\textsuperscript{34}. P values of less than 0.05 were considered to indicate statistical significance. The means of irradiated groups and the salicin treated groups were individually compared with those of control group. The irradiated group was compared with irradiated salicin treated groups

3 Results and Discussions

3.1 Lipid peroxidation

As compared to control, irradiation caused the severe increase in the lipid peroxidation product (MDA) level in the brain tissue. Salicin administration showed the ameliorative effect against irradiation by reducing MDA level in all irradiated salicin treated rats. From the data compiled in table 1, it was found that salicin showed the most suitable antagonistic effect against irradiation on brain of irradiated salicin post-treated group.

3.2 Electrophoretic protein pattern

Protein pattern in the control sample produced 9 bands with $R_n$ ranged between 0.09 – 0.98 (Mwts 5.21 – 212.46 KDa and B % values 0.20 – 25.09). There were no common bands but there were 2 characteristic bands appeared in irradiated salicin pre-treated Kamel et al. Ameliorative Effect of Salicin Against Gamma Irradiation group with $R_i$ 0.56 (Mwt 20.09 KDa and B % 21.13) and in irradiated salicin simultaneous treated group with $R_i$ 0.75 (Mwt 14.58 KDa and B % 10.85).

As shown in table 2 and illustrated in fig. 1, irradiation caused disappearance of 6 normal bands without appearance of abnormal bands. The 3\textsuperscript{rd} and 5\textsuperscript{th} bands might be deviated to be appeared with $R_i$ 0.22 and 0.43 (Mwts 113.48 and 30.53 KDa and B % 17.28 and 80.91). It caused quantitative mutation represented by increasing B % of the 9\textsuperscript{th} band ($R_i$ 0.98, Mwt 5.02 KDa and B % 1.81). Salicin administration resisted the qualitative mutagenic effect of irradiation in the irradiated salicin simultaneous treated and post-treated groups. It retained 4 normal bands with $R_n$ ranged between $R_i$ 0.23 - 0.98 (Mwts 5.08 - 112.21 KDa and B % 0.20 - 15.81) in irradiated salicin simultaneous treated group and with $R_i$ 0.45 - 0.99 (Mwts 4.75 - 27.06 KDa and B % 0.59 - 56.41) in irradiated salicin post-treated group. It was probable that the 5\textsuperscript{th} band was deviated to be appeared with $R_i$ 0.45 (Mwt 28.20 KDa and B % 37.70) in irradiated salicin simultaneous treated group. It could not prevent the quantitative mutation which was represented by increasing B % of the 3\textsuperscript{rd} and 7\textsuperscript{th} bands ($R_n$ 0.23 and 0.85, Mwts 112.21 and 10.65 KDa and B % 15.81 and 14.00) and by decreasing B % of the 6\textsuperscript{th} band ($R_i$ 0.63, Mwt 18.00 KDa and B% 11.99) in irradiated salicin simultaneous treated group. Also, it could not prevent the quantitative mutation which was represented by increasing B % of the 5\textsuperscript{th}, 6\textsuperscript{th}, 7\textsuperscript{th} and 9\textsuperscript{th} bands ($R_n$ 0.45, 0.65, 0.86 and 0.99 (Mwts 27.06, 17.68, 10.38 and 4.75 KDa and B % values 56.41, 0.59, 34.49 and 1.54) respectively.

It was found that the lowest SI value (SI = 0.17) was recorded with irradiated group and the highest SI value (SI = 0.70) with salicin treated group. Salicin improved the SI values in all irradiated salicin treated groups, and it showed the highest antagonistic effect against irradiation in irradiated salicin post-treated group (SI = 0.57). The overall results showed that salicin prevented the irradiation effect on number and arrangement of the bands in all irradiated salicin treated groups.

3.3 Electrophoretic lipoprotein pattern

As revealed in table 3 and illustrated in fig. 2, lipoprotein pattern in the control sample produced 3 bands with $R_n$ 0.22, 0.47 and 0.69 (B % 67.28, 18.91 and 13.82). There were no common bands appeared in all groups. Salicin alone caused severe qualitative alterations represented by disappearance of the normal bands with appearance of 3 abnormal bands with $R_i$ 0.25, 0.33 and 0.72 (B % 48.27, 31.12 and 20.61).

Irradiation caused severe abnormalities represented disappearance of all the normal bands without appearance of abnormal bands in the irradiated, irradiated salicin simultaneous treated and post-treated groups. Salicin administration could not prevent the irradiation effect.
which was represented qualitatively by disappearance of 2 normal bands and quantitatively by increasing B % of the normal band appeared with Rf 0.22 and B % 100.00 in the irradiated salicin pre-treated group and by disappearance of 2 normal bands with appearance of one abnormal band with Rf 0.35 (B % 29.73) in the irradiated salicin prepost-treated group. It was observed that all the bands were not matched with all bands of the other groups in the salicin treated, irradiated, irradiated salicin simultaneous treated and post-treated groups.

Table 1: Effect of irradiation, salicin and their combination in various treatment modes on the level of lipid peroxidation in spleen tissue of male rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Sal.</th>
<th>Irr.</th>
<th>Irradiated salicin treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre-treated</td>
</tr>
<tr>
<td>Brain</td>
<td>162.02</td>
<td>137.84a</td>
<td>239.15a</td>
<td>232.90a</td>
</tr>
<tr>
<td>(nmol/g)</td>
<td>± 2.28</td>
<td>± 2.17</td>
<td>± 6.55</td>
<td>± 6.24</td>
</tr>
</tbody>
</table>

a : Different from control at P < 0.05, b : Different from the irradiated group at P < 0.05.

Note : Sal. : salicin, Irr. : Irradiated

3.4 Electrophoretic esterase pattern

The electrophoretic esterase pattern in control of brain tissue produced 3 types with Rf 0.22, 0.48 and 0.64 (B % 36.85, 45.38 and 17.77). As shown in table 4 and illustrated in fig. 3, there were no common bands in all groups. Salicin alone caused qualitative mutation represented by appearance of one abnormal characteristic band with Rf 0.11 (B % 21.93) and decreasing B % of the 2nd normal type (Rf 0.49 and B % 19.00). Irradiation caused disturbances represented qualitatively by disappearance of 2 normal types of the enzyme and quantitatively by increasing B % of the 1st normal band (Rf 0.21 and B % 100.00).

Salicin administration prevented the qualitative or quantitative alterations occurred as a result of irradiation in the irradiated salicin post-treated group. It could not prevent the irradiation effect which was represented qualitatively by deviation of the 3rd type to be appeared with Rf 0.60 (B % 23.33) and quantitatively by increasing B % of the 1st band (Rf 0.21 and B % 51.38) and decreasing B % of the 2nd type (Rf 0.47 and B % 25.29) in the irradiated salicin pre-treated group, represented by disappearance of the 3rd type with deviation of the 1st and 2nd normal types of the enzyme to be appeared with Rf 0.23 and 0.48 (B % 55.23 and 44.77) in the irradiated salicin simultaneous treated group and represented by deviation of the 2nd and 3rd normal types of the enzyme to be appeared with Rf 0.45 and

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0.62 (B % 38.34 and 21.20) in the irradiated salicin prepost-treated group.

Table 3: Data of the electrophoretic lipoprotein pattern in brain tissue of control, irradiated and irradiated salicin treated groups in male rats

<table>
<thead>
<tr>
<th>Control</th>
<th>Salicin</th>
<th>Irradiated</th>
<th>Irradiated salicin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-treated</td>
<td>Simultaneous</td>
</tr>
<tr>
<td>Rf.</td>
<td>B. %</td>
<td>Rf.</td>
<td>B. %</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0.22</td>
<td>67.28</td>
<td>0.25</td>
<td>48.27</td>
</tr>
<tr>
<td>0.47</td>
<td>18.91</td>
<td>0.33</td>
<td>31.12</td>
</tr>
<tr>
<td>0.69</td>
<td>13.82</td>
<td>0.72</td>
<td>20.61</td>
</tr>
</tbody>
</table>

Rf.: Rate of Flow, B. %: Band Percent

Table 4: Data of the electrophoretic esterase pattern in brain tissue of control, irradiated and irradiated salicin treated groups in male rats

<table>
<thead>
<tr>
<th>Control</th>
<th>Salicin</th>
<th>Irradiated</th>
<th>Irradiated salicin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-treated</td>
<td>Simultaneous</td>
</tr>
<tr>
<td>Rf.</td>
<td>B. %</td>
<td>Rf.</td>
<td>B. %</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0.22</td>
<td>36.85</td>
<td>0.11</td>
<td>21.93</td>
</tr>
<tr>
<td>0.48</td>
<td>45.38</td>
<td>0.22</td>
<td>40.13</td>
</tr>
<tr>
<td>0.64</td>
<td>17.77</td>
<td>0.49</td>
<td>19.00</td>
</tr>
</tbody>
</table>

Rf.: Rate of Flow, B. %: Band Percent
It was found that the highest SI value (SI = 1.00) was noticed in irradiated salicin post-treated group. In the irradiated salicin simultaneous treated group, it was observed that all the bands were not matched with all bands of the other groups.

**Fig. 3:** Electrophoretic pattern showing effect of salicin against the irradiation effect on esterase pattern in brain tissue of male rats

### 3.5 Electrophoretic catalase pattern

As shown in table 5 and illustrated in fig. 4, 5 types of catalase enzyme were produced in the control sample with $R_0$ ranged between 0.21 - 0.97 (B % 8.11 – 47.12). There were 2 common bands appeared in all the groups with $R_0$ 0.45 and 0.97 (B % 8.47 and 15.17). Salicin alone caused alterations represented by appearance of one abnormal band with $R_i$ 0.18 (B % 23.47) with decreasing the B % of the 1st type ($R_i$ 0.22 and B % 6.12) and increasing the B % of the 2nd type ($R_i$ 0.44 and B % 25.69). Irradiation caused qualitative alterations represented by disappearance of the 1st type of the enzyme with deviation of the 3rd band to be appeared with $R_i$ 0.78 (B % 15.01) and quantitative mutation occurred by increasing B % of the 2nd band ($R_i$ 0.45 and B % 64.42).

Salicin administration could not prevent the irradiation effect which was represented by alteration occurred qualitatively by appearance of one abnormal band with $R_i$ 0.15 (B % 29.71) with deviation of the 3rd type of the enzyme to be appeared with $R_i$ 0.82 (B % 28.32) and quantitatively by decreasing B % of the band appeared with $R_i$ 0.22 (B % 9.44) in the irradiated salicin pre-treated group, by disappearance of the 1st and 4th types of the enzyme with appearance of one abnormal band with $R_i$ 0.17 (B % 31.52) and deviation of the 3rd type to be appeared with $R_i$ 0.82 (B % 38.60) in the irradiated salicin prepost-treated group and represented by disappearance of the 1st type with appearance of one abnormal band with $R_i$ 0.12 (B % 64.42). Salicin treatment minimized the qualitative irradiation effect in the irradiated salicin pre-treated group (SI = 0.73) more than the other irradiated salicin treated groups.

### 3.6 Electrophoretic peroxidase pattern

As shown in table 6 and illustrated in fig. 5, it was found that 5 types of peroxidase enzyme were produced in the control sample with $R_0$ ranged between 0.28 - 0.72 (B % 0.46 - 41.66). There were no common band appeared in all groups. Salicin alone caused no obvious qualitative or quantitative alterations. Irradiation caused severe disturbances represented qualitatively by disappearance of the 2nd, 3rd and 5th normal types of the enzyme and quantitatively by increasing B % of the 1st type ($R_i$ 0.28 and B % 58.28). Salicin could not remove the abnormalities which were represented qualitatively by disappearance of the 1st, 3rd and 5th types with increasing B % of the 2nd and 4th normal types of the enzyme ($R_0$ 0.35 and 0.58 and B % 34.34 and 65.66) in irradiated salicin pre-treated group and represented qualitatively by disappearance of the 3rd type of the enzyme with appearance of one abnormal characteristic band with $R_i$ 0.69 (B % 44.83) and quantitatively by decreasing the B % of the 1st type ($R_i$ 0.27 and B % 14.08) and increasing B % of the 2nd and 4th types of the enzyme ($R_0$ 0.38 and 0.69 and B % 25.82 and 44.83) in irradiated salicin prepost-treated group. In the irradiated salicin post-treated group, salicin could not prevent the mutagenic effect which was represented qualitatively by disappearance of the 5th type and deviation of the 4th type to be appeared with $R_i$ 0.56 (B % 61.82) and quantitatively by decreasing B % of the 1st type appeared with $R_i$ 0.27 and 14.47.

From the SI values, it was found that salicin minimized the irradiation effect on the band number and arrangement in the irradiated salicin prepost-treated groups (SI = 0.44) and post-treated groups (SI = 0.67). In the irradiated pre-treated group, it was observed that all the bands were not matched with all bands of the other groups. It showed the highest antagonistic effect in the irradiated salicin post-treated group.

### 4 Discussions

Kamel et al. Ameliorative Effect of Salicin Against Gamma Irradiation 20.99 deviation of the 3rd type to be appeared with $R_i$ 0.83 (B % 19.39) in the irradiated salicin simultaneous treated group. While in the irradiated salicin post-treated group, salicin could not overcome the mutation represented qualitatively by disappearance of 3 normal types with appearance of one abnormal band with $R_i$ 0.66 (B % 57.05) and quantitatively by increasing B % of the bands appeared with $R_0$ 0.45 and 0.96 (B % 20.65 and 22.30).

From the SI values, it was found that the lowest SI value (SI = 0.44) was observed with irradiated salicin prepost-treated group and the highest value (SI = 0.91) observed with salicin treated group. Salicin treatment minimized the qualitative irradiation effect in the irradiated salicin pre-treated group (SI = 0.73) more than the other irradiated salicin treated groups.
During results of the present study, the MDA level elevated significantly as a result of radiation exposure in the brain tissue. This was in accordance with the results obtained by Saada and Azab\(^3\) that showed that the MDA level increased due to production of ROS associated with the increase in lipid peroxidation. ROS are known to attack the highly unsaturated fatty acids of the cell membrane to induce peroxidation reactions, which considered a key process in many pathological events and is one of the reactions induced by oxidative stress\(^4\). The increase in intracellular ROS concentration leads subsequently to oxidative stress\(^4\) and decrease in activity of antioxidant enzymes with possible damage of cellular membranes\(^4\).

The present study showed that irradiation increased the MDA level. This was in agreement with Dixit et al\(^4\) who showed that the doses 2, 6 and 10 Gy of irradiation enhanced the MDA level. This may be due to reducing the antioxidant enzymes as superoxide dismutase, catalase and glutathione-S-transferase.

It was demonstrated that brain belonged to the most biosensitive organs to low doses of irradiation in rats\(^4\). The oxidative stress affects some brain regions more than others\(^5\).

Irradiation induced lesions tend to occur more frequently in the cerebral brain white matter. It caused necrosis in the cortex and subcortical area in the brain\(^6\). This because white matter tissue is more affected than gray matter tissue\(^7\). This means that the brain represents one of the most important targets of irradiation. So, it was selected to be under study during the current experiment.

The elevation of the levels of antioxidant enzymes in the irradiated animals when compared with the control suggests that these enzymes were up regulated to respond to the toxicity induced by the radiation\(^8\).

Proteins are the most complex compounds and at the same time the most characteristic of living matter. They are present in all viable cells; they are the compounds which, as nucleoproteins, are essential for cell division and, as enzymes and hormones, control many chemical reactions in the metabolism of cells. Thus, the separation and characterization of the individual proteins facilitate the study of the chemical nature and physiological function of each protein\(^9\).

Changes in the protein patterns of the tissues may reflect specialization and adaptation in the organisms. It is worthy to note that each protein is considered as reflect to the activity of specific gene through the production of enzyme, which act as a catalyst to produce the demanded protein; this type of produced protein is responsible for a specific biological character\(^10\). Proteins are major targets for oxidative damage due to their abundance and rapid rates of reaction with a wide range of radicals and excited state species\(^11\).

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It is worthy to note that each protein type has a biological role, due to this role, the DNA secrets enzymes which act as catalysts to produce the specific type of protein. Oxidative protein damage could also affect the activity of DNA repair enzymes. Another possible mutagenic effect of ROS involves their attack on lipids, to initiate lipid peroxidation. The peroxides can decompose to a range of mutagenic carbonyl products\(^12\).

The proteins in brains of all rats were separated electrophoretically and abundances were measured by using image analysis technique. Data in the present study indicated that specific protein bands in tissues of the irradiated rats differed (through disappearance in some protein bands or appearance of new ones). Disappearance of some protein bands in treated rats may be attributed to the effects of irradiation, which inhibits the synthesis and expression process of these deleted proteins (qualitative effect). In addition, even the band remained after irradiation, it usually differs in the amount of protein, and this may be explained by that irradiation could not inhibit the synthesis of this protein type, but it may be affected only on the quantitative level.

The representative electrophoretic profile of the proteins was carried out in serum and different tissue homogenates. This profile showed different types of mutations occurred as a result of radiation exposure. As compared to control, some normal proteins were represented by the fastest migrating band and had the highest staining intensity. There were some proteins showed the intermediate bands and the slowest migrating bands. On the other hand, there was type of proteins represented as the biggest protein band close to the well comb (origin). All these proteins differ from each other in the mobility, molecular weight, optical density and band intensity of each protein band.

In the present study, the similarity index between the control and all the irradiated samples and between the irradiated samples themselves recorded low values, indicating to apparent effect of the irradiation and the differences in the protein pattern. It was stated by many previous studies that the irradiation created a great genetic distance between the control and the irradiated samples that may be due to the activation of some genes. These genes produce different types of proteins not produced in the control. These protein types may lead to variation of the different biological processes.

The current study showed that there were different mutations was detected by the appearance of new proteins or by the quantitative decrease in abundance of normally occurring proteins. This was in agreement with the results reported by Giometti et al\(^13\) who reported that the electrophoresis can be used to detect the mutations reflected as quantitative changes in the protein expression.

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Table 5: Data of the electrophoretic Catalase pattern in brain tissue of control, irradiated and irradiated salicin treated groups in male rats

<table>
<thead>
<tr>
<th>Control</th>
<th>Salicin</th>
<th>Irradiated</th>
<th>Irradiated salicin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-treated</td>
</tr>
<tr>
<td>Rf.</td>
<td>B. %</td>
<td>Rf.</td>
<td>B. %</td>
</tr>
<tr>
<td>0.21</td>
<td>47.12</td>
<td>0.18</td>
<td>23.47</td>
</tr>
<tr>
<td>0.45</td>
<td>8.47</td>
<td>0.22</td>
<td>15.01</td>
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<td>0.85</td>
<td>21.13</td>
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<td>11.17</td>
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<td>26.36</td>
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<td>0.97</td>
<td>16.17</td>
<td>0.93</td>
<td>7.04</td>
</tr>
<tr>
<td>—</td>
<td>0.97</td>
<td>11.12</td>
<td>—</td>
</tr>
</tbody>
</table>

Rf.: Rate of Flow, B. %: Band Percent

Table 6: Data of the electrophoretic peroxidase pattern in brain tissue of control, irradiated and irradiated salicin treated groups in male rats

<table>
<thead>
<tr>
<th>Control</th>
<th>Salicin</th>
<th>Irradiated</th>
<th>Irradiated salicin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre-treated</td>
</tr>
<tr>
<td>Rf.</td>
<td>B. %</td>
<td>Rf.</td>
<td>B. %</td>
</tr>
<tr>
<td>0.28</td>
<td>35.62</td>
<td>0.27</td>
<td>35.91</td>
</tr>
<tr>
<td>0.36</td>
<td>12.62</td>
<td>0.37</td>
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</tr>
<tr>
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<td>0.46</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td>0.72</td>
<td>41.66</td>
<td>0.72</td>
<td>40.44</td>
</tr>
</tbody>
</table>

Rf.: Rate of Flow, B. %: Band Percent

Fig. 4: Electrophoretic pattern showing effect of salicin against the irradiation effect on catalase pattern in brain tissue of male rats

Fig. 5: Electrophoretic pattern showing effect of salicin against the irradiation effect on peroxidase in brain tissue of male rats

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During the current experiment, irradiation-dependent accumulation of oxidatively modified proteins was studied in the brain tissues. Alterations in the protein increased as a result of irradiation. This may be related to an extensive modification of lysine and arginine residues in histone molecules. The authors observed activation of histone-specific proteases in the nuclei of γ-irradiated rats. The lack of carbonyl accumulation in the nuclear proteins isolated from tissues of γ-irradiated animals may be explained by the degradation of oxidized histones by these proteases.

All lipoproteins carry all types of lipid, but in different proportions, so that the density is directly proportional to the protein content and inversely proportional to the lipid content. The lipoproteins were more susceptible to oxidative modifications resulting in small lipoproteins. The ROS can initiate one-electron oxidation or one-electron reduction reactions on numerous biological systems. The oxidative hypothesis classically admits the involvement of the lipoproteins oxidation radiolytically. There was natural binding between protein and lipoproteins in the rat tissues. These two tissues known to be involved in the processing of the lipoproteins. The lipoproteins-binding protein has previously been identified in adrenal cortical plasma membranes and concentration of the binding protein was strongest in kidneys. So the alterations in the protein pattern were associated with altering the lipoprotein pattern in these tissues.

The alterations in the lipoprotein pattern may refer to the disturbances in the cholesteryl esterase required or cholesterol hydrolysis. It was suggested that non-parenchymal liver cells possess the enzymic equipment (cholesteryl esterase) to hydrolyze very efficiently internalized cholesterol esters and this supported that these cell types are an important site for lipoprotein catabolism.

Salicin administration showed the protective effect against the irradiation. This may be due to its antioxidative effect against attack of the free radicals. It prevented the alterations in the proteins and hence the lipoproteins fractions in the brain tissues.

In the current experiment, polyacrylamide gel electrophoresis was used for separation of different enzymes, which help in explanation of different biological processes that occur inside the living organisms due to the deleterious effect of irradiation and the ameliorated effect of salicin.

According to the present data, the activities of CAT and GPx in brains of irradiated rats were altered. These results strongly suggested that irradiation has the capability to induce free radicals and oxidative damage as evidenced by perturbations in various antioxidant enzymes. Depletion of these enzymes activity could be due to the direct effect on the enzymes by irradiation-induced ROS generation, direct inhibition of the enzymes by irradiation.

Kamel et al. Ameliorative Effect of Salicin Against Gamma Irradiation
The present work evaluated the enzymatic activity of the AO enzymes enzymes as CAT and GPx and electrophoretic detection of oxidative damage in proteins (carbonyl groups) in brains of rats. This was in agreement with Ehrenbrink et al. The authors suggested that the patterns of activity and accumulation of damages can be sex-specific and related to the cycle of reproductive life of the rats.

Esterases with active thiol groups detected electrophoretically in brain of different animal species. It exhibits wide distribution among these species. The brain was selected to show the esterase activity because it is rich in esterase enzyme due to its role in the neurotransmission and communication of messages.

Electrophoresis plays a major role in identifying esterases. So, this technique was selected to identify the esterase types in male and female reproductive organs. The present study showed that irradiation caused alterations in the electrophoretic esterase pattern representing great differences in a number of zones of esterase activity and in substrate specificity between all treated groups compared to control.

During the current experiment, irradiation caused alterations in the electrophoretic esterase pattern. This may refer to effect of irradiation on the protein pattern. As regards changes in electrophoretic mobility demonstrated in the present study, it seemed that free radicals affect the integrity of the polypeptide chain in the protein molecule causing fragmentation of the polypeptide chain due to sulfhydryl-mediated cross linking of the labile amino acids as claimed by Bedwell et al. The changes in the fractional activity of different isoenzymes seemed to be correlated with changes in the rate of protein expression secondary to DNA damage initiated by free radicals.

During the current experiment, salicin administration showed the highest ameliorative effect against irradiation in the irradiated salicin treated rats. This may be due to trapping of these free radicals by salicin, thus preventing DNA damage. Salicin and salicylic acid belonged to the phenolic compounds which showed free radicals due to their ability to scavenge free radicals. So, they are able to overcome the disturbances in the esterase pattern in the tissues selected to be under study.

Salicin belonged to the phenolic glycosides which are characterized by their antioxidant activity in biological systems. The antioxidant activity of the phenolic compounds refers to their ability to scavenge free radicals. The authors suggested that the phenolic molecules undergo redox reactions because phenolic hydroxyl groups readily donate hydrogen to reducing agents. Ronca et al. showed that antioxidants suppressed hydroxyl radical production in the fenton reaction, probably by chelating the iron required in the generation of hydroxyl radicals. Results of the present study were in agreement.
with that reported by Das et al. who confirmed that elevation of MDA concentration might be a consequence of decreased production of antioxidants in the irradiated rats’ tissues. The antioxidant activities of salicin fraction varied markedly. This may be due to the differences in structures of phenolic compounds and primarily related to their hydroxylation and methylation patterns. The total antioxidant activity of the total aqueous extract of willow leaves was much more than salicin alone. This may be due to the aqueous extract of willow leaves contain other antioxidants such as tannins, flavenoids and proanthocyanidins in addition to salicin which could play some role in the increase of the total antioxidant activity. Salicin may induce elevation in activities of the antioxidants as glutathione peroxidase in the tissues homogenates which were selected to be under study. The antioxidants have a major role in the antioxidants defense mechanisms against irradiation injury.

So, the maintenance of normal electrophoretic protein and zymogram levels after the treatment with salicin may be due to trapping of these free radicals by this fraction, thus preventing DNA damage. Salicin was able to overcome the disturbances in the protein pattern in the tissues selected to be under study.

5 Conclusions

The study concluded that salicin showed radioprotective effect against the gamma irradiation effect on various electrophoretic patterns in brain tissue of male rats.

6 Competing interest

The study aimed to suggest new compounds obtained from the nature and act as radioprotector to minimize or resist the irradiation effect.

7 Author’s contributions

MSA and MALAK carried out literature review and draft the manuscript. HMS participated in collection of data and arranged in tabular form. IA and WMK carried out the experimental work. All authors read and approved the final manuscript.

8 References

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