Caffeine and Aspirin Protecting Albino Rats Against Biochemical and Histological Disorders Induced by Whole Body Gamma Irradiation

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ABSTRACT

Caffeine is an alkaloid (purine derivative) that contains flavonoids, whereas aspirin, natural component of mammalian tissue (acetylsalicylic acid) is one of the most commonly used non steroidal anti-inflammatory, and it is a necessary factor in the utilization of long-chain fatty acids to produce energy. Furthermore, it has been shown to protect cells from peroxidative stress. The objective of the present study is to evaluate the efficacy of caffeine (1,3,7-trimethyl xanthine) 80 mg/kg b.wt. and aspirin (acetylsalicylic acid) in the amelioration of the physiological and histological changes in stomach and intestine of rats exposed to gamma irradiation.

Male albino rats were divided into 8 groups. 1- Control group: rats not subject to any treatment, 2- Caffeine group: rats received caffeine (80ml/Kg body weight) via intraperitoneal injection for 21 days, 3- Aspirin group: rats received aspirin (150 mg/kg body) via intraperitoneal injection for 21 days, 4- Caffeine + Aspirin group: rats received caffeine and aspirin treatment, 5- Radiation groups: rats were whole body gamma irradiated at 8 Gy, 6- Caffeine + Radiation group: rats received caffeine for 21 days before whole body gamma irradiation at 8 GY, 7- Aspirin + Radiation group: rats received aspirin during 21 days before whole body gamma irradiation, 8- Caffeine + Aspirin + Radiation group: rats received caffeine parallel to aspirin for 21 days before whole body gamma irradiation. Animals were sacrificed 24 hrs post irradiation.

The results demonstrated that rats exposed to whole body gamma irradiation showed a significant increase in alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP) activities, and a significant decrease in total protein indicating liver injury. A significant increase in urea, creatinine, Na⁺, and K⁺ were recorded indicating kidney damage. Alteration of liver and kidney functions was accompanied by a significant increase in the content of thiobarbituric acid reactive substances (TBARS) associated with a significant decrease in glutathione (GSH) content, superoxide dismutase (SOD), and catalase (CAT) activities indicating oxidative stress. In addition, radiation caused inflammatory, fibrotic and cellular damage to the intestine and stomach. Administration of caffeine and aspirin resulted in significant improvement in hepatic and renal functions associated with reduction in oxidative stress and amelioration of the histological changes caused by gamma irradiation.

It could be concluded that the antioxidant properties of caffeine and aspirin might modulate γ-radiation-induced oxidative stress and histological disorders.

Key words: γ radiation, Caffeine, Aspirin, stomach, intestine.

INTRODUCTION

Radiation induces an inflammatory response in target and surrounding tissues which is characterized by accumulation of plasma proteins and leukocyte(1). The inflammatory reaction is a classical feature of radiation exposure and appears to be a key event in the development of the acute
radiation syndrome\textsuperscript{(2)}. Oxidative stress occurs due to excessive free radical production and/or low antioxidant defense, and results in chemical alterations of bio-molecules causing structural and functional modifications\textsuperscript{(3),4}. Reactive oxygen species, in turn, are capable of initiating and promoting oxidative damage in the form of lipid peroxidation\textsuperscript{(4)}. One of the major reasons for cellular injury after radiation exposure is the generation of free radicals and the possible increased levels of lipid peroxides in tissues. This leads to oxidative modifications of the cellular molecule\textsuperscript{(5)}. Radiation damage is largely caused by the overproduction of reactive oxygen species (ROS), including superoxide anion (O$_2^-$), hydroxyl radical (\textit{OH}), and hydrogen peroxide (H$_2$O$_2$), that overwhelm the levels of antioxidants, resulting in oxidative stress. One of the most important consequences of oxidative stress is lipid peroxidation\textsuperscript{(6)} with consequent damage of cellular membranes. Efficient defense and repair mechanisms exist in living cells to protect against oxidant species. Superoxide dismutase (SOD) catalyzes the reduction of O$_2^-$ to H$_2$O$_2$, the majority of which is broken down to oxygen and water by catalase (CAT). In addition to CAT, glutathione peroxidase in presence of adequate amount of reduced glutathione (GSH) can also break down H$_2$O$_2$\textsuperscript{(7)}.

Many structurally unrelated chemicals such as strong acids, alcohol or drugs damage the gastric mucosa and induce lesions in human and experimental animals. Human studies have shown that the use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with various gastrointestinal mucosal lesions\textsuperscript{(8)}. The mechanisms by which aspirin and other NSAIDs produce acute and chronic gastro-duodenal mucosal injury are incompletely understood\textsuperscript{(9)}. It has been suggested that the mechanism of aspirin-induced gastric lesion is mediated through lipid peroxidation\textsuperscript{(10)}. Also\textsuperscript{(11)} reported that, acetylsalicylic acid caused liver damage, and short –chain free fatty acid accumulation in rats, low birth weight\textsuperscript{(12)} reproductively and developmental toxicity\textsuperscript{(13)}, elevated lipid peroxidase in a rat's liver\textsuperscript{(14)}. Long-term treatment with aspirin (ASA) can significantly reduce the renal functional impairment that develops after high doses of irradiation\textsuperscript{(15)}. Also\textsuperscript{(11)} reported that, acetylsalicylic acid induced liver damage and short –chain free fatty acid accumulation in rats\textsuperscript{(16)} discerned that radiation and aspirin induced elevation in lipid peroxidase in rats' liver.

Caffeine (1,3,7-trimethylxanthine) is a major component of coffee and other beverages and is used as adjuvant analgesic in combination with drugs like acetaminophen, aspirin and ibuprofen. Though it has been shown to have several pharmacologically useful effects, it is regarded as radio sensitizer\textsuperscript{(16,17,18)}. However, several studies suggest that the presence of caffeine during radiation can offer significant protection. Moreover, caffeine had been shown to exert protective effect against toxic components of radiation damage in cell culture system\textsuperscript{(19)}. Caffeine has several biological and pharmacological, including, anti-inflammatory\textsuperscript{(20)}, anti-carcinogenic\textsuperscript{(21)} immune modulator and antioxidant activities\textsuperscript{(22,23)} and anti-apoptotic activity\textsuperscript{(24)}.

This study has been conducted to investigate the possible protective role of caffeine and aspirin against the toxic effects of exposure to whole body gamma-irradiation in albino rats.

**MATERIALS AND METHODS**

**Experimental Animals:**

Male albino rats *Rattusrattus* (10 ± 2 weeks old) weighing 120 ± 20 g were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). The animals were maintained under standard conditions of light, ventilation, temperature, and humidity and allowed free access to standard pellet diet and tap water. 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).

**Radiation Facility:**

Irradiation of rats was carried out using a Canadian Gamma Cell-40 (137 Cs), manufactured by Atomic Energy of Canada Ltd., located at the National Center for Radiation Research and
Technology (NCRRT), Nasr City, Cairo, Egypt. The animal’s whole body was exposed to gamma rays and received a dose of 8 Gy administered at a dose rate of 0.5 Gy/minute.

**Caffeine Treatment:**

Caffeine was purchased from Sigma chemical Co., St. Louis, MO, USA. The product is provided as a concentrated exudates, dissolved in saline. Caffeine was administered to rats via intraperitoneal injection at a dose of 80 mg/kg body weight (one hour prior to irradiation dose level of 8 Gy. Animals were sacrificed 24 hrs post irradiation.

**Aspirin Treatment**

Acetylsalicylic acid was purchased from ADWIC laboratory chemical – Egypt, was added to sterilized water (2.5 g/L) and was administered to rats via intraperitoneal injection before irradiation and all over the experiment.

**Animal Groups:**

Animals were divided into 8 groups 6 rats each:

1. Control: Rats of this group were neither treated with caffeine, aspirin, nor irradiation.
2. Caffeine: Rats of this group received caffeine via intraperitoneal administration (80 mg/kg body weight/day).
3. Radiation: Rats of this group were whole body gamma irradiated at 8 Gy.
4. Caffeine + Radiation: Rats of this group received caffeine via intraperitoneal administration (80 mg/kg body weight/day) before whole body gamma irradiation at 8 Gy.
5. Aspirin: Rats of this group received aspirin via intraperitoneal administration all over the experiment.
6. Caffeine + Aspirin: Rats of this group received caffeine via intraperitoneal administration (80 mg/kg body weight/day) parallel to aspirin treatment.
7. Aspirin + Radiation: Rats of this group received aspirin via intraperitoneal administration before irradiation (8 Gy) and all over the experiment.
8. Caffeine + Aspirin + Radiation: Rats of this group received caffeine via intraperitoneal administration (80 mg/kg body weight/day) parallel to aspirin treatment before whole body gamma irradiation at 8 Gy.

**Biochemical Analysis:**

At the end of the study, the animals were sacrificed. Blood samples were collected into heparin-treated tubes by cardiac puncture. Plasma samples were obtained by centrifugation at 3000 rpm for 10 min.

Liver function was evaluated in the plasma by the determination of the activity of aspartate amino transferase (AST), alanine amino transferase (ALT) according to (26). The activity of ALP and total proteins (TP) were determined according to (27) and (28), respectively. Kidney function was evaluated by the determination of urea and creatinine according to the methods of (29) and (30), respectively. Serum Na⁺ and K⁺ were estimated according to (31).

Malondialdehyde in plasma, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) according to (32). The activity of SOD was determined in blood erythrocytes according to the method of (33). The catalase activity was determined following the procedure described by (34). Blood glutathione was determined according to (35).

**Histological Methods:**

Intestine & stomach were removed and fixed in Bouin’s solution for 24 hours using (36). Samples were serially sectioned at a thickness of 4-5 um and stained applying the technique of (37) using hematoxylin and eosin. Tissue sections were examined under a research light microscope.
Statistical Analysis:

Experimental data were analyzed using one way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, 1999; ver.10.0) followed by Duncan test as post hoc ANOVA test. The significance among the samples was compared at P ≤0.05. Results were represented as mean ± Standard error (n =6).

RESULTS

The results obtained in the present study demonstrated that the intraperitoneal administration of caffeine (80mg/kg body weight/day) during 21 consecutive days resulted in no significant changes in all investigated parameters except for a significant decrease of K⁺. Aspirin treatment resulted in significant elevations of the activities of ALT, AST and ALP(Table 1,2& 3) as well as in the contents of urea, creatinine, and TBARS as well as a significant drop of T.P.,GSH, and the activities of SOD and CAT(Table 3).

In the current study, rats of radiation group, as well as, aspirin + radiation group showed alteration of liver functions manifested by a significant increase of ALT, AST and ALP activities and a significant decrease of T.P, compared to control group (Table 1). Moreover, alterations of kidney functions were illustrated as a significant increase of urea, creatinine, Na⁺ and decrease in K⁺, compared to control group (Table 2). Moreover, oxidative stress manifested by a significant increase of TBARS, associated with a significant decrease of GSH content and SOD and CAT activities was also recorded an compared to control group (Table 3).

In caffeine + aspirin group a significant drop of ALT& elevation of SOD and CAT were noticed, whereas a slight amelioration of AST, ALT, ALP and T.P (Table 1), urea, creatinine, Na⁺ and K⁺ (Table 2), TBARS, GSH, SOD and CAT (Table 3) compared with the aspirin group were detected. Animals of the caffeine+ radiation group showed significant amelioration of liver functions (Table 1), kidney functions (Table 2) and oxidative stress (Table 3), when compared with their respective values in the radiation group. In the same way, caffeine + aspirin + radiation group showed significant amelioration of liver functions (Table 1), kidney functions (Table 2) and oxidative stress (Table 3), when compared with their respective values in the aspirin+ radiation group.

Table (1): Influence of studied treatments on alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein (T.P) level in the serum of rats exposed to aspirin and radiation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>T. Protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.18±1.37</td>
<td>69.18±1.58</td>
<td>102.00±2.93</td>
<td>10.0±0.84</td>
</tr>
<tr>
<td>Caffeine</td>
<td>65.39±2.04</td>
<td>73.21±2.01</td>
<td>114.15±2.89</td>
<td>9.1±0.8</td>
</tr>
<tr>
<td>Aspirin</td>
<td>83.25±3.38ab</td>
<td>96.23±2.85ab</td>
<td>138.34±3.67</td>
<td>6.2±0.17</td>
</tr>
<tr>
<td>Radiation</td>
<td>94.21±2.95ab</td>
<td>100.7±3.12abc</td>
<td>187.18±3.57abc</td>
<td>5.8±0.14abc</td>
</tr>
<tr>
<td>Caffeine +Radiation</td>
<td>49.86±2.64d</td>
<td>44.29±4.08d</td>
<td>57.45±3.44d</td>
<td>8.6±1.33d</td>
</tr>
<tr>
<td>Aspirin +Radiation</td>
<td>61.28±1.44ad</td>
<td>98.08±3.07ab</td>
<td>161.26±3.01abd</td>
<td>6.4±0.47abd</td>
</tr>
<tr>
<td>Caffeine + Aspirin</td>
<td>54.15±3.01de</td>
<td>91.09±2.34bd</td>
<td>129.40±3.06de</td>
<td>7.3±1.04bd</td>
</tr>
<tr>
<td>Caffeine +Aspirin +Radiation</td>
<td>66.41±2.17cd</td>
<td>86.14±3.68cd</td>
<td>127.67±3.70def</td>
<td>8.6±1.33</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE (n=6).
P=0.05 not significant  P<0.05: significant
a:Significantly different compared to control.
b :Significantly different compared to caffeine treated.
c :Significantly different compared to aspirin .
d :Significantly different compared to radiation.
E:significantly different compared to caffeine & Rad.
f:significantly different compared to aspirin & Rad
G:significantly different compared to caffeine& aspirin.
Table (2): Influence of studied treatments on urea, creatinine, Sodium and potassium in the serum of rats exposed to aspirin, and radiation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Na⁺ (mEq/L)</th>
<th>K⁺ (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.12±1.08</td>
<td>1.12±0.09</td>
<td>112.97±1.68</td>
<td>13.18±0.57</td>
</tr>
<tr>
<td>Caffeine</td>
<td>45.14±1.36</td>
<td>1.72±0.16</td>
<td>109.82±1.44</td>
<td>9.55±0.42</td>
</tr>
<tr>
<td>Aspirin</td>
<td>46.52±1.57</td>
<td>2.03±0.66</td>
<td>128.49±1.18</td>
<td>10.37±0.64</td>
</tr>
<tr>
<td>Radiation</td>
<td>74.23±1.89</td>
<td>5.27±0.89</td>
<td>85.38±1.90</td>
<td>26.74±0.92</td>
</tr>
<tr>
<td>Caffeine + Radiation</td>
<td>55.19±1.06</td>
<td>3.79±0.79</td>
<td>137.74±1.35</td>
<td>19.57±0.87</td>
</tr>
<tr>
<td>Aspirin + Radiation</td>
<td>69.03±1.19</td>
<td>2.99±0.76</td>
<td>149.42±1.73</td>
<td>20.12±1.08</td>
</tr>
<tr>
<td>Caffeine + Aspirin</td>
<td>48.37±1.39</td>
<td>1.99±0.49</td>
<td>115.28±1.10</td>
<td>12.17±0.68</td>
</tr>
<tr>
<td>Caffeine + Aspirin + Radiation</td>
<td>39.63±1.28</td>
<td>1.89±0.54</td>
<td>143.09±1.18</td>
<td>8.41±0.19</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE (n=6)
P>0.05 not significant  P<0.05: significant
a:Significantly different compared to control.  b:Significantly different compared to caffeine treated.
c:Significantly different compared to aspirin.  d:Significantly different compared to radiation.
E:significantly different compared to caffeine & Rad.  F:significantly different compared to aspirin & Rad.
G:significantly different compared to caffeine & aspirin.

Table (3): Influence of studied treatments on thioborbituric acid reactive substances (TBARS) and glutathione (GSH) contents and superoxide dismutase (SOD) and catalase (CAT) activities in the serum of rats exposed to aspirin and radiation.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TBARS (nmol/ml)</th>
<th>GSH (mg/dl)</th>
<th>SOD (U/ml)</th>
<th>CAT (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106.04±3.40</td>
<td>16.12±1.18</td>
<td>93.50±3.78</td>
<td>28.17±0.80</td>
</tr>
<tr>
<td>Caffeine</td>
<td>108.53±3.96</td>
<td>15.57±1.56</td>
<td>84.90±2.95</td>
<td>27.09±0.67</td>
</tr>
<tr>
<td>Aspirin</td>
<td>156.13±8.1</td>
<td>10.44±0.84</td>
<td>79.41±2.05</td>
<td>13.94±0.09</td>
</tr>
<tr>
<td>Radiation</td>
<td>218.33±1.04</td>
<td>11.51±1.03</td>
<td>75.69±2.38</td>
<td>15.44±0.32</td>
</tr>
<tr>
<td>Caffeine + Radiation</td>
<td>179.78±4.48</td>
<td>13.38±0.91</td>
<td>82.19±2.34</td>
<td>25.13±0.83</td>
</tr>
<tr>
<td>Aspirin + Radiation</td>
<td>198.07±2.97</td>
<td>8.33±0.52</td>
<td>59.23±1.37</td>
<td>20.27±0.64</td>
</tr>
<tr>
<td>Caffeine + Aspirin</td>
<td>148.35±3.60</td>
<td>12.60±0.72</td>
<td>65.48±1.74</td>
<td>22.56±1.18</td>
</tr>
<tr>
<td>Caffeine + Aspirin + Radiation</td>
<td>134.81±2.63</td>
<td>14.22±0.88</td>
<td>67.41±3.05</td>
<td>35.15±1.92</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE (n=6)
P>0.05 not significant  P<0.05: significant
a:Significantly different compared to control.  b:Significantly different compared to caffeine treated.
c:Significantly different compared to aspirin.  d:Significantly different compared to radiation.
E:significantly different compared to caffeine & Rad.  F:significantly different compared to aspirin & Rad.
G:significantly different compared to caffeine & aspirin.
Histopathological Observation:

Sections of the small intestine of control animals showed serosa, muscularis of longitudinal fibers to the outside, and circular fibers to the inside. The submucosa with blood vessels, mucosa composed of muscularis mucosa of circular muscle layer, villi with a core of connective tissue containing lacteols, blood vessels and nerves and with a wall of columnar epithelium and goblet cells. Crypts of lieberkuhn lined columnar cells contain secretory granules (Fig.1 & Plate 1). Caffeine & aspirin treated group showed the typical distinct different intestinal layers as shown in Fig 2 & Plate 1. The mucosal surface showed normal architecture with normal goblet cells and the mucosa displayed numerous closely arranged tubular glands or crypts, and epithelial layer lined the mucosal surface and the glands. The intestine of animals exposed to 8 Gy γ–radiation showed thickened villus with discontinuous epithelium, mononuclear cellular infiltration of lamina propria, rupture and congestion of mucosa, atrophy, irregularity and ill-defined shape of crypts (Fig.3 & Plate 1). Administration of caffeine ameliorated the histopathological changes. The villi of mucosa were partially preserved and were comparable to that of the control group (Fig.4 & plate 1). In irradiated groups treated with aspirin, most of the columnar epithelial cells appeared to be normal, but there is separation of the epithelium from the underlying corium. Some villi showed loss of epithelial covering especially at the tip corium which demonstrated foci of cellular infiltration (Fig.5 & plate 2). Administration of caffeine & aspirin before exposure to gamma rays resulted in significant regeneration in the section showing well-defined different intestinal layers with highly developed crypts, definite epithelial lining, normal goblet cells and distinct fibrous coat (Fig. 6 & plate 2).

Sections of the stomach of control animals showed serosa, muscularis composed of longitudinal and circular muscle layers, sub mucosa with blood vessels, mucosa composed of two layered muscularis mucosa, connective tissue with gastric glands and columnar epithelium. The gastric glands are simple tubular and lie parallel to each other, their secretin cells are of two varieties granular or peptic cell with polygonal outline and secretory granules that has affinity to basic dyes, oxyntic or acidic cells with round outline and an affinity to acidic dyes (Fig.7 & Plate 2). In the case of caffeine & aspirin, all gastric mucosal cells appeared intact and had normal shape location, appearance and density. Surface mucous cells were columnar to cuboidal with varying amounts of apical mucous granules. Gastric pits were of expected depth and the gastric glands comprised intact mucous neck and endocrine cells (Fig. 8 & 9 Plate 2). Exposure to gamma rays revealed changes in the gastric mucosa. Gastric glands exhibited cytoplasmic degeneration and nuclear abnormalities. The apical cytoplasmic regions in most of those cells revealed foamy appearance similar to cloudy swelling (Fig.10 Plate 2). In radiation & aspirin treated animals, large degenerative regions were demonstrated and seen opening directly to the lumen of gastric glands. Numerous inflammatory cells were demonstrated in the lamina propria, which revealed the presence of some degenerative regions in the radiation & caffeine treated group. Administration of caffeine and aspirin after gamma rays ameliorated the histopathological changes where the mucosa was partially recovered if compared to that of the control group (Fig.12 & Plate 3).
Fig. (1): photomicrograph of a section in the small intestine of a control rat showing normal intestinal crypt and villi and normal components of all types of crypt base. (H & E 400 x)

Fig. (2): photomicrograph of a section in the small intestine treated with caffeine & aspirin showing normal structure. (H & E 400 x)

Fig. (3): photomicrograph of a section through rat intestine after exposure to 8 Gy gamma rays showing mononuclear cellular infiltration, rupture and congestion of mucosa. (H & E 400 x)

Fig. (4): photomicrograph of a section through rat intestine after exposure to 8 Gy gamma rays and treated with caffeine the villi of mucosa were partially recovered. (H & E 400 x)
Plate (2)

Fig. (5): Photomicrograph of a section through rat intestine after exposure to 8 Gy gamma rays and treated with aspirin. Some villi showed loss of epithelial covering especially at the tip corum. (H & E 400 x)

Fig. (6): Photomicrograph of a section through rat intestine after exposure to 8 Gy gamma rays and treated with caffeine & aspirin in apparent improvement and significant regeneration. (H & E 400 x)

Fig. (7): Photomicrograph of a section through control rat stomach showing intact muscle wall covered by serosa, muscularis, submucosa and mucosa with columnar epithelial cells. (H & E 400 x)

Fig. (8): Photomicrograph of a section through rat stomach treated with caffeine showing normal structure as control. (H & E 400 x)

Fig. (9): Photomicrograph of a section through rat stomach treated with aspirin showing normal structure as control. (H & E 400 x)

Fig. (10): Photomicrograph of a section through control rat stomach after exposure to 8 Gy gamma rays exhibited cytoplasmic degeneration and nuclear abnormalities. (H & E 400 x)
DISCUSSION

Exposure of mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs, which causes changes in the structure and function of cellular components. Oxidative stress with the subsequent production of reactive oxygen species (ROS) was postulated as one of the mechanisms of radiation toxicity. In the present study, whole body exposure of male albino rats to gamma radiation (8 Gy) has provoked an imbalance between oxidant and antioxidant species. Significant increases in the level of TBARS, accompanied by significant decreases of SOD and CAT activities as well as GSH content were recorded. The increase of TBARS level is probably due to the interaction of hydroxyl radical ('OH) resulting as a by-product of water radiolysis, upon exposure to ionizing radiation, with the polyunsaturated fatty acids present in the phospholipids portion of cellular membranes\(^6\). The significant decrease in the activity of SOD and CAT might also be attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation\(^38\). The ROS as chemically reactive molecules can modify most cell components such as lipids, nucleic acid, carbohydrates and proteins\(^39\). In the present study, 8 Gy gamma irradiation induced a significant decrease in total protein which could be attributed to that ROS, this may disturb cellular metabolism and directly or indirectly influence proteolytic-antiproteolytic balance of different tissues, thus can directly react with proteins including enzymatic proteins and provoke their modification\(^40,41\). The decrease of antioxidant by irradiation could be attributed to enhanced utilization of the antioxidant system in an attempt to detoxify radiation generated free radicals\(^42\). Excessive lipid peroxidation can increase GSH consumption\(^43\).

In the present study, gamma rays induced cellular alteration and significant elevation of AST, ALT and ALP. Radiation induced destruction of the hematopoietic systems causes depletion of peripheral blood elements which lead to loss of function. ALP plays an important role in maintaining the cell membrane permeability\(^44\). The present increase in the activity of ALP is in agreement with\(^45\) and may be due to the change in amino acid and catalytic activity. The increase in ALT, AST and ALP

**Fig. (11):** photomicrograph of a section through rat stomach after exposure to 8 Gy gamma rays and treated with aspirin showing numerous inflammatory cells demonstrated in the lamina propria. (H & E 400 x)

**Fig. (12):** photomicrograph of a section through rat stomach after exposure to 8 Gy gamma rays and treated with caffeine & aspirin showing partially recovered and comparable to that of the control. (H & E 400 x)
in irradiated rats was attributed to extensive breakdown of liver parenchyma with subsequent enzyme release and increase in permeability of the cell membrane that could enhance the movement of enzymes from their sites of production\(^{(46)}\). Exposure of animals to ionizing radiation 8 Gy revealed a significant decrease in the level of total protein that might result from leakage of proteins via the urine due to changes in kidney permeability\(^{(47)}\), or might result from enhanced protein degradation\(^{(47,48)}\). The accumulation of free radicals causes consecutive lipid peroxidation of the cell membrane and endoplasmic reticulum\(^{(49)}\). Treatment of rats with acetylsalicylic acid caused slight reduction in the impairment observed in stomach and intestine, a result which is in agreement with previous studies of\(^{(50,51,55,52)}\), prolonged treatment with acetylsalicylic acid resulted in a significant reduction of liver radiation injury after clinically fractionated irradiation\(^{(53)}\). Caffeine protected rats against the oxidative stress. Results of the present work showed an increase in GSH, CAT, SOD and decrease in MDA that may be due to caffeine free radical scavenging ability, by redox active sulphhydril group directly reacting with oxidant\(^{(53)}\). Radiation induced oxidative stress to the kidney was associated with a significant increase in urea & creatinin. The disturbance in Na' & K' levels induced by \(\gamma\)-irradiation could be attributed to a kind of stress exerted upon the Na' & K' pump mechanism that led to an imbalance in membrane permeability\(^{(54,55,18)}\). The intestine is well known to be a radio sensitive organ, after lower doses\(^{(56)}\). In the present study, the intestinal morphology is slightly modified in consistent with the observation made by-protrusion of crypts in the submucosa might be due to the loss of follicular structure. The blunting and/or loss of the villi might be attributed to direct action of radiation. Nevertheless, epithelial continuity was not disrupted suggesting that changes are functional. Radiation induced damage of intestinal cells has a dose dependent effect. Histopathological examination of the intestine after irradiation at 8 Gy showed epithelial sloughing of many villi and necrosis of basal cells. Similar results have been obtained for 5, 6 and 8 Gy\(^{(57,58,59,60)}\). This damage has been attributed by a number of authors to the release of free radicals by ionizing radiation\(^{(61,62)}\). Reduction of intestinal absorption after irradiation has been well documented\(^{(63)}\). It has been suggested that the decrease in intestinal absorption correlates with the number of functionally active cells on the absorptive surface when nutrients such as amino acids and glucose, which are actively transported through the mucosal membrane, are considered. In the present study, stomach damage was observed in irradiated group. Functional alteration in the stomach by radiation was previously reported\(^{(64,65)}\). The damage to the stomach occurred either after acute\(^{(66)}\) or fractionated doses\(^{(67)}\). Different histopathological alterations were reported including vasodilatation and edema, indicative of increase in micro-vasculature permeability\(^{(64)}\), and marked degenerative features including atrophic mucosa and ulceration\(^{(68,69)}\). In the current study, aspirin induced marked hepatic dysfunctions as evidenced by significant elevation of serum liver enzymes (ALT, AST, ALP) & significant decrease of T.P. The results are consistent with the findings of\(^{(70)}\). Aspirin produced a concentration dependent reduction in radiation induced DNA damage\(^{(68)}\). The decreased levels of ALT, AST and ALP after administration of caffeine may be due to the fact that caffeine stabilize the integrity of cell membrane, keeping the membrane intact and the enzymes enclosed\(^{(50)}\). Caffeine is likely to be factor in the protective efficacy of the irradiated rats\(^{(70)}\). Results of the present study revealed that caffeine could protect the stomach against the ulcerative damage produced by gamma-rays or aspirin treatment. Caffeine treatment has significantly ameliorated radiation and aspirin damage induced on liver and kidney functions, as well as structure of the intestine & stomach which were associated with amelioration of oxidative stress mediated through scavenging of ROS and/or reducing antioxidant depletion.

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