Modulator Effect of Turmeric on Oxidative Damage in Whole Body Gamma Irradiated rats

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ABSTRACT

Because of its penetrating power and its ability to travel great distances, gamma rays are considered the primary hazard to the population during most radiological emergencies. So, there is a need to develop medical countermeasures to protect the first responders and remediation workers from biomedical effect of ionizing radiation. Turmeric has been reported to have many beneficial health effects, including a strong anti-oxidant effect, anti-inflammatory and anti-microbial properties. In the present study, turmeric was investigated as a therapeutic agent against hazards induced by ionizing radiation on kidney, liver, urinary and serum calcium levels and blood counts. A daily dose of 0.5 g/kg body weight was used in whole body gamma irradiated female rats with 3 Gy. Radiation effects were followed up for four weeks post irradiation. The results revealed that the administration of turmeric post-irradiation resulted in a significant inhibition in the frequency of radiation induced oxidative damage. It could be concluded that definite turmeric dose exerts a vital modulator role against gamma irradiation hazards.

Key words: Irradiation, Turmeric, Kidney, liver, CBC, Serum and urinary Ca

INTRODUCTION

Turmeric is traditionally used a lot in the middle east as a liver protector. It is a stimulant of bile duct secretions, anti-flatulent, diuretic, for curing catarrh, aphrodisiac, anti-parasite, for the circulation, anti-fevered anti-inflammatory. In extreme cases, it is used for healing and disinfection of wounds (even in purulent ophthalmopathies) and for rheumatism or sprains. Also, Turmeric extract which is rich in curcuminoids is widely known for its anti-oxidant and antimicrobial properties, among others. In ancient times turmeric was much appreciated for its nutritional value. Nowadays it is used as a food, being the main constituent of curry, medicine and coloring \([1]\).

It is known that the damages caused by oxidation in the different cellular components are one of the main causes of many diseases, including ageing \([2]\). Ionizing radiation is an important environmental risk factor for various cancers and also a major therapeutic agent for cancer treatment. Exposure of mammalian cells to radiation induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage, as well as DNA–DNA and DNA–protein cross-links \([3]\).

Ionizing radiation interacts with biological systems to induce excessive oxygen free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, thereby leading to significant cellular damage. ROS also negatively affects intracellular concentration of antioxidants \([4]\). The major types of ROS or ROS-producing species generated by radiation are superoxide anion (\(O_2^-\)), hydrogen peroxide (\(H_2O_2\)), and hydroxyl (\(OH^-\)) radicals \([5]\). ROS present a paradox in their biological functions: on one hand, they prevent diseases by assisting the immune system, mediating cell signaling and playing an essential role in apoptosis. On the other hand, they can damage many biologically active molecules, leading to tissue damages and cell death, \([6]\), \([7]\). Other side effects of irradiation include; nausea, vomiting, diarrhea, body weight and hair loss, weakness etc. \([8]\).
Aim of the work

Ionizing radiation has been documented to disrupt the regulation of metabolic processes [4]. Therefore, the design of strategies capable of minimizing the damage caused by radiation is of great interest in radiation biology. So this work aims to use turmeric as a modulator against damage [1] caused to urinary and serum Ca levels, liver, kidney and blood count by 3.0 Gy gamma irradiation.

MATERIALS AND METHODS

Forty albino rats of about 150 -170 g weight (3-4 month during the growing stage) were adapted for one week in the animal house. They were divided equally into four groups, one group served as control which received no treatment. The second group received an acute whole body gamma radiation dose of 3.0 Gy (LD 50/30 ≈ 6.2 Gy for this species). The third group firstly received a daily oral dose of 1.0 g/Kg turmeric which causes death to most animals so the dose was reduced to be 0.5 g/Kg turmeric as suspension in 1 ml of water/day for 28 days. The fourth group received an acute whole body gamma radiation dose of 3 Gy then followed by daily oral injection with 0.5 g/Kg turmeric as suspension in 1 ml of water/day for 28 days. Both control and irradiated groups were injected daily with 1 ml water for 28 days.

Blood samples were collected from the venous plexus of the eye by standard venipuncture with glass capillary tubes from all rat groups at 3rd, 7th and 28th days post irradiation. One ml of venous blood was dispensed into EDTA tubes for Haematological studies using TC Hemaxa 1000 Cell counter (version 1:1) C.A 92807 USA. The rest were dispensed into anticoagulant free vacutainer tube to yield serum for other biochemical investigations. Twenty four hours urine was collected into metabolite cages with 50 µl/ml of 6 M HCl. The Sera were frozen at -20 °C until time of analysis. Total Ca was measured colourimetrically using cresophthalein complexone (The coefficient of variation of the method is 7.2%). The complex, formed between calcium and cresophthalein complexone in alkaline medium is measured spectrophotometrically while ionized Ca was measured by ion selective electrode [9]. Blood urea nitrogen (BUN) [10] and creatinine [11] were determined spectrophotometrically using an automated analyzer. Total proteins were measured spectrophotometrically using the Biuret reaction [12]. Albumin was determined by using bromocresol purple reaction [13]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured according to Reitman-Frankel colorimetric transaminase procedure [14].

For the statistical analysis of the data, two way analysis of variance F-test was carried out to explain the significant effect of the two factors (different treatments and different times). Also Duncan test have been done to investigate the significant difference between the different groups. SPSS program, version 17, has been used for all the statistical analyses.

RESULT AND DISCUSSIONS

Biochemical results are represented as histograms and tables. Histograms including (mean ± standard error (positive bar only)), F1 and F2 values and Duncan test 2 (for different time intervals). Changes in urine calcium, serum (total and ionized) calcium, blood urea nitrogen, creatinine, proteins and transaminases are present in figures (1-10). Table (1-3) represents Duncan test 1 (for different treatments) for the measured biochemical parameters. Hematological parameters (blood count) are represented as mean values± standard error, F1 and F2 values and Duncan results in tables (4-6).
Figure (1): Changes in serum total calcium levels in different groups

Figure (2): Changes in serum ionized calcium levels in different groups

Figure (3): Changes in urinary calcium levels in different groups
Table (1): Duncan test 1 of different treatments

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control</th>
<th>Irradiated</th>
<th>Turmeric</th>
<th>Irrad+turn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ca</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Ionized Ca</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Urinary Ca</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Duncan test showed that irradiation increases urinary Ca and decreases serum total Ca significantly but doesn’t affect serum ionized Calcium (Ca**+**). Treatment with turmeric improved the radiation effect on total and urinary Ca.

Radiation exposure can cause degeneration of connective tissues, including bone [15]. It increases the number of bone-resorbing osteoclasts and causes bone loss in cancellous tissue [16]. Irradiation causes reduced viability and increased apoptosis of marrow cells [17]. It causes oxidative damage to lipids within mineralized tissue and generation of reactive oxygen species (ROS) in marrow cells. Gamma irradiation leads to a rapid decrease in cancellous fractional bone volume [bone volume (BV) as a fraction of total volume (TV)] in post pubertal mice [16]. In vitro irradiation of osteogenic cells from the bone marrow leads to increased generation of ROS [18].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may mediate tissue degeneration diseases, such as osteoporosis [19]. It also leads to oxidative damage in blood and various soft tissues [20]. ROS can directly stimulate bone resorption by osteoclasts [21], [22], and the bone loss [23], [24]. The release of ROS and RNS can cause oxidative damage, which is associated with excess bone resorption by osteoclasts over bone formation by osteoblasts, reduced viability of the putative mechano-sensory cells (i.e., osteocytes), and osteoporosis [25].

Treatment with turmeric improved the radiation effect on total and urinary Ca. This effect may be due to stimulation of parathyroid gland. Parathyroid hormone (PTH) serves to protect the organism against acute fluctuations in extracellular ionized Ca and acts immediately to increase the acute retention of serum Ca at the distal renal tubule and to mobilize Ca from bone. Ca content in turmeric is about 183 mg/100 g [26]. The interface of serum Ca with the kidney (directly), the skeleton (directly), and the intestine (indirectly), are all driven by released PTH to restore the perturbation of a reduction in the serum Ca level.

Treatment with Turmeric improves the viability of bone marrow cells following irradiation. It may inhibit osteoclast differentiation and protects the bone marrow from DNA damage and apoptosis induced by tumor necrosis factor (TNF-α) or hydrogen peroxide (H₂O₂). Turmeric has a free radical scavenger activity, especially on the hydroxyl radical, which explains its capacity to protect DNA from damage in human cell cultures exposed to radiation [27]. Turmeric showed anti-inflammatory properties in animal models by inhibiting the activity of the enzymes cyclooxygenase-2 and lipoxygenase as well as the enzyme nitric oxide synthase [1]. In vitro assays indicated that turmeric inhibits the arachidonic acid-induced mouse edema [28]. For cases of arthritis and tendinitis in humans, clinical studies have demonstrated the beneficial effects of taking capsules containing turmeric rhizome extracts at doses of one or two 500mg capsules three times a day. This effect is reported to be due to inhibition of lysosomal enzymes (acid phosphatase and cathepsin D) as well as the inhibition of lipid peroxidation [1]. However, our results suggest that the potent antioxidant activity of turmeric mediates urinary and serum calcium disturbances because of irradiation.
Radiation induces an amount of injury in kidney which is directly proportion to the radiation dose and exposed volume of renal tissues which may lead to renal failure. Irradiation of the kidney occurs as a for m of direct injury, or as a consequence of irradiation on nearby structures, thus causing radiation nephritis. All components of the kidneys are affected, including glomeruli, mesangium, blood vessels, tubular epithelium, and interstitium. Cohen explains that renal injury caused by ionizing radiation is initiated by oxidative injury to DNA that is genotoxic injury [29]. It is established that tissue injury elicits acute inflammation whose features among others include swelling of the affected part. This is due to accumulation of exudates particularly fluid, proteins, and cells from local vessels unto the damaged part [30].

Duncan test showed that irradiated group has an insignificant increase in the level of BUN at the 3rd day of the experiment period compared to the control group. On the other hand, irradiated group show increase in the creatinine level significantly as compared to the control group. The group that
received irradiation and turmeric showed significant decrease in the creatinine level compared to the irradiated group.

The renal handling of urea represents an important example of the excretion of a substance in urine. Since in the nephrons, urea will be passively reabsorbed [31], [32]. Because the major pathway of nitrogen excretion is urea synthesis in the liver which is released into the blood and cleaned by the kidney, the reduction in animals receiving turmeric may be either due to lower rate of urea synthesis in the liver or higher rate of urea excretion in kidney. Since a number of studies indicated that Turmeric exhibit antioxidants activity because of its structure includes a phenol group, a β-di-ketone and anti-free radicals abilities [27] thus, it may stimulate the liver performance and urea synthesis. BUN in serum of the supplemented turmeric animal group did not changed significantly as compared with that of the control group. It could be explained that urea reabsorption varies markedly with the rate of urine flow and depends on the volume of water that is not reabsorbed [33]. Creatinine is an organic base formed during muscle protein metabolism as a degradation product of creatinine phosphate [34]. Like many other organic bases, creatinine is filtered at the glomerulus and eliminated from plasma by the kidney. It means that creatinine is filtered and not reabsorbed; therefore turmeric might have little influence on its excretion, whereas urea is filtered and reabsorbed partly in the nephrons. In addition the relation of urea to the water reabsorption may cause extra cellular contraction, which consequently results in higher concentration of substances such as creatinine in plasma. This may be the reason for significant high level of creatinine in the irradiated group.

Radiation injury of the liver is not a single entity and represents a continuum ranging from asymptomatic biochemical abnormalities to veno-occlusive disease and hepatic failure [35]. Radiation induced hepatitis has been reported after a threshold dose of 30-35 Gy. An acute pattern (< 3 months) of liver injury after radiation exposure is characterized by sinusoidal congestion, hyperaemia and fatty infiltration. The late chronic phase is characterized by peri-portal fibrosis and disorganization of lobular architecture [36]. The earliest electron microscopic changeafter hepatic irradiation is swelling of hepatocyte mitochondria without other subcellular abnormalities. Latter, sinusoidal, endothelial and kupffer cells swell with vaculation and intra-sinusoidal red blood cells appear deformed and entrapped [37]. These nuclear and cytoplasmic changes may be produced due to the lipid peroxidation and destruction of protein, DNA, cytoskeleton and organelles. Free-radicals generated by ionizing radiation, especially the hydroxyl radical (OH•), attack polyunsaturated fatty acids (PUFA) in cell membranes to yield highly destructive PUFA radicals (lipid hydroperoxy radicals and lipid hydroperoxides) that damage the cell membranes [38], [39].

![Figure 6: Changes in serum total protein levels in different groups.](image-url)
It is well accepted that hepatocytes are quite radio-resistant compared to other cells [40]. The development of radiation-induced liver disease (RILD) is considered to be a major dose limiting complication in abdominal irradiation [41]. The threshold dose for whole liver irradiation is assumed to be between 20 and 30 Gy [42]. Hepatic vein lesions and parenchymal cell death are the most prominent histological lesions [43]. Furthermore, liver irradiation above the threshold dose is not followed by a recovery phase, but leads to progressive liver fibrosis and cirrhosis at least in animal studies [44]. The pathophysiological mechanisms of hepatocellular cell death after irradiation are widely unknown. In fact, irradiation alone does not lead to apoptosis of hepatocytes. However, irradiation leads to susceptibility of hepatocytes to TNF-α mediated apoptosis [45]. TNF-α sensitize for DNA-damage-induced apoptosis via an NF-kappa-B independent mechanism [46].
Table (3): Duncan test 1 of different treatments

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>control</th>
<th>irradiated</th>
<th>turmeric</th>
<th>Irrad+turm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total protein</strong></td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td><strong>Globulin</strong></td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>a</td>
<td>d</td>
<td>b</td>
<td>C</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>a</td>
<td>d</td>
<td>b</td>
<td>C</td>
</tr>
</tbody>
</table>

The results of total proteins shows a significant decrease in total protein, albumin and globulin of the irradiated group and a significant increase after treatment with turmeric. The liver is the major source of serum proteins. The parenchymal cells are responsible for the synthesis of albumin, fibrinogen, and other coagulation factors and most of the alpha and beta globulin. So, liver injury caused by irradiation will alter the concentration of blood proteins. Radiation denaturants proteins which cause changes that render them inactive. The most prominent quantitative changes include decrease in the concentration of the albumin and gamma globulin fractions and an increase in the α- and β- globulins. The decrease in serum albumin was attributed to radiation damage of liver cells responsible for albumin synthesis and / or damage to the albumin molecule itself [50].

Studies on the effect of radiation on protein metabolism showed conflicting results [47], [54]. Some investigators noted a decrease in RNA and protein synthesis while other investigators reported an increase in RNA and protein synthesis in mouse and rat livers after whole body irradiation. This is attributed to variations in radiation doses schedules, post irradiation follow up periods, methodology of assay, biological end point considered and other factors. A decrease in the liver protein content was recorded 8 days after whole body radiation exposure to 450-600 Rad [47]. The changes in the concentration of serum proteins may occur due to alteration in synthesis, catabolism and distribution between intra- and extra- cellular spaces [48]. While whole body exposure to 600 Rad resulted in decrease of the serum protein content 1 hour and 6 days after irradiation. An increase was recorded 3 and 12 days after irradiation [49]. A decrease in albumin and gamma globulin concomitant with an increase in alpha- and beta- globulins was observed in mice [50]. Significant decrease of plasma protein content was observed in 2.2 Gygamma irradiated rats. This decrease continued up to the 5th days then started to increase from the 7th day and indicate moderate recovery on the 14th day [51].

Figure (10): Changes in serum ALT levels in different groups

Whole body gamma irradiation of mice with 600 Rad induced an increase in liver protein synthesis and liver protein content at 1 and 3 days after irradiation. On the 7th day, a decrease was recorded in liver protein content and liver protein synthesis [52]. The proteins levels in rats irradiated
with fractionated doses of 0.5 Gy up to 3.5 Gy showed an increase in the plasma levels of total protein, α1 and γ-globulins and decrease in the plasma albumin level and A/G ratio [53]. The percent of mortality of rats irradiated with doses 6.7, 7.17, and 8.13 Gy were followed up for 30 days after completing irradiation. The highest percent of mortality was observed in groups with lower (30 to 70%) and higher (130 to 150%) values of serum protein. Maximal numbers of surviving rats were found in the central region (90 to 110%) of serum protein [54]. The previous data show that studies on liver protein metabolism after irradiation are conflicting and the radiation exposure induced significant disturbances in serum total protein content and its fractions.

The result of transaminases shows significant increase in both serum aspartate amino-transferase (AST) and serum alanine aminotransferase (ALT) levels of irradiated groups than the control group. Turmeric significantly decreased their levels in irradiated groups but doesn’t reach the control level. Serum AST and serum ALT are present in the cytosol of the hepatocytes. The AST is also localized in the mitochondria. Whenever liver hepatocytes are damaged, these enzymes are released into the blood. A significant increase in AST and ALT activities indicates the damage to the cytosol and also to mitochondria. The earliest biochemical changes that can be seen after irradiation are an increase in the activity of aminotransferases. These changes may be due to drastic physiological effects caused by irradiation interaction with the cellular membrane, mitochondria or through the action of free radicals. The increase in ALT activities obtained in our results may be related to extensive breakdown of liver parenchyma with subsequent enzyme release, or to increase in permeability of the cell membrane that could enhance the movement of enzymes from their sites of production, while the increase in activities of AST can be attributed to the extensive destruction of the radiosensitive tissue of the hematopoietic system or attributed to changes in the physicochemical properties of the enzymes by radiation or due to enhanced gluconeogenesis and finally due to over-activation of the adrenal cortex. Also, at 3, 5, 7, 10, 14 and 30 days post irradiation with 600 R [55] and in mice at 1st, 3rd and 7th days after 600 Rad [52].

Turmeric is said to shrink engorged hepatic ducts, so it can be useful to treat liver conditions such as hepatitis, cirrhosis, and jaundice [56]. Turmeric might help to protect the liver from damage but there are reports show that turmeric extracts may damage the liver at high doses [57], [58]. It significantly protects the liver from injury by reducing the activity of serum AST, ALT, and ALP, as well as by improving the histological architecture of the liver [59]. Turmeric was effective in preventing and reversing cirrhosis, probably by its ability of reducing transforming growth factor-beta (TGF-beta) expression. Thus turmeric might be an effective anti-fibrotic and fibrolitic drug in the treatment of chronic hepatic diseases [60]. Turmeric prevents acute liver damage by at least two mechanisms: acting as an antioxidant and by inhibiting NF-kappaB activation and thus production of pro-inflammatory cytokines[61]. It was found that turmeric treatment reversed elevated serum marker enzymes, (AST), (ALT) and (ALP), increased lipid peroxidation, decreased glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in edematous, granulomatus, liver and heart tissues during inflammation, liver injury and cardiac necrosis, respectively [62].

It was reported that turmeric, a potent stimulator of stress-induced Hsp70 expression, exhibits protective effects against some forms of stress, such as heat or toxicant [63] and consequently irradiation.
Table (4: A-F): Mean values of RBCs indices

<table>
<thead>
<tr>
<th>A-RBCs (X10^6/cmm)</th>
<th>Control</th>
<th>Irradiation</th>
<th>Turmeric</th>
<th>Term.+Irrad.</th>
<th>DT2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1(TREATMENT)=25.6* F2 (TIME)=4.9*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>7.01±0.32</td>
<td>5.28±0.48</td>
<td>6.34±0.15</td>
<td>6.45±0.11</td>
<td>b</td>
</tr>
<tr>
<td>7 Days</td>
<td>6.95±0.35</td>
<td>3.98±0.35</td>
<td>5.43±</td>
<td>5.12±0.24</td>
<td>a</td>
</tr>
<tr>
<td>28 Days</td>
<td>5.71±0.36</td>
<td>5.73±0.31</td>
<td>6.43±0.09</td>
<td>6.20±0.20</td>
<td>b</td>
</tr>
<tr>
<td>DT1</td>
<td>c</td>
<td>a</td>
<td>b</td>
<td></td>
<td>b</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>B- Hb (g/dL)</th>
<th>Control</th>
<th>Irradiation</th>
<th>Turmeric</th>
<th>Term.+Irrad.</th>
<th>DT2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1(TREATMENT)=45.3* F2 (TIME)=0.84</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>18.0±0.91</td>
<td>14.4±0.9</td>
<td>15.35±0.41</td>
<td>14.98±0.47</td>
<td>a</td>
</tr>
<tr>
<td>7 Days</td>
<td>18.5±0.69</td>
<td>11.03±0.80</td>
<td>16.4±0.64</td>
<td>16.5±0.6</td>
<td>a</td>
</tr>
<tr>
<td>28 Days</td>
<td>18.25±0.85</td>
<td>15.07±0.53</td>
<td>15.85±0.39</td>
<td>15.53±0.15</td>
<td>a</td>
</tr>
<tr>
<td>DT1</td>
<td>c</td>
<td>a</td>
<td>b</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>C-MCV (fl)</th>
<th>Control</th>
<th>Irradiation</th>
<th>Turmeric</th>
<th>Term.+Irrad.</th>
<th>DT2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1(TREATMENT)= 15.52* F2 (TIME)= 6.9*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>76.5±3.67</td>
<td>60.48±1.52</td>
<td>68.5±1.45</td>
<td>65.75±0.85</td>
<td>a</td>
</tr>
<tr>
<td>7 Days</td>
<td>76.8±2.68</td>
<td>73.75±2.50</td>
<td>76.38±1.55</td>
<td>77.51±5.0</td>
<td>b</td>
</tr>
<tr>
<td>28 Days</td>
<td>77.5±2.33</td>
<td>70.95±1.86</td>
<td>75.5±3.77</td>
<td>76.98±2.10</td>
<td>b</td>
</tr>
<tr>
<td>DT1</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td></td>
<td>b</td>
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</table>

<table>
<thead>
<tr>
<th>D-MCH (pg)</th>
<th>Control</th>
<th>Irradiation</th>
<th>Turmeric</th>
<th>Term.+Irrad.</th>
<th>DT2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1(TREATMENT)=5.4* F2 (TIME)=24.4*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>33.0±2.55</td>
<td>24.75±1.70</td>
<td>23.0±1.47</td>
<td>23.13±0.78</td>
<td>a</td>
</tr>
<tr>
<td>7 Days</td>
<td>35.88±1.59</td>
<td>27.75±2.20</td>
<td>27.15±1.18</td>
<td>27.0±0.41</td>
<td>b</td>
</tr>
<tr>
<td>28 Days</td>
<td>33.25±1.93</td>
<td>24.25±0.95</td>
<td>26.0±2.27</td>
<td>24.75±0.85</td>
<td>a</td>
</tr>
<tr>
<td>DT1</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td></td>
<td>A</td>
</tr>
</tbody>
</table>
From table (4: A-C), it was found that irradiation significantly reduces RBCs, Hb and MCV levels than the control level and treatment with turmeric improve this reduction. Tables (4: D - E) show that each of the irradiation and turmeric reduce the levels of both MCH and MCHC levels so the treatment with turmeric doesn’t improve their reduced levels. Table (4: F) shows that HCT level decreased by irradiation and to less extent by treatment with turmeric only. The treatment with turmeric improved the decreased level but doesn’t reach the control level. Table (5) shows that WBCs level decreased by irradiation and to less extent by treatment with turmeric only. The treatment of the irradiated group with turmeric improves the decreased level but doesn’t reach the control group level. Table (6) illustrates that the platelets level reduced by the irradiation and the treatment with turmeric did not recover through 4 weeks.

The hematological and lymphoreticular systems are target systems susceptible to the effects of ionizing radiation, the severity of which occurs in a dose-dependent manner. The haematopoietic system is largely composed of undifferentiated rapidly dividing cells, making it more susceptible to the damage effects of ionizing radiation than are the tissues composed of highly differentiated more slowly dividing cells (central nervous system). Haematopoietic system mainly bone marrow is known to be one of the most radiosensitive and its damage may be critical for the survival due to haematopoietic syndrome [64].
Table (6): Platelets counts

<table>
<thead>
<tr>
<th>Platelets (10^3/cmm)</th>
<th>Control</th>
<th>Irradiation</th>
<th>Turmeric</th>
<th>Term.+Irrad.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F1(TREATMENT)=56.7*</td>
<td>F2 (TIME)=14.1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Days</td>
<td>679.5±19.34</td>
<td>331.5±29.33</td>
<td>242.75±37.03</td>
<td>297.5±19.36</td>
<td>a</td>
</tr>
<tr>
<td>7 Days</td>
<td>683.25±15.8</td>
<td>199.75±52.3</td>
<td>538.25±41.74</td>
<td>654.75±52.18</td>
<td>b</td>
</tr>
<tr>
<td>28 Days</td>
<td>695.25±25.02</td>
<td>22.25±35.33</td>
<td>271.5±68.64</td>
<td>195.75±38.76</td>
<td>a</td>
</tr>
<tr>
<td>DT1</td>
<td>b</td>
<td>a</td>
<td>a</td>
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The present study demonstrated that whole body gamma irradiation with 3Gy significantly decreases red blood cells number, haemoglobin content and haematocrit, which is a sign of anemia. This may be attributed to lysis of circulating RBCs due to a decreased production of erythropoietin [65] and hemorrhages caused by structural changes in membrane proteins, modification in internal peptides as well as internal viscosity of RBCs [66]. The structural and functional integrity of the double layer membrane depend on the association with a network of peripheral proteins (the cytoskeleton) attached to the inner membrane surface. The role of the cytoskeleton is to restore the shape of the red blood cell after mechanical deformation during its passage through the capillaries [67]. Gamma irradiation of red blood cells induces alterations at three different functional units of the membrane: lipid bilayer, protein components and cytoskeleton at the membrane surface [68]. Free radicals formed during irradiation can cause a variety of membrane changes including lipid peroxidation, hydrolysis of phospholipids head groups, lipid-lipid crosslinks, disulfide bridge formation and amino acid residue damage in membrane proteins and lipid-protein crosslinks [69]. Radiation increases membrane cholesterol level, causes oxidation of membrane protein, thiol groups and lipid peroxidation, and impairment of membrane permeability barrier [70]. In addition, ionizing radiation was reported to cause oxidation of the sulph-hydryl groups to the corresponding dithiols and induce conformational changes of membrane proteins [66].

Radiation induces shortening in the lipid fatty acid chains by lipid peroxidation [71]. The production of hydroperoxides and cross-linkages in the membrane lipids can disorder the upper region of the bilayer favoring penetration of water and ending by hemolysis [72]. The combined effects of free radicals on the red blood cell membrane and cytoskeleton may contribute to the leak of hemoglobin out of the cells. The hemolysis of the red blood cells reflects the loss of integrity of the cells which can lead to the liberation of intracellular hemoglobin [66]. Moreover, radiation induced haemodilution shown by decreased haematocrit value.

The radiation induced damage in the membrane permeability can facilitate the diffusion of molecule within the cell membrane and decreasing the mean cell volume of the red cells. The change in the shape of red blood after exposure to gamma radiation altered cell permeability, and developed echinocytes and spherocytes with the progressive appearance of the regularly spaced spicules on cell surface [70].

It is known that because of their longer lifespan in the peripheral blood (3 months) and lower turnover rates, making red blood cell is not a very radiosensitive cell [73]. Neutrophils have a naturally short lifespan in the peripheral blood (12-48 hours) and depend upon constant replenishment by the bone marrow to adequately defend the body against infection. Lymphocytes are replication components that normally survive in the blood for 2-4 days and are highly radiosensitive [74]. Radiation induced depletion in lymphocytes is primarily due to apoptosis, although necrotic death occurs [75]. Platelet count decreases after irradiation and this may be due to inhibition of bone marrow activity or may be due to decreased production or increased consumption of platelet or due to the increased of platelets aggregation [76].

In the present investigation, a drastic reduction in leucocyte count after irradiation is in agreement with the findings of earlier workers [77]. The leucocyte number showed a drastic decline during the first 24 hours. This initial phase of rapid decrease is due to direct killing of lymphocytes while the slower fall at later intervals is due to the reduced number of new lymphocytes entering the peripheral.
blood. The peripheral lymphocytes exhibited a maximum depletion in the first few hours after irradiation elucidating an early cell killing effect of radiations on this cell type, which is the most radiosensitive in peripheral blood. The lymphocytes increased during the first 24 hours, which can be attributed to “abortive” rise in the neutrophils after irradiation [78]. A second peak of neutrophilic elevation was noted on the second week after irradiation, suggested that the first peak can be possibly due to hastening of maturation in bone marrow and for the second peak a mobilization phenomenon in response to radiation-induced tissue injury can be held responsible [79].

Theresults show that animals treated with turmeric after irradiation had significant improvement in RBCs, Hb, Hct WBCs, and platelets as compared to irradiated group. This may be due to radio resistance effects of Turmeric on the bone marrow cells. It suppresses hydroxyl radical production in the Fenton reaction. The beneficial effect of Turmeric on stabilized cellular membranes prolongs their lives and raises their osmotic resistance. Turmeric improves the turnover of fatty acids peroxidated by the free oxygen radicals produced during normal metabolism. Also, turmeric induces elevation in GSH content which has a major role in the antioxidant defense mechanisms against irradiation injury [80], [81].

CONCLUSIONS

Turmeric as naturally occurring antioxidants was studied to reduce the cellular damage induced by ionizing radiation. Turmeric ameliorates the oxidative damage of γ-radiation. Turmeric elevates the protein profile (total protein, albumin and globulins) of the body that caused by whole body γ-radiation of rats and improves its liver functions. Turmeric provides radio-resistance effect to bone marrow cells in animals received γ-radiation. Use of commercial turmeric may limit our study but these naturally occurring antioxidants provide an extended window of protection against low-dose or low-dose-rate irradiation. It could be concluded that curcuma can be used as a radio protector to the professional radiation and remediation workers who may be expose to low level of ionizing radiation especially if it is administered after irradiation for therapeutic purpose.

REFERENCES


-592-
56- C. Sinadinos, Certified Clinical Herbalist, the world wide web, Turmeric For Liver & Digestive Health, Home of the electronic version of "A Modern Herbal" by Maud Grieve www.ottanical.com (1995)
79- L.O. Jacobson, , Marks, E.K. and Lovenz, E., Radiology, 52, 391. (1949)