

CHAPTER IV

RESULTS

This chapter contains a summary of the data of the changes in the levels of the cytokines, IL-1 β , IL-6 and IL-10, as well as PGE₂ and the level of MDA as a measure of oxidative stress and glutathione as an indicator of antioxidant defense. These parameters were determined in the livers, brains and testicles of male Swiss albino mice irradiated with either 2Gy or 6Gy of x-rays.

Results of the liver:

1. Interleukin-1 β :

Irradiation with x-rays resulted in significant elevation of the pro-inflammatory cytokine IL-1 β . One day after irradiation with 2Gy the level was 92.8 % above control, which further increased to reach more than 3-fold that of control after 7 days, $p < 0.05$ [Table 1, Figure 10]. The effect of whole body irradiation with 6Gy was more prominent as the level of IL-1 β was more than 3-fold and more than 6-fold the control value one and 7 days after irradiation respectively. As could be seen, the increase was dependent on radiation dose. The results of the 6Gy dose were significantly higher than the 2Gy by 74% and 102.2% after one and seven days respectively, $p < 0.05$.

Treatment with ectoine affected the levels of IL-1 β in all tested samples. When irradiated mice were treated with ectoine the extent of change was dependent on the radiation dose and duration of drug administration. Treatment of mice irradiated with 2Gy for one day resulted in a significant increase in IL-1 β of 33% while continued treatment for 7 days caused a significant decrease of 43.9%, $p < 0.05$. The effect of ectoine on mice irradiated with 6Gy was more apparent as IL-1 β was 31.8% after one day and 74.6% after 7 days below the irradiated levels.

Table (1): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-1 beta in the liver.

		1Day	7 days
Control (pg/g tissue)		18.70 ± 0.84	
2Gy	Irradiated(pg/g tissue)	36.30 ± 2.78 ^a	58.64 ± 3.77 ^{a*}
	Irradiated – Ectoine (pg/g tissue)	48.28. ± 1.44 ^{ab}	32.90 ± 1.95 ^{ab*}
6 Gy	Irradiated(pg/g tissue)	63.18 ± 4.41 ^{ab}	118.10 ± 3.36 ^{ab*}
	Irradiated - Ectoine (pg/g tissue)	43.12 ± 3.0 ^{ac}	30.14 ± 1.11 ^{ac*}

a: significantly different from control , p< 0.05

b: significantly different from Irradiation 2 Gy , p< 0.05

c: significantly different from Irradiation 6 Gy , p< 0.05

*: significantly different from 1 day , p< 0.05

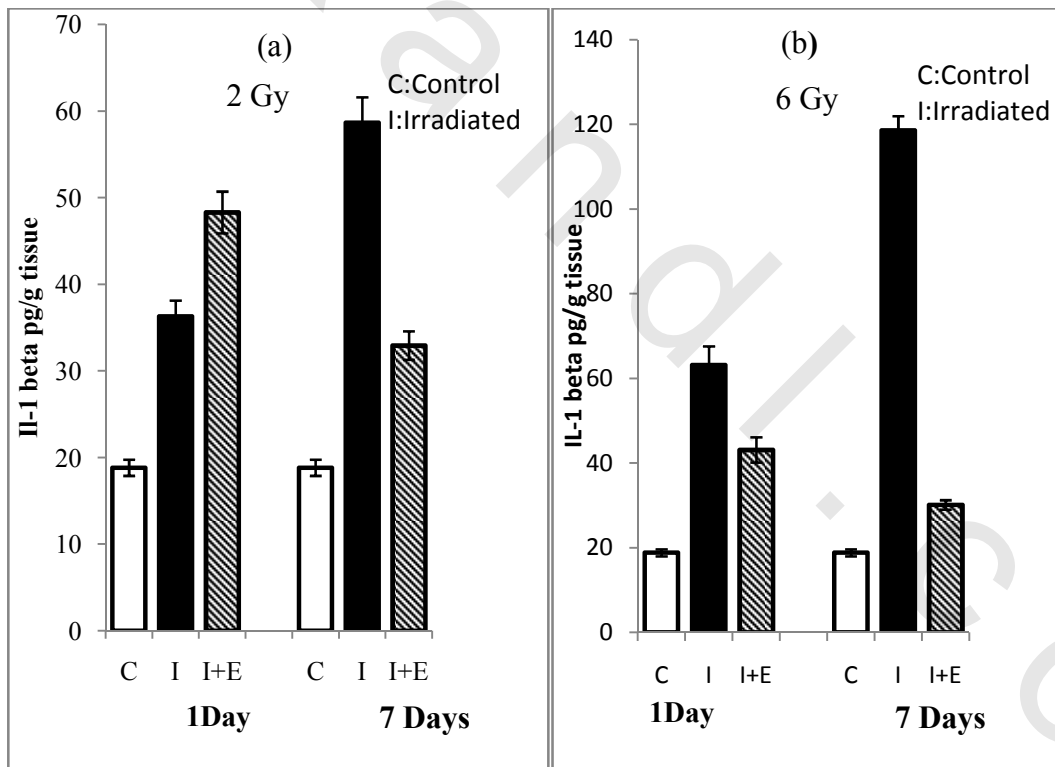


Figure (10): Changes in IL-1 beta in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

2. Interleukin -6:

The results of IL-6 were qualitatively similar to IL-1 β , with larger changes in the assayed concentrations in response to whole body x-irradiation or treatment with ectoine [Table 2, Figure 11]. One day after irradiation with 2 Gy the level of IL-6 was more than 3-fold the control value followed by a substantial increase to a value above 10-fold that of control after 7 days, $p < 0.05$. As may be expected, irradiation with 6 Gy gave higher values. The levels of IL-6 in the liver tissue, after one day and 7 days, were more than 8-fold and 16-fold that of control respectively. Again, the increase was dose-dependent, as the 6Gy caused significant increases of 157.8% and 55.6% after one and 7 days, $p < 0.05$, compared to the 2Gy dose.

Treatment of the 2Gy-irradiated mice with ectoine caused a significant increase of IL-6 after one day of more than 2-fold over the level of those who did not receive ectoine, $p < 0.05$. However, after seven days of irradiation, treatment with ectoine caused a significant decrease of 56.0% below the untreated group. Treatment of the groups, who received the higher x-irradiation of 6Gy, resulted in mean IL-6 values 19.6% after one day and 75.3%, after 7 days, less than those who did not receive the ectoine treatment, $p < 0.05$.

Table (2): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-6 in the liver.

		1 Day	7 Days
Control (pg/g tissue)		8.11 $\pm$ 1.0	
2Gy	Irradiated(pg/g tissue)	25.64 $\pm$ 1.48 ^a	83.60 $\pm$ 7.37 ^{a*}
	Irradiated - Ectoine (pg/g tissue)	54.57 $\pm$ 6.39 ^{ab}	36.82 $\pm$ 4.39 ^{ab*}
6 Gy	Irradiated (pg/g tissue)	65.97 $\pm$ 3.97 ^{ab}	130.13 $\pm$ 10.15 ^{ab*}
	Irradiated - Ectoine (pg/g tissue)	53.04 $\pm$ 2.27 ^{ac}	32.08 $\pm$ 4.14 ^{ac*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

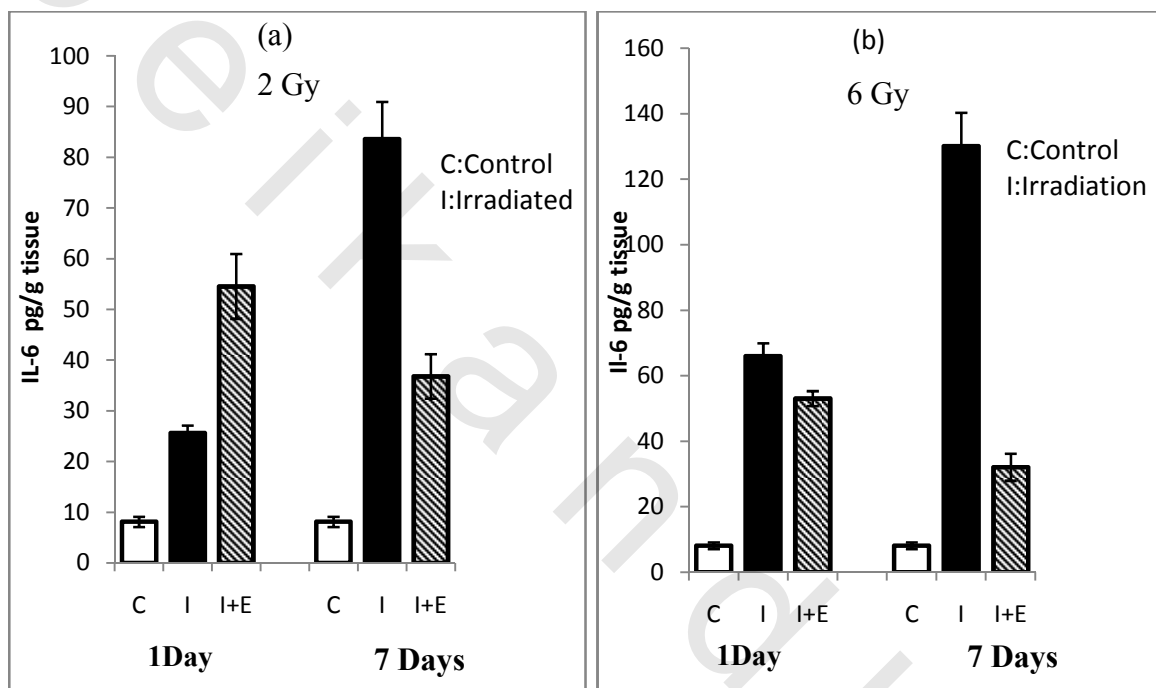


Figure (11): Changes in IL-6 in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

3. Interleukin-10:

Results of IL-10 are presented in Table [3] and Figure [12]. The level of this anti-inflammatory cytokine increased substantially as a result of exposure of the animals to whole body x-irradiation. Exposure to the 2Gy irradiation dose caused significant increases in the IL-10 concentration of 151.8% after one day and 581% after 7 days, above the mean control value, $p < 0.05$. These values were higher after the 6Gy dose reaching 443.6% and 945.5% after one day and 7 days respectively. It is clear that the changes in values were dependent on the radiation dose since the mean value for the animals receiving the 6Gy dose was higher than that of the 2Gy group by 115.6% after one day and by 53.3% after 7 days, $p < 0.05$.

Results of treatment with ectoine were qualitatively similar to those of IL-6. Although the level of IL-10 was higher by 73.6% above the untreated group after one day of radiation with 2Gy, such comparison showed a decrease of 53.8% after 7 days. Treatment with ectoine resulted in significant decreases of the mean IL-10 values below the untreated groups irradiated with 6Gy by 18.7% after one day and 74.6% after 7 days, $p < 0.05$.

Table (3): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-10 in the liver.

		1Day	7 days
Control (pg/g tissue)		10.98 ± 1.0	
2Gy	Irradiated (pg/g tissue)	27.73 ± 1.88 ^a	74.98 ± 5.17 ^{a*}
	Irradiated - Ectoine (pg/g tissue)	48.13 ± 3.64 ^{ab}	34.63 ± 3.21 ^{ab*}
6 Gy	Irradiated (pg/g tissue)	59.81 ± 5.06 ^{ab}	115.01 ± 6.35 ^{ab*}
	Irradiated - Ectoine (pg/g tissue)	48.65 ± 2.03 ^{ac}	29.24 ± 3.70 ^{ac*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation $p < 0.05$,

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

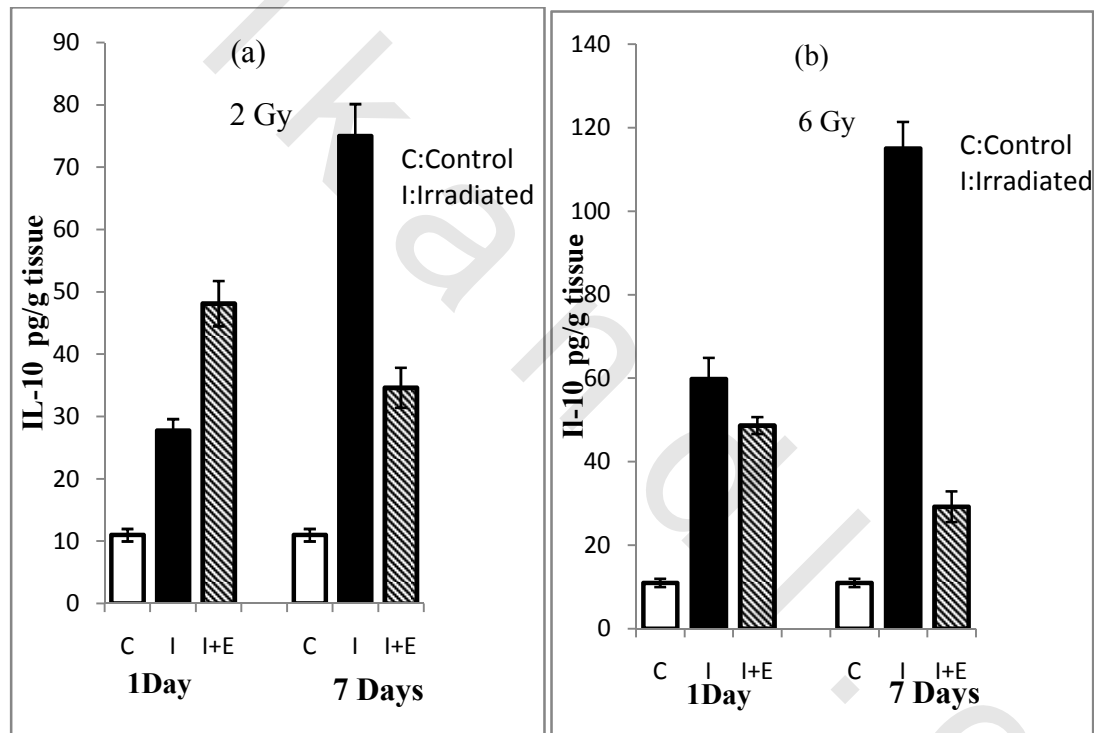


Figure (12): Changes in IL-10 in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

4. Prostaglandin E2:

The prostaglandin concentrations in mouse liver changed with the dose of radiation, the time after irradiation and treatment with ectoine [Table 4, Figure 13]. Because of the low level in control animals, such changes were relatively very large. PGE2 increased significantly by more than 3-fold that of control value one day after irradiation with 2Gy, which went up to more than 8-fold after 7 days, $p < 0.05$. The mean changes after the 6Gy irradiation dose were much larger, as there was a significant PGE2 increase from 7-fold after one day to near 32-fold the control value by the seventh day after exposure, $p < 0.05$. The effect of irradiation dose was clear, as the 6Gy resulted in a significant increase of PGE2 values of 94.7% above the mean values of the 2Gy after one day going up to 420.2% after 7 days.

Treatment with ectoine caused a significant increase in PGE2 levels in irradiated animals as compared to the control group, $p < 0.05$. Such increased levels reached 10-fold and more than 17-fold the control value one day and seven days after irradiation with 2Gy. The changes in PGE2 after the 6Gy irradiation dose were time dependent. While the mean level in treated animals reached 23-times the control value one day after irradiation, a significant decrease of 79.5% was observed after seven days.

Table (4): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on PGE2 in the liver.

		1Day	7 days
Control (ng/g)		2.13 ± 0.25	
2Gy	Irradiated(ng/g)	8.17 ± 1.19^a	$18.76 \pm 3.61^{a*}$
	Irradiated - Ectoine (ng/g)	22.14 ± 2.34^{ab}	$38.04 \pm 2.87^{ab*}$
6 Gy	Irradiated(ng/g)	15.91 ± 1.55^{ab}	$97.59 \pm 5.24^{ab*}$
	Irradiated - Ectoine (ng/g)	60.50 ± 5.27^{ac}	$13.82 \pm 2.36^{ac*}$

a significantly different from control , $p < 0.05$

b significantly different from Irradiation 2 Gy, $p < 0.05$

c: significantly different from Irradiation 6Gy, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

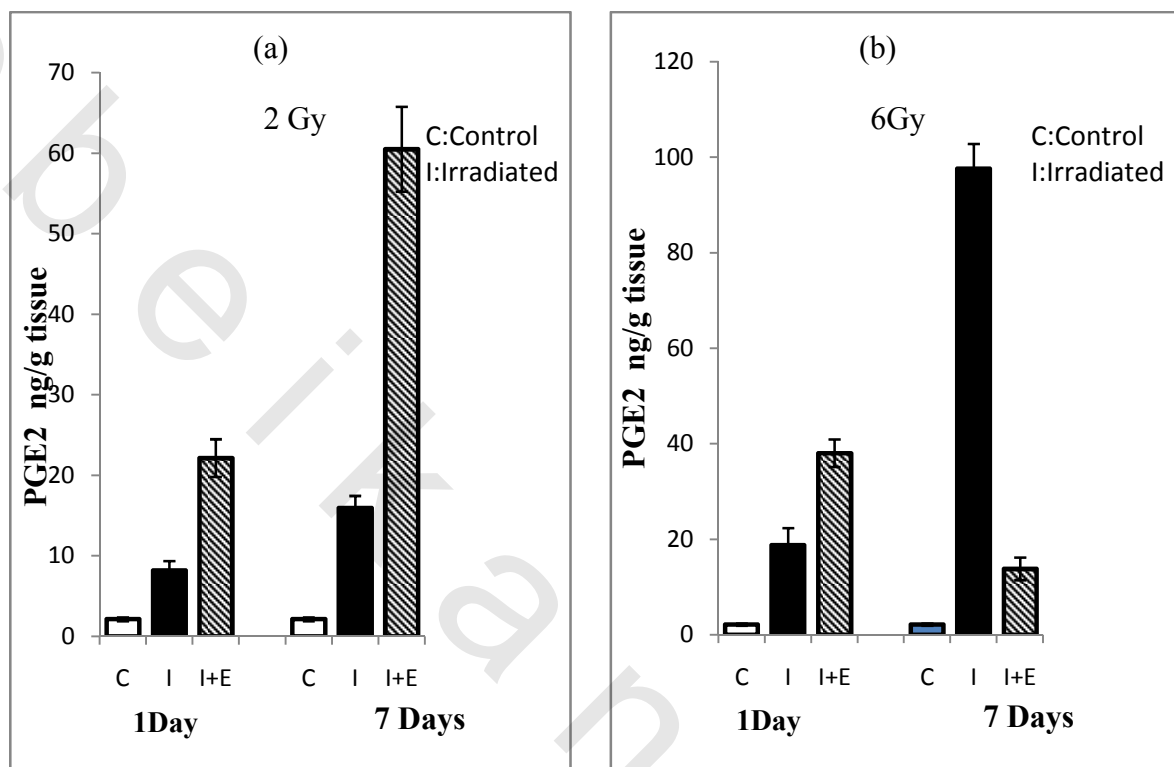


Figure (13): Changes in PGE2 in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

5. Oxidative stress and antioxidant defense:

A. Malondialdehyde:

Malondialdehyde, the principal product of lipid peroxidation is generally used as an indication of oxidative stress. After one day of irradiation by a 2Gy dose the mean level of MDA increased significantly to 3.8-fold that of control, but after seven days the level decreased by 7.2% although it was still 3.6-times the control level, $p < 0.05$ [Table 5, Figure14]. The 6Gy dose gave qualitatively similar results, but quantitatively higher levels. The level of MDA was 5.3-fold that of control after one day and only 3.9-fold after 7 days; a change of -25.5%.

Treatment with ectoine resulted in significant decreases in MDA levels in all irradiated groups, $p < 0.05$. MDA levels decreased by 12.8% and by 21% after one day and 7 days respectively, below the 2Gy irradiated group. However, the concentration of MDA decreased by 22.2% and 22.6% after 1 and 7 days of irradiation by 6Gy.

Table (5): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on MDA in the liver.

		1 Day	7 Days
Control (nmol/g tissue)		13.2 ± 2.1	
2Gy	Irradiated(nmol/g tissue)	50.8 ± 5.4 ^a	47.1 ± 3.6 ^{a*}
	Irradiated - Ectoine (nmol/g tissue)	44.3 ± 3.6 ^{ab}	37.2 ± 4.1 ^{ab*}
6 Gy	Irradiated (nmol/g tissue)	69.9 ± 7.0 ^{ab}	52.1 ± 3.6 ^{ab*}
	Irradiated - Ectoine (nmol/g tissue)	54.1 ± 6.2 ^{ac}	40.5 ± 3.8 ^{ac*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

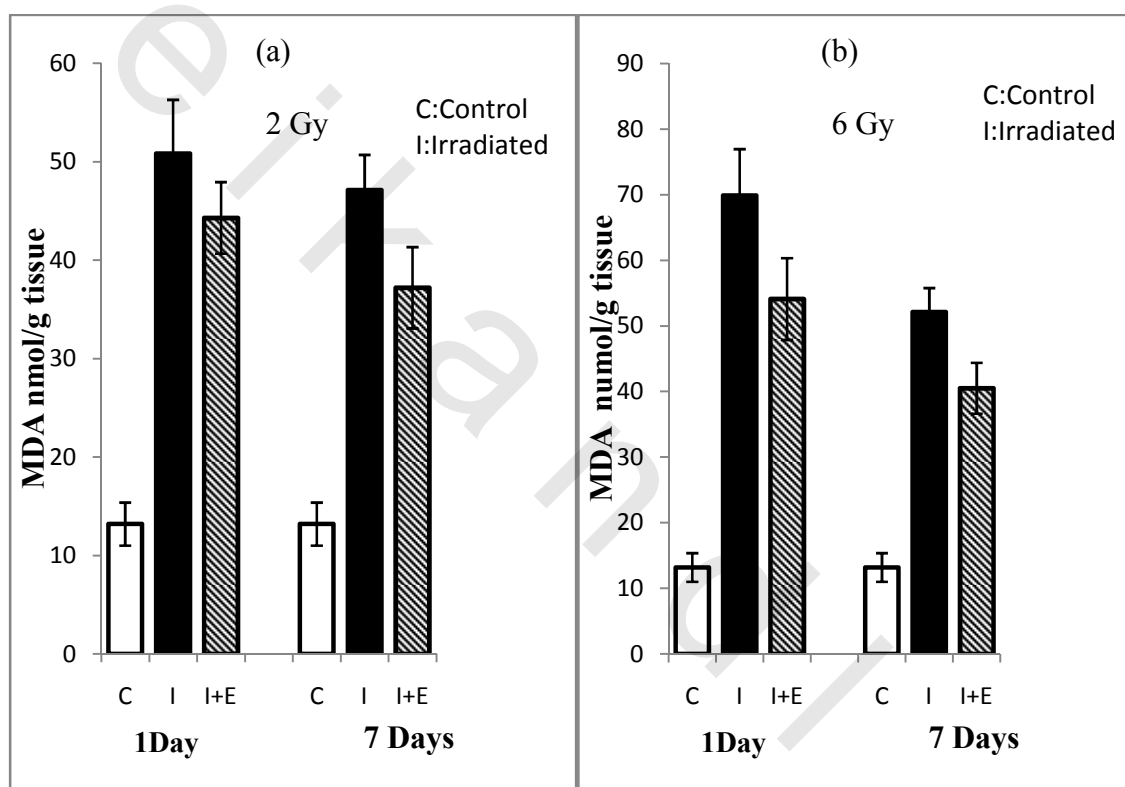


Figure (14): Changes in MDA in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

B. Glutathione:

The results of all forms of glutathione in the liver are presented in[Table (6,7) , Figure (15,16)]. There were practically no statistically significant differences in total glutathione levels either due to irradiation by the 2 Gy and the 6 Gy doses or due to treatment with ectoine. However, x-irradiation caused decreases in the reduced form. The 2 Gy dose resulted in a mean decrease of 15.3% after one day, which was corrected to a level 7.4% below that of control after 7 days. The effect of the 6 Gy dose was more prominent reaching 23.5% and 14.1% less than control after one and seven days respectively. By comparing the effects of the 2 radiation doses it could be seen that the 6Gy dose caused a further decrease below the 2Gy dose of 9.6% and 7.7% after one day and 7 days.

Treatment with ectoine caused a correction of the reduced glutathione level, as it caused an increase of 12.5% and 8.3% with the 2 Gy dose after one and 7 days. Such beneficial effect was higher with the 6Gy dose as the increases were 16.1% and 11.8%.

The oxidative stress by whole body x-irradiation was also reflected in the increased oxidation of glutathione. The oxidized form of glutathione (GSSG) doubled one day after irradiation with 2Gy. Such increased was halved by day 7 reaching only 48% above control. The 6Gy dose was stronger oxidizer, as the GSSG level increased by 132% above control after one day but again such increase went down to 64% after 7 days.

The beneficial effect of ectoine extended to the oxidized form of glutathione. Decreases in the levels of GSSG in the treated mice below the irradiated animals were found in all tested groups. Ectoine caused the concentration in animals irradiated with 2Gy to decrease by 24% after one day and by 16.2% after seven days as compared to the untreated groups. The effect was similar with the 6 Gy group as the decreases were 29.3% and 14.6%.

The values of the calculated redox potentials indicated that the liver cells were well in the reduced environment. The calculated values were within the range indicating that the liver cells were in the proliferative state. It is noted that the smallest value was that obtained after 24 hours following irradiation with 6 Gy. There were tendencies of shifting toward the control values after seven days.

Table (6): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on reduced glutathione in the liver.

		1 Day	7 Days
Control (μmol/g tissue)		4.05 ± 0.21	
2Gy	Irradiated(μmol /g tissue)	3.43 ± 0.24 ^a	3.75 ± 0.35 ^{a*}
	Irradiated - Ectoine (μmol /g tissue)	3.86 ± 0.17 ^{ab}	4.06 ± 0.33 ^{ab*}
6 Gy	Irradiated (μmol /g tissue)	3.10 ± 0.17 ^{ab}	3.48 ± 0.23 ^{ab*}
	Irradiated Ectoine (μmol /g tissue)	3.66 ± 0.18 ^{ac}	3.89 ± 0.26 ^{ac*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

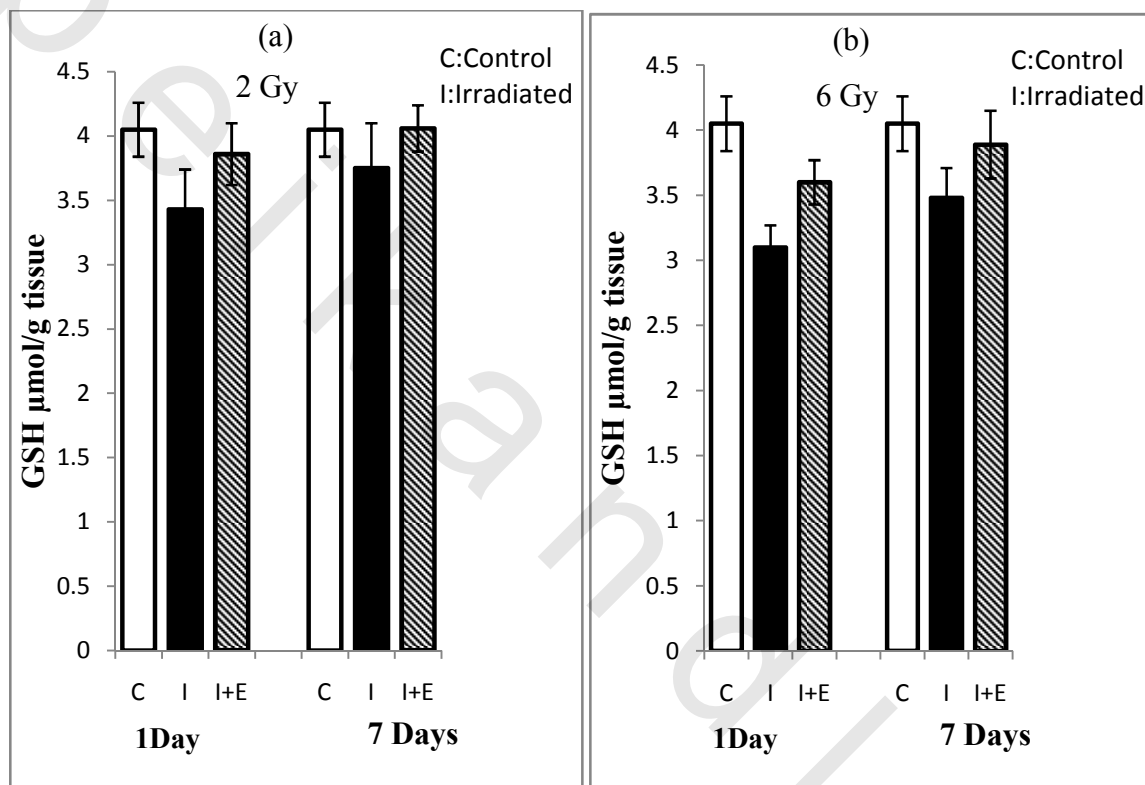


Figure (15): Changes in reduced glutathione in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

Table (7): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on oxidized glutathione in the liver.

		1 Day	7 Days
Control ($\mu\text{mol/g tissue}$)		0.25 ± 0.4	
2Gy	Irradiated($\mu\text{mol/g tissue}$)	0.50 ± 0.03^a	$0.37 \pm 0.04^{a*}$
	Irradiated - Ectoine ($\mu\text{mol/g tissue}$)	0.38 ± 0.04^{ab}	$0.31 \pm 0.02^{ab*}$
6 Gy	Irradiated ($\mu\text{mol/g tissue}$)	0.58 ± 0.03^{ab}	$0.41 \pm 0.02^{ab*}$
	Irradiated - Ectoine ($\mu\text{mol/g tissue}$)	0.41 ± 0.03^{ac}	$0.35 \pm 0.02^{ac*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

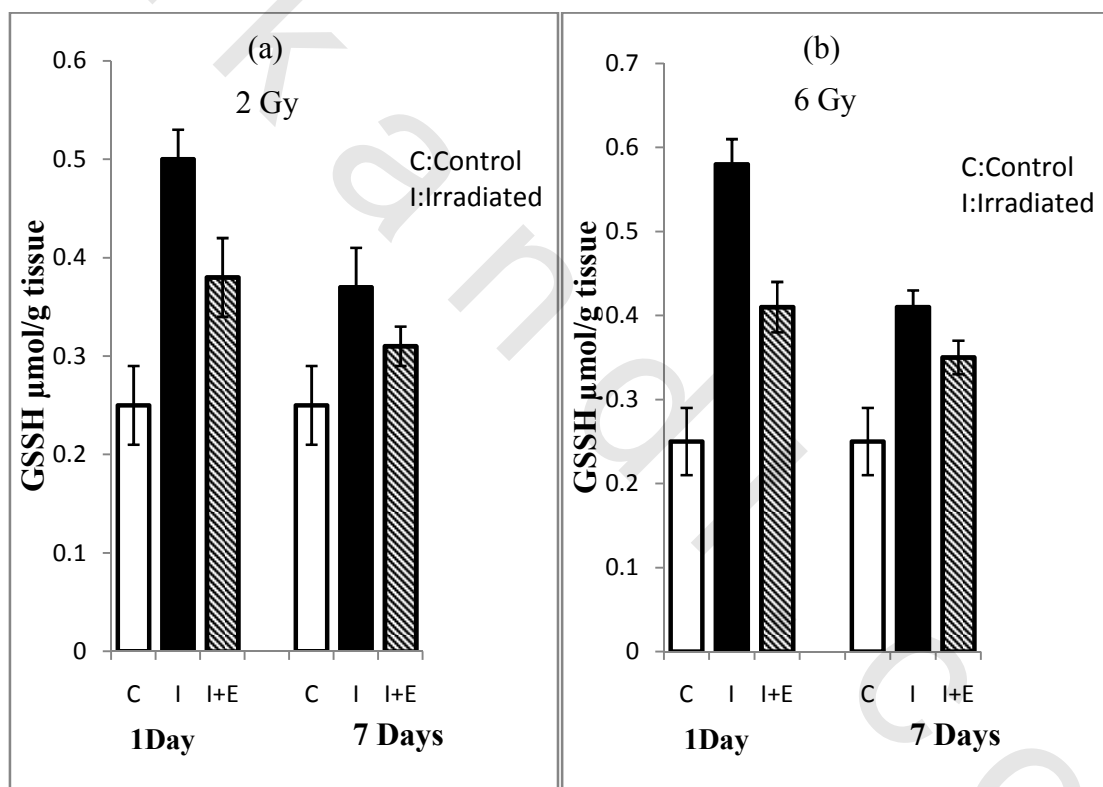


Figure (16): Changes in oxidized glutathione in mouse liver after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

Results of the brain:

1. Interleukin-1 β :

The concentration of IL-1 β increased in the brain as a result of whole body x-irradiation [Table 8, Figure 17]. The 2Gy dose caused significant increases over control levels of 33.9% after one day and 121.5% after 7 days, $p < 0.05$. Much higher increases were observed with the 6Gy dose reaching 404.3% after one day and 555.4% after 7 days. This means that the IL- β reached a level about 3.7-times and about 2.9-times those detected with 2Gy after one and seven days respectively, $p < 0.05$.

The effects of treatment with ectoine were dependent on both the radiation dose and time after irradiation, and in either case they were significantly higher than the mean level detected in the control group, $p < 0.05$. After the 2Gy dose such increases reached 108.6% and 61.3% after one and seven days, while with the 6Gy dose they were 92.5% and 58.2%. It should be noted, however, that the treatment with ectoine caused an additional increase of 55.8% over the irradiation level only one day after the 2Gy dose, $p < 0.05$. With the other irradiated groups administration of ectoine caused significant decreases in the levels of IL-1 β . With the same 2Gy dose a decrease of 27.2% was seen after 7 days. With the higher 6Gy the IL-1 β level decreased below the irradiated groups by 61.8% and 75.6% after one and seven days respectively, $p < 0.05$.

Table (8): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-1 β in the brain.

		1Day	7 days
Control (pg/g tissue)		3.72 ± 0.25	
2Gy	Irradiation (pg/g tissue)	4.98 ± 0.34^a	$8.24 \pm 0.38^{a*}$
	Irradiation + Ectoine (pg/g tissue)	7.76 ± 0.36^{ab}	$6.00 \pm 0.48^{ab*}$
6 Gy	Irradiation (pg/g tissue)	18.76 ± 1.13^{ab}	$24.38 \pm 1.35^{ab*}$
	Irradiation + Ectoine (pg/g tissue)	7.16 ± 0.51^{abc}	$5.96 \pm 0.24^{abc*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

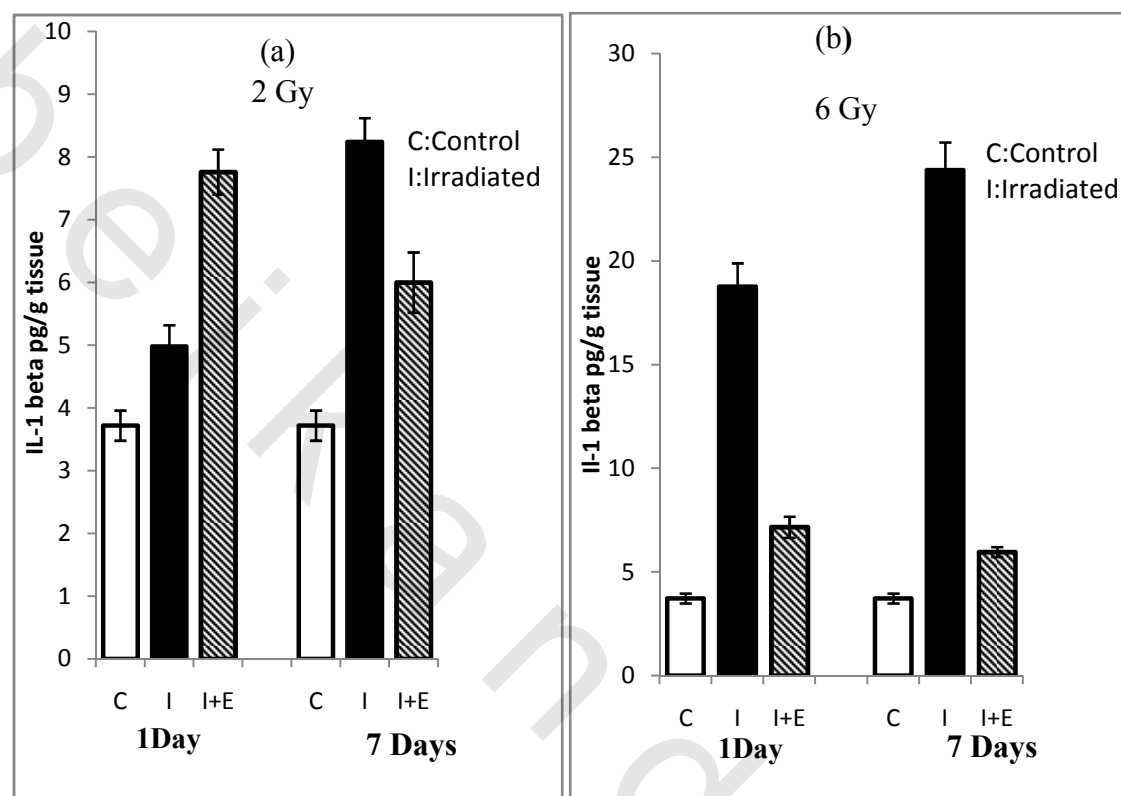


Figure (17): Changes in IL-1 beta in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

2. Interleukin-6:

Similar to what was found in the liver, IL-6 in the brain showed a substantial increase as a result of whole body x-irradiation [Table 9, Figure 18]. With the 2Gy such increases were 521.8% after one day and 919.4% after 7 days above control values, $p < 0.05$. With the 6Gy IL-6 reached levels more than 12-times the control value after one day and more than 17-times after 7 days, $p < 0.05$. It could be seen that the increases were dose-dependent since the 6Gy dose caused significant increases above the 2Gy of 103.4% and 66.9 % after one and 7 days respectively, $p < 0.05$. Again, similar to what was found in the liver ectoine caused a significant increase in the IL-6 levels in the 2Gy-treated group of 19.7% after one day, while the other irradiated groups showed significant decreases of 56.1% seven days after irradiation with 2Gy, 25.1% and 43.7% one and seven days after irradiation with 6Gy.

Table (9): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-6 in the brain.

		1Day	7 Days
Control (pg/g tissue)		2.11 ± 0.18	
2Gy	Irradiated (pg/g tissue)	13.12 ± 1.10 ^a	21.51 ± 1.38 ^{a*}
	Irradiated – Ectoine (pg/g tissue)	15.71 ± 1.16 ^{ab}	9.45 ± 0.76 ^{ab*}
6 Gy	Irradiated (pg/g tissue)	26.69 ± 2.18 ^{ab}	35.90 ± 2.92 ^{ab*}
	Irradiated Ectoine (pg/g tissue)	19.99 ± 2.15 ^{ab}	20.21 ± 1.95 ^{ab}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

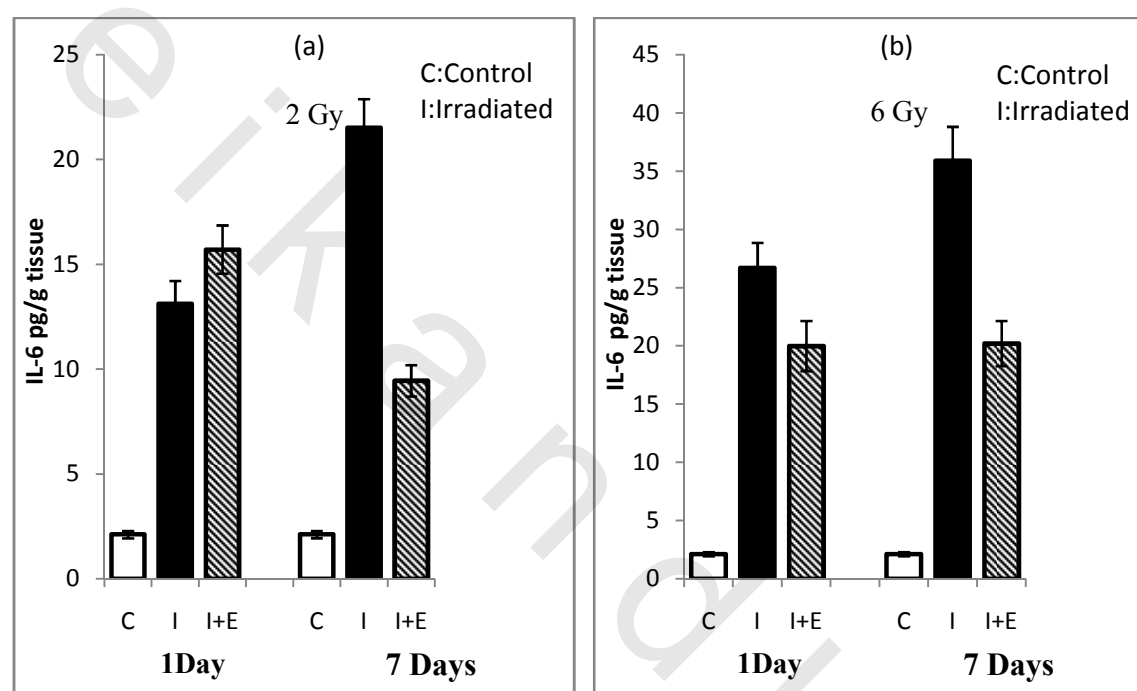


Figure (18): Changes in IL-6 in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

3. Interleukin-10

The anti-inflammatory IL-10 in the brain was much lower than in the liver, but showed a large increase as a result of exposure to whole body x-irradiation. At day one after the 2Gy dose its level was about 3.3-fold that of control value and went up to more than 6-fold after 7 days, $p < 0.05$ [Table 10, Figure 19]. The increase was even greater following irradiation with 6Gy, as the level exceeded 7.6-times and 12.1-times that of control after one day and 7 days respectively. The higher 6Gy dose caused significant increases of 128.1% and 99.8% over the 2Gy dose after one and 7 days, $p < 0.05$.

The pattern of change in IL-10 after treatment with ectoine was the same as seen with the other determined interleukins. A single dose of ectoine resulted in a slight non-significant increase over the irradiated group of 9.8% one day after irradiation with 2Gy. However, after 7 days and multiple dosing with ectoine IL-10 level decreased significantly by 56.1% below the mean of the group which did not receive ectoine. On the other hand, administration of ectoine caused a significant decrease in the IL-10 level of 27.0% and 57.8% below the 6Gy irradiated groups after one and seven days.

Table (10): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-10 in the brain.

		1Day	7 days
Control (pg/g tissue)		3.19 ± 0.39	
2Gy	Irradiated (pg/g tissue)	10.65 ± 0.76 ^a	19.39 ± 1.34 ^{a*}
	Irradiated - Ectoine (pg/g tissue)	11.70 ± 1.25 ^{ab}	8.52 ± 0.95 ^{ab*}
6 Gy	Irradiated (pg/g tissue)	24.29 ± 1.91 ^{ab}	38.74 ± 2.65 ^{ab*}
	Irradiated - Ectoine (pg/g tissue)	17.74 ± 1.70 ^{ac}	16.36 ± 1.66 ^{ac}

a: significantly different from control, $p < 0.05$

b: significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

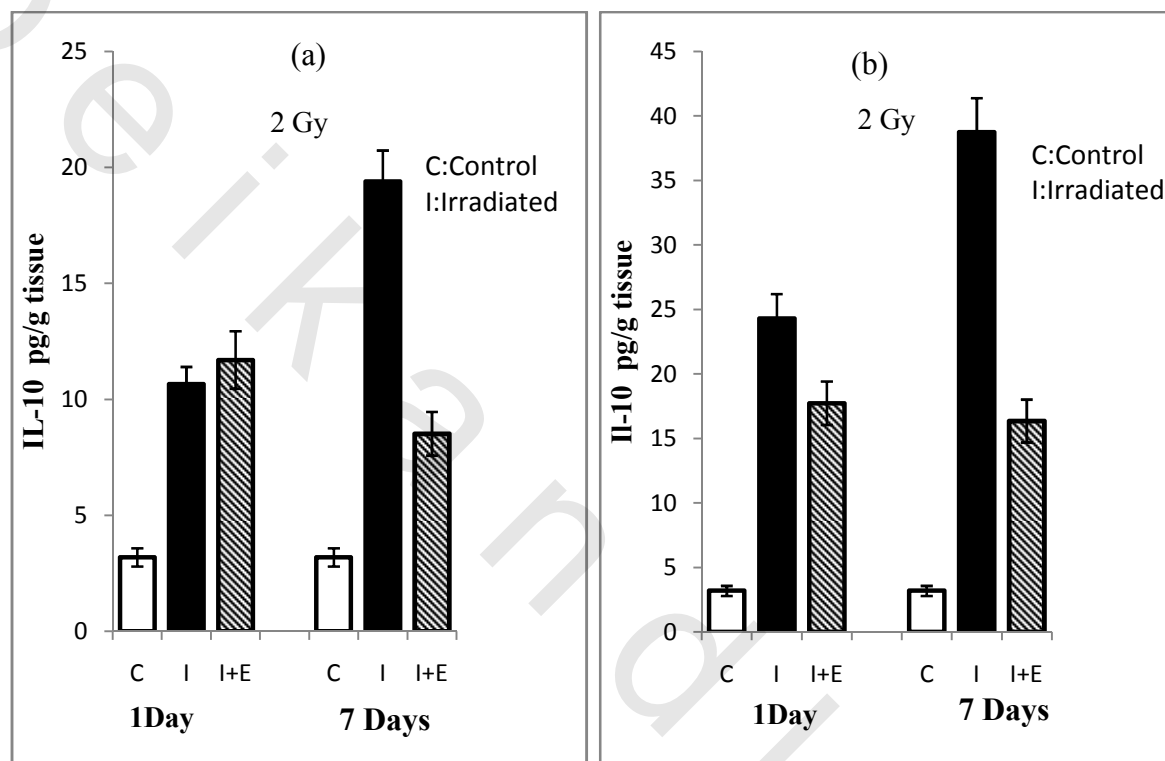


Figure (19): Changes in IL-10 in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

4. Prostaglandin E2:

The concentration of PGE2 in the whole brain of control mice was the highest of the 3 organs tested. Whole body X-irradiation caused a strong elevation in this parameter dependent on the radiation dose and the post-irradiation time [Table 11, Figure20]. The 2Gy dose caused significant increases of 202.8% after one day and 511.3% after 7 days over control values, $p < 0.05$. The increase in PGE2 was even higher with the 6Gy as the level reached 5.4-times and about 23.3-times that of control after one and seven days of irradiation. These data indicate that 6Gy dose of x-irradiation caused a significant increase in the level of PGE2 of 78.7% and 280.9% above the 2Gy after one and 7 days respectively, $p < 0.05$.

Unlike what was observed in the other determined parameters, administration of ectoine resulted in significant increases of PGE2 in the brain, except in one case. One day and seven days after irradiation with 2Gy the mean level in the brain showed significant increases of 150.1% and 169.9% above untreated irradiated mice, $p < 0.05$. An increase of 262.7% was also observed after one day of irradiation with 6Gy. However, after 7 days the level of PGE2 decreased by 79.5% as compared to the irradiated untreated group, $p < 0.05$.

Table (11): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on PGE2 in the brain.

		1Day	7 Days
Control (ng/g)		5.33 ± 1.13	
2Gy	Irradiated(ng/g)	16.14 ± 1.92 ^a	32.58 ± 4.12 ^{a*}
	Irradiated - Ectoine (ng/g)	40.36 ± 4.70 ^{ab}	87.93 ± 5.78 ^{ab*}
6 Gy	Irradiated(ng/g)	28.85 ± 3.53 ^{ab}	124.09 ± 6.12 ^{ab*}
	Irradiated - Ectoine (ng/g)	104.64 ± 5.20 ^{ac}	25.41 ± 3.63 ^{ac*}

a significantly different from control , $p < 0.05$

b significantly different from Irradiation 2 Gy , $p < 0.05$

c: significantly different from Irradiation 6Gy, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

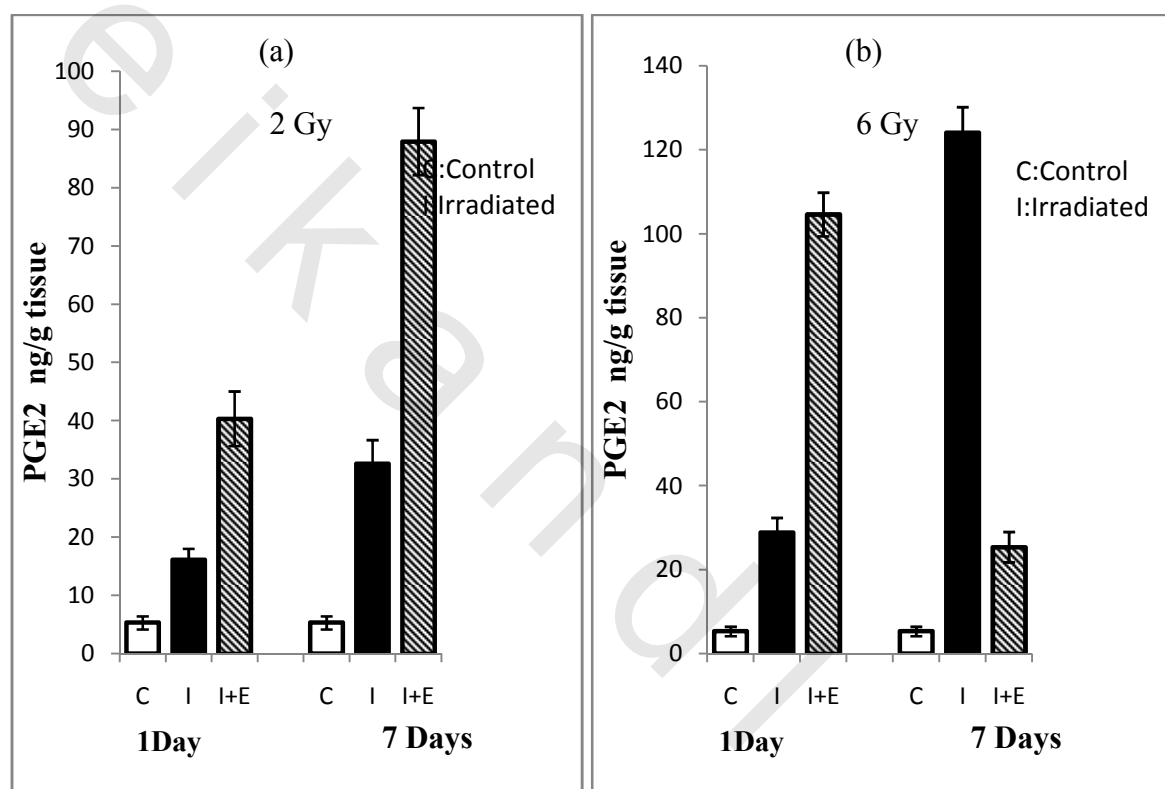


Figure (20): Changes in PGE2 in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

5. Oxidative stress and antioxidant defense:

A. Malondialdehyde:

The qualitative changes in brain MDA after whole body x-irradiation were similar to what was seen in the liver. One day after the 2Gy dose, the mean level of MDA was 681.4% higher than control, followed after 7 days by a decrease of one third, although it was still 420.9% higher than control (Table 12, Figure 21]. The effect of 6Gy was similar but more exaggerated. On the average, MDA showed concentrations 9.8-times the control after one day decreasing to 6.5-times the control after 7 days. This means that the 6Gy dose in both time points gave a mean level about 24 to 25% higher than the 2Gy.

Treatment with ectoine of mice irradiated with 2Gy caused a significant decrease in MDA of 10% after one day and 26.3% after 7 days, $p < 0.05$. With the 6Gy dose the decreases were 43.3% and 57.1% after the same time periods.

Table (12): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on MDA in the brain.

		1 Day	7 Days
Control (nmol/g tissue)		8.6 ± 1.2	
2Gy	Irradiated(nmol/g tissue)	67.2 ± 4.4 ^a	44.8 ± 3.6 ^{a*}
	Irradiated - Ectoine (nmol/g tissue)	60.5 ± 4.5 ^{ab}	33.0 ± 2.9 ^{ab*}
6 Gy	Irradiated (nmol/g tissue)	84.1 ± 5.5 ^{ab}	55.7 ± 3.6 ^{ab*}
	Irradiated - Ectoine (nmol/g tissue)	47.7 ± 2.88 ^{abc}	23.9 ± 3.36 ^{abc*}

a: significantly different from control, $p < 0.05$

b: significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

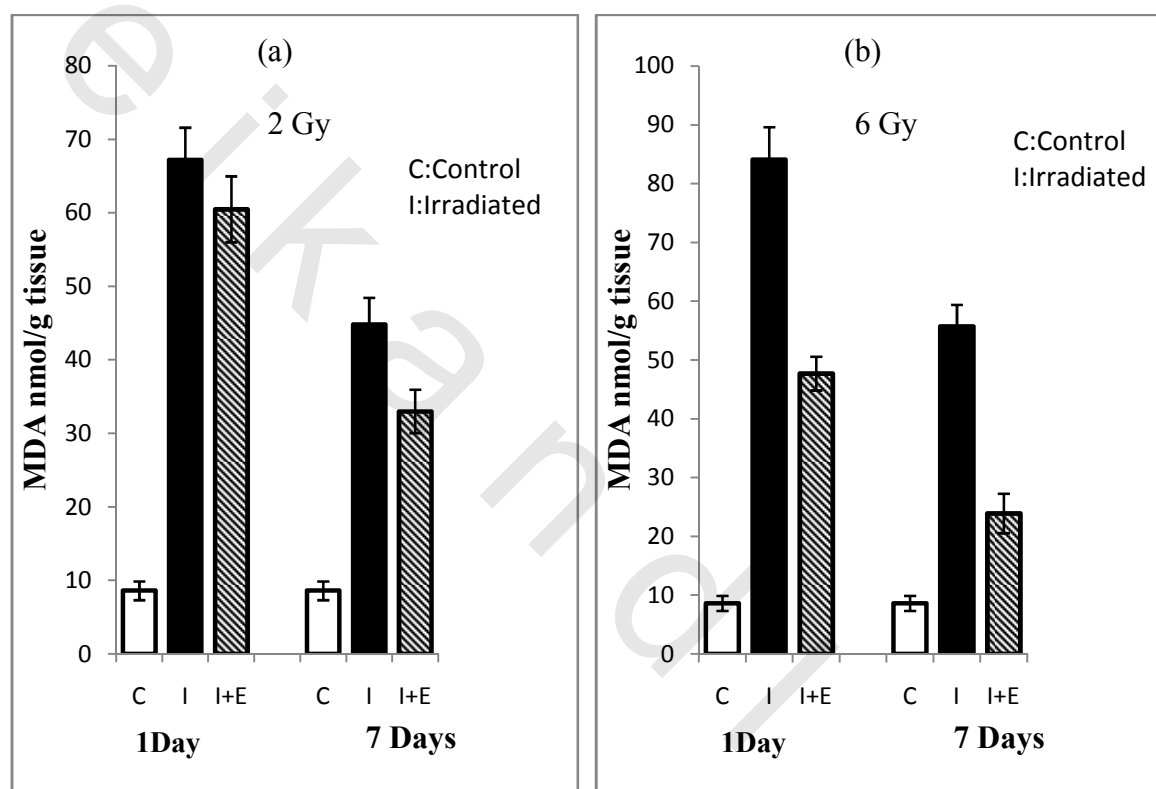


Figure (21): Changes in MDA in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

B. Glutathione:

As in the case of the liver there were practically no significant differences in the mean concentrations of total glutathione in the brains of all the irradiated groups or those treated with ectoine [Table(13,14), Figure(22,23)]. However, differences were noted in the reduced form, as its level was decreased following whole body x-irradiation. However, it is noteworthy that the effect of radiation was larger after one day and was attenuated after seven days, contrary to what was seen with interleukins or prostaglandin. The 2Gy dose caused 21.9% decrease after one day and 15.5% after seven days. The effect of the 6 Gy was larger as it reached -43.8% and -27.0% after one and seven days.

Treatment with ectoine was beneficial as it caused increases in GSH of 11.5% and 10.7% in the 2 Gy irradiated groups after one and seven days and 22.1% and 26,5% in the 6 Gy groups after the same time periods.

Table (13): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on reduced glutathione in the brain.

		1 Day	7 Days
Control (μmol /g tissue)		2.33 ± 0.27	
2Gy	Irradiated(μmol /g tissue)	1.82 ± 0.29 ^a	1.97 ± 0.22 ^{a*}
	Irradiated - Ectoine (μmol /g tissue)	2.03 ± 0.15 ^{ab}	2.18 ± 0.13 ^{ab*}
6 Gy	Irradiated (μmol /g tissue)	1.31 ± 0.07 ^{ab}	1.70 ± 0.24 ^{ab*}
	Irradiated – Ectoine (μmol g tissue)	1.60 ± 0.14 ^{abc}	2.15 ± 0.34 ^{abc*}

a: significantly different from control, p< 0.05

b significantly different from 2 Gy Irradiation , p< 0.05

c: significantly different from 6 Gy Irradiation , p< 0.05

*: significantly different from 1 day, p< 0.05

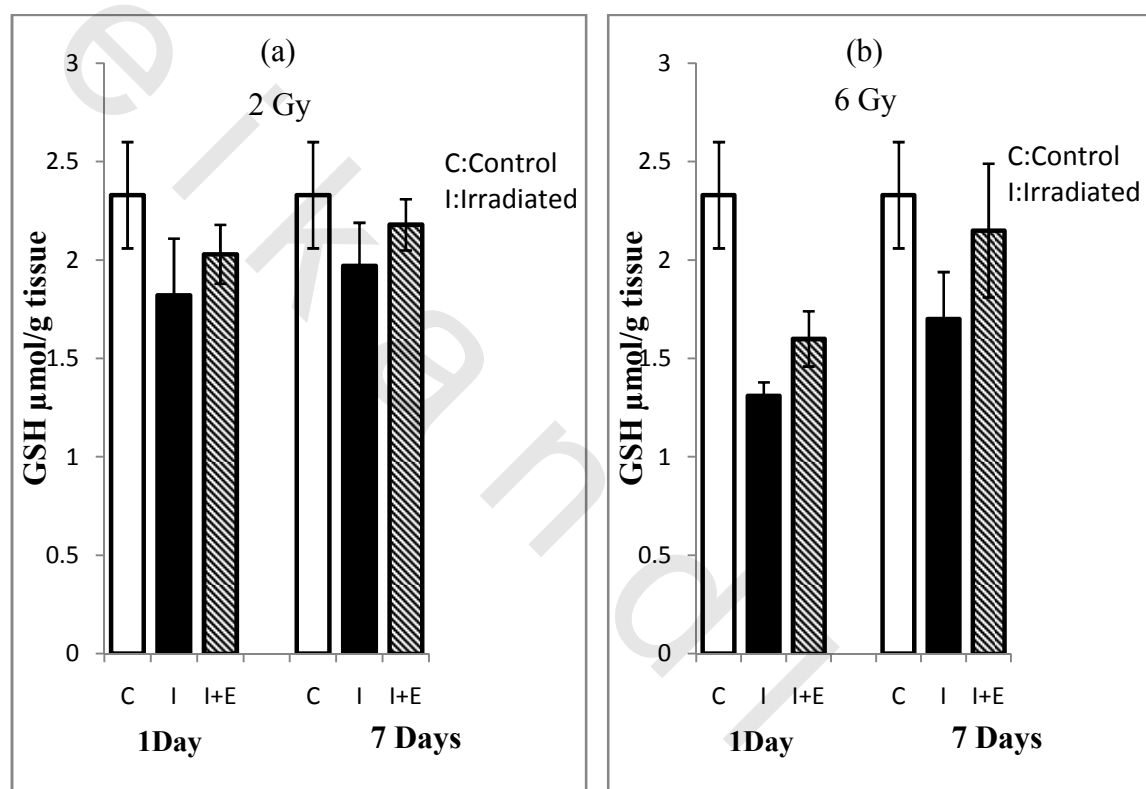


Figure (22): Changes in reduced glutathione in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

Results

The oxidized form of glutathione increased as a result of x-irradiation. Although the absolute values of such increases were not substantial, the percentage changes were relatively large. Irradiation with 2Gy caused a relative increase of 181.9% and 90.0% over control after one and seven days. As may be expected, the effect of the 6 Gy dose was larger reaching 236.4% and 118.8% within the same time frame. The percentage increases over the 2Gy were 19.4% and 14.3%.

Treatment with ectoine attenuated the relative increases in GSSG. The obtained values were approximately between 24% and 29% below the corresponding irradiated untreated groups.

Results of the calculated redox potential showed that the 6 Gy radiation dose caused the largest shift away from the control value after 24hours (-259.6 mV) with a tendency for going back toward normal after seven days.

Table (14): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on oxidized glutathione in the brain.

		1 Day	7 Days
Control ($\mu\text{mol/g}$ tissue)		0.11 ± 0.01	
2Gy	Irradiated($\mu\text{mol /g}$ tissue)	0.31 ± 0.03^a	$0.21 \pm 0.02^{a*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	0.22 ± 0.02^{ab}	$0.16 \pm 0.01^{ab*}$
6 Gy	Irradiated ($\mu\text{mol /g}$ tissue)	0.37 ± 0.02^{ab}	$0.24 \pm 0.17^{ab*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	0.28 ± 0.02^{abc}	$0.17 \pm 0.02^{abc*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

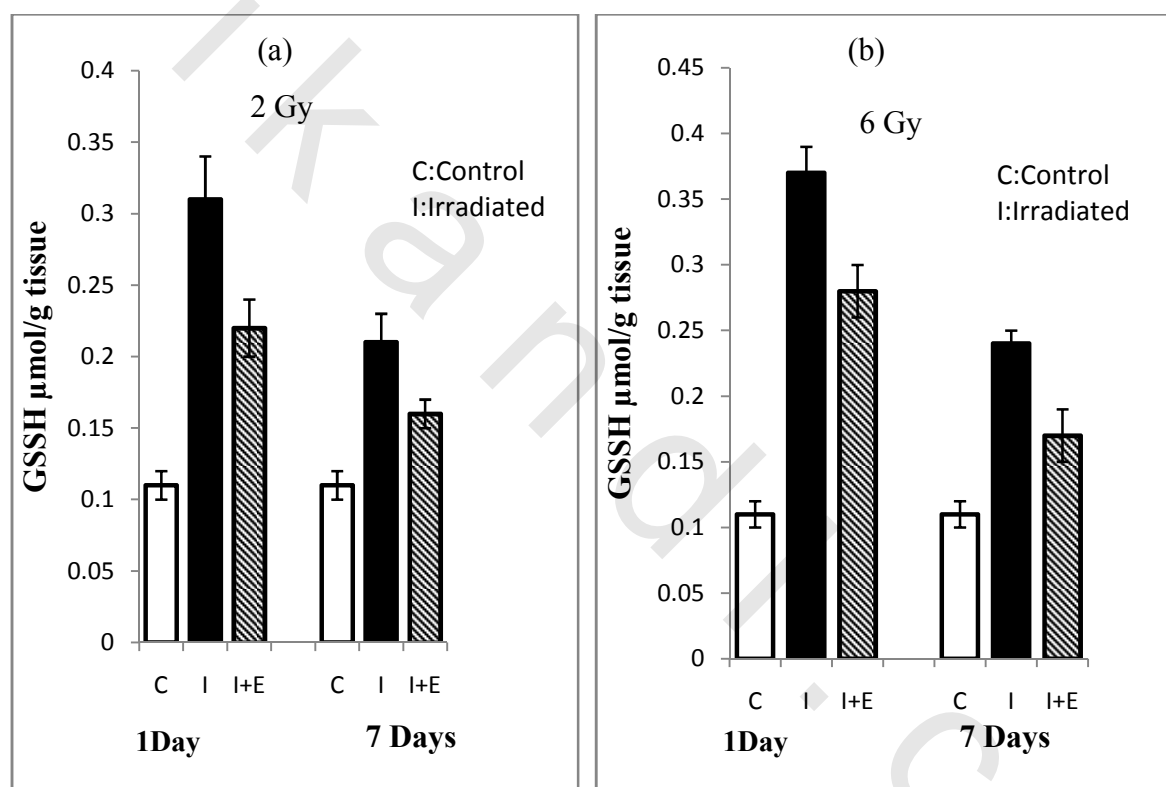


Figure (23): Changes in oxidized glutathione in mouse brain after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

Results of the testis:

1. Interleukin 1-β:

In common with the results of IL-1β in other tested tissues, whole body x-irradiation caused significant elevations in the values of this parameter above that of control, $p < 0.05$ [Table 15, Figure 24]. The 2Gy dose caused increases of 46.0% and 144.4% one and seven days post-irradiation. With the 6Gy the calculated values were 7.5-fold and 10.2-fold the control in these 2 time points. This means that the effect of radiation was dose-dependent as the 6Gy gave a mean value 414.1% and 319.5% above those obtained with the 2Gy dose one and seven post-irradiation respectively.

Treatment with ectoine affected the levels of IL-1β in all tested samples. When irradiated mice were treated with ectoine the extent of change was dependent on the radiation dose and duration of drug administration. Treatment of mice irradiated with 2Gy for one day resulted in significant increase of 41.3% while continued treatment for 7 days caused a significant decrease of 51.3% as compared to untreated irradiated mice, $p < 0.05$. The effect on the mice treated with 6Gy was more apparent as it was 70.8% after one day and 86.8% after 7 days below the irradiated levels.

Table (15): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-1 beta in the testis.

		1Day	7 days
Control (pg/g tissue)		1.26 ± 0.11	
2Gy	Irradiated(pg/g tissue)	1.84 ± 0.17 ^a	3.08 ± 0.24 ^{a*}
	Irradiated - Ectoine (pg/g tissue)	2.60 ± 0.14 ^{ab}	1.50 ± 0.16 ^{ab*}
6 Gy	Irradiated (pg/g tissue)	9.46 ± 0.53 ^{ab}	12.92 ± 1.33 ^{ab*}
	Irradiated - Ectoine (pg/g tissue)	2.76 ± 0.32 ^{abc}	1.70 ± 0.20 ^{abc*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

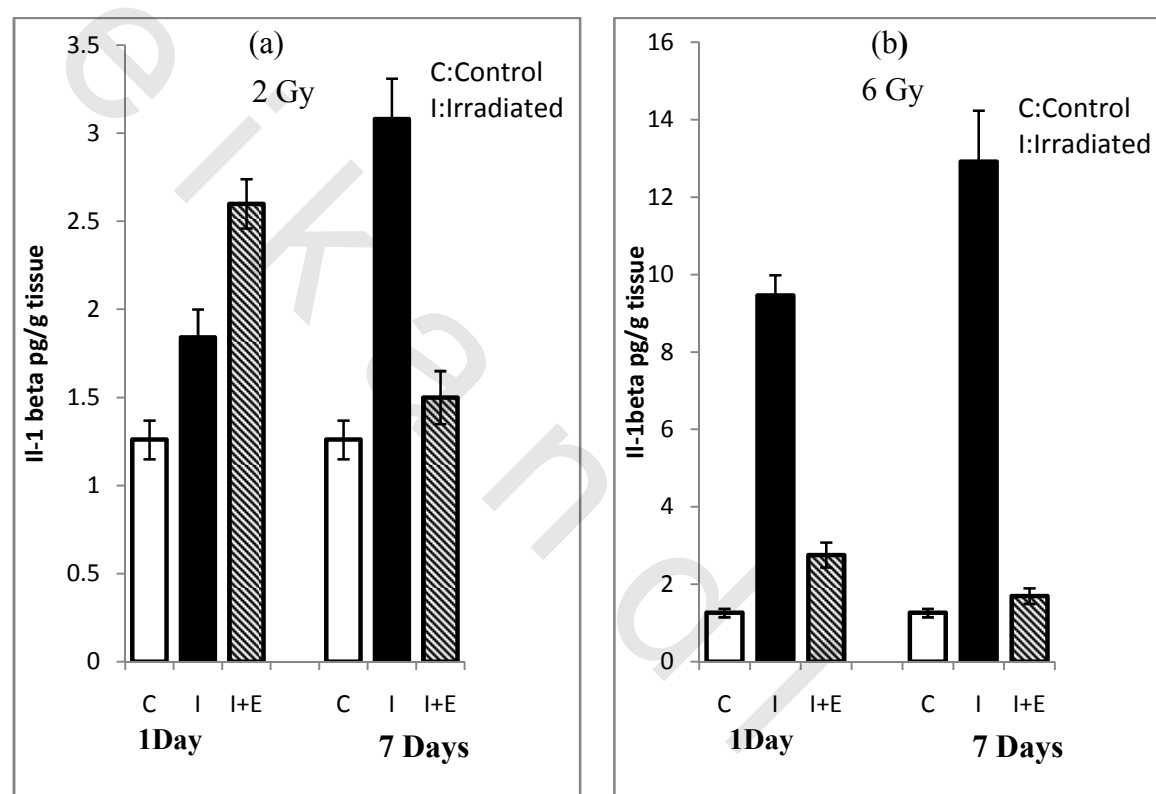


Figure (24): Changes in IL-1 beta in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

2. Interleukin-6:

The results of the effect of x-irradiation on the level of testicular IL-6 are presented in [Table 16, Figure 25]. One day after the 2Gy doses an increase of 88.2% was detected, which went up to 160.3% above control after 7 days, $p < 0.05$. The effect of the 6Gy was greater as IL-6 reached levels about 5.4-fold and 9.7-fold the control, one and seven days after irradiation respectively. By comparing the 2 radiation doses, it could be seen that the 6Gy dose caused increases over the 2Gy of 185.2% after one day and 272.9% after 7 days, $p < 0.05$.

Treatment of irradiated mice with ectoine affected the assayed levels of IL-6, depending on the radiation dose and duration of treatment. Treatment of the 2Gy caused a minimal non-significant increase in IL-6 of 3.1% over the irradiated untreated group. With all the other groups, ectoine caused significant decreases in IL-6 levels below the corresponding irradiated untreated groups. After 7 days of ectoine treatment the level of IL-6 in the group receiving the 2Gy decreased by 37.9%. With the 6Gy, the effect started as early as one day decreasing by 45.2% and reaching 57.6% after 7 days. However, such levels were still higher than control value, $p < 0.05$.

Table (16): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-6 in the testis

		1Day	7 Days
Control (pg/g tissue)		0.68 ± 0.11	
2Gy	Irradiated (pg/g tissue)	1.28 ± 0.13^a	$1.77 \pm 0.13^{a*}$
	Irradiated - Ectoine (pg/g tissue)	1.32 ± 0.11^{ab}	1.10 ± 0.09^a
6 Gy	Irradiated (pg/g tissue)	3.65 ± 0.48^{ab}	$6.60 \pm 0.73^{ab*}$
	Irradiated - Ectoine (pg/g tissue)	2.0 ± 0.15^{abc}	$2.80 \pm 0.36^{abc*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

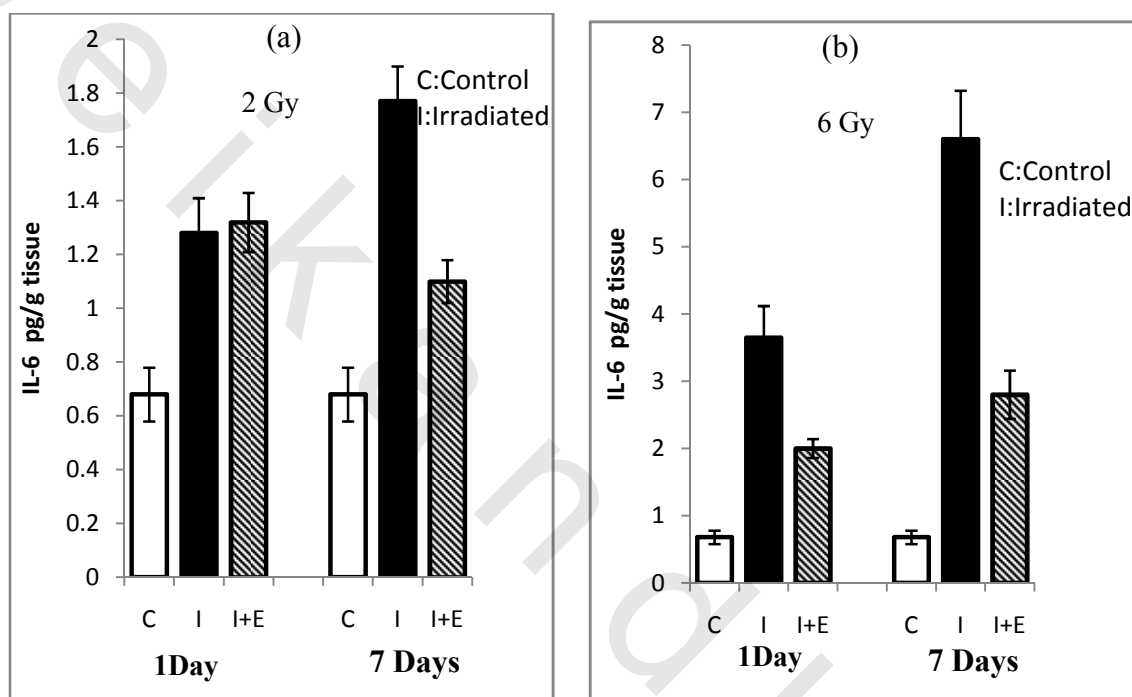


Figure (25): Changes in IL-6 in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

3. Interleukin 10:

Results of IL-10 in the testes are presented in [Table 17, Figure (26)]. The level of this anti-inflammatory cytokine increased significantly as a result of exposure of the animals to whole body x-irradiation. Exposure to the 2Gy radiation dose caused increases of 86.8% after one day and 216.9% after 7 days, above the mean control level. These values were higher after the 6Gy dose reaching 6.6-fold and 18.5-fold the control value after one day and 7 days, $p < 0.05$. It is clear that the changes were dependent on the radiation dose since the mean value for the animals receiving the 6Gy dose was higher than that of the 2Gy group by 252.5% after one day and by 483.9% after 7 days.

Results of treatment with ectoine were qualitatively similar to those of IL-6. Although the level of IL-10 was higher by 28.3% above the untreated group after one day of radiation with 2Gy, such comparison showed a decrease of 36.3% after 7 days. Treatment with ectoine resulted in significant decreases of the mean values of IL-10 below the untreated groups irradiated with 6Gy of 37.2% after one day and 67.9% after 7 days.

Table (17): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on IL-10 in the testis

		1Day	7 days
Control (pg/g tissue)		0.53 ± 0.15	
2Gy	Irradiated (pg/g tissue)	0.99 ± 0.08^a	$1.68 \pm 0.11^{a*}$
	Irradiated - Ectoine (pg/g tissue)	1.27 ± 0.08^{ab}	$1.07 \pm 0.11^{ab*}$
6 Gy	Irradiated (pg/g tissue)	3.49 ± 0.52^{ab}	$9.81 \pm 0.99^{ab*}$
	Irradiated – Ectoine (pg/g tissue)	2.19 ± 0.16^{abc}	$3.15 \pm 0.33^{abc*}$

a: significantly different from control, $p < 0.05$

b: significantly different from 2 Gy Irradiation, $p < 0.05$

c: significantly different from 6 Gy Irradiation, $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

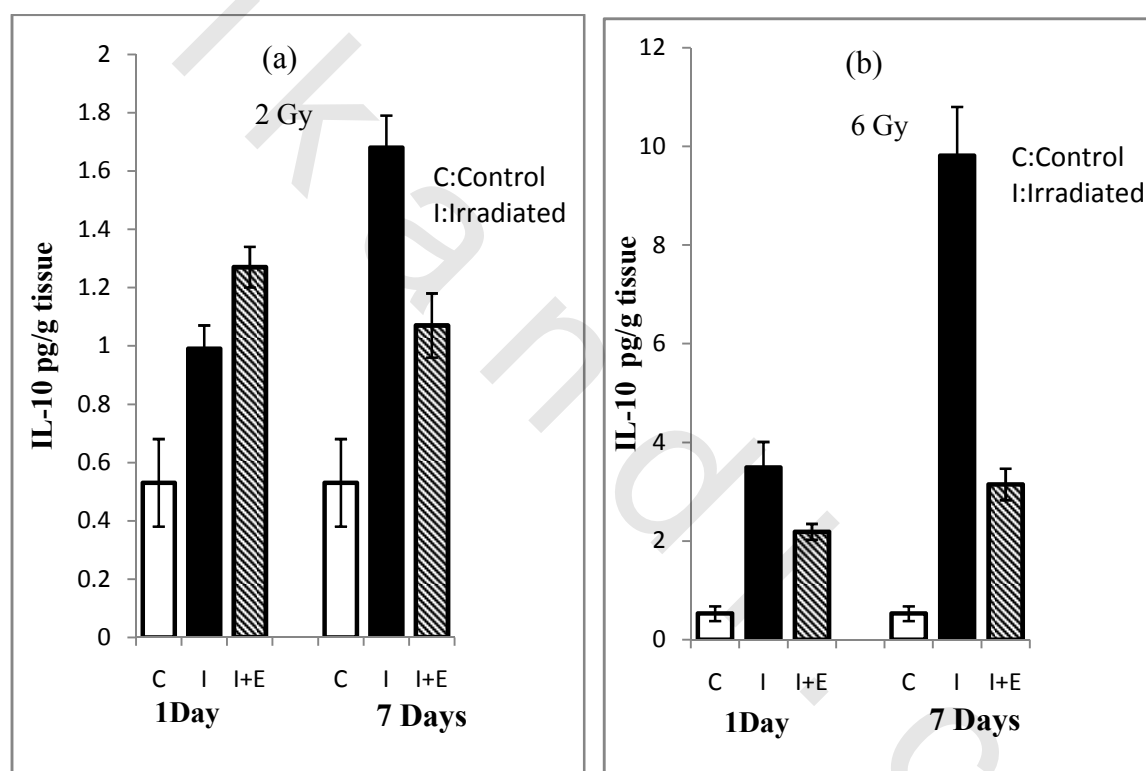


Figure (26): Changes in IL-10 in mouse testis after one day and 7days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

4. Prostaglandin E2:

Whole body X-rays caused a strong elevation in PGE2 dependent on the radiation dose and the post-irradiation time [Table 18, Figure 27]. Following the 2Gy dose the concentration of PGE2 in the testicles increased to about 3.2-times that of the control group after one day and about 4.8-times after 7 days. The level of PGE 2 was much higher with the 6Gy as the it reached 22.2-times and 51.1-times that of control after one and seven days of irradiation. These data indicate that 6Gy dose of x-irradiation caused a much higher increase in the level of PGE2 of 603.7% and 971.0% above the 2Gy after one and 7 days respectively, $p < 0.05$.

Treatment with ectoine caused an elevation in PGE2 one day and seven days after irradiation with 2Gy as the mean levels in the testicles showed increases of 717.1% and 144.4% over the irradiated untreated animals. To the contrary, treatment of the animals irradiated with 6Gy caused significant decreases of 61.4% and 87.0% after one and seven days as compared to the corresponding irradiated untreated groups, $p < 0.05$.

Table (18): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on PGE2 in the testis

		1Day	7 days
Control (ng/g)		0.26 ± 0.07	
2Gy	Irradiated(ng/g)	0.82 ± 0.10 ^a	1.24±0.12 ^{a*}
	Irradiated - Ectoine (ng/g)	6.70 ± 0.64 ^{ab}	3.03± 0.31 ^{ab*}
6 Gy	Irradiated(ng/g)	5.77 ± 0.67 ^{ab}	13.28 ± 1.88 ^{ab*}
	Irradiated - Ectoine (ng/g)	2.23 ± 0.21 ^{abc}	1.72 ± 0.28 ^{abc*}

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

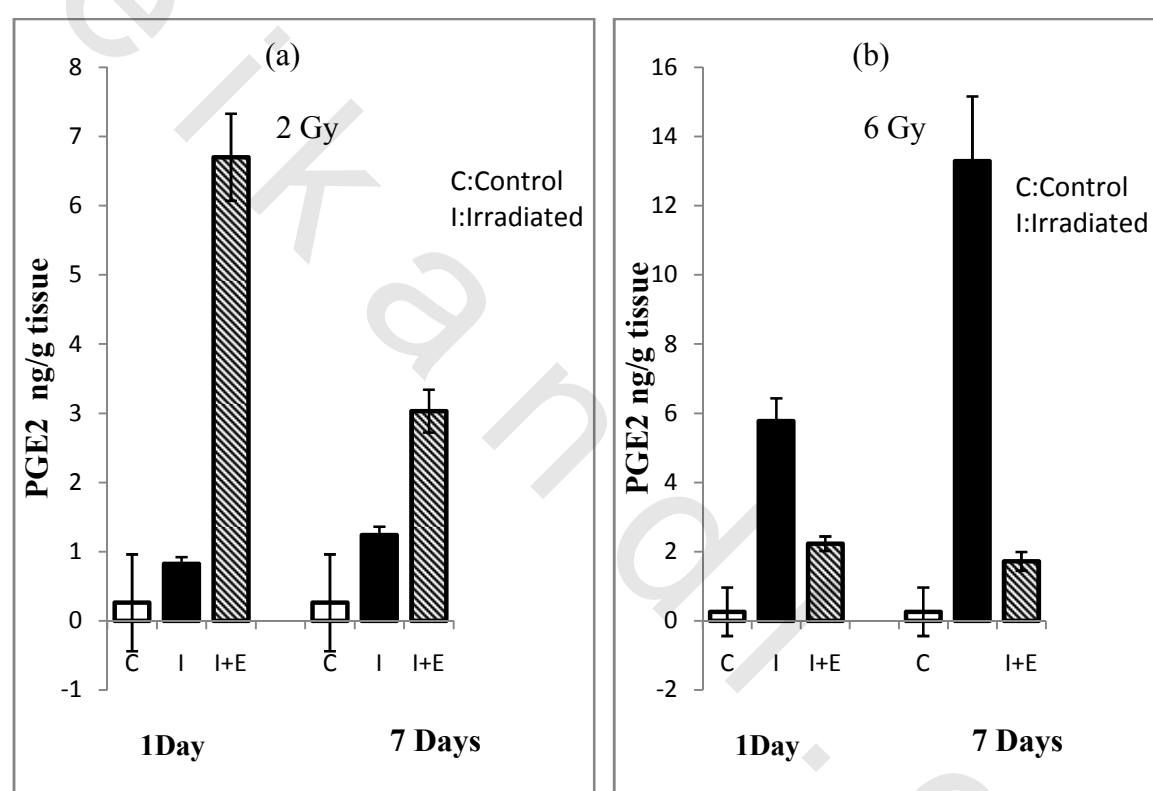


Figure (27): Changes in PGE2 in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

5.Oxidative stress and antioxidant defense:

A. Malondialdehyde:

One day after the whole body 2Gy dose, the mean level of MDA was about 6.9 times the control value, relatively declining after 7 days to 4.7-times. [Table 19, Figure 28]. The effect of 6Gy was similar but more exaggerated. On the average, MDA showed concentrations 9.7-times the control after one day decreasing to 4.6-times the control after 7 days, $p < 0.05$. This means that the 6Gy dose gave a mean level of MDA, in the testicles, 39.8% higher than the 2Gy after one day, $p < 0.05$. However, after 7 days MDA levels in both groups were not significantly different.

Treatment with ectoine of mice irradiated with 2Gy caused a significant decrease in MDA levels of 14.5% after one day and 25.4% after 7 days. With the 6Gy dose the decreases were 63.3% and 38.2% after the same time periods, $p < 0.05$.

Table (19): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on MDA in the testis

		1 Day	7 Days
Control (nmol/g tissue)		0.89 ± 0.12	
2Gy	Irradiated(nmol/g tissue)	6.15 ± 0.53^a	$4.18 \pm 0.41^{a*}$
	Irradiated - Ectoine (nmol/g tissue)	5.26 ± 0.68^{ab}	$3.12 \pm 0.31^{ab*}$
6 Gy	Irradiated (nmol/g tissue)	8.60 ± 0.63^{ab}	$4.11 \pm 0.42^{a*}$
	Irradiated - Ectoine (nmol/g tissue)	3.16 ± 0.34^{abc}	$2.54 \pm 0.35^{abc*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

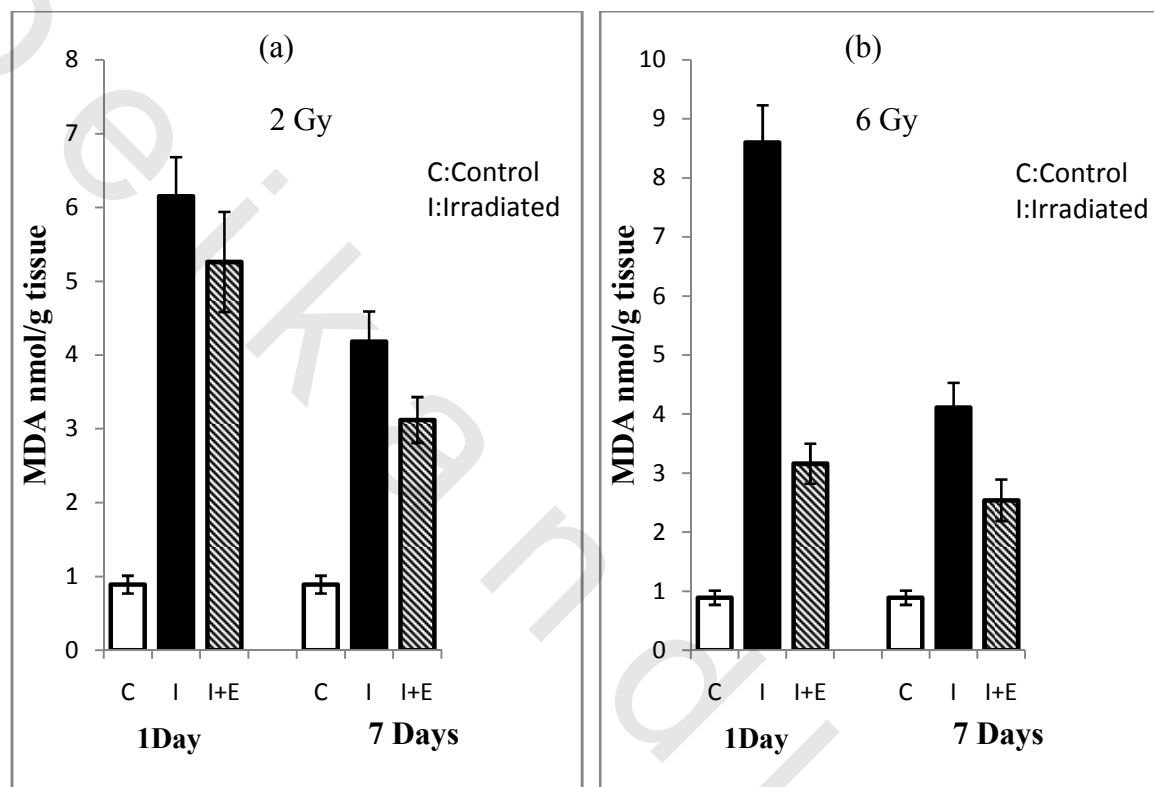


Figure (28): Changes in MDA in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

B. Glutathione:

The results of all forms of glutathione in mouse testicles are presented in [Table (20, 21) and Figure (29, 30)]. The effect of the 2Gy dose was minimal causing practically no change in total glutathione (less than 1%) after one day and about 4.3% after 7 days. However, after irradiation with 6Gy there was a decrease of 12.0% after one day and 20.1% after seven days. The effect of the higher dose was evident as the total glutathione values were 12.8% and 16.5% below those obtained with the 2Gy one and seven days after irradiation. Treatment with ectoine caused only small changes, which were still significantly below the control value.

The effect of radiation on reduced glutathione was more evident. One day after irradiation with 2Gy the results show a decrease of 30.2% below mean control value. The decrease was even greater with the 6Gy as it reached 51.7%. This was followed after seven days by a tendency toward recovery. The reduction in reduced glutathione was 23.2% seven days after irradiation with 2Gy and 47.1% after the 6 Gy dose. These data indicate that the decline in reduced glutathione was about 31% more with the 6 Gy than with the 2 Gy in both time points tested.

The effect of treatment with ectoine on testicular GSH was quite apparent. One day after the 2 Gy dose, the mean reduced glutathione was 11.7% higher than the untreated group although it was still 22.1% below the control level. After 7 days, GSH level was 14.4% higher than the irradiated untreated group, but lower than the control by 23.2%. Treatment with ectoine was more effective after irradiation with 6 Gy where it caused 16.9% increase in GSH level after one day and 31.9 % after 7 days. Despite such increases, the levels were far below the control value by 43.6% after one day and by 30.2% after 7 days.

The oxidized form of glutathione in the testes increased as a result of x-irradiation. Although the absolute values of such increases were not substantial, the percentage changes were relatively large. Irradiation with 2Gy caused a relative increase of 150.0% and 88.9% over control after one and seven days. As may be expected, the effect of the 6Gy dose was larger reaching 177.8% and 111.1% during the same time periods. The percentage increases as a result of irradiation with 6Gy were 19.4% and 14.3% over the 2Gy.

Treatment with ectoine attenuated the oxidative stress in the testes. The obtained values for GSSG were 20.0% below the corresponding irradiated untreated groups after one day and between 35.3% and 36.8% one and seven days after the 2Gy and the 6Gy radiation doses respectively.

The calculated redox potentials were all in the range between -275 mV and -243 mV indicating that the cells were in the proliferative stage. Within this range the treatment with ectoine tended to push the smaller values toward that of the mean control level.

Table (20): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on reduced glutathione in the testis

		1 Day	7 Days
Control ($\mu\text{mol/g}$ tissue)		1.72 ± 0.09	
2Gy	Irradiated($\mu\text{mol /g}$ tissue)	1.20 ± 0.14^a	$1.32 \pm 0.09^{a*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	1.34 ± 0.09^a	$1.51 \pm 0.05^{ab*}$
6 Gy	Irradiated ($\mu\text{mol /g}$ tissue)	0.83 ± 0.11^{ab}	$0.91 \pm 0.09^{ab*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	0.97 ± 0.12^{abc}	$1.20 \pm 0.01^{abc*}$

a: significantly different from control, $p < 0.05$

b significantly different from 2 Gy Irradiation , $p < 0.05$

c: significantly different from 6 Gy Irradiation , $p < 0.05$

*: significantly different from 1 day, $p < 0.05$

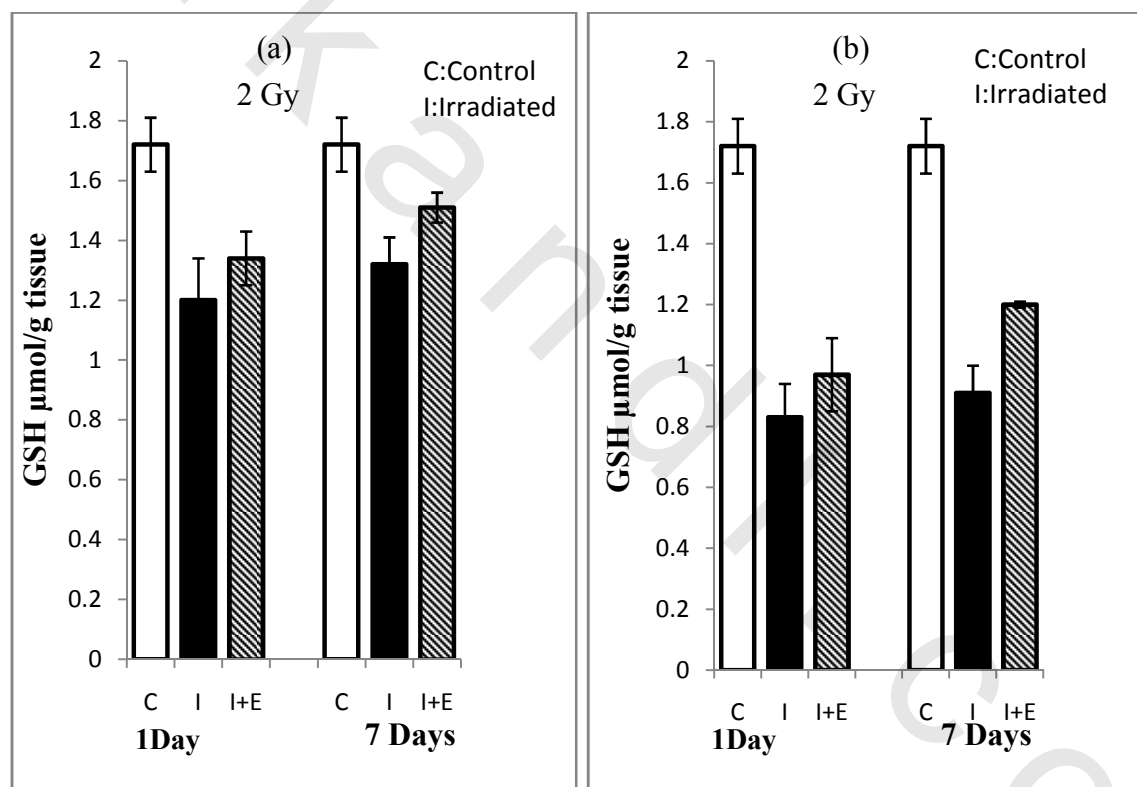
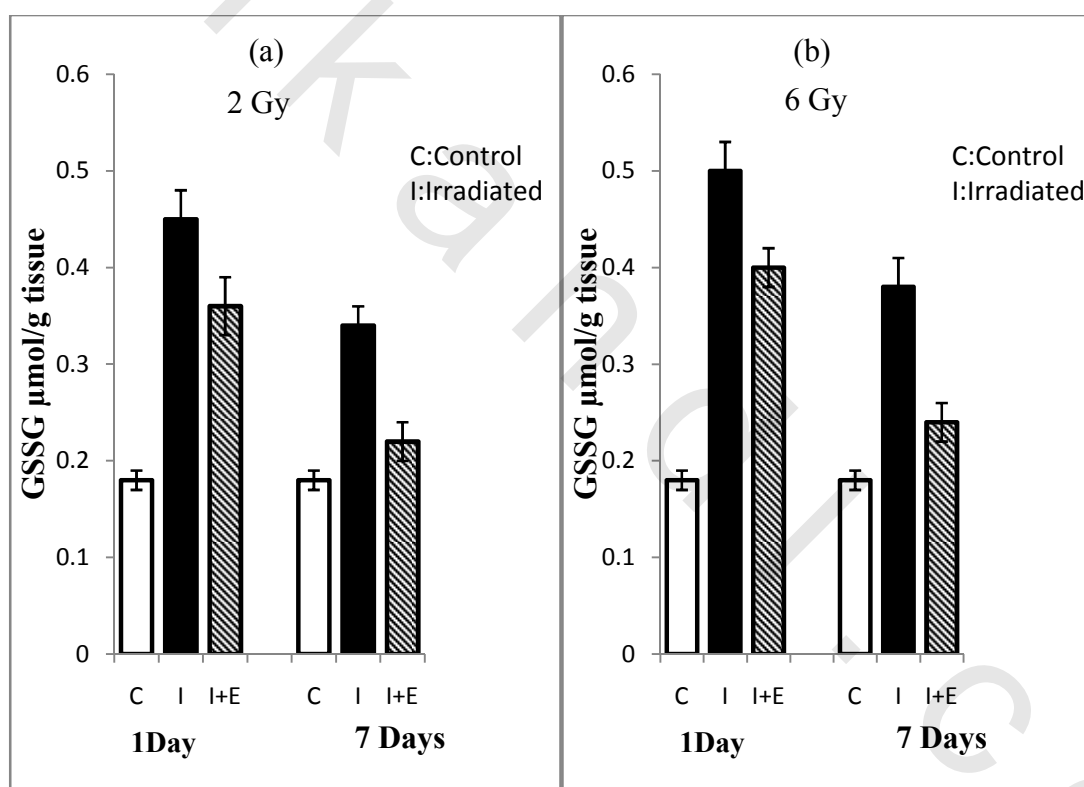


Figure (29): Changes in reduced glutathione in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.

Table (21): Effect of treatment of 2 Gy and 6 Gy whole body irradiated mice with ectoine on oxidized glutathione in the testis

		1 Day	7 Days
Control ($\mu\text{mol/g}$ tissue)		0.18 ± 1.0	
2Gy	Irradiated($\mu\text{mol /g}$ tissue)	0.45 ± 0.03^a	$0.34 \pm 0.02^{a*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	0.36 ± 0.03^{ab}	$0.22 \pm 0.02^{ab*}$
6 Gy	Irradiated ($\mu\text{mol /g}$ tissue)	0.50 ± 0.03^{ab}	$0.38 \pm 0.03^{ab*}$
	Irradiated - Ectoine ($\mu\text{mol /g}$ tissue)	0.40 ± 0.02^{abc}	$0.24 \pm 0.02^{abc*}$

a: significantly different from control, $p < 0.05$ b significantly different from 2 Gy Irradiation , $p < 0.05$ c: significantly different from 6 Gy Irradiation , $p < 0.05$ *: significantly different from 1 day, $p < 0.05$ **Figure (30):** Changes in oxidized glutathione in mouse testis after one day and 7 days of whole body X-irradiation with (a) 2 Gy and (b) 6 Gy and treatment with ectoine.