DISCUSSION
5. Discussion

When animals were irradiated with 3 Gy and the glyoxalase I mRNA expression was examined at different time intervals from 0 to 24h, the extent expression was found to depend on the post irradiation time (Figure 1). First it decreased at 3h. After showing relatively high at 6h, it again decreased at 12 and 24h. It seems the mode and magnitude of expression in the liver of irradiated mice depends on the post irradiation time. As mentioned earlier, further study on the mRNA expression was carried out at 6h as its maximum level was noticed at this interval.

Effect of different doses (upto 7 Gy) on the expression of glyoxalase I mRNA is shown in the Figure 3. The expression levels found to increase upto 5 Gy and declined beyond 5 Gy. The decrease at 7 Gy is clearly visible in Figure 3. It is important that the specific activity of Gly I also increased upto 5 Gy and then declined beyond 5 Gy (Table 5). Thus the pattern of mRNA expression and specific activity of glyoxalase I is similar.

Glyoxalase I is considered to be regulatory enzyme and biochemical indicator of proliferation. Its activity correlates with DNA synthesis (Sutrave and Rao, 1982). The change in the activity of glyoxalase I due to radiation ascribed to regeneration status of the organs/tissues of irradiated animals (Sharma and Kale, 1993). The reported changes in glyoxalase I activity have also been ascribed to the factors such as unscheduled DNA synthesis and activation of immune response which may be required for repair and later regeneration (Hooper et., 1988; Sharma and Kale, 1993). Therefore in the present study also increased activity of glyoxalase I probably reflects repair and regeneration process taking place in the liver. Recently glyoxalase I suggested to have antioxidant function (Choudhary et al., 1999). It is quite possible that increased level of glyoxalase I activity might be in response to the oxidative stress induced by radiation. It is known that pre­­ existed antioxidant enzymes get activated in response to the stress experienced by the animals (Hardmeier et., 1997). However, more amount of enzyme may be required to minimize the effect of oxidative stress. Therefore, it appears that overall expression of glyoxalase I is induced to overcome oxidative stress or repair and regeneration in the damaged tissue. However the process of induction is not known. The increased level of transcription of glyoxalase I reflects the requirement of the enzyme probably for repair.
and regeneration of liver. At higher doses the decrease in enzyme activity and level of transcription may be indicative of irreversible damage to the liver cells or the enzymes as well as induction process.

The specific activity of glyoxalase I and glyoxalase II are known to be inversely related. To see whether this also occurs in present system we have examined the specific activity of glyoxalase II in the liver of mice irradiated with same range of doses (0 – 7 Gy). The results are shown in Table 6. Unlike glyoxalase I, the specific activity of glyoxalase II progressively decreased. It may be mentioned that in the case of glyoxalase II, overall specific activity was above the normal level. It may result in non-accumulation of S-D lactoylglutathione in damage tissue, which is formed from catalytic action of glyoxalase I. S-D lactoylglutathione is known to be toxic to the biological systems (Kalapos, 1994). Although glyoxalase I and glyoxalase II are known to respond differently towards radiation (Sharma and Kale, 1993; Choudhary et al., 1999), the exact mechanism is not known. The radiation induced changes in the activities of the enzyme are attributed by several factors like change in population, change in the rate of synthesis or catabolism of enzymes, translocation from one organ to another and change in inhibitors or activators (Altman et al., 1970) However, liver cell are capable of dividing under the appropriate stimulus. The extent of damage depends on their capacity to divide and might be related to the change in the activity in liver.

In the present system it is quite possible, free radicals formed by radiolytic decomposition of water may react with enzymes and bring out the change in the structure and function (Van Sonntag, 1987). The accessibility of these free radicals, towards different sites, glyoxalase I and glyoxalase II may not be the same which may result in their differential radio-sensitivity.

As mentioned above, free radicals generated due to irradiation likely to cause oxidative damage. Since peroxidative damage is considered to be a measure of oxidative stress, it was examined in the liver of the mice which were irradiated with different doses (0 – 7 Gy). Interestingly no significant change in the level of peroxidation was found upto 5 Gy (Table 1). However, at 7 Gy peroxidative damage was significantly increased. The non-
significant increase in lipid peroxidation between 0 – 5 Gy might be due to increase in the specific activities of antioxidant enzymes such as SOD and GST (Table 13). The increased level of peroxidation at 7 Gy might have caused due to their inhibition. It may be mentioned that the increased oxidative stress at 7 Gy might have caused decline in both transcription and specific activity of glyoxalase I.

For sparsely ionizing radiation, the dose rate is one of the important factors which determines the biological consequences of a given absorbed dose (Hall, 1988). In the present study, the dose rate effect has been evaluated during radiation dose of 3 and 7 Gy. Animals were irradiated with three different dose rates 0.24, 0.06 and 0.015 Gy/sec. It was found that mRNA expression of glyoxalase I declined with decrease in dose rate. It could be seen from the densitometry analysis (Figure 5 & 6) the levels of expression was quite high at the dose rate (0.24 and 0.06 Gy/sec) compared to unirradiated control. However, the lowest dose rate adversely affected the expression which was reduced to 14% and 46% at 3 Gy and 7 Gy respectively. In numerous experiments using various biological systems, have shown decreasing radiation effect with decrease in dose rate as well as inverse dose rate effect (Tubiana et al., 1990; Koufen et al., 2000). Our present results has shown inverse dose rate effect because lowest dose rate probably detrimentally reduced the transcription of glyoxalase I. The transcription was brought down below normal level at the lowest dose rate. To confirm the possibility, under similar conditions i.e. same doses and dose rates, peroxidation was determined. As expected the peroxidative damage was increased with decrease in the dose rate (Table 2) which supports our assumption. To reconfirm further, we have also studied the dose rate effect on the specific activity of glyoxalase I. In case of glyoxalase I specific activity, the similar pattern as that of its mRNA expression was observed (Table 7). Further, mode of change of specific activity of Gly II was similar to Gly I (Table 7 & 8).

The biological effects are mainly caused by the free radicals (generated through radiolysis of water), interacts with biomolecules affecting their structure and function. The decreased radiation effect at higher dose rate is probably due to greater recombination of these extremely short lived and highly reactive free radicals. The chances of indirect radiation effect increases when the free radicals react with biomolecules without recombination, as it readily happens when dose rate decreased. A very similar mechanism
might have operated in the present system. The peroxidation is an important effect on membranes. The degradation products of this process have been shown to damage nucleic acids and proteins. The peroxidative effect is shown to be transferred from level of lipids to proteins and nucleic acids (Koufen, 2000). It is quite possible also in present case. Peroxidation might have transferred its effect through its degradation product to the nucleic acids and proteins, altering structure and function of glyoxalase I and mRNA as well as various regulatory proteins involved in expression of gene including glyoxalase I gene itself. This possibility also reflects from inverse dose rate response of antioxidant enzymes particularly SOD and GST (Table 14).

Preliminary irradiation of cells with low doses of ionizing radiations (conditioning doses) increases their resistance to subsequent exposure to higher doses (challenging doses). This adaptive response to radiations has demonstrated in wide range of systems from prokaryotic cells to mammals (Wolf et al., 1988; Cai and Liu, 1990; Liu et al., 1992; Farooqi and Kesavan, 1993; Azzam et al., 1994; Wojtcik and Streffer, 1994; Seong et al., 1995; Tiku and Kale, 2001). The time interval between the conditioning and challenging dose is one of the critical factors in determining the adaptive response (Youngblom et al., 1989). Moreover, very little information, if at all, is available on the biochemical aspect of adaptive response. With this backdrop, we tried to understand whether conditioning dose (0.5 Gy) modulates the mRNA expression of glyoxalase I and make it radio-resistant to subsequent exposure to higher doses (challenging doses: 3 Gy). The expression of mRNA was lowered in the liver of group of animals which were irradiated with conditioning dose (0.5 Gy) and then followed by challenging dose (3 Gy) compared to irradiated animals which received only 3 Gy (Figure 9 & 10).

In adaptive response, the conditioning dose is known to protect against the radiation damage induced by subsequent higher doses (Yamaoka et al., 1991). Induction of the protective mechanism by low dose of radiation has recently been demonstrated at the molecular level (Le et al., 1998). In the present study, it was also quit possible that the pre-exposure of animals to the conditioning dose (0.5 Gy) might have induced/activated the protective mechanism and rendered more resistance to the subsequent challenging dose (3 Gy). Therefore, the extent of damage in the liver of the mice which were irradiated with conditioning dose and subsequently with the challenging dose (0.5 Gy), is expected to be
less compared to the group of animals irradiated with single dose of 3 Gy. The lesser damage in the tissue needed lesser repair/regeneration. It is important that similar pattern as that of mRNA expression, was also seen in case of specific activity of glyoxalase I in the liver of mice irradiated with 0.5 Gy conditioning dose followed by challenging dose of 3 Gy (Table 11). Moreover, as expected the specific activity of glyoxalase II was found to be inversely related with the specific activity of glyoxalase I under similar conditions of irradiations (Table 12). Since the glyoxalase system is associated with repair/regeneration, lowered level and of glyoxalase I, mRNA expression and specific activity probably reflected the decreased levels of damage (Table 4). The increased level of specific activities of antioxidant enzymes might have contributed towards the minimizing the oxidative stress induced by irradiation in the tissue of mice (Table 16). Thus it appears that the response of transcription and activity of glyoxalase I is perhaps intimately linked with the radiation induced adaptive response.

The split dose effect is known to depend on the time interval between two exposures. The time intervals between two exposures are likely to allow cells to undergo repair and stimulate proliferation. We have also examined the response of mRNA expression of glyoxalase I in the liver of mice irradiated with two equal fractions of 3 Gy separated by 3, 6 and 12h. The mRNA level was found to be decreased compared with single dose of 6 Gy. Among three time intervals, the maximum expression was seen at 6h. The decrease in expression at 3 and 12h of time interval was almost same (Figure 7 & 8).

The single dose i.e. 6 Gy, however, enhanced expression of mRNA of glyoxalase I compared to group of animals which were exposed fractionated irradiation (3+3 Gy). In case of split dose it is likely that the damage was repaired during the time interval between exposure. In such events the resultant damage was expected to be lowered. This possibility was supported by lowered levels of peroxidation (Table 3) and increased in the level specific activities of antioxidant enzymes (Table 15). However, in case of the specific activity of glyoxalase I was found to be enhanced in animals irradiated with two equal fractions (3+3 Gy) than in the animals which received single dose i.e. 6 Gy (Table 9). At present we do not have explanation for this observation.

The glyoxalase system considered to be vital for biological functions. Its involvement in cell proliferation and detoxification is well established. Therefore, findings
of present work may have greater significance in understanding radiation effect. Since system is central pillar of electron theory of cancer, the present findings may have some significance in radiation therapy.