6. Summary & Conclusion

Silymarin, frequently used in the treatment of liver disease, is a flavonoid complex of sliybin, silydianin and silychristin. It is capable of protecting liver cells directly by stabilizing the membrane structures, including endoplasmic reticulum, by an effective decrease in membrane permeability and a change in lipid content in the membrane. Silymarin as a therapeutic agent seems to be well tolerated and largely free from adverse effects.

The present work was performed to investigate and assess the radioprotective effect of silymarin, applied before irradiation, against radiation-induced histological and histochemical changes of rat liver cells.

Male albino rats have been used and classified into four groups as follows:

1- Normal rats.

2- Rats irradiated at the dose 5 Gy of gamma rays.

3- Rats treated with silymarin as a single oral dose (70 mg/kg.wt) and then irradiated at 5 Gy of gamma rays.

4- Normal control rats (treated with silymarin as a single oral dose 70 mg/kg.b.wt. only) and not irradiated.

All these groups of rats were sacrificed at the same intervals; one, three, five, seven and fourteen days post treatment and the results of the present experiment revealed the following observations:

A) Histological and histopathological alterations:

The hepatocytes are polyhedral in shape, relatively large in size and exhibit distinct limiting membranes. Each hepatic cell has a centrally located nucleus with one or two prominent nuclei. Normally the hepatocytes appear sometimes binucleated with granular and homogenous cytoplasm. The hepatocytes are radially disposed in the liver lobules but in response to gamma irradiation, the hepatocytes showed different pathological alterations as follows:

On the first and third post irradiation days, the hepatocytes showed mild hydropic degeneration with loss of the normal radial structures, dilatation of the blood vessels which appeared congested with blood in some sort of haemorrhage together with fatty infiltration of the hepatic parenchyma. Increased cytoplasmic vacuolation, extensive haemorrhage with increased inflammatory leucocytic infiltration were also seen.

Passing to the fifth post irradiation day, the hepatocytes showed marked signs of degeneration where some apoptotic cells were seen distributed throughout the liver section accompanied by cytoplasmic vacuolation, fatty degeneration and marked dilatation of the blood vessels.

On the seventh post exposure day, some hepatocytes retained their normal appearance where some binucleated cells were seen, Küppfer cells were normal in shape and size. Dilatation of the blood vessels were also seen with the appearance of some necrotic cells.

On the fourteenth post exposure day, different signs of regeneration were seen where the hepatocytes appeared normal in shape and size with the appearance of multinuclear forms, Küpffer cells and blood sinusoids were normal with marked inhibition of the inflammatory reaction.

In the present work, silymarin administration one hour before irradiation, showed different signs of improvement in the hepatocytes where the liver cells appeared regular with intact cell membranes and prominent nuclei, indicating the prophylactic role of silymarin, as manifested on the first and third post treatment days.

On reaching the fifth post treatment day, the hepatocytes showed limited incidence of the inflammatory process with the appearance of some thick-walled hepatocytes with densely stained nuclei indicating progressive signs of proliferation. Moreover, the hepatocytes appeared healthy with some binucleated cells on the seventh post treatment day and such restoration process was continued till the fourteenth post treatment day where the hepatocytes retained their normal shape and organization, binucleate cells were prominent also.

Silymarin administration to normal control rats showed no detectable changes in the histological pattern of the hepatocytes which appeared normal in shape and size with one or two nuclei within with few tiny cytoplasmic vacuoles.

B) Histochemical Alterations:

* *General carbohydrates:*

- In whole body gamma irradiated rats, there was an obvious decrease in the total carbohydrate content one and three days post irradiation. This was followed by a marked increase in the carbohydrate material on the fifth and seventh post exposure days. Finally, a nearly normal carbohydrate pattern was seen fourteen days after exposure.

- Silymarin administration to experimental animals before irradiation showed a slight decrease in the carbohydrate material of hepatocytes one and three days post radiation exposure at a degree less than that of irradiated non-treated ones. Gradual and progressive increase in the PAS positive material is seen on the fifth, seventh and fourteenth days post silymarin administration and irradiation.

- Application of silymarin alone showed slight increase in the carbohydrate material from the first till the seventh post application day then a decrease in the PAS positive material could be seen at the fourteenth post administered day where its carbohydrate content appeared in a degree to some extent similar to that of the normal ones.

* *Total proteins:*

- Gamma irradiation showed a progressive and gradual increase in the total protein content of the hepatocytes from the first till the third post exposure days and a process of recovery began at the fifth day and continued till the fourteenth post exposure day.

- Silymarin application before irradiation showed a well marked increase in the protein content of the hepatocytes from the first till the fifth post exposure days in a degree exceeding that produced from irradiation only due to the double effect of silymarin and irradiation. This is followed by a phase of recovery on the seventh day and completed at the fourteenth day after silymarin administration and irradiation.

- Administration of silymarin alone caused an increase in the protein content of the hepatocytes and such increase was temporary and continued till the seventh day whereas the status in the fourteenth day was nearly normal.

**** Deoxyribonucleic Acid (DNA):***

- In gamma irradiated rats, the hepatocytes showed marked decrease in the DNA content and such decrease appeared more prominent on the first and third post exposure days. A gradual increase in the DNA content was seen at the fifth day and became more obvious at the seventh and fourteenth days post irradiation.

- In silymarin administered and irradiated rats, there was a marked improvement in the DNA picture of the hepatocytes throughout the entire period of the experiment indicating the prophylactic role of silymarin.

- Silymarin administration alone to normal control rats showed a slight increase in the DNA content of the hepatocytes from the first till the fourteenth day post application.

From these results we can conclude that, the prophylactic administration of silymarin showed its marked radioprotective role in liver cells which may be due to:

- Silymarin's ability to act as a free radical scavenger thereby preventing membrane permeability changes.

- Silymarin increases the hepatocyte protein synthesis by stimulating the activity of ribosomal RNA polymerase.

- Silymarin decreases the hepatic and mitochondrial glutathione oxidation induced by iron overload and is a mild chelator of iron.

- Silymarin protects against radiation-induced suppression of hepatic DNA and RNA synthesis.