DISCUSSION

In the present study, we evaluated hepatoprotective activity of some flavonoids on lysosomal enzymes activity in CCl_4 vapor induced liver damage in rats.

The flavonoids, viz; Silymarin, Chrysin and Quercetin did not cause any mortality and found to be safe upto 5000 mg/kg, p.o. b.w. Hepatoprotection offered by these flavonoids in CCl_4 vapour induced liver damage was significant and also delayed the development of liver damage induced by CCl_4 vapour via, necrosis, fibrosis to cirrhosis. Silymarin and Quercetin showed significant hepatoprotective activity throughout the course of study, whereas Chrysin has lesser activity. The combination of Quercetin and Chrysin also showed significant hepatoprotective activity, which was comparable to Silymarin.

All the flavonoids have been well documented for their powerful antioxidant status and there are several reports that the antioxidants prevent the free radical formation and liver damage.[142] These findings were supported by flavonoids under study showing antioxidant activity in the order Quercetin>Silymarin>Chrysin and offered hepatoprotection.

Lysosomes play an important role in cell death and tissue damage. They release hydrolytic enzymes that cause auto-digestion of cellular contents. Lysosomal membrane plays a vital role in maintaining the integrity of the cell. This is the first report on alteration in the activities of lysosomal enzymes in liver and sera by the flavonoids in different stages of liver injury leading to cirrhosis.

The ratio of total/free activity of lysosomal enzymes has been interpreted as an expression indicating the stability of lysosomal membrane.[143] A decreased value suggests decreased stability and/or an increased vulnerability of lysosomal membrane

(fragility) resulting in leakage of the lysosomal enzymes and consequently leading to cell injury.[144] This was supported in this study as decreased in the ratio of total/free activity of lysosomal enzymes was observed throughout the treatment period with CCl_4.

The increased lysosomal membrane fragility could be due to [145] low energy levels in the parenchymal cells of damaged liver because of reduced ATP generation by the damaged mitochondria,[146] enhanced lipid peroxidation of the lysosomal membrane by CCl_4 or both.[147] It has been reported earlier that in CCl_4 induced liver injury, mitochondrial function is impaired with the most frequent change being impaired function of the respiratory chain and ATP metabolism.[148] Study by Younes *et al*[148] supports the view of lipid peroxidation. In the present study these flavonoids prevented the development of cirrhosis by reversing the above-mentioned cellular changes as supported by histopathological studies. The increase in serum level of lysosomal enzymes may be as a result of increased fragility of liver lysosomal membrane allowing more enzymes to be leaked into the serum. It is also possible that increase in production and release of lysosomal enzymes from macrophages could also contribute to the increased serum lysosomal enzyme levels.[149] In the present study, the flavonoids increased the stability and/or decreased vulnerability of lysosomal membrane and prevented leakage of lysosomal enzymes. One of the possible mechanisms may be due to the antioxidant property of these flavonoids.

Liver regeneration is a fundamental mechanism by which the liver responds to injury. This process is regulated by endogenous growth factors and cytokines and it involves proliferation of all mature cells that exists within the intact organ. The growth-promoting effects of GH can be direct in selected target tissues, such as liver, or indirectly, via its endocrine mediator IGF-I. GH is the primary regulator of IGF-I

synthesis and secretion in hepatocytes, in turn, IGF-I regulates GH secretion through a classical negative feedback loop.[19, 20] It has been reported that both pituitary and serum concentrations of GH were significantly reduced in CCl_4- induced cirrhotic rats. CCl_4- induced acute liver injury in rats can be restored by exogenous GH-treatment.[102]

GH plays a critical role in liver regeneration, improves bioenergetics and decreases catecholamines in post infract rat hearts[28]. It has also been reported that adrenaline administration in immature cockerels lowered the levels of plasma GH.[150]

It has been reported that GH was able to inhibit the release of lysosomal enzymes from resting polymorphonucelar leucocytes.[106] Moreover, there is report suggesting paracetamol induced hepatocellular damage is associated with increased circulating catecholamines. Increased catecholamine levels may contribute to the pathophysiology of paracetamol-induced hepatotoxicity by compromising hepatic perfusion.[23]

Further it is reported that catecholamines may induce oxidative damage through reactive intermediate resulting from their auto-oxidation, irrespective of their interaction with adrenergic receptors, thus representing an important factor in the pathogenesis of catecholamines-induced cardiotoxicity.[24]

The mechanism of adrenaline action on the structure and function of the lysosomal-vacuolar cell apparatus were shown to produce a labializing effect on lysosomal membranes, increasing free activity of lysosomal enzymes and osmotic sensitivity of lysosomes in liver.[153]

It has been reported that late administration of COX_2 inhibitors minimize chloroform induced liver injury. Thus chrysin has been shown to induce an anti-inflammatory effect, most likely by inhibition of COX_2 expression and via IL-6 signaling.[152] Quercetin has been reported to inhibit catecholamines secretion from cultured bovine adrenal chromaffin cells.[153]

In the present work, Quercetin and combination (Quercetin+Chrysin) study showed significant increase in GH and decrease in catecholamines (adrenalin and nor-adrenalin) levels in plasma, whereas, silymarin and chrysin failed to modulate the hormonal levels.

In view of our findings it is understood of that the CCl_4 induced toxicity leads to the following sequence of events:

- First, there is increase in an oxidative stress that further leads to oxidative damages.
- Secondly, due to inhibition in the growth hormone level there was further increase in oxidative damage and increase in catecholamine (adrenaline and nor adrenaline) levels.
- Finally, decrease in growth hormone and increase in catecholamines cause hepatocytes dysfunction by increasing lysosomal enzymes free and decrease in total activities and ultimately cirrhosis.

It can be summarized that hepatoprotective activity of Quercetin is by its strong antioxidant potential. The ability to prevent the leakage of lysosomal enzymes and increased stability of lysosomal membrane is due to increase in GH and decrease in catecholamine levels by Quercetin. Silymarin and Chrysin prevented the leakage of

lysosomal enzymes and increased stability of lysosomal membrane may be due to their antioxidant potential alone.

In conclusion, besides from antioxidant activity, increase in GH and decrease in catecholamine levels play an important role in hepatoprotective activity against CCl_4 vapour induced hepatic damage by these flavonoids under study. This antioxidant potential and their ability to prevent leakage of lysosomal enzymes and increase stability of lysosomal membrane may promise these flavonoids having hepatoprotective potential.