Chapter 10

SUMMARY AND CONCLUSIONS

10.1. SUMMARY

The profile of glycoproteins in the pathology of Rheumatoic Arthritis was studied using Collagen Induced Arthritic rats. Albino rats were injected at the plantar surface of the left foot with type II collagen in Freund’s Incomplete Adjuvant. After the booster dose, animals with inflammation in the paw were screened. They were divided into 8 groups: 1) Normal/Control. 2) Arthritic. 3) Vitamin C treated. 4) Vitamin E treated. 5) Ginger treated. 6) Turmeric treated. 7) Sallaki treated. 8) Glucosamine treated. Treatments were continued for 30 days.

The status of inflammation was assessed by measuring the thickness of paw (oedema) using Vernier calipers on 8th, 15th, 22nd and 30th days. Animals were sacrificed on 30th day by cervical dislocation. Samples of blood were collected by cutting jugular vein. Tissues like liver, heart, kidney, testis and brain were collected and stored in ice cold. Tissues were analysed for carbohydrate components. Activities of glycosidases and antioxidants were studied using fresh blood/serum and tissues.

The dry, defatted tissues were hydrolysed and the extracts were used to estimate the carbohydrate fractions of Glycoproteins. Protein bound hexoses, Fucoses, Hexosamines and Sialic acids were estimated by appropriate methods.
Activities of α-Mannosidase, β-D Glucosidase, β-D Galactosidase, α-L Fucosidase and N acetyl-β-D Hexosaminidase were assayed using suitable p-Nitrophenyl glycosides as exogenous substrates. The specific activity of enzymes were determined. For antioxidant studies, the activities of Catalase and Superoxide dismutase were measured and the levels of Glutathione were determined. Concentrations of lipid peroxidation products like Conjugated Dienes and Malondialdehyde were also estimated.


Arthritic rats showed significant increase in the levels of protein bound hexoses and sialic acids in serum, liver, kidney and brain. But the levels of these sugars were decreased in heart and testis. Concentrations of fucose and hexosamines were increased in all the tissues except testis. It was obvious that the glycoprotein metabolism was altered in arthritis.

10.1.2. Effects of vitamins C and E, Ginger, Turmeric, Sallaki and Glucosamine on Carbohydrate components.

Treatments with vitamins C and E influenced the carbohydrate levels differently. Vitamin C supplemented tissues showed moderate, but significant variation in the levels of protein bound hexoses. No significant changes were produced in the levels of fucose, hexosamines and sialic acids in most of the vitamin C treated CIA tissues. Vitamin E supplementation in CIA rats resulted in significant decrease in the levels of protein bound hexoses, hexosamines and sialic acids towards normal. But, the concentrations of fucose were not affected by this vitamin.

The elevated levels of hexoses, fucoses and hexosamines were decreased significantly by ginger supplementation. There was no effect on sialic acid
level. Turmeric had no significant effect in rectifying the hexose and sialic acid levels whereas fucose and hexosamine concentrations were reduced towards normal in most of the tissues. (Though the sialic acid levels were reduced by turmeric, statistical significance was not accorded due to extreme variations). Sallaki reduced the hexose and hexosamine contents towards normal. But it did not bring any significant effect on fucose and sialic acid levels. The effect of ginger, turmeric and sallaki depended on the type of carbohydrate as well as the tissues involved.

Glucosamine reduced the enhanced levels of all the carbohydrate components in CIA rat tissues with relatively higher significance on hexosamine.

10.1.3. Changes in Glycosidase activity.

Activity of glycosidases also varied in tissues differently. Mannosidase activity was increased in all the tissues except heart where it was lowered. Glucosidase activity was elevated in liver, kidney and brain while it was decreased in other tissues. Galactosidase activity was increased in blood, testis and brain, but decreased in other tissues. Activity of fucosidase was raised in all the tissues except brain where it declined. Elevated levels of hexosaminidase was noticed for all the tissues in CIA rats.

10.1.4. Effects of vitamins C and E, Ginger, Turmeric, Sallaki and Glucosamine on Glycosidase activity.

The effects produced by various treatments on altered enzyme activities were also diverse. Vitamin C influenced all the enzymes to attain normal state of activity with more emphasis on mannosidase and hexosaminidase. Variations brought by vitamin E was less significant in the case of glucosidase and galactosidase. Highly significant decrease in mannosidase activity was
shown by vitamin E on CIA rats. It produced moderate effects on fucosidase and hexosaminidase activities.

Ginger caused decrease in the activity of all the three enzymes with a higher significance on hexosaminidase. The most significant effect of turmeric was on mannosidase and hexosaminidase activities followed by fucosidase and other enzymes. Reductions in the activities of mannosidase and fucosidase by sallaki were highly significant than those on hexosaminidase and glucosidase. There was no effect for this plant extract on galactosidase activity. Like turmeric, glucosamine also caused very highly significant regulatory effects on mannosidase and hexosaminidase followed by galactosidase and fucosidase. The variation in glucosidase activity was least significant.

10.1.5. Changes in Paw oedema.

CIA rats exhibited 147.95% of normal thickness in their oedematous paw. The reduction in paw oedema by all the 6 treatments were highly significant. Turmeric showed the best result reducing the oedema to 113.98% followed by sallaki (120.0%) and ginger (120.9%). Vitamin C reduced the paw oedema to 122.35%, vitamin E to 126.93% and glucosamine to 122.71%. Plant extracts were found to be more anti-inflammatory than vitamins and glucosamine.

10.1.6. Changes in Antioxidant status.

The activity of catalase was decreased significantly in all the tissues except testis where it was increased. Superoxide dismutase (SOD) activity was reduced in all the tissues of CIA rats. The levels of reduced glutathione (GSH) was also decreased in all the tissues. Concentrations of Conjugated Dienes (CD) and Malondialdehyde (MDA) were increased significantly in all the tissues. The antioxidant system was disturbed in inflammation.
10.1.7. Antioxidant effects of vitamins C and E, Ginger, Turmeric, Sallaki and Glucosamine.

All the treatments regulated the activities of catalase and SOD and the levels of GSH towards normal at different levels of significance. Elevated levels of CD and MDA in arthritic rats were decreased by all the treatments with high significance. This showed the antioxidant effect of the vitamins C and E, the plant products and glucosamine in the inflammation of RA.

10.2. CONCLUSION

Though inflammation primarily damages the cartilage at the joints, the consequent alterations in proteoglycan metabolism spreads to all organs of the body where the effects are reflected by increase in the levels of carbohydrates and by the enhancement of activities of glycosidases. These secondary effects on liver, heart, kidney, testis and brain reveals the involvement of these organs in the late pathology of the disease. The variations in the proteoglycan metabolism was found to be tissue/organ specific with particular pattern of change for each component monosaccharide or each glycosidase.

However, it may be realized that proteoglycan metabolism is a finely tuned orchestration in which glycosyltransferases, glycosidases and some monosaccharides perform as the components that are highly synchronized. Any component that is out of tune will disturb this metabolism leading to a disorder or disease. Treatments with Vitamins C and E, Ginger, Sallaki, Turmeric and Glucosamine corrected the altered activities of glycosidases as well as glycosyltransferases as inferred from this study. Since the activity of biosynthetic enzymes could not be studied due to lack of facilities, a definite conclusion could not be arrived at.
The pathology of CIA rats is almost similar to human RA and hence the former is widely accepted as a model for studying the pathogenesis of RA and for screening new drugs against it. This study was an attempt to establish the curative effects of various neutraceuticals like vitamin C, vitamin E, ginger and turmeric. As extracts of sallaki and also the glucosamine are widely used in the treatment of arthritis, they too were used in this work. Analysis of the glycoprotein levels and glycosidase activities in various tissues of CIA rats revealed that the glycoprotein metabolism is altered in arthritis. This derangement could be brought to near normal by the neutraceuticals as well as sallaki and glucosamine. The anti-inflammatory and antioxidative roles of these substances were also studied in CIA rats. They were found to be effective in decreasing inflammation via the removal of free radicals.

Even though the oxidative stress and the relief by the neutraceuticals, sallaki and glucosamine were uniform in all the tissues, variations in glycoproteins and glycosidase activity depended on the tissue and the parameter concerned. Hence the effects of treatments were also tissue specific. Certain treatments were more effective in one or the other tissue with respect to a parameter which got changed in another tissue or with a different parameter. The curative effect of neutraceuticals and other substances tested varied among themselves according to the tissue and the parameter involved. So it is recommended that a synergistic action of these neutraceuticals, sallaki and glucosamine would be a better choice in drug design and therapy against Rheumatoid Arthritis.