CHAPTER 8
SUMMARY AND CONCLUSION
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Our understanding of the cellular and molecular events that participate in the genesis of atherosclerosis has increased markedly in the past few years. This has led to the recognition of major risk factors and identification of specific events and proteins in the disease process which eventually provides numerous entry points for selective interference and inhibition of the process of atherogenesis.

In view of this most of the studies have indeed designed to test the hypothesis that a reduction in the major lipoproteins especially low density lipoproteins cholesterol level will lead to the reduction in morbidity and mortality from cerebrovascular and cardiovascular disease. Such studies result in substantial reduction in cardiovascular events. Further it is found that occlusive lesions of atherosclerosis in human can be clinically reversed after aggressive treatment with lipid lowering drugs (152-154).

The knowledge of the importance of inflammation, oxidative stress mediated endothelial dysfunction, oxidative modification of low density lipoproteins have provided the basis of antioxidant drug development. Results of large number of epidemiological studies have demonstrated an association between increased intake of antioxidant vitamins, such as vitamin E and vitamin C and reduced morbidity and mortality from coronary artery diseases.

Moreover recognition of the final thrombotic event as a result of lesion rupture and the role of coagulation factors and platelets have opened another line of approach to the prevention and treatment. Thus large number of newer drugs with antiplatelet aggregatory, anticoagulant and thrombolytic properties have emerged. The observation of hypercoagulability during hyperlipedemia has also provided
rationale that lipid lowering by diet or drug therapy appears to ameliorate these disturbances thus providing one possible mechanism where by such treatment reduces the risk of coronary heart diseases.

Though we have achieved tremendous progress in the therapeutic drug inventions, atherosclerotic disease remains uncontrolled. Changing life style, cessation of smoking, consumption of low fat diet and selective drug usage has proved to be effective in reducing the mortality. Thrombolytic therapy followed by antithrombotic treatment has been shown to reduce the further catastrophic events. But the various side effects attributed to hypolipedemic, hypocholesterolaemic and antithrombotic drugs currently in use are a major hindrance to the amelioration of the disease process. Hence newer agents having potent antiatherogenic activity, is well warranted. Present study is an attempt to test some animal origin and plant derived products in relation to various aspects of atherosclerotic disease process.

'Proteoglycans' a protein carbohydrate hybrid molecules have been isolated from different sources and shown it's efficacy as antiatherosclerotic in experimental animals. 'Atromid' and vessel are such preparation which have been used clinically. Present study tries to isolate proteoglycans from a different source in the view that it may have advantageous over previous one. More over many plants have been shown to possess hypolipidemic, antioxidant, antithrombotic properties in experimental animals and also in man (175-187). So search for newer agents are also extended to the plants.

Porcine pancreas tissue proteoglycans are found to be eluted with 0.4-0.9 Molar sodium chloride concentrations from the DE 52 ion exchange column. The PG 'A' fraction, (eluted with 0.4 M and 0.5 M NaCl) the PG'B' fraction (eluted with 0.6 M and 0.7 M NaCl) and PG 'C' fraction (eluted with 0.8 M and 0.9 M NaCl) containing
hexuronic acid when individually subjected to gel filtration chromatography on
sepharose 6 B column, have been found to purified further. The uronic acid and
protein containing fractions are eluted between V0 and Vt as single peak. These
peak materials are able to precipitate by the addition of 3 volume ethanol containing
potassium acetate. It is observed that all these three fractions contain protein, uronic
acid and hexosamin, suggesting that the fractions are a mixture protein and
carbohydrate. Further the alkali-borohydride treatment and subsequent gel filtration
shows a shift in the uronic acid elution profile in these fractions. The alkali-
borohydride treatment is known to disrupt the oligosaccharide protein linkage (200).
Probably this may be the reason for the shift in uronic acid. This indicate the intact
nature of the molecule.

In these proteoglycan fractions, PG ‘A’ is found to enhance the release of
lipoprotein lipase enzyme in the blood. The rabbit blood plasma obtained after PG
‘A’ treatment is found to liberate glycerol from TG rich serum in the enzyme assay.
When compares to heparin, The PG ‘A’ fraction possess more or less similar effect.
The other PG fractions are less effective.

The increased liberation of glycerol in the assay is an indication of elevated
level of enzyme in the plasma. Probably administration of PG ‘A’ may have
enhanced the release of LPL molecule into the blood in vivo. Heparin is known to
exert lipoprotein lipase releasing action from the blood capillaries and thus augment
catabolism of TG rich lipoproteins. Possibly PG ‘A’ may have acted in similar fashion
as heparin. Since increase in enzyme activity enhance lipoprotein metabolism, PG
‘A’ can be considered as an agents with lipid clearing activity.

In the anticoagulant studies in vivo administration of PG ‘A’ has delayed
coagulation time of rabbit blood. The two in vitro methods conducted to study
coagulation time i.e. plasma recalcification method and activated prothrombin time method suggest this findings. The activity of PG 'A' is less compared to heparin a known clinically accepted anticoagulant. This renders an anticoagulant property to this PG 'A' fraction. Considering the clinical complications of heparin and other anticoagulants presently in use, PG 'A' seems advantageous as it is comparatively less anticoagulant.

More over a dose dependent inhibitory effect on ADP induced platelet aggregation is noted in vitro in the PG 'A' treated blood samples. The inhibitory effect exerted by heparin is not dose dependent and beyond a particular concentration it shows a static effect. This indicate that PG 'A' fraction posses antiplatelet aggregatory effect. The other PG fractions are not effective in inhibiting ADP induced platelet aggregation.

Hence only this PG 'A' fraction is further characterised as it is found to posses promising antiatherogenic effect. When subjected to nitric acid digestion followed by gel filtration over sepharose 6 B column, the GAG fraction of this proteoglycan is found to have similar elution profile to heparan sulphate. In the agarose gel electrophoresis, the electrophoretic mobility of heparan sulphate GAG and the GAGS of the PG 'A' fraction are similar (Plate 3 i). Thus the PG 'A' fraction seems to be identical with heparan sulphate proteoglycan.

In the antioxidant studies it has been showed that 70% ethanol extract of Alpinia galanga, 70% ethanol extract of Kaempferia galanga and chromatographically purified acetone fraction (AF) of Rosa damascena posses significant free radical scavenging activity. The other test compounds are comparatively less active. The AF of R. damascena is most effective among the three. The AF is also showing dose dependent inhibitory effect on carrageenan
induced pedal oedema formation in mice \textit{in vivo}. This is a strong indication of the antiinflammatory potential of this plant extract. The \textit{A. glanga} and \textit{K. galanga} has been reported to posses antiinflammatory activity (191,192). The aetiology and pathogenesis of atherosclerosis are tightly linked with the ubiquitous protective mechanism associated with inflammation and repair. Therefore the ethanol extract of \textit{A. galanga}, ethanol extract of \textit{K. galanga} and AF of \textit{R. damascena} are selected for further studies to evaluate their antiatherogenic efficacy.

In the study of lipoprotein lipase releasing activity these plant extracts could not increase the liberation of glucose in the assay system. This indicate that their administration \textit{in vivo} does not enhance release of LPL into the blood. There fore it can be assumed that these plant extracts are not possessing lipoprotein lipase releasing activity.

The anticoagulant studies has performed to test the efficacy of these extracts as anticoagulant. The AF of \textit{R. damascena} is found effective in delaying coagulation time of rabbit blood. it is evidenced from plasma recalcification time study and activated prothrombin time study. Compared to heparin, it is less active. On the other hand 70% ethanol extracts of \textit{A. galanga} and \textit{K. galanga} don't delay the coagulation time.

In addition AF inhibit ADP induced platelet aggregation in a dose dependent manner. Compared to heparin and PG 'A' fraction, AF has significant activity. On the other hand \textit{K. galanga} has an activating effect while \textit{A. galanga} has no effect. These studies indicate that AF posses potent inhibitory activity on ADP induced platelet aggregation that is far better than heparinoids.

In order to see the mechanism of AF on ADP induced platelet aggregation malonedialdehyde (MDA) release in platelet suspension is assessed \textit{in vitro}. N-
ethylmaleimaid is known to induce arachidonate pathway in platelet. The MDA formation takes place in the cyclooxigenase pathway of the arachidonate metabolism. Present study reveal that AF inhibit MDA formation in platelets as evidenced from thiobarburate substance release study compared to indomethacine a known inhibitory agent of cyclo-oxigenase pathway in platelets, AF has comparable effect. Thus it could be possible to assume that AF exert its antiplatelet aggregatory effect through inhibition cyclo-oxigenase pathway in platelets.

Further studies to assess antiatherosclerotic efficacy in rabbit fed high fat high cholesterol diet. The proteoglycan fraction A (correspond to heparan sulphate) and AF of R. damascena are selected as these test materials posses promising antiatherogenic potential.

It has been observed that both AF and PG ‘A’ effectively inhibit the rise in blood lipid level of rabbits fed high fat high cholesterol diet over a period of 4 months. Interestingly significant reduction in the Low density lipoprotein level is observed in AF and PG ‘A’ treated group animals. PG ‘A’ shows comparatively good efficacy than AF. More over HDL level is increased in the treated group compared to control. As LDL positively and HDL negatively correlate with atherogenesis, both the tested extract could have protected the animals from hyper lipidemia. The triglyceride, phospholipids and total cholesterol fraction in the treated group show reduction compared to control. This suggest that both the tested extract effectively inhibit initial hyperlipidemia produced by high fat diet.

More over in the treated group animals, the fatty deposit in the aortal segments is less compared to control. It is evidenced from the deep stained area of aorta. The stained area is less in the AF treated groups, compared to control. Where as in the proteoglycan fraction treated group these is no fatty deposition. Thus PG
'A' treatment has protected the animals from the fatty deposition in the intima of arteries.

The AF is less active compared to PG 'A' treated animals. The hypolipidemic efficacy observed in these group may explain its antiatherogenic effect in rabbits. On the other hand in PG 'A' treated group, total inhibition of fatty area has observed.

Thus present study conclude that PG 'A' and AF possess hypolipidemic and antiatherosclerotic efficacy. PG 'A' shows comparatively better efficacy. Further studies on its structural functional characteristics may help to find new agents that clinically may be accepted.