

Protective Effect of *Aplotaxis Auriculata* Root on Glycoprotein Components in Diethylnitrosamine (Den) Induced Hepatocellular Carcinoma in Rat

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Abstract: Carbohydrates moieties of glycoproteins have also been implicated in the transport of metabolites across cell membranes and also observed a direct relationship between glycolproteins and tumorigenesis. In the present study to evaluate the glycoproteins as hexose, hexosamine and sialic acid have been measured in the plasma and liver tissues. This study shows that *Aplotaxis auriculata* root extract (AARE) administration decrease the glycoprotein synthesis in tumor cells. This may be due to the inhibitory action of AARE on the initiation of Diethylnitrosamine (DEN) activation/detoxification process. AARE offer promise as potential protective effect on carbohydrates moieties as glycoproteins against DEN treated rats.

Keywords: Glycolproteins, Diethylnitrosamine, *Aplotaxis auriculata*,

1. Introduction

Glycoproteins play a significant role in contributing to the surface properties of the cells and also important role in tumorigenesis and as mediators of immunological specificity. They also have a central role of functioning in biological systems such as stabilizing the conformation of glycoproteins on cellular membranes, assisting in cell-cell recognition, and interaction, and serving as chemical messengers in body fluids & tissues (Kurtul (2004). Glycoproteins are carbohydrate - linked protein macromolecules found in the cell surface, which is the principle component of animal cell. It contains oligosaccharide chains (glycans) covalently attached to their polypeptide chain. The oligosaccharide moieties of glycoproteins, hexose, hexosamine, fucose and sialic acid have an important role in protein stability, function and turnover (Wiese *et al.*, 1997; Pari and Rajarajeswari, 2010). Compositional analysis following acid hydrolysis is one method of identifying sugars, qualitatively and quantitatively (Crook 1993). The level of different types of glycoproteins are maintained within a narrow range in health, but is elevated in many pathological conditions viz. tuberculosis, autoimmune disease, cardiovascular disease, diabetes mellitus, cancer of cervix, uterus and breasts, trauma, prolonged bed rest and arthritis including psychiatric disorders (Nandave *et al.*, 2005).

Carbohydrates moieties of glycoproteins such as hexose, hexosamine, fucose and sialic acid have also been implicated in the transport of metabolites across cell membranes and also observed a direct relationship between glycolproteins and tumorigenesis (Thirunavukkarasu and Sakthisekaran, 2003). Sialic acid, one of the glycoprotein components is used as a tumor marker. it is acetylated derivative of neuraminic acid and exists as terminal component of nonreducing end of carbohydrate chains of glycoprotein. levels of sialic acid can be useful in early

detection of cancer indicating progress of the disease, degree of metastasis, and possible recurrence (Shanmugam and Nagarajan, 1985). Increased activity of sialyltransferase leads to an increased expression of sialic acid in cancer conditions. the influence of sialic acid on the oncogenicity of tumor cells has been studied by many investigators as the main determinant of the cell surface negative charge electromobility and the loss of contact inhibition. It also acts as an antigen-masking agent and as component of cell surface involved in the adherence of tumor cells to mesothelial membrane to form metastasis (Prasad, 1986; Sivagnanam *et al.*, 2012). In the present study to analysed the glycoproteins as hexose, hexosamine and sialic acid in plasma and liver tissues of control and experimental rats.

2. Material and Methods

Chemicals

Ethylene diamine tetraacetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA) and Diethylnitrosamine (DEN) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Plant material

The roots of the *Aplotaxis auriculata* were collected from the Koli hills, Tamil Nadu, during the month of January 2014. The plant material was identified and authenticated (TUH-87) by Dr. M. Jegadeesan Msc., Ph.D., Professor & Head of environmental & Herbal sciences, Faculty of sciences, Tamil University, Thanjavur. The plants were cut into small pieces and shade dried at room temperature for 15 days. The powdered plants were used for the preparation of extract.

Preparation of plant extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials were ground into a fine powder and used for extraction. Three hundred grams (300g) of the powdered plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytochemicals. For experiments 500mg/kg body weight of *Aplotaxis auriculata* root extract (AARE) was used. This effective dose was selected based on dose dependent studies of AARE carried out in our laboratory.

Dosage fixation

Different doses of *Aplotaxis auriculata* root extract (AARE) (50mg, 100mg, and 250mg, 500mg and 750mg/kg body weight) were treated for 4 weeks in rats. The effective dose of AARE was assessed based on the contents of liver and kidney lipid peroxidation (oxidative damage marker). Supplementations of *Aplotaxis auriculata* root extract (AARE) at doses of 250, 500mg and 750mg/kg body weight for 16 weeks were found to be effective in cancer rats. Among these doses, the minimal effective dose 500mg was fixed as therapeutic dosage for the subsequent studies.

Experimental Design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. Group 1: Normal control rats will be fed with standard diet and served as a control, which received saline. Group 2: Rats induced with hepatocellular carcinoma by providing 0.01% DEN through drinking water for 16 weeks (Woo-song *et al.*, 2001). Group 3: Rats treated with *Aplotaxis auriculata* root alone by oral gavage daily at a dose of 500 mg/kg body

weight (based on effective dosage fixation studies) for 16 weeks. Group 4: Rats pretreated with *Aplotaxis auriculata* root intragastrically at the dose of (500mg/kg body weight) for one week before the administration of DEN and continued till the end of the experiment (i.e., 16 weeks).

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Plasma was separated for the estimation of various biochemical parameters. The Liver was dissected out, washed in ice-cold saline, and weighed. A known weight of them was used for homogenate preparation and used for various biochemical analyses.

Biochemical estimation

Hexose level was estimated by the method of Niebes (1972). Hexosamine and Sialic acid content were estimated by the method of Wagner (1974).

3. Results

Fig 1 and 2 depicts the levels of hexose, hexosamine, and sialic acid in plasma and liver of control and experimental animals. In Group II cancer-bearing animals, the levels of these three parameters in liver and plasma significantly elevated ($P < 0.05$) as compared to that of the control group animals. But in Group IV drug-treated animals, these protein levels were reverted back to near normal condition as compared with Group II cancer animals. No significant changes of these levels were observed in Group III drug-alone-treated animals when compared to normal control group I animals.

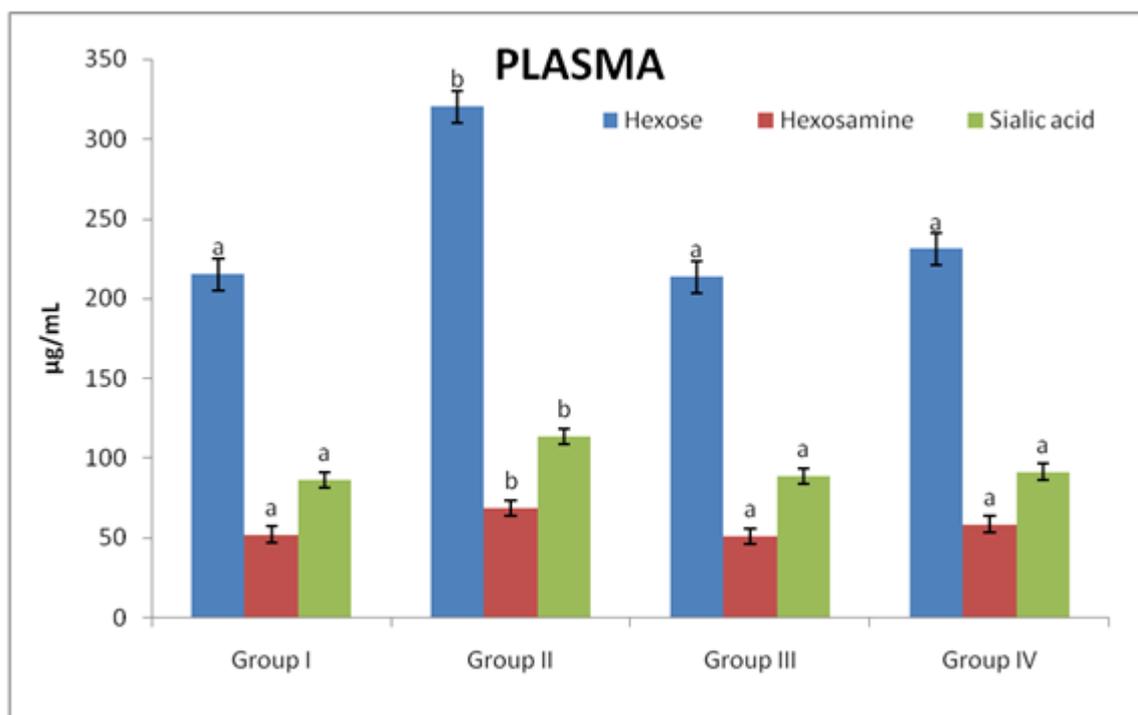


Figure 1: Effect of *Aplotaxis auriculata* root on plasma Hexose, Hexosamine and Sialic acid in experimental rats.

'b' is significant ($P < 0.05$) level different from group I, III and IV and same letter (a) are non significant in respective

group were comparison by Duncan's multiple range test (DMRT).

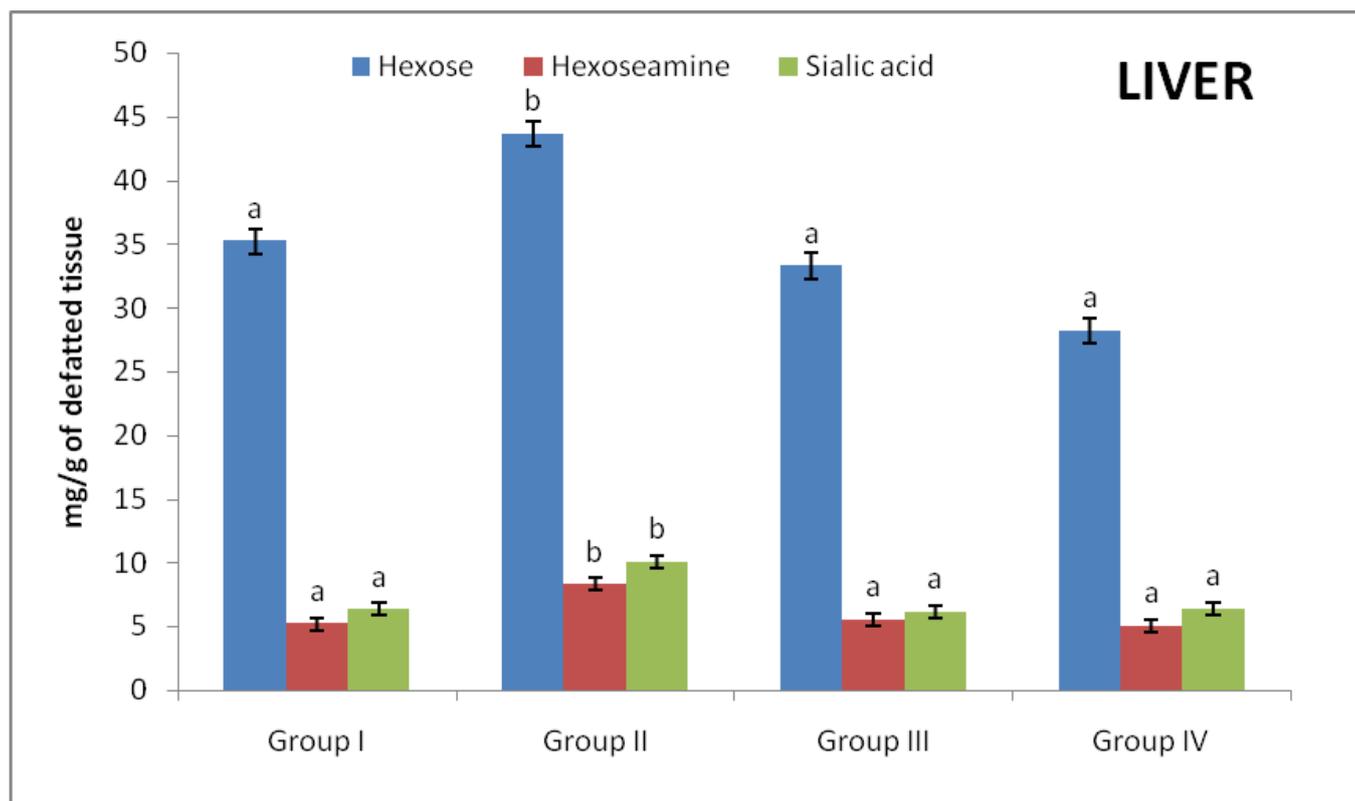


Figure 2: Effect of *Aplotaxis auriculata* root on liver Hexose, Hexosamine and Sialic acid in experimental rats.

'b' is significant ($P < 0.05$) level different from group I, III and IV and same letter (a) are non significant in respective group were comparison by Duncan's multiple range test (DMRT).

4. Discussion

The crucial role of cell surface and cell membrane constituents in neoplastic behaviours and the changes in serum and tissue glycoconjugates have long been associated with malignancies (Patel *et al.*, 1990). The presence of cancer-specific sialic acid-rich glycopeptides was first demonstrated in proteolytic digests derived from the surface of malignant cell (Van Beek, 1973). Thus, the combined evaluation of hexose, hexosamine and sialic acid residues of glycoproteins might help to establish a useful aid in strengthening the diagnosis and treatment monitoring of cancer patients (Patel *et al.*, 1994; Dube and Bertozzi, 2005).

Over expression of glycoconjugates in the cell surface of carcinogen treated experimental animals has been reported (Senthil *et al.*, 2007). A large number of experimental studies pointed out that glycoproteins were synthesized enormously in the tumor and liver tissues during cancerous conditions and subsequently entered into circulation (Thirunavukkarasu and Sakthisekaran, 2003). Over expression of glycoconjugates in the tumor cells with subsequent shedding into plasma could account for increased levels of plasma protein bound hexose, hexosamine and sialic acid were reported (Shimizu and Funakoshi, 1970). The increased levels of plasma glycoprotein components in cancer condition may be due to

the leakage of the disturbed membrane components from either disintegrating or dying neoplastic cells or as a consequent shedding of plasma membrane and due to increased synthesis by sequential addition of monosaccharide units to parent protein molecule catalysed by multiple glycosyltransferases such as sialyltransferase (NeuAc-T), galactosyltransferase (Gal-T), fucosyltransferases (Fuc-T A and Fuc-T B) (Kuhn and Schoentag, 1981; Manju *et al.*, 2002).

On drug treatment, glycoprotein components levels were reverted back to near normal levels. An increased expression of glycoprotein components in malignant liver tissue was decreased when compared to normal rats observed in our investigation is in line with previous reports (Thirunavukkarasu and Sakthisekaran, 2003; Sivagnanam *et al.*, 2012). This could be due to the cyto-stabilising property of the drug. Limtrakul *et al* (2005) showed that the flavonoids possess inhibitory action against carcinogenesis. Thus the flavonoids, alkaloids and other bioactive components of the drug may significantly alter the expression of glycosyltransferases thereby modulate glycoprotein synthesis and protected the structural integrity of cell surface and membrane, indicating its potent anticancer property.

This study shows that AARE administration decrease the glycoprotein synthesis in tumor cells. This may be due to the inhibitory action of AARE on the initiation of N-nitrosodiethylamine activation/detoxification process or alter cell membrane glycoprotein synthesis and structure. Thus the ethanolic extract of *Aplotaxis auriculata* shows

protective effect on carbohydrates moieties as glycoproteins against DEN treated rats. .

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