

Effect of Keelvayu Nivarana Churunam (KNC) Extract on Glycoproteins in Complete Freund's Adjuvant (CFA) induced Arthritic Rats

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ABSTRACT

Glycoproteins are important groups of compounds involved in the cellular function. They 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. 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 Keelvayu Nivarana Churunam (KNC) on glycoproteins as hexose, hexosamine and sialic acid in the plasma against CFA induced arthritis in albino rats. This study shows that KNC extract administration decrease the glycoprotein synthesis in arthritic rats. This may be due to the inhibitory action of KNC extract and offer promise as potential antiarthritic agent against CFA treated rats.

1. Introduction

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). 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)

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 Sakthisakaran, 2003). In the present study to analysed the glycoproteins as hexose, hexosamine and sialic acid in plasma and paw tissues of control and experimental rats.

2. Material and Methods

Collection of plant: The barks of *Withania sonifera*, *Simlax china* and roots of *Hemidimus indicus* and *Alpenia officinanum* were purchased from Traditional Medicinal shop, Thanjavur, Tamil Nadu, India. Healthy roots and

barks were washed several times with distilled water to remove the traces of impurities from the roots. Shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The barks of *Withania sonifera*, *Simlax china* and roots of *Hemidimus indicus* and *Alpenia officinanum* extracts were stored in refrigerator until used.

Preparation of plant extract: The fine powder of barks of *Withania sonifera*, *Simlax china* and roots of *Hemidimus indicus* and *Alpenia officinanum* used for extraction. Three hundred grams (300g) of the powered plants were extracted with ethanol (80%) using Soxlet Apparatus. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Preparation of Keelvayu Nivarana Churunam: Keelvayu Nivarana Churunam (KNC) prepared by the combination of Barks of *Withania sonifera*, *Simlax china* and roots of *Hemidimus indicus* and *Alpenia officinanum* taken in the ratio of 1:1:1:0.5 were used for further studies.

Complete Freund's adjuvant (CFA) induced arthritis

Arthritis was induced by a single injection of 0.1 ml CFA, (Sigma Aldrich, USA) into the sub-plantar surface of the left hind paw. Indomethacin and Keelvayu Nivarana Churunam were administered each day starting from 14th day after adjuvant injection till the 28th day (Newbould, 1963). Paw volume and body weight changes were measured during the experiment.

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 fed with standard diet and served as a control, which received saline. **Group 2:** Arthritis was induced by a single injection of 0.1 ml CFA into the sub-

plantar surface of the left hind paw. After 14 days treatment was started. **Group 3:** Rats treated with Keelvayu Nivarana Churunam was intragastrically at the dose of 100mg/100g body weight from 14th day after adjuvant injection till the 28th day. **Group 4:** Rats treated with Indomethacin intragastrically at the dose of 1mg/100g body weight from 14th day after adjuvant injection till the 28th day.

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.

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

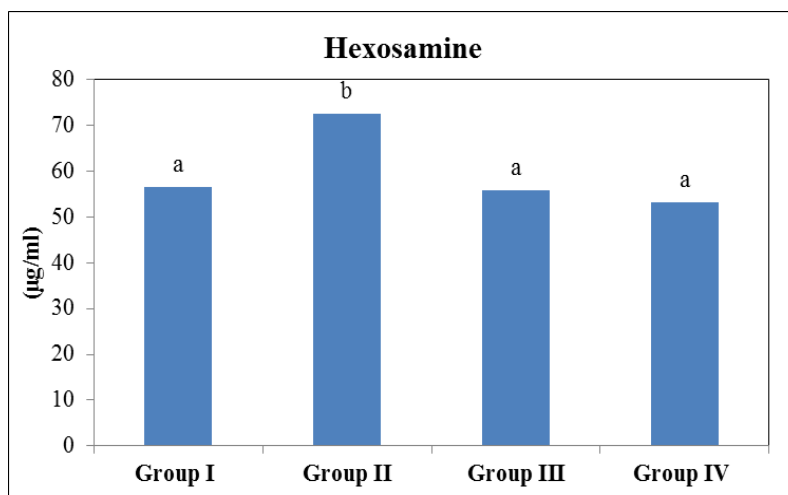
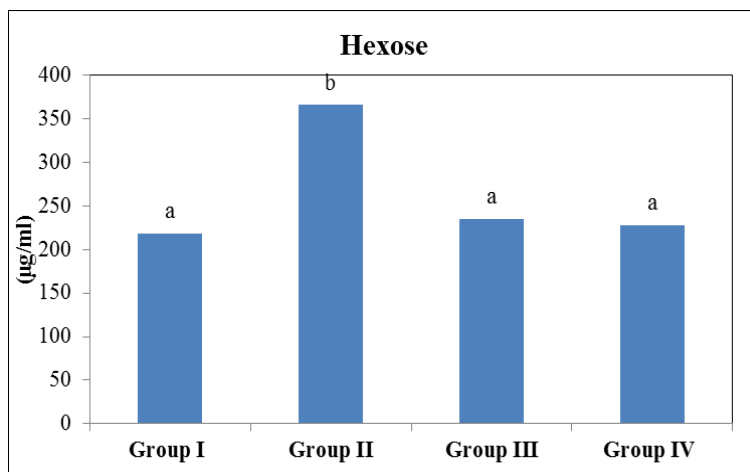
Table 1 and Fig 1 depicts the levels of hexose, hexosamine, and sialic acid in plasma of control and experimental animals. In Group II arthritic animals, the levels of these three parameters in plasma significantly elevated (P < 0.05) as compared to that of the control group animals. But in Group III KNC extract and IV standard as Indomethacin treated animals, these protein levels were reverted back to near normal condition as compared with Group II arthritic animals.

Table.1: Effect of Keelvayu Nivarana Churunam on glycoproteins in serum of control and experimental rats

Parameters	Group I (Normal)	Group II (Arthritis)	Group III (Arthritis + KNC extract)	Group IV (Arthritis +Std. Indomethacin)
Hexose (µg/ml)	218.32±15.26 ^a	365.78 ± 25.55 ^b	235.45±16.45 ^a	228.21±15.96 ^a
Hexosamine (µg/ml)	56.47±3.92 ^a	72.45 ±5.04 ^b	55.75±3.85 ^a	53.18±3.71 ^a
Sialic acid (µg/ml)	91.05±6.37 ^a	119.34±8.33 ^b	92.34±6.87 ^a	88.03±6.16 ^a

Values are expressed as Mean ± SD for six rats

Mean values within the row followed by different letters (Superscript) are significant (p< 0.05) level different from each other and same letter are non-significant were comparison by Duncan's multiple range test (DMRT).



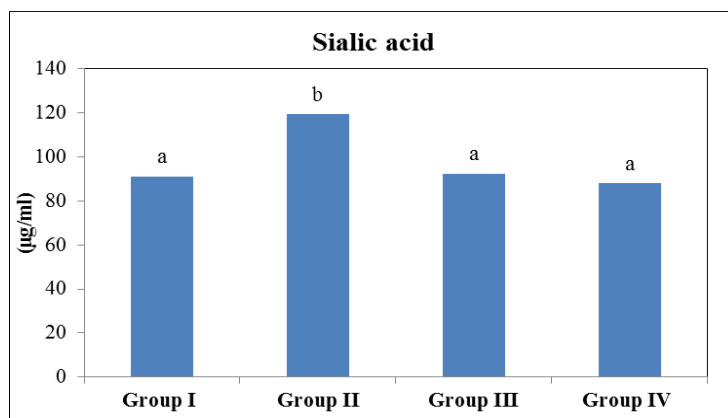


Fig 1 Effect of Keelvayu Nivarana Churunam on glycoproteins in control and experimental rats

4. Discussion

Glycoproteins can be simply defined as proteins that have carbohydrate moiety covalently attached to their peptide portion. The glycoproteins as a group have multiple and complex function and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes. These are organic compounds, composed of both a protein and carbohydrate monosaccharides, usually hexose, hexosamine and sialic acid, joined together covalently linked to polypeptide chain. The level of different types of glycoproteins are maintained within a narrow range in health, but is elevated in many pathological conditions including arthritis (Nandave et al., 2005).

During arthritic condition, synoviocytes activated monocytes, chondrocytes, and infiltrating neutrophils aids in the release of vacidhydrolases and altered the metabolism of glycoproteins. Data obtained from this study showed an increase in the levels of hexose, hexosamine, and sialic acid in

paw tissues of arthritic rats. The elevated glycoprotein level is attributed due to increased release of lysosomal enzymes during arthritic condition, which are found to amplify the metabolic turnover of structural macromolecules in connective tissue and cartilage proteoglycans. However, after KNC treatment, the glycoprotein levels were recouped to normal levels in arthritis-induced rats. This membrane stabilizing property of KNC could be due to its antioxidant property of its constituents, which has been already well established (Lee et al., 2005; Naik et al., 2005; Sivasankar et al., 2015).

This study shows that KNC administration decrease the glycoprotein synthesis in arthritis rats. This may be due to the inhibitory action of KNC on the initiation of inflammatory process or alter cell membrane glycoprotein synthesis and structure. Thus the extract of Keelvayu Nivarana Churunam shows strong anticancer potency.

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