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

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ABSTRACT:

Histology is that the microscopic study of animal and plant cell and tissues through staining and sectioning and examining them below a magnifier (electron or lightweight microscope). There square measure numerous ways won't to study tissue characteristics and microscopic structures of the cells. Microscopic anatomy studies square measure utilized in rhetorical investigations, autopsy, and diagnosis and in education. Additionally, microscopic anatomy is employed extensively in medication particularly within the study of pathological tissues to help treatment. The aim of this work was to attempt to assess the antiarthritic effect of Keelvayu Nivarana Churunam (KNC) through histological observation in structural alteration of bone on adjuvant-induced arthritis model. The cellular organization of bone proves the antiarthritic activity of KNC.

Key Words: *Keelvayu Nivarana Churunam, Bone, Histology*

INTRODUCTION

Arthritis causes disability, compromised quality of life, and premature mortality. It affects approximately 0.5%–1.0% of the global adult population, with an estimated annual incidence of 12.0–24.5 males and 23.9–54.0 females per 100,000., (Dugowson *et al.*, 1991; Gabrie, 2001). The incidence is largely consistent racially and geographically, and the peak age of onset lies between the ages of 45 and 65 years. The economic cost of arthritic related diseases is estimated to consume about 3% of gross domestic product in developed countries, and is somewhat lower in developing countries due to lower life expectancy (Jonsson and Husberg, 2000; Rat and Boissier, 2004). The present decade has therefore been declared the "Bone and Joint Decade" by the World Health Organization, (WHO 2003) in order to further our understanding of the impact of musculoskeletal diseases on society and individuals and to assist advancement on this front.

Histology is that branch of anatomy that studies tissues of animals and plants. In its broader facet, the word microscopic anatomy is employed as if it were a equivalent word for anatomy, as a result of its subject material encompasses not solely the microscopic structure of tissues however conjointly that of the cell, organs, and organ systems (Au, 2004. The body is composed of cells, intercellular matrix, and a fluid substance, extracellular fluid (tissue fluid), which bathes these components (Rocha and Monteiro, 1999). Extracellular fluid, which is derived from plasma of blood, carries nutrients, oxygen, and signaling molecules to cells of the body. Conversely, signaling molecules, waste products, and carbon dioxide released by cells of the body reach blood and lymph vessels by way of the extracellular fluid. Extracellular fluid and much of the intercellular matrix are not visible in routine histological preparations, yet their invisible presence must be appreciated by the student of histology (Selvanatha *et al.,* 2012).

One of the most deleterious effects induced by the chronic inflammation of RA is bone loss. It is against this background that we attempted to assess the antiarthritic effect of Keelvayu Nivarana Churunam (KNC) through histological observation in bone on adjuvant-induced arthritis model.

MATERIAL AND METHODS

Collection of plant: The roots of *Withania sonifera*, *Simlax china* and root bark of *Hemidimus indicus* and rhizome of *Alpenia officinanum* were purchased from Traditional Medicinal shop, Thanjavur, Tamil Nadu, India. Healthy roots, root barks and rhizome 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 roots of *Withania sonifera*, *Simlax china* and root bark of *Hemidimus indicus* and rhizome of *Alpenia officinanum* extracts were stored in refrigerator until used.

Preparation of Keelvayu Nivarana Churunam: Keelvayu Nivarana Churunam (KNC) prepared by the combination of roots of *Withania sonifera*, *Simlax china* and root bark of *Hemidimus indicus* and rhizome of *Alpenia officinanum* taken in the ratio of 1:1:1:0.5 were mixed with water 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).

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.

Histological studies

Histological studies of ankle joint carried out by the method of (Ochei and Kolhatkar, 2000). The various steps involved in the preparation of tissues for histological studies were fixation, dehydration, clearing, impregnation, embedding, section cutting, staining and mounting. Slides were viewed on a photograph of high-magnification images (40x) to find out the histological changes in bone

RESULT AND DISCUSSION

Histology is that branch of anatomy that studies tissues of animals and plants. This textbook, however, discusses only animal, and more specifically human, tissues. In its broader aspect, the word histology is used as if it were a synonym for microscopic anatomy, because its subject matter encompasses not only the microscopic structure of tissues but also that of the cell, organs, and organ systems (Au, 2004). The body is composed of cells, intercellular matrix, and a fluid substance, extracellular fluid (tissue fluid), which bathes these components (Rocha and Monteiro, 1999). Extracellular fluid, which is derived from plasma of blood, carries nutrients, oxygen, and signaling molecules to cells of the body. Conversely, signaling molecules, waste products, and carbon dioxide released by cells of the body reach blood and lymph vessels by way of the extracellular fluid. Extracellular fluid and much of the intercellular matrix are not visible in routine histological preparations, yet their invisible presence must be appreciated by the student of histology (Selvanathan *et al.,* 2012).

One of the highest advantage of using histopathological biomarkers in biological monitoring is that they permit examining exact target organs that are responsible for energetic functions, such as respiration, excretion, accumulations and biotransformation of xenobiotic (Velkova-Jordanoska and Kotoski, 2005). Numerous xenobiotics stimulate the action of definite enzymes that modify metabolism, further leading to apoptosis initially demonstrating as necrosis with inflammatory protective reactions (Velkova-Joudanoska, 2002; Roganovic-Zafirova and Jordanova, 1998; Roganovic-Zafirova *et al.,* 2003).

Bone loss often occurs in chronic inflammatory diseases and can be diagnosed in rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel diseases, systemic lupus erythematosus, psoriasis, and many others (Keller *et al.,* 2013). During the development of chronic inflammation, a large amount of body energy is diverted to the activated immune system, and this leads to signs and symptoms that may enhance bone loss. Anorexia, malnutrition,

muscle wasting, cachexia, and depression are directly or indirectly related to this persistent allocation of energy to the cytokine network activation. Decreased functional capacity and lack of exercises associated with joint pain and deformities impair the development of a healthy life and also contribute to progressive bone loss. An excellent and comprehensive review of the evolutionary and adaptive aspects of bone loss in chronic diseases and the concept of sick behavior can be found in a recent publication by Straub *et al.* (2015).

In the present study normal control rats shows no lesions in articular cartilage and vascularity formation in joint. Ankle joint synovial membrane structure is normal in control rat (Fig 1). Arthritis control rats observed that the chronic inflammation involve synovial hyperplasia with increased vascularity, oedematous inflammation, inflammatory cell infiltrate (involve T-lymphocytes) (Fig 2). Rats treated with KNC shows well protected articular cartilage with normal cellular characteristics. Decrease in inflammation with decrease in oedematous spaces, restructuring of synovial membrane with noticeable reduction of histological injury was observed as near to normal (Fig 3). Arthritis rats treated with standard as indomethacin observed mild vascularity otherwise synovial membrane structure re-establishing with the less oedema and inflammatory cell as compared to control were observed Fig 4). Present study agreement with Bhujade *et al.* (2015) who reported that AFCQ (Active Fraction of *Cissus quadrangularis*) possesses a significant anti-arthritic activity against CFA induced arthritis confirmed through histopathology. Another study of Madhavi, Patel and Kilambi Pundarikakshudu (2016) showed the anti-arthritic activity of a classical Ayurvedic formulation Vatari Guggulu in rats through histological changes in bone.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

CONCLUSION

In this study given valuable information on supplementation of Keelvayu Nivarana Churunam (KNC) to arthritic rats restored the histological alterations in joint knee bone. This study further supported the antiarthritic study of our work.

Fig 1 Histological observation of joint knee bone CFA in Normal control

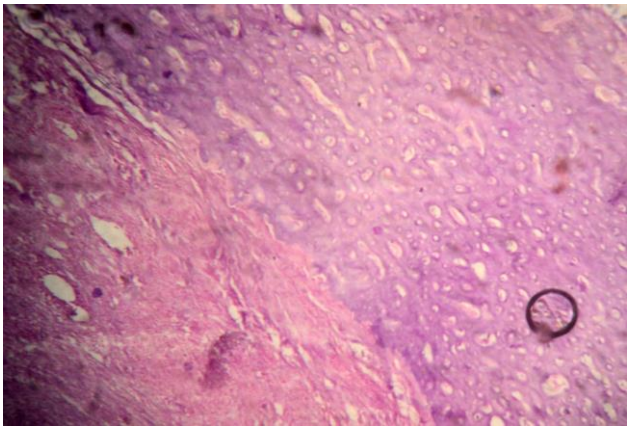


Fig 2 Histological observation of joint knee bone CFA in Arthritic rat

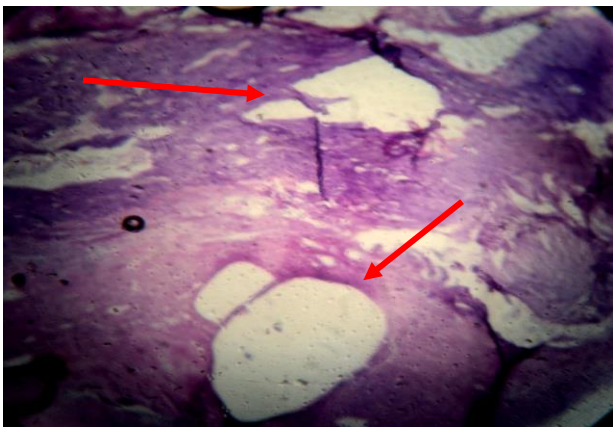


Fig 3 Histological observation of joint knee bone CFA in Arthritic rat and KNC treated

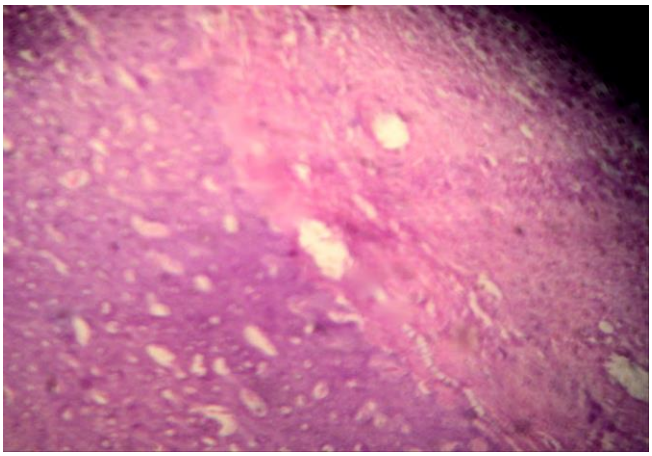
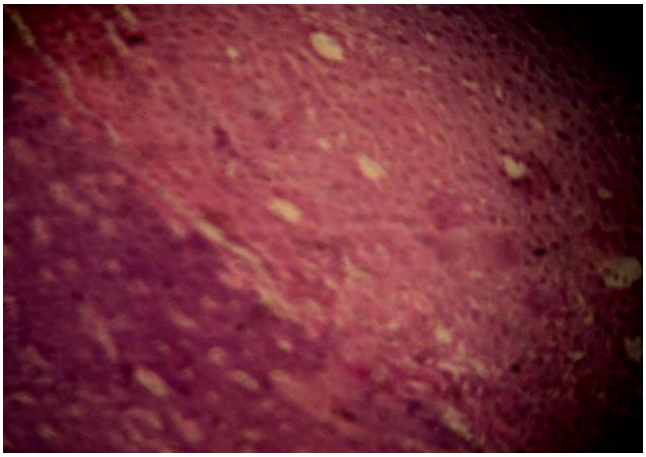


Fig 4 Histological observation of joint knee bone CFA in Arthritic rat andStandard treated



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