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EFFICACY OF GLYCOSAMINOGLYCANS IN PAPAIN INDUCED OSTEOARTHRITIS RAT MODEL IN RELATION TO HISTOLOGICAL LESIONS SCORING

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ABSTRACT

The aim of study was to evaluate efficacy of extracted Chondroitin Sulfate (CS) from chicken keel cartilages, its comparison with standard CS from shark origin alone and in combination with Glucosamine Sulfate (GS) in developed and standardized papain induced Osteoarthritis (OA) rat model. Control group (normal) received sterile normal saline solution while experimental group's papain intra-articularly. Induction of OA in relation to time was assessed on the basis of histological lesions scores. Statistical mean histological lesions score on 28th day of post papain injection in OA rats was 12.82±1.64. On the basis of data obtained, 29th day of post papain injection was decided as cut off point for starting the therapy for OA. Efficacy of treatments among control and OA groups (un-treated and treated) was assessed on the basis of histological lesions scores. Treatments started from 29th day were continued till 60th day of post papain injection. Histological lesions score was not reduced in cartilages of un-treated OA group. In treated groups, structural changes reduced and were found to be close to the normal (control) group. Highest histological lesions score was observed in un-treated OA group followed by GS treated, standard CS, extracted CS, extracted CS plus GS and standard CS plus GS. Maximum reduction in histological lesions score was noted in groups treated with combinations. Histological lesions score of group treated with standard CS (shark) was not significantly different from extracted CS (chicken) alone and extracted CS plus GS. CS extracted from chicken keel cartilages proved to be effective in reducing OA progression. Extracted CS in combination with GS was comparable with standard CS plus GS in efficacy. Chicken keel cartilage is found to be easily available and potential source of CS that may be used as therapeutic agent in OA.

Key words: Chondroitin Sulfate, Glucosamine Sulfate, Osteoarthritis, cartilage and histological lesions.

INTRODUCTION

Osteoarthritis (OA) is a group of mechanical diseases of joints in knee, hip, spine and hand (Lawrence *et al.,* 2008). It is a non-inflammatory disorder of joints with symptoms of severe pain, effusion, stiffness and reduced mobility. Associated muscles undergo atrophy and ligaments become stiff (McNulty *et al.,* 2012). The pathology of OA involves changes in articular cartilage. Cartilage deterioration results in formation of osteophytes and bony sclerosis. Initially, cartilage becomes hard which converts to soft with progression of disease (Felson *et al.,* 2000).

Animal experimental models have been used to assess OA or to reduce severity of lesions. Mankin Histological-Histochemical Grading System (HHGS) is one of the commonly used grading designs initially applied on human (Mankin *et al.,* 1971). Other currently-used histological grading schemes have been developed for accurate assessment of OA severity in rodent models (Xu *et al.,* 2010).

Successful and proper treatment of OA must control pain and reverse progression of disease. Treatment of OA is primarily by exercise and may be combined with some non-steroidal anti-inflammatory drugs (Zhang *et al.,* 2008). Pharmacological and biochemical studies reveal that Glucosamine Sulfate (GS) provides satisfactory results for the treatment of OA. The role of GS is to stimulate synthesis of glycosaminoglycans in the joint matrix (Bruyere *et al.,* 2008). Chondroitin Sulfate (CS) is also involved in the formation of cartilage matrix. Combined use of GS and CS has been successful and effective in the treatment of OA (Herrero-Beaumont *et al.,* 2007).

In general GS and CS are both obtained from animal sources like cartilaginous rings of bovine trachea, pork ears and shark cartilage. Pakistan is one of the major exporters of poultry meat products. Million tons of viscera are produced and discarded annually. By products like trachea and cartilages are rich source of CS. Therefore, in present study, effectiveness of CS extracted from chicken keel cartilage was evaluated and compared with CS from shark source in experimentally induced OA rat model. CS from both sources was also compared in combination with GS in relation to reduction in histological lesions score on 60th day of therapy.

MATERIALS AND METHODS

Efficacy of Chondroitin Sulfate (CS) extracted from chicken keel cartilages was compared with standard of shark origin (Curatech Pharma Pvt., Ltd.) alone and in combination with Glucosamine Sulfate (GS) in male Wister Osteoarthritis (OA) rats. OA was induced using papain 10mg/0.5mL (Sigma, Cat # P 3125) in buffered solution of 0.05M sodium acetate with enzyme activity of 31 I.U./mg intra-articulary in right knee joints of rats (n=35) as described by Khan *et al.* (2012). Severity of OA in relation to time was assessed on the basis of histological lesions scoring system (Khan *et al.*, 2013). Treatments were started from 29th day of OA (1st treatment day) till 60th day (last treatment day). Control (normal) and OA (un-treated) group of rats were offered standard feed throughout experimental period. Experimentally induced OA rats (n=30) were divided into six groups (n=5 each) and treated with GS alone, CS (standard), CS (sample), GS plus CS (standard) and GS plus CS (sample), respectively. GS was administered at the dose rate of 1.5g/kg/day (Cicala *et al.*, 2000). Whereas the dose of CS used throughout the treatment period was 1.2g/kg/day (Beren *et al.*, 2001). Guidelines laid by committee for Research and Ethical Issues of the International Association for the Study of Pain ® (IASP) were followed throughout the experiment.

Histological lesion scoring: Rats from treated, un-treated and control (normal) were sacrificed under high dose of anesthetic ether on 60th day and femorotibial joints were separated. Ligaments and tendons were removed with sharp razor and joints preserved in formalin solution (10%). The joints decalcified in formic acid (5%), paraffin embedded and sectioned (5µm thick) were stained with Hemtoxylin-Eosin as described by Schmitz *et al.* (2010). Semi quantitative histological lesions grading performed in accordance with scoring system of Mankin *et al.* (1971) was followed (Khan *et al.*, 2013). Briefly, histological sections dipped three times in Xylene solution (4 minutes), two times in 95 percent alcohol (1 minute), passed once through 70 % alcohol (1 minute) and rinsed with water (1 minute). Mayer Hemtoxylin solution was poured for 6 minutes, washed with water (8 minutes), dipped in 95% alcohol and then placed in 0.25% eosin Y solution for 1 minute. Finally, sections were dipped three times in 100% alcohol (5 minutes), xylene (5 minutes) and mounted. Histological sections were photographed by digital camera at 100 and 400X magnifications. Semi quantitative histological lesions scores for all groups of the rats were calculated following Mankin *et al.* (1971). Histological scores were expressed as mean ± SD. Difference between all groups was calculated using one way ANOVA forth Duncan's Multiple Range (DMR) post hoc test using SPSS software, version 10.0 (SPSS Chicago III, USA).

RESULTS

Efficacy of Glucosaminoglycans (GAGs) administered to Osteoarthritis (OA) groups was assessed by reduction in histological lesions score based on histopathological scoring system developed by Khan *et al.* (2013). Cartilages from normal rats (control) showed intact histological picture and was allocated zero grade (Fig. 1). Representative histological lesions in OA cartilage of rats were loss of superficial layer till mid zone, cleft formation, multiple patches of cell death, hyper-cellularity and matrix loss with statistical mean histological lesions score of 12.82±1.64 (28th day). In un-treated OA rats on 60th day lesions were not reduced and statistical mean lesions score was 12.24±1.32 (Fig. 2). Histological lesions observed in GS treated group were irregular superficial layer, disoriented columns, cluster formation of chondrocytes, disturbed chondrocyte columns, and hyper-cellularity with statistical mean histological lesions score of 3.96±1.21 (Fig. 3). Mean histological lesions score in standard CS treated group was 2.44±0.98 and representative lesions recorded were irregular superficial layer, slightly disturb architecture, mild cluster formation and few patches of cell death (Fig. 4). Histological lesions observed in extracted CS treated OA cartilage were almost same as of standard with histological lesions score of 2.28±1.33 (Fig. 5). Almost similar histological lesions were recorded in groups treated with standard CS plus GS and extracted CS plus GS. The lesions observed included irregular superficial surface, mild condensation of chondrones, mild disturbance of chondrocyte columns, mild chondrocyte cluster formation (Fig. 6 and 7).

Highest statistical mean histological lesions score was of un-treated OA group (12.24±1.32) followed by GS treated group (3.96±1.21), standard CS (2.44±0.98), extracted CS (2.28±1.33), extracted CS plus GS (1.27±0.49) and standard CS plus GS (0.94±0.55). On statistical analysis by DMR test mean histological lesions scores of groups treated with extracted CS alone, standard CS plus GS and extracted CS plus GS differed non-significantly. Maximum reduction in histological lesions was recorded in groups treated with combinations. Scores of extracted CS treated group was significantly different from normal group. Histological lesions score of group treated with standard CS was not significantly different from extracted CS alone and extracted CS plus GS. Score of group treated with GS alone was significantly different from all other groups. Results of histological lesions scoring are presented as bar diagram (Fig. 8).

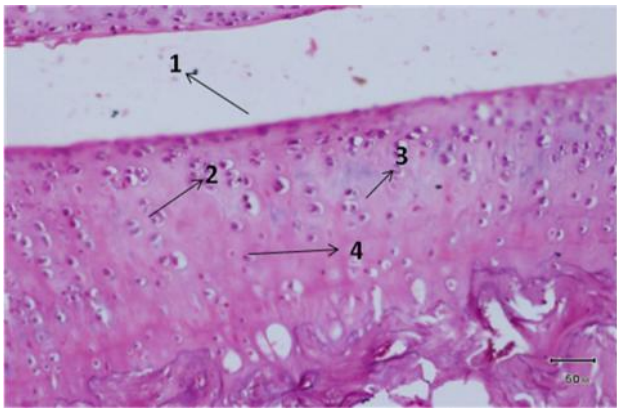

Fig. 1: Histological picture of rat cartilage of control group (100X) 1. Intact surface **2**. Normal population of chondrocytes **3**. Normal morphology of cells **4**.Continuous matrix

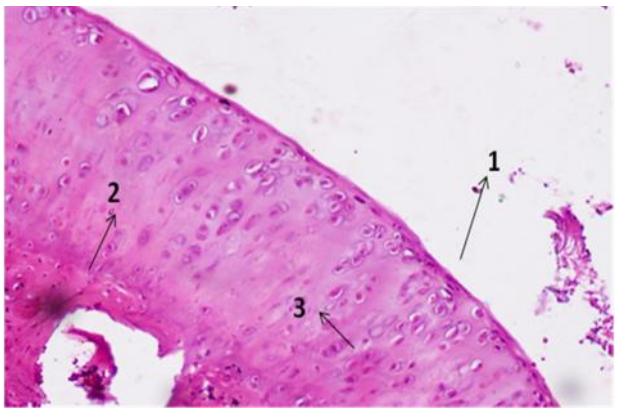

Fig 4: Histological lesions of OA at day sixty (60) post treatment with Standard CS (100X) 1. Slight roughness of superficial layer **2**. Architecture is slightly disturb **3**. Mild cluster formation.

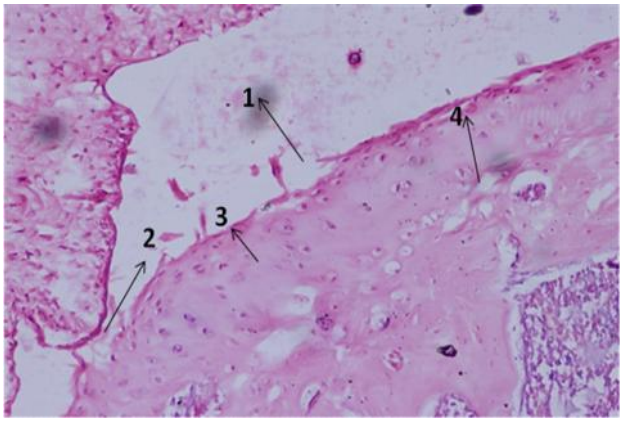

Fig. 2: Histological lesions of OA at day sixty (60) in un-treated group (100X) 1. Loss of superficial layer till deep zone **2**. Matrix loss **3**. Hypocellularity **4**. Cell death in patches

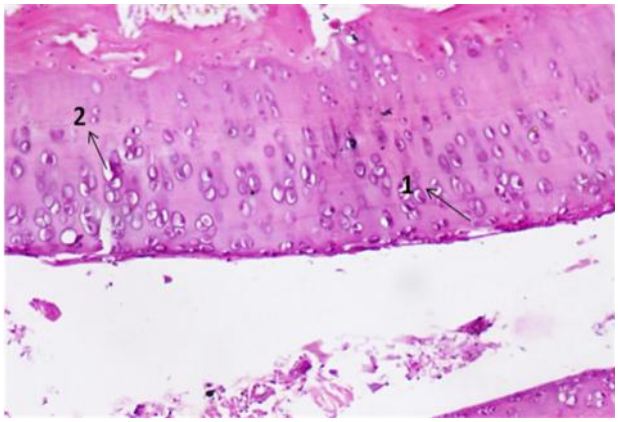

Fig 5: Histological lesions of OA at day sixty (60) post treatment with extracted CS (100X) 1. Superficial rough surface **2**. Mild cluster formation.

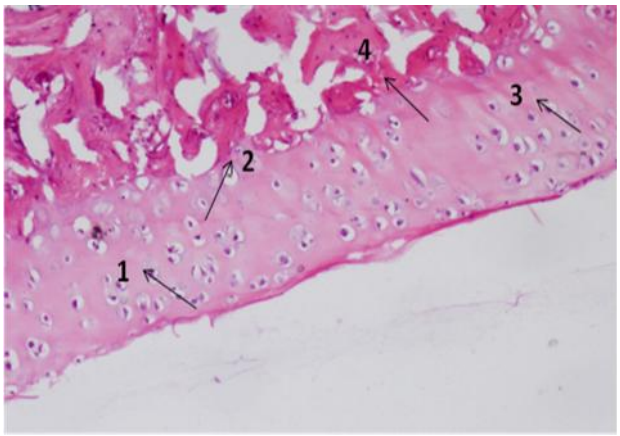

Fig. 3: Histological lesions of OA at day sixty (60) post treatment with glucosamine (100X) 1. Irregular superficial layer **2**. Disoriented columns **3**. Mild cluster formation **4**. Hypocellularity

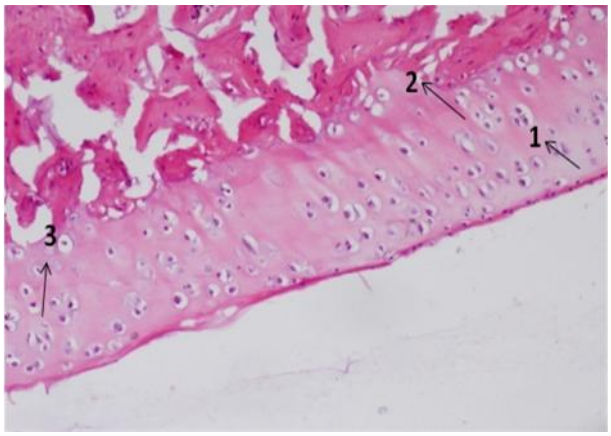

Fig. 6: Histopathology of OA at day sixty (60) post treatment with Extracted CS plus glucosamine (100X) 1. Mild condensation of chondrons **2**. Mild disturbance of columns **3**. Mild cluster formation

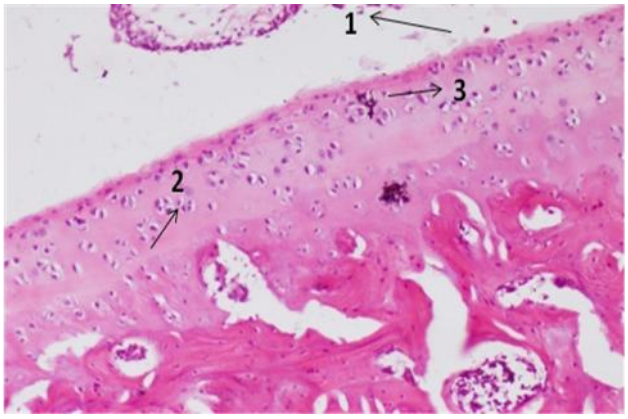

Fig. 7: Histopathological picture of OA at day sixty (60) post treatment with Standard CS plus glucosamine (100X) 1. Superficial rough surface **2.** Disturbance of columns **3**. Mild cluster formation

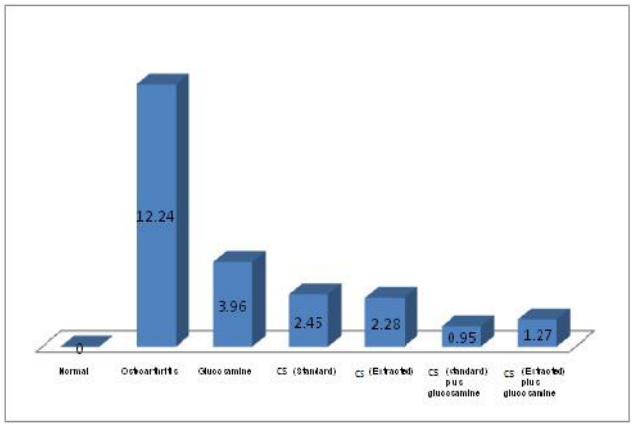

Fig. 8: Bar diagram of histological lesion scores in normal, OA un-treated and treated groups at 60th day

DISCUSSION

Effects of Chondroitin Sulfate (CS) extracted from chicken keel cartilage, standard CS (shark) and GS independently were evaluated on the basis of reduction in histological lesions. In other groups combinations of both extracted and standard CSs with GS were used for treatment of Osteoarthritis (OA) in rats till 60th day. OA was induced by intra-articular injection of papain enzyme in knee joints as demonstrated by Pomonis *et al.* (2005). Papain destroys the core protein, collagen chain and proteoglycan aggregates leading to loss of cartilage integrity (Murat *et al.*, 2007).

Groups treated with standard CS plus GS and extracted CS plus GS were found to be comparable and in close association with the scores in normal group. In vitro studies on efficacy of GS, an endogenous substance, had revealed better response in reversion of cartilage damage. GS is thought to be responsible for initiation of proteoglycan synthesis in articular cartilages (McAlindon *et al.*, 2000). Results on 60th day of therapy indicated that destruction of cartilage was reduced in rats receiving GS as treatment and was in agreement with Towheed *et al.* (2005).

CS another endogenous substance present abundantly in cartilages had frequently been tried to treat OA cases. It is thought to play role in the recovery of damaged cartilage owing to its proteoglycan synthesizing activity (McAlindon *et al.*, 2000). Different doses and time intervals had been used for the therapy of OA under experimental conditions. In present study, efficacy of CS alone in reduction of histological lesions was moderate which is in agreement with results already reported by Leeb *et al.* (2000). Highest efficacy for treatment of OA recorded in present experiment was of CS plus GS combinations which is comparable with the findings of Flood (2010). A study was conducted to compare efficacy of GS alone, CS alone and combination of GS with CS for the treatment of OA of the knee joint (Clegg *et al.*, 2006). The rate of response to treatment was higher in group receiving combined therapy in comparison to alone and results are in agreement with the findings of present research work. Monfort *et al.* (2008) summarized the data from relevant reports on action mechanisms of CS focusing beneficial effects of the drug. CS interferes with the progression of structural changes in joint tissues. Mathieu (2002) reported dose dependent inhibiting effect of ACS-4 and ACS-6 on the catabolism of proteoglycans and collagen. CS stimulates the synthesis of extracellular matrix macromolecules, produces chondrocytes and acts as chondoprotective agent.

Legendre *et al.* (2008) concluded that CS could repress expression of genes encoding proteolysis enzymes leading to cartilage degradation. It inhibits expression of pro-inflammatory genes. Data revealed that CS might exert chondroprotective and anti-inflammatory effects on articular cartilage. Lippiello *et al.* (2000) tested supplements of CS, glucosamine hydrochloride, manganese ascorbate alone and in combination to retard progression of cartilage degeneration in a rabbit instability model of OA. Histological lesions of the medial femoral condyles were measured on the basis of Mankin scoring system. In vitro, a combination of glucosamine hydrochloride and CS acted synergistically in stimulating glycosaminoglycan synthesis (96.6%). On the basis of the results presented above, it is concluded that CS extracted from chicken keel cartilages proves to be effective in reduction of OA progression. While extracted CS in combination with GS shows better efficacy than alone and results are comparable with combination of standard CS and GS. Chicken keel cartilage proved to be cheap, easily available and potential source for the isolation of CS used as therapeutic agent in OA.

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