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Chondroprotective effects of a new glucosamine combination in rats: Gene expression, biochemical and histopathological evaluation

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ABSTRACT

Aims: This study investigates the effect of a new combination of glucosamine hydrochloride, chondroitin sulfate, methylsulfonylmethane, *Harpagophytum procumbens* root extract (standardized to 3% harpagoside) and bromelain extract (GCMHB) on formalin-induced damage to cartilage tissue in the rat knee joint and evaluates this combination in comparison with another combination of glucosamine hydrochloride, chondroitin sulfate and methylsulfonylmethane (GKM).

Materials and methods: Animals in the control group were injected with formalin into the knee joint (FCG). Animals in the GCMHB-500 group were given 500 mg/kg GCMHB + formalin, and those in the GKM-500 group were given 500 mg/kg GKM + formalin. Finally, a healthy group (HG) was also used. GCMHB and GKM were administered to rats orally once a day for 30 days. At the end of this period, the rats were sacrificed and the levels of MDA, NO, 8-OH/Gua, and tGSH in the knee joint tissue were measured. Analysis of IL-1β and TNF-α gene expression was done and the tissue was evaluated histopathologically.

Key findings: MDA, NO and 8-OH/Gua levels and IL-1β and TNF-α gene expression were significantly lower in the GCMHB-500 group compared to the FCG group, whereas tGSH was significantly higher in the GCMHB-500 group than in the FCG group. No significant difference was found for the IL-1β, TNF-α and oxidant/antioxidant parameters between the GKM and FCG groups. The histopathological analysis showed that GCMHB could prevent damage to the cartilage joint, whereas GKM could not.

Significance: GCMHB may be used clinically by comparing with GKM in the treatment of osteoarthritis.

1. Introduction

Cartilage damage is a degenerative joint pathology observed in arthritis and osteoarthritis [1]. Osteoarthritis is the most common form of arthritis [2]. The role of free oxygen radicals and nitrogen products produced from the stimulated chondrocytes was shown to cause damage to the cartilage. These oxidants were reported to cause oxidative damage to the cartilage tissue by oxidizing lipids and deoxyribonucleic acid (DNA) [3–5]. It is also known that stimulated chondrocytes release cytokines such as Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [6], which initiate the oxidative stress reaction in the cartilage [3]. Combinations of glucosamine, chondroitin and manganese ascorbate mixtures are generally recommended in the treatment of osteoarthritis because mixtures of glucosamine, chondroitin and manganese ascorbate were shown to be more effective than each of

Abbreviations: GCMHB, glucosamine hydrochloride, chondroitin sulfate, methylsulfonylmethane, *Harpagophytum procumbens* root extract (standardized to 3 % harpagoside) and bromelain extract; GKM, glucosamine hydrochloride, chondroitin sulfate and methylsulfonylmethane; MDA, malondialdehyde; NO, nitric oxide; 8-OH/Gua, 8-hydroxyguanine; tGSH, glutathione; DTNB, [5,5′-dithiobis (2-nitrobenzoic acid)]; IL-1β, interleukin-1 beta; TNF-α, tumor necrosis factor alpha; FCG, formalin was injected into the knee joint of rats in the control group; GCMHB-500, treated with 500 mg/kg GCMHB mixture + formalin group; GKM-500, 500 mg/kg GKM mixture + formalin group; HG, healthy group; EDTA, ethylenediaminetetraacetic acid.

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them separately [7]. However, none of the treatment modalities can cure the disease completely.

Scientists were directed to develop more effective chondroprotective drug combinations because some drugs had insufficient effects, and allergic reactions developed against the other agents (such as hyaluronic acid) [1,8,9].

In this study, we examined the effects of GCMHB, a new glucosamine combination containing glucosamine hydrochloride (750 mg), chondroitin sulfate (600 mg), methylsulfonylmethane (600 mg), *Harpagophytum procumbens* root extract (standardized to 3% harpagoside) (97.5 mg) and bromelain (97.5 mg) in a formalin-induced cartilage damage model. A substance extracted from the plant *H. procumbens* root extract (standardized to 3% harpagoside), which is found in GCMHB, was reported to inhibit the functions of proinflammatory cytokines such as IL-β and TNF-α [10,11] and was also shown to be beneficial in experimental models of arthritis [12]. In addition, the bromelain found in GCMHB was shown to increase fibrinolysis and to suppress platelet aggregation [13,14]. The antioxidant properties of the *H. procumbens* root extract (standardized to 3% harpagoside) and bromelain were shown experimentally [15,16]. Formalin, which was chosen to induce cartilage damage in this study, is widely used in experimental models to form arthritis in rats [17]. Formalin causes an increase in IL-1β and TNF-α in the claw tissues of the rats, leading to oxidative stress [18,19]. No studies were found in the literature to assess the effects of GCMHB on formalin-induced cartilage damage in joints. Thus, the aim of our study was to investigate the gene expression and biochemical and histopathological effects of GCMHB on formalin-induced chondrotoxicity in the knee joints of rats. Moreover, we aimed to compare the effects of GCMHB and a combination of glucosamine hydrochloride, chondroitin sulfate and methylsulfonylmethane (GKM).

2. Materials and methods

2.1. Animals

Forty-eight male Albino Wistar rats weighing 275–285 g were used in this study. Rats were provided by the Medical Experiment and Research Centre of Recep Tayyip Erdogan University. The animals were brought to the Pharmacology Department Laboratory in groups and were fed there for 3 days at normal room temperature (22 °C) to adapt to the laboratory where the study would be carried out. Animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the local animal ethics committee of Recep Tayyip Erdogan University, Rize, Turkey (Ethics Committee Number: 2014/60, Dated: 29.09.2014).

2.2. Chemical substances

Nutraxin Artroflex, the commercially available form of GCMHB, was used in the study. Nutraxin Artroflex and the mixture of glucosamine hydrochloride, chondroitin sulfate and methylsulfonylmethane (GKM) were provided by BIOTA (Turkey). Thiopental sodium was obtained from İ.E ULAGAY. Formalin was acquired from the Pathology Department (Turkey).

2.3. Study groups

The rats used in the study were divided into four groups. Formalin was injected into the knee joint of rats in the control group (FCG). Two additional groups were treated with 500 mg/kg GCMHB mixture + formalin (GCMHB-500) and 500 mg/kg GKM mixture + formalin (GKM-500). A fourth, healthy group (HG) was also used.

2.4. Study procedure

2.4.1. Effects of GCMHB and GKM on formalin-induced cartilage damage in the knee joint of rats

The drugs were administered by using oral gavage in the following doses: 500 mg/kg of GCMHB mixture (glucosamine 175 mg/kg, chondroitin sulfate 139 mg/kg, methyl sulfonyl methane 139 mg/kg, bromelain 23 mg/kg, *Harpagophytum* extract 23 mg/kg) to the GCMHB-500 group (n = 12) and 500 mg/kg of GKM mixture to the GKM-500 group (n = 12). Similarly, distilled water was given to the HG group (n = 12) and FCG group (n = 12). 1 h after the administration of the GCMHB mixture, the GKM mixture or distilled water, one-time injection of 0.2 mL of 1% formalin was performed into the knee joints of all of the rats except those of the HG group [20]. GCMHB and GKM were administered daily in the doses mentioned above for 30 days. At the end of this period, the rats were sacrificed using a high dose of thiopental anesthesia. The knee joints of the rats were removed. Oxidant–antioxidant levels (malondialdehyde (MDA), total glutathione (tGSH), nitric oxide (NO)) and DNA oxidation were measured and the gene expression of IL-1β and TNF-α was performed in the cartilage tissue, which was then examined histopathologically. Six rats from each group were used for histological processing. The remaining six rats in each group were analyzed about the biochemical and gene expression. All results were compared with the FCG group.

2.5. Biochemical analysis of the cartilage tissue

Homogenates were prepared from the cartilage to measure the enzyme activity in the tissues. Supernatants obtained from this homogenate were used to measure MDA, NO and tGSH levels and to carry out DNA oxidation analysis according to methods defined in the literature. All spectrophotometric measurements were performed by the microplate reader (Bio-Tek PowerWave XS, USA). The results were expressed by dividing to g protein.

2.6. Preparing the samples

The 25 mg of the tissue was homogenized using a solution of 1.15% KCl (Merck, Germany). The homogenate was centrifuged at 4000 rpm for 30 min at +4°C. Supernatants were then used for for NO and MDA measurements. Tissues (25 mg) taken for tGSH analysis were washed with isotonic sodium chloride (İ.E. ULAGAY, Turkey) and subsequently brought to 2 mL total volume with phosphate buffer solution (0.213 g $NaH_2PO_4 \cdot 2H_2O$ (Merck, Germany) + 1.563 g $Na_2HPO_4 \cdot 2H_2O$ (Merck, Germany) + 0.038 g EDTA (Sigma-Aldrich, Germany) + 100 mL dH_2O, pH = 7,4) and then were homogenized in an icy environment. After that, the tissues were centrifuged at 1000 rpm for 15 min at a temperature of +4 °C. The supernatant was used as the sample for analysis. The protein concentration of the supernatant was measured using the method described by Bradford [21].

2.7. MDA analysis

According to the method defined by Ohkawa H et al., MDA forms a pink complex with thiobarbituric acid (TBA) at 95 °C, which can be measured using spectrophotometry at a wavelength of 532 nm [22]. The 0.1 mL homogenate was added to a solution containing 0.1 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid (Merck, Germany) 1.5 mL of 0.9% TBA (Sigma-Aldrich, Germany), and 0.3 mL dH_2O. The mixture was incubated at 95 °C for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (v/v, 15:1, Merck, Germany) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the 0.15 mL supernatant was measured at 532 nm by spectrophotometry. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Germany).