MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 9(2) pp. 138-144, February, 2021
Available online http://www.meritresearchjournals.org/mms/index.htm

DOI: 10.5281/zenodo.4562846

Original Research Article

Effect of a Proprietary Herbal Product "Arthrocon" on some Synovial Fluid and Serum Biomarkers in Horses with Naturally Occurring Osteoarthritis

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Abstract

Herbal extracts contain multiple biologically active chemicals, and indigenous medicinal plants have been used traditionally as a major source of drugs for the treatment of various illnesses, including osteoarthritis (OA). The concerns about side effects associated with conventional arthritis therapies have sparked a renewed scientific interest in traditional natural anti-arthritis remedies. Current study was performed to find out the potential beneficial effect of the originally compounded herbal tea "Arthrocon" on the damaged articular cartilage in equine naturally occurring OA using some markers of altered joint metabolism. The results suggest that the four monthsphytotherapy with herbal tea "Arthrocon" is clinically relevant for treatment of osteoarthritis in horses by its ability to reduce joint inflammation and the articular cartilage destruction.

Keywords: Articular degradation, Horse, Osteoarthritis, Phytotherapy

INTRODUCTION

Osteoarthritis (OA) is a common disease in horses, characterized by progressive articular damage, leading to constant pain and loss of performance. It affects 30% of sport horses and exerts a great impact on the animal welfare and equine industry (Souza, 2016). It was estimated that 60% of alllamenesses wererelated to osteoarthritis (OA) and approximately $145 million was spent on veterinary bills connecting to the problem (vanWeeren, 2016).

A single or repetitive joint injury produces immediate and progressive cell death and matrix disruption in articular cartilage. The resident chondrocytes are activated to produce inflammatory cytokines which recruit inflammatory cells and activate intracellular pathways for expression of metalloproteinases (MMPs) and other molecules degrading type II collagen. This progressive loss of the hyaline articular cartilage leads to long term release of extracellular matrix molecules in the synovial fluid (SF) (Bertuglia et al., 2016) including cross-linked C-telopeptide fragments of type II collagen (CTXII) and non-collagen complex oligomeric matrix protein (COMP). Pocollagen type II C-propeptide (PIICP), also referred to as CPII, is released as part of the secretion of newly synthesized type II collagen from the cells and could serve as a marker of cartilage regeneration.

Matrix metalloproteinases (MMPs) is a family of proteolytic enzymes taking part in degradation of extracellular matrix during normal and pathological tissue metabolism. MMP-1 is highly effective in disrupting specifically collagen type I, II, and III. MMPs' function is regulated by tissue inhibitors of metalloproteinases (TIMPs). TIMP-2 can serve either as an inhibitor or activator of MMPs (Bourboulia and Stetler-Stevenson, 2010).

Therefore, these kay molecules involved in the process of cartilage destruction and synthesis could serve as biomarkers of joint metabolism.

Conventional treatment with steroids and NSAIDs is associated with unsatisfactory results and well-known adverse effects. Therefore, new strategies are seeking by the scientists, including medicinal plants that could be given safely for a long period of time. Natural products can control inflammation through multiple pathways.

Mikaili et al. (2013) reported that the organosulfur compounds, such as allicin and diallyldisulfide, present in garlic are responsible for its medicinal effects. The active constituent of garlic (*Allium sativum*), allicin has exhibited significant anti-arthritic activity in vivo in albino rats and also antioxidant activity in vitro (Jayanthi et al., 2015). Diallyldisulfide is reported to have chondroprotective activity by repressing cytokine-responsive MMP induction (Williams et al., 2010).

Comphrey (*Symphytumofficinale*) has been effectively used as a topical cream for treatment of OA (Smith and Jacobson, 2011; Staiger, 2013). Its pharmacological components rosmarinic acid and tannin exhibit strong antioxidant properties. Levanon and Stein (1995) suggested the ability of tannic acid to augment glycosaminoglycan binding to collagen most possibly contributes to the structural reinforcement of synovial articulating surfaces.

In their review on the effectiveness of the devil's claw (*Harpagophytumprocumbens*) in the treatment of osteoarthritis Brien et al. (2006) concluded that this herb appears effective in the reduction of the main clinical symptoms and pain. Its main constituents are the iridoid glycosides, such as harpagoside, procumbide, harpagide, and phenol derivatives. In vitro studies of Fiebich et al. (2001) identified that both water and alcohol extracts of *Harpagophytum* inhibited the LPS-induced synthesis of PgE_2 and other proinflammatory cytokines.

Curcumin is an active constituent that is derived from the rhizome of turmeric (*Curcuma longa or domestica*). The anti-inflammatory effects of curcumin are believed to be the results of inhibiting pro-inflammatory signals such as prostaglandins, leukotrienes, cyclooxygenase-2, and metalloproteinases (Perkins et al., 2017; Dudics et al., 2018).

A clinical study showed that an unspecified dried extract of nettle (*Urticadioica*) combined with fish oil, vitamin E andzinc, in a proprietary product (Phytalgic®), decreased disease scores in patients with OA and reduces the use of analgesics or nonsteroidal anti-inflammatory drugs (Jacquet et al., 2009). The polar water extracts evaluated demonstrated no ability to reduce inflammation stimulated by LPS in vitro (Johnson et al., 2013). Qualitative and quantitative liquid chromatography tandem mass spectrometry analyses indicated that phenolic acids and flavonol glycosides (rutin, isoquercitrin, and kaempferol3-O-glucoside) are present in the aerial parts, while lignans (secoisolariciresinol, 9,9'-bisacetyl-neo-olivil and their glucosides) were detected in the root. Herb and root extracts expressed selective inhibition toward cyclooxygenase and lipoxygenase branches in human platelets (Franciškoviće et al., 2017). These observations suggest that stinging nettle is an interesting candidate for the development of phytopharmaceuticals or dietary supplements for cotreatment of various inflammatory diseases.

The aim of the study was to evaluate the therapeutic abilities of herbal tea "Arthrocon" on the naturally occurring osteoarthritis in horses using some biomarkers of altered joint metabolism in articular disorders.

MATERIALS AND METHODS

Animals

Eight working client owned horses (6 mares and 2 geldings) aged between 10 and 19 years with a mean body weight of 442kg were included in the study. The selection criteria were history of chronic lameness with one of the hind limbs, radiographic evidence for osteoarthritis of the hock joint, and intact opposite hock joint. The exclusion criteria were the presence of additional orthopaedic issues or general infections, orif horses received systemic medication, such assteroids, NSAIDs or dietary supplements. All owners voluntarily enrolled their horses to take part in this study. Informed consent has been obtained for client-owned animals included in this study. Animals were kept in one and the same nutritional and exercise regimen for the whole trial. They were fed with hay and pelleted grain according to their weight requirements. Horses were allowed to move freely in paddocks during the day and stabled at night.

Herbal product

"Arthrocon" is a supplement developed specifically for the study. It contains a mixture of herbs that are used widely in human to threat inflammation and pain: stinging nettle (*Urticadioica*), devil's claw (*Harpagophytumprocumbens*), garlic (*Allium sativum*), curcumin (*Curcuma longa*), and comfrey (*Symphytumofficinale*). Their primary active phytochemicals are glycosides, flavonoids, allantoin, minerals, ect. Each animal received 50g of "Arthrocon" daily for four months. The mixture was first steamed in 2 liters of water for 5 min. next given together with the water extract.

Samples

Blood and synovial fluid were drawn before and after treatment for quantitative assessment of some

Table 1. Concentrations of the C-telopeptide of type II collagen (CTXII) in the serum and synovial fluid (in pg/ml) before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	187.29	171.89	150.99	252.17		Between a and b	0.263
b	8	158.28	152.92	138.35	190.59		Between c and e	0.208
c	8	46.13	46.6	12.87	93.2		*Between c and d	**0.012**
d	8	**156.52***	143.85	92.34	278.02		**Between d and f	**0.017**
e	8	58.43	56.75	27.73	92.34		Between e and f	0.779
f	8	**60.93****	66.76	12.01	95.77			

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

Table 2. Concentrations of the Procollagen II C-terminal propeptide (PIICP) in the serum and synovial (in pg/ml) fluid before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis.

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	1127.79	1260.0	869.23	1301.3	217.48	Between a and b	0.138
b	8	903.41	926.78	793.41	1004.5	96.24	Between c and e	0.674
c	8	377.99	347.61	323.68	517.34	69.39	Between c and d	0.484
d	8	396.52	378.2	331.29	499.9	70.07	Between d and f	0.779
e	8	379.91	376.0	336.72	442.06	32.21	Between e and f	0.401
f	8	400.41	395.7	315.0	490.97	62.21		

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

biomarkers of inflammation and chondrocyte degradation and synthesis.

Blood was collected from jugular vein in heparin/EDTA tubes for determination of CBC and biochemistry profile in order to ensure the lack of other non-related diseases. Samples were centrifuged for 15 min at 1500 rpm, and plasma was separated and frozen at -25°C for further analysis.

Synovial fluid was collected from both hock joints in EDTA tubes by aseptic arthrocentesis after mild sedation of horses using 0.02mg/kg xylazine (Alfasan International, Holland) IV. It was frozen at -25°C for further analysis.

Biomarkers and analytical procedures

Six biomarker assays were performed on both serum and synovial fluid samples. Concentrations of the C-telopeptide of type II collagen (CTXII), Procollagen II C-terminal propeptide (PIICP), matrix metalloproteinase-1 (MMP-1), tissue inhibitors of metalloproteinase-2 (TIMP-2), cartilage oligomeric matrix protein (COMP) and prostaglandin E_2 (PgE_2) were measured by commercial equine enzyme linked immunosorbent assay (ELISA) kits (Cloud-Clone Corp., USA).

Statistics

The statistical analyses were performed with the aim of a computer program Statistica version 7.0 (StatSoft Inc., 2004, USA). The differences of variable between the initial and final periods as well as between healthy and diseased joints were determined by a non-parametric Wilcoxon Matched Pair Test. The value of $p<0.05$ was considered as a significant and was reported.

RESULTS

Clinical assessments

Clinical examination included observation for joint effusion, lameness scoring, and response to flexion. At the initial period 3 horses demonstrated 2/5 degree of lameness (AAEP scale), whereas the rest 5 showed 3/5 degree of lameness. Joint effusion was absent in all horses, but all reacted positively in flexion tests.

After treatment the lameness scoring decreased to 2/5 in 2 animals, 1/5 in 3 animals and 0/5 in 3 animals. There was not joint effusion and positive flex response was provoked in only 2 animals.

Table 3. Concentrations of the Matrix metalloproteinase 1 (MMP1) in the serum and synovial fluid (in ng/ml) before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis.

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	2.25	2.64	0.4	4.14	1.66	Between a and b	0.225
b	8	3.71	3.57	2.98	5.03	0.8	Between c and e	0.263
c	8	1.63	1.53	1.24	2.55	0.42	*Between c and d	**0.012**
d	8	**5.27***	5.66	1.67	8.49	2.33	**Between d and f	**0.036**
e	8	**1.41*****	1.4	1.19	1.65	0.16	***Between e and f	**0.036**
f	8	**4.42****	4.32	1.11	7.22	2.39		

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

Table 4. Concentrations of the Tissue inhibitors of metalloproteinase 2 (TIMP 2) in the serum and synovial fluid (in ng/ml) before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis.

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	288.42	285.3	231.82	391.83	52.37	Between a and b	0.674
b	8	278.72	251.08	189.87	411.39	89.38	Between c and e	0.208
c	8	332.35	340.34	289.8	357.53	27.14	*Between c and d	**0.012**
d	8	**245.91***	268.21	134.12	299.47	55.42	Between d and f	0.069
e	8	353.38	366.1	264.2	396.66	47.18	**Between e and f	**0.012**
f	8	**204.81****	199.03	154.6	306.46	48.53		

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

Table 5. Concentrations of the Cartilage oligomeric matrix protein (COMP) in the serum and synovial fluid (in pg/ml) before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis.

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	929.65	908.47	334.55	1454.2	402.36	Between a and b	0.263
b	8	1528.44	1505.91	616.81	2500.0	918.47	Between c and e	0.090
c	8	486.27	452.16	400.41	597.99	74.5	*Between c and d	**0.049**
d	8	**687.37***	659.15	372.19	983.74	229.96	Between d and f	0.069
e	8	433.34	419.23	381.6	541.54	48.49	Between e and f	0.123
f	8	508.61	522.72	315.74	616.8	95.28		

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

Table 6. Concentrations of the Prostaglandin E_2 (PgE_2) in the serum and synovial fluid before and after treatment with "Arthrocon", as well as in affected and unaffected hock joints in horses with osteoarthritis.

Sample	N	Mean	Median	Min.	Max.	SD	P value	
A	8	19.53	17.0	12.75	28.63	6.66	Between a and b	0.779
b	8	20.08	18.22	16.13	33.53	5.7	Between c and e	0.889
c	8	7.77	7.61	5.47	9.31	1.28	*Between c and d	**0.012**
d	8	**15.71***	14.54	13.59	23.73	3.33	**Between d and f	**0.012**
e	8	8.79	9.27	5.88	10.89	1.84	Between e and f	0.889
f	8	**7.5****	8.13	4.16	8.93	1.71		

a-Serum before treatment; b-Serum after treatment; c-Synovial fluid from healthy joints before treatment; d-Synovial fluid from diseased joints before treatment; e-Synovial fluid from healthy joints after treatment; f-Synovial fluid from diseased joints after treatment.

Biomarkers assessments

The changes in concentration of measured biomarkers in the serum and synovial fluid before and after treatment as well as in affected and unaffected joints are presented in tables 1 - 6.

Results showed that CTXII levels did not change in serum but increased in synovial fluid in osteoarthritis. Applied phytotherapy leaded to drop in CTXII concentrations in the affected joints close to quantities in non-affected joints (table 1). Osteoarthritis and herbal supplementation did not result in changes in serum and synovial fluid content of PIICP (table 2). MMP 1 quantities were elevated in synovial fluid of osteoarthritic joints. After treatment the levels decreased but remained still significantly higher in comparison to the healthy joints (table 3). Osteoarthritis resulted in lower content of TIMP 2 in synovial fluid of diseased joints in comparison to the healthy joints. Its levels remained lower in affected joints after treatment (table 4).Synovial fluid concentration of COMP increased in osteoarthritis, but herbal therapy leaded to insignificant decrease in its levels. Elevation in serum after treatment was also insignificant (table 5). PgE2 levels were elevated in synovial fluid of joints with osteoarthritis but phytotherapy decreased its concentrations (table 6).

DISCUSSION

Our result showed that OA is associated with significantly increase in synovial fluid (SF) concentrations of CTXII, a biomarker reflecting type II collagen degradation without concomitant changes in PIICP levels, a biomarker of type II collagen synthesis. Non-collagenous proteins like COMP were also increased in the SF of OA affected joints. These finding suggests an increased matrix turnover with progressive disruption in the articular cartilage during the OA. Similar results have been found by Bertuglia et al. (2016). They also reported a strong correlation of SF levels of COMP with the lameness score in OA. According to Skiöldebrand et al. (2005) synovial fluid COMP was correlated to length of time post injury, suggesting it is a suitable marker for studied on joint healing.

Frisbie et al. (2008) reported an elevation in both SF and serum biomarkers of articular cartilage and subchondral bone degradation (such as glycosaminoglycan, epitope ColCEQ, Col 1, C1, C2) and synthesis (epitope CS846, CPII, osteocalcin) in exercise and early OA in horses. They found out significant differences in the biomarkers concentrations between exercise and OA-affected joints with exercise alone horses returned to pre-study levels, whereas OA-affected horses did not. Opposite to this study, we found that these alterations in the SF did not correspond to concentrations of the same biomarkers detected in the serum. There is poor correlation between structural biomarkers in the serum and SF of OA-affected joints (Bertuglia et al., 2016), associated with the chronicity of the disease and low permeability between compartments.

Carboxypropeptide of type II collagen (CPII) was elevated in SF and serum of young horses with osteochondritis dissecans (McIlwright, 2005) but not in adult with OA, as we have found as well. A relation between CPII levels and severity of the disease was also shown in this study. According to the results of Kawcak et al. (2008) radiographic lysis in experimental OA was strongly correlated with CPII, whereas radiographic proliferation was strongly correlated with PgE_2 and WBC in SF.

OA also resulted in local increase MMP-1 activity with drop in TIMP-2 levels determining a disturbance in regulation of the molecules responsible for degradation of articular cartilage. MMP-1 is capable of cleaving intact collagen and its activity was significantly higher in SF of horses with OA of MCP joints than in the SF of age-matched healthy joints (Brama et al., 2004).

The concentration of proinflammatory cytokine PgE_2 was also found to be elevated in SF of OA affected joins. Similarly, in the study of Frisbie et al. (2008) synovial PgE_2 levels were increased for 90 days after experimental OA in horses but not in exercise control group. This finding leads to the conclusion that the inflammatory process persists during chronic development of the OA. PgE_2 activates osteoblasts to induce bone resorption in subchondral bone during the disease (Wiemer et al., 2011).

The application of herbal product “Arthrocon” resulted in significant drop in the SF concentration of the investigated biomarkers of joint inflammation (PgE_2) and cartilage matrix degradation (CTXII, COMP) in OA affected joints.

Thesynovial levels of MMP-1 and TIMP-2 regulation molecules were significantly different between injured and healthy joints and remained elevated and decreased, respectively at the post-treatment period comparatively to the initial one. This finding suggests that the OA joint is in lower grade but still catabolic state after 4 months of herbal application. Hu et al. (2011) investigated the effect of berberine on rat experimental OA and found out that berberine (*Rhizomacoptidis)* inhibits the expression of MMP-1, 3, and 13, and increased the TIMP-1 at mRNA level.

In an extensive review Li et al. (2017) reported that many herbs used in traditional Chinese medicine provide medical value, investigated using modern technologies, by modification of disease and symptoms in human OA.

Very limited number of scientific works investigated the clinical effect of herbs that we used on the equine OA assessed by specific markers in SF and serum for monitoring of cartilage degeneration and regeneration. Person et al. (Pearson et al., 1999) reported that herbal mixture “Mobility”, containing dandelion, devil’s claw,

stinging nettle, burdock, and comfrey, suppressed production of PgE_2 in the equine arthritic joints without significant alteration in synovial glycosaminoglycan and hyaluronic acid contents.

CONCLUSION

In conclusion, the four month oral application of herbal tea “Arthrocon” is clinically relevant for treatment of osteoarthritis in horses, because of its ability to reduce joint inflammation and the articular cartilage destruction.

ACKNOWLEDGEMENTS

We are thankful to d-r Dimitre Petlev and Mr. Gueorgui Hadjiyski from HerbaconGmbH,Berlin, Germany for the opportunity to perform this study.

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