

Effect of a Proprietary Herbal Product on Equine Joint Disease

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ABSTRACT. Osteoarthritis is a degenerative disease of the joints that affects a great number of horses, and accounts for a considerable economic burden on the industry. The condition is typically treated with NSAIDs and steroids, but these treatments may elicit negative side-effects when used over the long-term. Herbal remedies have increased in popularity over the past 3 years, but little scientific documentation exists to further the knowledge of these products for horses. This study investigated the effects of an herbal mixture "Mobility" on equine osteoarthritis. It was found that Mobility significantly suppressed production of prostaglandin E₂ in the arthritic joints. This observation provides a unique objective, scientific insight which may explain the anecdotal testimonials relating to the success of this product. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworthpressinc.com <Website: <http://www.haworthpressinc.com>>]*

KEYWORDS. Osteoarthritis, equine, degenerative joint disease, herbal products, prostaglandin E₂, GAGs

INTRODUCTION

Herbal medicine has a long and colorful history, dating back as far as 60,000 years (Solecki 1975). Trends in fashion and politics have

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seen the waxing and waning of herbal therapy over hundreds of generations (Griggs 1997), and currently we are experiencing yet another renaissance. Following historical pattern, this one too is borne largely of an increasing distrust in chemical/synthetic prophylactics, and a revitalized curiosity in the unique medicinal properties of plants (Balick et al. 1992; Cottrell 1996; Dossey and Swyers 1992; Driedger 1998; Murray 1996; Taylor 1996). A recent survey of more than 1000 trainers, owners, breeders and veterinarians in the Ontario horse industry highlighted their calls for more research into natural therapeutics (SEM 1998). A lack of information on alternative therapies was second only to respiratory disease as the number one horse-health issue facing the industry. And respondents claimed a strong desire to learn more about nutraceuticals and alternative therapies.

Lameness is a major cause of wastage among racehorses, and the condition accounts for a great economic burden on the racing industry (Rossdale et al. 1985). A wide variety of pathologic episodes in the equine joint can culminate in the common end stage of osteoarthritis (OA). The condition is typified by progressive deterioration of the articular cartilage, accompanied by changes in the bone and soft tissue of the joint, and eventual loss of joint function (Palmer and Bertone 1994; McIlwraith 1985). Osteoarthritis is typically treated with a regime of non-steroidal anti-inflammatory drugs (NSAIDs), and steroids. This method of treatment elicits large-scale, clinical improvement in equine patients (Higgins and Lees 1983; McIlwraith 1992), but there is some concern over potential side-effects to chronic use. Non-steroidal anti-inflammatory drugs used over the long-term have been implicated in gastric ulceration (Hunt, Lees, and Edwards 1985; Snow, Bogan and Douglas 1979; Snow et al. 1981) and inhibition of chondrocyte metabolism (Palmoski and Brandt 1980; Palmoski, Colyer and Brandt 1980). Reported side effects to some corticosteroids include reduced hyaluronic acid synthesis and chondrocyte metabolism, laminitis, osteoporosis of subchondral bone, loss of cartilage elasticity and hydration (Owen 1980), and a delay in healing which, together with reduced inflammation, causes an increased susceptibility to infection (Gabel 1977). Consumers and pharmaceutical companies are increasingly receptive to proactive, natural therapeutics (Cottrell 1996). This, coupled with a growing cynicism on the benefits of synthetic prophylactics leads many horse people to seek complimentary or alternative long-term treatments for their arthritic horses.

There are many testimonials to the anecdotal success of herbal remedies in mitigating various inflammatory disorders. However, despite a large battery of scientific documentation for herbs' actions in vitro and in some laboratory animals (Burger, Brandt, and Ferreira 1987; Lanhers et al. 1992; LeMoal et al. 1992a; LeMoal et al. 1992b; See et al. 1997; Wagner et al. 1988; Wagner, Willer, and Kreher 1989; Winship 1991; Murch, Simmons, and Saxena 1997), scientific information available on the use of herbs for horses is sparse. The purpose of this study was to quantify the effect of a proprietary herbal preparation on systemic and synovial metabolic indicators of inflammatory joint disease and repair.

MATERIALS AND METHODS

Horses

Six horses (4 Thoroughbred, 2 Standardbred) were selected for the double-blind, placebo controlled, cross-over study. The criteria for selection were clinical lameness, together with at least one other indicator of OA (i.e., history of chronic lameness, positive flexion, and/or positive radiographs). Horses and selection criteria are listed in Table 1. Horses were stabled at the Equine Research Centre, and fed a sweet feed and free-choice hay ration, which met the horses' nutritional requirements. Horses were given a minimum two week acclimatization period at the Equine Research Centre. Horses were randomly

TABLE 1. Selection Criteria at Beginning and End of Each Phase. (+, - indicates observed or not observed)

Horse #	History	Lameness			Flexion					
		A-0	A-28	B-28	A-0	A-28	B-28	A-0	A-28	B-28
1	+	+	nc*	nc	-	-	-	-	nc	nc
2	-	+	i@	nc	-	-	-	+	nc	nc
3	+	+	nc	nc	+	+	+	+	nc	nc
4	+	+	nc	nc	+	+	+	+	nc	nc
5	+	-/+	i	nc	+	+	-	+	w^	i

* no change, @ improved, ^ worse

assigned one of six “A” and “B” tubs of supplement, each of which was randomly filled with either herbal mixture or placebo (ground alfalfa meal), and blinded to researchers. In this way, each horse would have the opportunity to be on the control and test supplement.

During Phase A, horses received one half cup of “A” supplement mixed into their morning and evening grain ration. After 28 days, horses were given a 21 day “washing out” period, during which no supplement was fed. The horses were then crossed over, and the regime was repeated (Phase B) with the “B” supplements. Horses were turned out daily, and stabled at night. The experimental protocol was approved by the Equine Research Centre Animal Care Committee.

Herbal Supplement

“Mobility” is an herbal product developed specifically for horses by Selected Bioproducts (Guelph, Ontario). It is formulated to improve musculoskeletal function by reducing the inflammatory response in chronically inflamed tissue. The supplement contains a mixture of five herbs with historic application to the treatment of inflammation and pain: dandelion, devil’s claw, stinging nettle, burdock, and comfrey. The primary active phytochemicals in Mobility are glycosides, flavonoids, allantoin, mucilage, and sesquiterpenes (see Table 2).

Blood Parameters and Analytical Procedures

GAGs-Serum Assay

Blood was collected for assessment of serum glycosaminoglycans (SeGAGs) on Days 0, 14, and 28 of Phases A and B. Horses were sampled by jugular puncture, and blood was aspirated into one 10 ml silicone coated vacuum tube. Samples were centrifuged for 15 min at 1500 rpm, and serum was frozen at -13°C until being shipped on ice to the Equine Orthopaedic Research Laboratory, Colorado State University (CSU). Glycosaminoglycans were quantified by dimethyl methylene blue (DMMB) assay, as previously described (Alwan et al. 1991).

Blood Profiles

On Days 0 and 28, a second silicone coated tube was filled, together with one 7 ml heparin/EDTA tube, to be analyzed for complete blood

TABLE 2. Composition of "Mobility"

Plant	Scientific Name	Parts Used	Ref.
Dandelion	<i>Taraxacum officinalis</i>	roots, leaves	Tyler 1993; Tyler 1994; Self 1996; McCluggage 1996; Newall, Anderson, and Phillipson 1996 (p. 96)
Devil's Claw	<i>Harpagophytum procumbens</i>	tuber	Burger et al. 1987; Grahame 1981; Lahners et al. 1992; Larrey 1997; McLeod, Revell, and Robinson 1979; Moussard, Alber, Toubin, Thevenon, and Henry 1992; Whitehouse, Znamirowska, and Paul 1983; Newall et al. 1996 (p. 98)
Comfrey	<i>Symphytum officinalis</i>	leaves	Abbott 1988, Goldman, deFreitas, and Oga 1985; Hansen, Stoessel, and Rossi 1991; Mohammad, and Noorwala 1995; Winship 1991; Roitman 1981; Newall et al. 1996 (p. 87)
Burdock	<i>Arctium lappa</i>	leaves, seeds, pods, roots	Kato and Watanabe 1993; Tyler 1993; Self 1996; Tyler 1994; Newall et al. 1996 (p. 52)
Stinging Nettle	<i>Urtica dioica</i>	whole plant	Hirano, Homma, and Oka 1994; LeMoal et al. 1992; Mittman 1990; Wagner et al. 1989; Newall et al. 1996 (p. 201)

count (CBC: *WBC, RBC Hb, HCT, MCV, MCH, MCHC, RDW, Platelets, MPV, T.S. Protein, Segmented Neutrophil Count, Lymphocyte Count, Monocyte Count, Eosinophil Count, Basophil Count, Anisocytosis Rouleaux, Crenated, Poikilocytosis*) and equine serum profile (ESP: *GLDH, Creatinine Kinase, Haptoglobin, AST, Gamma-GT, Free Bilirubin, Alkaline Phosphatase, Conjugated Bilirubin, Total Bilirubin, Glucose, Cholesterol, Creatinine, Urea, Sodium, Phosphorus, Total Protein, Calcium, Albumin, Globulin, A:G Ratio, Potassium, Carbon Dioxide, Chloride*). This was performed in order to identify any possible non-related systemic- or side-effects. Complete blood count (CBC) was run on Technicon H*1 (Bayer Corp., Etobicoke, Ontario). Equine serum profile (ESP) was run through the Hitachi 911 Biochemical Analyzer (Boehringer Mannheim, Laval, Quebec).

Synovial Fluid Parameters and Analytical Procedures

Synovial fluid was collected from horses on Day 0, 14, and 28 of Phases A and B. Horses received 0.02 mg/kg BW of intravenous xylazine (Anased Injectable; Lloyd Laboratories, Shanandoah, IA). The arthritic joint, and corresponding opposite joint were surgically scrubbed. Synovial fluid samples were extracted by aseptic arthrocentesis, and collected in sterile silicone coated blood tubes.

Cytology

Volume of sample extracted from each horse varied across each sampling period. When quantity of synovial fluid permitted, cytological analysis was performed on fresh samples. Two hundred liters of fluid was spun in a cytocentrifuge (Shandon Cytospin 11; Shandon Inc., Pittsburgh, PA) at 1,000 rpm for 6 minutes, and resulting slides were stained with a Wright Hemastain (Technicon H*1; Bayer Corp., Etobicoke, Ontario) and fixed with xylol (Animal Health Laboratories, University of Guelph). From the smears, cellularity was determined as high, normal, or low. Cell differentials were ascertained, as were the presence or absence of red blood cells and polarized particles (cartilage fragments). The remaining synovial fluid sample was centrifuged at 1500 rpm for 15 minutes in order to precipitate cellular debris. They were frozen at -13°C and, upon completion of both sampling periods, were shipped on ice to the Equine Orthopaedic Research Laboratory, Colorado State University. There they were analysed for GAGs, hyaluronic acid (HA) and prostaglandin E_2 (PGE_2).

GAGs-Synovial Fluid Assay

Glycosaminoglycans (GAGs) were quantified using a dimethyl methylene blue (DMMB) assay, as described in Alwan et al., 1991. This is a spectrophotometric assay which uses the conjugation of 1,9-dimethyl methylene blue to GAGs and the absorbance is compared with that of a chondroitin sulphate (CS) standard.

HA Assay

Hyaluronic acid (HA) was quantified using the Alcian blue spectrophotometric assay, as described in Smith et al., 1980. This assay makes

use of a precipitate of Alcian blue and hyaluronic acid, and the resulting optical density is spectrophotometrically determined.

PGE₂ Assay

Prostaglandin E₂ (PGE₂) was quantified by an extraction process, followed by an ELISA assay with a commercially available kit (Titer-Zyme Prostaglandin E₂-catalog # 8-6801N, PerSeptive Biosystems, Framingham, MA).

Statistics

Paired differences were determined for each variable for each horse by subtracting the value of the variable at Day 14 from the value at Day 0 (Diff1), Day 28 from Day 0 (Diff2), and Day 28 from Day 14 (Diff3). Proc T test (SAS System for Elementary Statistical Analysis, SAS Institute Inc., Cary, NC) was then used to compare the means of the treated group and the control group. Affected and unaffected limbs were compared using the same method. A 5% confidence level was used to test for significant differences between groups. Data was reported as mean percent changes in parameters over time within treatment and control groups.

RESULTS

Selection Criteria

Changes in selection criteria are listed in Table 1.

Blood Profiles

Complete blood count was statistically unremarkable, with no significant differences in any of the hematology parameters examined.

Analysis of the equine serum profile showed no significant differences in biochemical profiles of horses on treatment or placebo.

SeGAG

There were no statistically significant differences in SeGAG levels in any horse while on the treatment or control supplements. While

there was considerable variation in the results, there was a clear trend for SeGAG to rise in the treatment group compared with the control group (see Figure 1). In the sampling interval between Day 0-14, the SeGAG concentration increased by 185%, while the control group increased only 10%. A similar trend was observed during the overall sampling (Day 0-28). Serum glycosaminoglycan (SeGAG) had an overall increase of 115%, while the concentration of control horses actually decreased by 41%.

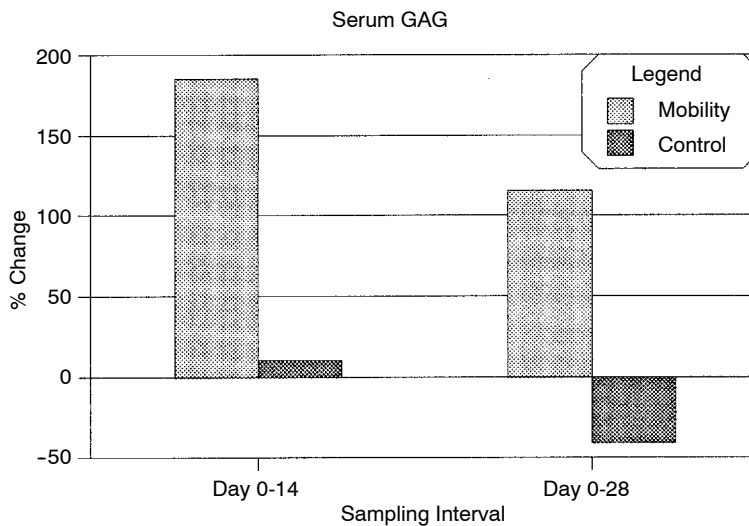
Synovial Fluid Cytology

Due to difficulty in obtaining sufficient quantity of sample, only 2 samples of 12 in Phase A, and 4 of 12 in Phase B, had cytology analysis performed on all three samples (i.e., Day 0, 14, and 28). Therefore, results were not considered to be representative, and further statistical analysis was abandoned.

SynGAG

There were no statistically significant differences between treatment and control horses in affected or unaffected limbs. There was,

FIGURE 1. Average Change in Serum GAG Levels



however, an observable trend to an increase in SynGAG in the treatment groups as compared with the control groups (see Figure 2). During the Day 0-14 sampling, the Mobility-treated, affected limbs a 5% increase in SynGAG, while the control affected limbs increased only 1%. In the unaffected limbs, this trend was more pronounced, with the concentration in the Mobility-treated limbs increasing by 6%, and the control limbs decreasing by the same amount. By Day 28, a trend was evident for the unaffected limbs (6% increase for Mobility-treated limbs, 2% decrease for control limbs), but none was observed for the affected limbs.

Prostaglandin E₂ (PGE₂)

There was a statistically significant increase in overall PGE₂ levels during the control phase from Day 0-14 ($p < 0.05$). During the same period, PGE₂ did not increase significantly when horses received Mobility. During the first 14 days, there were no statistically significant differences between arthritic and normal limbs regardless of treatment, but trends again were observed (see Figure 3). A 41.3% increase in PGE₂ levels in Mobility-treated, arthritic limbs was accompanied by a 91.7% increase in placebo-treated, arthritic limbs. There was no

FIGURE 2. Average % Change in Synovial Fluid GAG

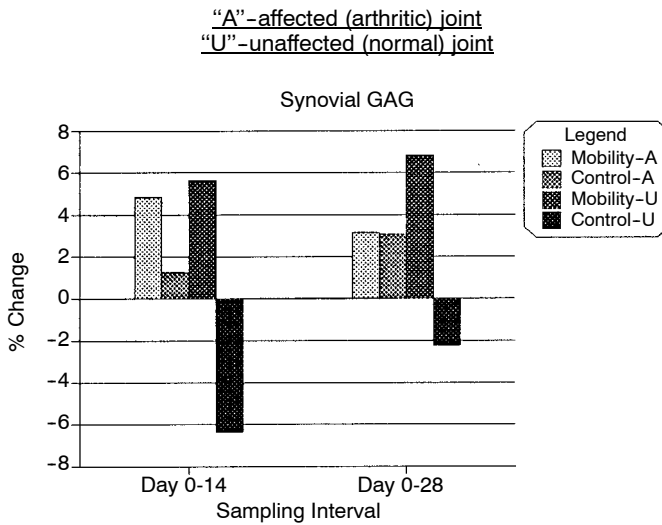
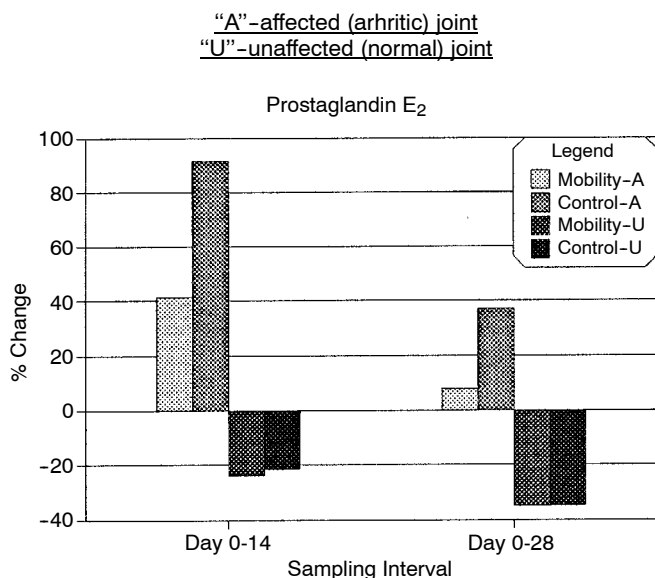


FIGURE 3. Average % Change in Synovial Fluid Prostaglandin E₂

difference between Mobility and control in normal limbs. Although increases in PGE₂ levels in the control arthritic joint were not statistically significant by the 28th day, the average increase in the Mobility arthritic joint was only 7.7% whereas in the control joint PGE₂ was still increased by 36.9%.

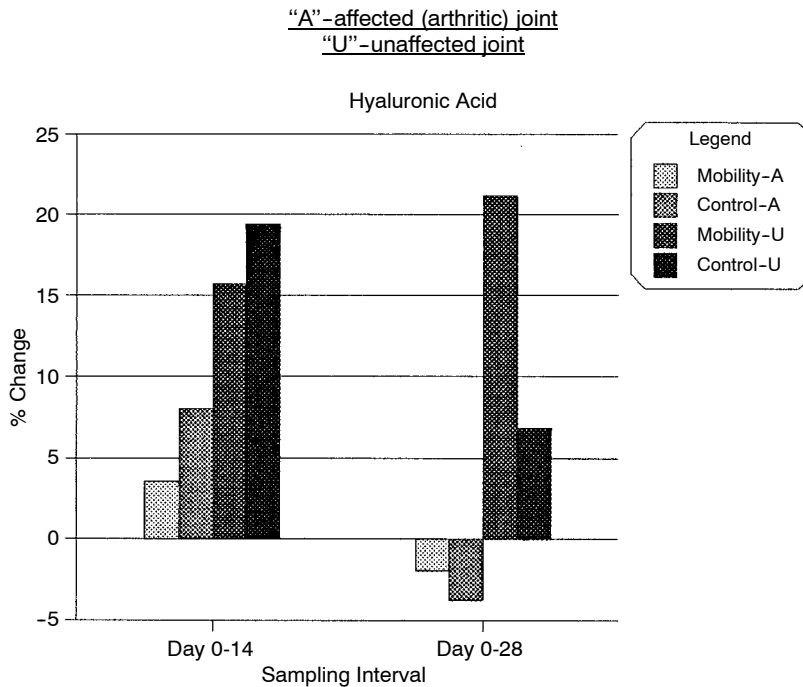
HA

There were no differences in HA levels between treatment and control phases within horses (see Figure 4).

DISCUSSION

Glycosaminoglycans (GAGs) are the major precursors to proteoglycans, which form the basis for cartilage matrix. There is some disagreement in the literature as to the aetiology of serum rise in GAG. Some researchers suggest that such a rise indicates an increase in articular cartilage degeneration (Sweet et al. 1988; Williams, Downey,

FIGURE 4. Average % Change in Synovial Fluid Hyaluronic Acid



and Thonar 1988). However, studies have shown that serum levels of GAG are significantly higher in growing children than in adults (Thonar et al. 1985). This suggests that the higher-than-normal levels of SeGAG may indicate an anabolic rather than a catabolic process, whereby articular proteoglycans are being manufactured from free GAGs. Both studies by Sweet et al. (1988) and Williams, Downey, and Thonar (1988) employed an acute degenerative process in which the increase in SeGAG was transitory and did not persevere. Thus, it is unlikely that a rise in SeGAG will reflect a net loss from cartilage unless that loss is dramatically high (Brandt 1989). Such a dramatic loss is not expected from the current study due to the non-acute nature of the horses' conditions. Therefore, the observed trend in increased GAG in the current study could indicate an increase in production of GAG.

The trend observed in SeGAG was similarly seen with SynGAG. The small sample size of the current study prohibited elucidation of

whether the rise was an experimental artifact, or if indeed there was an increase in availability of these proteoglycan precursors. The trend, however, provides an interesting stimulus for further research into the anabolic or catabolic characteristic of the perceived rise in SynGAG levels.

Levels of PGE₂ within the joint has significant anti-inflammatory consequences. Prostaglandin E₂ (PGE₂) has been shown to be a highly effective inflammatory agent (Salmon and Higgs 1987). The molecule is an oxygenation product of arachidonic acid—a reaction which is catalyzed by the enzyme cyclooxygenase. Prostaglandin E₂ (PGE₂) is a potent vasodilator, and hyperalgesic. The vasodilator actions occur through synergism between PGE₂ and other mediators including bradykinin and histamine, and the result is an increase in vascular permeability and erythema. This causes the characteristic hypersensitivity to pain observed in inflammatory conditions. In addition, there is important synergism between PGE₂ and the cytokine IL-1. This cytokine is present in articular joints, and its production is controlled by mRNA (Platt 1996). However, during OA, the level of IL-1 in articular cartilage is elevated and it promotes breakdown of the proteoglycans that make up the cartilage matrix (Platt 1996). It is often accompanied by an increase in PGE₂, and together they work to exacerbate the degenerative process by stimulating the release of metalloproteinases—enzymes which catalyze articular cartilage catabolism. By reducing the production of PGE₂, Mobility appears to inhibit the hypersensitivity to pain, and suppresses the ongoing degradation of articular cartilage.

This study did not take into account the possibility that Mobility may act as a preventive in degenerative joint disease, and it is possible that inclusion of glucosamine sulphate or chondroitin sulphate may strengthen the trends seen in GAG levels. These unanswered questions provide great potential for further investigation.

CONCLUSION

Supplementation with Mobility in arthritic horses in the current study did not have any effect on hyaluronic acid levels in synovial fluid, or on any of the hematological or biochemical blood parameters tested. However, Mobility supplementation did result in a reduction of PGE₂ production in arthritic joints in horses during the first two weeks of supplementation. This observation could explain the anecdotal tes-

timonials to the efficacy of Mobility in the management of OA in horses. The trends observed in SeGAG and SynGAG levels should be further investigated with a larger sample size to confirm their artifact nature, or to determine if the increase is an anabolic or catabolic process.

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