



Treatment of chronic arthritis in equines using anti-arthritic drug and 0.5 watt/cm² therapeutic ultrasound

NAVDEEP SINGH¹, V K SOBTI², S K MAHAJAN³ and K S ROY⁴

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141004 India

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Injuries due to accidents, racing or jumping may lead to arthritis in equines. Delay in the diagnosis and treatment invites severe pathophysiological changes in the joint resulting in secondary degenerative joint disease. A herbal preparation (HP) containing *Allium sativum*, *Cyperus rotundus* and *Zingiber officinalis* has remedial properties for arthritis. Therapeutic ultrasound which produces deep heat in the tissues and causes its micromassage (Downer 1976) has also proved beneficial in the treatment of acute arthritis in equines (Singh *et al.* 1996, Singh *et al.* 1997a, 1997b, 1997c). The combination of therapeutic ultrasound @ 1 watt/cm² and HP has proved helpful in arresting the degenerative changes in acute arthritis in equines (Singh and Singh 2004, Singh *et al.* 2005). The present study investigates the combined effect of HP and 0.5 watt/cm² therapeutic ultrasound in treatment of chronic arthritis in this species of animal.

The present study was conducted on 10 healthy donkeys aged 3–6 years weighing 70–100 kg. A fortnight before the start of the experiment, all the animals were given 2.5 ml of adsorbed tetanus toxoid intramuscularly. The animals were dewormed with albendazole @ 5mg/kg body weight. During experimental period the management and feeding practices were similar for all the animals. A day before the start of the experiment, the donkeys were clinically examined along with radiography of the carpal joints and declared fit for the experiment. The left carpal joint area was shaved and painted with povidone-iodine solution.

In all the animals the left carpal joint was prepared aseptically using soap and chlorhexidine and cetrimide solution. About 1 ml of lignocaine hydrochloride was injected subcutaneously in the depression felt over the intercarpal joint at the dorsomedial aspect of the carpus. This was followed by final preparation of the carpal joint and painting with povidone iodine antiseptic solution. In standing position,

the left knee was flexed and the intercarpal joint was entered in the depression on medial to extensor carpi radialis tendon with the disposable one inch, 20 gauze sterile needle. About 1 to 1.5 ml of synovia was aspirated with the syringe. After collection of synovia, 0.2 ml of turpentine oil and 0.2 ml of gentamycin was injected into the intercarpal joint through the same needle. The injection of 0.2 ml turpentine oil and 0.2 ml gentamycin was repeated 21 days after the first injection. The animals were left loose to move around freely on the subsequent days till the end of the experiment. Forty-three days after administration of first intra-articular injection, development of chronic arthritis was confirmed clinically and radiographically (Fig. 1).

All the 10 animals were randomly divided into groups A and B of 5 animals each. Group A animals were kept as control and were not given any treatment. Group B animals were given 20 g of HP daily for 25 days starting on the day of the development of chronic arthritis, i.e. from 43rd day. It was mixed in water and the suspension was fed with a syringe. The pulsed therapeutic ultrasound was also applied for 10 min daily up to 10 days starting on the same day. Before application of the transducer, liquid paraffin was applied on the affected joint. The intensity knob was fixed at 0.5 watt/cm². The transducer was brought in contact with the joint surface and the timer knob was set to 10 min time. A slow circulatory movement of the transducer was maintained all around the affected joint during application.

All the animals of groups A and B were sacrificed on day 42nd after induction of arthritis i.e. overall 84th day of the study. The affected carpus were harvested by cutting proximal and distal bones to the carpus. Skin around the joint was removed. The radiocarpal joints of affected joints were opened carefully avoiding any damage to the articular cartilage. The joint capsule and the articular cartilage were observed for any gross pathological changes. To study the histomorphological and histochemical changes, the tissue samples of articular cartilage and joint capsule of diseased joints were collected. Articular cartilage was collected from the base with a fine saw. All samples were fixed in Bouin's fixative.

After processing, 5 µ thick sections of collected tissues

Present address: ¹Associate Professor (nav2312@yahoo.com), Department of Veterinary and Animal Husbandary Extension Education.

²Former Professor-cum-Head, ²Associate Professor, Department of Veterinary Surgery & Radiology; ⁴Former Professor cum Head, Department of Veterinary Anatomy and Histology.

were obtained and stained with the following staining procedures:

- (a) Mayers haematoxylin and eosin (H & E) for routine histomorphological studies (Luna 1968)
- (b) Alcian blue/Periodic acid schiff (AB/PAS) (pH 1.0) for mucopolysaccharide (Luna 1968)
- (c) Masson's trichrome for connective tissue (Luna 1968)

Macroscopic findings: After 42 days of induction of chronic arthritis, the joint capsule of control animals was thickened with synovial membrane showing hemorrhages and blood spots. The articular cartilage was rough and at some areas, was pitted and eroded. These signs indicate that the degenerative joint disease has set in. The joints of group B animals showed less hemorrhages and little erosion of articular cartilage. The gross appearance of articular cartilage of the treatment group was close to normal with very few pinpoint erosions. It was whitish blue with slightly less shine. Gross findings in this study indicated that the joint tissue of treatment group gave more normal look than the control group. In equines, acute aseptic arthritis resulted in yellow to yellow brown discoloration of articular cartilage, haemorrhage and thickening of the joint capsule (Welch *et al.* 1991, Gustafson *et al.* 1992). The joint capsule has been reported to be thickened with haemorrhage in acute aseptic arthritis in donkeys with the articular cartilage grossly thinned and eroded (Singh *et al.* 1997a, 1997b, 1997c, Singh *et al.* 2005). The treatment with therapeutic ultrasound and HP separately as well as in combination resulted in the improvement of the condition of the joint (Singh *et al.* 1997a, 1997b, 1997c, Singh and Singh 2004, Singh *et al.* 2005). The treatment with HP and ultrasound was given for 25 and 10 days, respectively as suggested and standardized by Labana (1995).

Histomorphological findings: The lamina epithelialis of the synovial membrane of control (untreated) animals was necrosed. The subintimal layer showed infiltration of polymorphonuclear leukocytes, lymphocytes and macrophages. There was accumulation of exudate in the stromal tissue (Fig. 1). The sinusoids were dilated. All the above mentioned histopathological changes observed in synovial membrane confirmed the severity of arthritis produced by the turpentine oil. The synovial membrane of donkeys treated with combination of HP and 0.5 watt/cm² ultrasound showed synovial membrane at various stages of degeneration and regeneration. Some focal areas of necrosis were still observed but majority of the part appeared to be recovered from the experimental arthritis though there were still some inflammatory cells (Fig. 2). Bhatia (1990) observed proliferation of the synovial lining cells and marked regeneration in chronic arthritis of dogs and cats in which ultrasound therapy was administered @ 1.5 watt/cm² and 3 watt/cm², respectively. Singh *et al.* (2005) has reported advanced stage of healing of synovial membrane with near normal epithelial lining in donkeys having acute arthritis and

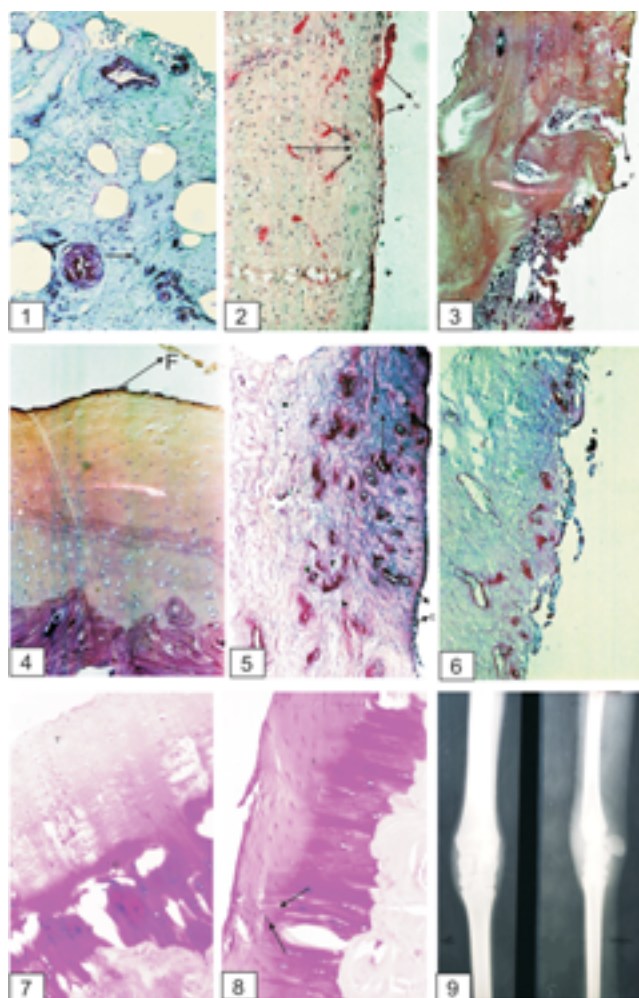


Fig. 1. The section of synovial membrane from group A animal showing cellular exudates (EX) and thickened arterial wall (B). Masson's trichrome. 70 ×; 2. The section of synovial membrane from group B animal showing focal necrosis (N) of the epithelium and increased vascularity (V) of subepithelial stroma. H&E. 140 ×; 3. The articular cartilage from radiocarpal joint of group A animal is showing somewhat changed morphology and fissures (F). Many lacunae are devoid of chondrocytes. H&E. 140 ×; 4. The articular cartilage of group B donkey showing all the four layers but they were lightly stained and the tangential layer showed slight fibrillation (F). H&E. 140 ×; 5. The epithelium (E) of synovial membrane of group A is showing mixed AB-PAS reaction whereas subepithelium is having a pocket showing increased alcianophilic reaction (R). AB-PAS. 140 ×; 6. Section of synovial membrane of group B donkey showing increased alcianophilic reaction of subepithelial layer (S). AB-PAS. 140 ×; 7. Section of articular cartilage from group A animal showing loss of neutral mucopolysaccharides in tangential layer (T) while they are increased in calcified layer (C). AB-PAS. 140 ×; 8. The intermediate layer (I) of articular cartilage of group B donkey is more PAS positive and the lacunae (L) are showing moderate content of acid mucopolysaccharides. AB-PAS. 140 ×; 9. The AP and Lateral views of carpal joint after 42 days of induction of arthritis showing increased soft tissue density and decreased joint space.

treated with combination of HP and 1.0 watt/cm² therapeutic ultrasound.

The articular cartilage of joint of donkey from control group animals was eroded to the extent that the articular surface of the bone got exposed (Fig. 3). Presence of fissures and fibrillation on the surface of articular cartilage reflected severe damage. All these lesions indicate towards the setting in of the degenerative joint disease (DJD). Such changes were reported earlier in different arthritic conditions in horses (Trotter *et al.* 1989, Gustafson *et al.* 1992, Singh 1994, Labana 1995, Singh 1998, Singh *et al.* 2005). In the cartilage of animals treated with HP and ultrasound @ 0.5 watt/cm², all the 4 layers were present although most of the lacunae were devoid of chondrocytes. Some fibrillation was also evident but overall picture indicates that there was some improvement in the arthritic condition (Fig. 4). Labana (1995) found the articular cartilage of donkeys suffering from chronic arthritis and treated with HP was near normal but a slight amount of fibrillation was still present in the tangential layer of the cartilage. A marked improvement was observed in the articular cartilage of animals treated with HP and 1.0 watt/cm² therapeutic ultrasound (Singh *et al.* 2005).

Histochemical findings: The AB-PAS stained section of synovial membrane of control group animals showed an increase in acid mucopolysaccharides and decrease in neutral mucopolysaccharides indicating that there was loss of glycogen due to degenerative changes in the synovial membrane (Fig. 5). The synovial membrane of animals treated with HP and 0.5 watt/cm² ultrasound also showed more of alcinoophilic reaction and decreased neutral mucopolysaccharides which indicated that the membrane is recovering from the damage done by the turpentine oil (Fig. 6).

The AB-PAS stained section of articular cartilage of control group animals showed that neutral mucopolysaccharides decreased progressively in tangential, intermediate and radiate layer with little or no acid mucopolysaccharides indicating severe degenerative changes in the articular cartilage (Fig. 7). Olsson (1971) reported that chondromalacia (softening of the articular cartilage) was the earliest sign of degeneration of articular cartilage due to decreased content of sulfated mucopolysaccharides in the ground substance as demonstrated by loss of alcinoophilic reaction. Fibrillation was the next stage as softened articular cartilage was unable to support collagen framework. Singh *et al.* (2005) has reported similar histochemical changes in the animals having acute arthritis. The section of cartilage of animals treated with HP and 0.5 watt/cm² therapeutic ultrasound showed increased content of neutral mucopolysaccharides in the upper layer while the lacunae showed moderate content of acid mucopolysaccharides (Fig. 8). This indicates that the cartilage was getting normal nutrition but the binding material was deficient.

From this study, it can be concluded that treatment with a

combination of HP and 0.5 watt/cm² therapeutic ultrasound resulted in healing of joint tissue and the articular cartilage and hence prevented the development of degenerative joint disease as observed in untreated animals.

SUMMARY

Acute aseptic arthritis was induced in 10 healthy donkeys aged 3–6 years weighing 70–100 kg. The animals were divided into 2 groups (A and B) of 5 animals each. Group A served as control whereas in group B, pulsed ultrasound therapy was applied for 10 min @ 0.5 watt/cm² for 10 days after induction of chronic arthritis along with HP @ 20 g daily orally for 25 days, post induction. The gross changes in synovial membrane and articular cartilage were mild in treated animals as compared to controls. Microscopically, the synovial membrane of control animals showed necrosed epithelial lining. The subintimal layer showed aggregation of polymorphonuclear leukocytes and macrophages. The synovial membrane of treated group showed near normal epithelial lining although there was focal necrosis and sloughing of epithelial lining. The AB-PAS reaction of synovial membrane of group A showed mixed reaction for acid and neutral mucopolysaccharides both in the intimal and sub intimal whereas the AB-PAS reaction of synovial membrane of group B showed slight sloughing of epithelial lining with more alcinoophilic reaction. Degeneration of articular cartilage was observed microscopically in control animals as marked by fibrillation and necrosis of perichondral tissue with lacunae devoid of chondrocytes. The articular cartilage of treated animals had mild changes in the superficial layer which was slightly rough and eroded. The lacunae were near normal with chondrocytes.

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