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Response of Ultrasound Therapy and Shortwave Diathermy on Oxidative Stress Parameters and Serum β-endorphins in Dogs Suffering from Hind Quarter Weakness

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ABSTRACT

Background: Hind quarter weakness (HQW) is the loss of bilateral motor function of the rear limbs due to dysfunction of neural or muscular system. Animals are mostly presented with focal or generalized pain, varying degrees of paresis, paralysis and inability to urinate. Serum β- endorphin is an endogenous hormone released during stressful and painful events and functions almost exclusively in an inhibitory fashion. Moreover, electrical stimulation has been demonstrated to release endorphins at low frequencies.

Methods: The present study was conducted to investigate the possible participation of endogenous opioid *i.e.* Serum β - endorphins and oxidative stress parameters in pain modulation and its variation in dogs suffering from hind quarter weakness subjected to ultrasound therapy and shortwave diathermy. Dogs were treated using therapeutic ultrasound (group I, n=6) and shortwave diathermy (group II, n=6) in combination with supportive drug therapy continued regularly for one week. Blood was collected on day 0, 3rd and 7th for oxidative stress estimation and regularly from the day of presentation till 7th day of treatment for serum β-endorphin estimation.

Result: LPO values decreased significantly (P<0.05) from day 0 to 3 in group I and from day 0 to 7 in group II. SOD values showed a significant (P<0.05) decrease between day 0 and 7 in group II cases. A continuous progressive increasing trend in GSH was noticed which was significant (P<0.05) from day 3 to 7 in both groups. CAT values showed a significant (P<0.05) increase from day 0 to 7 in group II and non-significant (P<0.05) increase in group I. Serum β-endorphin values showed a significant (P<0.05) increase from day 4th to 6th in group I and between day 0, 1st, 3rd and 5th in group II. The variation in values throughout the treatment was suggestive of reduction in stress response and indicates improvement in dogs suffering from HQW.

Key words: Dogs, Hind quarter weakness, Short wave diathermy, Serum β-endorphins, Therapeutic ultrasound.

INTRODUCTION

Painful and debilitating neurological disorders primarily involving the vertebral column and spinal cord are commonly encountered in dogs. Animals with spinal disorders are presented with focal or generalized pain, varying degrees of paresis, paralysis and inability to urinate (Nelson and Couto, 2004). Hind quarter weakness (HQW) is the loss of bilateral motor function of the rear limbs due to dysfunction of neural or muscular system.

Physiotherapy is a non-invasive technique that acts by decreasing pain, inflammation and swelling, improving blood supply, minimizing muscle atrophy, and thus promoting early recovery and back-to-normal or near-normal function (APTA, 2008). Physiotherapy is in fact complementary to conventional treatment and best used in collaboration (McGowan *et al.* 2007). Conventional treatment by corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) and nervine tonics yielded limited success in cases of HQW in dogs. However, when combined with other physiotherapeutic modalities *viz.* acupuncture, ultrasound and interferential, a higher success rate was noticed (Sharma 2005; Maiti *et al.* 2007).

β- endorphin is an endogenous morphine-like hormone produced primarily in the anterior lobe of pituitary gland. It

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is released during stressful and painful events and it functions almost exclusively in an inhibitory fashion. The peptide inhibits neuronal firing of peripheral somatosensory fibres, especially involved in nociception (Calza *et al*., 1989). Pert (1979) believed that the endogenous opioids may induce analgesia by specifically interfering with the processing of pain and preventing pain information from gaining access into the limbic structures that mediates pain experience. Stratton (1982) reported that discovery of endogenous opioid has shown to be involved in the process of pain modulation and play a significant role in inhibiting pain perception. Moreover, electrical stimulation has been demonstrated to release enkephalins and endorphins at low frequencies.

MATERIALS AND METHODS

The present study was conducted on twelve dogs irrespective of age, breed and sex suffering from HQW presented to the Department of Veterinary Surgery and Radiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. These were randomly divided in two groups (I and II) comprising of six dogs each on the basis of their subjection to one of the physiotherapeutic modalities along with supportive therapy.

Ultrasound therapy was conducted after clipping of hairs and applying ultrasound jelly to ensure perfect contact between transducer head and skin of the affected area. Sonication was performed at a frequency of 1 MHz, an intensity of
0.5 watt/cm^2 (SATA) for 5 minutes/day for 7 days in a pulsed mode (1:4). The transducer head of the ultrasound machine was moved slowly in a proximal distal linear fashion on the skin surface. Shortwave diathermy (200 mA intensity and 10 volts) using two pads at lumber region, was performed daily for 10 minutes for 7 days.

In addition to physiotherapy techniques, animals of both groups were treated with supportive medicinal therapy including analgesic and anti-inflammatory drug, Meloxicam (Melonex) @ 0.2 mg/kg body weight, intramuscularly; nerve stimulant, Methylcobalamin (Vit. B_{12}) @ 500 mcg/ml, Pyridoxine (Vit. B_6) @ 50 mg/ml and Nicotinamide (Vit. B_3) @ 50 mg/ml (Neurokind), intramuscularly; Calcium (Cipcal) @ 1 sachet per 20 kg body weight, orally; daily for 5 days; joint supplement, Glycosaminoglycans and Chondroitin Sulphate (Petjoint) @ 1 tablet per 20 kg body weight and corticosteroid, Methyl prednisolone succinate acetate (Depomedrol) @ 30 mg/kg body weight intramuscularly on first day and thereafter 15 mg/kg body weight on alternate days for a week.

Blood samples were collected from all the animals on 0, 3rd and 7th day in two EDTA containing polypropylene collection tubes. Blood samples were centrifuged at 3500 RCF for 15 minutes and used for estimation of oxidant-antioxidant balance parameter. One blood sample was used to prepare hemolysate and the other for RBC suspension. To prepare hemolysate, erythrocytes were washed thrice with normal saline solution and finally 10% hemolysate was prepared by adding chilled distilled water. From other blood sample, RBC suspension was prepared by adding equal volume of the erythrocytes and normal saline solution. Hemolysate and RBC suspension were kept at -70°C and used for oxidant-antioxidant assay within 6 hours. From hemolysate, SOD activity (Marklund and Marklund, 1974), CAT activity (Bergmeyer, 1983) and malondialdehyde (MDA) concentration (Placer *et al*., 1966) were estimated. MDA is a reliable marker of lipid peroxidation (LPO). The concentration of GSH in RBC suspension was estimated by the method of Prins and Loos (1969) and for that one ml of blood sample was collected from each animal daily from day 0 to 7th from the cephalic vein in clot activator serum tubes using disposable plastic syringes. Allow blood to clot for 10-20 minutes at room temperature. Centrifuge at 3500 RCF for 15 minutes. The data obtained was analyzed using two-way Analysis of Variance (ANOVA) followed by Duncan test using SPSS 16.0 version. The level of statistical significance for all comparisons was established at $P<0.05$. The values obtained were compared between and within the groups.

RESULTS AND DISCUSSION

The mean±SE values of oxidative stress parameters were presented in Table 1. A decreasing trend in lipid peroxidase (LPO) was noticed in both the groups till 7th day. This decrease was significant ($P<0.05$) from day 0 to 3rd in group I and from day 0 to 7th in group II. In comparison to group I, the values were significantly ($P<0.05$) higher in group II on 3rd and 7th day. Higher LPO levels are suggestive of enhanced oxidative damage to erythrocytes (Corry *et al*., 1970), either due to excess production of free radicals or compromised/ exhausted antioxidant defense in the affected animals. Similar findings of increased levels of LPO were reported in various disorders like inflammation (Lykkesfeldt, 2002), caprine scabies (De and Dey, 2010), sarcoptes in dogs (Camkerten *et al*., 2009), buffaloes (Dimri *et al*., 2008) and camels (Saleh *et al*., 2011). After the start of treatment, a continuous significant ($P<0.05$) decrease in LPO as compared to base value in all the treated groups suggested a decrease in excess free radicals.

A continuous progressive decreasing trend in SOD was noticed in both the groups till day 7. This decrease was significant ($P<0.05$) between day 0 and 7 in group II and non-significant in group I. In comparison to group I, the values were significantly ($P<0.05$) lower in group II on day of presentation. Various kinds of stressors increase lipid peroxidation levels and therefore SOD activity (Gaal *et al*., 1993; Lata *et al*., 2004). Superoxide dismutase (SOD) is a natural antioxidant of the body. SOD accelerates the dismutation of superoxide radicals (O^{2-}) to hydrogen peroxide (H_2O_2).

A continuous progressive increasing trend in GSH was noticed in both groups till 7th day. This increase was

Table 1: Effect of UST and SWD on oxidative stress parameters at different time intervals (Mean±SE).

Parameters	Group I			Group II		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
LPO (nmol MDA/g Hb)	3.47±0.16^{aB}	2.90±0.03^{aA}	2.66±0.20^{aA}	3.79±0.08^{aC}	3.66±0.04^{bB}	3.43±0.22^{bA}
SOD (U/mg Hb)	1.05±0.80^{bA}	0.99±0.04^{aA}	0.94±1.88^{aA}	1.10±0.19^{aB}	0.94±0.03^{aAB}	0.88±0.03^{aA}
GSH(µmol/g Hb)	0.57±0.08^{aA}	0.60±0.043^{aA}	0.85±0.02^{aB}	0.54±0.14^{aA}	0.59±0.03^{aA}	0.64±0.02^{aB}
CAT(U/mg Hb)	131.46±0.18^{aA}	133.05±0.40^{aA}	135.04±0.93^{aA}	136.56±16.94^{aA}	144.52±0.03^{aB}	151.10±1.79^{aC}

Values without a common superscript letter A-C are significantly different within the group (P<0.05).

Values without a common superscript letter a-c are significantly different between the group (P<0.05).

Table 2: Effect of UST and SWD on serum β- endorphin at different time intervals (Mean±SE).

B-EP(ng/ml)	Group I	Group II
Day 0	22.97±0.03^{aA}	19.70±0.23^{aA}
Day 1	23.51±0.52^{aA}	24.81±0.65^{aB}
Day 2	21.24±0.22^{aA}	28.32±0.34^{bB}
Day 3	25.04±0.31^{aA}	38.44±1.51^{bC}
Day 4	34.34±0.43^{aB}	39.19±0.98^{aC}
Day 5	42.73±0.26^{aC}	46.02±0.81^{aD}
Day 6	49.55±0.61^{aD}	46.70±0.76^{aD}
Day 7	45.26±0.77^{aD}	49.54±0.82^{aD}

Values without a common superscript letter A-D are significantly different within groups (P<0.05).

Values without a common superscript letter a-b are significantly different between groups (P<0.05).

significant (P<0.05) from day 3rd to 7th in both the groups. Reduced glutathione, one of the first line endogenous defense antioxidants effectively scavenge free radicals either directly or indirectly through enzymatic reactions and protects the cells against oxidative damage (Kosower *et al*., 1977; Fang *et al*., 2002).

CAT values showed a significant (P<0.05) increase from day 0 to 7th in group II and non-significant (P<0.05) increase in group I. Catalase (CAT) is the main scavenger of H_2O_2 at high concentration (Kono and Fridorich, 1982). It catalyzes the conversion of H_2O_2 to H_2O and molecular oxygen (Dringen, 2000). An increasing trend in CAT activity throughout the study might be due to increased activity of SOD which would have resulted into increased H_2O_2 production and thereby increased utilization of CAT for converting H_2O_2 into H_2O. This may be the reason behind lower CAT activity in dogs suffering from posterior paresis. The mean±SE values of serum β- endorphin values were presented in Table 2. The values of β- endorphin in group I showed a non-significant trend from day 0 to 3 and thereafter values increased significantly (P<0.05) from day 4 to 6. However, non-significant decrease in the values of β-endorphin was observed from day 6 to 7. In group II, the values showed a significant (P<0.05) trend between day 0, 1, 3 and 5. The values of β- endorphin in between group I and II are showing a significant (P<0.05) increase on day 2 and 3. Clement-Jones *et al*. (1980) found that low frequency electro acupuncture effectively alleviated recurrent pain and significantly increased the lumbar CSF β - endorphin levels. Xu *et al*. (1980) found that acupuncture significantly decreased the sensory discrimination with a correlation between the efficacy of amino acids and plasma content of opioid peptides.

CONCLUSION

From the above study, it was concluded that serum β-endorphin and reduced glutathione (GSH) levels were significantly increased after treatment in both the groups suggestive of reduction in stress response and indicate improvement in dogs suffering from posterior paresis.

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