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RESEARCH ARTICLE

Evaluation of Electro-Acupuncture Therapy on Markers of Oxidative Stress in Dogs Suffering from Hind Quarter Weakness

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ABSTRACT

The study was conducted to evaluate the markers of oxidative stress in dogs suffering from hind quarter weakness (HQW) and treated with conventional drug therapy (CDT, n=8, group II) alone, and along with electro-acupuncture therapy (EAT, n=8, group III). Eight healthy dogs were used as control (group I). The erythrocytic oxidant-antioxidant balance was recorded before and after the respective therapies. For oxidant level, lipid peroxidation (LPO) and antioxidant levels reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were evaluated in erythrocytes before (day 0) and after 3,7,14 and 28 days of therapy. In dogs with HQW, LPO in terms of malondialdehyde (MDA) production, was found significantly ($P<0.05$) increased as compared to healthy control on day 0. Thereafter, a significant ($P<0.05$) decrease in the level of LPO was noticed till day 28 post treatment. Amongst antioxidant enzymes, activities of GSH and CAT decreased significantly ($P<0.05$) whereas, the level of SOD increased significantly ($P<0.05$) as compared to healthy control. Excess free radicals production was lowered and antioxidant defense system was activated in dogs treated by EAT along with CDT followed by those treated by CDT alone. Results denote that incorporation of EAT in the therapeutic regimen might be helpful to curb the oxidative stress in the course of HQW in dogs. Hence, EAT was effective in minimizing oxidative stress in dogs suffering from hind quarter weakness.

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INTRODUCTION

Animals with neurological disorders primarily involving the vertebral column and spinal cord are commonly encountered in small animal practice, especially in dogs. Such animals are presented with the complaint of focal or generalized pain, varying degrees of paresis, paralysis and inability to urinate (Nelson and Couto, 2004). The most frequent manifestation of spinal cord affections is hind quarter weakness (HQW), which is the loss of bilateral motor function of the rear limbs due to dysfunction of neural or muscular system. Such animals show difficulty to bear weight, paresis or paralysis of limbs associated with urinary and faecal incontinence (McGowan *et al*., 2007). HQW is also manifested by the clinical signs such as dragging the rear part of the body with pain sensation while trying to walk, rigid hyperextension of both hind limbs, unable to stand or can stand only for short periods of time generally without the normal arched back (Nelson and Couto, 2004). It may result as a sequel to the spinal cord disorder either by fall, jump from height, road traffic accident, dog bite over the vertebral column, malicious blow by stick, rod, stone, crush by heavy object, fracture and myoclonus form of canine distemper (Hoerlein, 1971; Berg and Boudrieau, 1992).

Successful management of spinal cord injury in small animals involves diagnosing and relieving gross misalignments and other structural problems of the spine, minimizing cellular-level damage and stabilizing the vertebrae to prevent further injury. Once a patient is stabilized supportive care and rehabilitation strategies play an important role in the functional regeneration of spinal cord (Ettinger and Feldman, 2010). Physiotherapy is the use of non-invasive techniques that act 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 often perceived as an alternative therapy, whereas it is in fact complementary to conventional treatment and best used in collaboration with it (McGowan *et al*., 2007). Conventional treatment by corticosteroids, NSAIDs and nervine tonics yielded only limited success in cases of HQW in dogs, however when combined with other physiotherapeutic modalities viz. acupuncture, ultrasound and interferential, higher success rate was noticed (Sharma, 2005; Maiti *et al.,* 2007).

Free radicals are produced continuously by normal metabolic processes, but their rate of production increases during certain inflammatory or other disease conditions (Bernabucci *et al.,* 2005). Oxidative stress results when there is an imbalance between generating and scavenging activity of radicals, resulting in oxidative products and tissue damage (Saleh *et al.,* 2011). When free radicals generation overpowers the antioxidant defense, free radicals can interact with endogenous macromolecules leading to metabolic dysfunction and bimolecular oxidative damage (like damage to lipids, DNA, carbohydrates and proteins), which contribute to pathological changes in the tissues (Trouba, 2002, Valko *et al*., 2007). Estimation of antioxidant enzymes activities *viz.* superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) and level of oxidant (lipid peroxidation) in blood are indirect but reliable methods for assessment of free radicals activity and oxidative stress (Fang *et al.,* 2002).

Physiotherapeutic techniques have been found beneficial in the treatment and management of various degree of spinal trauma; pain, hind quarter weakness and posterior paresis in dogs, but their oxidant-antioxidant response have not been documented. The present study was aimed to evaluate the ameliorative effect of electro-acupuncture therapy (EAT) on markers of oxidative stress in dogs affected with clinical HQW disorder.

MATERIALS AND METHODS

Experimental design

A total of 16 dogs (2.5 months to 12 years of age) of either sex suffering from HQW presented to Referral Veterinary Polyclinic of Indian Veterinary Research Institute were included for the study. A set of 8 age-matched healthy dogs constituted the healthy control group (I), whereas, all the affected animals were randomly divided in two equal groups (II and III) of 8 dogs each. Dogs in group II were treated with conventional drug therapy alone. In addition to the conventional drug therapy, animals of group III were also treated with EAT. Conventional drug therapy was given for 14 days using Methyl prednisolone acetate (Depomedrol, Pfizer India Pvt Ltd. Mumbai-400102) @ 30 mg/kg body weight intramuscular on first day and later on 15 mg/kg body weight i/m on alternate days, Meloxicam (Melonex, Intas pharma, Chinubhai Center, Ashram Road, Gujarat-380009) @ 0.1 mg/kg body weight i/m daily, Gabapentine and Mecobalamine (Neurokind-G, Mankind Pharma Ltd., Okhla, New Delhi-110023) tablet, orally once daily and Vitamin B1, B6, B12 and D-Panthenol (Cyanocal-16, Ozone Pharmaceutical, New Delhi-110058), 2ml, I/M on alternate day. The electro-stimulation of acupoints, Bai Hui, BL-30, GB-34, ST-36, BL-67 and GV-1) was done using 55-100 mA intensity, 50 Hz frequency and 9 volts dense and disperse wave current with a electro-acupuncturescope. The EAT stimulation was given daily for 10 minutes for 14 days.

Blood collection and processing for assays of oxidant/ antioxidant balance

Three ml of venous blood sample was collected from each animal on day 0, 3, 7, 14 and 28 in two disposable plastic syringes containing heparin (20 IU/ml). Blood samples were centrifuged at 2000 rpm for 10 minutes and used for estimation of oxidant-antioxidant balance parameters. 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. RBC suspension was prepared by adding equal volume of the erythrocytes and normal saline solution. Hemolysate and RBC suspension were kept at -70^{o}C and used for oxidant-antioxidant assay within 6 hours. From hemolysate, SOD activity (Marklund and Marklund, 1974), CAT activity (Bergmeyer, 1983) and malonaldialdehyde (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).

Statistical analysis

The values were expressed as mean ± S.E. Statistical analysis was done by one way Analysis of Variance (ANOVA) for group, time and their interaction effects and followed by Duncan's multiple range tests using Statistical Package for Social Sciences software (SPSS 16.0, Chicago). The level of statistical significance for all comparisons was established at $P<0.05$.

RESULTS

The present study revealed that dogs suffering from HQW were in a state of significant oxidative stress and there was a significant altered oxidant-antioxidant balance. Effects of different treatments on oxidant-antioxidant parameters have been shown in tables 1-4. On day 0, the LPO in terms of MDA production was significantly ($P<0.05$) higher in all the dogs with HQW as compared to healthy control. Thereafter, LPO exhibited a continuous significant ($P<0.05$) decrease at all intervals in both the treated groups. The decrease was highest in group III followed by group II. The values differed significantly amongst the groups at corresponding intervals except group II on day 14 and group III on day 7 (Table 1).

Effects of different treatments on SOD level in HQW dogs and healthy control have been shown in table 2. SOD level was significantly ($P<0.05$) higher in dogs with HQW as compared to healthy control on day 0. Thereafter, SOD exhibited a continuous significant ($P<0.05$) decrease at all intervals in both the treated groups. The decrease was highest in group III followed by group II. There was a significant ($P<0.05$) difference in the SOD values

Table 1: Effect of different treatments on LPO (nmol MDA/g Hb)

Group	0 day	3 day	7 day	14 day	28 day
I	1.29^{aD} ±0.009	1.28^{aC}±0.007	1.29^{aC}±0.009	1.24^{aA} ± 0.006	1.26^{aB}± 0.007
II	3.63^{bE}±0.015	3.44^{dD}±0.031	3.04^{cC} ±0.559	2.03^{cB} ± 0.113	1.77^{dA} ± 0.047
III	3.63^{bE}± 0.012	3.31^{bD}±0.008	2.89^{bC} ±0.008	1.67^{bB} ±0.008	1.64^{bA}±0.007

Values with superscripts A, B, C, D, E differ significantly (P<0.05) in a row; Values with superscripts a, b, c, d differ significantly (P<0.05) in a column

Table 2: Effect of different treatments on SOD (U/mg Hb)

Group	0 day	3 day	7 day	14 day	28 day
I	0.32^{aA}±0.028	0.33^{aA}±0.025	0.34^{aAB}±0.017	0.36^{aB} ±0.023	0.35^{aB}±0.017
II	0.91^{bE}±0.023	0.86^{bD}±0.037	0.79^{cC}±0.031	0.52^{dB}±0.008	0.44^{cA}±0.008
III	0.92^{bE}±0.023	0.82^{bD}±0.065	0.75^{bC}±0.015	0.44^{bB}±0.008	0.40^{bA}±0.008

Values with superscripts A, B, C, D, E differ significantly (P<0.05) in a row; Values with superscripts a, b, c, d differ significantly (P<0.05) in a column

Table 3: Effect of different treatments on CAT (U/mg Hb)

Group	0 day	3 day	7 day	14 day	28 day
I	125.75^{bB}±1.38	124.75^{dAB}±0.70	125.13^{dB}±0.99	125.37^{dB}±0.91	124.00^{dA}±0.92
II	91.37^{aA}±0.51	91.50^{aB} ±0.53	94.00^{aC}±1.51	100.75^{aD}±0.70	114.75^{aE}±0.88
III	91.75b^{aA}±0.46	96.25^{cB}±1.66	103.50^{cC}±1.41	112.88^{bD}±0.83	122.00^{cE}±0.75

Values with superscripts A, B, C, D, E differ significantly (P<0.05) in a row; Values with superscripts a, b, c, d differ significantly (P<0.05) in a column

Table 4: Effect of different treatments on GSH (µmol/g Hb)

Group	0 day	3 day	7 day	14 day	28 day
I	0.97^{bD}±0.008	0.96^{cC}±0.009	0.95^{dBC}±0.007	0.95^{dAB}±0.007	0.94^{dA}±0.008
II	0.53^{aA}±0.010	0.54^{aA}±0.049	0.61^{aB}±0.051	0.70^{aC}±0.041	0.77^{aD}±0.008
III	0.53^{aA}±0.007	0.63^{bB}±0.013	0.75^{cC}±0.008	0.85^{cD}±0.008	0.90^{cE}±0.007

Values with superscripts A, B, C, D, E differ significantly (P<0.05) in a row; Values with superscripts a, b, c, d differ significantly (P<0.05) in a column

amongst the treated groups on day 14. On days 7 and 28, the SOD level in group II was found significantly higher than group III (Table 2).

The effects of different treatments on catalase (CAT) activity in dogs with HQW and healthy control have been shown in table 3. On day 0, the erythrocytes CAT level was significantly (P<0.05) lower in all the dogs with HQW as compared to healthy control. Thereafter, CAT exhibited a continuous significant (P<0.05) increase at all intervals in both the treated groups. The increase was highest in group III followed by group II. The values differed significantly amongst all the groups at corresponding intervals.

The effects of different treatments on GSH activity in dogs with HQW and healthy control have been shown in table 4. In all HQW affected dogs, a significant (P<0.05) decrease in erythrocytes GSH level was noticed on day 0 as compared with healthy control. In both the treated groups, GSH showed a continuous significant (P<0.05) increasing trend at all intervals except at day 3 in group II. The increase was highest in group III followed by group II. The GSH level differed significantly amongst all the groups at corresponding intervals.

DISCUSSION

In the present study, the oxidant/antioxidant balance shifted towards oxidative stress in all dogs suffering from HQW, as evidenced by an increase in the levels of LPO in terms of MDA. Free radicals are produced continuously by normal metabolic processes, but their rate of production increases during certain inflammatory or other disease conditions (Bernabucci *et al.,* 2005). Under normal conditions, free radicals are neutralized by efficient antioxidant systems (Nockels, 1996). 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. MDA is a breakdown product that is frequently quantified as a measure of lipid hydro peroxides. MDA assay has been found to be one of the better predictor of oxidative damage and often shows excellent correlation with other markers, such as isoprostanes, which are considered to be the most reliable markers of lipid peroxidation (Morrow, 2000). Similar findings of increased levels of LPO were reported in various disorders like inflammation (Lykkesfeldt, 2002), canine demodicosis (Dimri *et al.,* 2008a), caprine scabies (De and Dey, 2010), sarcoptes in dogs (Camkerten *et al.,* 2009), buffalo (Dimri *et al.,* 2008b) and camel (Saleh *et al.,* 2011). After the start of treatment, a continuous significant (P<0.05) decrease in LPO in all the treated groups suggested a decrease in excess free radicals. Highest decrease in LPO in group III followed by group II indicated that least oxidative damage to erythrocytes occurred when EAT was used as an adjunct therapy to conventional treatment, as compared to CDT alone.

It is well established that when the risk of oxidative damage increases, endogenous antioxidant protection also increases (Basha and Rani, 2003). 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), whereas CAT catalyzes the breakage of toxic H_2O_2 produced in the cell to O_2 and H_2O (Linares *et al.,* 2007).The present increase in SOD activity in all the dogs suffering from HQW as compared to control may be considered as a defense mechanism of the cortical neurons against the increase in the production of superoxide anions during the current state of oxidative stress. Increase in SOD activity might be attributed to up-regulation in its synthesis to counteract free radicals. The increase in SOD level in the present study was in agreement with the findings of Sathya *et al.* (2007) in dystocia affected buffalo and Dimri *et al.* (2008a) in canine demodicosis.

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). Increased activity of SOD might have resulted into increased H_2O_2 production and thereby increased utilization of catalase for converting H_2O_2 into H_2O. This may be the reason behind lower catalase activity in dogs suffering from HQW. Increase in SOD activity and decrease in catalase activities were also reported in vitiligo patients (Hanzneci *et al.,* 2005).

Reduced glutathione, one of the first line endogenous defense antioxidants is a tri-peptide with an active sulphydril (-SH) group and can react with different electrophilic compounds and effectively scavenge free radicals either directly or indirectly through enzymatic reactions and protects the cells against oxidative damage (Kosower *et al.,* 1977 and Fang *et al.,* 2002). Lower levels of GSH in group II and III in dogs suffering from HQW might be due to its enhanced utilization to neutralize excess free radicals. Similar findings were also reported in canine demodicosis (Dimri *et al.,* 2008a), canine sarcoptic mange (Camkerten *et al.,* 2009) and caprine sarcoptic mange (De and Dey, 2010).

After initiation of therapy, a continuous decrease in SOD, increase in CAT and GSH activity in both the treated groups was due to the decreasing antioxidant requirement at subsequent intervals. This was because of continuous decrease in oxidative stress following the treatment, as also evidenced by decreased LPO. Further, the critical evaluation of the results indicated that, when used in combination with conventional drug therapy, EAT was better in reducing oxidative stress and activating antioxidant defense system than CDT alone. Hence, it may be concluded that EAT in conjunction with conventional drug therapy can be used to counter free radical-mediated oxidative cell injury, induced by hind quarter weakness in dogs.

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