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Research Paper

Stimulation of Bladder Acupoints by Cloprostenol for Treating Back Soreness in Athletic Horses

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Abstract
Twenty-five Thoroughbred jumper geldings suffered back soreness with poor performance, and 5 control horses were assessed by archived computer data, clinical examination, and laboratory analyses of complete blood picture, serum enzymes, and cortisol level, before and after cloprostenol-pharmacopuncture. The 25 diseased horses before therapy showed significant increases in aspartate aminotransferase and creatine phosphokinase with clinical pains scored mild in 15 horses, moderate in 9 horses, and severe in one horse, without changes in the hormonal and hematological data. After therapy, they responded by an increase of heart rate (57.8 ± 4.3 bpm), body temperature (38.5 ± 0.7°C), respiration rate (28.3 ± 2.1 bpm), and capillary refilling time (CRT) (1.0 ± 0.0). On the 2nd day, a significant decrease in the mean levels of aspartate aminotransferase and creatine phosphokinase ($P = 0.001$) was detected, while on the 4th day, they mimed the level of the 5 controls, and on the 6th day, they showed a significant decrease ($P = 0.002$). The serum cortisol level showed a significant increase on the 6th day of treatment ($P = 0.013$). The blood picture showed significant increases in red blood cells, mean corpuscular volume, platelets, white

Abbreviations: AST, Aspartate aminotransferase; CPK, Creatine phosphokinase.

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blood cells, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume, platelet distribution width, lymphocytes, plateletcrit, and large platelet concentration ratio ($P < 0.05$) and nonsignificant changes in hematocrit, granulocytes, and midocytes. The improved blood parameters, enzymes, hormones, and performance progress after cloprostenol-pharmacopuncture proved its effectiveness in treating back soreness in athletic horses.

1. Introduction

The primary and/or secondary back pain in athletic horses is a common problem causing performance issues [1, 2]. The most common back pain classification is acute or chronic with mild, moderate, or severe degrees [3].

The use of nonsteroidal antiinflammatory drugs (NSAIDs) [4] glucosamine, chondroitin sulfate, or methylsulfonylmethane [3]; muscle relaxants [5]; regional perfusion of local anesthetics or antiinflammatory drugs [6]; extracorporeal shockwave therapy [7]; mesotherapy [8]; ultrasound-guided injections of the dorsal articular process [9]; systemic tiludronate therapy [10]; herbal medicine and acupuncture [11]; and rehabilitation modalities [12] produced an incomplete and short-lived improvement.

Prostaglandins are involved in acute immune reaction and inflammation. They are not made by glands but released from multiple different tissues into the bloodstream and exert their effects locally [13, 14]. The use of cloprostenol-pharmacopuncture as a new trend for treating back pain aims to alleviate discomfort and muscle spasm as fast as possible. The efficacy of cloprostenol-pharmacopuncture was assessed by the hematological, enzymatic, and hormonal blood alterations and performance progress.

2. Materials and methods

2.1. Animal of the study

The study was carried out on 25 Thoroughbred jumper geldings that suffered back soreness and 5 controls exempted back pain, at the Armed Forces Equestrian Club during the winter season from September to December 2017. They aged 8 to 15 years and weighted 400 to 500 kg. They were cared for and managed in accordance with the guidelines of the Animal Ethics Committee of Veterinary Medicine, Cairo University (No. CU/II/F/82/18). They were being fed balanced ration daily three times with a suitable amount of green fodder once a day and water ad libidum.

2.2. Clinical examinations

Recent or past secondary back pain that was related to lameness was excluded by horses' history, clinical examination, and archived computer data. Detailed history toward the onset and the duration of the clinical signs of back pain was also recorded.

The horses were examined at rest by inspection, palpation, and manipulation; during exercise by using loose rein and lunging on both directions; and during riding following the study by Allen et al [8]. The clinical back pains were scored, and the vital parameters before and after cloprostenol-pharmacopuncture therapy were recorded as well.

2.3. Hematological examination

Jugular vein blood samples from 5 control horses at rest and after exercise for hematology were collected in commercial blood Vacutainer tubes (5 ml, containing EDTA K2, Yuli Medical Instrument Co., Ltd, China) containing EDTA, and the other samples were collected from the 25 diseased horses before treatment and on the 6th day after therapy. They were analyzed within 2 to 3 hours according to the method followed by Weiss and Tvedten [15]. The complete blood picture was analyzed using a hematology analyzer device "Medonic CA 620 (Boule Medical AB, SE-126 13 Stockholm, Sweden)" by electronic impedance for cell counting and sizing and by a colorimetric method for hemoglobin measurement.

2.4. Enzymatic analyses

Serum aspartate aminotransferase (AST) and creatine phosphokinase (CPK) were colorimetrically estimated by using FUJI DRI-CHEM NX500 [FUJIFILM Company (Europe GmbH Medical Systems Division Heesenstr. 31. 40549 Düsseldorf, Germany)], as per the protocol described by Wu [16]. Samples were collected from control horses 6-hours after exercise for detection of CPK and 24 hours after exercise for detection of AST. Samples were also taken from the diseased horses at Day 2, 4, and 6 on the same timing after cloprostenol-pharmacopuncture therapy following the steps followed by Padilha et al [17].

2.5. Hormonal assay

Serum cortisol levels were measured using LIAISON XL [DiaSorin Company (S.p.A. Via Crescentino, snc 13040 Saluggia (VC) Italy)] by method based on the principle of solid phase linked antigen technique described by Tunn et al [18]. The samples were collected before treatment and on the 6th day after therapy in the stall in the morning 7:00 am, following the method used by Mircean et al [19].

2.6. Preparation for cloprostenol-pharmacopuncture therapy

During stall rest, the five selected bilateral bladder acupoints located at the dorsal midline, namely, BL-14, BL-16, BL-19, BL-23, and BL-26 [20], were aseptically prepared and disinfected with 70% alcohol followed by Betadine (El-Nile Co. for Pharmaceuticals and Chemical Industries,Cairo, Egypt.

licensed by Mundipharma AG-Basel-Switzerland) (povidone iodine 10%).

2.7. The technique of inoculation of cloprostenol

A solution of synthetic prostaglandin $F_2\alpha$ (Estrumate; 250 μg/ml cloprostenol; Vet Pharma Friesoythe GmbH, Germany) was inoculated perpendicularly using a 1-ml syringe with a hypodermic needle of size 27G × 1/2″, 0.4 × 13 mm (Shandong Zibo Shanchuan Medical Instrument, China), for a depth of 1 cm, and then 0.2 ml of the solution was injected in each acupoint. The horses were allowed to walk for half an hour and then prescribed to stall rest on the day of injection. The exercise started the next day of the injection and continued for 6 days.

2.8. Competition rate post—cloprostenol-pharmacopuncture

The 25 horses entered the competition after 6 days of cloprostenol-pharmacopuncture therapy in the show jumping season 2017/2018 under the supervision of the Egyptian Equestrian Federation, and the results were recorded.

2.9. Statistical analyses

The data were analyzed using PASW statistics, version 18.0, software (SPSS Inc., Chicago, IL, USA) [21]. The enzymatic data analyses between control horses and self-control horses were carried out using the independent sample *t* test. Hematological and hormonal data were analyzed using the paired sample *t* test. The differences between enzymatic data of the 25 diseased horses on Day 2, 4, and 6 were analyzed using the sample *t* test, and all values were expressed by means ± standard error. The effects were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Clinical results

The reasons for the back issue in the presented horses might be related to the unfitted saddle, changed training program, exposure to fans, or improper riding. The back soreness was a primary type that was determined in soft tissue only and not detected in the articular or osseous structures of the spine, with different pain scores: 15 horses had mild pain, 9 reacted moderately, one horse responded severely, and 5 were normal. The inspection revealed symmetrical gluteal and pelvic muscles, with no lumps, scars, or saddle marks on the withers and saddle region. Palpation and manipulation showed localized pain areas under the saddle and lumbar region of different degrees. The dorsal sacral ligament showed slight swelling and pain with no evidence of fibrosis or thickening. The tail and croup regions were normal.

The horses resisted turning either in one direction or both, guarding their spine to bend laterally. They showed abnormal gait during backing up and down a slope. During lunging, the horses tended to lean out of the circle with elevated heads and also exhibited difficulty to transfer from one gait to another. Acute pain was found in five horses that struggled to be ridden and moved away, and the onset of pain signs lasted for 1 to 3 days. The chronic pain was recognized after protracted times more than three days in the remaining 20 horses that accepted mounting and ridding, but they resented turning in one or both directions.

Ten minutes subsequent to acupoint stimulation with the injectable solution, the horse's bodies reacted with excessive sweating, engorged superficial subcutaneous blood vessels, body muscle flaccidity, protruded and relaxed penis, dropped head, slow and staggering steps, urination, and defecation. In addition, they showed signs of mild stress which included pupil dilation and increases in heart rate (57.85 ± 4.301 bpm), body temperature (38.5 ± 0.7°c), respiration rate (28.333 ± 2.123 bpm), and CRT (1.00 ± 0.00 second) (Table 1).

3.2. Hematological results

The effects of cloprostenol-pharmacopuncture on the hemogram of Thoroughbred jumper horses (Table 2) showed significant increases ($P < 0.05$) in red blood cells, mean corpuscular volume, platelet count, white blood cells, hemoglobin, lymphocytes, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean platelet volume, platelet distribution width, plateletcrit, and large platelet concentration ratio. In addition to these significant increases, no significant differences were seen in the levels of hematocrits, granulocytes, and midocytes before and after treatment ($P > 0.05$).

3.3. Enzymatic results

The data of self-control horses before treatment at rest and after exercise which were statistically compared with the five control horses revealed significant increases in both AST and CPK results where $P < 0.05$, as shown in Table 3.

The comparison of AST and CPK levels after treatment and after exercise on Day 2, 4, and 6 with AST and CPK levels of control horses and the self-control group had displayed significant differences from the Day 2 ($P = 0.001$); on Day 4, the results showed no significance ($P = 0.188$) as it did mime the values obtained from the control horses and became significant on Day 6 ($P = 0.002$), as shown in Figs. 1 and 2.

Table 1 The vital parameters before and after cloprestenol-pharmacopuncture. All injected Horses showed signs of mild stress which included increases in heart rate (57.85 ± 4.301 bpm), body temperature (38.5 ± 0.7 °C), respiration rate (28.333 ± 2.123 bpm), and capillary refilling time CRT (1.00 ± 0.00) second.

	Before injection	After injection
Heart rate	42.85	57.85 ± 4.301
Temperature	37.7	38.5 ± 0.7
Respiratory rate	14.85	28.333 ± 2.123
Capillary refilling time	1.00 ± 0.00	2

Table 2 The CBC picture before and after 6 days of cloprostenol-pharmacopuncture. Samples were taken before therapy and day 6 after therapy, the results show significant increases ($P < 0.05$) in RBCs, MCV, PLT, WBCs, HGB, LYMF, MCH, MCHC, MPV, PDW, PCT, LPCR and no significant differences were seen in the levels of HCT, GRAN and MID all the differences in values present within the normal range.

Parameters	Before treatment	After treatment	P value
RBCS	6.86 ± 0.12	8.1 ± 0.108	0.04[a]
MCV	44.92 ± 0.85	45.81 ± 0.83	0.00[a]
HCT	31.11 ± 0.79	31.85 ± 0.67	0.13[b]
PLT	188.28 ± 11.9	196.2 ± 12.12	0.01[a]
WBC	7.49 ± 0.29	7.85 ± 0.26	0.04[a]
HGB	11.70 ± 0.19	12.19 ± 0.25	0.00[a]
MCH	17.2 ± 0.28	18.37 ± 0.25	0.00[a]
MCHC	38.29 ± 0.19	39.57 ± 0.16	0.02[a]
LYMF	2.24 ± 0.13	3.49 ± 0.14	0.00[a]
GRAN	4.52 ± 0.29	4.64 ± 0.29	0.11[b]
MID	0.74 ± 0.08	0.83 ± 0.06	0.12[b]
MPV	7.05 ± 0.29	7.49 ± 0.29	0.00[a]
PDW	12.55 ± 0.55	13.97 ± 0.38	0.01[a]
PCT	0.15 ± 0.01	0.18 ± 0.01	0.00[a]
LPCR	24.51 ± 2.43	25.44 ± 2.5	0.00[a]

CBC = complete blood picture; GRAN = Granulocyte; HCT = Hematocrit; HGB = Hemoglobin; LPCR = Large Platelet Concentration Ratio; LYMPH = Lymphocytes; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration; MCV = Mean Corpuscular Volume; MID = Midocyte; MPV = Mean Platelet Volume; PDW = Platelet Distribution Width; PCT = Plateletcrit; PLT = Platelet count; RBC = Red Blood Cells; WBC = White Blood Cells.
[a,b] Superscripts data are significantly different at P value ≤0.05.

3.4. Hormonal results

Serum cortisol levels were significantly higher ($P = 0.013$) than the levels obtained before treatment (151.51 ± 6.19) and on Day 6 after treatment (399.22 ± 6.46) (Table 4).

3.5. Competition results

Competition records showed 9 champion horses, 7 reached advanced positions, and the other 9 horses to have competition progress.

Table 3 AST and CPK values in the control and 25 diseased-horses before therapy at rest and after exercise.

Parameters		5 Control horses	25 diseased-horses before therapy
AST (U/L)	At rest	190.60 ± 3.64[a]	242.36 ± 4.31[b]
	After exercise	203.20 ± 5.43[b]	267.68 ± 5.51[b]
CPK (U/L)	At rest	97.60 ± 7.45[a]	180.88 ± 10.21[b]
	After exercise	118.80 ± 14.11[s]	218.40 ± 6.67[b]

AST = aspartate aminotransferase; CPK = creatine phosphokinase.
[a,b] Superscripts indicate significant difference at $P < 0.05$.
Number of 25 diseased jumper geldings with back pain and 5 control horses exempted back pain.

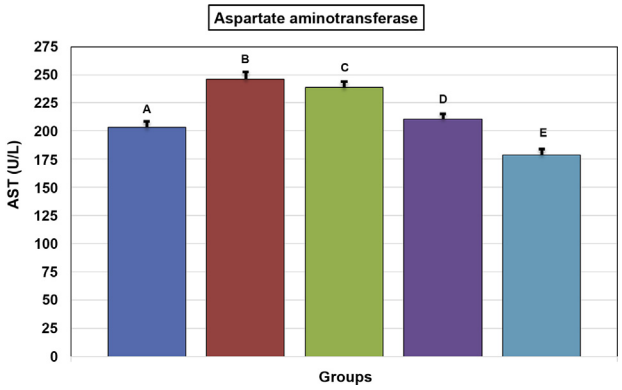

Figure 1 The level of AST in the 5 control horses (A), 25 diseased-horses before therapy and after exercise, AST level showed significant increase than control horses which indicates muscle injury (B), 2 days following cloprostenol-pharmacopuncture therapy, the results showed significant decrease in the mean levels of AST (C), 4 days after therapy, AST level mimed the level of the 5 controls (D) and 6 days after therapy showed a significant decrease (E). All samples were taken 6-hours after exercise and results considered significant at $P < 0.05$.

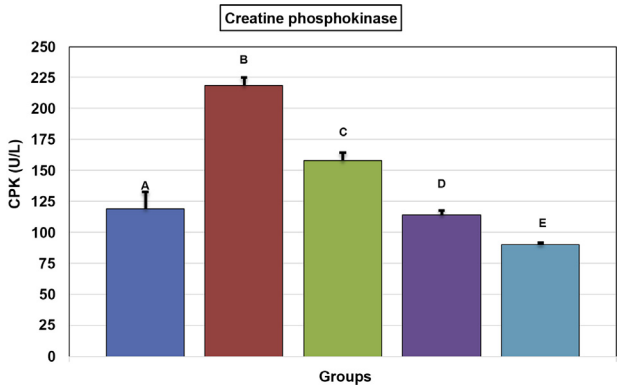

Figure 2 The level of CPK in the 5 control horses (A), 25 diseased-horses before therapy and after exercise, CPK level showed significant increase than control horses which confirm muscle injury (B), 2 days following cloprostenol-pharmacopuncture therapy, the results showed significant decrease in the mean levels of CPK (C), 4 days after therapy, CPK level mimed the level of the 5 controls (D) and 6 days after therapy showed a significant decrease (E). All samples were taken 24-hours after exercise and results considered significant at $P < 0.05$.

4. Discussion

In the study, geldings have been chosen to avoid disagreement in the results where metabolic and stress/inflammatory responses are reliant on the sex, in accordance with the study by Radom-Aizik et al [22]. Moreover, the aforementioned horses have different features than the same breed living in other countries and they are put under different weather conditions and training regimens.

Back soreness is related to primary soft-tissue issues with different pain degrees; the pain scores are normal, mild, moderate, and severe. This classification is concurrent with that mentioned in the study by Mathews [23]. In addition, the

Table 4 Serum cortisol level before treatment and after 6 days of cloprostenol-pharmacopuncture, samples were taken at the morning 7 am, the levels after treatment at day 6 were significantly higher than the levels obtained before treatment where $P < 0.05$.

Serum cortisol level	Mean	Number	Std. deviation	Std. error mean
Before treatment	151.5120	25	30.95756	6.19151
After treatment	399.2240	25	32.27654	6.45531

Std. = Standard.

etiological factors that have been mentioned in the study are similar to those stated in the study by Denoix and Dyson [24].

Administration of various medicines and alternative therapies to horses with back pain failed to provide sufficient pain management in the affected horses [25], whereas using cloprostenol-pharmacopuncture in the diseased horses has proved dramatic success in the treatment of back pains.

Cloprostenol creates pressure or mild irritation to the acupoint and keeps the point stimulation for a longer period of time, and these comprise the benefits of both acupuncture and injection as mentioned in the study by Xie and Priest [20].

Cloprostenol does not only participate in the initiation but also actively contribute to the resolution of inflammation, pain relief, and muscle relaxation in the diseased horses. The role of cloprostenol in acute and chronic inflammation opens chances for the design of new antiinflammatory drugs, in concurrence with the study by Ricciotti and FitzGerald [13]. The clinical signs exhibited by the horses after cloprostenol-pharmacopuncture therapy is related to its powerful local action that leads to vasodilation and inhibition of blood platelet aggregation. This role is associated with their dependent receptors and receptor-independent mechanisms in concurrence with the study by Scher and Pillinger [14]. Furthermore, the roles in the contraction of the smooth muscles of the intestine, urinary bladder, and blood vessels were in concurrence with the studies by Anderson et al [26] and Robert [27].

The bladder meridian was chosen because it is one of the major acupuncture meridians that have a unique effect on balancing the other acupuncture meridians [28] and very effective in treating back pain [11]. The five selected bladder acupoints are potent points that are commonly used and cover all back regions from the withers to the lumbosacral joint [20].

The significant increases of blood indices confirmed the data published in the National Institutes of Health Conference which stated that acupuncture increases red blood cell and white blood cell count, treats thrombocytopenia, and increases immunity, also corresponding with World Health Organization recommendations that endorse acupuncture for treating leukopenia [29]. The study found increased levels of hematocrits, granulocytes, and midocytes before and after treatment, but this difference is nonsignificant and also, the increases of hematological variables are within the normal values in accordance to the study by Aiello et al [30].

The 25 jumper horses have shown significant increase in AST and CPK levels at rest and after exercise (self-control) than the control horses ($P < 0.05$), whereas the obtained results indicate muscular damage that corresponds to clinical evaluation and confirms back soreness in these horses as mentioned in the study by Hodgson et al [31] that muscle injury is determined by elevated levels of AST and CPK and that the activity of these enzymes is used to monitor the effect of exercise on athletic horses.

The increase in the CPK activity can be the first symptom of overtraining, especially when it is accompanied by an increase in the AST activity. Moreover, the gradual increase in resting values of CPK can indicate muscle microdamages and is apt to make a decision to reduce the work competence for athletic horses. Therefore, the effect of physical effort on the serum activity of these enzymes depends on the fitness level, intensity, and duration of exercise [32].

AST, CPK, and serum cortisol levels after cloprostenol-pharmacopuncture have explained how synthetic prostaglandin $f_2\alpha$ treats pain and how horses responded differently to therapy and may be one of the reasons that let the diseased horses regain their athletic performance. The significant increases in serum cortisol levels after the end of the therapy are attributed to its effect on the central and peripheral nervous systems to activate the body's endogenous or natural pain relief mechanisms. It stimulates the release of several neurotransmitters, chemical secretions that cause a favorable reaction in another nerve, a muscle, or a gland. It also causes a release of β-endorphins and adrenocorticotropic hormone from the pituitary gland that induces the release of cortisol from the adrenal gland; this is in agreement with several studies [11-33]. Moreover, several investigators stated that cortisol is released as a result of stress conditions [19, 34].

Competition records together with the improvement of hematological, enzymatic, and hormonal data of the presented horses after therapy proved the efficacy of cloprostenol in the treatment of back soreness which is related to soft-tissue issues in athletic horses. However, the alterations are attributed to the release of splenic erythrocytes under the influence of catecholamines during exercise as stated in the study by Ricketts [35]. Therefore, it is highly recommended to use cloprostenol-pharmacopuncture therapy for back soreness in horses, as stall resting of horses with back pain causes muscle loss and further complications and slows the horses' return to work [8].

In conclusion, cloprostenol is effective in treating back soreness in athletic horses. The successful results recommend its application for human patients suffering from identical back issues. Prospectively, this research will have a positive effect on animal and human medicine and welfare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jams.2019.07.001.

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