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HEMATOLOGICAL AND BIOCHEMICAL INVESTIGATIONS IN CASE OF ACETAMINOPHEN ADMINISTRATION IN HORSES

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Abstract

Acetaminophen is one of the most used analgesic agent for the relief of acute and chronic pain in humans. Equine analgesia poses a common challenge to clinicians, so acetaminophen could be considered as an alternative to common non-steroidal anti-inflammatory drugs used in horses.

The purpose of this research was to observe the safety of treatment with acetaminophen, and it was carried out at the USAMV Cluj-Napoca. In order to initiate treatment with acetaminophen, a dose of 20 mg/kg was administered orally, once every 12 hours, for a period of 14 days, in two horses of approximately 500 kg, aged 16 and 17 years, from Lipizzan breed. We administered the commercial product Paracetamol Terapia, containing 500 mg acetaminophen each tablet, for human use. The horses in this study were monitored throughout the treatment, from a clinical point of view, and complementary examinations were made (the gastric mucosa was monitored with gastroscopy). The haematological analyses were performed with the Abacus Junior Vet5, and for the biochemical analyses a Skyla vb1+ analyser was use. In this study during the treatment, no significant haematological or biochemical changes were observed after acetaminophen administration.

Key words*: acetaminophen, pain, anti-inflammatory drug, exams.*

INTRODUCTION

Pain management in horses represents a real challenge encountered often by clinicians. In human medicine, acetaminophen is used for its analgesic and antipyretic effect, therefore emerged an alternative to replace non-steroidal anti-inflammatory drugs (NSAID) that are so commonly used in horses. The studies that have already been completed are pointing out that acetaminophen has a high absorption rate in horses and because of its mechanism of action, there were no signs of side effects when it was used in the correct doses. Acetaminophen, used in horses that showed different sort of pain-inducing diseases showed encouraging results, becoming more a current topic, as the pain from an orthopaedic condition is different from an abdominal pain (Jones E. et al., 2007). Moreover, when used as an adjuvant, it proved to enhance the analgesic effect of other drugs.

The purpose of the research carried out under the guidance of the department of Toxicology and Internal Medicine, from the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, is to observe the safety of acetaminophen treatment in horses. The horses monitored in this research were clinically evaluated before the acetaminophen administration, haematological, and biochemical analyses were carried out.

Acetaminophen, often known as paracetamol, is a medicine used to treat fever and moderate pain. It was synthesized in 1873, by reducing p-

nitrophenol in acetic acid medium. Its therapeutic properties were not recognised at the time of its discovery, until Bernard Brodie and Julius Axelrod discovered that the metabolite, acetanilide had the analgesic effect.
The relatively large safety margins make it a drug of choice in the management of moderate pain in humans (Graham et al., 2013). This substance gained interest as an alternative to non-steroidal anti-inflammatory drugs because its mechanism of action has fewer gastrointestinal side effects compared to NSAIDs. It has been found, that when administered together with other non-steroidal anti-inflammatory drugs, especially COX-2 inhibitors, acetaminophen potentiates their analgesic and anti-inflammatory action compared to their solitary use (Latimer et al., 2011).
Recently there have been studies proving the effectiveness of acetaminophen treatment in horses, acetaminophen is reported to have 91% bioavailability (Neirinckx et al., 2010). Its use is cited both as a single agent, especially in cases of lameness, West et al., in 2011, has demonstrated efficacy as an adjunct treatment for laminitis in one pony and in combination with NSAIDs for pain control in general, it is an effective analgesic agent when combined with NSAIDs in a model of inducible foot pain (Foreman et al., 2016). Recent research on this protocol makes this topic novel, making acetaminophen a serious candidate for equine analgesic medication. In this paper, we want to present data's on the adverse effects of acetaminophen and see it's safety in adult horses.

MATERIALS AND METHODS

Acetaminophen was administered to two horses of approximately the same weight (500-550 kg) presenting normal body score. Both horses are belonging to the Lipizzan breed, a 16-year-old male (Siglavy-Capriola XX-25) and a 17-year-old female (Maestoso XLVII-27). Before starting the treatment with acetaminophen, the horses were examined from a clinical and paraclinical point and performed biochemical and haematological analyses. Following the examinations performed, the horses were declared clinically healthy.
In our protocol, we administered acetaminophen, in a dose of 20 mg/kg, orally once every 12 hours for 14 days. Considering that there is no approved commercial product containing acetaminophen, for veterinary use, we used Paracetamol Terapia 500 mg tablets, for human use. Prior to administration the tablets were ground in a mortar to obtain a powder. This pulvis was mixed with a 3% glucose solution and was administered orally with a 20 mL syringe whose tip was previously removed.
To perform the haematology examination, the blood was collected on day 0, before the first administration of acetaminophen, on day 7, and day 14, which was also the last day of the treatment. Blood sample collection was performed by puncturing the left jugular vein, after performing antisepsis and a light manual haemostasis. The collected blood was stored in vacutainers with a purple and green lid. The haematological analyses were performed using an Abacus Junior Vet 5 device.
Blood biochemistry was performed using a Skyla vb1+ device. The serum is introduced inside the rotor with reagents, where the chemical reactions take place, then at the end of the process the results of the biochemical analyses are displayed. A single sample is sufficient for the analysis of several biomarkers in a panel.

RESULTS AND DISCUSSIONS

Before starting the treatment protocol with acetaminophen, we carried out a series of analyses at the department of Pathology and Clinical Medicine of Faculty of Veterinary Medicine in Cluj-Napoca. The obtained values and required parameters are shown in the Table 1, below.
We observed slightly elevated values of urea, total protein, serum glucose and triglycerides. These values can be attributed to the advanced age of the horses, to the diet or to some conditions existing before the research was carried out. We administered acetaminophen to older horses, with the approximately same age, breed, and weight, to have a picture of the effects of acetaminophen in this category, where we found no studies.

Table 1. The biochemical values before starting the treatment protocol

The analysed parameters	Mollys results	Samans results	Reference interval
Urea (mg/dL)	51.9	44.2	18-42
Creatinine (mg/dL)	1.19	0.91	0.79-1.808
Total bilirubin (mg/dL)	1.43	3.43	<2.63
Total proteins (g/dL)	7.14	7.28	5.3-6.5
Albumin (g/dL)	2.12	2.23	1.8-2.73
Globuline (g/dL)	1.62	1.67	1.32-2.14
Glucose (mg/dL)	121.0	100.4	50-90
Triglycerides (mg/dL)	87.6	76.4	8.75-35
Phosphorus (mg/dL)	3.68	3.45	3.1-5.26
Calcium (mg/dL)	9.15	10.1	8-13
Gamma-glutamyltransferase (U/L)	43.3	46.2	10-60

The patients were clinically healthy during the administration of the substance, their appetite and peristalsis was also normal.

The haematological and biochemical analyses did not reveal significant changes or detect a liver dysfunction attributed to a potential hepatotoxicity resulting from the administration of acetaminophen. Compared to the results presented in other studies, which demonstrate a decrease in the value of platelets during treatment, in this study the blood platelets decreased too, possibly due to platelet aggregation. In humans, Fischereder et al. (1994) reported thrombocytopenia in 3.4% of humans, presenting acute acetaminophen toxicity in a retrospective evaluation suspecting a direct toxic effect on platelets. More studies are needed to the changes in platelet counts. Maintenance of serum protein values close to the upper limit or even slightly exceeding this threshold, has also been reported in other studies (Mercer, 2018) where the same treatment protocol was used. Thus, an interaction between the mechanism of action of acetaminophen and protein production can be considered.

Table 2. Haematological analyses

HAEMATOLOGY ANALYSES	MOLLY				SAMAN			
	Day 0	Day 7	Day 14	Ref. int.	Day 0	Day 7	Day 14	Ref. int.
Erythrocyte (RBC) 10^{12}/l	6.08	6.38	7.14	6.8-12.9	7.18	6.95	6.89	6.8-12.9
Hemoglobin (HGB) g/dL	10.6	11.8	13.5	11-19	12.2	12.2	11.5	11-19
Hematocrit (HCT) %	37.27	39.31	43.06	32-53	40.54	39.29	38.06	32-53
VEM / MCV Fl	61	62	60	37-59	56	57	55	37-59
MCH Pg	17.5	18.5	18.9	12.3-19.7	16.9	17.5	16.7	12.3-19.7
MCHC g/dL	28.5	29.9	31.4	31-39	30.0	31.0	30.2	31-39
Platelet 10^9/l	108	61	59	100-400	127	88	111	100-400
Leukocyte (WBC) 10^9/l	10.16	13.14	11.25	5.4-14.3	11.02	10.04	9.80	5.4-14.3
Limphocyte (LYM) 10^9/l	5.05	4.85	5.27	1.5-7.7	3.87	3.64	3.18	1.5-7.7
Monocyte (MON) 10^9/l	0.44	0.55	0.06	0-1.5	0.01	0.22	0.23	0-1.5
Neutrophil (NEU) 10^9/l	4.14	7.46	5.55	2.3-9.5	6.08	5.48	5.85	2.3-9.5
Eozinophil (EO) 10^9/l	0.49	0.26	0.34	0.1-1	0.95	0.64	0.49	0.1-1

The blood tests from Molly on day 0 reveals a mild, microcytic, hypochromic and regenerative anaemia, probably due to a post haemorrhagic background. On the 7th and 14th day of treatment, the values normalized, thus suggesting the presence of a pre-existing condition. At the end of the treatment, the erythrogram values were within reference

intervals. The values displayed in the leukogram did not show significant changes during the treatment, being within normal limits at each test (Table 2).

The parameters of the erythrogram did not show significant changes in any value in case of Saman. The only changes reported were in the case of platelets, on day 7, when the displayed value dropped below the normal limits of the species, most likely due to platelet agglutination. The values displayed in the leukogram showed no significant changes throughout the treatment, being within normal limit at each test (Table 2).

The biochemical analyses of Molly showed that the albumin and glucose level remained within the normal limits, the total proteins were close to the upper limit. The liver enzymes represented by ALP, AST and GGT did not show values outside of the reference ranges during treatment. The GLDH enzyme was measured only at the end of the research, but its value was also within the reference limits of the species (8.8 U/L, Ref. int 0-20 U/L). The value of creatine kinase, an enzyme with importance in the metabolism of skeletal muscles, produced by the metabolism of creatine-phosphate in muscles in a constant rate, depending on the muscle mass (Jose H. Salazar, 2014) did not suggest damage throughout the administration of acetaminophen. The role of serum urea is to measure renal dysfunction, also providing information about the liver function or the dietary protein intake (Jose H. Salazar, 2014). In this study urea showed values above the normal limits on day 0 (30.8 mg/dL) and on day 7 (36.4 mg/dL) but on day 14 dropped to 24.4 mg/dL, situating between the normal range (10.0-30.0 mg/dL). The creatinine values were maintained in the reference range throughout the treatment. There were also no significant changes in the electrolytes throughout the treatment, they were constantly within normal limits. As an observation, we noticed an apparently low value of serum calcium, but that can be attributed to the reference range of the device, this value is only relatively low, compared to other kits (8-13 mg/dL), yet in other similar studies calcium decrease was observed too, may have been due to improvement in protein binding from rising total protein concentrations, or to increased dietary intake with supplemental feeding provided during the study period (Mercer et al., 2018)

The analyses in case of Saman showed that the total proteins were above the reference limits, characteristic of the species (5.6-7.9 g/dL), with a value of 8.1 g/dL on the first day, respectively 8.2 g/dL on day 7, following which on day 14 it normalized (6.9 g/dL), so mild hyperproteinaemia is noted at the start of acetaminophen administration, with serum total protein values subsequently normalizing. Comparing these results with the results presented in other articles based on the same protocol, it confirms a potential correlation between the administration of acetaminophen and the increase of total protein values. The value of serum albumin decreased gradually during the study period but was always within the biological limits (2-4.30 g/dL). The glucose value did not undergo significant changes, neither the hepatic enzymes, or creatine kinase, remaining constant within the reference range, as in a pilot study, made by Mercer et al., in 2018. The value of total bilirubin decreased gradually during the treatment. As in the first patient, the glutamate dehydrogenase value was only measured on day 14 of treatment and it was found also within the normal reference intervals (4.5 U/l, Ref. int 0-20 U/L). The serum urea values were normal on day 0 and day 7, but on day 14 they increased, yet the creatinine situated between the normal limits, also the electrolytes. The data is presented in Tabel 3.

Table 3. Biochemical analyses

BIOCHEMICAL ANALYSES	MOLLY				SAMAN			
	Day 0	Day 7	Day 14	Ref. int.	Day 0	Day 7	Day 14	Ref. int.
Albumin (ALB) [g/dL]	3.0	2.6	2.8	2.1-4.3	3.2	2.9	2.7	2.1-4.3
Total proteins (TP) [g/dL]	7.6	7.5	7.6	5.6-7.9	8.1	8.2	6.9	5.6-7.9
Glucose (GLU) [mg/dL]	81	66	84	63-136	85	87	72	63-136
Alkaline phosphatase (ALP) [U/L]	191	159	165	0-326	240	203	165	0-326
Aspartate-aminotransferaze(AST)[U/L]	434	306	342	92-610	390	299	334	92-610
Gamma-glutamyltransferaze (GGT) [U/L]	14	16	16	0-42	13	16	17	0-42
Total bilirubin (TBIL) [mg/dL]	1.5	1.5	2.9	0.0-3.5	2.9	2.3	1.5	0.0-3.5
Creatinkinaza(CPK) [U/L]	272	217	258	0-350	205	160	221	0-350
Uree(BUN) [mg/dL]	30.8	36.4	24.4	10.0-30.0	25.2	24.1	31.7	10.0-30.0
Creatine (CREA) [mg/dL]	1.24	1.38	1.00	0.70-2.00	0.91	0.83	1.23	0.70-2.00
Serum bicarbonate (tCO2) [mmol/L]	26.3	27.6	33.6	20.0-33.0	27.1	29.9	29.2	20.0-33.0
Calcium (Ca) [mg/dL]	10.3	9.6	10.2	11.5-14.2	9.9	9.9	9.6	11.5-14.2
Sodium (Na) [mmol/L]	135	138	138	126-146	132	139	138	126-146
Potassium (K) [mmol/L]	3.6	3.6	3.5	2.5-5.2	2.7	2.4	3.5	2.5-5.2

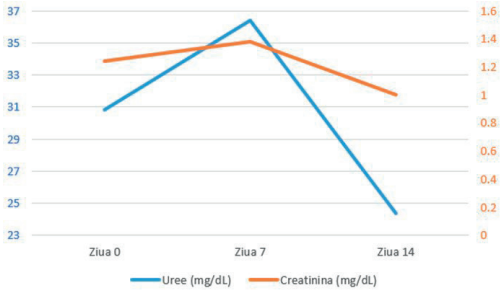

Figure 1. The uree value analyses in Day 0, 7 and 14 (Molly)

The urea value from Molly's blood according to the biochemical analyses was elevated in day 7 but it dropped to the normal limits in day 14, we could observe slightly increased creatinine values too in day 7 (Figure 1).

In Saman case both urea and creatinine were elevated in day 14, but after another 7 days after stopping the administration of acetaminophen both normalized (Figure 2).

The urea and creatinine elevations in both cases, raise questions about the metabolization and elimination of acetaminophen in elder horses, because these modifications were not reported in middle-aged horses.

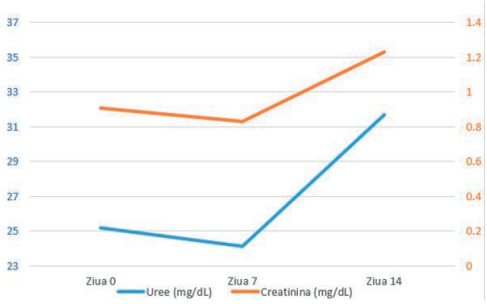

Figure 2. The uree value analyses in Day 0, 7 and 14 (Saman)

CONCLUSIONS

In this study during the treatment, no significant haematological changes were observed after acetaminophen administration in a dose of 20 mg/kg, orally once every 12 hours, for 14 days. The decrease in platelets may be attributed to platelets aggregation, and further investigation is needed to see the correlation.

The biochemical analyses revealed a slight increase in proteinemia during treatment, these results were seen in other studies too, probably the increase is a consequence of acetaminophen mechanism of action.

The increased values of urea and creatinine could be seen probably due to the age of the patients or nutrition factors, these increases were not reported in other studies, so they need to be investigated.

The liver function was not affected during the treatment. The horses did not show clinical signs indicating a hepatopathy (as weight loss, jaundice, colic, photosensitivity, etc.) and the liver enzyme values remained within the physiological ranges. Thus, following the use of this protocol, no cases of hepatotoxicity have been reported.

Acetaminophen can be recommended for elderly horses with chronic conditions for its anti-inflammatory and analgesic effect. This could be an option in case of chronic laminitis, chronic osteoarticular conditions or in case of conditions that do not allow venous access (phlebitis, severe dehydration, etc.).

Following this study, we showed that acetaminophen has a high bioavailability in horses with chronic conditions, without the presence of adverse reactions in the administered dose. It can be administered instead of non-steroidal anti-inflammatory drugs, or a combined protocols could be used.

Further multi-way studies are needed to be able to correlate the action of acetaminophen with the changes in proteinemia and platelet count. Due to the lack of research acetaminophen is not recommended to administer to pregnant mares, and to sport horses participating in the equestrian competitions.

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