

Comparative Study of the Efficacy of Flunixin, Ketoprofen and Phenylbutazone in Delman Horses with Mild Colic

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ABSTRACT

This study aimed to evaluate the efficacy of flunixin, ketoprofen and phenylbutazone on serum biochemistry, plasma catecholamines and serum cortisol in Delman horses with mild colic. During the study period, 32 horses were evaluated due to mild colic. Flunixin, ketoprofen, and phenylbutazone were administered intravenously at the recommended dose rates of 1.0; 2.2 and 4.4 mg/kg, respectively. Administration of the NSAIDs commenced on Day 1 and continued every 12 h for 12 days. Blood samples collected between days 2, 5, 9 and 13 to evaluate AST, ALP, GGT, creatinine, urea, epinephrine, norepinephrine, and cortisol level. The data analysis performed using Multivariate analysis of variance (Manova) followed by Duncan

multiple range test. The results showed a significant alleviation in all observed variables on Day 13, although the use of various NSAIDs showed no significant difference.

Keywords: serum biochemical, catecholamine, cortisol, colic, NSAIDs

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INTRODUCTION

Adult horse deaths by 50% were caused by digestive diseases, such as colic, diarrhea, or enterotoxemia. The incidence of colic is estimated at 13.6% per year in horses aged 6 months or more in 28 US states.¹ Although 75% of colic cases recover in less than 24 hours, 67% of horses with colic were examined by veterinarians, of which 85% receive intensive treatment. The probability of a horses' death rate is very high when referred to an animal clinic.²

Colic is a general term used for symptoms of abdominal pain that can be caused by a variety of different conditions. Colic is a symptom that must be anticipated by horse owners because it can cause horse death. In some cases, colic can cause death within hours.³ Other studies have reported the colic associated with food material, parasites infections, dental diseases, access to water, but the cause of the majority of colic cases is unknown.⁴

Flunixin, ketoprofen, and phenylbutazone belong to a group of nonsteroidal anti-inflammatory drugs (NSAIDs) compounds. Other compounds such as salicylates (aspirin), propionic acid (ibuprofen, phenopropfen, ketoprofen and naproxen), pyrazolone (phenylbutazone), anthranilic acid (meclofenamic acid), and aminonicotinic acid (flunixin) are also included as NSAIDs derivatives. The use of NSAIDs is as an anti-pyretic, anti-inflammatory, and analgesic compound.⁵

The efficacy of NSAIDs is still the first choice for reducing colic symptoms.⁶ This study aimed to evaluate the efficacy of flunixin, ketoprofen and phenylbutazone on serum biochemistry, plasma catecholamines and serum cortisol in Delman horses with mild colic.

MATERIALS AND METHODS

Ethical approval

This study approved by Indonesian Horse Veterinarian Association and according to the standard operating procedure for colic treatment.

Animals

All Delman horses with mild colic examined at each enclosed between June 29 to August 21, 2019, were eligible for inclusion in this study. To be included in the study, a blood sample for the measurement of selected variables had to have been collected from the affected horse within 30 minutes. For eligible horses (age, sex, breed, heart rate at initial evaluation, and suspected mild colic) enrolled to evaluate the blood sample.

Administration of the NSAIDs

Administration of the NSAIDs commenced at 07.00 hours on Day 1, and continued every 12 h for 12 days. Flunixin meglumine (Flumine, Jaapharm Canada Inc); ketoprofen (Ketofen, Zoetis) and phenylbutazone (Phenylbute, Phoenix Pharm) were administered intravenously via an indwelling 6.5 mm catheter (Equivet Stallion, North Yorkshire, UK) in the jugular vein, at the recommended dose rates of 1.0; 2.2 and 4.4 mg/kg, respectively.

Serum evaluation

Each blood sample for serum measurement was allowed to clot before centrifugation (1500 X g for 10 minutes), and an aliquot of the serum was stored at -80°C until analyzed using the clinical chemistry analyzer Hitachi 902[®] (Roche Diagnostics, USA) for serum biochemical level⁷ and validated using radioimmunoassay for serum cortisol.⁸ Each blood sample for measurement of plasma epinephrine and norepinephrine concentrations was centrifuged immediately after collection, and plasma aliquots were stored at -80°C until analyzed using highly-performance liquid

chromatography (HPLC).⁹ All blood samples collected between days 2, 5, 9 and 13 to determine the trend after NSAIDs administration.

Statistical analysis

All data on the following variables i.e. aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), creatinine, urea, epinephrine, norepinephrine, and cortisol level compared between days 2, 5, 9 and 13 after administered NSAIDs, respectively. The data analysis performed using Multivariate analysis of variance (Manova) followed by Duncan multiple range test ($p < 0.05$) (IBM, USA).

RESULTS AND DISCUSSIONS

During the study period, 32 horses were evaluated due to mild colic. NSAIDs administered in horses with colic i.e. flunixin ($n=11$), ketoprofen ($n=11$), and phenylbutazone ($n=10$). There were no significant differences ($p > 0.05$) between the administration of flunixin, ketoprofen, and phenylbutazone on the following variables: AST (Figure 1), ALP (Figure 2), GGT (Figure 3), creatinine (Figure 4), urea (Figure 5), epinephrine (Figure 6), norepinephrine (Figure 7), and cortisol level (Figure 8).

On the other hand, the results showed significantly different ($p < 0.05$) between days 2, 5, 9 and 13 (Figure 1-8). Compared to the first day when the initial diagnosis of mild colic in horses followed by a pre-treatment blood collection, all variables showed an alleviation trend in levels. The alleviation of serum biochemical, plasma catecholamine and serum cortisol levels were highly significant on Day 13.

Non-steroidal anti-inflammatory drugs (NSAIDs) are important veterinary medicines for most mammalian species. In recent years, the use of NSAIDs has increased rapidly to handle clinical cases.¹⁰ NSAIDs can reduce pain and inflammation without immunosuppressive and metabolic side effects associated with corticosteroids (Figure 8). Flunixin meglumine is used in veterinary practice as an analgesic, antipyretic, and anti-inflammatory drug. The use of flunixin in ruminants for the treatment of various inflammatory conditions such as endotoxemia, mastitis, and musculoskeletal disorders.¹¹ This drug performs a mechanism of action by inhibiting the enzyme cyclooxygenase (Cox). Cox enzymes play a role in the arachidonic acid cascade and convert it into prostaglandins. Flunixin inhibits the formation of prostaglandins and inflammatory mediators by inhibiting Cox.^{12,13}

Ketoprofen [(3-benzophenyl) -propionic acid] is a propionic acid derivative that has anti-inflammatory, analgesic, and antipyretic activity. Ketoprofen is quickly eliminated from the body for 2-4 hours so it is necessary to give ketoprofen more often to be able to maintain its therapeutic concentration in the blood.¹⁴ Ketoprofen performs first pass metabolism in the stomach so that it will affect the bioavailability of drugs in plasma.¹⁵

Phenylbutazone is metabolized in the liver to oxyphenbutazone, active metabolites that are eliminated more slowly from the body than phenylbutazone. The metabolic activity of the liver in gastrointestinal disorders affects the serum levels of liver enzymes, i.e., AST, ALP, and

GGT (Figure 1-3). Oxyphenbutazone inhibits phenylbutazone metabolism. The liver's capacity to metabolize phenylbutazone is not optimal at relatively low doses of the drug, producing dose-dependent kinetics.¹⁶ Phenylbutazone is widely used in horses for various musculoskeletal disorders. Phenylbutazone also has an endotoxin antagonistic effect on intestinal motility. Phenylbutazone inhibits prostaglandin synthesis at low plasma concentrations in horses (5-15 $\mu\text{g/mL}$).⁶ This difference may be due to species differences in the structure of Cox.¹⁷ An initial dose of 4.4 mg/kg every 12 hours on the first day of therapy is followed by a decrease in dose and an increase in the dosing interval for subsequent therapy.¹⁸ Due to the accumulated withdrawal time of long-standing phenylbutazone and oxyphenbutazone, chronic therapy must be at the lowest possible dose and the longest dose interval that may still control pain.¹⁹

Decreased creatinine (Figure 4) and urea (Figure 5) levels after Cox-1 and Cox-2 inhibition occur in peripheral tissues.^{20,21} This inhibition interferes with the process of converting arachidonic acid into prostaglandins. Prostaglandins are the main protective component in the hemodynamic function of the kidneys. 63% of NSAIDs are metabolized through glucuronidation and 34% through sulfation in the liver. Water-soluble metabolites will be excreted through the kidneys. *N-Acetyl p-benzoquinone* (NAPQI) is a reactive intermediate formed when oxidation $< 5\%$ paracetamol by the cytochrome P-450 enzyme. The decrease in urea in the results of this study was obtained because the effect of NSAIDs was converted to inactive metabolites, non-electrophilic, and decreased toxic effects for the liver and kidneys. The decrease in NAPQI will prevent tubular damage which can be characterized by a decrease in serum creatinine and urea levels.²²

CONCLUSION

It can be concluded that there were no significant on the following variable: AST, ALP, GGT, creatinine, urea, epinephrine, norepinephrine, and cortisol after administered flunixin, ketoprofen, and phenylbutazone. The significant result showed between days 2, 5, 9 and 13 compared with pre-treatment. The highest alleviation showed on the 13th day.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

All authors declare that they have no competing interest.

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ABBREVIATIONS

ALP: alkaline phosphatase; AST: aspartate aminotransferase; Cox: cyclooxygenase; GGT: gamma-glutamyltransferase; HPLC: highly-performance liquid chromatography; Manova: multivariate analysis of variance; NAPQI: *N-Acetyl p-benzoquinone*; NSAIDs: nonsteroidal anti-inflammatory drugs; U/L: unit per liter; $\mu\text{mol/L}$: micromole/liter; mmol/L : millimole/liter; pg/mL : picogram/milliliter; $\mu\text{g/dL}$: microgram/deciliter

FIGURES

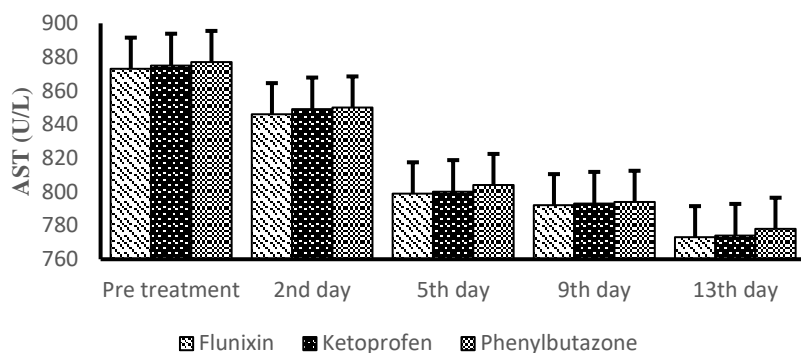


Figure 1: The AST values between days 2, 5, 9 and 13

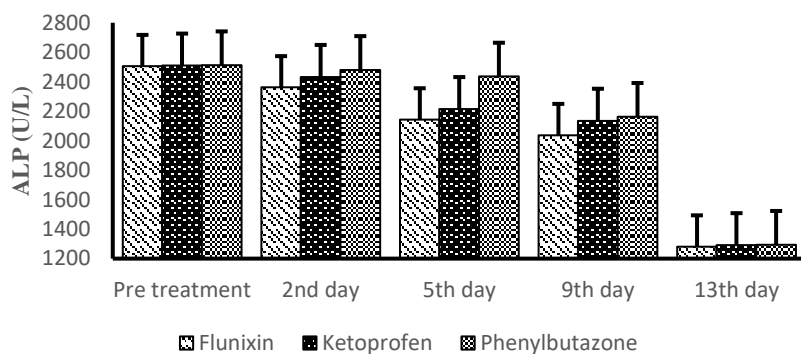


Figure 2: The ALP values between days 2, 5, 9 and 13

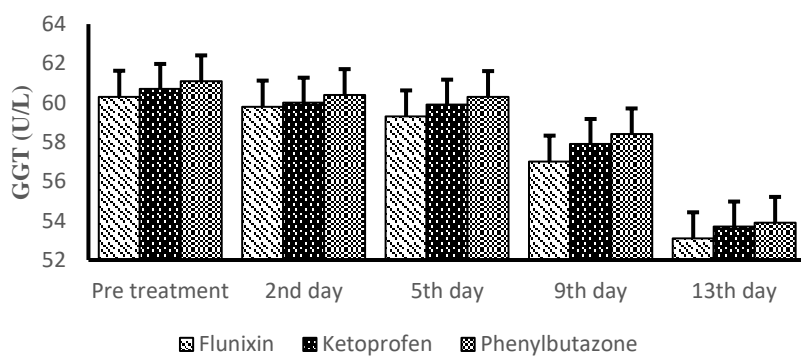


Figure 3: The GGT values between days 2, 5, 9 and 13

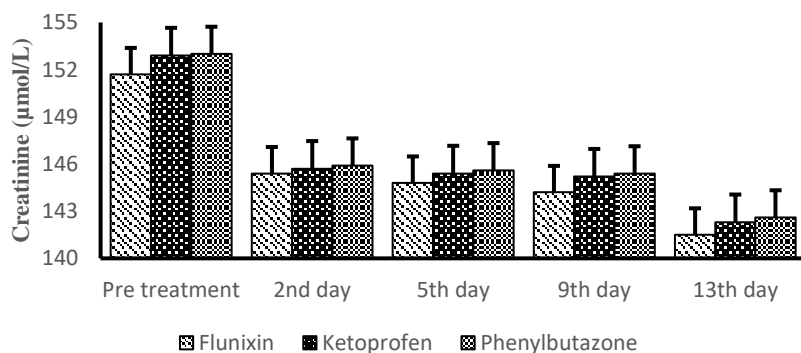


Figure 4: The creatinine values between days 2, 5, 9 and 13

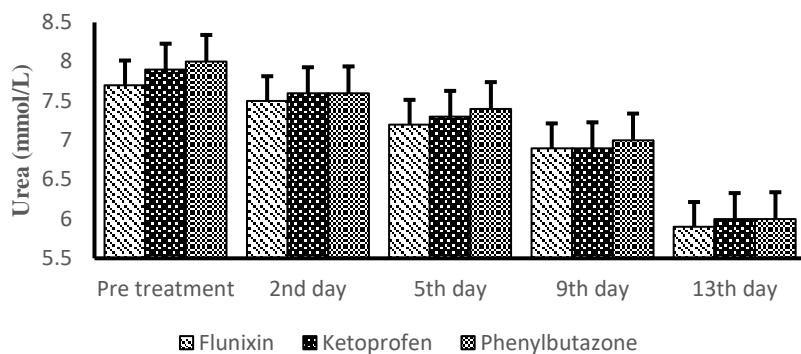


Figure 5: The urea values between days 2, 5, 9 and 13

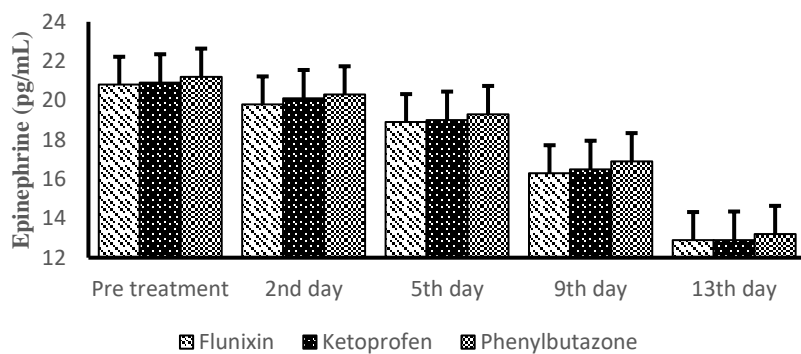


Figure 6: The epinephrine values between days 2, 5, 9 and 13

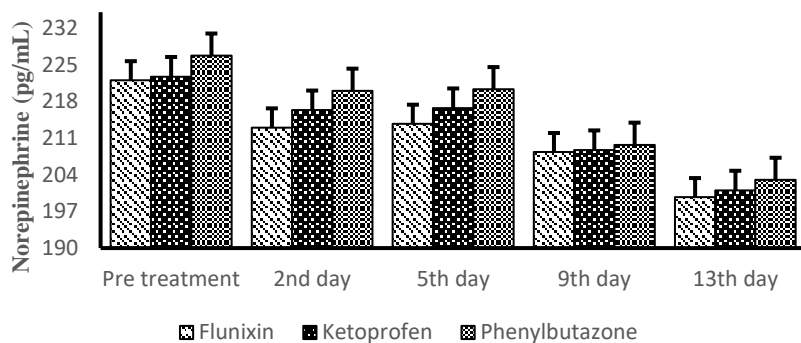


Figure 7: The norepinephrine values between days 2, 5, 9 and 13

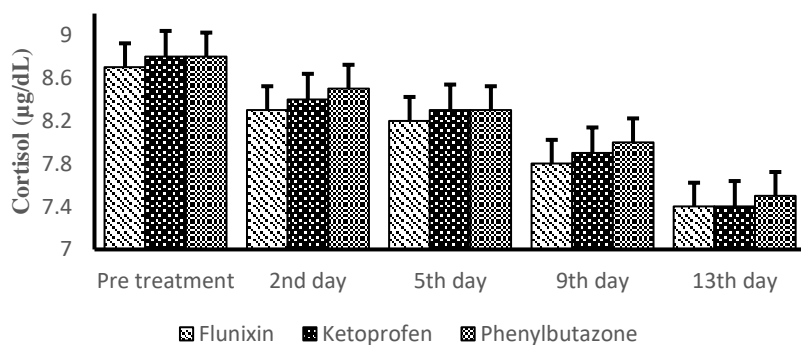


Figure 8: The cortisol values between days 2, 5, 9 and 13