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Safety of Antitheilerial Drug Buparvaquone in Rams

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ABSTRACT

***Background*:** Theileriosis is a tick-borne disease caused by *Theileria* strains of the protozoan species. Buparvaquone is the mostly preferred drug in the treatment theileriosis, while it is safety in sheep, has not been detailed investigated. It has been hypothesized that buparvaquone may show side effects and these effects may be defined some parameters measured from blood in sheep when it is used at the recommended dose and duration. The aim of this research was to determine the effect of buparvaquone on the blood oxidative status, cardiac, hepatic and renal damage and bone marrow function markers.
***Materials, Methods & Results*:** In this study, ten adult (> 2 years) Akkaraman rams were used. Healthy rams were placed in paddocks, provided water *ad libitum*, and fed with appropriate rations during the experiment. Buparvaquone was administered at the dose of 2.5 mg/kg (IM) intramuscularly twice at 3-day intervals. Blood samples were obtained before (0. h, Control) and after drug administration at 0.25, 0.5, 1, 2, 3, 4 and 5 days. The blood samples were transferred to gel tubes, and the sera were removed (2000 *g*, 15 min). During the study, the heart rate, respiratory rate, and body temperature were measured at each sampling time. In addition, the animals were clinically observed. Plasma oxidative status markers (Malondialdehyde, total antioxidant status, catalase, glutathione peroxidase, superoxide dismutase), serum cardiac (Troponin I, creatine kinase-MBmass, lactate dehydrogenase), hepatic (Alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase, total protein, albumin, globulin) and renal (Creatinine, blood urea nitrogen) damage markers and hemogram values (white blood cell, red blood cell, platelet, hemogram, hematocrit) were measured. Buparvaquone caused statistically significantly ($P < 0.05$) increases in the troponin I and blood urea nitrogen levels and fluctuations in alkaline phosphatase activity, but there was no any statistically significance difference determined in the other parameters.
***Discussion*:** In this study, buparvaquone was administered two times at a dose of 2.5 mg/kg (IM) at 3-day intervals. Although the result was not statistically significant ($P > 0.05$), it was determined that buparvaquone gradually increased the levels of the main oxidative stress marker, MDA, by approximately 2.8 fold. CAT and GPX levels were also found to have decreased by 2.2 fold. Buparvaquone may cause lipid peroxidation by producing free radicals. Some other antiprotozoal drugs may affect the oxidative status and may increase MDA level and decrease SOD level. In this study, MDA, which is an indicator of lipid peroxidation *in vivo*, was used to partially detect developing lipid peroxidation. Changes in the levels of reduced GPX and CAT enzymes could be attributed to their use in mediating the hydrogen peroxide detoxification mechanisms. The absence of significant changes in the TAS levels in this study suggests that buparvaquone may partially induce oxidative stress by producing hydrogen peroxide, but no significant changes occurred in the oxidative stress level because of the high antioxidant capacity of sheep. In this study, buparvaquone caused a statistically significant increase ($P < 0.05$) in the level of Tn-I, which is a marker of specific cardiac damage ($P < 0.05$), whereas there was no statistically ($P > 0.05$) significant increase in CK-MBmass. Tn-I and CK-MB levels, which are used to define heart damage in humans, have been successfully used to determine heart damage in sheep. In this research study, the statistically significant increases in Tn-I but not CK-MBmass levels could be considered indicative of mild cardiac damage.

Keywords: ram, buparvaquone, safety.

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INTRODUCTION

Theileriosis is a tick-borne disease caused by *Theileria* species. Ovine theileriosis is an important parasitic disease, especially in tropical and subtropical regions [27]. Buparvaquone is the most important drug used in the treatment of theileriosis [13,27]. Although some studies have shown that buparvaquone does not have carcinogenic, mutagenic, or teratogenic effects [17], no directly safety studies have been reported in the animals.

Antiprotozoal drugs may affect the oxidative status [5]. Malondialdehyde (MDA) is the main biological marker of lipid peroxidation in oxidative stress [6,12,20,24]. Reactive oxygen species (ROS) are targeted for inactivation by antioxidants [glutathione peroxidase (GPX), catalase(CAT), superoxide dismutase (SOD)] in living cells [19,30]. The total antioxidant status (TAS) measured in biological materials describes the total activity of antioxidant substances against free radicals *in vivo* [10,25].

Drugs used at the prescribed dose and duration of treatment may cause side effects [3]. Troponin I (TnI), creatine kinase-MB (CK-MB) mass, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels are used to determine cardiac damage [9,15,29]. The alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), AST and proteins levels are measured as indicators of liver damage, while alkaline phosphatase (ALP) activity is used to determine bile duct damage. Blood urea nitrogen (BUN) and creatinine concentrations are affected by changes in renal function [14,28].

In this study it was aimed to determine the effect of buparvaquone on parameters of blood oxidative status, the heart, liver, kidney, and bone marrow function.

MATERIALS AND METHODS

Animals and experimental procedure

In this study, ten adult (> 2 years) Akkaraman rams were used. Healthy rams were placed in paddocks, provided water *ad libitum*, and fed with appropriate rations during the experiment. Buparvaquone was administered at a dose of 2.5 mg/kg intramuscularly twice at 3-day intervals. Blood samples were obtained before (0 h, control) and 0.25, 0.5, 1, 2, 3, 4, and 5 days after drug administration. The blood samples were transferred to gel tubes, and the sera were removed (2000 *g*, 15 min). During the study, the heart rate, respiratory rate, and body temperature were measured at each sampling time. In addition, the animals were clinically observed.

Measurements

MDA (MDA-586 Kit)[1], TAS (Total Antioxidant Status Kit)[2], SOD (Superoxidase Dismutase Assay Kit)[3], GPX (Glutathione Peroxidase Assay Kit)[3] and CAT (Catalase Assay Kit)[3] were determined using ELISA reader (MWGt Lambda Scan 200)[4] according to the kit procedure. The serum CK-MB and Tn-I levels were measured using a chemiluminescent immunoassay (Advia Centaur XP)[5] while LDH, ALP, ALT, AST, GGT, total protein, albumin, globulin, BUN, and creatinine concentrations were measured using an autoanalyzer (ILAB-300)[6]. Hemogram tests (white blood cell, red blood cell, platelet, hemoglobin, hematocrit) of heparinized blood samples were performed using a blood cell counting device (BC-2800 Auto Hematology)[7].

Statistical analysis

The results of the study were presented as mean ± SE. Data were assessed by ANOVA and post hoc Tukey test (SPSS 22.0)8. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS

No negative clinical observations were detected in the animals during the study. The effects of buparvaquone on plasma oxidative status parameters are presented in Figure 1 and Table 1, and the effects on organ damage markers are presented in Table 2. The effects of Tn-I and CK-MB mass levels are shown in Figures 2 and 3. Buparvaquone caused a statistically significant ($P > 0.05$) increase in Tn-I and BUN levels and fluctuations in ALP activity. The effect of buparvaquone on vital (pulse, respiration rate, body temperature) and hemogram parameters is presented in Table 3. Buparvaquone caused statistically significant fluctuations in body heat and heart rate ($P < 0.05$).

Table 1. Effect of buparvaquone on the oxidative status parameters (mean ± SE).

Parameter	Day 0	Day 0.25	Day 0.5	Day 1	Day 2	Day 3	Day 4	Day 5
TAS (mmol/L)	0.63 ± 0.08	0.86 ± 0.09	0.95 ± 0.11	0.94 ± 0.06	0.99 ± 0.10	0.95 ± 0.14	0.97 ± 0.08	0.82 ± 0.08
SOD (U/mL)	0.034 ± 0.01	0.035 ± 0.01	0.032 ± 0.01	0.031 ± 0.01	0.027 ± 0.01	0.027 ± 0.01	0.025 ± 0.01	0.024 ± 0.01
CAT (nmol/min/mL)	134.00 ± 31.68	86.45 ± 12.46	84.45 ± 16.07	143.90 ± 35.64	90.22 ± 13.56	94.48 ± 43.98	92.72 ± 24.61	60.09 ± 12.93
GPX (nmol/min/mL)	266.53 ± 23.75	241.19 ± 4.50	249.47 ± 16.17	240.55 ± 15.18	251.25 ± 15.01	267.80 ± 21.57	267.55 ± 19.30	337.58 ± 54.64

TAS: total antioxidant status; SOD: superoxide dismutase; CAT: catalase, GPX: glutathione peroxidase. There was no any statistically significance determined in the same line ($P > 0.05$).

Table 2. Effect of buparvaquone on the heart, liver and kidney functions parameters (mean ± SE).

Parameter	Day 0	Day 0.25	Day 0.5	Day 1	Days 2	Days 3	Days 4	Days 5
LDH (U/L)	503.20 ± 40.61	468.30 ± 31.53	469.20 ± 35.24	525.10 ± 36.09^{a}	513.10 ± 35.41	497.30 ± 38.33	541.20 ± 29.26	556.50 ± 25.26
ALP (U/L)	45.50 ± 8.07^{b}	60.90 ± 8.97^{ab}	57.90 ± 8.33^{ab}	62.00 ± 6.25^{ab}	82.30 ± 7.14^{a}	69.10 ± 7.69^{ab}	72.60 ± 9.07^{ab}	63.80 ± 7.86^{ab}
AST (U/L)	108.50 ± 11.8	101.70 ± 10.50	102.20 ± 7.18	112.00 ± 6.67	105.30 ± 4.29	88.70 ± 4.05	96.80 ± 4.01	93.00 ± 4.09
ALT (U/L)	22.40 ± 2.23	22.40 ± 1.86	23.20 ± 1.98	25.10 ± 2.07	22.90 ± 1.87	21.30 ± 1.60	23.30 ± 1.78	22.50 ± 1.73
GGT (U/L)	67.70 ± 4.10	66.80 ± 3.92	66.00 ± 3.67	68.40 ± 3.57	64.60 ± 3.60	65.10 ± 4.06	67.70 ± 4.02	65.10 ± 3.78
TP (g/dL)	8.00 ± 0.23	7.65 ± 0.26	7.59 ± 0.20	8.00 ± 0.20	8.24 ± 0.19	7.66 ± 0.30	8.02 ± 0.17	8.02 ± 0.20
ALB (g/dL)	2.19 ± 0.07	2.09 ± 0.06	2.06 ± 0.06	2.16 ± 0.04	2.12 ± 0.03	2.00 ± 0.04	2.10 ± 0.05	2.09 ± 0.05
GLB (g/dL)	5.81 ± 0.23	5.56 ± 0.25	5.53 ± 0.19	5.84 ± 0.22	6.12 ± 0.21	5.61 ± 0.31	6.02 ± 0.13	5.93 ± 0.22
CR (mg/dL)	0.73 ± 0.05	0.74 ± 0.05	0.74 ± 0.04	0.79 ± 0.04	0.72 ± 0.04	0.70 ± 0.03	0.75 ± 0.04	0.81 ± 0.05
BUN (mg/dL)	11.24 ± 0.67^{c}	11.53 ± 0.45^{bc}	10.88 ± 0.84^{c}	14.06 ± 0.91^{abc}	13.82 ± 1.07^{abc}	13.07 ± 0.98^{abc}	15.32 ± 0.92^{ab}	16.69 ± 1.14^{a}

LDH: lactate dehydrogenase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma glutamyltransferase; TP: total protein; ALB: albumin; GLB: globulin; CR: creatinine; BUN: blood urea nitrogen. Different letters in the same line (a, b, c) are statistically significant ($P < 0.05$).

Table 3. Effect of buparvaquone on the hemogram parameters (mean ± SE).

Parameter	Day 0	Day 0.25	Day 0.5	Day 1	Days 2	Days 3	Days 4	Days 5
BT (°C)	39.61 ± 0.12^{ab}	39.81 ± 0.21^{a}	39.67 ± 0.16^{ab}	39.24 ± 0.16^{ab}	39.28 ± 0.14^{ab}	39.12 ± 0.11^{b}	39.09 ± 0.13^{b}	39.26 ± 0.11^{ab}
Pulse (pcs/min)	87.60 ± 7.16^{ab}	114.80 ± 8.58^{ab}	100.80 ± 6.44^{ab}	82.40 ± 8.43^{b}	97.60 ± 4.66^{ab}	95.60 ± 4.67^{ab}	96.00 ± 8.06^{ab}	89.40 ± 6.03^{a}
RR (pcs/min)	113.60 ± 10.36	86.80 ± 8.60	80.40 ± 8.29	76.00 ± 6.33	93.20 ± 9.83	103.60 ± 13.21	94.40 ± 5.84	91.60 ± 6.06
WBC (10^9/L)	10.66 ± 1.17	9.11 ± 1.15	10.27 ± 1.03	10.73 ± 1.33	10.03 ± 0.99	9.77 ± 1.07	8.99 ± 1.02	8.64 ± 0.92
Lymphocyte (10^3µL)	3.26 ± 0.34	2.78 ± 0.43	3.13 ± 0.36	3.29 ± 0.34	3.35 ± 0.37	3.32 ± 0.37	3.38 ± 0.35	6.00 ± 2.7
Monocyte (10^3/µL)	1.16 ± 0.19	0.83 ± 0.12	1.10 ± 0.15	1.02 ± 0.17	0.77 ± 0.07	0.95 ± 0.15	0.72 ± 0.06	0.79 ± 0.07
Granulocyte (10^3/µL)	6.24 ± 0.79	5.50 ± 0.77	6.54 ± 1.12	6.42 ± 1.06	5.91 ± 0.71	5.50 ± 0.72	4.89 ± 0.76	4.55 ± 0.66
RBC (10^{12}/L)	10.17 ± 0.57	9.50 ± 0.55	9.57 ± 0.69	9.92 ± 0.62	9.64 ± 0.62	9.63 ± 0.63	10.06 ± 0.64	10.03 ± 0.59
MCV (fl)	33.41 ± 1.64	32.3 ± 2.37	33.78 ± 1.60	34.08 ± 1.59	34.01 ± 1.61	33.88 ± 1.54	33.31 ± 1.52	33.31 ± 1.63
MCH (pg)	10.32 ± 0.19	10.09 ± 0.20	10.32 ± 0.21	10.21 ± 0.22	10.02 ± 0.18	10.07 ± 0.21	9.89 ± 0.19	9.63 ± 0.16
MCHC (g/dL)	31.37 ± 0.88	30.42 ± 0.78	30.96 ± 0.76	30.37 ± 0.73	29.94 ± 0.82	30.15 ± 0.80	30.43 ± 0.82	29.57 ± 0.79
PLT (10^3/µL)	477.70 ± 23.28	425.90 ± 24.53	446.00 ± 30.82	487.00 ± 33.83	473.50 ± 31.18	488.00 ± 28.67	467.70 ± 27.27	471.10 ± 35.91

BT: body temperature; RR: respiration rate; WBC: white blood cell; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: Platelet count. Different letters in the same line (a, b) are statistically significant ($P < 0.05$).

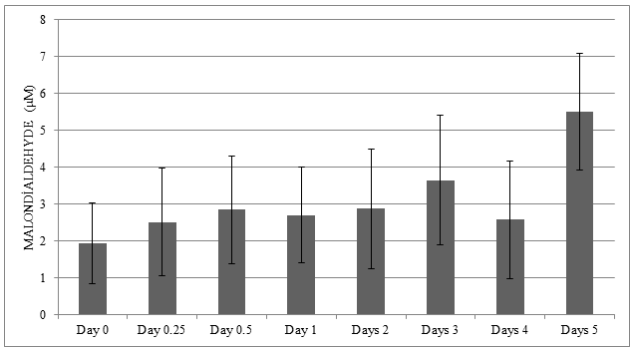

Figure 1. Effect of buparvoquone on the serum plasma malondialdehyde (µM) levels (mean ± SE, $P > 0.05$).

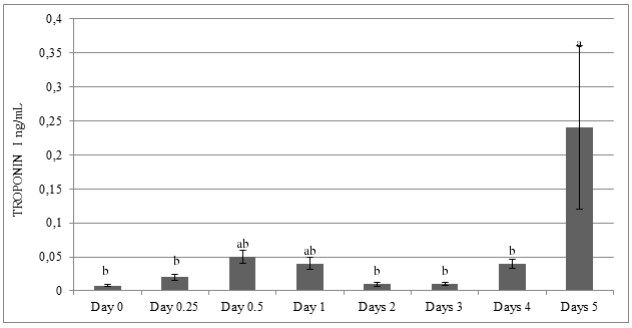

Figure 2. Effect of buparvoquone on the serum troponin I (ng/mL) levels (mean ± SE, $P < 0.05$). [a,b]Within groups statistical significant.

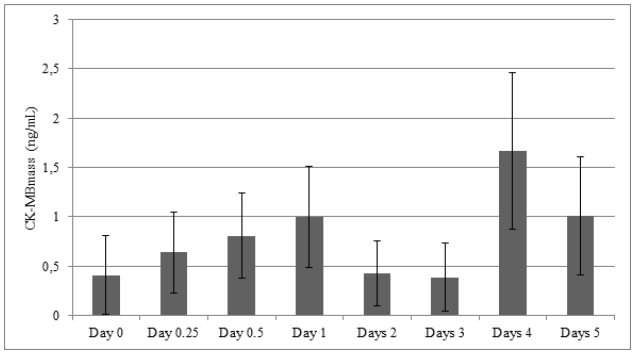

Figure 3. Effect of buparvoquone on the serum CK-MBmass (ng/mL) levels (mean ± SE, $P > 0.05$).

DISCUSSION

Buparvaquone is one of the most frequently used drugs in theileriosis treatment in the animals. Although no carcinogenic, mutagenic, and teratogenic effects have been observed with this drug [17] and the standard dose is accepted as safe, the European Medical Agency and the US Food and Drug Administration do not provide detailed safety information in the animals. The use of buparvaquone is recommended as an extra-label treatment for theileriosis in sheep [31].

In this study, buparvaquone was administered two times at a dose of 2.5 mg/kg (IM) at 3-day intervals. Although the result was not statistically significant ($P > 0.05$), it was determined that buparvaquone gradually increased the levels of the main oxidative stress marker, MDA, by approximately 2.8 fold (Figure 1). CAT and GPX levels were also found to have decreased by 2.2 fold (Table 1). It has been reported that buparvaquone may cause lipid peroxidation by producing free radicals [18]. Some other antiprotozoal drugs have been reported to affect the oxidative status [5] and may increase MDA level [1,11,16] and decrease SOD level [5]. In this study, MDA (Figure 1), which is an indicator of lipid peroxidation *in vivo* [24], was used to partially detect developing lipid peroxidation. Changes in the levels of reduced GPX and CAT enzymes (Table 1) could be attributed to their use in mediating the hydrogen peroxide detoxification mechanisms [26,30]. The absence of significant changes in the TAS levels in this study (Table 1) suggests that buparvaquone may partially induce oxidative stress by producing hydrogen peroxide, but no significant changes occurred in the oxidative stress level because of the high antioxidant capacity of sheep.

In this study, buparvaquone caused a statistically significant increase ($P < 0.05$) in the level of Tn-I (Figure 2), which is a marker of specific cardiac damage ($P < 0.05$), whereas there was no statistically ($P > 0.05$) significant increase in CK-MBmass. It has been determined that antiprotozoal drugs may increase Tn-I and CK-MB levels [4]. Tn-I, a troponin subtype, is the most specific marker used clinically to diagnose heart damage [21] and increases in circulating levels are observed within 4-6 h after damage [9,15,29]. The CK-MB level peaks within 24 h after damage and falls to normal levels within 3 days. TnI and CK-MB levels increase simultaneously in heart damage and are used as primer markers in the diagnosis of acute myocardial damage [9]. Tn-I and CK-MB levels, which are used to define heart damage in humans, have been reported to be successfully used to determine heart damage in sheep. Furthermore, experimental studies conducted in sheep determined that the heart failure markers (Troponin I, CK-MB) increased in the first 6 h after administration [4,7]. In this research study, the statistically significant increases in Tn-I but not CK-MBmass levels could be considered indicative of mild cardiac damage. Furthermore, we determined that buparvaquone caused statistically ($P < 0.05$) significant changes in the reference values of the BUN, ALP (Table 2), pulse, and body temperature (Table 3) [2,22,23]. It has been reported that antiprotozoal drugs cause changes in the cardiac, liver, renal function, and hemogram parameters in sheep compared to the reference values [4,5,8].

CONCLUSIONS

The present result suggests that the administration of buparvaquone at the prescribed doses and duration in rams does not have significant adverse effects on the oxidative status, heart, liver, and kidney damage markers as well as on hemogram parameters. However, we propose that it is necessary to pay attention to potential heart and kidney damage, especially in seriously diseased animals or when used prolonged usage.

MANUFACTURERS

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***Ethical approval*.** The study protocol was approved by Ethical Committee of Faculty of Veterinary Medicine, Selcuk University, Turkey (No.2017/45).

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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