



Effect of Halofuginone on Blood Thiobarbituric Acid Reactive Substances, Testosterone and 13,14-dihydro-15-keto-Prostaglandin F2 α Levels in Male Yearling Sheep

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The primary aim of this study was to evaluate the effect of halofuginone on serum of thiobarbituric acid reactive substances (TBARS) and plasma testosterone, and 13,14-dihydro-15-keto-prostaglandinF2 α (PGM) levels in male yearling sheep. It was also evaluated the effects of halofuginone on routine blood biochemistry and hemogram values.

Methodology: Ten male yearling sheep were treated at a dose of 0.1 mg/kg of halofuginone (PO, SID) for 15 days. Blood samples were collected before treatment (day 0, control) and on treatment days 5, 10, and 15. Hemogram parameters and the levels of TBARS, PGM, troponin I, creatinine kinase-MB, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ -

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glutamyl transferase, total protein, blood urea nitrogen, creatinine, and calcium were determined.

Results: No statistically significant differences ($P>0.05$) in the levels of TBARS, testosterone or PGM were observed. The erythrocyte count, hematocrit, hemoglobin, total protein and calcium levels were lower ($P<0.05$) following the halofuginone treatment, whereas levels of troponin I, aspartate aminotransferase and alanine aminotransferase were higher ($P<0.05$), compared with the control values.

Conclusion: Halofuginone might cause anemia and organ damage, whereas it has no significant effect on indicators of fertility, oxidative status, or inflammation at the dosage and duration of treatment in male yearling sheep.

Keywords: Halofuginone; safety; male yearling sheep.

1. INTRODUCTION

The plant alkaloid, 7-bromo-6-chloro-3-(3-[3-hydroxy-2-piperidinyl]-2-oxopropyl)-4(3H)-quinazolinone, otherwise known as halofuginone, has been isolated from *Dichroa febrifuga*, and has also been produced synthetically [1]. It has been reported that halofuginone can be effective in the treatment of malaria and theileriosis [2,3]. Previous studies have also shown that it prevents collagen synthesis in the treatment of fibrosis and scar tissue development, and that it might prevent tumorigenesis and metastases of some cancers [1,4,5].

Halofuginone has been used for many years in veterinary medicine. The lactate form has been approved for the treatment of cryptosporidiosis in calves, and the hydrobromide form has been approved for the treatment of coccidiosis in poultry [6]. Cryptosporidiosis is a protozoan infection that causes diarrhea. It can be fatal in calves, and causes severe symptoms in cats, dogs, lambs, and goats [7,8,9,10]. Although the use of halofuginone has not been approved by the European Medicines Agency [6] for such purposes, studies have described the use of 0.1 mg/kg of halofuginone orally for 7 days for the treatment of cryptosporidiosis in goats and lambs [11,12].

Free radicals are unstable, short-lived atoms or molecules. In living cells, free oxygen radicals, such as superoxide and hydroxyl radicals etc, are continuously produced. Both intracellular and extracellular free oxygen radicals are harmful to cells. Antioxidant substances inactivate free oxygen radicals, thus preventing such harmful effects. However, when the oxidant-antioxidant balance is disrupted in cells or tissues, lipid peroxidation initiates. In the lipid peroxidation, the products of the oxidative degradation of fatty acids, such as thiobarbituric acid-reactive substances (TBARS), accumulate in the blood

and tissues, high levels of which are an indicator of oxidative damage [13,14,15]. Halofuginone has demonstrated antioxidant effects in previous study [16].

The male sex hormone, testosterone, is synthesized in the Leyding cells. Testosterone becomes active when it is transformed to dihydrotestosterone by the 5α -reductase enzyme. The hormone exerts androgenic effects on the genitals, and stimulates anabolic processes in skeletal muscle and bone [17]. Previous studies have shown that long-term halofuginone use can cause testicular atrophy and reduce fertility [6].

The synthesis of prostaglandins F, E, and G from arachidonic acid is mediated by cyclooxygenase. The various classes of prostaglandins exhibit a wide range of effects in vivo. Non-steroidal anti-inflammatory drugs exert their analgesic, antipyretic, and anti-inflammatory effects primarily through the inhibition of prostaglandin synthesis [18]. Previous studies have shown that the plasma level of 13,14-dihydro-15-keto-prostaglandin F 2α (PGM), a metabolite of prostaglandin F 2α , increases in inflammatory states, and is an indicator for the treatment of inflammation [19,20]. Halofuginone has been reported to may exhibit anti-inflammatory effects [21].

The long-term or high-dose use of certain drugs can result in adverse effects that cause abnormal variation in hematological and blood biochemistry parameters that resemble various pathological conditions. Drug-related changes in hematological values can be indicators of bone marrow disorders, and can also be caused by fluid-electrolyte imbalances, certain chronic diseases, or infections. Anemia may be caused by hemorrhage, hemolysis, or hypoplasia/aplasia. The main function of hemoglobin in red blood cells (RBCs) is to carry

oxygen to tissues. The hematocrit value is a measure of the total relative number of RBCs in the blood [22,23,24].

In recent years, level of cardiac troponin I (cTn), which increases following cardiac cell necrosis, has been used for the diagnosis of acute myocardial damage. The level of cTn is considered to be a more specific indicator of cardiac damage than creatinine kinase-MB (CK-MB) [25]. The level of alkaline phosphatase (ALP) increases in bile duct disorders, and those of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ -glutamyltransferase (GGT) increase as a result of liver damage, whereas the level of total protein decreases in liver failure. Non-ionized calcium in the blood binds to albumin, which results in an apparent reduction in calcium when levels of albumin are reduced. Increased blood urea nitrogen (BUN) and creatinine values are considered to be indicators of renal failure [26,27].

In addition to the antioxidant and anti-inflammatory effects of halofuginone [16,21], the general side and adverse effects, including testicular atrophy [6] should be considered; it has been hypothesized that use of halofuginone in male yearling sheep may affect hemogram parameters, organ damage markers, and the levels of testosterone, PGM, and TBARS.

The aim of this study was to determine the effects of halofuginone in male yearling sheep when administered orally at the dosage recommended for lambs and goats for twice the recommended duration of treatment.

2. METODOLOGY

This study was approved by the Ethics Committee at Selçuk University Veterinary Faculty (2012-077). Ten male yearling Akkaraman sheep (45-56 kg, <2 years, Bahri Dagdas International Agricultural Research Institute, Konya, Turkey) were given 0.1 mg/kg of halofuginone (Halocur[®] Oral Solution, Intervet, Istanbul, Turkey) orally once daily for 15 days. Blood samples were collected before treatment (day 0, control) and on treatment days 5, 10, and 15. Serum TBARS (Cayman Chemical, Ann Arbor, MI, USA) and plasma testosterone (Eastbiopharm, Hangzhou, China) and PGM (Cayman Chemical, Ann Arbor, MI, USA) levels were measured by ELISA plate reader (MWGt Lambda Scan 200, Bio-Tek Instruments,

Winooski, VT, USA). Serum cTn levels were determined with the immunoassay method (Siemens Advia Centaur XP, Ireland). The hematocrit, the hemoglobin concentration, and the white blood cell (WBC), RBC, and platelet counts were measured using hemacell counter (BC-2800 Auto Hematology Analyzer, Mindray Bio-Medical Electronics, Shenzhen, China). The plasma CK-MB and serum ALP, ALT, AST, GGT, total protein, BUN, creatinine, and calcium levels were determined with autoanalyzer (ILab-300 plus, Instrumentation Laboratory, Milano, Italy).

The differences between the data collected before and during treatment were evaluated using an analysis of variance (ANOVA) and a Scheffe posthoc test (SPSS, version 19.0, IBM, Armonk, NY, USA), and $P < 0.05$ was accepted as the level of statistical significance.

3. RESULTS

No differences in clinical features were observed in the animals following the initiation of the halofuginone treatment. The serum TBARS, and plasma testosterone and PGM levels before and during halofuginone treatment were not significantly different ($P > 0.05$; Graphics 1, 2 and 3). The results of the hemogram and routine blood biochemistry analyses are presented in Tables 1 and 2, respectively. Following halofuginone treatment, RBC count, hemoglobin concentration, hematocrit, and serum levels of total protein and calcium decreased ($P < 0.05$), and the serum levels of cTn, AST, and ALT increased ($P < 0.05$), compared to the pre-treatment values for those parameters (Tables 1 and 2). The serum level of creatinine significantly decreased ($P < 0.05$) on day 15 of halofuginone treatment, compared to the control and day 10 levels (Table 2).

4. DISCUSSION

Halofuginone has been approved for the treatment of cryptosporidiosis in calves and coccidiosis in poultry [6]. In addition, halofuginone may be used in the treatment of cryptosporidiosis in the lambs and kids [9,10]. Previous studies have shown that halofuginone might also be useful for the prevention of some types of tumors [5].

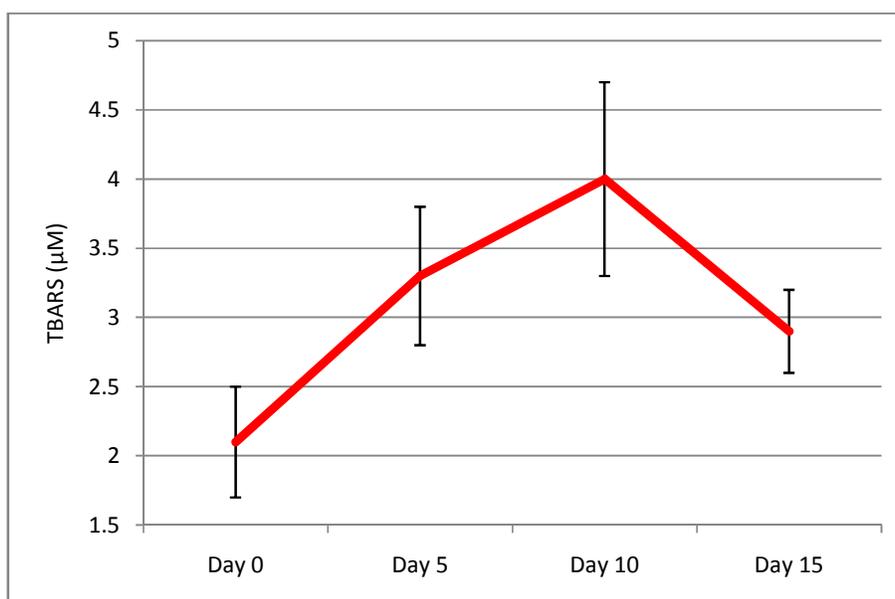
In the present study, no statistically significant difference ($P > 0.05$) in the level of TBARS, which are indicators of oxidative stress, was detected during halofuginone treatment, compared with the pre-treatment values (Graphic 1). Previous

studies have shown that halofuginone may prevent oxidative damage in some organs by reducing level of malondialdehyde, which is an indicator of oxidative stress [16,21,28]. In the current study, the parameters measured, the differences in dosage, and/or the duration of treatment might have been suboptimal for demonstrating the antioxidant effects of halofuginone in sheep.

It is also observed that halofuginone treatment had no significant effect ($P>0.05$) on the plasma level of testosterone (Graphic 2). However, previous studies have shown that halofuginone can cause testicular atrophy and decreases fertility [5,6]. The lack of an observable difference in testosterone levels in the present study may

have been due to the duration of halofuginone treatment [6] or interspecies differences in the effects of halofuginone.

Halofuginone and extracts of *Dichroa febrifuga* have been shown to exhibit anti-inflammatory effects [21,29]. Although a gradual decrease ($P>0.05$) in the plasma level of PGM, an indicator of inflammation, was detected during halofuginone treatment, no statistically significant change in the plasma level of PGM was observed during the treatment period in the current study (Graphic 3). However, the effect of halofuginone on the plasma level of PGM may be more pronounced in the presence of inflammation.



Graphic 1. Effect of halofuginone (0.1 mg/kg, PO, SID, 15 days) on serum thiobarbituric acid reactive substances (TBARS) levels (mean±SE)

Table 1. Effect of halofuginone (0.1 mg/kg, PO, SID, 15 days) on the hemogram parameters (mean±SE)

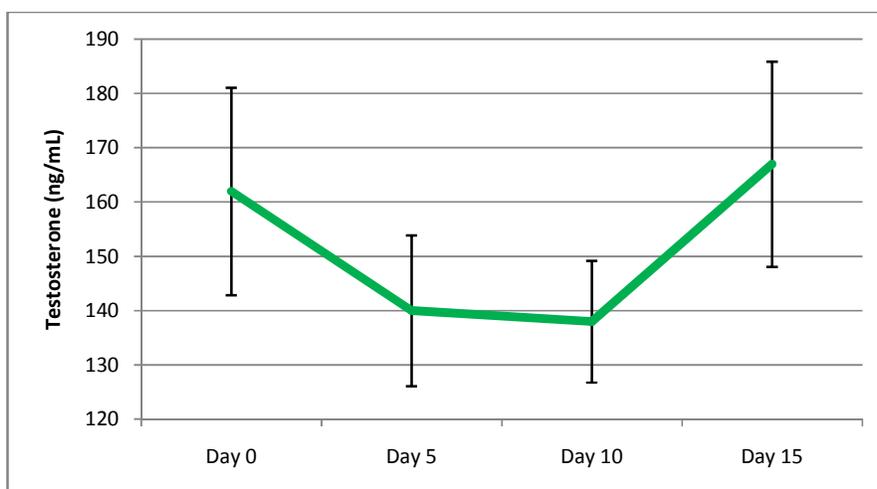
Parameters	Day 0	Day 5	Day 10	Day 15
WBC ($\times 10^9/L$)	9.40±0.54	9.24±0.60	8.46±0.64	8.66±0.42
RBC ($\times 10^{12}/L$)	10.3±0.21 ^a	8.36±0.35 ^b	8.46±0.43 ^b	8.11±0.23 ^b
Platelet ($\times 10^9/L$)	365±17.0	370±16.0	350±19.6	368±20.6
Hemoglobin (g/L)	95.5±1.24 ^a	80.9±2.90 ^b	83.1±2.94 ^b	82.2±2.76 ^b
Hematocrit (%)	33.6±0.54 ^a	26.2±0.76 ^b	27.2±0.95 ^b	26.8±0.81 ^b

WBC: White blood cell; RBC: Red blood cell, ^{a, b}: The different letters on the same line indicate statistically significant differences ($P<0.05$)

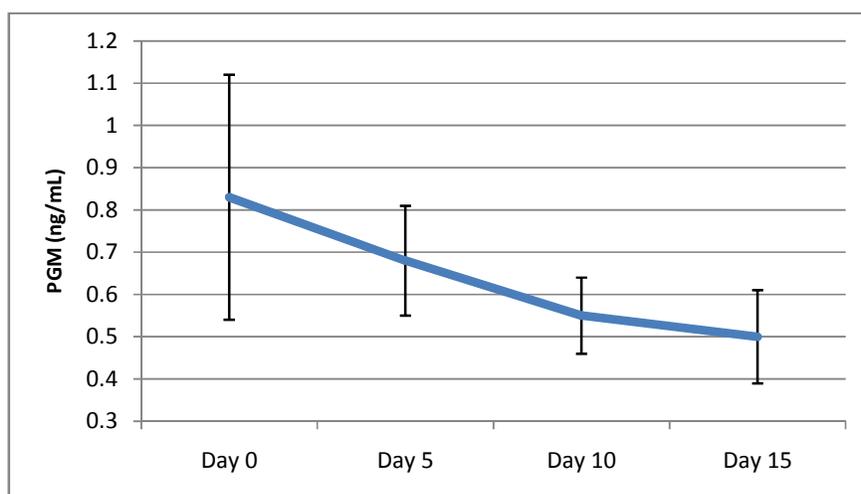
Table 2. Effect of halofuginone (0.1 mg/kg, PO, SID, 15 days) on routine blood biochemistry parameters (mean±SE)

Parameters	Day 0	Day 5	Day 10	Day 15
Troponin I (ng/mL)	0.06±0.02 ^c	0.29±0.09 ^{bc}	1.34±0.24 ^a	0.71±0.14 ^b
CK-MB (U/L)	204±11.9	178±12.3	189±9.14	161±9.10
ALP (U/L)	168±17.5	209±23.1	196±21.9	181±21.6
ALT (U/L)	15.3±1.26 ^c	17.6±1.48 ^c	32.1±2.10 ^b	46.0±4.07 ^a
AST (U/L)	86.7±3.58 ^c	90.8±3.29 ^c	163±8.34 ^d	206±13.6 ^a
GGT (U/L)	38.7±1.80	37.7±2.06	39.7±1.77	37.1±1.89
TP (g/dL)	6.80±0.17 ^a	6.29±0.22 ^{ab}	6.32±0.11 ^{ab}	5.85±0.23 ^b
Creatinine (mg/dL)	0.72±0.02 ^a	0.66±0.02 ^{ab}	0.70±0.02 ^a	0.57±0.03 ^b
BUN (mg/dL)	43.6±0.74	43.7±2.40	47.4±2.17	44.7±4.06
Calcium (mg/dL)	9.40±0.10 ^a	8.77±0.24 ^{ab}	8.62±0.10 ^b	8.31±0.15 ^b

CK-MB: Creatinine kinase-MB; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: γ -glutamyltransferase; TP: Total protein; BUN: Blood urea nitrogen. ^{a, b, c} The different letters on the same line indicate statistically significant differences ($P < 0.05$)



Graphic 2. Effect of halofuginone (0.1 mg/kg, PO, SID, 15 days) on plasma testosterone levels (mean±SE)



Graphic 3. Effect of halofuginone (0.1 mg/kg, PO, SID, 15 days) on plasma 13,14-dihydro-15-keto-prostaglandinF2 α (PGM) levels (mean±SE)

Halofuginone treatment reduced ($P<0.05$) the RBC count, the hemoglobin concentration, and the hematocrit in yearling sheep in the present study (Table 1). Halofuginone might reduce the hemoglobin level [6], and hemogram parameters are interrelated [23]; hence, a reduction in the RBC count should correlate with reductions in the hemoglobin concentration and the hematocrit. The development of anemia, reduced hematocrit and a reduction in the hemoglobin concentration can be the result of bone marrow depression. A previous study showed that halofuginone is a mutagen in poultry [5]. However, whether halofuginone causes hemolytic or bone marrow toxicity is unclear. Although no statistically significant differences ($P>0.05$) in the WBC count were observed in the current study, the observed decreases in other hemogram parameters (Table 1) indicate that the halofuginone treatment may had a depressive effect on the bone marrow in yearling sheep.

In the current study, treatment using halofuginone increased ($P<0.05$) the serum level of cTn, a specific indicator of cardiac damage that has been shown to increase following cardiac cell necrosis [25], throughout the study period (Table 2). Adverse cardiac effects of halofuginone treatment have not been reported. However, halofuginone has demonstrated anti-fibrotic and anti-angiogenic effects [30,31], effects on the heart should be considered, and it is possible that sheep may be more sensitive to such effects.

The serum levels of ALT and AST, biomarkers of liver damage [26], increased gradually in yearling sheep during halofuginone treatment, reaching a statistically significant difference ($P<0.05$) at days 10 and 15 (Table 2). Although previous studies revealed no adverse effects of halofuginone on the liver, hepatotoxicity has been reported for a wide range of drugs [32]. Certain drugs can cause elevations in the plasma levels of tissue damage indicators, even at the recommended dosage rates [22, 32]. The serum levels of total protein, an indicator of liver function [26], and calcium also decreased ($P<0.05$) on day 15 of this study (Table 2). Halofuginone has been reported to cause hypoproteinemia [5], and calcium is bound by serum albumin in the blood, reduced serum calcium correlates with hypoproteinemia [27,33]. The reduced levels of total protein and calcium observed in this study suggest that halofuginone might have had negative effects on the liver in yearling sheep. Thus, liver failure should be

considered as a potential adverse reaction in halofuginone treatment, especially with regard to long term use.

In this study, halofuginone treatment also reduced ($P<0.05$) the serum level of creatinine (Table 2), which is an indicator of renal damage. A previous study has shown that halofuginone increases the level of creatinine in the urine [5], and malnutrition-inflammation complex syndrome can cause hypoproteinemia [34]. Thus, the hypoproteinemia observed in the yearling sheep might have been the result of malnutrition caused by halofuginone and/or an increase in the excretion of creatinine in the urine.

5. CONCLUSION

In conclusion, the long-term or high-dose use of halofuginone might lead to anemia and adverse cardiac and hepatic effects in male yearling sheep. However, halofuginone has no notable effect on fertility, oxidative status, or inflammation at the dosage and duration of treatment used.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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