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RESEARCH ARTICLE

Effect of combined application of ivermectin and praziquantel on oxidative stress and selected biochemical parameters in sheep

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İvermektin ve prazikuantelin kombine uygulamasının koyunlarda oksidatif stres ve seçilmiş biyokimyasal parametreler üzerindeki etkisi

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Öz

Amaç: Bu çalışmanın amacı sağlıklı koyunlara oral yolla ivermektin + prazikuantel kombinasyonu uygulamasının oksidatif durum, karaciğer ve böbrek fonksiyon parametrelerine etkisini belirlemektir.

Gereç ve Yöntem: Araştırmada 18 adet Anadolu Merinosu ırkı koyuna önerilen dozda ivermektin + prazikuantel tablet (1 tablet/koyun) oral yolla uygulandı. Uygulamadan önce (0. gün) ve sonrasında 24 saat aralıklarla dört gün kan örnekleri alındı. Plazma 8-hidroksi-2-deoksiguanozin, malondialdehid, süperoksit dismutaz, glutasyon peroksidaz ve katalaz düzeyleri ticari kitler ile ELISA okuyucusunda belirlendi. Serum aspartat aminotransferaz, alanin aminotransferaz, alkalen fosfataz, kreatinin ve kan üre nitrojen düzeyleri otoanalizör ile ölçüldü.

Bulgular: Araştırmanın ilk 2 gün plazma 8-hidroksi-2-deoksiguanozin düzeyleri, 3. ve 4. gün değerlerinden yüksek olduğu belirlenirken ($p<0.05$), plazma glutasyon peroksidaz düzeylerinde ise istatistiki dalgalanmalar gözlendi ($p<0.05$). Araştırmanın 3. gün kan üre nitrojen değerinin 0. günden yüksek olduğu belirlendi ($p<0.05$).

Öneri: Sonuç olarak sağlıklı koyunlara önerilen dozda ivermektin + prazikuantel uygulamasının oksidatif strese neden olmadığı, karaciğer ve böbrek fonksiyon parametrelerine belirgin etkisinin olmadığı ifade edilebilir.

Anahtar kelimeler: İvermektin, prazikuantel, oksidatif stres, biyokimyasal parametreler, koyun

Abstract

Aim: Purpose of this research was to determine the combined effect of the oral administration of ivermectin and praziquantel on the oxidative status, liver and kidney function parameters in healthy sheep.

Materials and Methods: Ivermectin + praziquantel tablets at the recommended dose (1 tablet/sheep) were administered orally to 18 Anatolian Merino sheep, and blood samples were obtained before administration (day 0) and at 24-hour intervals for 4 days thereafter. Plasma 8-hydroxy-2-deoxyguanosine, malondialdehyde, superoxide dismutase, catalase, and glutathione peroxidase levels were measured with commercial kits using an ELISA reader. Serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and blood urea nitrogen levels were measured in an autoanalyzer.

Results: While the plasma 8-hydroxy-2-deoxyguanosine levels in the first 2 days were higher than the values on the 3rd and 4th days ($p<0.05$), statistical fluctuations were observed in the plasma glutathione peroxidase levels ($p<0.05$). An increased level of blood urea nitrogen was present on day 3 compared to day 0 ($p<0.05$).

Conclusion: It can be stated that the recommended dose of ivermectin + praziquantel does not cause oxidative stress and has no significant effect on their liver and kidney function parameters in healthy sheep.

Keywords: Ivermectin, praziquantel, oxidative stress, biochemical parameters, sheep

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Introduction

Ivermectin has been approved for use in horses, donkeys, cattle, sheep, and pigs as ectoparasitic and antinematodal purposes. Praziquantel is used in veterinary medicine against various cestode parasites in horses, sheep, dogs, and cats. Commercially available ivermectin (10 mg) plus praziquantel (250 mg) oral tablets are licensed for use against gastrointestinal helminths, liver flukes, nasal worms, scabies, lice, and ticks in sheep, and the general recommended dose of the combined drug is 1 tablet per sheep. Although the combination of the drugs shows activity against many endoparasites and ectoparasites in sheep and is generally well tolerated in the target animals, it can cause gastrointestinal side effects (Yazar 2018, Yazar 2022). Research on the safety of the combined use of ivermectin and praziquantel in the target species was not found in the literature, though studies have reported that the administration of avermecitin B1a causes oxidative stress in the liver of pigeons to increase MDA and decrease SOD and GPX levels (Zhu et al 2013). In addition, scholars have stated that ivermectin may lead to nephrotoxicity based on causing lipid peroxidation in experimental animals (Salman et al 2022). In another study, exposure of rainbow trout to different doses of eprinomectin led to a decrease in antioxidant enzyme (CAT, GPX, and SOD) activities and an increase in MDA levels in liver tissue (Alak et al 2017). Report has also indicated that emamectin causes oxidative stress in liver tissue and an increase in the level of 8-hydroxy-2-deoxyguanosine (8-OHDG) (Temiz 2020). In addition, the application of abamectin or emamectin to Oreochromis niloticus fish might cause elevations in plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (Firat and Tutus 2020). Furthermore, another report indicated that serum AST and blood urea nitrogen (BUN) levels did not change while ALT levels increased after the oral administration of praziquantel to hamsters (Zaparina et al 2021).

Living cells continuously yield free oxygen radicals (reactive oxygen species [ROS]) during metabolism (Yazar and Tras 2002). ROS are mostly produced from the mitochondria, plasma membrane, etc (Ayala et al 2014), and those generated in vivo are detoxified by some enzymes. Superoxide dismutase (SOD) converts the superoxide radical to hydrogen peroxide and oxygen; in the case of SOD deficiency, hydroxyl radical formation increases. The hydrogen peroxide formed by SOD is then detoxified and converted into water by catalase (CAT) or glutathione peroxidase (GPX) (Yazar and Tras 2002). Oxidative stress is defined as the presence of active oxygen species that exceed the available antioxidant buffering capacity, where ROS can damage biological molecules, such as lipids and DNA. In other words, oxidative stress can cause tissue damage due to the imbalance caused by the overproduction of oxidant compounds and insufficient antioxidant defense mechanisms (Czerska et al 2015). ROS can directly damage lipids, and lipid peroxidation leads to a wide variety of products, such as malondialdehyde (MDA), which is the most mutagenic (Ayala et al 2014) and one of the most frequently analyzed markers of oxidative stress (Czerska et al 2015). Exposure of living organisms to oxidative stress results in modifications to DNA; 8-hydroxy-2-deoxyguanosine (8-OHDG), which is produced during the guanine oxidation in degraded DNA, is considered the most important indicator of oxidative stress (Dabrowska and Wiczkowski 2017, Paredes-Sanchez et al 2018) and has been measured in both physiological fluids and cells (Dabrowska and Wiczkowski 2017). Factors that cause an increase in ROS production also led to an increase in 8-OHDG levels (Yokus and Cakir 2002).

Organ damage can occur in living organisms for various reasons and can be determined by the measurement of selected serum parameters. While serum AST, ALT and alkaline phosphatase (ALP) levels increase in the development of liver damage, serum BUN and creatinine levels increase in kidney damage (Turgut 2000).

In this study, considering the separate effects of macrocyclic lactones (Alak et al 2017, Abdel-Daim and Abdellatief 2018) and praziquantel (Zaparina et al 2021) on oxidative stress and/or serum biochemical parameters, we hypothesized that the administration of a combination of ivermectin and praziquantel to sheep might affect their oxidative status and liver and kidney function parameters.

Thus, this study aims to determine the effect of the combined oral administration of ivermectin and praziquantel on the oxidative status (8-OHDG, MDA, GPX, CAT, and SOD) and the liver (AST, ALT, and ALP) and kidney (creatinine and BUN) function parameters in healthy sheep.

Material and Methods

Totally 18 Anatolian Merino sheep (52-58 kg, 4-5 years) were used in the study and the recommended dose of ivermectin + praziquantel tablet (1 tablet/sheep, Dicromec tablet, Anadolu Ilac, Konya, Turkey) was administered orally to each sheep (Yazar 2018). Blood samples were taken before administration (day 0) and at 24-hour intervals for four days afterwards. After removing serum/plasmas from blood samples, plasma 8-OHDG (Sheep OHDG ELISA kit, BT-lab Bioassay Technology Laboratory, Shanghai, China), MDA (Sheep MDA ELISA kit, BT-lab), SOD (Sheep SOD ELISA kit, BT-lab), GPX (Sheep GPX ELISA kit, BT-lab), and CAT (Sheep CAT ELISA kit, BT-lab) levels were determined in an ELISA reader (MWGt Lambda Scan 200, Bio-Tec Instruments, Winooski, VT, USA) with commercial kits. Serum ALP, ALT, AST, BUN and creatinine levels were measured with autoanalyzer (BT 3000 plus, Rome, Italy).

Study results were presented as mean ± standard error. Data were evaluated with ANOVA and Tukey test (SPSS 22.0). p<0.05 level was accepted statistically significant.

Results

Plasma 8-OHDG, MDA, SOD, GPX and CAT levels are presented in Figure 1. While it was determined that plasma 8-OHDG levels on the 0th and 1st days of the study were higher than the 3rd and 4th day values (p<0.05), statistical fluctuations were observed in the plasma GPX levels (p<0.05). There was no statistical difference in MDA, SOD and CAT levels during the study (p>0.05).

Serum ALP, ALP, AST, BUN and creatinine levels are presented in Table 1. While it was determined that the BUN value was higher than the 0th day on the 3rd day of the study (p<0.05), there was no statistical difference in other values (ALP, ALT, AST, creatinine) during the trial (p>0.05).

Discussion

Commercially existing ivermectin (10 mg) plus praziquantel (250 mg) oral tablets are approved for use against most gastrointestinal helminths and ectoparasites in sheep. Combined drug is usually well tolerated in the target animals, but it can cause gastrointestinal side effects (Yazar 2018). Previous research on the effects of the combination of ivermectin and praziquantel on oxidative stress and selected organ (liver and kidney) function parameters in sheep was not identified in the literature. However, scholars have stated that the administration of an ivermectin + praziquantel oral paste to pregnant horses does not cause significant negative effects on the hemograms and routine biochemical parameters, and the combination can be considered safe to use during pregnancy (Mercier et al 2003).

In the current study, decreased plasma 8-OHDG levels were identified last 2 days after administration of the tablets

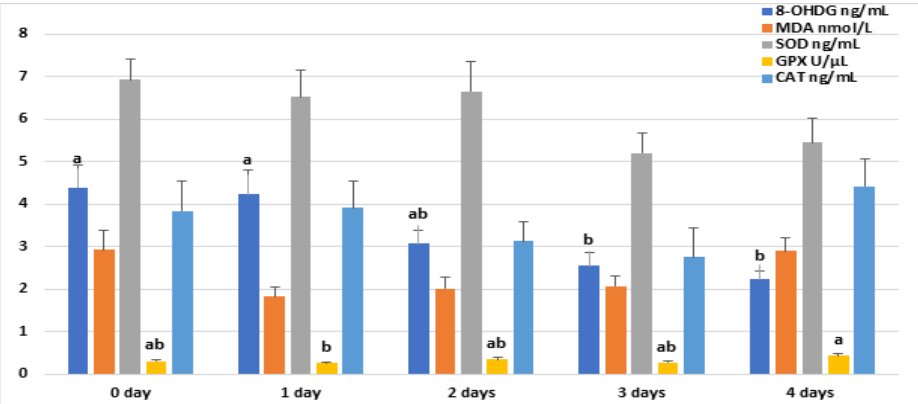

Figure 1. The effect of ivermectin+praziquantel combination on plasma oxidative status parameters in sheep

Table 1. Effect of ivermectin + praziquantel combination on serum biochemical parameters of sheep (mean ± standard error)

Parameters	0. day	1. day	2. days	3. days	4. days
ALP U/L	51.00±10.67	63.16±13.08	45.27±11.65	55.11±11.79	64.55±13.72
ALT U/L	48.61±9.75	57.00±11.86	63.94±9.93	65.61±13.73	61.05±14.11
AST U/L	76.66±4.81	91.16±4.08	86.94±6.27	92.55±4.64	93.83±4.77
BUN mg/dL	16.68±0.66^{c}	21.13±0.73^{ab}	21.92±0.73^{ab}	23.70±0.75^{a}	20.40±0.70^{b}
Creatinine mg/dL	1.25±0.05	1.32±0.05	1.36±0.04	1.71±0.27	1.23±0.04

$^{a, b, c}$: Different letters in the same column are statistically significant (p<0.05, tukey test)

(p<0.05, Figure 1); in reviewing the literature, no sources on the effect of the combination of ivermectin and praziquantel on 8-OHDG levels were found. Oxidative stress in living organisms leads to DNA modifications, and 8-OHDG is produced due to the resultant damage; thus, 8-OHDG levels measured in biological fluids or tissues are considered a biomarker of oxidative DNA damage (Di Minno et al 2016, Dabrowska and Wiczkowski 2017). Reports have indicated that the levels of 8-OHDG in brain (Temiz 2022) and liver (Temiz 2020) tissue increased after the oral administration of emamectin to mice. In addition, scholars have reported that the application of abamectin to Schizothorax prenanti fish led to an increase in the level of 8-OHDG in liver tissue (Hong et al 2020). In contrast, eprinomectin caused a decrease dose dependent manner in the 8-OHDG levels in the liver tissue of rainbow trout (Alak et al 2017). Considering the effect of the combination of ivermectin and praziquantel on 8-OHDG levels in this study, it can be stated that it has no damaging effect on DNA.

No statistical differences in the plasma MDA, SOD, and CAT levels were identified in this study (p>0.05, Figure 1), while statistical fluctuations were observed in plasma GPX levels (p<0.05, Figure 1). The literature review did not reveal any previous research on the effect of the combination of ivermectin and praziquantel on oxidative stress parameters. MDA produced from lipid peroxidation, due to oxidative stress, has been identified as the most mutagenic product formed (Ayala et al 2014) and is considered one of the most analyzed markers of oxidative stress (Czerska et al 2015). Defense against developing oxidative stress is provided by some antioxidant enzymes (Yazar and Tras 2002, Paredes-Sanchez et al 2018). Scholars have indicated that serum MDA levels did not change after ivermectin administration to rats (Omshi et al 2018). In another study, after the oral administration of different doses of avermecitin B1a to pigeons, the level of MDA in the liver tissue homogenate increased in a dose-dependent manner, though the levels of SOD and GPX decreased (Zhu et al 2013). Further studies have indicated that after the administration of emamectin to rats, the levels of brain tissue MDA increased (Madkour et al 2021, Noshy and Azouz 2021), while the levels of some antioxidant enzymes (CAT, SOD, and GPX) in brain tissue decreased (Madkour et al 2021, Noshy and Azouz 2021, Temiz 2022). Further, administering high oral doses of abamectin to rats increased liver and kidney tissue MDA levels while decreasing SOD, GPX, and CAT levels (Abdel-Daim and Abdellatief 2018). Scholars have also reported that after the application of abamectin to Schizothorax prenanti fish (Hong et al 2020) or eprinomectin to rainbow trout (Alak et al 2017), liver tissue SOD, GPX, and CAT levels decreased and MDA levels increased. One can state that the combination of ivermectin and praziquantel does not cause oxidative stress in sheep.

In the current study, no change was detected in the creatinine level, one of the kidney function parameters (p>0.05); on the other hand, the BUN value on day 3 was higher than on day 0 (Table 1) and was close to the reference values (8–20 mg/dL) determined for BUN in sheep (Bülbül 2013). Previous studies have indicated that after the application of ivermectin (Salman et al 2022), abamectin (Abdel-Daim and Abdellatief 2018, Salman et al 2022), and emamectin (Madkour et al 2021) to experimental animals, increases in serum kidney function parameters were observed. Researchers have also reported that serum urea levels did not change after the oral administration of praziquantel to hamsters (Zaparina et al 2021). In the current study, no statistical differences (p>0.05) were found in the liver function parameters (ALP, ALT, and AST; Table 1). Scholars have reported that the levels of urea, creatinine, ALP, ALT, and AST did not change after the oral administration of ivermectin to rats (Omshi et al 2018), while others have revealed increases in serum ALP, ALT, and AST levels after orally administering high doses of abamectin to rats (Abdel-Daim and Abdellatief 2018). In another study, while the serum AST level did not change after the oral administration of praziquantel to hamsters, the ALT level increased (Zaparina et al 2021). One can note that the differences in the findings of these research studies may depend on variations in the types of animals, drug types, or doses used. Considering the study's duration and measurements, one can state that the combined application of ivermectin and praziquantel to sheep does not negatively affect the liver and kidney functions.

Conclusion

In conclusion, the recommended dose of the combined application of ivermectin and praziquantel to sheep does not lead to DNA damage and oxidative stress and is safe for liver and kidney functioning.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

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