

A Comparative Study on the Effects of Eprinomectin and Ivermectin on Plasma Antioxidant Level and Lipid Peroxidation in Cows

Ali BILGILI * Asım KART ** Yılmaz CIGREMİS *** Basak HANEDAN **** Dincer ERDAG ***

* Department of Pharmacology and Toxicology, College of Veterinary Medicine, University of Ankara, 06110, Ankara - TURKEY

** Department of Pharmacology and Toxicology, College of Veterinary Medicine, University of Kafkas, 36100, Kars - TURKEY

*** Department of Biology, Faculty of Art and Science, University of Kafkas, 36100, Kars - TURKEY

**** Department of Internal Disease, College of Veterinary Medicine, University of Ankara, 06110, Ankara - TURKEY

Yayın Kodu (Article Code): 2009/038-A

Summary

Avermectins including Ivermectin and Eprinomectin are broad spectrum endectocides. These drugs are considered to have no serious adverse effects at therapeutic range. However, use of avermectins in overdose in some animal species and at the therapeutic range in humans could result in some adverse effects including mainly nervous system effects and some general symptoms. This study was aimed to investigate effects of Eprinomectin and Ivermectin on plasma malondialdehyde (MDA) and glutathione (GSH) levels in lactating cow. Ten Holstein breed lactating dairy cows were divided into 2 groups. Before the application of drugs, blood samples were collected from the jugular vein in each group at 0 hour as control, and then Group 1 and 2 received a single pour-on application of Ivermectin (0.5 mg/kg body weight) and Eprinomectin (0.5 mg/kg body weight), respectively. Blood samples were then collected again from the jugular vein at timed-intervals (at 1, 4, 8, 24 and 36 hours and on 2, 3, 4, 5, 6, 8, 10, 12, 15, 20 and 25th days) to measure plasma MDA and GSH concentrations. Eprinomectin and Ivermectin treatment caused a transient decrease in GSH levels which is followed by transient increase ($P<0.05$). However, no difference was observed in MDA levels at all sampling points following the Eprinomectin and Ivermectin treatment compared to control samples ($P>0.05$). It was concluded that Eprinomectin and Ivermectin cause decreased GSH concentrations without alterations in MDA level leading to a decrease in the defense mechanism against oxidative stress although the decrease is not enough to cause lipid peroxidation.

Keywords: *Eprinomectin, Ivermectin, MDA, GSH*

İneklerde Eprinomectin ve İvermektin'in Plazma Antioksidan ve Lipid Peroksidasyonu Üzerine Etkisinin Karşılaştırmalı Araştırılması

Özet

Avermektinlerden İvermektin ve Eprinomektin geniş spektrumlu endektositlerdir. Bu ilaçlar tedavi dozlarında ciddi yan etkileri olmayan ilaçlar olarak kabul edilir. Fakat avermektinler yüksek dozlarda hayvanlarda ve sağaltım dozunda insanlarda sinir sistemi ve genel semptomlarla karakterize bazı yan etkileri vardır. Bu çalışmada İvermektin ve Eprinomektin'in sağıldaki ineklerde plazma malondialdehid (MDA) ve glutatyon (GSH) seviyelerine etkisi araştırılmıştır. 10 adet Holştayn ırkı süt veren inek 2 gruba ayrıldı. Grup 1 ve grup 2 ye sırasıyla tek doz olarak dökme tarzında İvermektin (0.5 mg/kg vücut ağırlığı) ve Eprinomektin (0.5 mg/kg vücut ağırlığı) uygulandı. İlaç uygulamasından önce kan örnekleri 0. saatte kontrol uygulaması olarak vena jugularis'ten alındı. Kan örnekleri ilaç uygulamasını takiben belirli zaman aralıklarında (1, 4, 8, 24 ve 36. saatlerde ve 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, ve 25. günlerde) yine jugular ven'den toplandı. Eprinomektin ve İvermektin GSH seviyesinde geçici bir düşmeye ve takiben yine geçici bir artışa sebep oldu ($P<0.05$). Fakat MDA seviyelerinde herhangi bir değişikliğe rastlanmadı ($P>0.05$). Sonuç olarak, Eprinomektin ve İvermektinin plazma GSH seviyesinde geçici bir düşmeye sebep olarak antioksidan kapasitede bir düşüşe neden olabileceği fakat bu düşüşün lipid peroksidasyonuna yol açmayacak derecede olduğu sonucuna varıldı.

Anahtar sözcükler: *Eprinomektin, İvermektin, MDA, GSH*



İletişim (Correspondence)



+90 474 242 68 00/1126



asimkart@hotmail.com

INTRODUCTION

Ivermectin and Eprinomectin are widely used anthelmintic drugs belonging to avermectins of macrocyclic lactones. They are both effective in controlling internal and external parasites, and they are often called as endectocides due to their broad spectrum against endo- and ectoparasites including nematodes and arthropods ¹. Both are semi-synthetic derivatives of avermectin B1 produced by soil bacterium *Streptomyces avermilitis*. Eprinomectin (4"-epiacetyl-amino-4"-deoxy-ivermectin B1) is an Avermectin used as a topical endoectocide in cattle. In contrast to other members of Avermectins, Eprinomectin can be safely used in lactating cows due to the lowest residue level in milk which is below the allowed level of 30 ng/ml ¹⁻³.

The low milk residue of Eprinomectin was reported to be associated with physicochemical properties of Eprinomectin. Other than Eprinomectin, many other members of Avermectin family including Ivermectin have a milk to plasma ratio greater than or equal to 1. However, Eprinomectin has milk to plasma ratio lower than 0.2 ⁴. This is reported to be due to chemical structure of Eprinomectin where the unsaturated C bond at 20-22 and 4"-epi-acetyl-amino substituents in the structure confer the low milk to plasma partitioning by altering solubility and membrane interactions. On the other hand, Ivermectin are saturated at the C 20-22 and show equal or greater milk concentration than the plasma ².

Although mechanism of action of these compounds is not fully determined, avermectins including Ivermectin and Eprinomectin act on gamma-amino-butyric acid (GABA)-related and non-GABA related chloride channels in the central nervous system (CNS) of nematodes and insects. In mammals, it is known that GABA-mediated nerve transmission is confined to CNS. In addition, mammals have blood-brain barrier which limits the passage of avermectins to the CNS due to large molecular structures of these drugs. Therefore, mammals are resistant to the toxic effects of avermectins in normal therapeutic doses ^{1,5}. However, reports also indicate that avermectin toxicity may be seen following over dosage of avermectins. The toxic signs in response to avermectins are related to general indefinite

symptoms and also include nervous symptoms such as ataxia and tremors ^{6,7}.

Pesticides are responsible for an important part of mortality and morbidity among many drugs and chemicals. They could result in oxidative stress and generation of free radicals which play an important role in the toxicity. In addition, pesticides interfere with some antioxidant systems including glutathione system ^{8,9}. Use of Eprinomectin and Ivermectin for antiparasitic therapy is generally known to be safe and effective at the therapeutic range ¹. To our knowledge, however, their effects on the antioxidant mechanism and oxidative stress are unknown. Oxidative stress is a term defining the imbalance between prooxidant and antioxidant state in favor of prooxidants in the organism. Excessive generation of reactive oxygen species (ROS) or a decrease in antioxidant mechanism due to endogenous or exogenous sources could lead to the oxidative stress. Lipid peroxidation occurs as a result of oxidative damage leading to the breakdown of cellular membranes and other lipid containing structures ^{10,11}. In addition, ROS could interact with many cellular components of the cell including structural carbohydrates, enzymes, proteins and nucleic acids leading to pathological alterations and damage to these structures ¹⁰. Glutathione is the most important non-enzymatic antioxidant peptide playing some important roles in many cellular processes. In addition to free radical scavenging activity, GSH serves in detoxification of xenobiotics, regulation of signal transduction, cell proliferation, synthesis of deoxyribonucleotides, immune response, gene expression, apoptosis and leukotriene and prostaglandin metabolism ^{12,13}. Therefore, GSH deficiency could play an important role in the pathophysiology of many diseases.

In this study, it is aimed to investigate and monitor plasma levels of GSH and MDA following pour-on application of Ivermectin and Eprinomectin in lactating cows.

MATERIAL and METHODS

Ten clinically healthy Holstein breed lactating dairy cows were divided into 2 groups as group 1 (Ivermectin applied group) and group 2 (Eprinomectin applied group). All animals were kept under close watch for a month without giving

any drugs. During this period, animals were daily controlled and examined for any health problems, and no health problem was encountered. The animals were fed with alfalfa and pasture grass ad libitum and 8 kg of concentrate pelleted feed 2 times a day after milking. An approval was obtained from the institutional Ethics committee of Adnan Menderes University for the study. Eight ml of blood samples were collected in EDTA containing tubes. Before the application of drugs, blood samples were collected from the jugular vein at 0 hour. These blood samples served as control to compare the expected alteration in GSH and MDA levels after the application of the drugs on the following days. After obtaining the control samples, group 1 received single pour-on application of Ivermectin (Hektamec, Hektas, Kocaeli, Turkey, containing 5 mg/ml Ivermectin, pour-on solution at a dose of 0.5 mg/kg, 1 ml/kg) along the midline of the back. Similarly, Group 2 received pour-on administration of Eprinomectin (Eprinex®, Topkim, Istanbul, Turkey, pour-on solution containing 5 mg/ml Eprinomectin), along the midline of the back (at a dose of 0.5 mg/kg, 1 ml/10 kg body weight). Blood samples were then collected again from the jugular vein at 1, 4, 8, 24, 36 hours and on 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, and 25th days. The blood samples were centrifuged at 3000 rpm for 20 min to separate plasma. The samples were stored at -30°C until further analysis. The GSH

concentrations of plasma samples were determined according to Ellman ¹⁴. GSH reacts with 5.5 dithiobis-2-nitrobenzoic acid, and the product has a maximal absorbance at 410 nm. The results were expressed as $\mu\text{mol/L}$. The MDA levels of plasma samples were assayed spectrophotometrically at 535 and 520 nm according to Uchiyama and Mihara ¹⁵. A standard calibration curve was drawn by using 1.1.3.3-tetramethoxypropane. The results were expressed as $\mu\text{mol/L}$.

Statistical comparison of the data obtained at different sampling points within the individual groups was analyzed by repeated measurement ANOVA which is followed by Tukey's post hoc test using a computer program SPSS version 10.0 for Windows (SPSS Inc., Chicago, IL). P values less than 0.05 were considered significant. Data are presented as means \pm SD.

RESULTS

Plasma levels of GSH and MDA vs. time curve in group 1 and 2 were presented in Fig 1 and 2, respectively. Following the application of Eprinomectin to group 2, no change was observed in plasma GSH level for the first 24 hrs (at 1, 4, 8 and 24 hrs) compared to plasma samples obtained before Eprinomectin treatment (0 hour sample). However, plasma GSH levels of Eprinomectin-

Fig 1. Plasma GSH concentrations vs. Time profile in Eprinomectin- and Ivermectin treated groups following pour-on treatment in lactating cow (n=5). (*) Indicates significant difference at time points compared to 0 hour control in Eprinomectin treated group at $P<0.05$. (#) Indicates significant difference at time points compared to 0 hour control in Ivermectin treated group at $P<0.05$

Şekil 1. Dökme tarzı Eprinomektin ve İvermektin uygulaması sonrası süt veren ineklerde plazma GSH konsantrasyonu-zaman profili (n=5). (*) Eprinomektin uygulanan grupta 0. saat kontrole göre istatistiksel olarak anlamlı fark olduğunu göstermektedir, $P<0.05$. (#) İvermektin uygulanan grupta 0. saat kontrole göre istatistiksel olarak anlamlı fark olduğunu göstermektedir, $P<0.05$

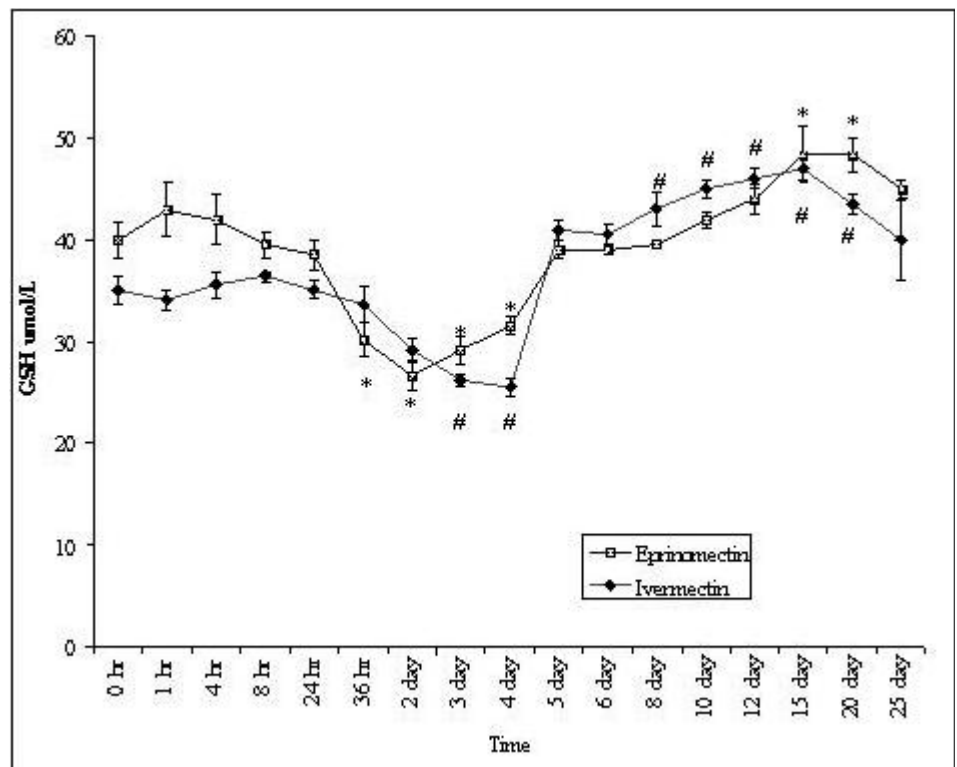
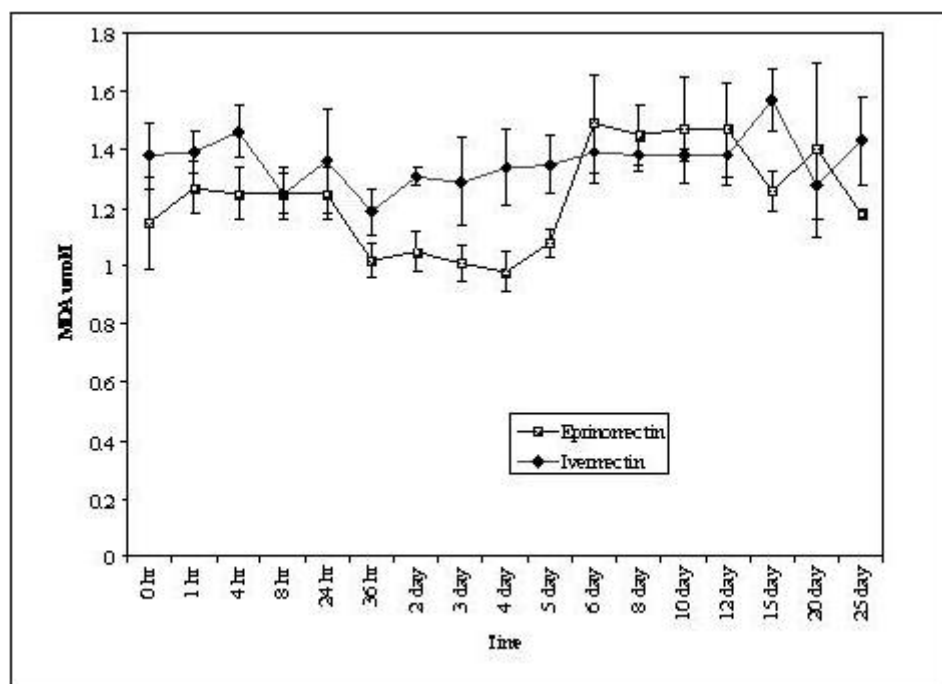


Fig 2. Plasma MDA concentrations vs. Time profile in Eprinomectin- and Ivermectin treated groups following pour-on treatment in lactating cow (n=5). No difference was observed in MDA levels at all sampling points following Eprinomectin and Ivermectin treatment compared to 0 hour control samples, at $P < 0.05$

Şekil 2. Dökme tarzı Eprinomektin ve İvermektin uygulaması sonrası süt veren ineklerde plazma MDA konsantrasyonu-zaman profili (n=5). Eprinomektin ve İvermektin uygulaması sonrası MDA seviyesinde 0. saat kontrole göre istatistiksel olarak anlamlı bir fark gözlenmedi, $P < 0.05$



treated group were significantly decreased at 36 hr ($P=0.004$), on day 2 ($P=0.000$), 3 ($P=0.001$) and 4 ($P=0.032$) compared to that of control obtained before the application of the Eprinomectin (0 hour sample). On day 5, 6, 8, 10 and 12, plasma GSH levels in group 1 were similar to that of control. However, there was an increase in GSH of Eprinomectin treated group on days 15 and 20. GSH decreased significantly ($P < 0.05$) in Ivermectin treated group on days 3 ($P=0.005$) and 4 ($P=0.014$) which is followed by an increase in GSH level on days 8, 10, 12, 15 and 20 after the drug application (Fig 1). No difference was observed in MDA levels in both groups at all sampling points compared to the blood samples obtained before the drugs administration ($P > 0.05$).

DISCUSSION

In the present study, cows received pour-on Eprinomectin and Ivermectin treatment at the therapeutic doses showed a transient reduction in plasma GSH levels which is followed by a transient increase, whereas no alteration was observed in plasma MDA concentration before and after the treatments at all sampling points. The reduced GSH at several time points in both Eprinomectin and Ivermectin treated groups suggest a decrease in non-enzymatic part of antioxidant defense system. Administration of experimentally very high

concentration of Ivermectin to the rats for LD₅₀ studies indicated that Ivermectin causes centrilobar necrosis in liver ¹⁶. In human, a single dose of 150 or 200 µg/kg of Ivermectin resulted in elevation in alanine amino transferase activity (ALT) and some hematological alterations including increased total leukocyte count and prolongation of prothrombin time ¹⁷. It was reported that acaricides including Ivermectin are able to inhibit recombinant glutathione S-transferase (GST) activity which catalyses conjugation of GSH with exogenous and endogenous molecules in the detoxification and metabolism ¹⁸. Avermectins at therapeutic level are safe in mammals. However, transient decrease in GSH level in the present study was observed as the adverse effect of these tested chemicals. Similarly, several pesticides were reported to decrease blood and lymphocyte GSH following exposure. The lymphocyte GSH concentration was reported to play important role in pesticide induced oxidative stress and immune function ⁹.

In addition, avermectin could alter humoral and cellular immune response. Sajid et al.¹⁹ reported that Ivermectin in rabbits at therapeutic doses caused immunostimulatory effects by increasing leucocyte count, macrophage engulfment percentage, plaque forming cell. Increase in cellular immune activity could be a source for the free radical generation ¹⁰ which could be counteracted by GSH against oxidative stress leading to utilization and

reduction of GSH from the available GSH pool.

Glutathione is mainly synthesized in the liver and released into blood ²⁰. Avermectins undergoes limited metabolism in the liver and the highest concentration found in the liver following administration ⁵. Eprinomectin and Ivermectin reach the peak plasma concentration approximately on day 2 following pour-on administration ^{2,4}. In the present study, following the drug application the decrease in GSH levels was observed at 36 h, on days 2 and 3 in Eprinomectin treated group and on days 3 and 4 in the Ivermectin treated group. The decrease in GSH level in both groups correlates well with the peak plasma level of Eprinomectin and Ivermectin in terms of the highest available effect. There was also a transient increase in GSH in both groups. The transient increase in GSH in both groups following reduction could be attributed to compensatory mechanism to replenish the GSH stores and to counteract the altered redox state. The adaptive changes in GSH following oxidative stress are reported in many studies especially in chronic type of oxidative stress conditions ^{21,22}.

Cells have several types of defense system including enzymatic and non-enzymatic antioxidant systems against oxidative damage. Among these mechanisms, GSH plays an important role in scavenging of hydroxyl radicals and singlet oxygen ²⁰. If there is an over production of free radical, the antioxidant capacity can be overwhelmed by excessive level of pro-oxidants leading to lipid peroxidation. MDA can be utilized to estimate lipid peroxidation. Malondialdehyde is an end product produced following lipid peroxidation and considered to be one of the indicators of lipid peroxidation ²³. In the present study, unaltered MDA levels indicate lack of lipid peroxidation. The most plausible explanation for unaltered MDA could be associated with transient decrease in GSH and other available antioxidant systems which are not used up by ROS. Therefore, the concentration of free radicals generated are not enough to induce lipid peroxidation due to transient decrease in GSH, or GSH and other antioxidant system were able to compensate the oxidative state. Another mechanism could be related to some antioxidant elements found in the diet such as vitamin E which could protect against lipid peroxidation. However, decreased GSH concentrations without alterations in MDA levels

at particular time points following Eprinomectin and Ivermectin treatments indicate that these drugs could decrease the defense mechanism against oxidative stress although the decrease is not enough to cause lipid peroxidation.

In conclusion, Eprinomectin and Ivermectin can cause decreased plasma GSH concentrations without alterations in plasma MDA level leading to a decrease in the defense mechanism against oxidative stress although the decrease is not enough to cause lipid peroxidation.

REFERENCES

1. **Taylor MA:** Recent developments in ectoparasiticides. *Vet J*, 161, 253-268, 2001.
2. **Alvinerie M, Sutra JF, Galtier P, Mage C:** Pharmacokinetics of eprinomectin in plasma milk following topical administration to lactating dairy cattle. *Res Vet Sci*, 67, 229-232, 1999.
3. **Shoop WL, Egerton JR, Eary CH, Haines HW, Michael BF, Mrozik H, Eskola P, Fisher MH, Slayton L, Ostlind DA, Skelly BJ, Fulton RK, Barth D, Costa S, Gregory LM, Campbell WC, Seward RL, Turner MJ:** Eprinomectin: A novel avermectin for use as a topical endectocide for cattle. *Int J Parasitol*, 26, 1237-1242, 1996.
4. **Shoop WL, DeMontigny P, Fink DW, Williams JB, Egerton JR, Mrozik H, Fisher MH, Skelly BJ, Turner MJ:** Efficacy in sheep and pharmacokinetics in cattle that led to the selection of eprinomectin as a topical endectocide for cattle. *Int J Parasitol*, 26, 1227-1235, 1996.
5. **Courtney HR:** Veterinary Pharmacology and Therapeutics. In, Adams HR (Ed): Antinematodal Drugs. Ames, Iowa State University Press, 1995, p. 916-932.
6. **Frischke H, Hunt L:** Alberta. Suspected ivermectin toxicity in kittens. *Can Vet J*, 32, 245, 1991.
7. **Edwards G:** Ivermectin: Does P-glycoprotein play a role in neurotoxicity? *Filaria J*, 2 (Suppl 1): S8, 2003.
8. **Banerjee BD, Seth V, Ahmed RS:** Pesticide-induced oxidative stress: Perspectives and trends. *Rev Environ Health*, 16, 1-40, 2001.
9. **Seth V, Banerjee BD, Bhattacharya A, Pasha ST, Chakravorty AK:** Pesticide induced alterations in acetylcholine esterase and gamma glutamyl transpeptidase activities and glutathione level in lymphocytes of human poisoning cases. *Clin Biochem*, 34, 427-429, 2001.
10. **Droge W:** Free radicals in the physiological control of cell function. *Physiol Rev*, 82, 47-95, 2002.
11. **Stehbens WE:** Oxidative stress, toxic hepatitis, and antioxidants with particular emphasis on zinc. *Exp Mol Pathol*, 75, 265-276, 2003.
12. **Sen CK:** Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol*, 55, 1747-1758, 1998.
13. **Lu SC:** Regulation of hepatic glutathione synthesis: current

concepts and controversies. *Faseb J*, 13, 1169-1183, 1999.

14. Ellman GL: Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82, 70-77, 1959.

15. Uchiyama M MM: Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem*, 86, 271-278, 1978.

16. Dadarkar SS, Deore MD, Gatne MM: Comparative evaluation of acute toxicity of ivermectin by two methods after single subcutaneous administration in rats. *Regul Toxicol Pharmacol*, 47, 257-260, 2007.

17. Ali BH, Bashir AA: Ivermectin in human filariasis: A mini-review. *Vet Hum Toxicol*, 32, 110-113, 1990.

18. da Silva Vaz I, Jr., Torino Lermen T, Michelon A, Sanchez Ferreira CA, Joaquim de Freitas DR, Termignoni C, Masuda A: Effect of acaricides on the activity of a *Boophilus microplus* glutathione S-transferase. *Vet Parasitol*, 119, 237-245, 2004.

19. Sajid MS, Iqbal Z, Muhammad G, Sandhu MA, Khan MN,

Saqib M, Iqbal MU: Effect of ivermectin on the cellular and humoral immune responses of rabbits. *Life Sci*, 80, 1966-1970, 2007.

20. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND: Glutathione metabolism and its implications for health. *J Nutr*, 134, 489-492, 2004.

21. Shi MM, Iwamoto T, Forman HJ: Gamma-Glutamylcysteine synthetase and GSH increase in quinone-induced oxidative stress in BPAEC. *Am J Physiol*, 267, L414-421, 1994.

22. van Klaveren RJ, Hoet PH, Pype JL, Demedts M, Nemery B: Increase in gamma-glutamyltransferase by glutathione depletion in rat type II pneumocytes. *Free Radic Biol Med*, 22, 525-534, 1997.

23. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP: Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radic Biol Med*, 26, 202-226, 1999.