RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

The Effects of Flumethrin and Flumethrin+Vitamin C Application on Oxidative Stress Biomarkers in Chios Sheep

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SUMMARY

Present study was undertaken to study the effects of flumethrin and flumethrin+vitamin C treatment on oxidative stress biomarkers in Chios sheep. Twenty Chios sheep were divided into 2 equal groups. A pour on flumethrin to doses of 0.2 ml/kg body weight was applied on dorsal skin and vitamin C to give an intramuscular injection amount of 0.15 ml/kg in twenty Chios sheep. Results showed that flumethrin treatment unchanged malondialdehyde levels (MDA), but with vitamin C treatment significantly decreased MDA levels in whole blood on day seven. The activities of erythrocyte catalase (CAT) and superoxide dismutase (SOD) were significantly increased in both groups whereas whole blood reduced glutathione (GSH) concentration decreased in flumethrin treatment. On the other hand, plasma nitric oxide levels (NO_x) decreased in flumethrin with vitamin C application. The present study determinated that application of flumethrin with vitamin C could be no adverse effect on oxidant/antioxidant status in Chios sheep.

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Flumetrin ve Flumetrin + Vitamin C Uygulamalarının Sakız Irkı Koyunlarda Oksidatif Stress Parametreleri Üzerine Etkileri

ÖZET

Bu çalışma, flumetrin ve flumetrin + vitamin C uygulamalarının sakız ırkı koyunlarda oksidatif stress parametreleri üzerine etkilerinin belirlenmesi amacıyla gerçekleştirildi. 20 sakız koyunu 2 eşit gruba ayrıldı. Flumetrinin dökme çözeltisi 0.2 ml/kg dozunda sırt derisi üzerine ve vitamin C 0.15 ml/kg dozunda intramusküler olarak 20 sakız koyuna uygulandı. Yedinci gün sonunda flumetrin uygulaması kandaki malondialdehid düzeyini değiştirmedi fakat vitamin C ile birlikte flumetrin uygulaması malondialdehid düzeyini önemli bir şekilde azalttı. Eritrosit katalaz (CAT) ve süperoksid dismutaz (SOD) aktiviteleri her iki grupta da önemli derecede artarken kan redükte glutatyon (GSH) konsantrasyonu flumetrin grubunda azaldı. Diğer taraftan, flumetrin ve vitamin C uygulaması plazma nitrik oksit seviyelerini (NOx) azalttı. Sunulan çalışma ile flumetrin ve vitamin C'nin birlikte uygulanmasının sakız ırkı koyunlarda oksidan/antioksidan dengeye olumsuz bir etkisinin olmadığı belirlendi.

INTRODUCTION

Oxidative stress can be defined most simply as the imbalance between the production of free radicals capable of causing peroxidation of the lipid layer of cells and the body's antioxidant defense.[1] Under normal conditions, the free radicals generated and detoxified by the antioxidants present in the body and there is equilibrium between the generated free radicals and present antioxidants. However, owing to free radicals overproduction or inadequate antioxidant defense, this equilibrium is hampered favouring the free radicals upsurge that culminates in oxidative stress. The free radicals readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA.[2,3]

Flumethrin is a fat-soluble pyrethroid insecticide used in the control of ectoparasites on cattle, sheep, goats, horses, and dogs. Flumethrin was absorbed rapidly, but not completely, after oral administration in all species investigated. The concentrations in the tissues of rats two days after dosing were three- to 50-fold lower than those in the blood; the lung contained higher concentrations than other tissues, and the central nervous system had the lowest concentrations. Elimination was mainly in the faeces. The main metabolite was flumethrin acid, which was distinctly less toxic than the parent substance in acute and four-week dietary studies in rats and did not induce reverse mutations in bacteria.[4]

Stendel et al.[5] reported that pour-on flumethrin could be recovered from all hair samples of cattle taken on Day 1 following application from dorsal, lateral, ventral and distal body regions in concentrations ranging from 670 to 1 micrograms active ingredient g^{-1} hair, depending on the distance from the site of application. On Days 3, 5, and 10 after treatment, the corresponding concentrations were 125.0-1.5, 23.0-1.0, and 44.0-0.9 micrograms active ingredient g^{-1} hair, respectively. This result suggests flumethrin is more effective acaricidal action and it has remained on body for a longtime.

Although the rat metabolism studies with flumethrin are useful for identifying metabolites and provide useful information on the mammalian metabolism of orally, intravenous or intragastric administered flumethrin, they do not fully reflect exposure from topical application which is relevant to the approved uses on cattle, horses, goats or sheep. However, flumethrin has frequently used as clinical medicine to control ectoparasitic infestation and there have been no published articles investigating the use of pour-on flumethrin and flumethrin+vitamin C in Chios sheep. This study was conducted in order to investigate the effects of pour-on flumethrin and flumethrin+vitamin C treatment on oxidative stress parameters in Chios sheep.

MATERIALS and METHODS

Chemicals

Flumethrin is a registered trademark of Ultraback®, Alke, Turkey which contains 10 mg flumethrin per milliliter of the ready-to-use solution. Vitamin C is an injection solution (Sanovel, Turkey) which contains 200 mg ascorbic acid per milliliter. All the other chemicals and reagents were of analytical reagent grade purchased from commercial sources.

Animals and experimental design

In this study, twenty Chios sheep of about 40-50 kg bodyweight were treated by pour-on application of 10 ml of the product Ultraback® on dorsal skin and vitamin C to give an intramuscular injection amount of 0.15 ml/kg. In clinical examination of animals performed before drug treatment, ectoparasite infestation was not observed. Animals were divided into two groups of 10 each: the animals were applied one application of flumethrin and flumethrin+vitamin C, respectively. Animals were ensured from the animal husbandry in region of Afyonkarahisar, Turkey. Blood samples were collected into tubes containing heparin as anticoagulant prior to treatment and following drug administration on day seven. All the animals were carefully monitored in a period. The experimental protocols were approved by the Animal Care and Use Ethical Committee at Afyon Kocatepe University (91-09).

Biochemical analyses

Blood samples were separated to plasma and erythrocytes. Whole blood malondialdehyde (MDA) levels were measured by the double heating method of Draper and Hardley.[6] The method is based on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid with MDA. Whole blood reduced glutathione (GSH) concentrations were assayed by colorimetric method of Beutler et al.[7] using dithiobis nitrobenzoic acid. Erythrocytes were prepared according to Witterbourn et al.[8] and erythrocyte hemoglobin levels were determined as described by Fairbanks and Klee.[9] Cu-Zn superoxide dismutase activity (SOD) in erythrocytes was measured

by the previously detailed method of Sun et al.[10]. Catalase (CAT) in erythrocytes was measured spectrophotometrically as described by Luck.[11] The total antioxidant activity (AOA) was determined using the method described by Koracevic et al.[12] Plasma nitric oxide (NO_x) concentration was measured by a modified method of Griess assay, described by Miranda et al.[13] Shimadzu UV-1601 (Kyoto, Japan) visible spectrophotometer was used for determination of biochemical analysis.

Statistical analyses of data

Statistical analyses were performed with the SPSS 10.1 computer program (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± SEM. Significant differences between groups were analyzed by paired t test. The significance of the results was ascertained at $p<0.05$.

RESULTS

There were no differences in the mean of whole blood MDA, plasma AOA and NO_x levels in flumethrin treatment. The mean levels of whole blood GSH ($P<0.001$) decreased and erythrocyte SOD and CAT ($P<0.05$) activity levels increased in animals on day seven (Table 1).

There were no differences in the mean of whole blood GSH and plasma AOA levels in flumethrin+vitamin C treatment. The mean levels of whole blood MDA ($P\leq0.001$) and plasma NO_x ($P<0.01$) were decreased whereas erythrocyte SOD and CAT ($P<0.05$) activity levels were increased in animals on day seven (Table 2).

DISCUSSION

Lipid peroxidation (LPO) is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function and inactivation of a several membrane bound enzymes.[14] The presence of MDA is considered as an indicator of free-radical damage through membrane LPO.[3,15] Many environmental chemicals such as pesticides may also cause LPO in organism.[16] In this study, MDA was not found to be high level on day seven. This data suggest that pour on flumethrin application did not affect MDA concentration in blood on day seven. This result is inconsistent the results reported Yousef et al.[17] that oral exposure of deltamethrin (1.28 mg/kg/bw) which is phyretroid insecticide, following a thirty days significantly induced plasma thiobarbituric acid-reactive substances in male rats. Similarly, Yarsan et al.[18] reported that MDA levels increased in deltamethrin groups were given at orally 1.5, 2.5 or 7.5 mg/kg/bw, especially for the subchronic and chronic periods in mice. Flumethrin+vitamin C treatment decreased MDA concentration in animals on day seven. This result suggests that vitamin C attenuated to produce of free radicals and thus, reduced the LPO in blood. It is well known that vitamin C has a reducing potential low enough to react with most of the physiologically important radicals and oxidants,[19] enabling vitamin C to act as a powerful hydrosoluble antioxidant in body fluids,[20] scavenging reactive oxygen and nitrogen species.[21]

GSH is responsible for the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including the GSH peroxidases.[22] Raina et al.[23] reported that cypermethrin through chronic dermal application decreased GSH activity in blood of rats. Similarly, increased activity of GSH was reported in rats at the 48th hour than in the earlier period after chlorfenvinphos exposure.[24] In this study, flumethrin treatment in animals decreased GSH activity on day seven and this may be due to either the inhibition of GSH synthesis or increased utilization of GSH for detoxification of free radicals. However, flumethrin+vitamin C treatment was not affected GSH concentration in animals.

Pyrethroid insecticides are metabolized in liver via cytochrome P450 oxidative pathways yielding reactive oxygen species.[25] Oxidative stress takes advantage of the available mitochondrial electron to make molecular oxygen, resulting in excess superoxide production in most tissues.[26] These superoxide anions are converted to hydrogen peroxide and water with the help of a group of SOD.[27] Catalase enzyme hydrolyses hydrogen peroxide to molecular oxygen and water.[28] In this study, flumethrin and flumethrin+vitamin C treatment increased erythrocyte SOD and CAT activities in Chios sheep on day seven. This result may indicate that enhances the scavenging ability of erythrocytes to handle excessive free radicals.[26]

Antioxidant capacity is an important factor for all physiological standards and for performance in human and all animals.[29,30] This study showed that both flumethrin and flumethrin+vitamin C treatment unchanged plasma antioxidant capacity in Chios sheep on day seven. High production of NO has been suggested as a cause of tissue injury.[31] Stimulation of tissue production of NO is also associated with adverse events such as the production of the potent oxidant peroxynitrite following radical-radical reaction with superoxide.[32] In the present study flumethrin did not af-

fect on plasma whereas treatment with vitamin C decreased plasma NO_x in Chios sheep. This result may indicate that vitamin C prevented to NO production in Chios sheep.

In view of this data, it can be concluded that pour on flumethrin with vitamin C treatment has decreased MDA and plasma NO_x in a week and this combination has no adverse effects on oxidant/antioxidant status in Chios sheep. Moreover, future studies should be carried out to understand the long term effects profile of flumethrin+vitamin C treatment on oxidative status in animals ■

Table 1. Effects of flumethrin treatment on levels of MDA and GSH concentration in whole blood and CAT, SOD and AOA, NO_x activities in erythrocyte and plasma (Mean±SEM).
Çizelge 1. *Flumetrin uygulamasının tam kan MDA, GSH; eritrosit CAT, SOD ve plazma AOA, NO_x düzeyleri üzerine etkileri (Ortalama ± Standart Hata).*

Parameters (n:10)	0. day	7. day	P value
MDA (nmol/ml)	5.20±1.68	5.36±0.15	0.924
GSH (mg/dl)	30.49±2.30	14.84±0.34***	0.000
CAT (k/gHb)	150.20±9.87	203.73±14.73*	0.025
SOD (U/mgHb)	27.51±1.75	43.03±6.32*	0.029
AOA (mmol/L)	3.79±0.21	4.28±0.17	0.109
NO_x (µmol/L)	49.51±4.58	45.55±4.35	0.632

In the same line values with different show stars statistically significant differences in whole blood GSH (P<0.001), erythrocyte CAT and SOD (P<0.05). *P<0.05; ***P<0.001.

Table 2. Effects of flumethrin+vitamin C treatment on levels of MDA and GSH concentration in whole blood and CAT, SOD and AOA, NO_x activities in erythrocyte and plasma (Mean±SEM).
Çizelge 2. *Flumetrin + vitamin C uygulamasının tam kan MDA, GSH; eritrosit CAT, SOD ve plazma AOA, NO_x düzeyleri üzerine etkileri (Ortalama ± Standart Hata).*

Parameters (n:10)	0. day	7. day	P value
MDA (nmol/ml)	5. 03±0.21	2.91±0.18***	0.001
GSH (mg/dl)	23.94±4.85	15.11±0.53	0.128
CAT (k/gHb)	129.54±17.07	212.90±28.37*	0.014
SOD (U/mgHb)	30.57±3.11	52.26±6.16*	0.030
AOA (mmol/L)	4.09±0.21	3.82±0.22	0.525
NO_x (µmol/L)	56.55±3.06	46.27±2.97**	0.008

In the same line values with different show stars statistically significant differences in whole blood MDA (P≤0.001), erythrocyte CAT and SOD (P<0.05), plasma NO_x (P<0.01). *P<0.05; **P<0.01; ***P≤0.001.

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1. **Cochranc CG** (1991) Cellular injury by oxidants. *Am J Med*, 92: 235–305.
2. **Farber JL (**1994) Mechanisms of cell injury by activated oxygen species. *Environ Health Persp*, 102: 17–24.
3. **Kaur G, Alam MS, Jabbar Z, Javed K, Athar M** (2006) Evaluation of antioxidant activity of Cassia siamea flowers. *J Ethnopharmacol*, 108: 340-348.
4. **Kaya S, Bilgili A** (2000) Insecticides. In: Kaya S, Pirincci I, Bilgili A (Eds): Veterinary Pharmacology, Medisan Press, Ankara, 535-537.
5. **Stendel W, Hamel HD, Sieveking HU, Brühne D** (1992) Analytical determination of the distribution of flumethrin on the body surface of cattle following topical pour-on application. *Vet Parasitol*, 42: 137-43.
6. **Draper HH, Hardley M (**1990) Malondialdehyde determination as index of lipid peroxidation. *Method Enzymol*, 186: 421-431.
7. **Beutler E, Gelbart T, Pegelow C** (1986) Erythrocyte glutathione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. *J Clin Invest*, 77: 38-41.
8. **Witterbourn CC, Hawkins RE, Brain M, Carrel W** (1975) The estimation of red cell superoxide dismutase activity. *J Lab Clin Med,* 55: 337–341.
9. **Fairbanks VF, Klee GG (**1987) Biochemical aspect of haematology. In: Tiez NW, Saunders WB (Eds): Fundamentals of Clinical Chemistry, 803–804. Philadelphia.
10. **Sun Y, Oberley LW, Li Y** (1988) A simple method for clinical assay of superoxide dismutase, *Clin Chem*, 34: 497–500.
11. **Luck H** (1955) Catalase. In: H.U. Bergmeyer (Eds): Methods in Analysis, Academy Press, London.
12. **Koracevic F, Koracevic G, Djordjevic V, Andrejevic S, Cosic V** (2001) Method for the measurement of antioxidant activity in human fluids. *Clin Pathol*, 54: 356–361.
13. **Miranda KM, Espey MG, Wink AD (**2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide,* 5: 62-71.
14. **Gutteridge JM, Halliwell B** (2000) Free radicals and antioxidants in the year 2000: a historical look the future. *Ann NY Acad Sci*, 899: 136-147.
15. **Kucukkurt I, Ince S, Fidan AF, Ozdemir A** (2008) The effects of dietary supplementation of different amount of *Yucca schidigera* powder (sarsaponin 30®) on blood and tissue antioxidant defense systems and lipid peroxidation in rats. *J Anim Vet Adv*, 7: 1413-1417.
16. **Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaiee A** (2004) Pesticides and oxidative stress: a review. *Med Sci Monitor*, 10: 141– 147.
17. **Yousef MI, Awad TI, Mohameda EH** (2006) Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology,* 227: 240–247.
18. **Yarsan E, Bilgili A, Kanbur M, Celik S** (2002) Effects of deltamethrin on lipid peroxidation in mice. *Vet Hum Toxicol*, 44: 73 –75.
19. **Buettner GR** (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch Biochem Biophys,* 300: 535-543.
20. **Frei B, England L, Ames BN** (1989) Ascorbate is an outstanding antioxidant in human blood plasma. *P Natl Acad Sci USA,* 86: 6377-6381.
21. **Halliwell B** (1996) Vitamin C: antioxidant or pro-oxidant in vivo? Free Radical Research, 25: 439-454.
22. **Cathcart RF** (1985) Vitamin C: the nontoxic, nonrate-limited, antioxidant free radical scavenger. *Med Hypotheses,* 18: 61–77.
23. **Raina R, Verma KP, Pankaj NK, Prawez S** (2009) Induction of oxidative stress and lipid peroxidation in rats chronically exposed to cypermethrin through dermal application. *J Vet Sci,* 10: 257-259.
24. **Lukaszewicz-Hussain A, Moniuszko-Jakoniuk J** (2005) A low dose of chlorfenvinphos affects hepatic enzymes in serum and antioxidant enzymes in erythrocytes and liver of the rat. *Pol J Environ Stud,* 14: 199–202.
25. **Flodström S, Wärngård L, Ljungquist S, Ahlborg UG** (1988) Inhibition of metabolic cooperation in vitro and enhancement of enzyme altered foci incidence in rat liver by the pyrethroid insecticide fenvalerate. *Arch Toxicol,* 61: 218-223.
26. **Andreyev AY, Kushnareva YE, Starkov AA** (2005) Mitochondrial metabolism of reactive oxygen species. *Biochemistry Moscow*, 70: 200-214.
27. **Fridovich I** (1995) Superoxide radical and superoxide dismutases. *Annu Rev Biochem*, 64: 97-112.
28. **Gaetani GF, Kirkman HN, Mangerini R, Ferraris AM** (1994) Importance of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood,* 84: 325-330.
29. **Draper HH, McGirr LG, Hardley M** (1986) The metabolism of malondialdehyde. *Lipids,* 21: 305-307.
30. **Prior R, Cao G** (1999) Antioxidant capacity and polyphenolic components of teas: implications for altering in vivo antioxidant status. *Proc Soc Exp Biol Med*, 220: 255-261.
31. **Bohloli M, Uzun H, Aytac E, Toklu AS, Paksoy M, Durak H, Ipek T** (2007) Hyperbaric oxygen (HBO) therapy after partial hepatectomy: an experimental study on oxidative stress in rats. *Scand J Lab Anim Sci*, 34: 131-140.
32. **Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA** (1994) Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem*, 269: 26066-75.