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Deltamethrin residues and its toxic effect in milk and serum of cows and buffaloes

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ABSTRACT

The present work aimed to evaluate deltamethrin toxicity and to estimate the pesticide residue in milk and serum of dairy cows and buffaloes after different periods of deltamethrin withdrawal. 240 blood and 240 milk samples were collected from dairy cows and buffaloes in El-Behaeira Governorate farms during summer 2020 before spraying with deltamethrin (50 mg/L) with a quantity of (1 ml/ liter water) two times a week apart and after cessation of spraying for 1, 7, and 21 days. The obtained results proved that deltamethrin residues were not detected in milk and serum before spraying and after 7 and 21 days of withdrawal. However, Deltamethrin residues exceeded the permissible limit in cow's milk and in buffaloes' milk after one day of withdrawal. All cow's buffaloe's milk samples were contaminated with deltamethrin after one day of withdrawal. Deltamethrin residues ranged from 0.05 to 0.10 ppm in cow's milk and from 0.07 to 0.10 ppm in buffaloes milk after one day of deltamethrin withdrawal. Cows and buffaloes exhibited a significant elevation in serum alanine aminotransferase, aspartate aminotransferase activities, urea, creatinine, and lipid peroxide (malondialdehyde) concentrations while the total protein, albumin, globulin, and antioxidants activities declined significantly after 1 day and 7 days of withdrawal. However, all biochemical parameters significantly improved after 21 days of treatment cessation.

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INTRODUCTION

Pesticide detection in the ruminants' milk hazards public health because of the excessive consumption of milk and dairy products throughout the world. Therefore, many countries have set the permissible limits of pesticide residues in milk to protect the consumers **(LeDoux 2011).** The animal body system contamination with pesticide residues affects the animal directly and humans indirectly through the food of animal origins **(Stefanelli et al. 2009).** Pyrethroids still contaminating the environment resulting in the contamination of milk despite banning much of them. The consumption of contaminated milk causes a potential risk to human health **(Abdel-hameed et al. 2015).** Deltamethrin as a most potent insecticide is widely used in veterinary med-icine as an acaricide to control animal infestations (**Côté et al. 2014).** Pyrethroids can cause health problems in humans and animals respecting alteration in various haematobiochemical profiles and oxidative stress indices during the reduction of the pests population **(Dar et al. 2015).**

The current study aimed to clarify the toxicological effect of topical application of deltamethrin on liver and kidney functions, lipid peroxidation, and antioxidative biomarkers of Egyptian cows and buffaloes from dairy farms in El-Behaeira Governorate and to monitor the withdrawal time of the pesticide residues in dairy cow's and buffaloe's milk and serum collected in summer 2020.

MATERIAL AND METHODS

Sampling

A total of 240 Blood and 240 milk samples were collected from thirty healthy Egyptian (Baladi) lac-tating cows and thirty Egyptian buffaloes aged 4±0.5 years old from private dairy farms in El-Beheira Governorate during summer 2020. Blood (n=30) and milk (n=30) samples were collected one day before spraying with Butox 50- EC with the active ingredient of deltamethrin 50 mg/L obtained from Intervet company (1 ml/ liter water) on the back from the shoulder to sacrum two times with a week apart and after 1, 7, and 21 days of treatment cessation. Fresh milk samples were collected in sterile McCartney bottles. Blood samples were withdrawn from the jugular vein. The serum was separated by centrifugation of blood at 3000 rpm for10 min. Samples were kept frozen at – 20 °C for further analysis.

Estimation of Deltamethrin residues

10 ml of milk or 10 ml of blood samples and 10 ml of acetonitrile (1 % ACS) were placed in a 50 ml centrifuge tube. The mixture was manually shaken for 1 min. Vortex mixing for 2 min, 4 gm of magnesium sulfate, and 1 gm of sodium chloride were added. Samples were immediately shaken for 1 min to prevent agglomerates from forming during MgSO4 hydration and centrifuged at 4000 rpm for 10 min. For sample clean-up, the supernatant was transferred into a containing primary-secondary amine (PSA) tube. The supernatant was filtrated by syringe filter and transferred to HPLC vials for analysis with an Agilent 1100 HPLC system (USA) **(Anastassiades et al., 2003).**

Biochemical Studies

Serum transaminases (AST and ALT) were determined according to **Reitman and Frankel (1957).** Serum urea and creatinine were determined according to **Patton and Crouch (1977) and Henry (1974),** respectively. The serum total protein and albumin were estimated according to **Doumas et al. (1981) and Drupt (1974)** respectively. Globulin was calculated as the difference between total protein and albumin according to **Latner (1975)**. Malondialdehyde (MDA) level determination was based on the spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid with MDA as described by **Draper and Hadley (1990**). Serum glutathione (GSH) concentrations were assayed by the colorimetric method of **Beutler et al. (1986)** using dithio (bis) nitrobenzoic acid. Serum Cu-Zn Superoxide Dismutase (SOD) and catalase (CAT) activities were measured spectrophotometrically as described by the method of **Sun et al. (1988)** and **Aebi (1984),** respectively.

Statistical analysis

Data were statistically analyzed using analyses of variance (F-test) followed by Duncan's multiple range test. A probability at a level of 0.05 or less was considered significant. Standard errors were also estimated using interna-

tional business machine statistical package for social sciences (IBM SPSS) statistics program version 20.

RESLUTS

In the current study, deltamethrin was not detected in serum and milk samples of cows and buffaloes before spraying and after 7 and 21 days of withdrawal. The mean values of deltamethrin concentration exceeded the maximum residue limit (MRL) in cow's milk samples (30 µg/kg) after 1 day of withdrawal and in buffaloes' milk (0.05 ppm) after 1 day of withdrawal and deltamethrin metabolites were not detected. Furthermore, deltamethrin concentrations were higher in buffaloes' serum and milk samples collected after one day of withdrawal (Table 1).

Table (1) Deltamethrin residue in mg/kg (ppm) in cows' and buffaloes' serum and milk.

Residue	Sample	Time / Animal	Before spraying	Days of Withdrawal 1	7	21
	Serum	Cow	0 ±0	0.068±0.009	0 ±0	0 ±0
		Buffalo	0 ±0	0.128±0.021	0 ±0	0 ±0
	Milk	Cow	0 ±0	0.070 ±0.009	0 ±0	0 ±0
		Buffalo	0 ±0	0.088±0.006	0 ±0	0 ±0

Data were represented as means ± SE. n=30 .

100% of cow's milk and 100% of buffaloes' milk were contaminated with deltamethrin after one day of withdrawal and were above the permissible limit. Deltamethrin levels ranged from 0.05 to 0.10 ppm in cow's milk and from 0.07 to 0.10 ppm in buffaloes milk (Table 2).

Table (2) Minimum, Maximum, Mean, of deltamethrin level (ppm) in cows' and buffaloes' milk after one day of cessation of treatment

Samples	+Ve samples %	Min.	Max.	Means ± SE	Over MRL %	MRL (ppm)
Cows' milk	100 %	0.05	0.10	0.070±0.009	100 %	0.03 ppm a
Buffaloes' milk	100 %	0.07	0.10	0.088±0.006	100 %	0.05 ppm b

Data are expressed as means of 30 samples ±SE
a=European Commission Regulation (EC) (2017). b= Codex Alimentarius internatonal food standards (2015).

The liver enzymes (alanine aminotransferase and aspartate aminotransferase) activities, as well as urea and creatinine levels, increased significantly (P <0.05), while the total protein, albumin, and globulin were decreased significantly (P <0.05) in serum after 1 day and the 7 days of deltamethrin withdrawal. However, liver and kidney functions were significantly improved after 21 days of deltamethrin withdrawal in both cows and buffaloes (Table 3).

Table (3). Effect of deltamethrin on the hepato-renal function of cows and buffaloes

Parameters	Time / Animals	Before spraying	Days of Withdrawal 1	7	21
ALT (U/L)	Cow	31.63±0.69c	41.23±0.62 a	38.18 ±0.43 b	32.52±0.51 c
	buffalo	23.98 ± 0.08c	66.35±0.90 a	47.66±0.88 b	26.79±1.28 c
AST (U/L)	Cow	25.00±0.84 b	34.89±0.49 a	33.17±0.44 a	26.52±0.33 b
	buffalo	37.76±0.39 c	49.08±0.53 a	41.33±0.36 b	38.90±0.07c
Urea (mg/dl)	Cow	20.56±0.29 d	35.02±0.21 a	31.11±1.22 b	22.90±0.67 c
	buffalo	24.67±1.45 d	42.68±0.32 a	38.40±0.35 b	28.67±1.86 c
Creatinine (mg/dl)	Cow	0.95±0.11 b	1.71±0.06 a	1.64±0.30 a	0.81±0.07 b
	buffalo	1.77±0.04 c	2.03±0.02 a	1.90±0.01 b	1.83±0.04 bc
T.Protein (g/100 ml)	Cow	7.36±0.27 a	4.54±0.08 c	5.47±0.26 b	7.33±0.18 a
	buffalo	8.51±0.22 a	6.21±0.18 c	7.33±0.28 b	7.81±0.16 ab
Albumin (g/100 ml)	Cow	3.90±0.05 a	3.53±0.04 b	3.72±0.04 ab	3.70±0.10 ab
	buffalo	4.07±0.12 a	3.65±0.04 b	3.82±0.04 b	3.87±0.04 a
Globulin (g/100 ml)	Cow	3.46±0.22 a	1.00±0.12 b	1.74±0.28 b	3.64±0.26 a
	buffalo	4.44±0.11 a	2.55±0.18 c	3.50±0.31 b	3.95±0.13 ab
A/ G	Cow	1.13±0.06 c	3.62±0.49 a	2.25±0.36 b	1.03±0.10 c
	Buffalo	0.92±0.01 b	1.45±0.10 a	1.11±0.10 b	0.98±0.03 b

Values are represented as means of 30 samples ± SE Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

Deltamethrin exposure induced a significant reduction (P <0.05) in the non-enzymatic antioxidant, glutathione, and the enzymatic antioxidants (superoxide dismutase and catalase) activities in serum while the lipid peroxide (malondialdehyde) level was significantly increased (P <0.05) after 1 day and 7 days of deltamethrin withdrawal. On the other hand, antioxidant activities significantly enhanced while lipid peroxidation significantly decreased after 21 days of cessation of deltamethrin dermal application (Table 4).

Table (4) Effect of deltamethrin on antioxidants activity and lipid peroxidation in cow's and buffaloe's serum

Parameters	Time / Animals	Before spraying	Days of Withdrawal 1	7	21
MDA (nmol/ml)	Cow	2.85±0.08 c	6.19±0.24 a	5.05±0.16 b	3.22±0.18 c
	buffalo	3.52±0.27 c	6.48±0.30 a	5.64±0.32 ab	5.10±0.47 b
GSH (µmol/ml)	Cow	25.29±0.36 a	12.38±0.31 c	14.85±0.43 b	23.93±0.61 a
	buffalo	33.47±0.32 a	20.27±0.43 d	26.27±0.64 c	30.00±0.58 b
SOD (U/ml)	Cow	113.57±1.79 a	56.10±2.08 c	69.36±1.50 b	108.33±1.76 a
	Buffalo	94.67±2.40 a	25.88±1.61 d	37.64±1.89 c	79.00±1.15 b
CAT (U/ml)	Cow	69.85±0.99 a	32.20±1.33 d	40.34±2.04 c	63.93±1.59 b
	buffalo	152.98±2.06 a	127.33±2.73 c	137.00±3.61b	141.31±0.66 b

Values are represented as means of 30 samples ± SE Mean in the same row with different letters are significantly different (Duncan multiple range test (P < 0.05).

In the present study, cows and buffaloes exhibited a significant

DISCUSSION

Deltamethrin as alpha cyano-pyrethroids causes a long-lasting protraction of the nerve membrane during excitation. The primary mechanism of action of deltamethrin is interference with neuronal voltage-gated calcium and sodium channels **(Soderlund 2012).**

After one day of withdrawal deltamethrin concentration in cow's and buffalo's milk was above the MRL of deltamethrin in raw milk (0.05 ppm) according to European Commission Regulation (EC) (2017), and in cattle milk (30 µg/kg) according to Codex Alimentarius international food standards (2015) and was also above the acceptable daily intake (ADI) (0 -0.01 mg/kg body weight/day) according to **FAO/WHO (2010).**

The lipophilic properties of deltamethrin facilitate increased absorption that could intoxicate non-target organisms **(Oliveiraa et al. 2018).** Therefore, the lower levels of deltamethrin in cow's milk than buffalo's milk observed in the present study (Tables 1-2) may be due to higher acidity, fat, and total protein content in buffalo's milk as recorded by **Yoganandi et al. (2014**) who found that the average fat content in cow and buffalo milk was 4.68% and 6.38%, respectively. The average protein content in cows' and buffaloes' milk was 3.32% and 3.87% respectively. Egyptian buffalo's milk contained higher amounts of fat, protein, and total solids and ash **(Ca, K, Na, P, and Fe)** contents than cow's milk in Qena governorate **(Hamad, and Baiomy 2010).**

Sajid (2015) observed that deltamethrin residue exceeded the MRLs (50 ppb) in milk. Besides, **El Bahgy et al. (2018)** detected the highest levels of deltamethrin in cow's and goat's milk on the second day then on the first week and the fifteenth-day after exposure and were beyond the maximum residual limits (50 µg/kg), while it was within MRLs at the 21st and 35th days after exposure. Also, **Marzouk et al. (2016)** found that deltamethrin disappears within the 21st day after application in milk. Nearly similar to the present study, **El Nabarawy and Alam (2008)** found that the examined raw buffaloes' milk samples had deltamethrin residues on the third days and one-week post deltamethrin spray which were more than permissible limit but on the second, fourth, and sixth-week post spray were less than permissible limit (0.05 ppm) as recommended by Codex Alimentations Commissible Limit (2004).

In agreement with the present study, deltamethrin could be considered hepatotoxic, nephrotoxic. **El Nabarawy and Alam (2008)** found that deltamethrin spraying induced a significant increase in liver enzyme activities (AST and ALT) and urea accompanied by a significant reduction of total protein, albumin, and globulin in serum, while creatinine insignificantly increased on the third days, first, second, fourth, and sixth-week after deltamethrin exposure. The elevation in the liver enzyme activities in plasma indicates liver damage and an alteration in liver function. **Sethi et al. (2016)** found that protein and lipoprotein binding accounted for the presence of 90% of deltamethrin in adult plasma. According to Emam (2002), the recorded reduction in total protein and albumin may be attributed to liver damage and the inability of the liver to synthesize protein due to the drastic effect of sprayed deltamethrin.

Deltamethrin induces oxidative stress in humans and laboratory animals **(Yousef et al. 2006).** Lipid peroxidation is the procedure of oxidative degradation of polyunsaturated fatty acids in biological membranes that may impair membrane function, decrease membrane fluidity, and deactivate various membrane-bound enzymes. Thus, deltamethrin metabolization with liver enzymes' may damage the liver **(Gutteridge and Halliwell 2000).**

Ince et al. (2010) observed that deltamethrin dorsal dermal application for a short time caused oxidative damage and may damage antioxidant status in Anatolian water buffaloes. The decline in catalase and superoxide dismutase activities may be due to the exhaustion of these enzymes to control the increased oxidative stress. Consequently, malondialdehyde MDA was significantly increased on day 7.

CONCLUSION

It was concluded that the remarkable oxidative stress, hepatotoxicity, and nephrotoxici-

ty induced by deltamethrin topical application could be recovered after 21 days of pesticide cessation in both cows and buffaloes. Deltamethrin residues were not detected in cow's and buffaloes' serum and milk after 7 and 21 days of withdrawal. Deltamethrin levels were beyond the permissible limit in buffaloe's and cow's milk after one day of cessation of deltamethrin application and were higher in buffaloes' milk. Thus, the best risk management tool is the monitoring of the withdrawal time.

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