Journal of Animal Research: v.6 n.2, p. 27-32 April 2016

DOI: 10.5958/2277-940X.2015.00162.X

Evaluation of Biochemical Profile Alterations and Oxidative Stress in Mice Model on Oral Exposure to Deltamethrin

Anupama Sharma*, J.P.S. Gill and J.S. Bedi

School of Public Health and Zoonoses, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA

**Corresponding author: A Sharma; Email:* anupamasharma.vph@gmail.com

Received: 21 January, 2016 **Accepted:** 29 February, 2016

ABSTRACT

Present study was conducted to observe the biochemical alterations as well as oxidative stress in swiss albino male mice on exposure to low dose of deltamethrin through oral route for 15, 30 and 60 days. Three doses of deltamethrin *viz.,* 0.5 *mg/kg bwt/ day*, 1 *mg/kg bwt/day* and 1.5 mg/kg bwt/day dissolved in groundnut oil were administered orally. Control group was administered groundnut oil alone. Results indicate that the serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine increased significantly ($P < 0.05$) on exposure to all the doses of deltamethrin as compared to control groups whereas TP and albumin decreased significantly indicating hepatic and kidney dysfunction. It was observed that deltamethrin causes severe oxidative stress to mice as observed by measuring the level of malondialdehyde (MDA), a marker for analyzing peroxidation of the lipid in the cells. The levels of MDA as well as superoxide dismutase (SOD) were found to be significantly high in the treatment group at all doses and exposure periods as compared to the control group indicating the oxidative stress. Therefore, the present study indicates significant alterations in biochemical profile as well as oxidative stress in mice exposed to the pesticide.

Keywords: Deltamethrin, mice, biochemical, oxidative stress

The usage of pesticides has been inevitably increased from the past few decades in agriculture as well as in vector control programmes. Their indiscriminate use leads to various adverse health effects. Long term exposure to low doses of pesticides may lead to the chronic toxicity in terms of DNA damage (Ali *et al.* 2008; Undeger *et al.* 2002), immunotoxicity (Rabideau 2001), neurotoxicity, mutagenicity, reproductive toxicity, haematological and endocrinal disorders in humans (Ritz *et al.* 2000; Mourad T. 2005). Pesticides induce the generation of reactive oxygen species (ROS) at cellular level (Tope and Panemangalore 2007). Endogenous antioxidant enzymes such as superoxide dismutase (SOD), Glutathione peroxidase, catalase, etc. act as scavengers for these ROS. Any alterations in the activity of these scavengers exacerbate the toxicological events which may lead to lipid peroxidation (Bukowska *et al.* 2000). Pyrethroid insecticides are among the most commonly used classes of insecticides worldwide. These are the synthetic analogues of the pyrethrins which are extracted from the flower of the genus *Chrysanthemum* (Kim *et al.* 2004). Pyrethroids are successively replacing organophosphorus pesticides due to their advantage of photostability, high effectiveness even at lower concentrations, easy disintegration and low toxicity in birds and mammals (Bradbury and Coats 1989). Various studies conducted in the past have reported the alterations in biochemistry, hematology and reproduction due to pyrethroids (Yousef *et al.* 1998; El-Demerdash *et al.* 2004). Deltamethrin, a commonly used pyrethroid, is effective against a broad spectrum of insects with a wider margin of safety (Ismail and Mohamed, 2012). Deltamethrin has been reported to induce chromosomal aberrations, micronuclei and sperm abnormalities in mice (Bhunya and Pati, 1990). During the metabolism of pyrethroids, ROS are produced which cause oxidative stress in the intoxicated animal (Yousef *et al.* 2006). The main sources of general population exposure to this pesticide are contaminated food and water, and deltamethrin is readily absorbed by the oral route (Barlow *et al*. 2001). Agricultural and pest control operations act

as a continuous source of exposure of deltamethrin to humans which gets accumulated in the body tissues due to its lipophilic nature (Sayeed *et al.* 2003). The biomarkers of oxidative stress as well as biochemical alterations can serve as an important tool to study the subchronic toxic effects of pesticide in the body. Therefore, the present study was undertaken to observe the biochemical alterations as well as the oxidative stress in mice exposed to low dose of deltamethrin so as to correlate the possible toxic effects in humans.

MATERIALS AND METHODS

Chemicals

Technical grade Deltamethrin with 98.50% purity was obtained from Kilpest India Limited, Bhopal, India. Oral LD_{50} for deltamethrin in mice is reported to be 30-50 mg/kg body weight (EMEA, 2001).

Animals and experimental design

Seventy two Swiss Albino male mice were divided into 12 groups each consisting of six animals. Animals were kept under controlled conditions of temperature (22 ± 1°C) and humidity (60 ± 5%). They were provided with *ad libitum* pelleted feed (Ashirwad industries, Chandigarh, India) and drinking water. A twelve-hour day and night cycle was maintained. Animal were administered daily dose of deltamethrin dissolved in groundnut oil through oral route. The experimental protocol met the national guidelines on the proper care and use of animals in the laboratory research. The institutional animal ethics committee (IAEC) approved the experimental protocol. The doses used in the study are in the multiples of (maximum residual limit (MRL) values of deltamethrin found in various food products *i.e.* 0.5 mg/kg for fat, 0.1 mg/kg for chicken (USEPA 2010).

The animals were grouped as follows:

Collection of samples

Mice were sacrificed after 24 hr of completition of trial period (15/ 30/ 60 days) under ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) anaesthesia intraperitoneally. Blood samples were collected directly from the heart and were divided in two parts both for serum biochemistry as wells as oxidative stress analysis.

Table 1. Grouping of animals for experiment

Group	Treatment	Dose (mg/kg/day)	Duration of exposure
Group I_a	Deltamethrin (dose 1)	1.5	15 days
Group I_b	Deltamethrin (dose 2)	1	
Group I_c	Deltamethrin (dose 3)	0.5	
Group II_a	Deltamethrin (dose 1)	1.5	30 days
Group II_b	Deltamethrin (dose 2)	1	
Group III_c	Deltamethrin (dose 3)	0.5	
Group III_a	Deltamethrin (dose 1)	1.5	60 days
Group III_b	Deltamethrin (dose 2)	1	
Group III_c	Deltamethrin (dose 3)	0.5	
Group C_A	Vehicle control	-	15 days
Group C_B	Vehicle control	-	30 days
Group C_C	Vehicle control	-	60 days

For serum biochemistry the one part of blood samples was collected in the vaccutainer without an anticoagulant. Serum thus separated was collected and stored at refrigeration temperature till further analysis. For analysis of oxidative stress, another half of the blood samples was collected in vaccutainer containing EDTA as anticoagulant. Plasma was separated by centrifugation at 2000 rpm for 15 minutes and erythrocytes pellet was stored at refrigeration temperature till further analysis.

Biochemical parameters

Total six biochemical parameters were estimated using auto analyzer (BIOTRAN BTR 830) diagnostic reagent kits (ortho clinical diagnostics) supplied by Johnsons and Johnsons Ltd., New Delhi, India. Liver function was evaluated from the serum samples by measuring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and albumin (ALB). Renal function was evaluated by measuring the level of blood urea nitrogen (BUN) and creatinine.

Oxidative stress analysis

Separation of erythrocytes: Freshly collected heparinised blood samples (approx. 1ml each) were centrifuged (R24,

Remi centrifuge) at 2000 rpm for 15 min. After removal of the Plasma and buffy coat, erythrocyte pellet was washed thrice with 0.15 M NaCl. The 33% dilution of the packed RBC was made in phosphate buffer saline (PBS, pH 7.4) (Yagi *et al.*, 1989). The washed erythrocyte pellets were suspended in PBS (pH 7.4) and kept at 4°C till further analysis. Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA, *nM MDA/ml*) production as per the method of Rehman (1984). To 1 ml of 33% packed erythrocytes, one ml of 10% w/v trichloroacetic acid (TCA) was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 min. One ml of 0.67 % w/v of thiobarbituric acid (TBA) was added to 1 ml of supernatant and kept for 10 min in boiling water bath, cooled and diluted with 1 ml of distilled water. Blank was made by adding all the reagents except the packed erythrocytes. The absorbance was read at 535 nm. Calculation was done using the molar extinction coefficient (EC) of MDA-TBA complex at 535 nm, *i.e*., 1.56 x 10^8 /M/cm. The activity of superoxide dismutase (SOD, U/gm of protein) in erythrocyte lysate and tissue homogenates was determined by method of Marklund and Marklund (1974). In a cuvette, 1.5 ml of 100 mM buffer, 20 µl of 1% hemolysate or 10 percent tissue supernatant, 0.5 ml of 6 mM EDTA and 1ml of 0.6 mM pyrogallol solution was added. The rate of auto-oxidation of pyrogallol was taken from the increase in absorbance at 420 nm in a spectrophotometer, every min after a lag of 30 sec up to 4 min. For the test appropriate amount of enzyme was added to inhibit the auto-oxidation of pyrogallol to about 50%. A unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of the auto-oxidation of pyrogallol observed in blank. The activity of superoxide dismutase was expressed as SOD units/gm protein.

Statistical analysis

The results were presented as means (Standard deviations (S.D.) from individual magnitudes). Statistical differences were analyzed by one way ANOVA wherein differences were considered to be significant at $P<0.05$. Comparisons were done both on the basis of level of exposure *i.e.* dose of deltamethrin as well as duration of exposure *i.e.* number of days of exposure.

RESULTS

Results indicate that the serum concentrations of AST, ALT, BUN and creatinine increased significantly ($P < 0.05$) whereas that of TP and albumin decreased significantly, on exposure to all the doses of deltamethrin as compared to control groups. Enzymatic activities of AST and ALT in serum were significantly increased in deltamethrin treated mice as compared to the control group indicating the liver damage and thus alterations in the liver function. The highest level of both these enzymes was found at dose 1 (1.5 mg/kg body weight) as compared to the control group as well as the other two doses (dose 2 and 3) at 15 days of exposure (Table 2). Significantly lower levels were found in serum for TP and ALB the serum of treated mice as compared to control group at 15 days exposure. Similarly exposure to deltamethrin for 30 days has also lead to significant increase in the levels of AST and ALT in mice as compared to control. TP and albumin levels decreased instead, in the treated group (Table 3). Similar trend was observed when the levels of these enzymes and protein were compared with the control group and at different doses at 60 days of exposure (Table 4). In the present study, significantly higher levels of BUN were found in the sera of the treated mice at 15, 30 and 60 days of exposure as compared to control.

Table 2. Changes in the biochemical parameters in the mice exposed to deltamethrin for 15 days

Parameter	C_A	I_a	I_b	I_c
AST (IU/L)	111.16 ± 27.58	254 ± 45.25*	224.5 ± 19.09	120 ± 1.41*
ALT (IU/L)	34 ± 17.22	95 ± 21.21*	89 ± 9.89	43.5 ± 0.70
BUN (mg/dl)	17.55 ± 4.67	20 ± 1.41	17.5 ± 6.36	18 ± 7.07*
CREAT (g/dl)	0.28 ± 0.18	0.5 ± 0.282*	0.45 ± 0.49	0.45 ± 0.21*
TP (g/dl)	4.52 ± 2.08	4.27 ± 0.03*	4.05 ± 0.49	4.3 ± 0.28*
ALB (g/dl)	2.45 ± 1.49	2.35 ± 0.07*	2.4 ± 0.14*	2.4 ± 0.42*

Note: results are expressed in mean ± standard deviation

**significantly differ from control*

Table 3. Changes in the biochemical parameters in the mice exposed to deltamethrin for 30 days

Parameter	C_B	II_a	II_b	II_c
AST(IU/L)	135.5 ± 13.69	323.5 ± 229.06	281.5 ± 7.77*	255 ± 34.5*
ALT(IU/L)	39.17 ± 17.98	103.25 ± 94.91	36.5 ± 6.36	34.5 ± 4.94
BUN(mg/dl)	18 ± 8.46	30.5 ± 3.69	20.5 ± 0.70*	27 ± 1.4
CREA (g/dl)	0.4 ± 0.19	0.425 ± 0.15	0.4 ± 0.14	0.4 ± 0.14
TP (g/dl)	5.5 ± 3.27	5.22 ± 0.78*	5.45 ± 0.63	5.42 ± 1.52
ALB (g/dl)	2.3 ± 1.10	2.12 ± 0.05*	2.25 ± 0.35	2.26 ± 0.34

Note: results are expressed in mean± standard deviation

*significantly differ from control

Table 4. Changes in the biochemical parameters in the mice exposed to deltamethrin for 60 days

Parameter	C_C	III_a	III_b	III_c	
AST(IU/L)	129.83 ± 23.85	185 ± 38.18*	209 ± 35.35*	205.5 ± 55.86*	
ALT(IU/L)	37 ± 8.65	61 ± 0	48.5 ± 7.78	47.5 ± 2.12	
BUN(mg/dl)	20.67 ± 3.72	18.5 ± 2.12*	17.5 ± 0.70	21.5 ± 3.53*	
CREA(g/dl)		0.33 ± 0.21	0.35 ± 0.35	0.35 ± 0.07	0.3 ± 0.14
TP(g/dl)		4.87 ± 1.64	3.95 ± 1.20	4.1 ± 1.13	4.3 ± 0.70
ALB(g/dl)		2.33 ± 0.70	2.01± 0.01*	2.15 ± 0.07	2.15 ± 0.02

Note: results are expressed in mean± standard deviation

**significantly differ from control*

Similar difference was found at different dose levels also. In the present study, the higher levels of creatinine in the serum of treatment group animals indicate the ongoing renal dysfunction. This difference was found to be significantly higher in the treatment group at all dose levels and exposure periods used in this study. The result of oxidative stress analysis is depicted in table 5. In the present study, it was observed that deltamethrin causes severe oxidative stress to mice as observed by measuring the level of MDA which is a marker for analyzing peroxidation of the lipid in the cells. The levels of MDA were found to be significantly high in the treatment group as compared to the control group. When the levels of antioxidant enzyme, *i.e.* SOD were measured, these were found to be significantly decreased in the samples from the treatment group as compared to control group.

DISCUSSION

Biochemical alterations

Cellular damage due to toxic chemicals may sometimes be accompanied with alteration in the cell membrane permeability which may leads to cellular leakage (Awad *et al.* 1998). The increase in serum AST and ALT activities in this study (Table 1,2,3) is in agreement with the findings of Yousef *et al.* (1998, 2003), El-Demerdash *et al.* (2004), Yousef *et al.* (2006), Tewari and Gill, (2014) and is indicative of hepatic damage. The decrease in the total proteins as well as albumin is in agreement with El-Demerdash *et al.* (2004) and Yousef *et al.* (2006) in rats. The reduction in plasma protein, particularly albumin in animals exposed to pesticides may be attributed to the changes in the protein and free amino acid metabolism and their synthesis in liver (Rivarola and Balegno 1991).

Also, as confirmed by the increase in the activities of ALT and AST, the damage caused by deltamethrin to liver may also be correlated to the decrease in the level of these proteins. Serum creatinine level is a parameter commonly used to measure the renal damage. The increase in the level of creatinine in blood is related to the lower clearance by the kidneys due to their reduced ability to filter these waste products and to excrete them out from the body via urine. The elevated blood creatinine level is a specific and sensitive indicator of impaired kidney function (Cameron, 1996).

Blood urea nitrogen is an important parameter to assess the renal damage and thus the renal dysfunction. Elevated blood urea nitrogen level is correlated with increased protein catabolism in the body or its impaired excretion through the kidneys. It may also be due to more efficient conversion of ammonia to urea as a result of increased in the synthesis of enzymes involved in urea production (Rodwell, 1979 add recent one). The findings in the present study show reduction in the total protein as a result

Table 5. Changes in the level of oxidative stress markers in the mice exposed to deltamethrin for 15, 30 and 60 days

	Control			Treatment								
Days →	**15 days**	**30 days**	**60 days**	**15 days**			**30 days**			**60 days**		
↓ Marker	C_A	C_B	C_C	I_a	I_b	I_c	II_a	II_b	II_c	III_a	III_b	III_c
TBA	5.0 ± 1.15	4.52 ± 1.58	5.10 ± 0.35	6.47 ± 0.87*	5.01 ± 0.74*	5.76 ± 0.79*	8.53 ± 4.23*	8.07 ± 5.54*	5.43 ± 1.08*	5.95 ± 0.69	5.27 ± 0.97	5.79 ± 1.98
SOD	1.10 ± 0.14	0.85 ± 0.019	0.98 ± 0.01	0.08 ± 0.055*	0.07 ± 0.005	0.10 ± 0.06	0.18 ± 0.05*	0.07 ± 0.008	0.07 ± 0.015	0.63 ± 0.010*	0.54 ± 0.044*	0.51 ± 0.015*

of exposure to deltamethrin, and as urea is the end product of protein catabolism, the elevated level of BUN in the present studies is consistent with these explanations.

Oxidative stress

Lipid peroxidation has been extensively used as a specific marker for measuring oxidative stress in the body. In the present study, the increase in the level of thiobarbituric acid, which is a marker for lipid peroxidation, indicates oxidative stress caused due to exposure to deltamethrin. This finding is in agreement with the studies done in the past (Yousef *et al.* 2003, El-Demerdash *et al.* 2004 and Yousef *et al.* 2006). The decrease in the level of SOD is in agreement with the similar results observed in the previous studies (Kale *et al.* 1999 and Yousef *et al.* 2006). This decrease may be due to the overproduction of oxygen free radicals or the direct action of pesticide on the synthesis of SOD which is a free radical scavenging enzyme in the body (Oruc and Uner 2000). Superoxide anion, hydroxyl radicals and H_2O_2 are the three metabolites that are generated in the body of intoxicated animal during pyrethroid metabolism. The α-cyano pyrethroids such as deltamethrin form cyanohydrins that are further decomposed to cyanides and aldehydes. Cyanide ions are mainly converted to thiocyanate and CO_2 and the major metabolic reaction involves ester cleavage and hydroxylation at 4-position and formation of lipophilic conjugate 2[R]-2-(4-chlorophenyl) isovalerate. The aldehyde and other lipophilic conjugates may produce oxidative stress in pyrethroid toxicity (Kale *et al.* 1999).

CONCLUSION

Present study indicated the toxic potential of deltamethrin to cause alteration in the functioning of liver as well as kidneys even at lower doses which necessitates further insight to study the toxicity at much lower dose levels for longer period of time. The accumulation of these pyrethroids in the body throughout the lifetime can cause permanent alterations in the vital organs of the body. Therefore it is imperative to study the toxic potential of deltamethrin at the levels to which human beings are usually exposed.

ACKNOWLEDGEMENT S

The authors are thankful to GADVASU for providing necessary funding to complete the research work. Authors are also thankful to DST -INSPIRE, for providing funds.

REFERENCES

Ali, T., Bhalli, J.A., Rana, S.M. and Khan, Q.M. 2008. Cytogenetic damage in female Pakistani agricultural workers exposed to pesticides. *Environ. Mol. Mutagen*., **49**: 374–380.

Awad, M.E., Abdel-Rahman, M.S. and Hassan, S.A. 1998. Acrylamide toxicity in isolated rat hepatocytes. *Toxicol. In Vitro.,* **12**: 699–704.

Barlow, SM, Sullivan and FM, Lines, J. 2001. Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. *Food Chem. Toxicol*., **39**(5): 407–422.

Bhunya, S.P. and Pati, P.C. 1990. Effect of deltamethrin, a synthetic pyrethroid, on the induction of chromosome aberration, micronuclei and sperm abnormalities in mice. *Mutagenesis,* **5**(3): 229-232

Bradbury S.P. and Coats J.R. 1989. Comparative toxicology of the pyrethroid insecticides. *Rev. Environ. Contam. Toxicol.,* **108**: 133–177.

Bukowska, B., Chajdys, A., Duda, W. and Duchnowicz, P. 2000. Catalase activity in human erythrocytes: effects of phenoxy-herbicides and their metabolites. *Cell Biol. Int.,* **24**: 705–711.

Cameron, J.S. 1996. Kidney Failure: The Facts. Oxford University Press, New York.

Ismail, M.F. and Mohamed, H.M. 2012. Deltamethrin-induced genotoxicity and testicular injury in rats: Comparison with biopesticide. *Food Chem. Toxicol.,* **50**: 3421–3425.

El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S. and Baghdadi, H.H. 2004. Role of α-tochopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hematobiochemical parameters and semen quality of male rats. *J. Environ. Sci. Health. - B,* **39**: 443–459.

EMEA. 2001. Committee for veterinary medicinal products. Deltamethrin, Summary report 3, London, UK.

Kale, M., Rathore, N., Jone, S. and Bhatnagar, D. 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.,* **105**: 197–205.

Kim, I.Y., Shin, J.H., Kim, H.S., Lee, S.J., Khang, I.H. and Kim, T.S. 2004. Assessing estrogenic activity of pyrethroid insecticides using in vitro combination assays. *J. Reprod. Dev.,* **50**: 245–255.

Marklund, S. and Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.,* **47**(3): 469-474.

Mourad, T. 2005. Adverse impact of insecticides on the health of Palestinian farm workers in the Gaza strip. *Int. J. Occup. Environ. Health,* **11**: 144–149.

Oruc, E.O. and Uner, N. 2000. Combined effects of 2,4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of Oreochromis niloticus. *Comp. Biochem. Physiol., C* **127**: 291–296.

Rabideau, L.C. 2001. Pesticide mixture induce immunotoxicity: Potentiation of apoptosis and oxidative stress. MSc Thesis Department of Biomedical Sciences and Pathobiology, Virginia Maryland Regional College of Veterinary Medicine, p.170.

Rehman, S.U. 1984. Lead induced regional lipid peroxidation in brain. *Toxicol. Lett.,* **21**: 333-337.

Ritz, B. and Yu, F. 2000. Parkinson's disease mortality and pesticide exposure in California 1984–1994. *Int. J. Epidemiol.,* **29**: 323–329.

Rivarola, V.A. and Balegno, H.F. 1991. Effect of 2,4-dichlorophenxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicol.Lett.,* **56**: 151–157.

Rodwell, E.W. 1979. In: Harper, H.A., Rodwell, E.W., Mayer, P.A. (Eds.), In Review of Physiological Chemistry, 17th ed. Lange Medical Publications, California, pp. 401–404.

Sayeed, I., Parvez, S. and Pandey, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in fresh water fish, Channa punctatus Bloch. *Ecotoxicol. Environ. Saf.*, **56**: 295-301.

Tewari, A. and Gill, J.P.S. 2014. Assessment of hemato-biochemical parameters on exposure to low level of deltamethrin in mouse model. *Vety. world,* **7**: 152-157.

Tope, A.M. and Panemangalore, M. 2007. Assessment of oxidative stress due to exposure to pesticides in plasma and urine of traditional limited-resource farm workers: Formation of the DNA-adduct 8-hydroxy-2-deoxy-guanosine (8-OHdG). *J. Env. Sci. Health (B),* **42:** 151-155.

Undeger, U. and Basaran, N. 2002. Assessment of DNA damage in workers occupationally exposed to pesticides mixtures by the alkaline comet assay. *Arch. Toxicol.,* **76**: 430–436.

United States Environmental Protection Agency. 2010. Deltamethrin. Human Health Assessment Scoping Document in Support of Registration Review Washington, D.C. 20460. Office of prevention, pesticides and toxic substances, pp. 1-27.

Yousef, M.I., Ibrahim, H.Z., Yacout. H.M. and Hassan, A.A. 1998. Effects of cypermethrin and dimethoate on some physiological and biochemical parameters in Barki sheep. *Egypt. J. Nutr. Feeds,* **1**(1): 41–52.

Yousef, M.I., El-Demerdash, F.M. and Al-Salhen, K.S. 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. *J. Environ. Sci. Health - B,* **38**(4): 463–478.

Yousef, M.I., Awad, T.T.I. and Mohamed, E.H. 2006. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicol.,*: 240–247.