

Research Article

Deltamethrin-induced Alterations in Sperm Morphology and Spermatogenesis Impairment in Adult Sprague-Dawley Rats

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Abstract: The aim of this investigation was to assess Deltamethrin-induced alterations in sperm quality and testicular toxicity. Sixty adult male Sprague-Dawley rats were assigned Four Groups (A-D). A acted as control and received deionised distilled water, B-D were exposed orally to 6.25, 12.5 and 25 mg/kg/day of Deltamethrin Emulsifiable concentrate for 35 days. The results showed significant ($p < 0.05$) reduction in bodyweight at 25 mg/kg/day with no effect on vital organs weights. Accessory sex organ weights declined significantly ($p < 0.05$). A concentration-dependent reduction ($p < 0.05$) in the testes weight (2.81 ± 0.52 to 2.56 ± 0.22 g) epididymal weight (2.36 ± 0.12 to 2.18 ± 0.22 g) occurred. Histopathological evaluation of the testes showed degenerative changes and impairment of spermatogenesis accompanied by cellular losses at 12.5 and 25 mg/kg/day. The most affected spermatogenic elements were the primary spermatocytes. Post testicular toxicity was confirmed from epididymal sperm quality with reduction in motility and viability, increased percentage dead sperm cells and percentage abnormality in the sperm morphology including retention of cytoplasmic droplet and sperm head abnormality. There was elevation in the concentration of Aspartate aminotransferase which was positively and significantly ($p < 0.01$) correlated with Deltamethrin concentration ($r = 0.9060$), $R^2 = 0.8209$, Alanine aminotransferase (ALT) $r = 0.9015$, $R^2 = 0.8127$ and Alkaline phosphatase (ALP) $r = 0.9099$, $R^2 = 0.828$. It was concluded that Deltamethrin has the potential to impair fertility and general reproductive health by inhibiting spermatogenesis and thus, limiting sperm production potential. Periodic evaluation of reproductive health especially in males constantly exposed to Deltamethrin due to their occupation is recommended.

Keywords: Deltamethrin, pyrethroid insecticide, reproductive toxicity, sperm morphology, testis

INTRODUCTION

Non-persistent pesticides (also referred to as “contemporary-use pesticides”) are chemical mixtures that are currently available for application as insecticides, herbicides, fungicides or rodenticides, as opposed to pesticides that have been banned from use in most countries. Three common classes of non-persistent pesticides in use today include organophosphates, carbamates and pyrethroids. Although environmentally non-persistent, their extensive use as pest control in these various settings results in a majority of the general population being exposed to some of the more widely used pesticides at low levels. Deltamethrin is a broad spectrum insecticide, a Type II synthetic pyrethroid containing α -cyano group and is used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control (Gajraj and Joshi, 2009). Exposure among the general population occurs primarily through the ingestion of foods that contain low levels of Deltamethrin residue or through inhalation and/or dermal exposure in or around the home and in other indoor environments. Pyrethroids are less toxic to

animals and mammals, but mimic hormonal activities and are thus classified as endocrine disrupting compounds. An endocrine disrupter is an exogenous substance or mixture that alters function (s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations.

Toxicity and reduction in the weight of testes and accessory sex organs, as well as, marked reduction in epididymal and testicular sperm counts and fertility has been reported in Deltamethrin-exposed males (Gajraj and Joshi, 2009). Histopathological examination revealed severe degenerative changes in seminiferous tubules. Also reported was loss of germ cells such as spermatids, vacuolation of primary spermatocytes and eventually reduction in sperm count (Wang *et al.*, 2009).

No signs of maternal toxicity were detected at 4.0 mg/kg/day while significant adverse effects were only seen on testicular and epididymal absolute weights and the diameter of seminiferous tubules (Andrade *et al.*, 2002). Ben Slima *et al.* (2012) maintained that in utero and lactational exposure to Deltamethrin induced subtle changes in reproductive behavior and physiology of male offspring of rats without maternal toxicity.

Abd El-Aziz *et al.* (1994) reported that a combination of diazinon and deltamethrin decreased the weights of most genital organs and motility as well as increased percentage of dead and morphologically abnormal spermatozoa in rats. Also decreased was the plasma testosterone level and conception rate in non-treated females mated with treated males. Several authors have reported reduction in testis weight, epididymal sperm count, motility and viability in mice treated with Deltamethrin (Oda and El Maddawy, 2012). Also reported is increased percentage dead spermatozoa, spermatozoa with abnormal morphology (Abd El-Aziz *et al.*, 1994; Salem *et al.*, 1988) Inhibition of spermatogenesis and germ cell degeneration has also been reported in rats (Issam *et al.*, 2009; El-Gohary *et al.*, 1999; Oda and El Maddawy, 2012).

Due to the broad spectrum and widespread use of Deltamethrin in Nigeria in agriculture, home pest control, protection of foodstuff and disease vector control, the general population may be exposed through ingestion of food contaminated with low levels of the pesticide residue or through inhalation and/or dermal exposure in or around the home and other indoor environments. This investigation was carried out to assess reproductive toxicity of Deltamethrin, a pyrethroid insecticide, on spermatogenesis and the posttesticular effect on reproduction in the Niger Delta region of Nigeria.

MATERIALS AND METHODS

Experimental location: The experiment was carried out in the Animal House of the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, (Coordinates 4°48'14"N 6°59'12"E). The experiment was conducted from July to October 2012.

Experimental animals: Sixty sexually matured male Sprague-Dawley rats (mean weight 230± g) were obtained from the Department of Biochemistry, University of Port Harcourt and Port Harcourt, Nigeria. The rats were housed individually in plastic cages under standard conditions (12 hL:12 hD, room temperature 26±2°C, relative humidity 54%) and acclimated for fourteen days prior to the commencement of the experiment. All animals were fed standard rodent pellet and cool clean water *ad libitum*. All experiments were conducted according to the institutional animal care protocols at the University of Science and technology, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

Experimental design and procedure: Sixty adult Sprague-Dawley rats were assigned four groups (A-D) each with fifteen rats and exposed to Deltamethrin by oral gavage for 35 days. Group (A) received deionised

distilled water and acted as control, Group B received Deltamethrin Emulsifiable Concentrate (EC) diluted to 6.25 mg/kg/day with deionised distilled water. Group (C) 12.5 mg/kg/day and Group (D) 25 mg/kg /day. All animals were observed daily for behavioral changes; signs of intoxication, mortality, morbidity as well as food and water intake. Animals were weighed daily and the weights recorded to the nearest 0.01 g.

Data collection: Data was collected on body and organ weights, testicular histology, sperm quality and morphology, as well as, hepatic toxicity biomarker enzymes.

Morphometry of the testis: Twenty four hours prior to the end of the investigation feed was withheld and all rats were weighed and euthanized by the use of Ethyl Ether, dissected and the reproductive organ removed intact. The testes and epididymides were freed of all adhering tissues and weighed according to the method of Orlu and Egbunike (2010), the paired testes, left and right testis weights and the weights of Seminal vesicles and Prostate were also recorded to the nearest 0.01 mg. Weight of testes, epididymides and the accessory glands were calculated as organ means and standard deviation at the various concentrations evaluated.

Testicular histology and histometry: Known weights of the testis were fixed in Bouins' fixative solution for 24 h, washed in 70% ethanol and, thereafter, dehydrated in increasing concentrations of ethyl alcohol, cleared in three changes of chloroform and embedded in paraffin. Histological sections 5 µm thick were mounted on clean slides smeared with Mayer's egg albumin and stained with Hematoxylin and Eosin (H and E) according to Orlu and Egbunike (2009). The effect of Deltamethrin on spermatogenesis in the treated rats was evaluated on 20 randomly selected seminiferous tubules per animal. The seminiferous tubules of the experimental rats were observed at low power (X10) and thereafter critically analyzed at X100 oil immersion lens with a digital Microscope Model DB1-180M. The photomicrographs of the epithelium captured with CCD Camera were processed with a DN-2 Microscopy Image Processing Software (Orlu and Ogbalu, 2012).

Sperm collection and analysis: The cauda epidididymis was opened and the spermatozoa collected for estimation of motility and analysis of sperm viability and morphology.

Evaluation of sperm motility: Sperm motility was evaluated in fresh activated semen diluted in 2 mL of physiological saline and dropped on clean glass slide. This was examined under a cover slip with a light

microscope. Only progressive straight line movement across the field of vision was counted and expressed as a percentage of spermatozoa motility (Orlu and Ogbalu, 2011).

Spermatozoa viability: The viability of rat spermatozoa at different concentrations of Deltamethrin exposure was determined in a thin smear stained with freshly prepared Nigrosin-eosin supervital stain. The vital stain determines whether the membrane is intact, if the membrane is damaged or broken, the dye is able to stain the sperm head. If the membrane is intact the cells remain unstained. Dead/non-viable cells were identified at $\times 100$ magnification under oil immersion as those with total or partially stained cytoplasm. Percentage viability of the spermatozoa (cells that remained unstained) was calculated from 200 cells per slide as the number of viable cells/number of viable+number of non-viable cells $\times 100$ (Orlu and Ogbalu, 2011).

Spermatozoa morphology: The morphology of the spermatozoa evaluated as incidence of abnormal sperm head, tail, midpiece or retention of the cytoplasmic droplet was also assessed using the same slides at $\times 100$ magnification (Orlu and Ogbalu, 2011).

Biochemical analysis: Blood samples for biochemical analysis were collected as reported in Orlu and Gabriel (2011a, b).

The samples were collected individually by carotid bleeding into sterile tubes and the serum separated at 2500 g for 10 min and stored for determination of Alanine aminotransferase (ALT) (Bergmeyer *et al.*, 1986b) and Aspartate aminotransferase (AST)

(Bergmeyer *et al.*, 1986a) Alkaline Phosphatase (ALP) (McComb and Bowers, 1972).

Statistical analysis: All data collected from this investigation were subjected to ANOVA and Students't-test using the XLSTAT 2011 statistical software for determination of means and standard deviation. Following determination of normality of the data, they were further subjected to Pearson's Correlation analysis and linear regression to determine the effect of varying concentrations of Deltamethrin on the experimental rats.

RESULTS

Body weight and vital organs: The effect of intraperitoneal administration of varying concentrations of Deltamethrin on the bodyweight and vital organs of Sprague-Dawley rats is presented in Table 1. Bodyweight was significantly ($p < 0.05$) reduced at 25 mg/kg/day. No significant difference was observed in the weights of the vital organs-Liver, Heart and Kidney.

Reproductive organ weight: Deltamethrin administration induced significant ($p < 0.05$) weight reduction of the reproductive organs, as well as, accessory sex organs. There was concentration-dependent reduction in the testes weight from 2.81 ± 0.52 g at the control to 2.56 ± 0.22 g at 25 mg/kg bodyweight/day. The epididymal weight was also significantly ($p < 0.05$) reduced from 2.36 ± 0.12 g at the control to 2.18 ± 0.22 g. The accessory sex glands also experienced weight reduction as the concentration of Deltamethrin increased (Table 2).

Table 1: Body weight and vital organ weights of deltamethrin-treated sprague-dawley rats (Mean \pm S.D.)

| Parameters weights | Concentration of deltamethrin (mg/kg bodyweight) | | | |
|--------------------|--|--------------------|--------------------|---------------------|
| | 0.00 | 6.25 | 12.50 | 25.00 |
| Bodyweight (g) | 239.36 \pm 28.55 | 213.82 \pm 12.47 | 213.58 \pm 13.27 | 212.52 \pm 39.64* |
| Liver (g) | 7.44 \pm 1.422 | 7.462 \pm 2.563 | 8.318 \pm 1.484 | 7.248 \pm 1.522 |
| Heart (g) | 0.622 \pm 0.043 | 1.068 \pm 0.058 | 0.978 \pm 0.088 | 0.686 \pm 0.056 |
| Kidney (g) | 1.716 \pm 0.460 | 1.144 \pm 0.504 | 1.022 \pm 0.465 | 1.754 \pm 0.552 |

All values are Means \pm S.D.; *: Values are significant at $p < 0.05$

Table 2: Effect of deltamethrin on reproductive organs of sprague-dawley rats

| Reproductive organ weight | Concentration of deltamethrin (mg/kg bodyweight) | | | |
|------------------------------|--|-------------------|-------------------|---------------------|
| | 0.00 | 6.25 | 12.50 | 25.00 |
| Paired testes weight (g) | 2.81 \pm 0.12 | 2.78 \pm 0.22 | 2.71 \pm 0.21 | 2.16 \pm 0.22* |
| Right testis weight (g) | 1.27 \pm 0.16 | 1.35 \pm 0.25 | 1.18 \pm 0.35 | 1.22 \pm 0.19 |
| Left testis weight (g) | 1.54 \pm 0.078 | 1.43 \pm 0.26 | 1.64 \pm 0.78 | 1.34 \pm 0.17 |
| Paired epididymal weight (g) | 2.69 \pm 0.16 | 2.52 \pm 0.57 | 2.36 \pm 0.72 | 2.11 \pm 0.46** |
| Right epididymal weight (g) | 1.04 \pm 0.10 | 1.22 \pm 0.20 | 1.10 \pm 0.27 | 0.96 \pm 0.26 |
| Left epididymal weight (g) | 1.32 \pm 0.17 | 1.47 \pm 0.25 | 1.42 \pm 0.52 | 1.22 \pm 0.20 |
| Seminal vesicles (g) | 0.416 \pm 0.025 | 0.402 \pm 0.031 | 0.368 \pm 0.018 | 0.312 \pm 0.012** |
| Prostate (g) | 0.366 \pm 0.086 | 0.308 \pm 0.042 | 0.264 \pm 0.089 | 0.186 \pm 0.068** |
| PVG (g) | 0.672 \pm 0.082 | 0.590 \pm 0.054 | 0.474 \pm 0.096 | 0.455 \pm 0.102 |

All values are Means \pm S.D.; *: $p < 0.05$, **: $p < 0.01$; PVG: Paired vesicular gland

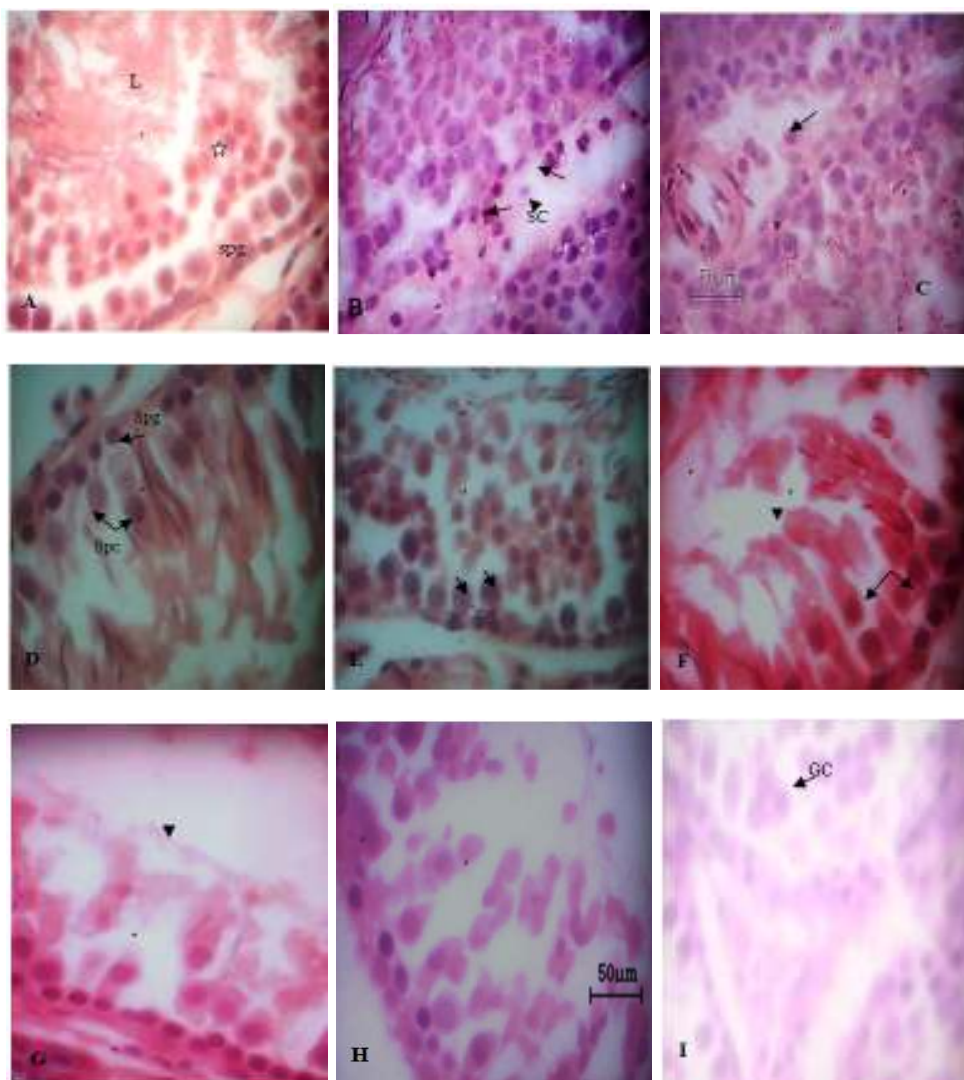


Fig. 1: A-I: Shows the seminiferous epithelium of sprague-dawley rat treated with varying concentrations of deltamethrin and prepared according hematoxylin-eosin proptocol. A-C shows seminiferous epithelium of control rats with full complement of spermatogenic elements. (spg) spermatogonia, Sertoli Cells (SC) primary spermatocytes, spermatids (*) and Lumen (L) filled with spermatozoa and interstitial tissue populated with leydig cells. 1D-seminiferous epithelium of rat treated with 6.25 mg/kg/bodyweight of deltamethrin for 35 days. The basal compartment is still intact and well populated with spermatogonia, reduction in the number of primary spermatocytes. The lumen is still filled with spermatozoa at late stage. 1E same as D at early stage of spermatogenesis and the interstitial space devoid of leydig cells. Figure 1F-seminiferous epithelium of rats treated with 12.5 mg/kg/day of deltametrin for 35 days. The basal compartment is intact with mitotic spermatogonium and the adluminal compartment is occupied by primary spermatocytes. There is depression of the luminal spermatozoa. Figure 1G-I seminiferous epithelium of rats exposed to 25 mg/Kg/day showing degeration of the seminiferous epithelium and loss of primary spermatocytes. Lumen is completely devoid of spermatozoa. Figure 1H loss of primary spermatocytes and spermatozoa as well as vacuolation. Figure 1I: presence of a Giant Cell (GC) with multiple nuclei

Testicular histological assessment: Evaluation of the seminiferous tubules of the control rats administered deionised distilled water orally revealed an intact epithelium with full complements of spermatogenic cells and lumen filled with mature spermatozoa (Fig. 1A to C). The basal compartments are lined with spermatogonia, some undergoing first maturation division (Fig. 1B) to produce premeiotic spermatocytes.

The adluminal compartment is populated with primary spermatocytes and round spermatids. The interstitial tissue is also populated with Leydig cells. Figure 1D shows seminiferous epithelium of rat treated with 6.25 mg/kg bodyweight of Deltamethrin for 35 days. The basal compartment is still intact and well populated with spermatogonia, reduction in the number of primary spermatocytes. The lumen is still filled with



Fig. 2: The spermatozoa morphology of sprague-dawley rats exposed to deltamethrin

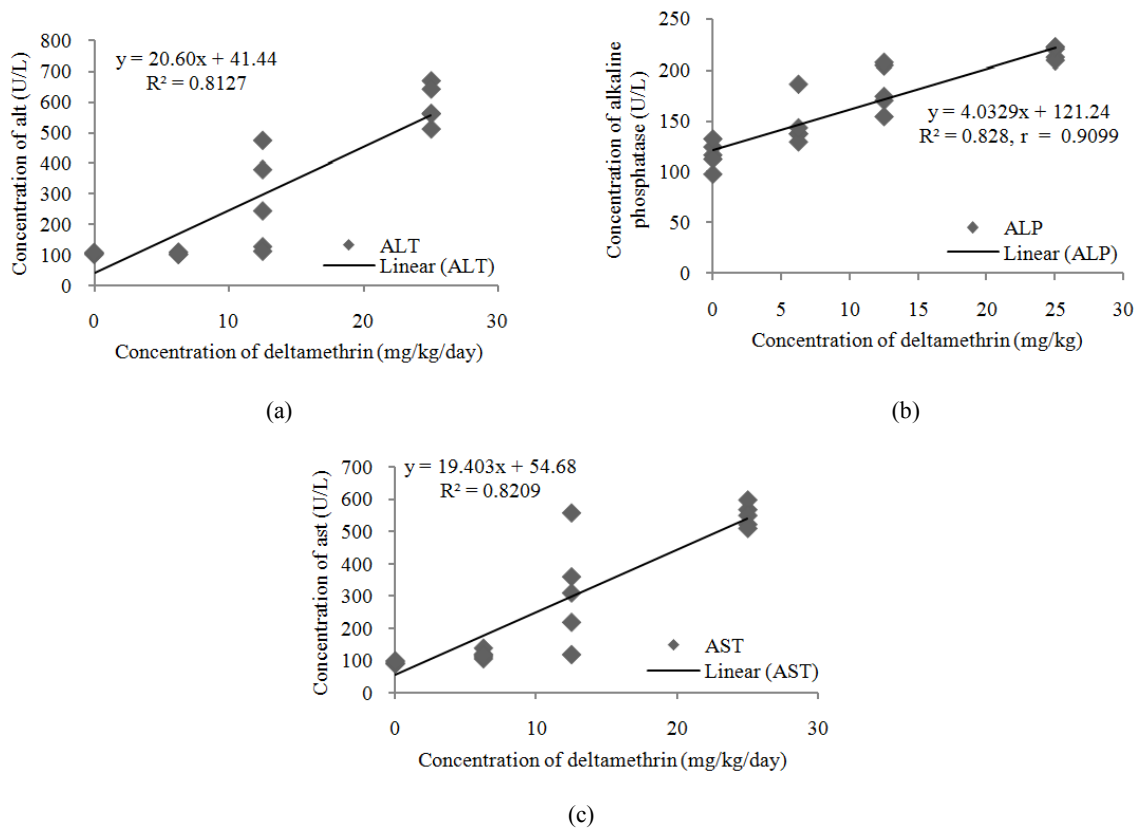


Fig. 3: Regression of hepatic enzymes: Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) and Aspartate aminotransferase (AST) levels in deltamethrin-treated sprague-dawley rats

spermatozoa at late stage. Figure 1E same as D at early stage of spermatogenesis and the interstitial space devoid of Leydig cells. Figure 1F shows seminiferous epithelium of rats treated with 12.5 mg/kg/day of Deltamethrin for 35 days. The basal compartment is intact with mitotic spermatogonium and the adluminal compartment is occupied by premeiotic primary spermatocytes. There is depression of the luminal spermatozoa (Fig. 1G). Degeneration of the seminiferous epithelium and loss of primary spermatocytes. Lumen is completely devoid of spermatozoa (Fig. 1H). Loss of primary spermatocytes and spermatozoa (Fig. 1I). Presence of a giant cell with multiple nuclei.

Sperm vitality and morphology: Cells were treated with the vital stain Nigrosin-Eosin. Figure 2A shows live epididymal spermatozoa from the control rat (Fig. 2B). Posttesticular spermatozoa from rat exposed to 6.25 mg/kg/day of Deltamethrin showing abnormal sperm head (hooked) (Fig. 2C). Dead spermatozoa from rat exposed to 12.5 mg/kg/day, as well as, immature sperm cells at Pachytene and Diplotene undergoing first maturation division (Fig. 2D). Dead and immature spermatozoa with Cytoplasmic Droplet (CD).

Cells were treated with the vital stain Nigrosin-Eosin. Figure 1A shows live epididymal spermatozoa from the control rat. Figure 2B: Posttesticular spermatozoa from rat exposed to 6.25 mg/kg/day of Deltamethrin showing abnormal sperm with hooked (Hh). Figure 2C Dead spermatozoa from rat exposed to 12.5 mg/kg/day, as well as, immature sperm cells at Pachytene and Diplotene (D) undergoing first maturation division. Figure 2D: Dead and immature spermatozoa with Cytoplasmic Droplet (CD).

Enzyme analysis: The effect of Deltamethrin administration on liver enzyme biomarkers is shown in Fig. 3a to c. The elevation in the concentration of Aspartate aminotransferase was positively and significantly ($p < 0.01$) correlated ($r = 0.9060$, $R^2 = 0.8209$) with the concentration Deltamethrin.

Alanine aminotransferase (ALT) and Alkaline Phosphatase (ALP) concentrations also increased with increased concentration of the pesticide, $r = 0.9015$, $R^2 = 0.8127$ and $r = 0.9099$, $R^2 = 0.828$ for ALT and ALP respectively.

DISCUSSION

The result of this investigation revealed that Deltamethrin induced significant ($p < 0.05$) systemic toxicity reflected as the reduction in bodyweight of treated rats causing an 11.86% differential in the bodyweight of the control and treated rats. There was no significant change in the weights of vital organs (Liver, Kidney and Heart).

Testicular toxicity was indicated as a 23.13% significant ($p < 0.01$) reduction in the relative weight of

the testes at 25 mg/kg/day, 21.56% significant (< 0.01) reduction in epididymal weight. The weights of the accessory sex organs were significantly reduced: seminal vesicle by 25% and Prostate by 49.18%. This result is in agreement with the observations of Oda and El Maddawy (2012) who reported reduction in relative weight of testis, epididymis and accessory sex organs following oral gavage with 0.6 mg/kg of Deltamethrin. Also in agreement are the reports of Ben Slima *et al.* (2012) on mice, Akbarsha *et al.* (2000) and Salem *et al.* (1988). Reduction in the bodyweight of treated rats is attributed to reduced feed intake and feed conversion efficiency, while reduction in the reproductive and accessory sex organs is an irrevocable proof of reproductive toxicity. Reduction in testicular weight would impact negatively not only on sperm concentration but also on sperm production potential. This is because the weight of the testis actually depends on the mass of differentiated spermatogenic elements, thus, reduction in weight of the testis is directly caused by the decreased number of germ cells, due to inhibition of spermatogenesis, as well as, steroidogenic enzyme activity. Both adversely impact on sperm production and its efficiency as earlier reported (Orlu and Egbunike, 2009) and testicular weight is linearly and positively correlated with sperm production and reserve potential (Orlu and Egbunike, 2010). Histopathological evaluation of the testes showed degenerative changes and impairment of spermatogenesis accompanied by cellular losses as the concentration of Deltamethrin increased to 25 mg/kg/day. The most affected spermatogenic elements were the primary spermatocytes, an indication that the meiotic phase of spermatogenesis was targeted. Wang *et al.* (2010) and Grewal *et al.* (2010) also reported degenerative changes in seminiferous epithelium of mice treated with synthetic pyrethroids.

Post testicular toxicity was confirmed from epididymal sperm morphology reflected as increased percentage dead sperm cells and percentage abnormality in the sperm morphology. Increase in the percentage of dead spermatozoa has also been reported in mice following administration of cypermethrin (Wang *et al.*, 2009). Also in agreement with the result of this investigation is the report of Salem *et al.* (1988). However, Al-Hamdani and Yajurvedi (2010) reported reduction in sperm count by cypermethrin without effect on fertility. In this investigation higher concentration of Deltamethrin induced retention of Cytoplasmic Droplet. Presence of the Cytoplasmic droplet is an indication of failure of maturation of spermatozoa during transit through the epididymis. Spermatozoa released from the luminal compartment following spermiation contain a cytoplasmic droplet which is lost during their transit through the epididymis before reaching the cauda epididymis. Spermatozoa with retained cytoplasmic droplets lack the capacity to fertilize. This abnormality is also reflected as loss or inhibited motility. Perhaps, the mechanism of posttesticular toxicity of Deltamethrin on male

reproduction is the induction of retained cytoplasmic droplets and subsequent loss of motility. This is similar to the observation (Akbarsha *et al.*, 2000) on Wistar strain male albino rats administered with the organophosphate insecticides malathion or dichlorvos.

Enzymes are informational constitutive or inducible proteins and their presence in tissues and organs, as well as, the variation in the concentration of the constitutive forms can be used as biomarkers in the evaluation of the effects of substrate, metabolites and pollutants on the integrity of vital organs and, indeed, the general health status of the organism. Increase in the enzymes Aspartate aminotransferase and Alanine aminotransferase and Alkaline Phosphatase is an indication of oxidative stress induced by Deltamethrin administration.

CONCLUSION

Based on these observations it can be concluded that although Deltamethrin synthetic pyrethroid insecticide is quite potent and toxic to insects, mammals are not susceptible to its toxicity at doses used. However, prolonged and consistent exposure of mammals to Deltamethrin has the potential to impair fertility and general reproductive health by inhibiting spermatogenesis, limiting sperm production potential as well as negatively impacting on the sperm quality of affected organisms especially sperm motility. Periodic evaluation of sperm quality and reproductive health particularly in males constantly exposed to Deltamethrin due to their occupation is recommended.

REFERENCES

Abd El-Aziz, M., A. Sahlab and A. Menha, 1994. Influence of diazinon and deltamethrin on reproductive organs and fertility of male rats. *Dtsch Tierarztl Wochenschr*, 101: 230-232.

Akbarsha, M.A., P.N. Latha and P. Murugaian, 2000. Retention of cytoplasmic droplet by rat cauda epididymal spermatozoa after treatment with cytotoxic and xenobiotic agents. *J. Reprod. Fertil.*, 120(2): 385-90.

Al-Hamdani, N.M.H. and H.N. Yajurvedi, 2010. Cypermethrin reversibly alters sperm count without altering fertility in mice. *Ecotox. Environ. Safe.*, 73(5): 1092-1097.

Andrade, A.J., S. Araujo, G.M. Santana, M. Ohi and P.R. Dalsenter, 2002. Reproductive effects of deltamethrin on male offspring of rats exposed during pregnancy and lactation. *Regul. Toxicol. Pharm.*, 36: 310-317.

Ben Slima, A., F. Ben Abdallah, L. Keskes-Ammar, Z. Mallek, A. El Feki and R. Gdoura, 2012. Embryonic exposure to dimethoate and/or deltamethrin impairs sexual development and programs reproductive success in adult male offspring mice. *Andrologia*, 44(1): 661-666.

Bergmeyer, H.U., M. Herder and R. Rej, 1986a. International Federation of Clinical Chemistry (IFCC) scientific committee, analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytical concentration of enzymes, Part 2. IFCC method for aspartate aminotransferase. *J. Clin. Chem. Clin. Biochem.*, 24: 497-510.

Bergmeyer, H.U., M. Horder and R. Rej, 1986b. International Federation of Clinical Chemistry (IFCC) scientific committee, analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytical concentration of enzyme, part 3. IFCC for alanine aminotransferase. *J. Clin. Chem. Clin. Biochem.*, 24: 481-495.

El-Gohary, M., W.M. Awara, S. Nassar and S. Hawas, 1999. Deltamethrin-induced testicular apoptosis in rats: the protective effect of nitric oxide synthase inhibitor. *Toxicology*, 132(1): 1-8.

Gajraj, A. and S. Joshi, 2009. Assessment of reproductive toxicity induced by deltamethrin in male rats. *Iran. J. Toxicol.*, 2(3): 194-202.

Grewal, K.K., G.S. Sandhu, R. Kaur, R.S. Brar and H.S. Sandhu, 2010. Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. *Toxicol. Int.*, 17: 94-8.

Issam, C., H. Samir, H. Zohra, Z. Monia and B.C. Hassen, 2009. Toxic responses to deltamethrin (DM) low doses on gonads, sex hormones and lipoperoxidation in male rats following subcutaneous treatments. *J. Toxicol. Sci.*, 34: 663-670.

McComb, R.B. and G.N. Bowers, 1972. Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. *Clin. Chem.*, 18: 97-104.

Oda, S.S. and H. El Maddawy, 2012. Protective effect of vitamin E and selenium combination on deltamethrin-induced reproductive toxicity in male rats. *Exp. Toxicol. Pathol.*, 64(7-8): 813.

Orlu, E.E. and G.N. Egbunike, 2009. Daily sperm production of the domestic fowl (*Gallus domesticus*) determined by quantitative testicular histology and homogenate methods. *Pak. J. Biol. Sci.*, 12(20): 1359-1364.

Orlu, E.E. and G.N. Egbunike, 2010. Breed and seasonal variation in the testicular morphometry, gonadal and extra gonadal sperm reserves of the barred plymouth rock and Nigerian indigenous breeds of the domestic fowl. *Pak. J. Biol. Sci.*, 13(3): 120-125.

Orlu, E.E. and O.K. Ogbalu, 2011. Effect of sublethal concentrations of *Lepidagathis alopecuroides* (Vahl) on sperm quality, fertility and hatchability in gravid *Clarias gariepinus* (Burchell, 1822) broodstock. *Res. J. Environ. Toxicol.*, 5(2): 117-124.

- Orlu, E.E. and U.U. Gabriel, 2011a. Effect of sublethal concentrations of *Lepidagathis alopecuroides* aqueous extract on the efficiency of spermatogenesis in gravid broodstock fresh water African catfish *Clarias gariepinus*. Res. J. Environ. Toxicol., 5(1): 27-38.
- Orlu, E.E. and U.U. Gabriel, 2011b. Liver and plasma biochemical profile of male *Clarias gariepinus* (Burch 1822) broodstock exposed to sublethal concentrations of aqueous leaf extracts of *Lepidagathis alopecuroides* (Vahl). Am. J. Sci. Res., 26: 106-115.
- Orlu, E.E. and O.K. Ogbalu, 2012. Partial inhibitory effect of ethanol extract of *Lepidagathis alopecuroides* (Vahl) on spermatogenesis in sprague-dawley rats. Int. J. Anim. Vet. Adv., 4(3): 214-220.
- Salem, M.H., Z. Abo-Elezz, G.A. Abd-Allah, G.A. Hassan and N. Shaker, 1988. Effect of organophosphorous (dimethoate) pyrethroid (deltamethrin) pesticides on semen characteristics in rabbits. J. Environ. Sci. Health., 23: 279-290.
- Wang, X.Z., S.S. Liu, Y. Sun, J.Y. Wu, Y.L. Zhou and J.H. Zhang, 2009. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, 72: 599-611.
- Wang, H., Q. Wang, X.F. Zhao, P. Liu, X.H. Meng, T. Yu, Y.L. Ji, H. Zhang, C. Zhang, Y. Zhang and D.X. Xu, 2010. Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Arch. Toxicol., 84: 53-61.