Ultrastructural Studies on the Glyphosate- Induced Toxicity in the Testicular Tissue of Adult Male Albino Rats Ameliorated by N- Acetylcysteine

Asmaa R. Hashim[1], Dina W. Bashir[1], Noha A.E. Yasin[1], El-Gharbawy S.M[1]

[1] Cytology and Histology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Correspondence author; Dina Wagih Mohamed Bashir, Email: dr_dina50@yahoo.com, tel. +201227294677

1. Abstract

Glyphosate (GLP) is a globally spread herbicide that causes many public health disorders through induction of oxidative stress. The present study aimed to investigate the harmful effect of GLP at the testicular ultrastructural level and to evaluate the possible protective effect of N-acetylcysteine (NAC) against such toxicity. Thirty adult male albino rats were divided into 3 groups (10 rats/group).Group I served as **a** negative control group received distilled water only, Group II orally administered (375 mg/kg) GLP and Group III orally administered NAC (160mg/kg, one hour before GLP) plus GLP (the same dose of group II) daily for 6 weeks. Testes were collected and fixed in glutaraldehyde then were processed for transmission electron microscopy. Examination of ultrathin sections of testicular tissue vacuolation, mitochondrial degeneration, nuclear condensation, increase intercellular spaces and deformed spermatozoal head**s** and tails with excess residual bodies. On the other hand, examination of ultrathin sections of testicular tissue from NAC co-treated rats revealed partial disappearance of most degenerative changes induced by GLP. In conclusion, GLP exerts some deleterious effects on testicular structure and the concurrent administration of NAC can ameliorate these detrimental effects.

Key words: • *Glyphosate; NAC; Testis; Ultrastructure; Sertoli; Spermatozoa.*

2. Introduction

Previous studies have reported that male reproductive efficacy has been declined by 50% to 60 % at the last 60 years [1] . Mammalian exposure for environmental pollutant one of the most important reasons for reproductive dysfunction [2]. Herbicides are known as environmental pollutants used in plants involved in food production[3].

Glyphosate (GLP) is the main component of most widely used herbicides [4-6] . Additionally, it is used in a large scale in Africa[7].GLP based herbicides have been claimed to induce depletion of the antioxidant defense systems and redox imbalance [8]. Some studies showed that GLP has many adverse effects on male reproductive system inducing histopathological alterations in testis [9-11] including testicular degeneration, marked reduction in sperm cell generations as well as vacuolation of the seminiferous tubules [11].

N-Acetylcysteine(NAC) is the synthetic form of the sulphur-containing amino acid cysteine after its acetylation [12]. NAC is considered a substrate for glutathione synthesis which is a crucial endogenous antioxidant [13]. NAC has been known to prevent cell damage[14] . It is commonly

used as the drug of choice for treatment of paracetamol overdoses, pneumonia, emphysema, cystic fibrosis, and bronchitis [15]. Its antioxidant role because of its capability of scavenging of free radicals as hydroxyl (OH), nitrogen dioxide (NO2) and carbonate radical(CO) [12] Recent studies have shown that NAC regenerates testicular damage induced by paranonylphenol (an environmental pollutant)[16] and chlorpyrifos (an organophosphate pesticide) [17].

Testis, is the most important male genital organ which responsible for fertility. It is very sensitive to xenobiotic exposure so great attention should be paid for its affection by adequate and accurate manner. Despite, the extensive use of GLP as herbicide and its harmful effect on reproduction, its potential toxic effect on the testicular ultrastructure was not clearly observed. Therefore, our study aimed to investigate the effect of GLP on the fine structure of testicular tissue and assessed the potential protective effect of NAC on the adult male albino rats.

3. Materials and Methods

2.1. Chemicals

Roundup (GLP 48% WSC Monsanto Co.) was purchased from the Central Agricultural Pesticide Laboratory (CAPL) in Dokki, Giza, Egypt. N-acetyl cysteine (NAC) (600 mg concentration) in crystalline form was obtained from El-Mekawy Company.

2.2 Experimental protocol and animal grouping

2.2.1. Experimental animals

Thirty adult male albino rats (weighted 180 - 200 gm and aged 4 months) were obtained from Animal Health Research Institute's breeding unit, Dokki, Egypt. They were maintained in the animal house of pharmacology department at the Faculty of Veterinary Medicine, Cairo University. Rats were kept in plastic cages under the same laboratory conditions of humidity, temperature, and exposed to 12h light/dark cycle. The feeding was standard rodent laboratory diet and drinking distilled water *ad libitum*. The protocol was ethically approved by Institutional Animal Care and Use Committee (IACUC) of Faculty of VeterinaryMedicine, Cairo University (protocol no. Vet CU 160720196).

2.2.2. Experimental design

After acclimation (2 weeks), rats were randomly divided into 3 equal groups, each group contained 10 rats (n=5 rats per cage). Group I (GPI) (untreated control group); rats were received distilled water only, group II (GPII) (GLP-exposed group); rats were received GLP by oral gavage in a dose of 375 mg/kg/day for 6 weeks in accordance with**[18]** while, group III (GPIII) (NAC co-treated group); rats were received NAC by oral gavage (160 mg/kg/day) in accordance with**[19]** one hour prior to treatment with GLP (375 mg/kg/day) daily for 6 weeks.

2.3. Sample collection and preparation

Finally, at the end of the experiment, rats were euthanized, and testicles were collected and fixed in 5% glutaraldehyde for transmission electron microscopy examination.

2.4. Transmission electron microscopy (TEM)

Small testicular specimens from all groups were immediately fixed in 5% glutaraldehyde in phosphate buffer for a few hours**[20]**, post fixed in osmium tetroxide for one hour, rinsing in 0.1 M phosphate buffer (PH 7.3), then using gradual dilutions of ethanol for drying out and insertion in open araldite mixture**[21].** Semi-thin sections of 1µm thickness were cut and stained with toluidine blue **[22].** Ultra-thin sections were cut using ultra microtome and stained with uranyl acetate and lead citrate. Then photographed with Transmission Electron Microscope TEM-109 of SEO Company in Faculty of Agriculture Cairo University Electron Microscopy Unit**,** Egypt.

4. Results

Transmission electron microscopy observations:

Examination of ultrathin sections of testicular tissue from GPI revealed normal architecture of seminiferous tubules with the apparently regular course of spermatogenic and Sertoli cells. Sertoli cells' cytoplasm contained smooth endoplasmic reticulum (sER), lipid droplets, numerous spherical mitochondria and large, indented, euchromatic nuclei **(Fig. 1A).** Additionally, normal shaped primary spermatocytes; the largest spermatogenic cells, had large, spherical nuclei with dispersed granular chromatin and dense aggregates of coiled chromosomes on the periphery. Their cytoplasm contained many rounded mitochondria **(Fig. 1B)**. Furthermore, spermatozoal heads appeared pyriform in shape with elongated electron dense nuclei **(Fig. 1C)**. As regards the spermatozoal tail, cross sections of the principle pieces were observed with characteristic peripheral fibrous sheath; formed from dorsally and ventrally arranged longitudinal columns attached by ribs around the dense fiber**s (Fig. 1D).** Moreover, interstitial tissue revealed Leydig cells with numerous mitochondria and oval euchromatic nuclei and endothelial cells with euchromatic nuclei lining interstitial blood capillaries **(Fig. 1E).**

In contrast to the above mentioned results, testes of rats received GLP (GPII) exhibited several degenerative changes involving most germ cells; disintegration of the basal lamina, shrunken heterochromatic nuclei of myoid cells with scalloped nuclear envelop and detachment of Sertoli and spermatogenic cells from the basal lamina. Some Sertoli cells revealed degenerated mitochondria with complete loss of cristae, loss of inter Sertoli Junctional complex and large, indented, heterochromatic nuclei (**Fig. 1F**). While others revealed many cytoplasmic vacuoles, numerous autophagic bodies, lysosomes, dilated sER and large, oval notched nuclei with clumps of peripheral heterochromatin**.** There were wide vacuoles in between Sertoli cells **(Fig. 1G)** and spermatocytes with signs of germ cell exfoliation and depletion**.** Additionally, Spermatogenic cells showed destructed mitochondrial cristae (degenerated mitochondria), cytoplasmic vesicles with electron dense materials (lysosomes), cytoplasmic vacuolations with intercellular spaces **(Fig. 1H)** and large, irregular, heterochromatic nuclei(**Fig. 1F& 1H**). Moreover, primary spermatocyte**s** exhibited large, spherical nucleu**i** with abnormal chromatin patterns, rupture of nuclear envelop, vacuolated cytoplasm, and degenerated mitochondria. Intercellular spaces were also detected **(Fig. 2A).** Round spermatid showed swollen mitochondria**;** some with complete loss of cristae while

others with partial loss of cristae and karyolytic nuclei. Single large electron-lucent space attached to the anterior pole of the nucleus was developed (absence of acrosomal granule) and intercellular spaces were noted**(Fig. 2B).** Early spermatid**s** at Golgi phase revealed degenerated mitochondria, presence of acrosomal granule**s**, absence of acrosomal vesicle**s** and disintegrated nuclear envelop with shrunken karyolytic nucle**i**. Also, wide intercellular spaces were observed **(Fig. 2C).**Regards spermatozoa, they displayed several alterations including deformed heads with wrinkled acrosomal caps and excess residual cytoplasm with degenerated mitochondria as residual bodies (**Fig. 2D)**. Additionally, cross sections through the middle piece of spermatozoal tail were encountered with irregular disorganized mitochondrial sheath and destructed flagellar membrane. Moreover, other sections retained excessive residual cytoplasm (**Fig. 2D &2E**).The principle piece exhibited disintegration of its circumferential rib and flagellar membrane **(Fig. 2E)**. Concerning Leydig cells; they showed heterochromatic nuclei and their cytoplasm exhibited numerous vacuoles, Moreover, increased vacuolation of interstitial tissue was dominant **(Fig. 2F)**.

Ultra-structurally, NAC co-treated group revealed partial disappearance of most degenerative changes induced by GLP since Sertoli cells, spermatogenic cells and myoid cells restore their normal histological structure. Their nuclei appeared with nearly normal chromatin patterns, apparently normal mitochondria as well as intercellular spaces decreased to acceptable limit compared to those of GPII. Only some vacuoles were observed in their cytoplasm (Fig. 3A&3B). Furthermore, primary spermatocytes appeared nearly normal with intact nuclear envelop, large, spherical, euchromatic nuclei and most mitochondria resumed to their normal histological appearance (Fig. 3C).In addition, round spermatids (Fig. 3D) and early spermatids at Golgi phase (Fig.3E) nearly regained their normal structure but some mitochondria still degenerated. Juxta-nuclear chromatoid bodies were also observed in both (Fig. 3D&3E). Concerning spermatozoa, heads began to restore their normal shape (Fig. 3F). Cross sections of tails revealed nearly normal principle pieces with normal circumferential ribs and flagellar membranes. Additionally, some cross sections of middle pieces appeared nearly normal while others still degenerated(Fig.3G).Moreover, the interstitial tissue revealed less vacuolation and partial recovery of Leydig cell's nucleui (Fig. 3H).

5. Discussion

GLP is a non-selective systemic herbicide commonly used for killing undesired plants and weeds [23].

Previously, it is expected that GLP is very specific on plant metabolism but recently, it has been found to have adverse effects on both animals and humans [24]. Further studies showed that GLP adversely affect the male genital system[10, 11, 25, 26].

The current study revealed numerous ultrastructural alterations in the testicular tissue sections from GLP-exposed rats in comparison with the control group. These alterations were in the form of; disintegration of the basal lamina with

shrunken heterochromatic nuclei of myoid cells and detachment of spermatogenic and Sertoli cells from the basal lamina. The disintegration of the basal lamina might be due to shrinkage of seminiferous tubules or the contractility of myoid cells [27]. Meanwhile, the detachment of the spermatogenic cells may be due to decrease in spermatogenic cell population[28]. Moreover, [29]speculated that the pathological changes of seminiferous epithelium may cause the disruption of Sertoli and germ cells, which results in impaired spermatogenesis and may also lead to germ cells loss. [30] reported that the spermatogonia are highly sensitive to toxicants because of their mitotic activity. Moreover, the defects of spermatogonia will affect the development of the following stages of spermatogenesis [31]. However, the intact basement membrane is necessary for nutritive and supportive functions for both germ and Sertoli cells[32]and for normal structure and functional integrity of the seminiferous tubules [33].As shown by other authors that lamina propria plays an important role in the maintenance [34]and differentiation of epithelia [35]. Therefore, any alteration of the basement membrane following administration of toxic substances, leads to changes in germ cells, might contribute to degeneration of germinal epithelium.

The ultrastructural examination of primary spermatocytes from GLP-exposed rats showed their nuclei with abnormal chromatin patterns and rupture of nuclear envelop. However, chromatin condensation, disintegration and karyolysis as a sequence of cytoplasmic damages and a characteristic stage of apoptotic pathway [36-38]. Karyolysis can occur because of enzymatic digestion and/or denaturation of nuclear proteins [39]Generally, GLP has been claimed to induce depletion of the antioxidant defense systems and redox imbalance [8]. It was reported that the oxidative stress has been postulated as one of the mechanisms leading to testicular damage [40]

The present study demonstrated many mitochondrial degenerations in the cytoplasm of the spermatids in addition to irregular disorganized mitochondrial sheath of spermatozoa. These lesions are considered as early features for cellular injury induced by free radicals' excessive exposure and apoptosis [41].In addition to, absence of acrosomal granules and acrosomal vesicles in some early spermatids, spermatozoa exhibited many deleterious abnormalities and sloughing including deformed heads with wrinkled acrosomal caps, excess residual bodies, destructed flagellar membrane. These alterations may be due to disturbance at

spermatocytogenesis and altered Sertoli cells. Moreover, retained excessive residual cytoplasm is either due to disturbance at the mechanism of cytoplasmic extrusion by degenerative changes occurred during the spermatogenesis [42]or due to excessive Reactive oxygen species (ROS) production associated with sperm DNA damage [43] ROS cause defective sperm function as a result of lipid peroxidation of the poly unsaturated fatty acids in the head and mid-piece which consequently alter sperm morphology and lead to decreased motility and ineffective spermatozoon oocyte fusion [44, 45].

Our study demonstrated many morphological changes in Sertoli cells after GLP administration in the form of cytoplasmic vacuolation which is an early morphological indicator to its damage resulting from dilatation and vesiculation of sER [46] that may be due to changes in osmolarity by disruption of Na^+/ K^+ pump leading to ingress of water into the cell[38]. However, Sertoli cells, are target cells for pesticides [47] and more sensitive to testicular disturbance than germ cells; thus they are the primary site of alteration subsequently leading to spermatogenic disruption [48]. In addition, there was an extensive coalescence of vacuoles between Sertoli cells and spermatocytes which may be attributed to interrupted inter-Sertoli junctional complex leading to germ cells exfoliation and depletion; as Sertoli cells became unable to support germ cells. Generally, reactive oxygen species (ROS) are the causative agents for cell membranes oxidative phosphorylation leading to disruption of junctional complex integrity[49]. [50] added that the testicular tissues are very sensitive to reactive oxygen species effects. However, Sertoli cells form the sites of attachment of germ cells and provide physical support to them[51, 52]. Such intercellular spaces, as a result of germinal cells loss, in turn affect spermatogenesis [53].The intercellular spaces assimilated progressive degenerative changes disrupting the plasma membrane integrity because of the oxidative stress [54]. Additionally, Sertoli cells displayed numerous autophagic bodies which might be due to increased autophagy throughout testicular tissue to eliminate the accumulated GLP and its metabolites via intracellular breakdown of lysosomes.

Leydig cells showed heterochromatic nuclei and their cytoplasm exhibited numerous vacuoles. Increased vacuolation of interstitial tissue was also apparent in GLP administrated group. However, the change in Leydig cells character was an evidence of non functioning cells as the fate of these phenomena could lead to fatty degeneration [55]. The oxygen-induced

damage mediated by lipid peroxidation may also damage membrane integrity with increased cell membrane permeability, thus leading to structural damage of DNA and cell death. Moreover, increased oxidative stress is very harmful for Leydig cells especially that these cells locate close to the blood vessels which expose them to a high risk of exogenous toxicants[56] .The change in the structure of Leydig cells could affect the testosterone level [57]. Also, [58] regard the decline in serum testosterone level to the damage of Leydig cells. [59] suggested that the decreased testosterone level reflected on Sertoli cells function leading to loss of germ cells, loss of contact between them, decrease the thickness of the seminiferous epithelium and finally, destruction of testicular tissue.

On contrary, to the GLP only exposed rats; the NAC co-treated rats revealed a well noticed improvement in which Sertoli cells, sprematogonia, primary spermatocytes, myoid cells, Leydig cells and spermatozoa showed disappearance of most degenerative changes appeared to a great extent. Also decrease of intercellular spaces which may be due to the return of sprematogonia and Sertoli cells to their organized state within the seminiferous tubules and the Na^+/K^+ pump restore its normal function. These effects may be attributed to the role of NAC as a cytoprotective by scavenging reactive oxygen species and reducing lipid peroxidation. However, previous studies showed that NAC possess antioxidant effects [12,13]. Administration of antioxidants enhanced the testicular functions, sperm motility and fertilizing ability in rats [60]. Furthermore, antioxidants have been experimentally proved and used as effective protection against free radical induced tissue damage and oxidative stress [61]. These ameliorating changes may be supported and explained by that reported previously; where NAC has been known to prevent cell damage[14] NAC has been also found to regenerate testicular damage induced by paranonylphenol; an environmental pollutant[16] and chlorpyrifos; an organophosphate pesticide[17]. These results are in accordance with [62] who co-treated the toxicity of titanium dioxide nanoparticles with NAC. Also, histological improvement in seminiferous tubules might be due to ability of NAC to preserve and enhance the proliferation, maturation and differentiation of spermatozoa. This may confirm that the exposure of rats to NAC prior to GLP has an ameliorating effect in attenuating the adverse effects of toxicity induced by GLP.

6. Conclusion

GLP disrupts male fertility through degenerative alterations, in testicular tissue,

fortunately these alterations can be ameliorated by NAC; although the NAC did not lead to complete recovery. Therefore from our study we advise to decrease the use of GLP as can as possible and if it is necessary to use it; NAC administration is recommended in such cases. Regarding the still noticed mild alterations in our study may need further investigations to overcome it completely.

7. References

1. Oehninger, S. (2001). Strategies for the infertile man. In *Seminars in reproductive medicine* (Vol. 19, No. 03, pp. 231-238). Copyright© 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.
2. Mathur, P. P., & D'cruz, S. C. (2011). The effect of environmental contaminants on testicular function. *Asian journal of andrology*, *13*(4), 585John, S., et al., Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. 2001. 12(9): p. 500-504.
3. John, S., Kale, M., Rathore, N., & Bhatnagar, D. (2001). Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *The Journal of nutritional biochemistry*, *12*(9), 500-504.
4. Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*(1), 3.
5. Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., ... & Straif, K. (2015). International Agency for Research on Cancer Monograph Working Group ILF. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncol*, *16*(5), 490-491.
6. Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., ... & Vandenberg, L. N. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental Health*, *15*(1), 1-13.
7. Gianessi, L., & Williams, A. (2011). Overlooking the obvious: the opportunity for herbicides in Africa. *Outlooks on pest management*, *22*(5), 211-215.
8. Cavalli, V. L. D. L. O., Cattani, D., Rieg, C. E. H., Pierozan, P., Zanatta, L., Parisotto, E. B., ... & Zamoner, A. (2013). Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. *Free Radical Biology and Medicine*, *65*, 335-346.
9. Dallegrave, E., Mantese, F. D., Oliveira, R. T., Andrade, A. J., Dalsenter, P. R., & Langeloh, A. (2007). Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. *Archives of Toxicology*, *81*(9), 665-673.
10. Razi, M., Najafi, G., Feyzi, S., Karimi, A., Shahmohamadloo, S., & Nejati, V. (2012). Histological and histochemical effects of Glyphosate on testicular tissue and function. *Iranian journal of reproductive medicine*, *10*(3), 181.
11. Owagboriaye, F. O., Dedeke, G. A., Ademolu, K. O., Olujimi, O. O., Ashidi, J. S., & Adeyinka, A. A. (2017). Reproductive toxicity of Roundup herbicide exposure in male albino rat. *Experimental and Toxicologic Pathology*, *69*(7), 461-468.
12. Samuni, Y., Goldstein, S., Dean, O. M., & Berk, M. (2013). The

chemistry and biological activities of N-acetylcysteine. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1830*(8), 4117-4129.

13. O'Leary, A. J., Drummond, S. E., Edge, D., & O'Halloran, K. D. (2018). Diaphragm muscle weakness following acute sustained hypoxic stress in the mouse is prevented by pretreatment with N-acetyl cysteine. *Oxidative Medicine and Cellular Longevity*, *2018*.
14. Jiao, Y., Ma, S., Wang, Y., Li, J., Shan, L., Liu, Q., ... & Liu, H. (2016). N-Acetyl cysteine depletes reactive oxygen species and prevents dental monomer-induced intrinsic mitochondrial apoptosis in vitro in human dental pulp cells. *PloS one*, *11*(1), e0147858.
15. Saleem, T. H., Abo El-Maali, N., Hassan, M. H., Mohamed, N. A., Mostafa, N. A., Abdel-Kahaar, E., & Tammam, A. S. (2018). Comparative Protective Effects of N-Acetylcysteine, N-Acetyl Methionine, and N-Acetyl Glucosamine against Paracetamol and Phenacetin Therapeutic Doses–Induced Hepatotoxicity in Rats. *International journal of hepatology*, *2018*.
16. Malmir, M., Faraji, T., Noreini, N. S., & Mehranjani, S. M. (2018). Protective antioxidant effects of N-acetylcysteine on testicular tissue and serum testosterone in paranonylphenol-treated mice (A stereological analysis). *Reprod Syst Sex Disord*, *7*(225), 2.
17. Kheradmandi, R., Jorsaraei, S. G. A., Feizi, F., Moghadamnia, A. A., & Neamati, N. (2019). Protective effect of N-acetyl cysteine on chlorpyrifos-induced testicular toxicity in mice. *International Journal of Fertility & Sterility*, *13*(1), 51.
18. Tizhe, E. V., Ibrahim, N. D. G., Fatihu, M. Y., Igbokwe, I. O., George, B. D. J., Ambali, S. F., & Shallangwaa, J. M. (2013). Haematogical changes induced by subchronic glyphosate exposure: ameliorative effect of zinc in Wistar rats. *Sokoto Journal of Veterinary Sciences*, *11*(2), 28-35.
19. Turkmen, R., Birdane, Y. O., Demirel, H. H., Yavuz, H., Kabu, M., & Ince, S. (2019). Antioxidant and cytoprotective effects of N-acetylcysteine against subchronic oral glyphosate-based herbicide-induced oxidative stress in rats. *Environmental Science and Pollution Research*, *26*(11), 11427-11437.
20. Karnovsky, M. J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for uc in electron micrwcop. *Ceil Biol*, *27*.
21. Mollenhauer, H. H. (1964). Plastic embedding mixtures for use in electron microscopy. *Stain technology*, *39*, 111.
22. Richardson, K. C., Jarett, L., & Finke, E. H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain technology*, *35*(6), 313-323.
23. Walsh, L. P., McCormick, C., Martin, C., & Stocco, D. M. (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environmental health perspectives*, *108*(8), 769-776.
24. AVDATEK, F., et al., Protective Effect of N-Acetylcysteine on Testicular Oxidative Damage, Spermatological Parameters and DNA Damage in Glyphosate-Based Herbicide-Exposed Rats. 2018. 11(3): p. 292-300.
25. Romano, R. M., Romano, M. A., Bernardi, M. M., Furtado, P. V., & Oliveira, C. A. (2010). Prepubertal exposure to commercial formulation of the herbicide

glyphosate alters testosterone levels and testicular morphology. *Archives of toxicology*, *84*(4), 309-317.
26. Antoniou, M., Habib, M. E. M., Howard, C. V., Jennings, R. C., Leifert, C., Nodari, R. O., ... & Fagan, J. (2012). Teratogenic effects of glyphosate-based herbicides: divergence of regulatory decisions from scientific evidence. *J Environ Anal Toxicol S*, *4*(006), 2161-0525.
27. Abdrabou, M. I., Elleithy, E. M., Yasin, N. A., Shaheen, Y. M., & Galal, M. (2019). Ameliorative effects of Spirulina maxima and Allium sativum on lead acetate-induced testicular injury in male albino rats with respect to caspase-3 gene expression. *Acta Histochemica*, *121*(2), 198-206.
28. Meng, X., Lindahl, M., Hyvönen, M. E., Parvinen, M., de Rooij, D. G., Hess, M. W., ... & Pichel, J. G. (2000). Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science*, *287*(5457), 1489-1493.
29. Xie, B. G., Li, J., & Zhu, W. J. (2014). Pathological changes of testicular tissue in normal adult mice: A retrospective analysis. *Experimental and therapeutic medicine*, *7*(3), 654-656.
30. DE ROOIJ, D. G., & RUSSELL, L. D. (2000). All you wanted to know about spermatogonia but were afraid to ask. *Journal of andrology*, *21*(6), 776-798.
31. Elshennawy, W. W., & Elwafa, H. R. A. (2011). Histological and ultrastructural changes in mammalian testis under the effect of hydrocortisone. *Journal of American Science*, *7*(9), 38-48.
32. Siu, M. K., & Cheng, C. Y. (2004). Dynamic cross-talk between cells and the extracellular matrix in the testis. *Bioessays*, *26*(9), 978-992.
33. Morales, E., Horn, R., Pastor, L. M., Santamaria, L., Pallarés, J., Zuasti, A., & Canteras, M. (2004). Involution of seminiferous tubules in aged hamsters: an ultrastructural, immunohistochemical and quantitative morphological study. *Histology and histopathology*.
34. Davidoff, M. S., Breucker, H., Holstein, A. F., & Seidl, K. (1990). Cellular architecture of the lamina propria of human seminiferous tubules. *Cell and tissue research*, *262*(2), 253-261.
35. Lustig, L., et al., Passive immunization with anti-laminin immunoglobulin G modifies the integrity of the seminiferous epithelium and induces arrest of spermatogenesis in the guinea pig. 2000. 62(6): p. 1505-1514.
36. Susin, S. A., Daugas, E., Ravagnan, L., Samejima, K., Zamzami, N., Loeffler, M., ... & Brothers, G. (2000). Two distinct pathways leading to nuclear apoptosis. *The Journal of experimental medicine*, *192*(4), 571-580.
37. Abdel Hameed, T. F. (2004). Light and electron microscopic studies on the effect of orally-adminstered formalin on liver and kidney of guinea pig. *JOURNAL-EGYPTIAN GERMAN SOCIETY OF ZOOLOGY*, *45*(C), 203-224.
38. Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic pathology*, *35*(4), 495-516.
39. Kumar, V., Abbas, A. K., Fausto, N., & Aster, J. C. (2014). Robbins and Cotran pathologic basis of disease, professional edition e-book. Elsevier health sciences .
40. El-Dakdoky, M. H., & Helal, M. A. (2007). Reproductive toxicity of male mice after exposure to nonylphenol. Bulletin of

environmental contamination and toxicology, 79(2), 188-191.
41. Makhlouf, M., Eldien, H., Zagloul, D., Dief, E. A., & ElHaliem, N. G. A. (2008). The effect of lead acetate on testicular structure and protective effect of vitamin E in adult albino rat. Egypt. J. of Histol, 31(2), 406-418.
42. Malekinejad, H., Mirzakhani, N., Razi, M., Cheraghi, H., Alizadeh, A., & Dardmeh, F. (2011). Protective effects of melatonin and Glycyrrhiza glabra extract on ochratoxin A—induced damages on testes in mature rats. *Human & experimental toxicology*, *30*(2), 110-123.
43. CHEN, S. S., HUANG, W. J., CHANG, L. S., & WEI, Y. H. (2004). 8-Hydroxy-2′-deoxyguanosine in leukocyte DNA of spermatic vein as a biomarker of oxidative stress in patients with varicocele. The Journal of urology, 172(4 Part 1), 1418-1421.
44. Baumber, J., Ball, B. A., GRAVANCE, C. G., Medina, V., & DAVIES-MOREL, M. C. (2000). The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. *Journal of andrology*, *21*(6), 895-902.
45. Dandekar, S. P., Nadkarni, G. D., Kulkarni, V. S., & Punekar, S. (2002). Lipid peroxidation and antioxidant enzymes in male infertility. *Journal of Postgraduate Medicine*, *48*(3), 186.
46. Creasy, D. M. (2001). Pathogenesis of male reproductive toxicity. *Toxicologic pathology*, *29*(1), 64-76.
47. El-Gerbed, M. S. (2013). Histopathological and ultrastructural effects of methyl parathion on rat testis and protection by selenium. *Journal of Applied Pharmaceutical Science*, 3(8), S53.
48. Russell, L. D. (1993). Morphological and functional evidence for Sertoli-germ cell relationships. The Sertoli Cell.
49. Fouad, A. A., Qureshi, H. A., Al-Sultan, A. I., Yacoubi, M. T., & Ali, A. A. (2009). Protective effect of hemin against cadmium-induced testicular damage in rats. *Toxicology*, *257*(3), 153-160.
50. Guney, M., Ozguner, F., Oral, B., Karahan, N., & Mungan, T. (2007). 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. *Toxicology and industrial health*, *23*(7), 411-420.
51. Richburg, J. H. (2000). The relevance of spontaneous-and chemically-induced alterations in testicular germ cell apoptosis to toxicology. *Toxicology letters*, *112*, 79-86.
52. Sawada, H., & Esaki, M. (2003). Electron microscopic observation of 137 Cs-irradiated rat testis: production of basal laminae for germ cells, despite their absence. *Microscopy*, *52*(4), 391-397.
53. Anniballo, R., et al., Criteria predicting the absence of spermatozoa in the Sertoli cell-only syndrome can be used to improve success rates of sperm retrieval. 2000. 15(11): p. 2269-2277.
54. El Ghazzawy, I. F., Meleis, A. E., Farghaly, E. F., & Solaiman, A. (2011). Histological study of the possible protective effect of pomegranate juice on bisphenol-A induced changes of the caput epididymal epithelium and sperms of adult albino rats. *Alexandria Journal of Medicine*, *47*(2), 125-137.

55. Wang, S. M., Wang, D. W., Peng, R. Y., Gao, Y. B., Yang, Y., Hu, W. H., ... & Gao, Y. (2003). Effect of electromagnetic pulse irradiation on structure and function of Leydig cells in mice. *Zhonghua nan ke xue= National journal of andrology*, *9*(5), 327-330.
56. Boekelheide, K. (1993). Sertoli cell toxicants. *The Sertoli Cell*.
57. Ross, M. H., Kaye, G. I., & Pawlina, W. Histology a Text and Atlas, pp: 142–4, 661–8. 2003.
58. Hajioun, B. (2014). Effects of cell phone radiation on testosterone levels and testicular changes in rats treated with garlic (Allium sativum L.) hydroalcoholic extract.
59. Monsees, T. K., Franz, M., Gebhardt, S., Winterstein, U., Schill, W. B., & Hayatpour, J. (2000). Sertoli cells as a target for reproductive hazards. *Andrologia*, *32*(4-5), 239-246.
60. Suzuki, N., & Sofikitis, N. (1999). Protective effects of antioxidants on testicular functions of varicocelized rats. *Yonago Acta medica*, *42*(7), 87-94.
61. Lampe, J. W. (2003). Spicing up a vegetarian diet: chemopreventive effects of phytochemicals. *The American journal of clinical nutrition*, *78*(3), 579S-583S.
62. Elnagar, A. M. B., Ibrahim, A., & Soliman, A. M. (2018). Histopathological effects of titanium dioxide nanoparticles and the possible protective role of N-acetylcysteine on the testes of male albino rats. *International journal of fertility & sterility*, *12*(3), 249.

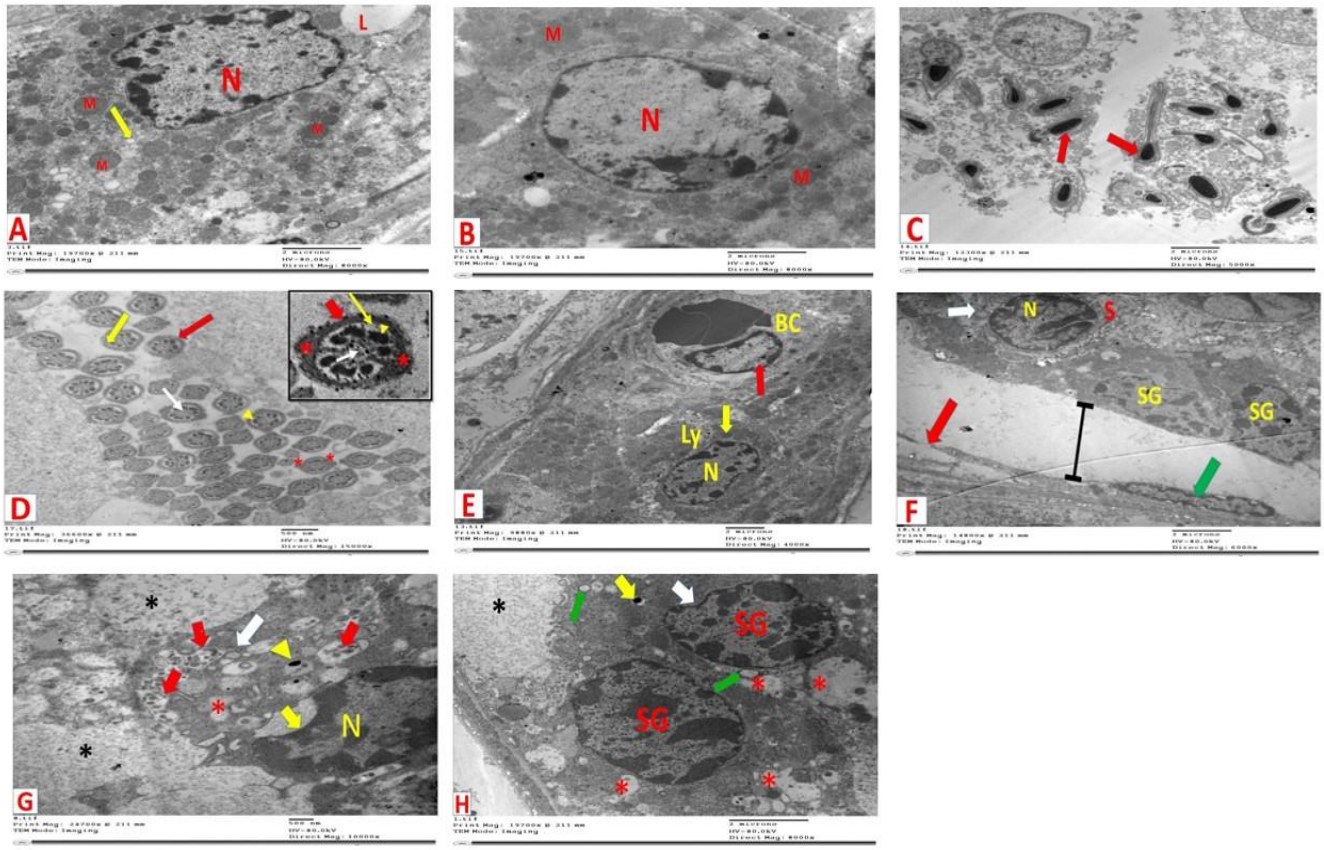

Fig. 1. Transmission electron micrographs of testicular tissue sections of rats. **(A:E)** Control group (GPI) revealing **(A)** Sertoli cell with numerous spherical mitochondria (M), sER (yellow arrow), lipid droplets (L) and large, indented, euchromatic nucleus (N) **(B)** Primary spermatocyte with large, spherical, euchromatic nucleus (N) and numerous spherical mitochondria (M) **(C)** Normal pyriform heads of spermatozoa (red arrows) with elongated electron dense nuclei **(D)** Cross sections through the tail of spermatozoa indicated principle piece showed axoneme (white arrow), 9 outer dense fibers (arrow head), ventral and dorsal columns (2 stars), circumferential ribs (yellow arrow) and flagellar membrane (red arrow). **(E)** Interstitial tissue with normal shaped Leydig cell (Ly) with oval euchromatic nucleus (N) and numerous mitochondria (yellow arrow), endothelial cell nucleus (red arrow) lined blood capillary (BC). **(F:H)** GLP-exposed group (GPII) illustrating **(F)** Disintegrated basal lamina (red arrow), shrunken heterochromatic nucleus of myoid cell with scalloped nuclear envelop (green arrow) and detachment of Sertoli (S) and spermatogonia (SG) from basal lamina (black line segment). Sertoli cell (S) with degenerated mitochondria (White arrow) and indented heterochromatic nucleus (N).Notice: spermatogonia (SG) showed large irregular heterochromatic nuclei**(G)** Sertoli cell showed large, oval heterochromatic nucleus (N) which appeared notched (yellow arrow), dilated sER (white arrow) numerous autophagic bodies (red arrows), lysosome(arrow head)and cytoplasmic vacuolation (red stars). Wide intercellular vacuoles (black stars) were noted**(H)** Spermatogonia (SG) revealed heterochromatic nuclei with irregularity of their envelopes (White arrow), cytoplasmic vesicles with electron dense materials(lysosomes) (yellow arrow), degenerated mitochondria (green arrows), cytoplasmic vacuolations (red stars) and increase intercellular spaces (black star).

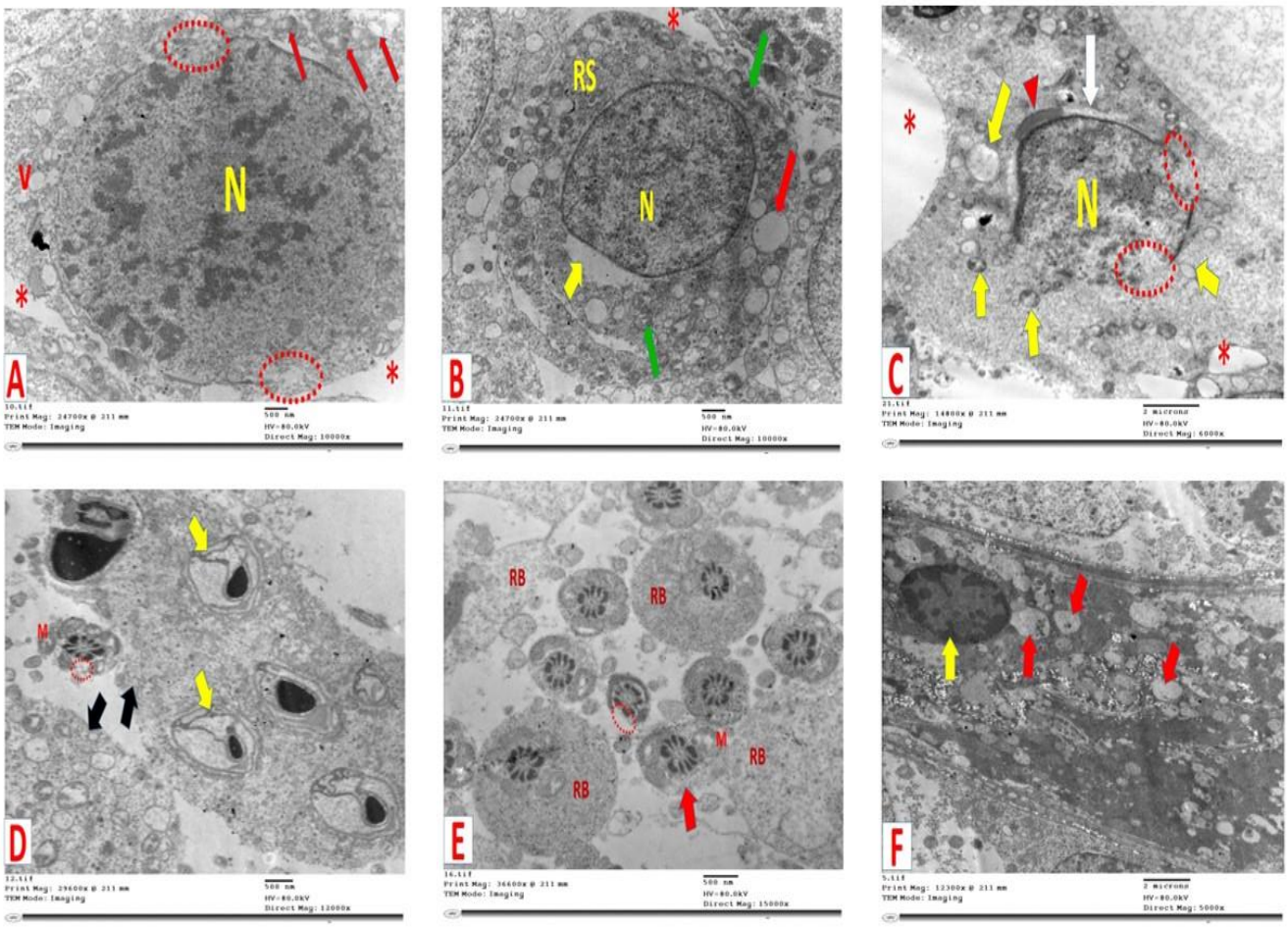

Fig. 2. Transmission electron micrographs of testicular tissue sections from GLP-exposed group (GPII) showed **(A)** primary spermatocyte showing large spherical nucleus with abnormal chromatin patterns (N),rupture of its nuclear envelop (dotted red circle), cytoplasmic vacuoles (V),mitochondria(red arrows) at different stages of degeneration and Intercellular spaces (stars) were also observed. **(B)**Round spermatid (RS) showing absence of acrosomal granule (yellow arrow), karyolytic nucleus (N) and swollen mitochondria (red arrow), others at different stages of degeneration (green arrows). Also, intercellular spaces (star) were detected. **(C)** Early spermatid at Golgi phase showing disintegration of nuclear envelop (dotted red circle) with shrunken karyolytic nucleus (N),presence of acrosomal granule(arrowhead),absence of acrosomal vesicle (white arrow) and mitochondria (yellow arrows) at different stages of degeneration. Wide intercellular spaces (stars) were also noticed. **(D)** Sections of spermatozoa showing deformed heads with wrinkled acrosomal cap (yellow arrows). Excess residual cytoplasm with degenerated mitochondria as residual bodies (black arrows) were also observed. Notice: cross sections through middle piece of tail showing irregular disorganized mitochondrial sheath (M) and destructed flagellar membrane (dotted red circle). **(E)** Cross sections through middle pieces of spermatozoa showing disorganization (red arrow) of mitochondria (M) and excess residual bodies (RB). Principle piece showing disintegration of its circumferential rib and flagellar membrane (dotted red circle). **(F)** Interstitial tissue showing Leydig cell with heterochromatic nucleus (yellow arrow) and increase vacuolation of Leydig cell cytoplasm and in interstitial tissue (red arrows).

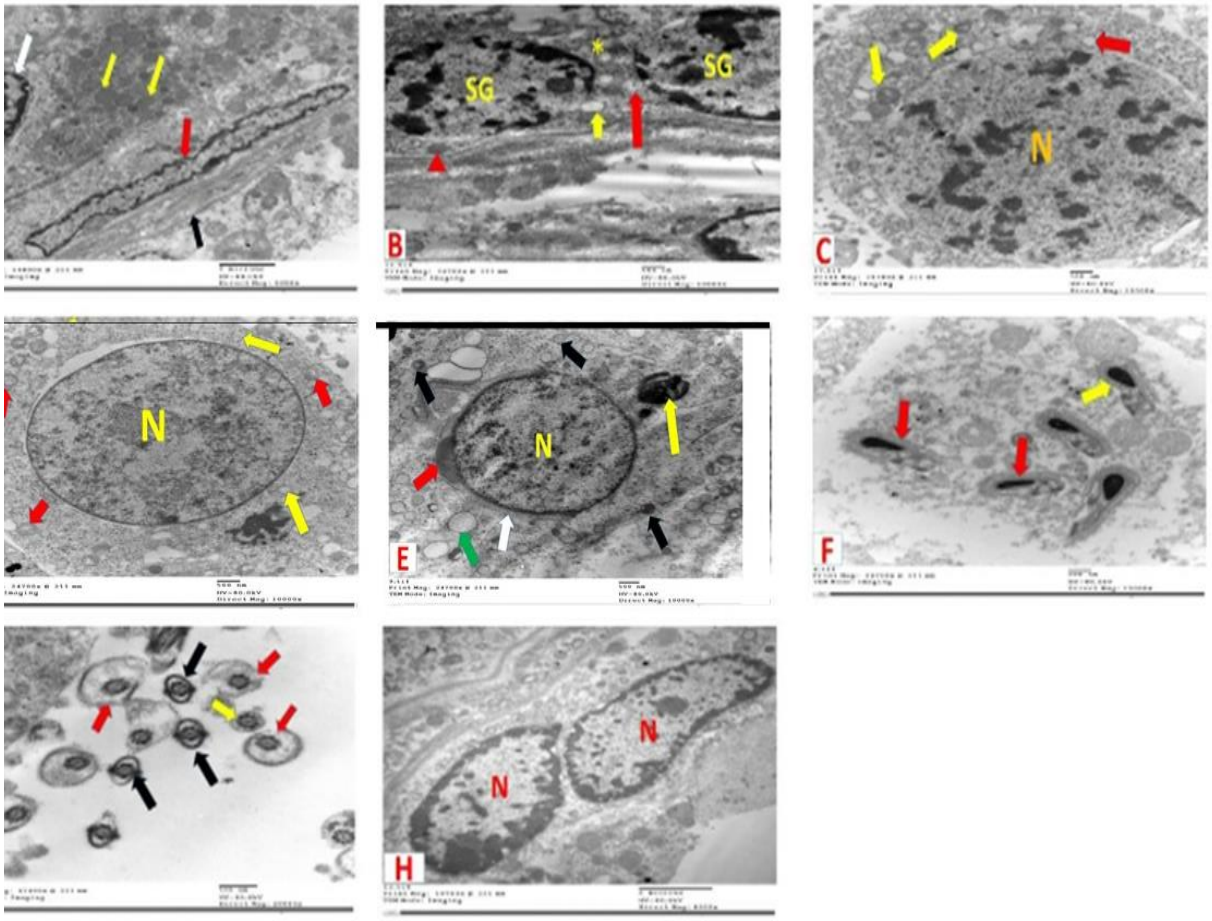

Fig. 3. Transmission electron micrographs of testicular tissue sections from NAC co-treated group (GPIII) showed **(A)** Myoid cell with normal nucleus (red arrow) and Sertoli cell showing normal mitochondria (yellow arrow) and nearly normal euchromatic nucleus (white arrow) rested on an intact basal lamina (black arrow). **(B)** spermatogonia (SG) with nearly normal nuclei, reduction in the intercellular space**s** (red arrow) and mitochondria showing different stages of recovery; degenerated (yellow arrow), partial recovered (star)and normal mitochondria (arrowhead). **(C)** Primary spermatocyte partially recovered with large spherical euchromatic nucleus (N) and most mitochondria with their normal histological appearance (yellow arrows), few still degenerated (red arrow) **(D)** Round spermatid exhibited large round euchromatic nucleus (N). Some mitochondria appeared nearly normal (red arrows) while others still degenerated (yellow arrow). **(E)** Early spermatid at Golgi phase had nearly normal structure: nearly normal nucleus (N), acrosomal granule (red arrow), acrosomal vesicle (white arrow) and mitochondria nearly normal (black arrows), others were degenerated (green arrow). Juxta-nuclear chromatoid bodies (arrowheads) were also observed in **D and E. (F)** Heads of spermatozoa began to restore their normal shape (red arrows), others appeared normal (yellow arrow) **(G)** Cross sections through spermatozoal tails revealed nearly normal principle pieces with normal circumferential ribs and flagellar membranes (black arrows). Some middle pieces appeared nearly normal (yellow arrow) while others still degenerated (red arrows). **(H)** Nearly normal interstitial tissue showing less vacuolation, normal shape and chromatin content of Leydig cells nucleus (N)