

PATHOLOGICAL ALTERATIONS IN MALE AND FEMALE ALBINO RAT (*RATTUS NORVEGICUS ALBINUS*) TREATED WITH HERPHOSATE

Nema M. EL-Abd

Plant Protection Research Institute, A.R.C., Dokky ,Giza, Egypt

(Received: Aug. 10, 2014)

ABSTRACT: *Herphosate was used at LC₅₀ (9.6% Herphosate bait crushed maize) and ½ LC₅₀ (4.8% Herphosate bait crushed maize) for 15 days offered to albino rat *Rattus norvegicus albinus* to show the toxicological effect. Histopathological and abnormal forms of sperm rat were studied at ½ LC₅₀. Cut sections were made for both untreated and treated rats either of male or female. After mating no pregnancy occur of all coupled that treated with herphosate (LC₅₀ & 0.5LC₅₀). Deformations of the sperm and all defects were observed. Abnormal forms may include deformed heads, middles, tails and immature forms of sperm. Histopathological samples of untreated and treated ovaries and testis were taken from rats. Ovary seemed with neoplasm of surface epithelium lining which send papillary like projection to the peneath tissues. Ovary also showed fibro adenocarcinoma with infiltration of eosinophil and tubular adenocarcinoma. Testis appear with necrosis of spermatogenesis series cells lining seminiferous tubules with congested blood vessels and devoid of sperm, some of blood vessels thrombosis were noticed.*

Key words: *Herphosate, LC₅₀, 1/2LC₅₀, toxicity, histopathological effects, albino rat, male and female.*

INTRODUCTION

Glyphosate (Nphosphonomethyl glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with commercial crops grown around the globe. It was discovered to be a herbicide by Monsanto Chemist (Franz 1974). Glyphosate's mode of action is to inhibit an enzyme involved in the synthesis of the aromatic amino acids tyrosine tryptophan and phenylalanine. It is absorbed through foliage and translocate to growing points. Because of this mode of action, it is effective on actively growing plants; it is not effective as a pre-emergence herbicide. Glyphosate was quickly adopted by farmers, even so when Monsanto introduced glyphosate-resistant crops, enabling farmers to kill weeds without killing their crops (Jaworski 1972). Glyphosate consumption in water caused hepatic, kidney failures and large mammary tumors in females and problems in males (Séralini *et al.*, 2012). The LC₅₀ of glyphosate for male and female albino rats

and acute toxicity were studied by (EL-Abd 2008).

Therefore, this work aimed to study the pathological effect of sub lethal dose, of herphosate on albino rat and their offspring.

MATERIALS AND METHODS

Pesticide tested: Herphosate used as SL9.6 & 4.8%. It is obtained from Monsanto company USA. It used post emergence herbicide.

Chemical name: Isopropylamine salt of N –(phosphonomethyl) glycine.

Experimental animals: Healthy and sexually mature, three month old male and female white albino rats were used as an experimental animals (*Rattus norvegicus albinus*). Agouza strain (190-200g) body weight were obtained from (Helwan farm, Cairo, Egypt). They were allowed to adaptation under the laboratory conditions. They were provided with water and balanced diet which consisted of crushed

maize throughout the adaptation and experimental period.

Toxicological and histopathological study: Lethal medium concentration LC₅₀(9.6 %) for herphosate were assayed according to the method of(Finney,1971). Mating have been made between either treated and untreated female and male and both of them, (Table 1 and 2).

Histopathological examinations:

Five individuals of control and treated animals were sacrificed at the end of experiments (15 days). The organs (testis and ovaries) were removed, washed and placed in formalin solution for histopathological study. The remaining rats were kept under laboratory condition for mating. Five females were killed at 2 and 4 days before the end of fertility period . Autopsy samples were taken from the testis and ovary of sacrificed rats and fixed in 10% formalin saline solution overnight then washed and soaked in tap water for 12 hours, dehydrated through ascending grades of ethanol concentrations ,cleared in xylene and embedded in paraffin- wax or paraplast. Serial sections of 3 micron thick were cut by sledge microtome. The sections were stained with hematoxylin and eosin stain and mounted on the glass slides for microscopic examination (Banchroft *et al.* 1996).

Deformation and Abnormal forms of sperm :

200ul of semen was taken by automatic micro pipette in a plastic tube containing 800 ul of normal saline (Na cl 0.9%) and few drops of formalin (40%) were added to keep sperm. The sperms were appearance by hemocytometer according to method described by Robb *et al* (1978). Morphology analysis evaluated the shape of the sperms and all its defects .

Statistical Analysis:

All the data were calculated as Mean± SD and analyzed by T- tests (Motulsky, 1987).

RESULTS AND DISCUSSION

Histopathological examination :

Histopathological effect of Herphosate in comparative with control were shown in figs. (1-10). As seen in these figures, it was clear that herphosate has carcinogenic effect (Figs. 5-7) on the female where it cause, neoplasm (tumor) of surface epithelium lining ovary which send papillary like projection to the peneath tissues (Fig. 5) in treated rats receiving of herphosate. Animals showed tubular adenocarcinoma (Fig. 6) ,Ovary fibro adenocarcinoma with infiltration of eosinophil (Fig.7). Thus herphosate caused ovary cancer for female rat . These results are not accordance with Mink *et al* (2012). The last authors reviewed relevant methodological and bio monitoring studies of glyphosate. Seven cohort studies and fourteen case-control studies examined the association between glyphosate and one or more cancer outcomes, they found no consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or children) or any site-specific cancer and exposure to glyphosate. But Cox(2002)mentioned that all products containing glyphosate are linked to cancer.Séralini *et al.*, 2012,proved that Glyphosate consumption in water caused hepatic, kidney failures and large mammary tumors in females and problems in male .

Herphosate caused pathological alterations on the male testis as it resulted necrosis of spermatogenesis series cells lining seminiferous tubules with congested blood vessels (Fig. 2), it prevented sperm formation (Fig. 3). Clair *et.al.* (2012) found that the pesticide has an endocrine impact at very low environmental doses, but only a high contamination appears to provoke an acute rat testicular toxicity. This does not anticipate the chronic toxicity which is insufficiently tested, and only with glyphosate in regulatory tests. Roundup exposure Leydig cells are damaged. Within 24-48h this formulation is also toxic on the other cells, mainly by necrosis , by contrast

Pathological alterations in male and female albino rat (rattus

to glyphosate alone which is essentially toxic on Sertoli cells. Later, it also induces apoptosis at higher doses in germ cells and in Sertoli/germ cells. Sirinathsinghiii (2012). found that glyphosate exposure reduced testosterone levels in mouse leydig cells. Amanda, (2005) cleared that pesticides cause lasting damage to rats sperm.

Cox,(2002) cleared that all products containing glyphosate are caused reproductive problems such as reduced sperm and increased numbers of dead Sperm and abnormal forms sperm.

Both LC₅₀ and LC₂₅ofHerphosate showed absence of embryos when males treated and crossed with either treated or untreated females with insignificant

differences . Therefore, we can conclude that the Herphosate may cause some sort of male sterility . This may be due to deformation in

Sperms caused by herphosate (fig 9-10).

DE Liz Oliveira *et al* (2013),found that acute Roundup exposure at low doses (36 ppm, 0.036 g/L) for 30 min induces oxidative stress and activates multiplestress-response pathways leading to Sertoli cell death in prepubertal rat testis.

Thus this pesticide can play a role in reducing population of rodents through its male sterility effect by using it as a bait . Great warning should be taken ,that it could affect human health.

Mating:

Table (1): Effect of LC₅₀ of Herphosate on the rat offspring's production.

Cross	Mean number of embryos ± SD
♂ _u × ♀ _u	6.00± 1
♂ _u × ♀ _t	0.00
♂ _t × ♀ _u	0.00
♂ _t × ♀ _t	0,00

U: untreated

t: treated

Table (2): Effect of 0.5 LC₅₀ofHerphosate on the rat offspring production.

Cross	Mean number of embryos ± SD
♂ _u × ♀ _u	6.00±1
♂ _u × ♀ _t	0.00
♂ _t × ♀ _u	0.00
♂ _t × ♀ _t	0.00

U: untreated

t: treated

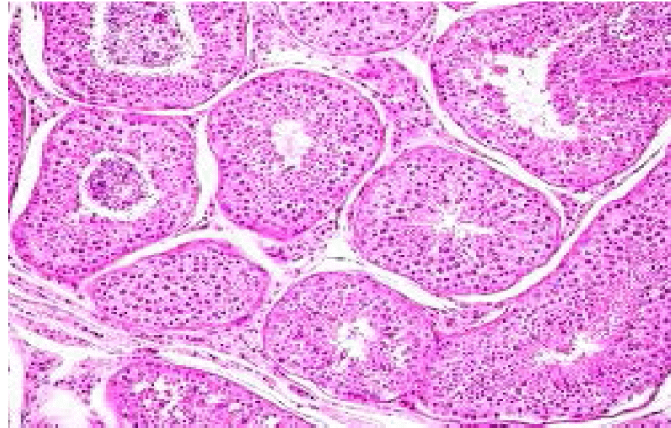


Fig. 1: The testis of control showed normal histological structure of the active mature seminiferous tubules and interstitial leydig cells . .H&E× 200

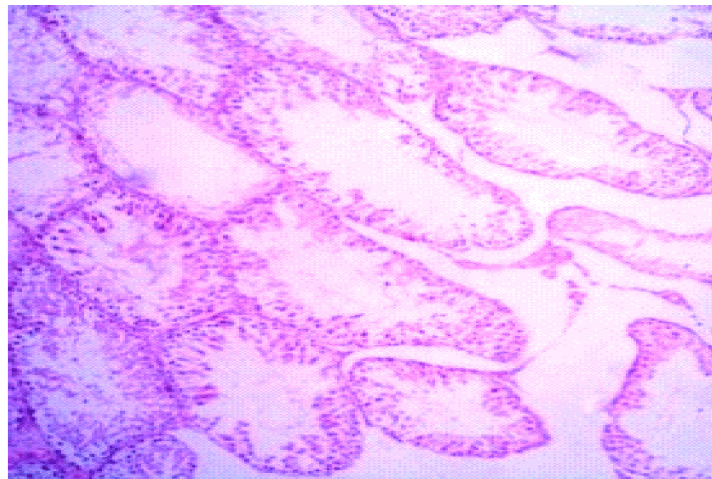


Fig. 2: Testis showing necrosis of spermatogenesis series cells lining seminiferous tubules with congested blood vessels . .H&E×400

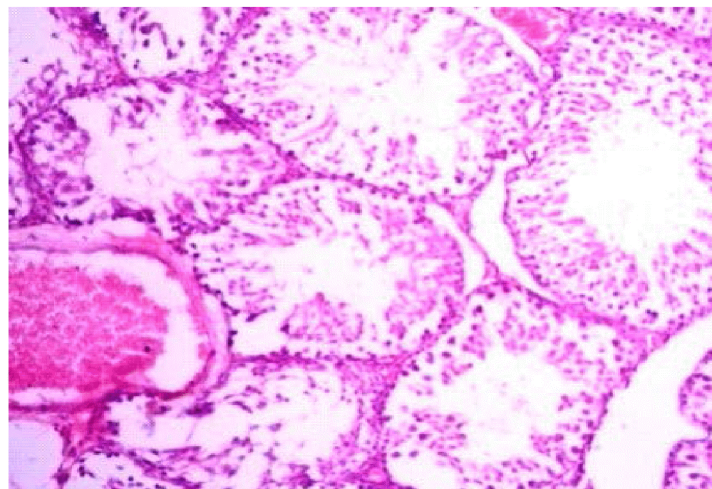


Fig. 3: Testis showing necrosis of spermatogenesis series cells and devoid of sperm, some of blood vessels thrombosis were appeared on the left side . H&E× 400

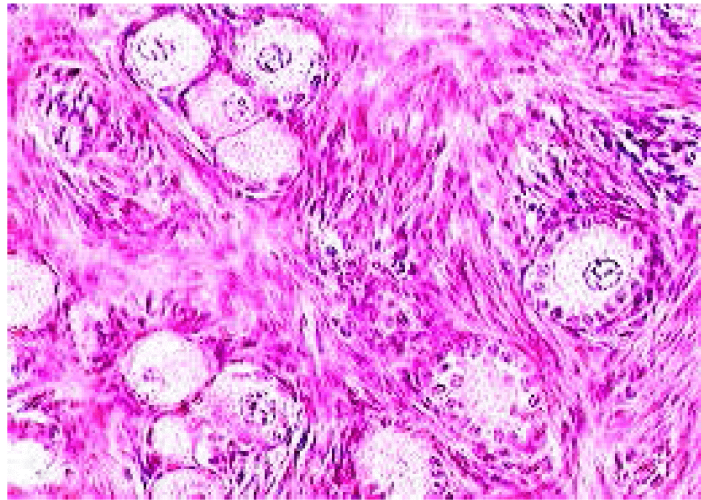


Fig. 4: Histological structure of ovary control rat.H&E×400

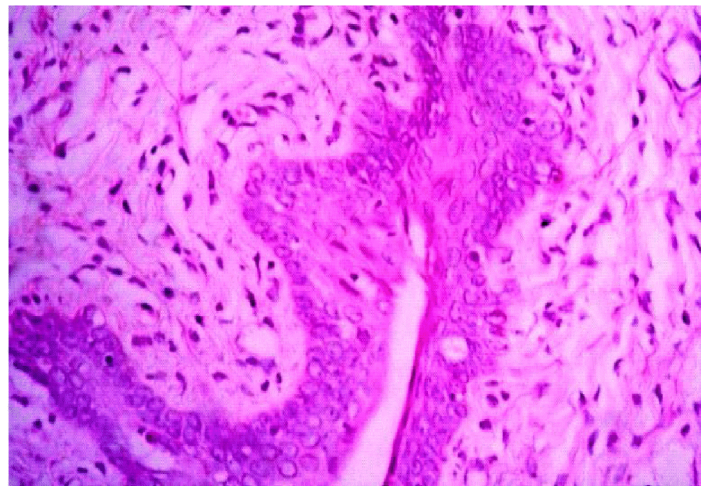


Fig. (5): Ovary showing neoplasm of surface epithelium lining ovary which send papillary like projection to the beneath tissues. H&E×400

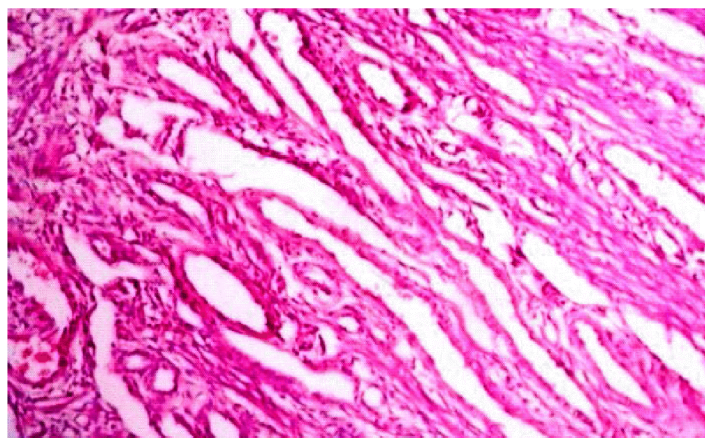


Fig.6 : Ovary showing adenocarcinoma (tubular) . H&E×400

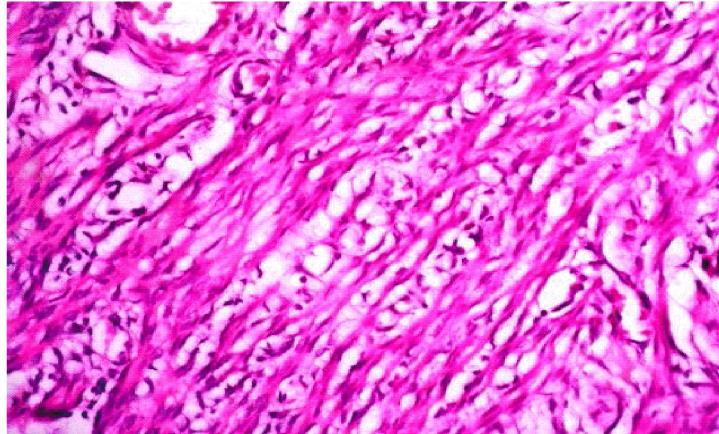


Fig.7 : Ovary showing fibro adenocarcinoma with infiltration of eosinophils H&E×400

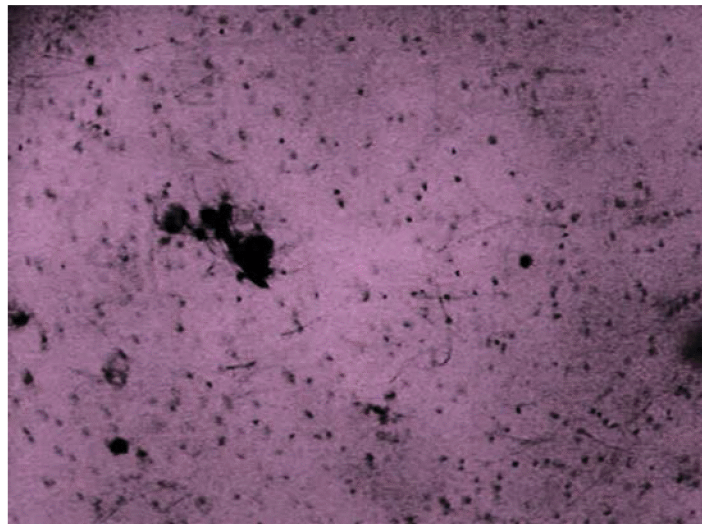


Fig. 8: A normal looking sperm has an oval head and a tail seven to fifteen times longer than the head.(×40)

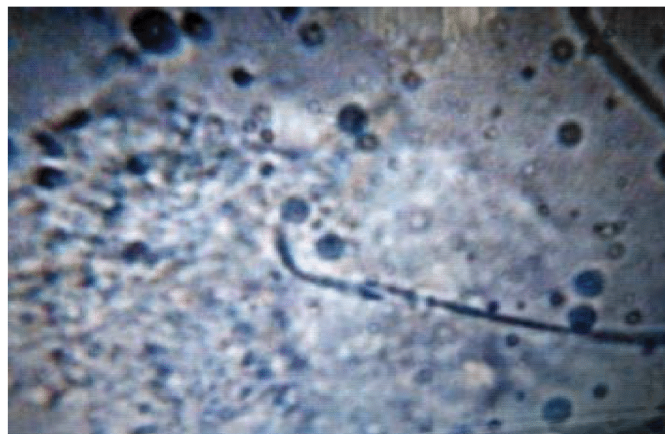


Fig.9: Abnormal forms including defective tails ×40.

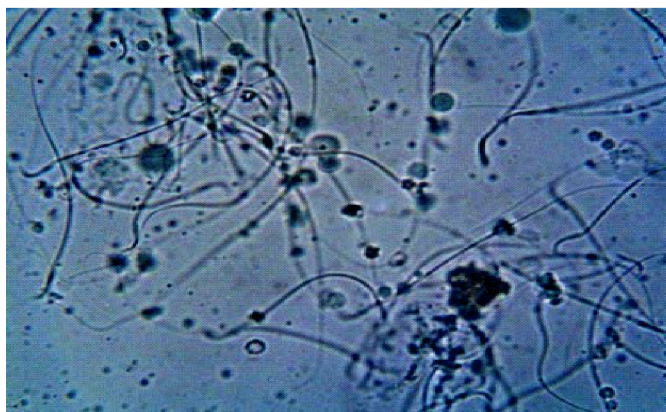


Fig. 10: Abnormal forms including defective heads, .x40)

ACKNOWLEDGMENT

The authoress is very indebted to Prof. Dr. Karima Mahmoud Azzam, Professor of Malacology and Head of the Department of Harmful Animals Research, Plant Protection Research Institute, for her kind help, valuable suggestions, kindly advice and encouragement throughout this investigation and reviewing the manuscript.

Thanks due to my colleague Al- Shaimaa Fathy for her kind help through the work.

REFERENCES

- Amanda, G. (2005). Pesticides causes lasting damage to rats sperm. Heath Day New N. Y. Sci. (3).
- Banchroft, J. D., A. Stevens and D.R. Turner (1996). Theory and practice of histological techniques. Fourth Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
- Clair, E., R. Mesnage, C. Travert and G.E. Seralini (2012). Glyphosate - based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro and testosterone decrease at lower levels. *Toxi. In vitro*. Mar. 26(2): 269-279.
- Cox, C. (2002). Facts about glyphosate. *Journal of pesticide Reform*. 18 (3)1-15.
- De Liz Oliveira, V.L., D. Cattani, C.E. Heinz Rieg, P. Piero Zan, L. Zanatta, E. Benedetti, D. Wilhelm, F.R. Mena Barreto, R. Pessoa and A. Zamoner (2013). Round up disrupts male reproductive functions by triggering calcium mediated cells death in rat testis and sertoli cells. *Free Radic Biol. Med. Dec.* 65: 335-346.
- EL-Abd, N. M. (2008). Harmful impacts of some pesticides on albino rat *Rattus norvegicus albinus*. PhD. Thesis, Fac. Agric. Minufiya Univ. P.149.
- Finney, D.J. (1971). Probit analysis. 3rd Ed. Combridge Univ. Press. London.
- Franz, JE, "N-phosphonomethyl-glycine phytotoxicant compositions", issued 1974-03-26, assigned to Monsanto Company.
- Jaworski, E.G. (1972). Mode of action of N-phosphonomethyl glycine inhibition of aromatic amino acids biosynthesis. *J. Agric. food Chem.* 20(6)1195-1198.
- Mink, P.J., J.S. Mandel, B.K. Scurman and J.L. Lundin (2012). Epidemiologic studies of glyphosate and cancer. *Regul. Toxicol. Pharmacology*. Aug. 63(3): 440-452.
- Motulsky, H.I. (1987). T.ease (tm) T-tests with ease, ISI. Soft were. Institute for scientific Information.
- Robb, G., R. Amann and G. Killian (1978). Daily sperm production and epididymal reserves of pubertal and adult rats. *J. Reproduction and fertility*, 54: 103-107.
- Séralini, G.E., E. Clair, M. Robin, G. Steeve, D. Nicolas, M. Manuela, H. Didier and S. d. V. Joël (2012). Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize, *Food Chem. Toxicol.*, 50: 4221-4231.
- Sirinath Singhii, E. (2012). Glyphosate kills rat testis cells. *The Institute of Science in Society*. 21(9):32.

التغيرات المرضية في ذكور وإناث الفأر النرويجي الألبينو المعاملة بمبيد الهيرفوسيت

نعمة محمد العبد

معهد بحوث وقاية النبات – مركز البحوث الزراعيه - مصر

الملخص العربي

تم استخدام مبيد الهيرفوسيت بتركيز النصف مميت ونصفه لمدته 15 يوم على الفئران البيضاء لدراسات السمية والتزاوج وتم استخدام فقط نصف التركيز النصف مميت في الدراسات الهستوباثولوجيه ودراسه التشوهات والاشكال الغير طبيعيه للحيوان المنوى.

تم اجراء التزاوجات بين الجنسين سواء المعامله وغير المعامله للفأر . سببت المعامله بتركيز النصف مميت ونصف التركيز النصف مميت عدم تكون الاجنه فى كل التزاوجات . كما ظهرت اشكال غير طبيعيه للحيوان المنوى وبعض العيوب تواجدت فى الرأس والعنق والذيل واشكال غير ناضجه للحيوان المنوى.

تم اخذ عينات الهستوباثولوجى من خصيه ومبيض الفئران التى تم ذبحها.

ظهر تورم فى سطح الخلايا الطلائية المبطنه للمبيض والتى ترسل حلمات مثل النتوءات الى انسجه بنيس . كما ظهر فى المبيض ورم سرطانى مع ارتشاح لخلايا الايزنوفيل وورم سرطانى انبوى.

ظهر فى الخصيه موت للخلايا المنتجه للحيوانات المنويه الموجوده فى الانابيب المنويه مع احتقان الاوعيه الدمويه وخلو خلايا الخصيه من الحيوانات المنويه وانتشار جلطات فى الأوعية الدمويه.