

HISTOPATHOLOGICAL EFFECTS OF GLYPHOSATE HERBICIDE ON DIFFERENT ORGANS OF MALE AND FEMALE ALBINO RATS

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ABSTRACT

The effect of major herbicide used worldwide glyphosate was studied on some organs of mature male and female albino rats (*Rattus norvegicus*). The LC₅₀ was determined for glyphosate was 9.7% as crushed maize bait. Animals were fed on bait treated with (1/2 LC₅₀) for 15 days. The organs were collected two weeks after treatment and fixed in 10% formalin saline. The results revealed that the liver of male showed necrosis of some hepatocytes with infiltration of mononuclear inflammatory cells, while the liver of females showed necrosis of some hepatocytes and dilation of blood vessels surrounded by fibrin with activation of vankopfer cells. Hemorrhage, infiltration of mononuclear inflammatory cells and necrosis of cells lining renal tubes were observed in the kidney of male, while in female ,granular degeneration changes of cells lining renal tubes with infiltration of mononuclear inflammatory cells and hyper cellularity of glomeruli with thrombosis of some blood vessels were recorded . The lung of male showed congestion of blood vessels and thickening of its wall, hyper proliferation of cells lining bronchial, infiltration of mononuclear inflammatory cell and thickening of interstitial wall, whereas hemorrhage, swelling of endothelial cells lining blood vessels, infiltration of mononuclear inflammatory cells and emphysema were observed in the lung of female.

Keywords: Glyphosate, *Rattus norvegicus* , histopathology.

INTRODUCTION

Glyphosate (N-phosphonomethyl glycine), the active ingredient in round up herbicides, inhibits the 5-enolpyruvylshikimate-3-phosphate synthase proteins (EPSPS) enzyme, thereby starving plants of aromatic amino acids (Steinrucken and Amrhein, 1980; Haslam, 1993 and Hammond *et al.*, 2004).

Toxicity evaluation of herbicides is generally performed on mammalian physiology through the long-term study of only their active principle, rather than the formulation used in agriculture, as the case for glyphosate (Williams *et al.*, 2000). It is important to note that glyphosate is only able to efficiently penetrate target plant organisms with the help of adjuvants present in the various commercially used round up formulations (Cox, 2004, Monosson, 2005, Cox and Sorgan, 2006 and Mesnage *et al.*, 2010) . El-Abd(2014) found that Herphosate caused neoplasm (tumor)of surface epithelium lining ovary of females albino rats treated with 1/2 LC₅₀ .Ovary also showed fibro adenocarcinoma with infiltration of eosinophil and tubular adenocarcinoma..

The liver and pancreas of mice were affected, as highlighted by disturbances in sub-nuclear structure (Malatesta *et al.*, 2008). Glyphosate and round up consumption in water caused hepatic, kidney failures and large

mammary tumors in females and organic problems in males (Séralini *et al.*, 2012).

Séralini *et al.*, (2007) and De Vendômois *et al.*, (2009) found that the alterations in kidney and liver functions of male rats which fed with the glyphosate. Séralini *et al.* (2012) revealed that the rat treated with glyphosate after two years suffered from hepatorenal failure which could be explained by the fact that the herbicide acted directed on liver and kidney.

The paraquat herbicide caused pulmonary fibrosis lead to death, meanwhile liver, kidney, heart and CNS were affected in male albino rat Karakani *et al.*, 2006). Smith and Heath (2014) reported lung cells degenerative changes consisting of swelling of mitochondria and vacuolation of lamellar bodies. Also disruption of the rough endoplasmic reticulum after 24 h. with paraquat herbicide, but after three days alveolar capillaries are congested with blood and there is alveolar pulmonary oedema.

The present work aims to study the effects of the glyphosate herbicide on some organs of male and female albino rats .

MATERIALS AND METHODS

I- Pesticide:

Herphosate herbicide SL 48% , obtained from Monsanto com. USA. It is used postemergence herbicide.

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

Commune name: Glyphosate .

Determination of half lethal concentration dose for rat (LC₅₀):

Mortality percentages were recorded up to 28 days after rats treatment with glyphosate (1.25 , 2.5 , 5 ,10 and 20 ml / kg of body weight) and LC₅₀ was calculated according to Finney (1971).

I I- Animal groups:

The present experiments were carried out on albino mature male and female rats *Rattus norvegicus* (190-200gm)which obtained from animal farm (Ministry of Health). Rats were kept at temperature 34 C0. throughout the period of the experiment, water and food were supplied *ad libitum*.

Feeding method:

Animals were divided into 2 groups (each 10 rats). The first group represented the control, which was fed on crushed maize and water only without any pesticide addition.. The second group was fed on crushed maize mixed with 1/2 LC₅₀ of herphosate. The consumed amount of bait was estimated daily for 15 days.

Non- choice feeding method:

Each animal was supplied with 50 gm of Glyphosate bait daily for five successive days. The daily consumed amount of bait was estimated. The treated bait was removed and the survivor rats were fed on untreated crushed maize and observed for 15 days. During this period , mortality rate was recorded.

Free choice feeding method:

Each animal was supplied with 25 gm of Glyphosate bait and 25 gm of untreated crushed maize daily for five days, then removed and untreated

crushed maize only introduced to the animals which observed for 15 days and mortality was recorded to determine the acceptability of Glyphosate according to Palmateer (1974).

The consumed amount of treated bait and untreated (diet) were recorded for 5 days .

$$\text{Acceptance \%} = \frac{\text{Treated bait consumption (gm)} \times 100}{\text{Treated bait consumption (gm)} + \text{Untreated (Diet) consumption (gm)}}$$

Organs collection:

After 15 days, samples were taken from the liver, kidney and lung of sacrificed rats and fixed in 10% formalin saline solution over night then washed in tap water for 12 hours. Serial concentrations of ethyl alcohol were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 3 micron thickness by slide microtome. The obtained tissue sections were mounted on the glass slides and stained by hematoxylin and eosin stain (Banchroft *et al.*, 1996) for histopathological examination.

RESULTS AND DISCUSSION

1- Acute toxicity and acceptance:

The LC₅₀ of glyphosate of male and female albino rats was calculated 28 days after treatment (Table,1). As shown in this table , LC₅₀ value was 9.7 mg/kg b. w. for glyphosate. Consumption of treated bait in free choice and non-choice feeding was represented in Table (2) which indicated that consumption of treated baits was 13gm in non-choice feeding and 7 gm in free choice feeding ,consequently the acceptance rate recorded 65% .

The rate of acceptance indicated that the rats accepted the bait, and that means glyphosate is suitable to be used in rat baits.

Table (1): Acute toxicity (LC₅₀) of glyphosate herbicide on male and female rats through 28 days.

Herbicide	Group rats (10 rats)	Dose (ml/kg)	Mortality %	LC ₅₀ (mg/kg)
Glyphosate	1	1.25	10	9.7
	2	2.50	20	
	3	5.00	30	
	4	10.00	50	
	5	20.00	70	

Table (2)Effect of Glyphosate bait on *Rattus norvegicus albinus* using non choice and free choice method feeding test for 5 days .

Feeding method	Average treated bait consumption (g)	Mortality %	Time to death (day)	
			Range	Mean
Non- choice	13 ± 3 (g)	70	1-3	2
Free- choice	7 ± 2 (g)	60	12.5	10- 15

2-Histopathological effect of herphosate on liver, kidney and lung organs:

As shown in Fig. (1) the liver of male albino rat showed necrosis of some hepatocytes with infiltration of mononuclear inflammatory cells, while the liver of female showed necrosis of some hepatocytes and dilation of blood vessels surrounded by fibrin with activation of vankopfer cell (Fig. 2) . While histological structure of normal (control) liver had no alteration observed and normal histological structure of the center veins, hepatic cords and sinusoids Fig. 3).

Hemorrhage, infiltration cells and necrosis of cells lining renal tubules was observed in male kidney treated with herphosate (Fig. 4) .While the kidney of female showed granular degeneration of cells lining renal tubes with infiltration of mononuclear inflammatory cell and hypercellularity of glomeruli with thromobosis of some blood vessels, (Fig. 5). In control group ,no alteration was observed in kidney and normal histological structure of the glomeruli and renal tubules in cortex (Fig. 6).

The lung appears with congestion of blood vessels and thickening of its wall, hyper proliferation of cells lining bronechiol, infiltration of mononuclear inflammatory cell and thickening of interstitial wall compared to the normal histological structure of lung (Fig 7), while in female it showed heamorrhage, swelling of endothelial cells lining blood vessels, infiltration of mononuclear inflammatory cells and emphysema (Fig. 8) compared with the normal histological structure of lung which had no change in alveolar cells, blood vessel and bronchiole (Fig. 9). The lung cell showed degenerative changes consisting of swelling of mitochondria and vacuolation of lamellar bodies. There was also disruption of the rough endoplasmi creticulum after 24 h., but after three days alveolar capillaries were congested with blood and there was alveolar pulmonary oedema. Neutrophil polymorphs are scattered about the lung.

The liver is the first target organ for toxicological prospects due to its role in detoxification, biotransformation and excretion of xenobiotics (Katzung, 1990).

In the present study, the animals treated with glyphosate suffered from congestion of cells displayed by the liver, this may be due to damage to the hepatocyte induced by the glyphosate. Séralini *et al.*,(2007) and De Vendômois *et al.*, (2009), found alterations in kidney and liver functions that may be the signs of early chronic diet intoxication, by herbicide residues of the glyphosate feed. The liver and pancreas of mice were affected, as highlighted by disturbances in sub-nuclear structure (Malatesta *et al.*, 2008). Toxic effect of very low dilutions of round up on apoptosis, mitochondrial function, and cell membrane degradation inducing necrosis of hepatocytes, and other cell lines (Benachour and Seralini, 2009; Benachour *et al.*, 2007; Gasnier *et al.*, 2010; Peixoto, 2005). Glyphosate consumption in water caused hepatic failure (Séralini *et al.*, 2012)

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The present results revealed that the inflammatory infiltration found in the kidney in treated animals may be due to the effect of the glyphosate. These results agree with those of Seralini *et al.* (2012) in the rat which treated with glyphosate after two years and caused renal failure.

Death in male rats was mostly due to the development of severe hepatorenal insufficiencies, confirming the first signs of toxicity (De Vendômois *et al.*, 2009). In females kidney, ion leakages were evidenced at the biochemical levels, when severe nephropathies were evidenced in dead males afterwards, at the anatomopathological level.

The disturbed kidney parameters may be induced by the reduction of phenolic acids in this study, since caffeic and ferulic acids are beneficial in the kidney as they prevent oxidative stress (Srinivasan *et al.*, 2005; Rehman and Sultana, 2011).

The present results revealed that the lung congestion of blood vessels and thickening of their wall, hyper proliferation of cells lining bronchioles, infiltration of mononuclear inflammatory cells and thickening of interstitial wall, which agree with the findings of Karakani *et al.* (2006) who reported that pulmonary fibrosis in rats treated with paraquat herbicide is the usual reason of deaths in the cases with intoxication. The intraalveolar mononuclear cells may be the first few profibroblasts to infiltrate the lung by paraquat herbicide. (Smith and Heath, 2014).

REFERENCES

- 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.
- Benachour, N. and Seralini, G.E. (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem. Res. Toxicol.*, 22: 97–105.
- Benachour, N.; Sipahutar, H.; Moslemi, S.; Gasnier, C.; Travert, C. and Seralini, G.E. (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch. Environ. Contam. Toxicol.* 53, 126–133.
- Cox, C. (2004): Herbicide factsheet– Glyphosate. *J. Pestic. Reform*, 24: 10–15.
- Cox, C. and Sargan, M. (2006): Unidentified inert ingredients in pesticides: implications for human and environmental health. *Environ. Health Perspect.*, 114: 1803–1806.
- De Vendômois, J.S.; Roullier, F.; Cellier, D. and Seralini, G.E. (2009): A comparison of the effects of three GM corn varieties on mammalian health. *Int. J. Biol. Sci.*, 5: 706–726.
- El-Abd, N.M. (2014): Pathological alterations in male and female albino rat (*Rattus norvegicus albinus*) treated with Herphosate. *Minufiya J. Agric. Res.* 39(5): 1711-1718.
- Finney, D.J. (1971): Probit analysis. 3ed. Cambridge Univ. Press. London.

- Gasnier, C.; Benachour, N.; Clair, E.; Travert, C.; Langlois, F.; Laurant, C.; Decroix- Laporte, C. and Séralini, G.E. (2010): Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J. Occup. Med. Toxicol.*, 5: 29.
- Hammonda, B.; Dudekb, R.; Lemena, J. and Nemeth, M. (2004): Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn food and *Chemical Toxicol.*, 42 : 1003–1014.
- Haslam, E. (1993): *Shikimic Acid: Metabolism and Metabolites*. J. Wiley and Sons, Chichester, England.
- Karakani, A.M.; Ghazi-Khansari, M. and Sotoudeh, M. (2006): Lisinopril ameliorates paraquat - induced lung fibrosis. *Clin Chim Acta*, 367(1-2):170-4.
- Katzung, B.G. (1990): *Basic and clinical pharmacology*, 3rd ed, Appleton and Lung Connecticut, USA.
- Malatesta, M.; Perdoni, F.; Santin, G.; Battistelli, S.; Muller, S. and Biggiogera, M. (2008): Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol. In Vitro*, 22: 1853–1860.
- Mesnager, R.; Clair, E. and Séralini, G. E. (2010): Roundup in genetically modified crops: regulation and toxicity in mammals. *Theorie in der Ökologie.*, 16: 31–33.
- Monosson, E. (2005): Chemical mixtures: considering the evolution of toxicology and chemical assessment. *Environ. Health Perspect.*, 113: 383–390.
- Palmateer, S. D. (1974). Laboratory testing of albino rats with anticoagulant rodenticides. *Proc. 6 th Vert. Pest. Conf. Anaheim. Calif.*, March 5(7): 63- 72 .
- Peixoto, F. (2005): Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. *Chemosphere*, 61: 1115–1122.
- Rehman, U. M. and Sultana, S. (2011): Attenuation of oxidative stress, inflammation and early markers of tumor promotion by caffeic acid in Fe-NTA exposed kidneys of wistar rats. *Mol. Cell. Biochem.*, 357:115–124.
- Séralini, G.-E.; Cellier, D. and de Vendomois, J.S. (2007): New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. *Arch. Environ. Contam. Toxicol.*, 52: 596–602.
- Séralini, G.E.; Clair, E.; Robin M.; Steeve G., Nicolas, D.; Manuela, M.; Didier, H., and Joël, S. d. V. (2012): Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize, *Food Chem. Toxicol.*, 50: 4221–4231.
- Smith, P. and Heath, D. (2014): The pathology of the lung in paraquat poisoning. *J. Clin. Path.*, 28, Suppl. (Roy. Coll. Path.), 9: 81-93.
- Srinivasan, M.; Rukkumani, R.; Ram Sudheer, A. and Menon, V.P. (2005): Ferulic acid, a natural protector against carbon tetrachloride-induced toxicity. *Fundam. Clin. Pharmacol.*, 19: 491–496.

- Steinru" cken, H.C. and Amrhein, N. (1980): The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochemistry Biophysica Research Communication, 94: 1207–1212.
- Williams, G.M.; Kroes, R. and Munro, I.C. (2000): Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul. Toxicol. Pharmacol., 31: 117–165.

**التأثيرات الهستوباثولوجية لمبيد الحشاش الجليفوسيد على أعضاء مختلفة لذكور
واناث الفئران البيضاء
نعمة محمد العبد
معهد بحوث وقاية النبات -الدقى -جيزة-مصر**

تم دراسة تأثير مبيد الحشاش الاكثر استخداما" عا لميا" الجليفوسيت على بعض اعضاء ذكور واناث الفئران البيضاء (الفأر النرويجي) . تم تقدير التركيز نصف المميت لجليفوسيت وهو ٩٧% .
تم تغذية الفئران بطعم جريش الذرة الذى يحتوى على نصف التركيز النصف مميت لمدة ١٥ يوم. تم تجميع العينات من الفئران النافقة والمذبوحة بعد نهاية التجربة من المعاملة ووضعها فى فورمالين ١٠% لثشريحها وتم دراسة التغيرات الهيستوباثولوجية (النسيجية المرضية) على الأعضاء.
اوضحت النتائج حدوث موت لبعض خلايا الكبد مع ارتشاح للخلايا الليمفاوية فى كبد ذكور الفئران المعاملة بينما حدث موت لخلايا الكبد مع اتساع للاوعية الدموية المحاطة با خلايا مع تليف لخلايا فينكوبيفر فى اناث الفئران المعاملة. و حدث نزيف مع ارتشاح وموت للخلايا المبطنة للاوعية الناقلة فى كلىة ذكور الفئران المعاملة ولكن فى كلىة اناث الفئران البيضاء حدث تحطم حبيبي و تغيرات فى الخلايا المبطنة للانابيب الكلوية مع ارتشاح فى الخلايا الليمفاوية مع زيادة الخلايا و حدوث جلطات فى الاوعية الدموية. وجد أيضا احتقان فى الاوعية الدموية فى رئة ذكور الفئران المعاملة مع تغلظ لجدار الاوعية وزيادة تكاثر الخلايا المبطنة للشعب الهوائية مع ارتشاح للخلايا الليمفاوية و تغلظ لجدار الخلايا بينما حدث نزيف وتغلظ للخلايا المبطنة للاوعية الدموية وارتشاح للخلايا الليمفاوية والامفيزيم فى رئة اناث الفئران المعاملة.