



## Histopathological Changes in The Tongue, Palate and Parotid Gland After Exposure To Glyphosate

Atef I. Ahmed<sup>(1)</sup> and Badawy M. Abd-El-Hameed<sup>(2)</sup>

Codex : 03/1810

Aadj@azhar.edu.eg

### KEYWORDS

*Glyphosate, Tongue, Palate,  
Parotid salivary gland*

### ABSTRACT

Nowadays, many herbicides are used for pest resistance purposes. One of these is glyphosate. Although, the glyphosate is a broad-spectrum herbicide, it may be toxic to humans. The purpose of the present study was to determine the glyphosate-induced histopathological changes in the parotid salivary gland, tongue, and palate after sub-chronic exposure of rats to glyphosate. The present investigation was carried out on twenty healthy adult male Sprague-Dawely rats. The rats were exposed to single dose of the glyphosate (15 mg/kg body weight) for the period of 2 weeks which was the duration of the study. At the end of the experiment, the rats were sacrificed and sections of the parotid salivary glands, tongue and palatal mucosa were taken for histopathological preparation and examination. The results demonstrated that, in the experimental glyphosate treated group, there are remarkable changes in the tongue, palate or the parotid salivary gland. It was concluded that glyphosate may had adverse effect on tongue, palate and parotid salivary glands.

### INTRODUCTION

The glyphosate is a broad-spectrum herbicide widely used to kill unwanted plants both in agriculture and in nonagricultural landscapes. The products containing glyphosate are toxic to animals, including humans. The symptoms include eye and skin irritation, cardiac depression, gastrointestinal pain, vomiting, and accumulation of excess fluid in the lungs. Feeding of glyphosate for three months caused reduced weight gain and diarrhea. Glyphosate-containing products have caused genetic damage in human blood cells, fruit flies, and onion cells. <sup>(1)</sup>

The glyphosate is an aminophosphonic analogue of the natural amino acid glycine. Like all amino acids, it exists in different ionic states depending on pH. There are many regulatory and scholarly reviews which have evaluated the relative toxicity of glyphosate as an herbicide. It was found that “the available data is contradictory and far

1. Assistant Professor, Department of Oral Biology, Faculty of Oral and Dental Medicine, Al-Azhar University, Assiut, Egypt.
2. Lecturer, Department of Oral Biology, Faculty of Dentistry, Assiut University, Egypt.

from being convincing” with regard to correlations between exposure to glyphosate formulations and risk of various cancers, including non-Hodgkin lymphoma (NHL). Also, increased risk of NHL in workers exposed to glyphosate formulations was observed<sup>(2)</sup>. Generally, the glyphosate was classified as “probably carcinogenic in humans” based on epidemiological studies, animal studies, and in vitro studies<sup>(3-5)</sup>.

Reports on glyphosate, was concluded that “the substance is unlikely to be genotoxic (i.e. damaging to DNA), or to pose a carcinogenic threat to humans. Furthermore, other report clarified that while other, probably carcinogenic, glyphosate-containing formulations may exist, studies “that look solely at the active substance glyphosate do not show this effect<sup>(6)</sup>. In addition, it was concluded that “glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet, even at doses as high as 2,000 mg/kg body weight orally<sup>(7)</sup>. On the other hand, other review found no support for a causal relationship between glyphosate exposure and the risk of NHL or of multiple myeloma<sup>(8)</sup>.

Glyphosate has low persistence and, because repeated applications of this herbicide are practiced for the control of weeds in agricultural fields, large quantities find their way into water bodies. The indiscriminate use of the herbicide therefore makes it a potential source of danger to animals, not only in grazing fields but also in the water bodies<sup>(9)</sup>.

Glyphosate had also been reported to induce oxidative stress in animals<sup>(10)</sup>. Oxidative stress has been implicated in the molecular mechanisms of glyphosate toxicity<sup>(11)</sup>. The body responds to oxidative stress by evoking the enzymatic defense system within the body<sup>(10)</sup>. The aim of this study was to visualize the glyphosate-induced histopathological changes in the parotid salivary

gland, tongue, and palate after sub-chronic exposure of rats to glyphosate.

## MATERIALS AND METHODS

Twenty healthy adult male Sprague-Dawley rats, 160–200 g body weight, were obtained, housed for 2 weeks before the commencement of the experiment which lasted for 2 weeks. They were fed appropriately using standard rat chow (Growers mash, maize offal and ground nut cake in the ratio of 4:2:1, respectively) and water was provided ad libitum. The rats used for the study were randomized into control and experimental group. Group I served as the control and were administered 2 ml/kg of distilled water daily. Group II served as the experimental and were administered glyphosate 15mg/kg body weight and distilled water for a period of two weeks at the concentration of 1:2 glyphosate and distilled water, respectively. The rats were monitored for clinical signs and death.

At the end of the experiment, the rats were sacrificed and sections of the parotid salivary glands, tongue and palatal mucosa were taken for histopathological preparation and examination. The samples collected were fixed in 10 % buffered neutral formalin. They were processed for histopathological assessment and viewed under light microscope<sup>(12)</sup>.

## RESULTS

### Parotid gland

The parotid glands of the rats of the control group showed well known histologic features with no observable microscopic lesions. In the experimental group there were atrophic and degenerated acinar cells. Glands in this group also showed proliferation of the ducts, thick capsule and increase number of degenerated ducts in some specimens(Fig. 1A, B).



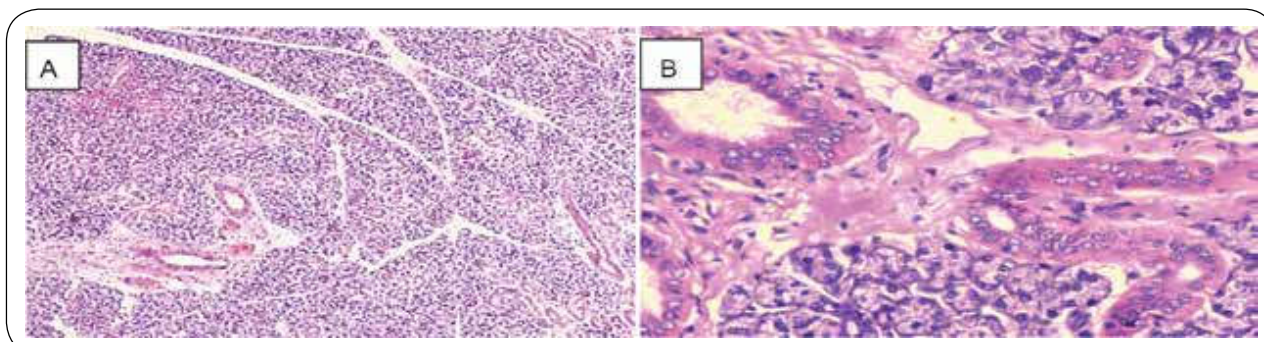


Fig. (1) Photomicrograph of the parotid gland. A & B) H & E stained sections of rats exposed to glyphosate. Degenerated acinar and ductal cells were observed.

### Palate

No observable microscopic lesions were noted in the palatal mucosa of the rats of the control group (Fig. 2A). But, in the experimental group, moderate palatal hyperkeratosis were observed. Mucosa in this group showed degenerated epithelial cells and the stratum granulosum was widened and prominent. The underlying connective tissue was composed of collagen bundles often degenerated and separated (Fig. 2B).

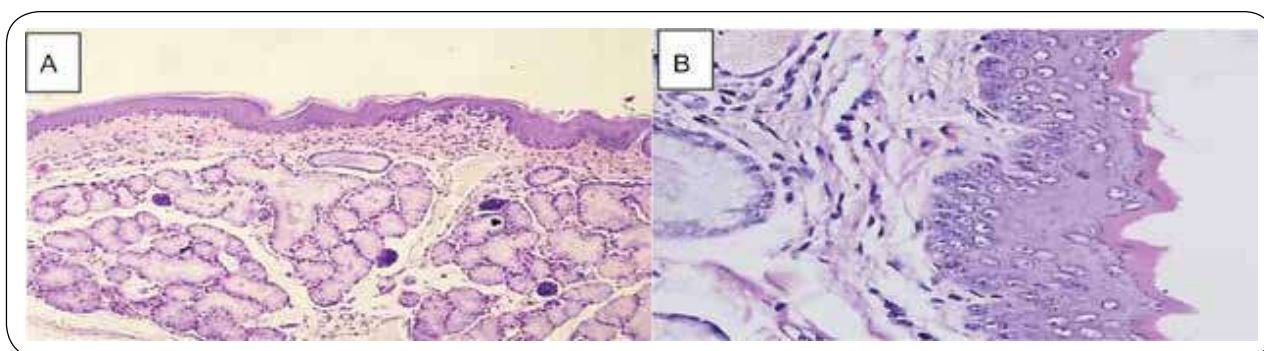


Fig. (2) Photomicrograph of the palate A) H & E stained sections of rats exposed to distilled water only. No observable microscopic lesions were noted. B) H & E stained sections of rats exposed to glyphosate. Palatal hyperkeratosis, degenerated epithelial cells were noted. The collagen bundles were degenerated and separated.

### Tongue

The tongue of the rats of the control group showed normal histologic features with no observable microscopic lesions (Fig.3A). The dorsal surface revealed prominent filiform papillae. These papillae are regular in the size and shape. The connective tissue and muscles were well formed. In the experimental group there was irregular appearance with focal hyperkeratosis of the covering epithelium. In some areas, hyaline degeneration in the basal and parabasal layers was observed. The underlying connective tissue was granular and atrophic with, mononuclear cells infiltration. Regeneration of the muscle fibers was also seen (Fig. 3B).

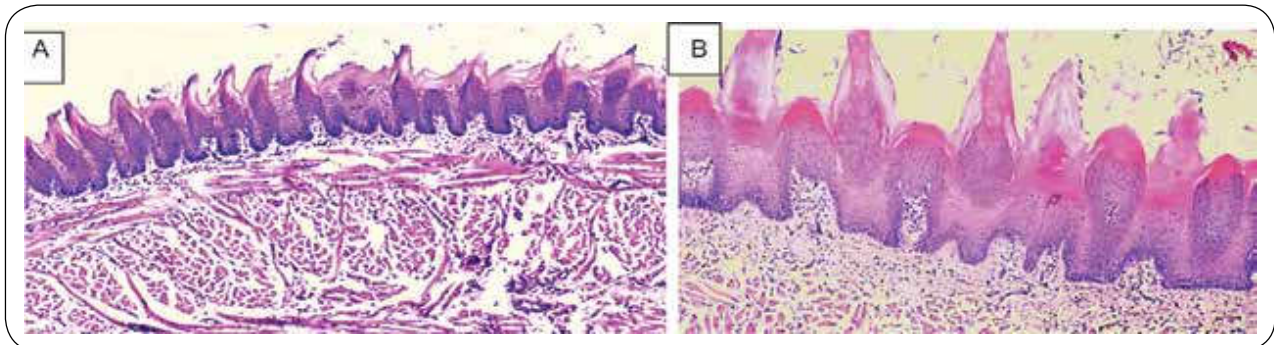


Fig. (3) Photomicrograph of the tongue A) H & E stained sections of a rat exposed to distilled water only. No observable microscopic lesions were noted. B) H & E stained sections of a rat exposed to glyphosate. Irregular appearance and focal hyperkeratosis were seen. The connective tissue was granular and the muscle fibers were degenerated.

## DISCUSSION

Several animal studies revealed that, feeding of glyphosate caused reduced weight gain, diarrhea, and salivary gland lesions. Lifetime feeding of glyphosate caused excess growth and death of liver cells, cataracts and lens degeneration, and increases in the frequency of thyroid, pancreas, and liver tumors. Glyphosate-containing products have caused genetic damage in human blood cells, fruit flies, and onion cells<sup>(1)</sup>.

Histologically, the rats had no observable microscopic changes in their parotid glands, tongue and palatal mucosa in the control group in the present study. But, the histological examination of parotid glands specimens exposed sub-chronically to glyphosate in the experimental group showed degenerated acinar and ductal cells which involved the glands which might be sequel to the oxidative damage in these cells. Beuret et al. 2004; Vivian and Claudia has been reported that the pathophysiology of glyphosate toxicity through oxidative stress<sup>(10-11)</sup>. Similarly, Kammon et al. recorded degeneration of glandular acini, mild necrosis of the glandular cells and mild degeneration of beta islets of Langerhans with distension of interlobular septa of pancreas probably induced by oxidative stress in chickens<sup>(13)</sup>.

The histopathologic lesions seen in the palate of the rats sub-chronically exposed to glyphosate in the experimental group were palatal hyperkeratosis and degeneration of the epithelial cells. The underlying

connective tissue was composed of collagen bundles often degenerated and separated. These effects might be through the direct toxic effect of glyphosate on the cell function (Kammon et al.)<sup>(13)</sup>, which might involve reactive free radicals, oxidative stress or both (Alden and Frith)<sup>(14)</sup>. Histopathological examination in the tongue in the present study showed hyperkeratosis of the covering epithelium, hyaline degeneration in the basal and parabasal layers. The underlying connective tissue was granular and atrophic with, mononuclear cells infiltration. Regeneration of the muscle fibers were seen in the glyphosate treated group. These findings are similar to the result of previous study by Ayoola, who reported neuronal degeneration, mononuclear cells infiltration and severe spongiosis in catfish exposed to acute concentrations of glyphosate<sup>(9)</sup>.

Glyphosate appears to be a potent immunosuppressive agent. The probable immunosuppressive effect of glyphosate was reported by Emmanuel et.al and Blackley<sup>(15-16)</sup>. The findings of this study agree with Emmanuel et.al who concluded that glyphosate caused degeneration in the mucosal epithelial cells and the glands in the stomachs, kidneys, brains, pancreas and livers in the rats. Additionally, there were mononuclear cells infiltration into the interstices and tubular necrosis in the kidneys and depopulated splenic cells<sup>(15)</sup>. Finally, the findings of this study conclude that had adverse effect on tongue, palate and parotid salivary glands.



## REFERENCES

1. Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Benbrook CM. "Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement". *Environmental Health*. (February 17, 2016), 15 (19): 13.
2. Schinasi L, Leon ME. "Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis". *International Journal of Environmental Research and Public Health*. 2014; 11 (4):4449–527.
3. Guyton KZ, Loomis D, Grosse Y, Guha N, Scoccianti C, Mattock H, Straif K. "Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate". *The Lancet. Oncology*. 2015; 16 (5): 490–1.
4. Mink PJ, Mandel JS, Scourman BK, Lundin JI. "Epidemiologic studies of glyphosate and cancer: a review". *Regulatory Toxicology and Pharmacology*. (Aug 2012), 63 (3): 440–52.
5. Chang, Ellen T, Delzell, Elizabeth. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers". *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes*. (2016), 51 (6): 402–434.
6. Sribanditmongkol P, Jutavijittum P, Wunnapak K, Durongkadech P "Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report". *The American Journal of Forensic Medicine and Pathology*. (Sep 2012). 33 (3): 234–7.
7. Mann RM, Hyne RV, Choung CB, Wilson SP. "Amphibians and agricultural chemicals: Review of the risks in a complex environment". *Environmental Pollution*. (2009) 157 (11): 2903–2927
8. Acquavella, John, Garabrant, David, Marsh, Tom, Weed, Douglas L. "Glyphosate epidemiology expert panel review: a weight of evidence systematic review of the relationship between glyphosate exposure and non-Hodgkin's lymphoma or multiple myeloma". *Critical Reviews in Toxicology*. September 2016; 46 (sup1): 28–43. doi:10.1080/10408444.2016.1214681.
9. Ayoola SO. Histopathological effects of glyphosate on Juvenile African Catfish ( *Clarias gariepinus*) *Am-Eur J Agric Environ Sci*. 2008;4(3):362–367.
10. Vivian DCL, Claudia BRM. Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comp Biochem Physiol C: Toxicol. Pharmacol*. 2007;147(2):222–231.
11. Beuret CJ, Fanny Z, Maria SG. Effects of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses. *Reprod Toxicol*. 2004; 19(4):501–504.
12. Baker J, Silverton RE and Pillister CJ (2000) Dehydration, impregnation, embedding technique and section preparation. *Introduction to Medical Laboratory Technology*, 2000; 7th edition, pp. 199–242.
13. Kammon AM, Brar RS, Sodhi S, Banga HS, Singh J, Nagra NS. Chlorpyrifos chronic toxicity in broilers and effect of vitamin C. *Open Vet J*. 2011;1:21–27.
14. Alden CL, Frith CH. Urinary system. In: Hashek WM, Rousseaux CG (ed) 1992. *Handbook of toxicologic pathology*. Academic Press, San Diego, pp. 316–379.
15. Emmanuel Vandi Tizhe, Butcher Danladi Jonathan George. Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate. *Comp Clin Path*. 2014; 23(5): 1535–1543.
16. Blakley BR. Effect of round up and tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*. 1997; 39:204–206