Effect of the herbicide Glyphosate on renal tissues in adult female rats
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Abstract

Introduction:
Glyphosate is a herbicide with great toxicity, and is more commonly known as 'Roundup' and is formulated as isopropylamine salt. The aim of this study was to investigate the possible effects of the herbicide glyphosate on the renal tissue of female rats.

Materials and Methods:
50 female rats were randomly divided into 5 groups of control, sham and experimental groups 1, 2, and 3. The three experimental groups 1, 2 and 3 received intraperitoneal glyphosate 50, 100 and 200 mg/kg body weight daily for 14 days, respectively. Then, the rats were anesthetized and their kidneys were removed and weighted. Tissue section was prepared and studied by light microscope. Urea nitrogen, uric acid and creatinine in the blood were measured.

Results:
Proximal tubule diameter in groups 2 and 3 compared to that of the sham and control groups and distal tubule diameter in group 2 compared to that of the control group were significantly increased. Urea nitrogen, uric acid and creatinine levels increased in the Experimental Groups. Other tissue changes such as lymphocytic infiltration, congestion of the kidney and renal cells damage in the experimental group 3 was harder than that of the groups 1 and 2.

Conclusion:
Glyphosate causes inflammation and damage to the renal tissue. So this herbicide should be handled with caution.

Keywords: Glyphosate, Kidney, Rats

Introduction
Glyphosate is a widely used herbicide for controlling weeds and is generally known by the trade names of Roundup or Sting. This pesticide was registered in 1970 by Monsanto Company in the USA and is categorized under phosphorus pesticides (1). Glyphosate is a common herbicide that is easily absorbed through the foliage of weeds and is transported to the roots through xylem and phloem and destroys the root tissue. Depending on the amount of glyphosate used by the farmers, the half-life of glyphosate in the soil varies between 1 and 147 days while the average duration is estimated at 47 days (2). Glyphosate destroys plants by interfering with synthesis of the amino acids

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phenylalanine, tyrosine and tryptophan. Through this interference, glyphosate prevents chorismate—the raw material for the formation of aromatic amino acids—from forming by inhibiting the enzyme 1-5-enolpyruvylshikimate-3-phosphate involved in the formation of 5-enolpyruvylshikimate-3 (3). The impact velocity of phosphorus pesticides is extremely high and causes severe poisoning and side effects such as dizziness, diarrhea, impaired vision, body tremors, numbness and fatigue in humans and warm-blooded animals (4). This herbicide also causes mild irritation in the mouth, gastrointestinal weakness, sore throat, dysphagia, gastrointestinal bleeding and blood pressure drop (5). People exposed to glyphosate have a higher risk for developing cancer (6). Studies have shown that this herbicide passes through human placenta cells and causes endocrine disorders (7). In rats, this pesticide increases antioxidant levels in the mother’s and the fetus’ serum and decreases intestinal activity and liver enzymes (8-9). Kidney is the main organ in the body responsible for excreting metabolic waste including most toxins and foreign substances such as insecticides, drugs, food additives, etc. produced or consumed by the body (10).

Given the heavy use of glyphosate as a strong herbicide and its risks for farmers, growers, the environment and other organisms, the present study was conducted to investigate the effect of glyphosate on the renal tissue of rats in order to warn consumers of this herbicide about its proper user guide and safety issues.

**Materials and Methods**

This research is an experimental study observing all the ethical issues regarding the manner of working with laboratory animals. For the purpose of this study, 50 adult female rats weighing between 180 and 200 grams and aged between 110 and 120 days were procured from the Laboratory Animal Center of the Islamic Azad University of Jahrom. The rats were housed in Azad University of Jahrom’s Animal House for a week in order to adapt to the environment.

The animals were fed with rat feed provided by Shiraz Livestock and Poultry Manufacturing Company. Room temperature was 22±2°C and relative humidity was 55-50%. The photoperiod was taken to be 12 hours of light and 12 hours of darkness. The rats were randomly divided into 5 groups of 10 each including the negative control group, the sham control group (receiving 1 ml normal saline solution as a glyphosate solvent) and the experimental groups 1, 2 and 3 (each receiving 50, 100 and 200 mg/ kg of glyphosate, respectively).

Concentration was determined using the LD50 method and the lethal dose was estimated at 800 mg per kg based on the present study. The rats were intraperitoneally injected with 41% glyphosate for two weeks. Blood collecting and dissecting the animals were both carried out between 10 and 12 o’clock in the morning. Twenty four hours within the final injection, the animals were anesthetized by ether and their blood sample was collected directly from the heart using a syringe. The collected blood was transferred to a test tube and was kept in a hot water bath for 30 minutes. It was then centrifuged at 2500 rpm for 10 minutes and its serum was separated using a sampler and then transferred into an Eppendorf tube. The serum levels of urea nitrogen, uric acid and creatinine were measured in the laboratory.

The rats’ renal tissue was removed from their bodies, weighed on a digital scale with an accuracy of 001.0 g (AND model, Japan) and then deposited in 10% formalin solution. After the stabilization, dehydrating, clearing and molding stages were completed, tissue sections with a thickness of 5 microns were prepared with a microtome and then stained using Hematoxylin - Eosin. Slides were observed using the
Dino software installed on the microscopic monitoring system attached to the computer and then the kidney nephron components were measured. Data were analysed using SPSS software v.18 and through the ANOVA and Duncan tests. The statistically significant level was set at p-values less than 0.05.

Results
The findings of the study show that the weight of the rats in all three experimental groups dramatically decreased compared to the negative controls (p<0.05). The weight of the rats’ left and right kidneys increased in all three experimental groups compared to the negative controls, but the increase was not statistically significant (Table 1).

The proximal tubule diameter in the experimental groups 2 and 3 and the distal tubule diameter in experimental group 2 significantly increased compared to the negative controls (p<0.05). The loop of Henle diameter decreased significantly in experimental group 2 compared to the sham controls (p<0.05) (Table 1). Blood urea nitrogen levels in all the three experimental groups and uric acid level in experimental group 3 significantly increased compared to the negative controls. Although serum creatinine levels in the experimental groups had increased compared to the negative controls, this increase was not statistically significant (Figure 4). Diameters of other parameters measured including the cortex, medulla, capsule, glomerulus, and corpuscle and collecting tubule did not show a significant change compared to the negative controls (Table 1).

Bladder urea nitrogen levels in all the three experimental groups and uric acid level in experimental group 3 significantly increased compared to the negative controls. Although serum creatinine levels in the experimental groups had increased compared to the negative controls, this increase was not statistically significant (Figure 4). Diameters of other parameters measured including the cortex, medulla, capsule, glomerulus, and corpuscle and collecting tubule did not show a significant change compared to the negative controls (Table 1).

Table 1: Comparison of parameters measured in the different groups of rats after receiving different doses of glyphosate

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Negative Control</th>
<th>Sham Control</th>
<th>Dose 1 50mg/kg</th>
<th>Dose 2 100mg/kg</th>
<th>Dose 3 200mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>106.4±4.44a</td>
<td>105.7±6.12a</td>
<td>104.3±4.26a</td>
<td>103.0±3.27a</td>
<td>100.6±1.98a</td>
</tr>
<tr>
<td>Slaughter weight</td>
<td>157.2±3.56a</td>
<td>159.9±4.68a</td>
<td>154.6±2.91a</td>
<td>150.4±3.24b</td>
<td>146.8±5.33b</td>
</tr>
<tr>
<td>Right kidney weight</td>
<td>0.590±0.029a</td>
<td>0.692±0.013b</td>
<td>0.651±0.017ab</td>
<td>0.623±0.020a</td>
<td>0.623±0.019a</td>
</tr>
<tr>
<td>Left kidney weight</td>
<td>0.565±0.030a</td>
<td>0.640±0.007a</td>
<td>0.635±0.016a</td>
<td>0.596±0.024a</td>
<td>0.643±0.023a</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.23±0.007a</td>
<td>0.218±0.009a</td>
<td>0.232±0.009a</td>
<td>0.207±0.006a</td>
<td>0.221±0.009a</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.416±0.029a</td>
<td>0.37±0.040a</td>
<td>0.416±0.015a</td>
<td>0.377±0.029a</td>
<td>0.369±0.022a</td>
</tr>
<tr>
<td>Capsule</td>
<td>0.041±0.002a</td>
<td>0.037±0.001a</td>
<td>0.038±0.001a</td>
<td>0.040±0.001a</td>
<td>0.042±0.001a</td>
</tr>
<tr>
<td>Glomerulus</td>
<td>0.39±0.014a</td>
<td>0.41±0.018a</td>
<td>0.409±0.007a</td>
<td>0.404±0.01a</td>
<td>0.412±0.011a</td>
</tr>
<tr>
<td>Corpuscle</td>
<td>0.47±0.012a</td>
<td>0.47±0.013a</td>
<td>0.487±0.009a</td>
<td>0.488±0.015a</td>
<td>0.501±0.013a</td>
</tr>
<tr>
<td>Proximal</td>
<td>0.166±0.002a</td>
<td>0.21±0.004a</td>
<td>0.173±0.002a</td>
<td>0.186±0.003b</td>
<td>0.186±0.005b</td>
</tr>
<tr>
<td>Henle</td>
<td>0.059±0.002ab</td>
<td>0.062±0.005b</td>
<td>0.058±0.002ab</td>
<td>0.053±0.002a</td>
<td>0.058±0.001ab</td>
</tr>
<tr>
<td>Distal</td>
<td>0.124±0.005a</td>
<td>0.134±0.005ab</td>
<td>0.133±0.003ab</td>
<td>0.143±0.005b</td>
<td>0.140±0.006ab</td>
</tr>
<tr>
<td>Collecting tubule</td>
<td>0.13±0.003a</td>
<td>0.137±0.003a</td>
<td>0.139±0.005a</td>
<td>0.141±0.006a</td>
<td>0.148±0.007a</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>19.20±1.624a</td>
<td>20.80±1.593a</td>
<td>28.30±1.513b</td>
<td>26.80±1.412b</td>
<td>29.88±1.961b</td>
</tr>
<tr>
<td>uric acid</td>
<td>2.82±0.374a</td>
<td>2.98±0.394a</td>
<td>3.79±0.333ab</td>
<td>4.52±0.435ab</td>
<td>4.81±0.788b</td>
</tr>
<tr>
<td>creatinine</td>
<td>0.420±0.073a</td>
<td>0.360±0.050a</td>
<td>0.510±0.040a</td>
<td>0.50±0.033a</td>
<td>0.455±0.052a</td>
</tr>
</tbody>
</table>

Means in each row with at least one letter in common are not significantly different at the 5% level.
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Figure 1:

Photomicrograph of the renal tissue in experimental group 3, magnification ×400

Hematoxylin-Eosin stain

A: Lymphocytic infiltration within tubules

Figure 2:

Photomicrograph of the renal tissue in experimental group 3, magnification ×400

Hematoxylin-Eosin stain

A: Glomerular Damage
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Discussion
Organophosphorus pesticides are major pesticides used in agriculture and animal husbandry. Every year, many people are poisoned on account of external contact with these pesticides or eating the meat of animals immersed in an organophosphorus pesticide solution. The present research was carried out due to the abundance of these pesticides along with their associated risks and the devastating damages posed to human and livestock health caused by their absorption through skin contact or eating (11).

This study revealed that the final weight of the rats in the experimental groups 1, 2 and 3 had considerably decreased compared to the negative control group. Previous studies have shown that glyphosate reduces the final weight of Wistar rats as well as their fetal weight (12). Another study in line with the present research also showed that glyphosate reduces the final weight of rabbits (13).

Certain studies have indicated that glyphosate causes excessive increase of lipid peroxidation in rabbits, interferes with amino acid synthesis via the shikimate pathway and disrupts endogenous metabolites (14). The weight loss observed in experimental groups 1, 2 and 3 is therefore likely caused by the fact that glyphosate interferes with the process of amino acid production and causes excessive lipid peroxidation and thus weight reduction; however, these changes depend on the doses used of the pesticide.

According to Table 1, the left and right kidney weight shows an insignificant increase compared to the negative control group. Studies conducted on organophosphorus pesticides other than glyphosate also show an increase in kidney weight (15). Another study that investigated the overall weight and the weight of the different organs of several living organisms revealed that feeds poisoned...
with glyphosate increases kidney weight (16). According to results of some other studies, different species of rodents eating glyphosate-contaminated feed encounter weight loss in some organs such as the liver and weight gain in their kidney, heart and brain (17).

With its devastating genetic effects, glyphosate causes damage and inflammation in the kidneys and overgrowth in some renal cells (18). The weight gain observed in the present study can be attributed to the abnormal growth of certain cells. The insignificant increase observed in the present study regarding kidney weight might be caused by the variety of doses used, the longer duration of the test and the different genders and types of animals used in previous studies. Based on Table 1, results of the measurement of the collecting tubules’ diameter indicate their increase in experimental groups 1, 2 and 3 compared to the negative controls. Findings of the study reveal no significant changes in the distal convoluted tubule diameter while the proximal convoluted tubule diameter has significantly increased in experimental groups 2 and 3 compared to the negative controls. Findings also reveal that the Bowman’s capsule diameter insignificantly decreased in experimental groups 1 and 2 compared to the negative control group while no significant changes occurred in other parts of the kidney.

Based on previous studies, organophosphorus pesticides including glyphosate produce free radicals in the cells that cause structural changes in cell proteins and unsaturated lipid peroxidation in the cells. These complications can cause cell necrosis and neoplastic changes. A study conducted by Vidagazagar et al. in 2004 also confirms the findings of this study. Some organophosphorus pesticides, however, increase protein metabolism (20). Kidneys are the central part for uptake and degrading proteins and peptides with low molecular weight through the proximal and distal convoluted tubules (19).

Possamai et al. argued in their study of 2007 that although the mechanisms of producing toxic effects are not properly identified in certain organophosphorus pesticides, their direct and indirect damages through free radicals produced by glyphosate can be regarded as contributing factors; they also determined that kidney, lung, diaphragm and quadriceps femoris are the most sensitive organs affected by oxidative damage. Given that the amount of lipid is increased by certain phosphorous pesticides in the kidney, lipase activity also increases.

Affected by phosphorous pesticides, lipid peroxidation also increases and damages the tissue (23). In previous studies conducted on fish kidney, it was revealed that glyphosate changes the Bowman's space and leads to the accumulation of transparent droplets and pyknotic bodies in the epithelial cells of the proximal and distal convoluted tubules (24). In another study conducted by Ayoola et.al, it was revealed that at higher doses, glyphosate leads to cellular accumulation, lymphocytic infiltration necrosis, bleeding and deformation of the kidney cells in different parts of the renal tissue, which is in line with findings of Omoniyi’s 2002 study conducted on a certain type of fish (24-25).

According to the photomicrographs pictured before (figures 1, 2 and 3), these changes are quite evident in the present study. In addition, certain organophosphorus pesticides have adverse effects on the kidney tissue and interfere with the activity of the energy-dependent sodium and potassium pumps and increase their activity and decrease urine volume (26-27). Any disruption in the flow of urine and its consequences is called obstructive uropathy. Complete obstruction and blockage of the urine in the urinary tract has a significant damaging effect on the renal function from a urologic point of view. Any obstruction can ultimately lead to hydronephrosis atrophy and even
complete kidney failure. In addition, obstruction can lead to the development of infections and consequently double the damage caused by obstruction. Understanding the effects of obstruction on renal function is important for its treatment and prognosis. Nevertheless, the precise mechanisms of renal change have not yet been clarified and numerous researchers are studying the subject (28-29). Researchers have stated that pressure increase in the obstructed part then increases the reversed flow pressure, directly damages the renal parenchyma and causes several dysfunctions such as dysfunctions in the energy metabolism oxidative balance, hemodynamics and the excretory function of the kidney (30). There is a direct relationship between the renal tubular damage and glomerular atrophy (31). An increase in glomerular volume also increases glomerular filtration and the renal function, therefore, the sodium and potassium pump dysfunction, the urine volume change and also the insignificant increase in glomerular volume observed in the present study are only reasonable and cannot be overlooked; in fact, they could be one of the possible reasons for the significant increase in the proximal convoluted tubule diameters in experimental groups 2 and 3. In addition, the accumulation of pyknotic and transparent objects could be yet another reason for the diameter increase. This diameter increase could also be attributed to the diameter increase in the glomerulus and the collecting duct and glomerulus changes.

The insignificant results obtained in the present study regarding renal tissue changes in the majority of the experimental groups and differences existing with previous studies could be attributed to the method of conducting the tests, including the dose of pesticide used, gender and species of laboratory animals studied, duration of pesticide injection and method of pesticide use –hence the slight difference of our findings compared to previous studies. Undoubtedly, this does not reject the adverse effects of glyphosate on the renal tissues; rather, through the aforementioned dysfunctions, glyphosate causes pathological and histomorphometric changes in certain parts of the kidney –however statistically insignificant. It is therefore necessary for those working with this herbicide to abide closely by the safety issues regarding its use in order to avoid possible damage to body tissues.

**Conclusion**

Based on the present research, it can be concluded that glyphosate has a possible toxic effect on body weight and on the different parts of the renal tissue; this herbicide should thus be applied with meticulous care while observing the safety issues so as to avoid renal damages that are, to a certain extent, irreparable.

**Conflicts of interest**

The authors declare to have no conflicts of interest in this study.

**References:**