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The Effect of Glyphosate on Human Sperm: In Vitro Approximation

Efecto del glifosato sobre los espermatozoides humanos: una aproximación in vitro

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Abstract

Introduction Glyphosate is an herbicide used to eradicate illicit crops; however, its use is controversial due to different health problems associated with it. The present study aims to evaluate the effects of glyphosate on human sperm in vitro.

Methods Twenty-two semen samples from healthy normozoospermic men were included; 11 semen samples were incubated with Panzer (INVESA S.A., Antiquia, Colombia) and 11 with Roundup (Monsanto Company, MO, USA). The changes in motility and viability were observed. Functional seminal parameters were evaluated as well.

Results The samples exposed to glyphosate showed less motility and viability; a decrease in the potential of the mitochondrial membrane was observed, and an increase in the lipoperoxidation of the membrane was evidenced.

Conclusion Based on the present results, we concluded that glyphosate has cytotoxic potential for exposed people and may affect their fertility.

Keywords
- sperm
- fertility
- reproduction
- glyphosate
- herbicide

Resumen

Introducción El glifosato es un herbicida utilizado ampliamente para la erradicación de cultivos ilícitos; sin embargo, su uso es polémico debido a diferentes problemas de salud asociados con él. El objetivo del presente estudio fue evaluar los efectos del glifosato sobre los espermatozoides humanos *in vitro*.

Métodos Se incluyeron 22 muestras de semen de hombres sanos normozoospérmicos, de las cuales 11 se incubaron con Panzer y 11 con Roundup, y se evaluaron los cambios en la movilidad y la viabilidad espermática, además de valorar los parámetros seminales funcionales.

Resultados Las muestras expuestas al glifosato presentaron una menor movilidad y viabilidad, una disminución en el potencial de la membrana mitocondrial, y un aumento en la lipoperoxidación de la membrana.

Conclusiones El glifosato es potencialmente citotóxico para las personas que estén expuestas, y puede afectar su fertilidad.

Palabras clave
- espermatozoides
- fertilidad
- reproducción
- glifosato
- herbicida

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Introduction

Glyphosate (N-(phosphomethyl)-glycine) is an herbicide used for agricultural purposes in the eradication of unwanted plants, and, in recent decades, it has been widely used for the elimination of illicit crops, a controversial issue in countries like Colombia, due to the different health problems associated with its use.[1–3] Since 1984, pesticides have been used in Colombia to help with the War on Drugs' illicit crops. Due to its high effectiveness and supposed safety for human health, the first to be used on a large scale was glyphosate.[4] The other two herbicides that had been tested previously were paraquat and triclopyr.[5] Paraquat was discarded due to the possibility of intoxication in drug users, as it happened in Mexico in the 90s,[6] and generating severe adverse effects on the respiratory system.[7] On the other hand, glyphosate, the active ingredient in Roundup (Monsanto Company, MO, USA) and Panzer (INVESA S.A., Antioquia, Colombia), inhibits the enzyme 5-enolpyruvylshikimate-3-phosphatephoshase synthetase, essential in the formation of aromatic amino acids as phenylalanine, tyrosine, and tryptophan in plants.[8]

In 2014, a Brazilian study reported that prolonged exposure of a group of zebrafish to different concentrations of glyphosate caused decreased motility, mitochondrial function, and sperm DNA integrity, changes that affected their fertility.[9] In rats, glyphosate exposure has been observed to increase the percentage of sperm with abnormal morphology and to decrease the expression of two proteins used as markers of sperm nuclear quality (protamine 1 and histone 1).[10] Glyphosate has even been reported to increase the reactive oxygen species (ROS).[11]

Additionally, when treating human sperm in vitro with glyphosate at a concentration of 0.36 mg/L, the alteration of progressive motility 1 hour posttreatment was observed,[12] while the decrease in sperm motility after 1 and 3 hours postincubation and mitochondrial dysfunction 1 hour after treatment were observed after incubating them with glyphosate at a concentration of 1 mg/L.[13]

However, the available information regarding humans' potential exposure and the relation with adverse reproductive effects is insufficient.[3,14] Therefore, the objective of the present study was to determine the in vitro effect of glyphosate on conventional (motility and viability) and functional (mitochondrial membrane potential, membrane integrity, membrane lipoperoxidation, and reactive oxygen species) parameters in human sperm.

Methods

Semen Samples

The present experimental in vitro study included 22 semen samples from healthy individuals with normal seminal parameters (normozoospermic), collected by masturbation in a sterile container after a period of sexual abstinence of 2 to 7 days.

Ethical approval for this study was obtained from the bioethics committee of the school of medicine at Universidad de Antioquia, and all men signed an informed consent form.

Sperm motility and viability ($n = 22$) were quantified for each seminal sample following the guidelines established in the manual for seminal analysis published by World Health Organization in 2010,[15] before and after incubating them with each of the two glyphosate brands—Roundup and Panzer—in vitro ($n = 22$). On the other hand, the effect of glyphosate on mitochondrial membrane potential ($n = 3$), the integrity of the membrane ($n = 3$), membrane lipoperoxidation ($n = 3$), and levels of ROS of human sperm ($n = 3$) were intracellularly quantified.

Direct Incubation of Human Sperm with Glyphosate

Twenty-two semen samples were processed, 11 for each glyphosate brand, and compared with the control (phosphate-buffered saline [PBS]). Sperm parameters were quantified at the initial time (0 hours) and 1 hour after incubation. Glyphosate was diluted to a 10 mg/mL (real concentration of the active ingredient glyphosate 3.6 mg/mL), a dose significantly lower than the one that is considered toxic.

Three different concentrations of glyphosate were evaluated in terms motility and viability: 1 mg/mL, 0.01 g/mL and 0.1 g/mL. During the functional parameters test, the intermediate concentration (0.01 g/mL) was selected.

Mitochondrial Membrane Potential

Sperm cells, both control and sperm cells plus glyphosate, were incubated with propidium iodide (PI, 0.25 mg/mL, Molecular Probes Inc, Eugene, OR, USA) and 3,3' dihexyloxacarbocyanine (DIOC6, 10 nM, Molecular Probes) for 30 minutes; sperm were washed once by centrifuging at 300 g/5 minutes. The pellet was resuspended in PBS, and the reading was performed by flow cytometry as previously standardized.[16–18]

Membrane Integrity Evaluation

Following the previously established protocol, sperm were incubated with PI at a final concentration of 0.25 mg/mL and Sybr 14 (1 µM, LIVE/DEAD Sperm-Viability Kit, Molecular Probes Inc.) for 30 minutes, washed once with PBS, the pellet was resuspended in PBS, and the reading was performed by flow cytometry.[16–18]

Membrane Lipoperoxidation Evaluation

Sperm were incubated with BODYPI C11 dye (Molecular Probes Inc.) for 30 minutes, washed once with PBS, the pellet was resuspended in PBS, and the reading was performed by flow cytometry.[16–18]

Intracellular ROS Production

Sperm were incubated with 2, 7 Dichloro-dihydro-fluorescein diacetate (DCFH-DA, 1 µM, Sigma-Aldrich, St Louis, MO, USA) and PI (0.25 mg/mL) for 30 minutes, washed 3 times, the pellet was resuspended in PBS, and the reading was performed by flow cytometry following the previously established protocol.[16–18]

Statistical Analysis

To compare the semen parameters before and after treatment, the Friedman test was performed. Data were analyzed

using the Prism 6.0 (GraphPad Software, San Diego, CA, USA) statistical software; statistical significance was defined as $p < 0.05$. Numeric values were expressed as the median ± range (minimum to maximum).

Results

The concentrations of 0.01 g/mL and 0.1 g/mL showed a significant decrease in the conventional sperm parameters (motility—total and progressive—and viability) with respect to controls samples, ►**Table 1**, ►**Figures 1** and **2**.

The concentration of 0.01 also showed alterations in the functional parameters of sperm, decreasing mitochondrial membrane potential and viability, thus increasing the number of dead cells and increasing membrane lipoperoxidation, ►**Table 2**. In addition, ROS were affected by glyphosate incubation in Panzer (0.2%-0.01–0.44, $p < 0.05$) and Roundup (1%-0.87–1,2-) when compared to the control samples (55% -41.3-68,7-).

Discussion

In the present study, an in vitro evaluation of the effect of glyphosate on human seminal parameters was performed. Taken together, the findings of this study evidence that the initial sperm motility of seminal samples are significantly affected when they are in contact with glyphosate at a concentration of 10 mg/mL, which is consistent with the results previously reported by Anifandis et al., who were the first to evaluate the effects of glyphosate on conventional and functional parameters.[12,13] Additionally, glyphosate is recognized for its effect on testosterone levels, a hormone that plays an essential role during spermatogenesis, so that it could harm both sperm production and its maturation.[19] On the other hand, the present work shows that glyphosate affects mitochondrial membrane potential and lipoperoxidation of the membrane. Decreased mitochondrial membrane potential could be associated with loss of motility, with mitochondria being the primary source of energy.[20] At the same time, the increase in lipoperoxidation generates

Table 1 Effect of Panzer and Roundup herbicides on functional parameters at time 0 and 1 hour after incubation. A: Panzer, and B: Roundup

A.								
	Panzer							
	Time 0				1 hour			
	Control	1 mg	0.01 g	0.1 g	Control	1 mg	0.01 g	0.1 g
	Motility I							
Median	50.00	40.00	4.000	0.000	43.00	31.00	0.000	0.000
Range	13–66	0–64	0–44	0–0	13–62	0–52	0–24	0–0
	Total motility							
Median	54.00	50.00	11.00	0.000	48.00	39.00	0.000	0.000
Range	15–69	0–66	0–57	0–0	16–77	0–54	0–26	0–0
	Viability							
Median	76.00	58.50	19.50	0.000	69.00	56.00	0.000	0.000
Range	63–90	0–80	0–62	0–0	33–83	0–67	0–43	0–0
B.								
	Roundup							
	time 0				**1 hour**			
	Control	**1 mg**	**0.01 g**	**0.1 g**	**Control**	**1 mg**	**0.01 g**	**0.1 g**
	Motility I							
Median	53.00	46.00	4.000	0.000	42.00	32.00	0.000	0.000
Range	41–68	0–63	0–46	0–0	16–60	0–45	0–15	0–0
	Total motility							
Median	57.00	51.00	11.00	0.000	50.00	39.00	0.000	0.000
Range	50–73	0–75	0–57	0–0	19–77	0–82	0–18	0–0
	Viability							
Median	79.00	65.00	7.500	0.000	69.00	55.00	0.000	0.000
Range	65–87	0–89	0–79	0–30	46–83	00–80	0–46	0–0

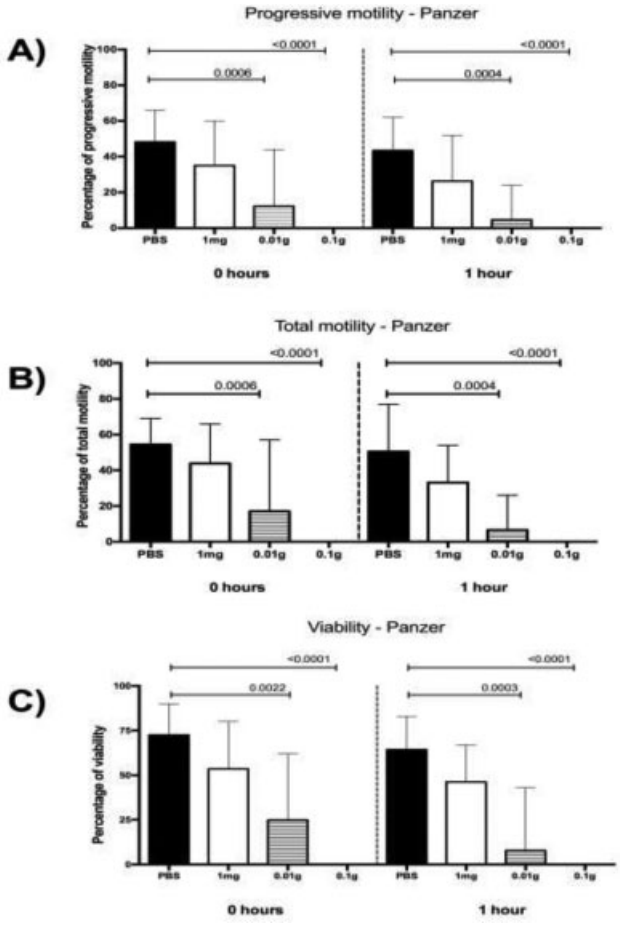

Fig. 1 Effects of Panzer herbicide with glyphosate at different concentrations (1 mg, 0.01 g, and 0.1 g) on (**A**) progressive motility (%), (**B**) total motility (%) and (**C**) sperm viability (%) respectively to the time of measurement compared with the initial sample in phosphate-buffered saline as a control.

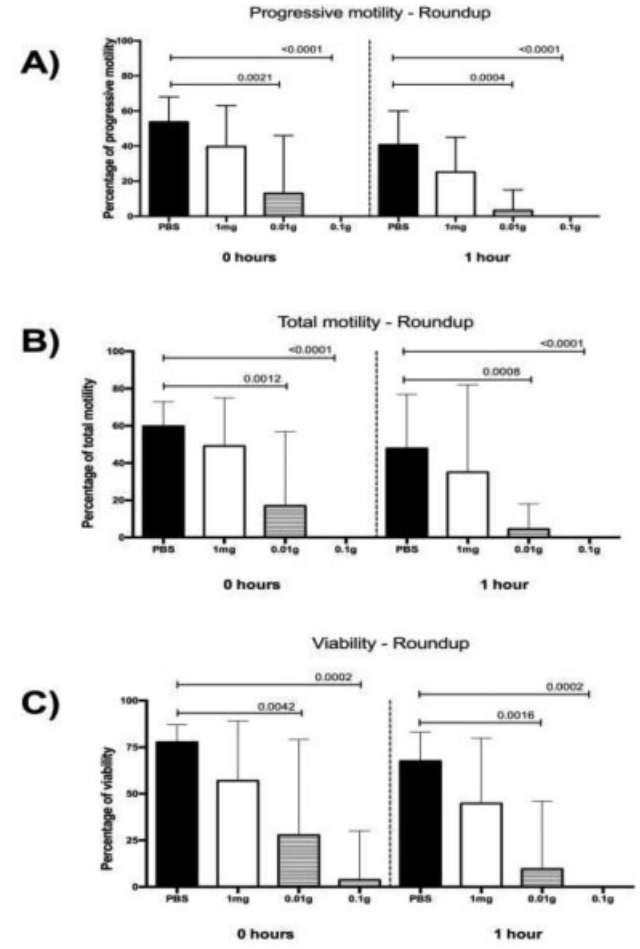

Fig. 2 Effects of Roundup herbicide with glyphosate at different concentrations (1 mg, 0.01 g, and 0.1 g) on (**A**) progressive motility (%), (**B**) total motility (%) and (**C**) sperm viability (%) respectively to the time of measurement compared with the initial sample in phosphate-buffered saline as a control.

Table 2 Consolidated effect of Panzer and Roundup herbicides (0.01 g) on functional parameters

High mitochondrial membrane potential (%)			
	Control	**Panzer 0.01 g**	**Roundup 0.01 g**
Median	46	10.9	5.5
Minimun–Maximun	43.6–48.4	5.6–16.2	15.4–17.5
Viability (%)			
Median	60	8.1	5.1
Minimun–Maximun	58.8–61.2	0.06–16.2	4.8–6.1
Lipoperoxidation (%)			
Median	2.4	17.5	17.4
Minimun–Maximun	1–4.1	4.6–33.1	15.3–18.5

irreversible oxidative damage in male reproductive cells since it leads to a considerable increase in the permeability of cell membrane, making them more susceptible to oxygen free radicals, driving them even to total lysis, which would explain increased dead cells and decreased viability.[18]

On the other hand, studies in different animal models report that glyphosate affects sperm quality.[9,21–24] Glyphosate has been observed to affect motility, mitochondrial membrane integrity, and DNA integrity in zebrafish, primarily.[9] When exposed to glyphosate, the testosterone levels, sperm motility, concentration, viability, and epididymal weight of rats were found to be considerably lower.[22] Furthermore, a study that evaluated the relationship between rabbits and glyphosate resulted in a reduction in body weight, libido, ejaculate volume, sperm concentration, and increased the number of abnormal sperm cells and viability.[21] Additionally, when evaluating the effect of glyphosate on the seminal parameters of yellow-tailed tetra fish (*Astyanax lacustris*), a widely used species for human consumption, alteration of motility, concentration, and sperm viability was observed.[24] Finally, when evaluating the effect of glyphosate exposure in other fish (*Jenynsia multidentata*), an oxidative

imbalance was reported due to the increase in oxygen free radicals and a decrease in motility and sperm concentration.[23]

The strengths of our study are that all semen analyses—conventional and functional— were performed in the same laboratory, using the same glyphosate dilution and methodology. The limitations of our study include small sample size.

Conclusion

In conclusion, the present work results allow us to postulate that glyphosate, both Roundup and Panzer, affects the conventional and the functional parameters of human sperm in vitro. Although the negative effect generated by Panzer is slightly lower than that observed with Roundup, both have the cytotoxic potential for people who are exposed and may be affecting their fertility. Finally, additional studies are required to establish the potential role of glyphosate on fertility and human semen parameters in vivo in men exposed to glyphosate.

Conflict of Interests

The authors have no conflict of interests to declare.

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