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Effects of Glyphosate on Cognitive Development, Sensory Sensitivity and Locomotor Balance in Male Rats

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Abstract: Glycophate is the active ingredient in Roundup® hebicide, which is commonly used in agriculture. Exposure to the herbicide glyphosate may affect the development of Parkinson Disease in humans. pre-natal and post-natal exposure to glyphosate exerts neurological effects and neuropsychiatric effects, even at low concentrations. This study aimed to examine the effects on cognitive, sensory and motor development in male rats exposed to the active ingredient glycophate in Roundup® at various concentrations. In this study using test animals in the form of male rats with an age of 10 weeks, then the rats were divided into 4 treatment groups, namely the normal group, 100 µL, 50 µL, and 25 µL Roundup®, induction was given orally every day for 4 weeks. Then at the last week, the body weight of the rats was measured and several tests were conducted to see the effect of glycophate induction on rat cognition through the morris water maze and y maze tests, sensory development through the hot plate test, and rat motor development through the hanging wire test. The results showed that glyphosphate administration affected the body weight of the rats, and affected the cognitive development of the rats. The 50 µL dose treatment group showed the most visible effect of glycophosphate exposure on cognitive tests compared to other groups. In the sensory test, the 50 µL ml dose treatment group also had low sensitivity compared to other groups, and low motor ability compared to other groups. Glycophate was seen to affect the cognitive, sensory and motor development of the rats.

Keywords: Cognitive test, glycophate, locomotor test, sensory tets.

Introduction

Parkinson's disease is categorized as a neurological condition that progresses over time. According to epidemiological research, glyphosate exposure may have an impact on a person's development of Parkinson's disease (Pu et al., 2020). The main component of Roundup®, glyphosate [N-(phosphonomethyl) glycine] is the most extensively used herbicide worldwide. According to a previous study, individuals who were exposed to glyphosate had a 33% higher chance (odds ratio=1.33) of dying young from Parkinson's disease (PD) than those who were not exposed (Cattani et al., 2017). Glyphosate is an organophosphate chemical that is commonly used as a crop desiccant and a broad-spectrum, non-selective, post-emergent herbicide. Approximately 60% of the global market for non-selective herbicides is made up of commercial formulations that contain glyphosate (IBAMA, 2014; IARC, 2015).

Additionally, a recent study demonstrated that glyphosate exposure during pregnancy and lactation has neurological and neuropsychiatric consequences. Exposure to neurotoxins during the development of the central nervous system (CNS) can result in loss of cognitive function and damage to brain regions such as the hippocampus and prefrontal cortex, even at low concentrations (Ainge et al., 2007; Chen et al, 2012; Mogensen et al., 2007). Only as people age does exposure to these neurotoxins become more noticeable (Rice and Barone, 2000).

According to certain research, exposure to glycophate may cause deficiencies in motor development and delays in the development of innate reflexes. When subjected to alterations in the cholinergic and dopaminergic systems, adult rats exhibit decreased locomotor activity, sociability, learning, and short- and long-term memory (Ait-Bali et al., 2020). According to the preceding description, the purpose of this paper's research is to determine how male rats exposed to various concentrations of the active chemical ingredient in roundup® will develop cognitively, sensoryly, and motorly.

Methods and Materials

Tools and materials

The tools used in this study were rat cages (30cm x 20 cm x 25 cm), rat drinking bottles, 100µL micropipettes, 100µL tips, latex gloves, masks, tissues, rat sonde needles, Morris Water Maze Test equipment, Y-maze Test equipment, Hot Plate Test equipment, Wire Hanging Test equipment, handphone cameras, digital scales, stopwatches and stationery. The materials used in this study were male white rats aged 10 weeks, rat feed (Rat-bio), rat drinking water, 70% alcohol, and Roundup®.

Animal treatment

Male rats aged 10 weeks were acclimatized for 7 days before treatment. During the acclimatization period, rats were fed ad libitum with standard animal feed (Rat-bio). Rats were kept in plastic cages covered with woven wire to cover the cage, and the bottom was filled with sawdust. The cages were positioned in an area with ample natural light and ventilation. The cage had a 12-hour light-dark cycle and a temperature of approximately 25˚C. In addition to unlimited food and water, each day's recommended dosage of roundup® was given orally.

Treatment induction

Four groups of rats were given varying dosage treatments. One treatment group was administered 100 µL, 50 µL, and 25 µL of Roundup®, whereas the other was not administered any roundup. The study therapy, which lasted for four weeks or thirty days, examined the effects of glycophate on cognitive development, sensory sensitivity, and locomotor balance. A previously published method served as the model for the glycophate induction protocol (Larsen et al, 2016).

Measurement body weight of animal models

Throughout the trial, body weight measurements were made once a week to assess the rats' post-treatment weight.

Cognitive test procedure

During the final week of treatment, cognitive tests were administered. The Y-Maze and Morris Water Maze tests are the cognitive assessments that will be administered.

Morris Water Maze (MWM)

Used to evaluate rats' spatial learning and memory. The rats were tested in a pool that measured 116 cm in circumference and 55 cm in height, with water that was between 21 and 23 °C. The pool was split into four sections, labeled N, S, E, and W zones. In quadrant 2, there was a platform measuring 11 cm in diameter. The rats were taught for two days in a row prior to the spatial memory test. The rats were submerged in the water with their backs to the pool wall for every attempt. The rats were taught to locate the leading platform in 60 seconds and to spend 15 seconds there. The 60-second delay would be recorded and the rat would be gently guided to the platform if it was unable to locate it in that amount of time. The rats are carefully dried and warmed after testing, and then they are put back in their cage. Following completion, testing was carried out on the third day, placing the platform below the water's surface and timing how long it took the rat to get there. The rat's capacity for spatial memory improves with decreasing latency.

Y-Maze Test

Spatial memory can also be evaluated using the Y-maze test. Three arms measuring 40 cm in length, 8 cm in breadth, and 15 cm in height, each angled 1200 degrees from the center, make up the apparatus utilized in this Y-maze. Every arm will have a label, and all entry points from the arm will be sealed off. Two arms will be exposed during the test, with one arm serving as the beginning point. For five minutes, rats are free to investigate the two exposed arms. The mice are evaluated once more after a two-hour rest period. Three exposed arms were employed in this test, though. Five minutes were given to the rats to explore. Each arm's exploration time as well as the total time in all three would be noted. While rats with weak spatial memory did not significantly vary from those with superior spatial memory, the former would investigate the new arm more frequently.

Sensory sensitivity testing

The rats were put on a heated plate that was between 50 and 60ºC. The rats' first reactions, such as licking, paw movements, small jumps, or jumping to avoid the heat, were timed. Repetition was done three times within 10 seconds.

Locomotor balance testing

A measure of motor function, the hanging wire test evaluates the strength and grasping abilities of the forelimb. Rats were hung by their forelimbs on a wire between two poles that were 45 cm apart on a foam cushion in the manner that was described by Hattori et al. (2000). With a maximum testing period of 300 s, the time it took the rat to fall from the wire was measured in s (seconds). Each rat will be tested three times, with a five-minute break in between. The fall latency for that particular time point was determined by averaging the results of the three separate tests.

Analysis data

Data analysis using SPSS For Windows, the resulting data, if normally distributed and homogeneous, were analyzed with One Way Anova ($P<0.05$) if significantly different, followed by Duncan's post-hoc test.

Result and Discussion

Rat Body Weight

The results obtained by the group of test animals treated with a dose of 100 µL Roundup® had a low average body weight compared to the other groups.

Studies carried out by Mesnage et al. (2014) revealed that rats given low doses of glyphosate had alterations in the way hormones that control appetite functioned. The endocrine system, which regulates hunger, is disrupted, leading to a decrease in appetite. As a result, the body can use more resources to manufacture energy in order to survive this energy crisis. For instance, phosphocreatine hydrolysis provides a phosphate group to adenosine diphosphate (ADP) for the synthesis of ATP, making creatine and phosphocreatine significant energy metabolites (Holtzman et al., 1996).

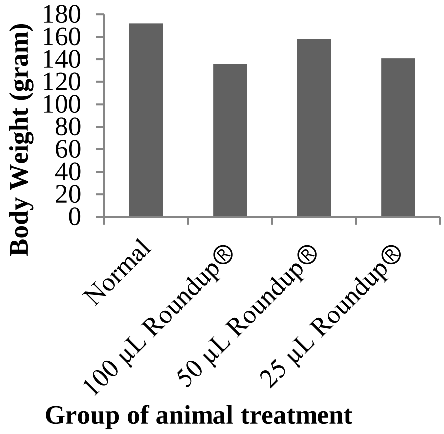

Figure 1. Rat Body Weight

Regarding this, it was found that brains treated with glycophate had lower amounts of creatine and phosphocreatine, which may indicate that the phosphocreatine interaction with ADP was activated to meet ATP requirements. Li et al. (2016) found that brain tissue treated with glycophate had higher concentrations of ketone 3-hydroxybutyrate. Ketones are the primary substitute fuel for glucose in the brain, so exposure to glyphosate can replenish the deficiency in energy supply by using them (Cunnane et al., 2016).

Morris Water Maze (MWM)

The group administered 50 µL Roundup® took on average longer than the other groups to finish the Morris Water Maze Test, a cognitive test. Rats and other animal models are used as test subjects for the Morris Water Maze Test, which assesses spatial learning and memory. Neurons treated with glyphosate exhibited reduced dendritic complexity as well as the production and maturation of synaptic spines

(Luna et al., 2021). Furthermore, glyphosate exposure was found to reduce the development of synapses in hippocampus neurons (Baier et al., 2017).

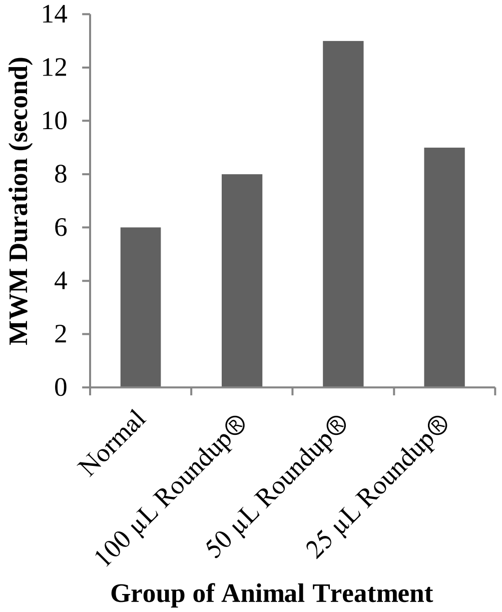

Figure 2. Morris Water Maze Test average duration

Glyphosate exposure during pregnancy affects memory and spatial awareness, leading to cognitive impairment. The relationship between synaptic protein expression and synaptic organization in the hippocampus following glyphosate exposure was examined by Luna et al. in their study from 2021. They found that glyphosate decreased the expression of synapsin-1, PSD-95, and CaMKII as well as PSD-95 clustering in the hippocampus. All things considered, exposure to glyphosate has a significant effect on synapse formation, lowers the expression of synapse proteins in the hippocampus, and may even precipitate a decline in brain connectivity and cognitive performance.

Y-Maze Test

Based on the result group that was given exposure to glycophate showed the smallest percentage of Y-maze calculation compared to the normal group. The dose groups of 100 µL Roundup®and 25 µL Roundup®showed the same y-maze test value.

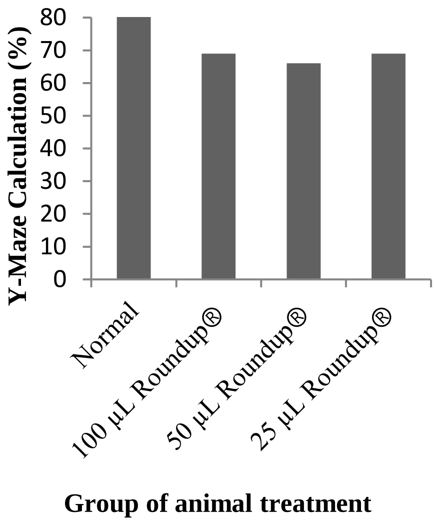

Figure 3. Y- Maze Test average calculation

The Y-Maze Test was also used to assess the test animals' capacities for spatial learning and memory. According to earlier research, the substantia nigra, cerebral cortex, and hippocampus of rats are among the brain regions in which glycophate can cause oxidative stress (Astiz et al., 2009; Cattani et al., 2014). In addition to changes in the regulation of emotional and cognitive functions, oxidative stress will lead to a functional decrease in connectivity between the prefrontal cortex and several limbic system areas, particularly the dopaminergic and serotoninergic systems (Wahlstrom et al., 2010; Naneix et al., 2012; Tombak, 2013). This will decrease recall ability for spatial memory and be consistent with behavioral changes. Similarly, Faria et al.'s study from 2020 indicated that exposure to glyphosate hampered social and exploratory activity, which is linked with elevated anxiety.

Sensory Sensitivity (Hot Plate Test)

The results obtained show that the normal group has a good sensitivity response to heat by giving a reaction in a fast time and a more diverse reaction response when compared to the treated group. The treatment group seemed to take longer in giving a response and fewer reactions were given when given a stimulus in the form of heat.

Table 1. Sensory sensibility of animals test

Group	Rat Reaction			
	Foot Licking	**Moving the Claws**	**Small Leap**	**Avoiding the Heat**
Normal	1st Second	4th Second	5th Second	6th Second
100 µL Rounduo®	3rd Second	4th Second	6th Second	8th Second
50 µL Rounduo®	-	-	7th Second	9th Second
25 µL Rounduo®	4th Second	-	-	10th Second

The alterations noted are supported by studies by Bridi et al. (2017), which found that administering glycophate can lessen aggressive behavior. Changes in serotonergic neurotransmission may be the cause of this decline in violent behavior (Sobjak et al., 2017). When an unpleasant signal is present, glyphosate exposure also causes avoidance (Pereira et al., 2018). The most comprehensive discussion of the link between glyphosate exposure and delays in sensorimotor development that is, delays in the development of innate reflexes was found in a prior study conducted by Ait-Bali et al. (2020).

Locomotor Balance (Hanging Wire Test)

The results obtained in the locomotor balance test show that the treatment group has a significant difference with the normal group, this difference shows the length of time the rats can survive on the wire. The normal group appears to have better balance when compared to the three treatment groups, this can be seen from the duration of staying on the wire which is longer when compared to the treatment group

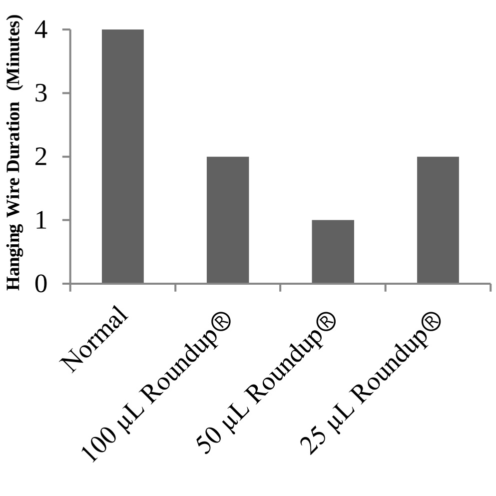

Figure 4. Average Hanging Wire Test Duration

Ait Bali et al., (2017) found that mice's locomotor activity changed after an acute exposure to glycophosphate. Additionally, a number of studies have shown a favorable correlation between the loss of dopaminergic neurons and DA receptors in the substantia nigra and the reduction in locomotor activity Gallo et al., 2015. It has been demonstrated that the mesolimbic and nigrostriatal dopaminergic systems, which are important in motor control, are susceptible to herbicides. One common definition of a significant risk factor for Parkinson's disease (PD) is exposure to pesticides (Hatcher et al., 2008).

Conclution

The administration of glycophosphate in the roundap has an impact on the rats' body weight as well as their cognitive, sensory, and motor development. Following testing with the Morris Water Maze and Y-Maze, the 50 µL dosage treatment group demonstrated the impact of glycophate administration in Roundap on the cognitive development of rats. In the sesoris test, the 50 µL dose treatment group also showed a small response and in a long time compared to other groups. In the locomotor test, the 50 50 µL dose treatment group had a lower balance duration when compared to other groups. So that the dose of 50 µL is the dose that has the most effect on the cognitive, sensory, and motor development of test animals. Inducing test animals using glycophate in roundap is also cheaper and easier to do than other inducers.

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