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Effect of Glyphosate on Male Fertility, Cytogenicity and Biochemical Parameters in RATS

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ABSTRACT

Oral administration of round up in rats doses of 49 and 490 mg/kg b.wt. daily for 65 successive days respectively, significantly decreased the weight of sexual organs, sperm cell concentration and sperm motility while the sperm abnormalities were increased. The levels of creatinine, cholesterol, uric acid and bilirubin in serum were significantly increased.Round up significantly increased the activities of serum AST, ALT and AP. The tested herbicide showed mutagenic potential as measured by a positive response in the micronucleus assay. The small dose tested caused slight toxicity to the bone marrow proliferation as determined by insignificant increased rate of bone marrow cells while the large dose significantly increased the rate of bone marrow proliferation as determined by the increase the rate of ratio in tested groups over that of the control.

INTRODUCTION

Glyphosate compounds are the major herbicides affecting pests of agricultural crops and on which farm animals can consume (**Danielsen and Larsen, 1990)**. The toxicity, metabolism, teratogenicity, cytogenicity, residual effect, carcinogenicity of Roundup and its effect on male fertility were previously investigated (**Brewster *etal*;1991; Goldsworthy *et al*., 1991; Talbot *et al*.,1991; Wester *et al*., 1991; Rank *et al*.,1993; Ashby,1994; Cox,1995; Hammond *et al*., 1996 and Yousef *et al*.,1996).**

Samsel and Seneff (2013) said that glyphosate is the most cause for diseases and condition associated with infertility.

The study regarding the effect of Roundup on male fertility and cytogenicity are rare. Therefore, the present work was deigned to elucidate the effect of commonly used herbicide Roundup on the fertility of male rats, cytogenicity and some biochemical parameters.

MATERIAL AND METHODS

Roundup: N-(phosphonomethyl) glycine $C_3H_8NO_5P$ was obtained from Central Agricultural Pesticide Laboratory of Egypt.We used 1/10 & 1/100 of LD_{50} of Roundup

Animals

Forty five mature male rats were used. Rats were fed on ordinary ration and water adlibitum.

I- **sexual organs weight and epididymal sperm character:**

Thirty mature male rats (150-180g) were divided into 3 groups. The first group is kept as control, whereas the second and third groups were orally administrated 49 and 490mglkg b.wt. daily for 65 successive days, respectively to cover a complete spermatogenic cycle (**Hershberger *et al*.,1969**).

The sexual organs weight and epididymel sperm characters were determined according to (**Bearden and fluquary, 1980**).

Blood sample was obtained from each rat, left to clot and the serum was separated for biochemical analysis. The activities of AST,ALT and AP were determined according to the method of **Reitman and Frankel (1957) and Roy(1970).**Creatinine, uric acid, cholesterol and bilirubin levels in serum were estimated as explained by **Husdan and Rapopart (1968), Ramesh *et al*.(1978), Richmond (1973) and Walters and Gerarde (1970) respectively.**

II-Cytogenicity effect:

In order to assess the possible mutagenic effect of Roundup, the micronucleus test was performed to detect chromosomal damage associated with the treatment.

For the micronucleus investigation, 3 groups of 5 mature male rats each were used**,** the first one is kept as control, the second and third groups were orally administrated Roundup in doses of 49 and 490 mg/kg b.wt. daily for 30 successive days.

Following the protocol established by **Solamon *et al*.(1980),** bone marrow cells of rats were extruded with a pin into a clean dry glass slid and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried, fixed in absolute methanol and stained with Giemsa stain in phosphate buffer PH 6.8.The polychromatic erythrocytes (PCE,1000/animal) were screened for micronucleus and the changes in the mitotic activity (**Hart and Engberg-Pederson,1983**) and **(Al-Bekairi *et al*.,1991)** were assessed on the basis of ratio **PCE** (polychromatic erythrocyte) /**NCE** (Normochromatic erythrocyte) .

Statistical analysis:

Data are presented as mean ± (SE or SD).

Differences between means are tested for significance using the students T test as Described by Snedecor and Cochran (1973)

RESULTS AND DISCUSSION

I- Effect of roundup on sexual organs weight, epididymal sperm characters:

Oral administration of roundup in doses of 49 and 490mg/kg b.wt. respectively for 65 successive days showed a significant decreased in the weight of testis, seminal vesicles, prostate gland, sperum cell concentration and sperm motility, while the sperm abnormalities were increased (Table 1). These toxic effect may by due to damage of testosterone producing cells at concentration 1/10 of agricultural use1 hr. after exposure. After 48hrs of exposure, Roundup induces apoptosis in germ cells and in sertoli cells (involving DNA Fragmentation) as proved by **Clair *et al*.(2012)**.

Moreover, Roundup may act as endocrine disruptor for both male and female by altering aromatase activity, oestrogen regulated genes and testosterone levels in rats, moreover induce oxidative stress and activate multiple stress-response pathways leading to cell death in prepuberty rat testis. **Cavalli, *et al*.(2013)** and **Romano *et al*.(2010)**.Similar observation have been regested by **Yousef *et al*.(1995)** and **(1996)**

The effect of Roundup significantly increased the level of creatinine, cholesterol, uric acid and bilirubin in serum. The tested substance significantly increased the activities of serum AST, ALT and AP(Table2).

Our results agree :

Kitchin and Brown (1990) reported that given of Roundup to female sprague dawley rats increased the activity of ALT in serum.

Raquel *et al.*(2012) and **Gilles *et al.*(2014)** recorded that Roundup administration to male and female rats with 50 or 500 mg/kg b.wt. significantly increased AST,ALT, Urea and creatinine.

Renal and hepatic impairment are frequent sequelae of glyphosate intoxication and usually effect reduced organ perfusion (Talbot *etal.*,1991).

II- Cytogenetic:

Limited information is available on the genetic activity of roundup. In present study, the tested glyphosate caused chromosomal breakage in vivo as shown by rat micronucleus test. Treatment with Roundup had a dose dependent mutagenic effect as it increased the incidence of micronuclei formation by increasing the dose level. **Rank *et al*.(1993)** mentioned that Roundup at concentration 1.44 and 2.88 mg/liter significantly increased the chromosome aberration after treatment in the onion anaphase tellophase test of onion.

In contrast, the two tested doses of Roundup (49 and 490mg/kg b.wt) showed a significant increase in PCE/NCE ratio. According to **Guyton (1991)** enhancement of the mitotic activity of bone cells and the increased production of red blood cells should be a sign of toxicity or damage of some organs of the body, enough to enhance the production of PCEs in bone marrow, there by attempting to supply the demand for RBCs in the body. In a study conducted by **Ashby (1994)** found that Roundup had a mutagenic and carcinogenic effect in both sexes of mice.

Sahdeo *et al*. (2009) and **Cavas and Konen(2007)** mentioned that glyphosate was given I/P to mouse at concentration 25 and 50mg/kg b.wt. significantly increase CAS and MN induction at both treatment and time. The cytotoxic effect of Roundup were also observed by significant decrease in miotic (MI).

The impairment of fertility in male rats and the mutagenic effect caused by Roundup indicated the importance of protection of male animals and human from their exposure to studied substance.

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Table (1): the effect of 65 successive oral administration of Roundup on sex organs weight, and epididymal sperm characters of mature male rats.(mean±, n=10).

Group	Dose Mg/k b.wt.	Weight of sexual organs/gm ** Testis	Semin Vesicle	Prostat	Epidi	Sperm cell characters Con. 10^6	Motility %	Abnormality %
Control	-	1.73 ±0.07	0.67 ±0.05	0.41 ±0.03	0.70 ±0.32	520 ±2	82 ±2.3	1.8 ±0.4
Round up	49	1.526 ±0.08	0.5 ±0.02	0.2*** ±0.02	0.582 ±0.07	480*** ±1.1	±3.16	1.7 ±007
	490	1.04*** ±0.04	0.158*** ±0.014	0.072* ±0.01	0.38 ±0.02	224*** ±3.99	52*** ±3.7	5.8*** ±0.4

*= significant at P < 0.05 ***= significant at P < 0.001

Table (2): the effect of oral administration of Roundup on enzymatic activity and biochemical constituents in se
(mean±SE/SD, n=10) *= significant at P < 0.05 ***= significant at P < 0.001

Group	Dose mg/	Creatinine mg/L	Uric Acid mg%	Cholesterol Mg/dl	Bilirubin Umol/L	ALT U/L	AST U/L	AP U/L
Control	-	110.1 ± 2.2	32.6 ± 3.2	92.74 ±22.5	88.5 ±8.9	78.4 ±4.5	43.6 ±1.2	36.6 ±0.6
Roundup	49	195.3** ± 3.5	76.1** * ±2.4	92.05 ±17.3	179.9* ** ±3.1	79.0 ±4.2	47.4* ±0.7	36.2 ±0.5
	490	289.4*** ± 2.5	80.7** * ±2.4	133.69*** ±36	304.5* ** ±21.8	97.8** * ±7.1	63.4** * ±2.8	50.5** * ±0.6

Table (3): The effect of oral administration of Roundup on the incidence of MPCE and on relation of PCE to NCE in rats (n=5)

Group	Dose mg/kg b.wt.	PCE screened	MPCE NO.	Mean/1000 PCE (MPCE±SE)	%	NCE screened	PCE/NCE ratio
Control	-	5000	28	5.6 ±2.6	0.56	2183	2.29 ±0.4
Roundup	49	5000	32	8.4 ±0.6	0.84	1850	2.72 ±0.2
	490	5000	382.17	76.14*** ±1.84	7.6	318.31	2.324 ±0.07

***= significant at P < 0.001

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