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CHROMOSOMAL ABERRATIONS INDUCED BY GLYPHOSATE ISOPROPYLAMINE HERBICIDE AND TRIALS FOR DIMINUTING ITS TOXICITY USING SOME CHEMICAL INACTIVATORS AND ANTIOXIDANT

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SUMMARY

Fifty apparently healthy Newzealand white male rabbits (with average weight of 1329 gm) were used in the chronic and acute toxicological studies of Roundup (Glyphosate Isopropylamine) herbicide. Fifteen rabbits were divided into three equal groups, which were used in the chronic cytogenetical (genotoxicological) study, where the group one as a control, the group two administered 750 ppm glyphosate Isopropylamine herbicide in drinking water for 60 days, and the group three administered the herbicide (with the same above dose) along with 312 ppm vitamin-E for 60 days. Results demonstrated the presence of significant structural and numerical chromosomal aberrations in the bone marrow cells of Roundup treated rabbits, and these cytogenetic effects could be partially reduced by vitamin-E treatment.

In the second acute toxicity study, thirty five rabbits were divided into seven equal groups, the group one as a negative (normal) control, the remaining six groups of rabbits were orally administered with one gm glyphosate salt/kg.b.w., once a day, for 6 days, but these groups were administered the different proposed glyphosate-salt inactivators as follow : the group two administered the herbicide only (positive control), the group three administered calcium phosphate (350mg/kg.b.w., orally), the group four administered vitamin-E (100 mg/kg.b.w., orally), the group five administered charcoal (350mg/kg.b.w., orally), the group six given calcionate (32mg/kg.b.w.I/M.inj.) and the group seven administered the mixture of the above four treatments (with the same above doses). Liver histochemical, serum biochemical, haematological and liver histopathological studies were carried out. Results indicated that the charcoal is the most active inactivator (from the tested treatments) against Roundup toxicity, that it normalizing (or improving) the levels of the majority

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of measured parameters, followed by calcium phosphate then vitamin-E inactivators. Oppositely, the calcionate treatment promote the toxicity of Roundup (based on the measured parameters) followed by the mixture of four treatments (perhaps due to that the calcium therapy may promotes the toxicity of glyphosate salt). So that calcium therapy should be contraindicated in case of Roundup toxicity.

INTRODUCTION

The acid glyphosate herbicide is the N-(phosphonomethyl) Glycine, Roundup is the formulated salt of Glyphosate which chemically named Mono(Isopropylammonium) of N-(phosphonomethyl) Glycine (FAO, WHO and IPCS, 1986).

It has been recorded that glyphosate herbicide activates the oxidation reactions through enhancing some of the NAD- dependent dehydrogenases in rat liver. Also, it interferes with the phosphorylation reaction (ATP-synthesis) through activation of the mitochondrial Adenosine-Tri-phosphatase (ATP-ase) enzyme of the hepatocytes and consequently it interferes with the energy metabolism, and the uncoupling of the oxidative phosphorylation suggested to be the major lesion in Glyphosate intoxication Olorunsogo et al. (1979). On the other hand, Glyphosate salt (Roundup) herbicide induced oxidative stress through activation of Glucose-6-phosphate Dehydrogenase (G6PD) enzyme, Helal (1993) and Lioi et al. (1998).

Another aspects of the toxicological properties of Glyphosate salt (Roundup) herbicide, that it could induce significant reduction of lymphocytes, gamma-globulins and the specific antibodies against bacterial (e.g.Pasteurellosis) and viral (e.g.Rift valley fever) diseases, that it is an immunosuppressive agent (Helal, 1993).

Concerning the Genotoxicological properties of Glyphosate (Roundup) herbicide, two opposite conclusions around this aspect were found, Williams et al. (2000) concluded that glyphosate or Roundup is non carcinogenic, non-teratogenic or non-developmentally toxic for humans and animals. Whereas , Vigfusson and Vyse (1980), Lioi et al. (1998) and Kooffreh (1999) stated that Roundup could induced genotoxicity in vitro, and in plants.

Many glyphosate-salt inactivators in soil and/or in plants could be proved such as : kaolinite, bentonite, illite, charcoal, clay (saturated with aluminium or iron) and muck. Phosphate compete with glyphosate for its adsorption site (Sprankle et al., 1975). So that the objective of the current study is a trial for reducing the Roudup toxicity (in vivo) by using some of its chemical or physical inactivators (which used in soil or plants), in addition to studying its genotoxic (cytogenetic) effects on the chromosomes and trial for reducing such lesions by one of the antioxidants.

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MATERIALS AND METHODS

Animals :

Fifty apparently healthy Newzealand white male rabbits with average weight of 1329 ± 64 grams were used in the current study through two experiments, 15 rabbits were divided into 3 equal groups in separated cages used for the first experiment as chronic toxicity (chromosomal aberrations) and 35 rabbits were divided into 7 equal groups in separated cages for the 2nd. experiment (acute toxicity) and treatment trials of the herbicide toxicity were carried out. All rabbits were kept under observation and accommodation for 2 weeks and given ready feed for rabbits throughout the experimental periods.

The Herbicide :

The formulated Glyphosate Isopropylamine salt (Roundup) herbicide (Monsanto Agricultural Co., USA) with 480gm glyphosate salt/Litre.

Drugs, Chemicals and kits :

1- Calcium monohydrogen phosphate, anhydrous, water insoluble (El.Nasr Co.)
2- Vitamin-E. (di-alpha-tocopheryl acetate) as a soft gelatin capsule (Farco Pharm. Co.).
3- Calcionate (each 10ml ampoule contain : 0.5 gm calcium gluconate and 0.3416 calcium levulinate) (Memphis Co.).
4- Activated charcoal (El-Nasr Co.).
5- Colchicine (El-Nasr Co.)
6-Giemsa stain (El-Nasr Co.).
7- Kis for determination of some serum biochemical constituents (El-Nasr Co)
8- ATP (Sigma Co.) for preparation of mitochondrial ATP-ase enzyme -incubation medium.

Methods :

I) Experiment-I (chronic toxicity):

Fifteen male rabbits were divided into 3-groups, the first group served as a non treated control rabbits, the second and the third groups of rabbits were orally administered with Glyphosate Isopropylamine (Roundup) herbicide with a dose level of 750 ppm in drinking water for 2-months, but the 3rd. group was orally administered with 312 ppm of vitamin-E (along with Roundup administration). After two months of treatments, all rabbits were intramuscularly injected with 0.5% colchiciene 90 minutes prior to sacrificing. The bone marrow cells samples were obtained from the femur of rabbits and the chromosomes of bone marrow cells were prepared according to Giri et al. (1986), stained with Giemsa stain, and examined under 1000 magnification oil immersion objective. For each animal, 50 metaphases were scanned for the structural and numerical chromosome aberrations (Lasley, 1978).

II) Experiment-II (acute toxicity and treatment trials) :

1- Treatments : Thirty-five rabbits were divided

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into 7 equal groups, the 1st. group of rabbits was served as a normal (-ve) control, the 2nd. , 3rd. , 4th. , 5th., 6th. and 7th. group were orally administered with the Roundup herbicide by the dose level of 1gm Glyphosate isopropylamine salt/ kg.b.w., once a day, for 6 consecutive days. The 2nd, group was Roundup intoxicated only and served as a positive (+ve) control group, but the remaining five groups of rabbits were given different treatments along with Roundup intoxication as follow : the 3rd, group was orally administered 350 mg calcium phosphate / kg. b.w., the 4th. group was orally administered with 100 mg vitamin-E/kg.b.w., the 5th. group was orally administered 350 mg activated charcoal/kg.b.w., the 6th. group was intramuscularly injected 32 mg calcionate/kg.b.w. and the last 7th. group was administered the mixture of the four above treatments (by the same doses and routs). All treatments along with the herbicide administration achieved once a day, for 6 consecutive days.

2- Sampling and methods of investigations : After 6 days of treatments, all rabbits were sacrificed at the 7th. day from starting the experiment, and the following samples were taken :

a- Blood samples (with heparin anticoagulant) were taken for differential leucocytic counts (Schalm, 1975).

b- Serum samples were prepared from all rabbits of the 7 groups for determining some serum biochemical constituents as follow : Alanine aminotransferase (ALT) enzyme activity (Reitman and Frankel, 1957), Total Bilirubin (Jendrassiki, 1938), total protein (Doumas et al., 1971), albumin (Rojkin et al,. 1974), the globulins is the difference between total protein and albumin, total lipid (Schmit, 1964) and the serum creatinine (Henry, 1974).

c- Fresh (unfixed) liver tissue samples (Kiernan, 1981) and the cryostat Freezer Plate-method (Sheehan and Hrapchak, 1980) were used for Frozen-sectioning of liver tissue prior to the histochemical staining of the mitochondrial Adenosine-Tri-phosphatase (ATP-ase) enzyme (Mg^{2+} activated, lead method) (Wachstein and Meisel, 1957). The activity of the mitochondrial ATP-ase enzyme in the liver was semiquantitatively estimated microscopically which appeared as fine to coarse, brownish to black precipitate of lead sulfide (the end product of the histochemical reaction of ATP-ase enzyme).

d- Fresh liver tissue samples from the rabbits of the seven groups were immediately put in 10% neutral formaline saline for the histopathological technique (Lillie and Fulmer, 1976), and the histological changes were microscopically examined for histopathological lesions.

The statistical analysis : The t-student test and the F-test through the analysis of variance (ANOVA) were the statistical methods used for analyzing the obtained data according to Snedecor and Cochran (1969).

RESULTS

I) Chromosomal aberrations :

A) Structural chromosomal aberrations :

The Glyphosate Isopropylamine salt (Roundup) herbicide (by the dose level of 750 ppm for 60

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days) could significantly induced the following structural chromosomal aberrations in the bone marrow cells : Centromeric Attenuations (CA), Pulverizations (P), Chromosomal Gaps (G) and Chromosomal Breaks(B). But vitamin-E treatment of Roundup intoxicated rabbits could only normalizing the pulverizations and chromosomal breaks as indicated in the table (1) and illustrated in the Figs. (1,2,3 and 4).

B) Numerical Chromosomal Aberrations :

The Roundup chronic toxicity could induced significant numerical chromosomal aberrations such as: Hypoploidy, polyploidy and periploidy. Treatment of Roundup intoxicated rabbits with vitamin-E could only normalizing the polyploidy, as indicated in the Table (1) and illustrated in the Figs. (3 and 4).

II) Histochemical study :

The semiquantitative estimation of the mitochondrial Adenosine -Tri-phosphatase (ATP-ase) enzyme activity in the liver of the different groups are arranged from intense reaction (+++++) to weak reaction (+) as follows: (1)-intense (+++++) ATP-ase activity by calcionate treatment of Roundup intoxicated rabbits (2) Moderate to strong (++++) ATP-ase activity by the group treated with the mixture of the four treatments against Roundup toxicity (3) moderate (+++) enzyme activity could induced by the only Roundup intoxicated rabbits (+ve control) (4) submoderate to moderate (+++) enzyme activity by vitamin-E treatment (5) submoderate (++) enzyme activity by calcium phosphate treatment (6) weak to submoderate (++) enzyme activity by charcoal treatment (7) weak (+) ATP-ase reaction by the five control (normal) rabbits, as tabulated in table (2) and illustrated in Fig. 5 (A,B, C,D,E, F and G).

III) Serum biochemical constituents (table, 3):

Alanine aminotransferase (ALT) enzyme activity: Only calcionate treatment (against Roundup toxicity) could induced significant increase of ALT- enzyme activity than that of normal (-ve) control (and without significant change with the +ve control which treated with Roundup only). Other treatments against Roundup could normalizing the ALT-enzyme level.

2- Total Bilirubin: The significant increase of total bilirubin by Roundup intoxicated rabbits could normalized by only charcoal treated rabbits. Also, the rabbits treated with the mixture of the 4 treatments could reduced the total bilirubin than that of only Roundup intoxicated rabbits.

3-Serum creatinine : The serum creatinine levels could be normalized by all tested antidotes except by calcionate treated animals, where the creatinine was significantly increased than both normal (-ve) and positive (Roundup treated) control groups of rabbits.

4- Total lipids : the serum total lipids could be normalized only by calcium phosphate treatment of Roundup intoxicated rabbits. No significant changes of total lipids between all tested antidotes and that of +ve control animals (except in case of calcionate treated animals which showed significant hyperlipedemia than that of the all other groups).

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5- Albumin and total proteins : The serum albumin and total proteins are non-significantly changed between all groups of rabbits.

6- Globulins : The globulins was significantly decreased by only Roundup intoxicated rabbits, and oppositely it significantly increased by either charcoal or calcionate treated animals than both controls. The other tested antidotes could normalizing the globulin level.

VI) The Differential Leucocytic Counts (table, 4):

1- Neutrophils : The neutrophil percentages was significantly decreased by all tested antidotes than negative control rabbits except by calcionate treated group, where it significantly increased. No significant change of neutrophil percentage between negative and positive control rabbits.

2- Lymphocytes : The only Roundup or the calcionate treated groups of rabbits showed significant decrease of lymphocyte percentage than that of the negative control rabbits. The charcoal and the (mixture of four treatments) treated groups showed significant increase of lymphocyte percentage than either negative or positive control rabbits, but either calcium phosphate or vitamin-E treated groups could normalizing the lymphocyte percentage.

3- Monocytes : The monocyte percentage was significantly decreased by all tested antidotes except by calcionate treated group of rabbis, where it significantly increased than that of the negative control rabbits. No significant changes of monocyte percentage between negative and positive control rabbits.

4- Eosinophils and Basophils : No significant changes of either easinophil or basophils percentages between all of the seven groups of rabbits.

V) The histopathological study :

The Roundup intoxicated rabbits showed focal areas of coagulative necrosis and hydropic degeneration of the hepatocytes, small pycnotic nuclei, congestion of the central viens and focal areas of haemorrhages (Fig. 6-A).

The Roundup intoxicated rabbits along with calcium phosphate, vitamine-E or charcoal treatments showed varying degrees of hydropic degeneration, congestion of the blood vessels and focal haemorrhagic areas. The lowest degree of these lesions was illustrated in the liver of the charcoal treated rabbits (Fig. 6-B).

The most prominent severe lesions of the all treatments was detected for calcionate treated group, then followed by rabbits received the mixture of the four tested antidotes, where the liver of calcionate treated rabbits showed severe coagulative necrosis, vacuolar degeneration and focal areas of haemorrhages (Fig. 6-C).

The rabbits received the mixture of 4-treatments showed coagulative necrosis, vacuolar degeneration, but less severe than that of calionate treated rabbits, additionally, this group showed focal areas of leucoytic infiltration and pycnotic nuclei of some hepatocytes (Fig. 6-D).

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Table (1): Effects of Roundup (Glyphosate Isopropylamine) herbicide and vitamin-E treatments on the chromosomal aberrations (Structural and Numerical) of the bone marrow cells of male Newzealand white Rabbits.

Groups (and doses per ppm in drinking water)	Centromeric attenuations (CA)	Pulverization (P)	Gap (G)	Break (B)	Deletion (D)	Total structural aberrations	Hypoploidy	Polyploidy	Ploidy	Total numerical aberrations
Groups (and doses per ppm in drinking water)	0.80± 0.49	0.00± 0.00	0.80± 0.49	0.00± 0.00	0.40± 0.40	2.00± 0.63	1.20± 0.49	0.00± 0.00	0.40± 0.40	1.60± 0.40
Control Roundup treated rabbits (570 ppm)	** 16.40± 1.33	* 2.80± 1.20	* 8.00± 2.10	* 5.60± 2.32	NS 2.00± 0.89	** 34.80± 2.42	** 10.00± 1.10	* 4.00± 1.26	* 6.40± 2.14	** 20.40± 1.33
Roundup (750ppm) + vitamin-E (312ppm)	** 13.60± 1.72	NS 1.20± 0.80	** 8.40± 1.47	NS 1.60± 0.74	NS 0.80± 0.49	** 25.60± 0.93	** 9.20± 0.80	NS 3.60± 1.60	* 4.40± 1.33	** 17.20± 2.42

N.B. : (1) * = significant change (at $P < 0.05$) , (2) ** = highly significant change (at $P < 0.01$),
(3) NS = Non significant change, (4) the two Roundup intoxicated groups are compared with the normal control rabbits.

Table (2): The semiquantitative Histochemical Activity of the Mitochondrial Adenosine -Tri-Phosphatase (ATP-ase) enzyme (Mg2+ activated, lead method) in the liver of Roundup intoxicated rabbits and with different tested Antidotes.

Groups	(1) -ve control	(2) +ve control	(3)	(4)	(5)	(6)	(7)
Dose of Glyphosate isopropylamine (Roundup)	0.00	1gm /kg.b.w	1gm /kg.b.w	1gm /kg.b.w	1gm /kg.b.w	1gm /kg.b.w	1gm /kg.b.w
Doses of tested antidotes (Inactivators)	0.00	0.00	Calcium phosphate (oral 350 mg/kg. b.w)	Vitamin. E (oral 100 mg/kg. b.w.)	Charcoal (oral 350 mg/kg. b.w.)	Calcionate (1/K.inj.of 32mg/kg. b.w)	The mixture of the four tested antidotes
The activity of mitochondrial ATP. ase enzyme	+	+++	++	++±	+±	+++++	+++±

N.B. : 1) + = weak ATP-ase enzyme reaction. 2) ++ = submoderate ATP-ase reaction.
3) +++ = Moderate enzyme reaction. 4) ++++ = Strong enzyme reaction.
5) +++++ = Intense enzyme reaction.

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Table (3): Effect of Glyphosate Isopropylamine salt (Roundup) and the different tested inactivators (antidotes) on some serum biochemical constituents in rabbits of the different groups.

Roundup (dose)	Doses of treatment	ALT enzyme activity (U/L)	Total billrubin (mg/dl)	Albumin g/dl	Globulins	Total protein g/dl	Total lipids (mg/dl)	Creatinine g/dl
0.00 (-ve control)	0.00	ae 20.44± 0.114	a 0.227± 0.070	a 3.911± 0.331	a 2.950± 0.288	a 6.861± 0.567	a 5.441± 0.459	ad 1.250± 0.102
1gm/kg.b.w (+ve control)	0.00 (+ve control)	bd 24.889± 0.537	b 1.307± 0.081	a 3.895± 0.199	b 2.538± 0.206	a 6.433± 0.850	b 7.273± 0.389	b 1.590± 0.097
1gm/kg. b.w	Calcium phosphate 350mg/kg. b.w. (oral)	a 19.635± 0.965	b 1.287± 0.102	a 3.898± 0.261	a 3.261± 0.145	a 7.159± 0.433	ab 6.678± 0.568	ad 1.320± 0.145
1g/kg. b.w.	Vitamin-E (1.00mg/kg. b.w. (oral)	a 19.635± 0.540	b 1.274± 0.136	a 3.903± 0.148	ac 3.046± 0.236	a 6.949± 0.649	b 7.786± 0.566	ad 1.300± 0.141
1g/kg. b.w.	Charcoal 350/kg. b.w. (oral)	a 17.91± 1.704	a 0.302± 0.102	a 4.136± 0.226	c 3.316± 0.319	a 7.452± 0.313	b 7.718± 0.860	a 1.140± 0.127
1g/kg. b.w.	Calcionate 32mg/kg b.w(1/M inj.)	d 25.867± 0.772	c 2.225± 0.137	a 3.565± 0.111	c 3.308± 0.258	a 7.393± 0.368	c 11.023± 0.425	c 2.200± 0.159
1g/kg. b.w.	The above four mixture treatments (as the same above doses)	eb 23.717± 2.213	d 1.523± 0.090	a 3.737± 0.086	ab 2.836± 0.206	a 6.573± 0.580	b 7.538± 0.545	db 1.490± 0.164
F-test		*	*	N.S	*	N.S	*	*
LSD (at P ≤ 0.05)		3.601	0.099	----	0.324	---	1.625	0.270

N.B.: (1) LSD = Least significant difference between groups (at p <0.05) (2) * = significant differences beween means (at P < 0.05), (3) N.S. = Non significant changes beween means of treatments. (4) The different litters in columns denote significant changes between treatments (at P < 0.05) and vise versa.

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Table (4): Effects of Roundup (Glyphosate Isopropylamine salt) and the different tested inactivators (antidotes) on the differential leucocytic counts in rabbits of the different groups.

Roundup (dose)	Doses of treatments	Neutrophil (%)	Lymphocy-tel (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
0.00 (-ve control)	0.00	ab 19.782± 2.332	a 60.572± 3.04	ab 10.512± 1.019	a 7.12± 1.145	a 2.014± 0.286
1gm/kg.b.w (+ve con-trol)	0.00 (+ve control)	ae 25.586± 3.396	bd 52.48± 1.985	ad 13.914± 1.673	a 4.268± 1.011	a 3.752± 0.833
1gm/kg. b.w	Calcium phosphate 350mg/kg. b.w. (oral)	bc 18.116± 1.620	a 64.324± 1.738	bce 8.264± 1.823	a 5.89± 0.833	a 3.316± 0.292
1g/kg. b.w.	Vitamin-E (100mg/kg. b.w. (oral)	cdf 13.486± 0.274	a 67.296± 0.768	bce 9.048± 0.489	a 6.448± 0.350	a 3.722± 0.286
1g/kg. b.w.	Charcoal 350/kg. b.w. (oral)	df 11.696± 1.915	c 75.148± 3.546	ce 6.60± 0.780	a 4.232± 0.722	a 2.324± 0.464
1g/kg. b.w.	Calcionate 32mg/kg b.w(1/M inj.)	e 31.484± 0.928	d 44.922± 2.117	d 14.032± 1.06	a 7.29± 1.456	a 2.272± 0.277
1g/kg. b.w.	The above four mixture treatments (as the same above doses)	f 10.068± 0.591	c 75.85± 1.224	e 6.65± 0.344	a 4.616± 0.657	a 2.816± 0.433
F-test		*	*	*	N.S	N.S
LSD (at P ≤ 0.05)		6.169	7.837	3.430	---	---

N.B.: (1) LSD = Least significant difference between groups (at p <0.05) (2) * = significant differences between means (at P < 0.05), (3) N.S. = Non significant changes between means of treatments. (4) The different litters in columns denote significant changes between treatments (at P < 0.05) and vise versa.

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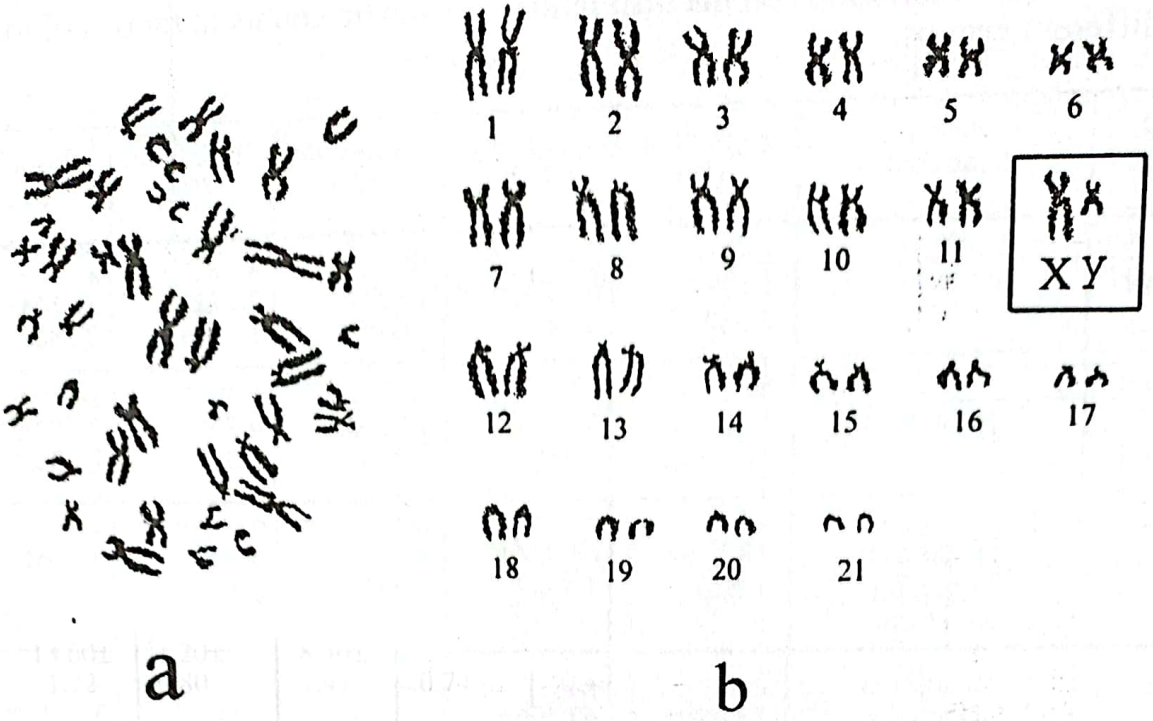

Fig. (1) (a) A metaphase spread of the chromosomes of the bone marrow cell of the control male rabbit, b) the detailed karyotypic profile of the metaphase spread of (a) (X 1000)

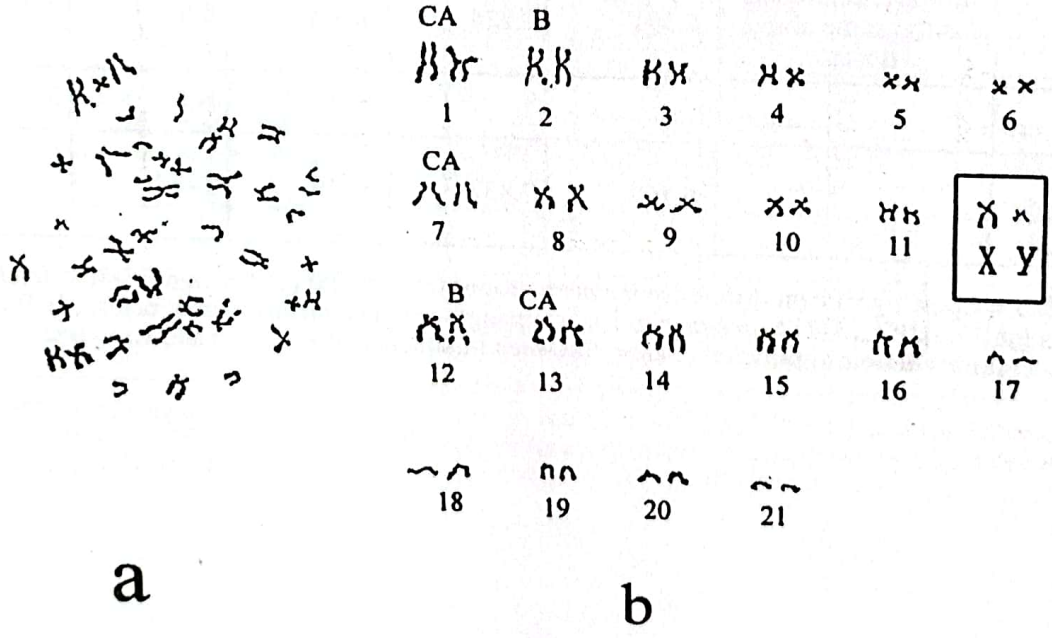

Fig.(2): a) A metaphase spread of the chromosomes of the bone marrow cell of rabbit administered 750 ppm glyphosate isopropylamine herbicide in drinking water for 60 days showing Centromeric attenuations (CA) and Breaks (B). b) the detailed karyotypic profile of the metaphase spread of (a) (X 1000).

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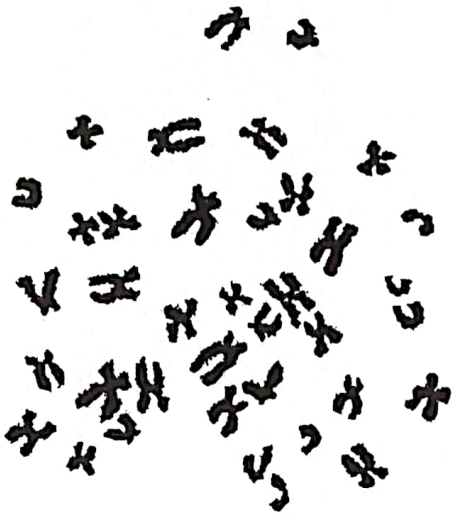

Fig. (3): A metaphase spread of the chromosomes of the bone marrow cell of male rabbit administered with 750 ppm Glyphosate isopropylamine in drinking water for 60 days showing Hypoploidy (2n = 38) (X 1000).

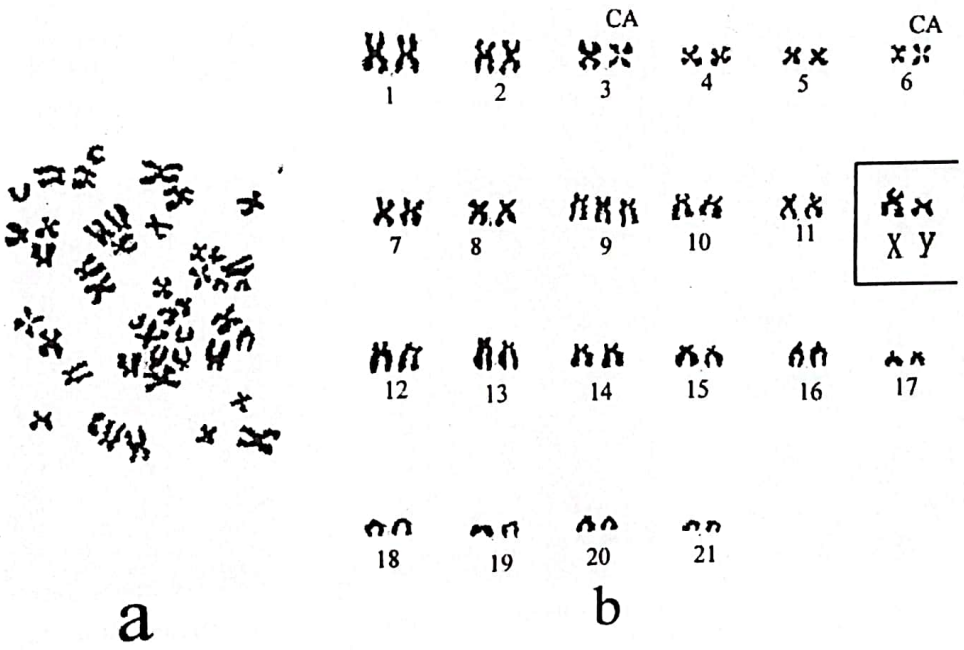

Fig. (4): a) A metaphase spread of the chromosomes of the bone marrow cell of male rabbit administered 750 ppm Glyphosate Isopropylamine and 312 ppm vitamin-E in drinking water for 60 days showing Centromoric attenuations (CA) and Trisomy. b) The detailed karyotypic profile of (a) (2n = 45) (X 1000).

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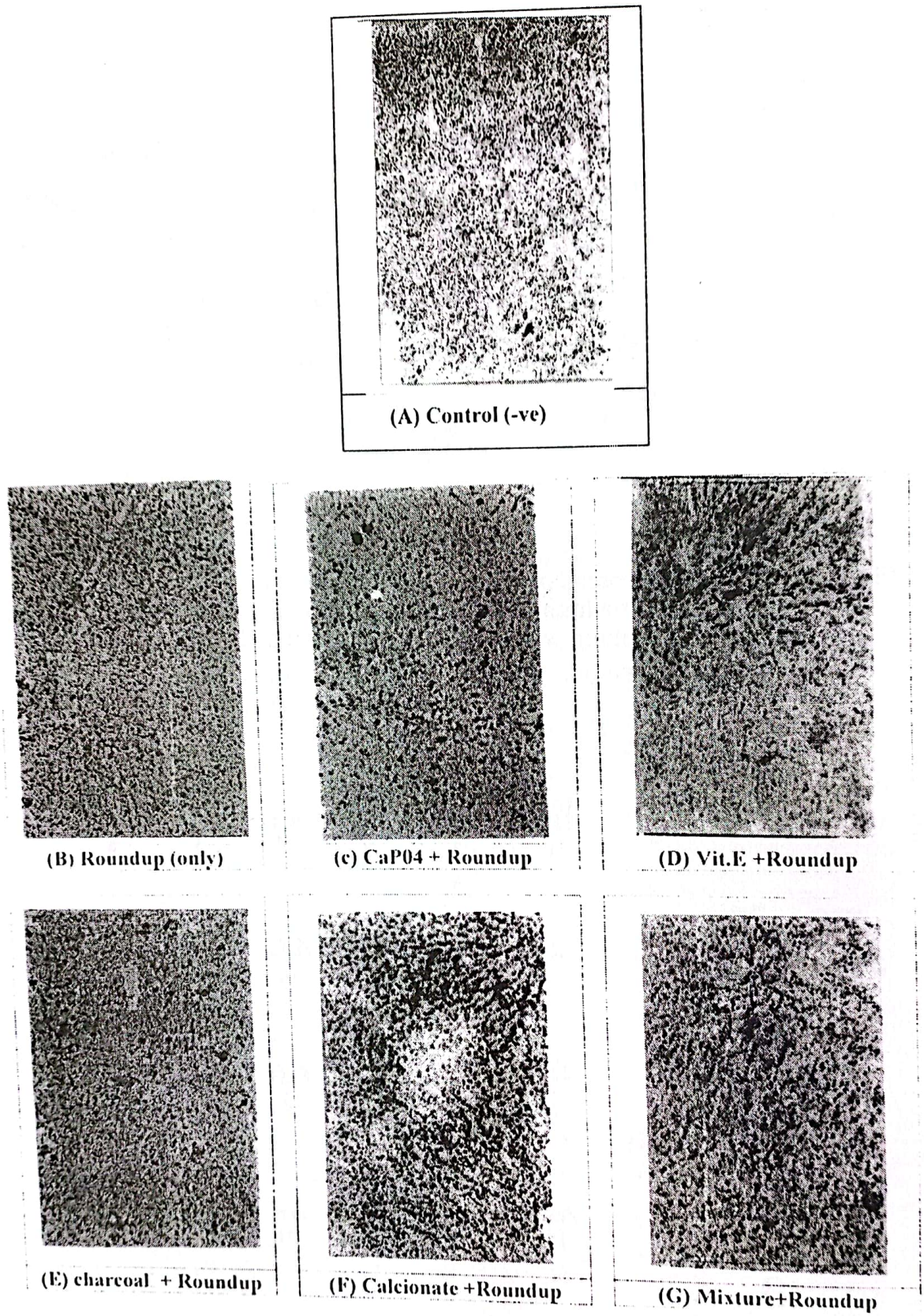

Fig. (5): The cryostat sections in liver of rabbits administered glyphosate isopropylamine (Roundup) herbicide (1gm/kg.b.w.) along with tested antidotes demonstrating the activity of mitochondrial ATP-ase enzyme as a fine to coarse brownish black lead sulfide ppt. as follow : A- Control rabbit with weak (+) activity, (B) Roundup poisoned rabbit with moderate (+++) activity, (C) calcium phosphate treated rabbit with submoderate (++) activity, (D) vit. E treated rabbit with moderate to submoderate (+++) reaction, (E) charcoal treated rabbit with weak to submoderate (++) reaction, (F) calcionate treated rabbit with intense (+++++) activity and (G) : rabbit with mixture of four treatments with moderate to strong (+++±) ATP-ase activity (X 200)

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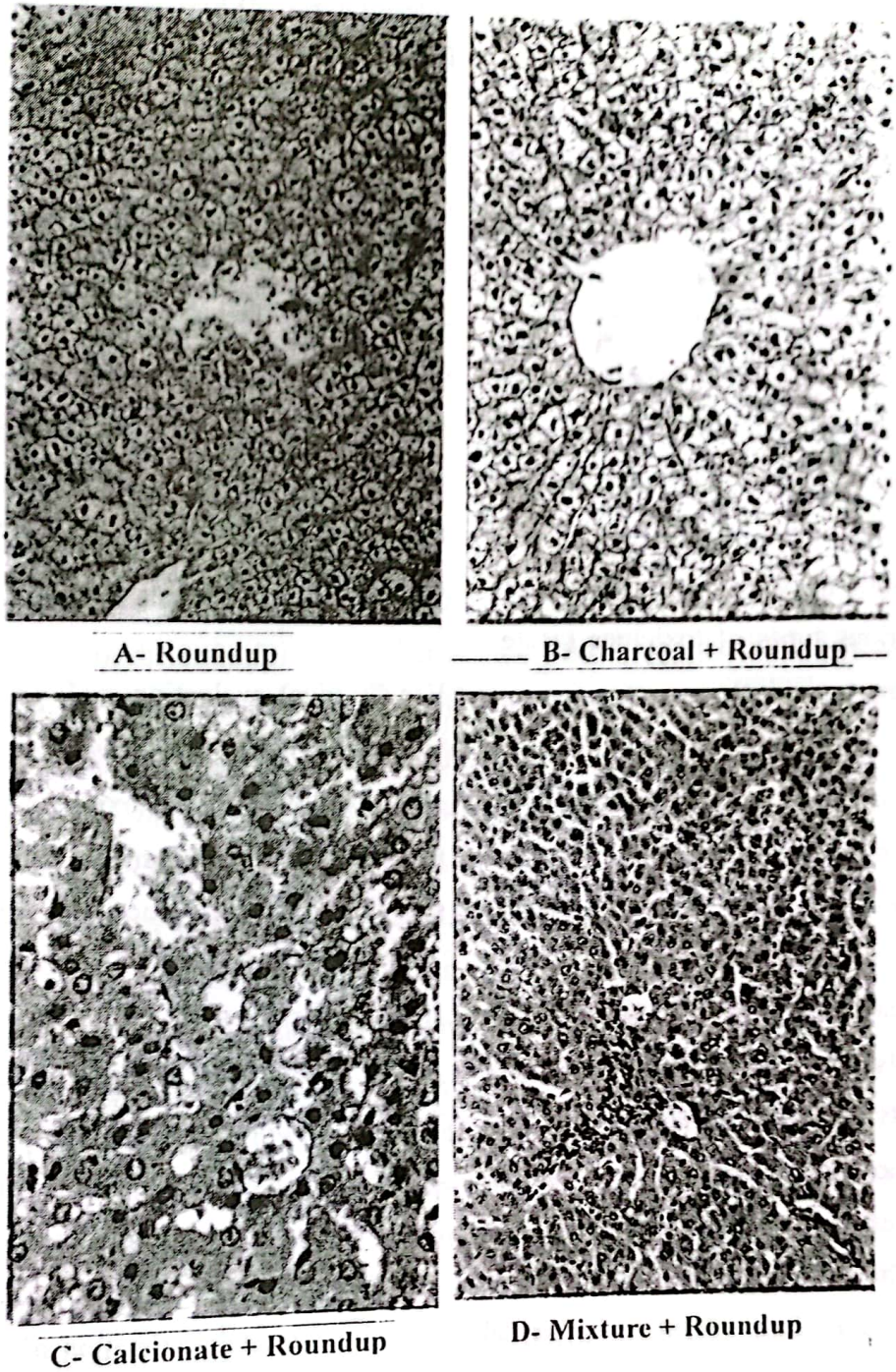

Fig. (6): Sections in the liver of rabbits administered 1gm glyphosate isopropylamine salt (Roundup) herbicide /Kg.b.w., once a day, for 6 days and treated with different tested antidotes, showing : A- Liver section of Roundup intoxicated rabbits with hydropic (vacuolar) degeneration, focal areas of necrosis and small pycnotic nuclei of hepatocytes. B) Liver section of rabbits treated with Roundup and 350 mg/kg.b.w. charcoal with hydropic degeneration of hepatocytes. C) Liver section of rabbit treated with Roundup and 32 mg calcionate (I/M inj.)/kg.b.w. with diffuse coagulative necrosis, focal haemorrhagic areas and vacuolar degeneration. D) Liver section of liver of rabbit treated with Roundup and mixture of four treatments with focal areas of necrosis, congestion of blood vessels, focal leucocytic infiltration and small pycnotic nuclei (H & E X 200).

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DISCUSSION

The pesticides are broadly used for pest control in agriculture despite the negative impacts they may pose to the environment. Animals may get these pesticides in their bodies by eating the treated plants, also human may get pesticides, directly by eating treated plants or eating meat or animal food products with considerable residual amounts in their tissues. The continuous using of such pesticides lead to possible hazards to animals and humans through various types of toxicities (acute, chronic or long term toxicities).

Roundup herbicide (the formulated isopropylamine salt of glyphosate) used in Egypt as a broad spectrum weed killer. Many Roundup (glyphosate) inactivators used in soil, plants or in vitro studies. Such inactivators as phosphate which competes with glyphosate for its adsorption site, and as the quantity of phosphate increased, the adsorption of glyphosate decreased (Sprankle et al,. 1975). So we used the calcium phosphate in the current study. The metal chelating properties of glyphosate concerning the mode of its herbicidal activity was of considerable value, that glyphosate interfere with several metal ions in plants and its herbicidal activity is reduced if it is supplied together (Grossbard and Atkinson, 1985). So we testing the calcionate drug (a source of calcium) as a proposed inactivator *in vivo*. Also, the physical inactivator by adsorption through many glyphosate adsorbants in the soil such as kaolinite, illite, charcoal, muck, etc..were recorded by Sprankle et al., (1975). So we used charcoal in the current study. The physiological inactivation by antioxidant such as vitamin-E (Murray et al,. 1993) that glyphosate (Roundup) toxicity induced oxidative stress (Olorunsogo et al., 1979 and Helal, 1993). So we used such proposed glyphosate inactivators as a trial in order to (at least) reducing the acute or chronic toxicities of this herbicide in the body.

For (*in vivo*) evaluating such proposed inactivators in the present work, we used some of the already well established toxicological properties of glyphosate isopropylamine (Roundup) such as : the degree of activation of the mitochondrial ATP-ase enzyme by glyphosate biochemically or histochemically such as recorded by Olorunsogo et al., (1979) and Helal(1993), respectively. The immunosuppression properties of Roundup through its reduction of the circulating lymphocytes and consequently the immunoglobulins (Helal, 1993). The suppression of the liver and kidney functions through estimation of some serum biochemical parameters were used by many authers for evaluating the toxic chemicals, drugs or infectious agents (Kachman and Moss, 1976), in addition to the histopathological changes which may induced by Roundup toxicity (Helal, 1993), or along with the different treatments.

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For evaluating the cytogenecity of Roundup herbicide in the present chronic study, Roundup could induced the following statistically significant structural chromosomal aberrations : Centromeric attenuations, chromosome gaps, chromosome breaks, and pulverization, but vitamin-E treatment of Roundup intoxicated rabbits could only normalizing both chromosomal breaks and pulverization. Concerning the numerical chromosomal aberrations, Roundup could significantly induced : hypoploidy, polyploidy and periploidy, but vitamin-E treatment of Roundup toxicity could only normalizing the polyploidy. Williams et al. (2000) recorded that glyphosate (Roundup) herbicide has no genotoxic effects in animals and human beings, but many other researches were recorded such cytogenetic changes (genotoxic effects). Vigfusson and Vyse (1980) and Lioi et al. (1998) could induced a mutagenic effect as sister chromatid exchange *in vitro* in bovine and human lymphocytes respectively by Roundup (glyphosate) herbicide. Lioi et al. (1998) could induced also a statistically significant chromosomal aberrations in the bovine lymphocytes. Also, Rank et al. (1993) could induced disturbance in the spindle fibres *in vitro* by glyphosate, Kooffreh (1999) could induced bridges, laggards, acentric fragments and disturbances in the spindle fibres, but *in vivo* study Clements et al. (1997) could induced erythrocyte DNA-damage in Tadpole by Roundup. In plants, Njagi and Gopalan (1981) could induced premature chromosome condensation and pycnotic nuclei, also Ezhova et al. (1992) could relating the epigenetic and cytogenetic changes induced by glyphosate to the reduction of fertility in vicia faba plant. Based on our study, it could be concluded that Roundup herbicide may induced structural and numerical chromosomal aberrations (i.e. it is a genotoxic agent) , and these effects could partially reduced by the vitamin-E antioxidant treatment. Further mutagenicity and carcinogenicity studies should be carried out.

For evaluating the tested proposed inactivators (antidotes) against Roundup toxicity, the present histochemical study showed that the tested antidotes could be arranged according to the strength of their activations to the mitochondrial ATP-ase enzyme as follow : negative (normal) control (the lower ATP-ase activation), charcoal, calcium phosphate, vitamin-E, positive (Roundup treated) control, the mixture of the four treatments and the calcionate (the highest ATP-ase activation). According to Olorunsogo et al. (1979) the activation of mitochondrial ATP-ase enzyme in hepatocytes is the most important side reaction in glyphosate intoxication (with dose dependent). Accordingly charcoal should be considered the most effective inactivator in reducing Roundup toxicity from this aspect , in contrast the calcionate or the mixture of the four treatments could promote the Roundup toxicity perhaps due to calcium therapy (in the two groups) which suggested to be the reason for increasing the Roundup toxicity. Concerning the

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phytotoxicity in plants, Grossbard and Atkinson (1985) mentioned that in short term studies, the to calcium, sodium and potassium increased the phyotoxicity but zinc and iron decreased it, but previousely, Phillip (1975) found that calcium, iron, and aluminum reduced the phytotoxicity of glyphosate in plants.

The present serum biochemical study revealed that either calcionate or the mixture of four treatment (against Roundup toxicity) and the group treated with Roundup only could induced a significant increase of ALT-enzyme activity and the serum concentrations of total bilirubin and creatinine, while the other treatments could normalizing these parameters, suggesting that either calcionate or the (mixture of the four treatments) and the group treated only with Roundup could induced liver dysfunction according to Zimmerman (1984) and Kidney dysfunction according to Hayes (1989).

The serum total lipids increased significantly by all Roundup treated groups of rabbits, that the all treatments against Roundup failed to normalizing the hyperlipidemia induced by this herbicide, and the highest level of total lipid was showed by calcionate treatment, and this hyperlipidemia indicated a regulatory disturbances in lipid metabolism according to (Stroev, 1986).

The serum globulins was significantly decreased by Roundup treated rabbits, in contrast, it significantly increased by either charcoal or calcionate treated groups (against Roundup) than normal control. The globulins may decreased due to the hypogamma-globulinemia which may induced by Roundup toxicity (Helal, 1993), or it may be increased due to hyper alpha- or beta - globulinemia as a result of some chemical or toxic agents (Grant and Kachman, 1976).

The lymphocyte percentage was significantly decreased by the groups of rabbits treated with Roundup (only) and in calcionate treated rabbits, but calcium phosphate and vitamin-E treated rabbits (against Roundup) could normalizing the lymphocyte percent. Oppositely, charcoal and the mixture of four treatments could induced significant increase of the lymphocyte percentage, this indicated that all tested inactivators (except calcionate) succeed to abolish the lymphocytopenia induced by Roundup. Approximately (in most cases) the opposite pattern of lymphocytes could be detected for neutrophils and monocytes. The decreased levels of circulating lymphocyte percentages by Roundup toxicity in rabbit was detected by Helal (1993) as one of the parameters used for measuring its immunosuppressive action of Roundup. The increased levels of neutrophils and monocytes by calcionate treated rabbits perhaps following the tissue injury which may induced by coagulative necrosis and vacuolar or hydropic degeneration of the hepatocytes, as

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indicated for this group by the current histopathological study, where the neutrophils eliminate the foreign materials via phagocytosis and may increase in response of tissue injury, infectious agents, parasites and inflammatory or foreign materials (Jain, 1986).

The most severe and diffuse histological changes in the liver could be induced by calcionate treated rabbits (along with Roundup) followed (according to the degree of severity) by the group of rabbits treated with the mixture of the four treatments, where the liver of the two groups of rabbits showed focal or diffuse coagulative necrosis, small pycnotic nuclei of some hepatocytes and focal areas of haemorrhages (as recorded by the rabbits of calcionate group). In addition to the above lesions (with less severe) there were focal areas of leucocytic infiltration which could showed by the group treated with the mixture of four treatments, and this may explain the significant increase of lymphocyte percentage by this group. But charcoal, calcium phosphate and vitamin-E treated rabbits showed hydropic degeneration of hepatocytes with varying degrees, which become less severe or focal by charcoal treatment. The hydropic degeneration could also detected for only Roundup treatment which associated also with focal areas of coagulative necrosis and small pycnotic nuclei of the hepatocytes such as previousely recorded by Helal (1993).

Based on the present genotoxicological, histochemical, biochemical, haematological and histopathological studies, it could be concluded that Roundup (Glyphosate Isopropylamine salt) herbicide could induced significant structural and numerical chromosome aberrations, which in turn, could (partially) reduced by vitamin-E antioxidant treatment. Also, the charcoal should be considered the most active inactivator, which could reduced the most toxic effects induced by Roundup, followed (in order of their inactivation) by calcium phosphate then the antixodiant vitamin-E. Oppositely, the calcionate could enhance the Roundup toxicity, and the calcium preparations should be contraindicated in case of the Roundup toxicity.

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