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Cytogenetic Study on the Effect of Bentazon and Glyphosate Herbicide on Mice

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Genotoxicity, Bentazon, Glyphosate, chromosomal aberrations, micronucleus

ABSTRACT:

Genotoxic effect of acute and chronic doses of Bentazon and Glyphosate herbicides were estimated using micronucleus (MN), nuclear budding, mitotic index (MI) and chromosomal aberration assays. Five groups of Swiss male albino mice were used in acute exposure of 1/5 and 1/10 LD_{50} of both herbicides after 24, 96 hours of exposure compared to the control. For chronic exposure 1/20 of LD_{50} of each herbicide was for two months. Genotoxicity of acute doses at different duration of Bentazon and Glyphosate were confirmed through significant increased number of aberrant cells, different types of chromosomal aberration, micronucleus and nuclear budding. Chronic dose of Bentazone induced more genotoxic effects than Glyphosate.

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1. INTRODUCTION

An herbicide is any agent used to bring about plant death. Herbicides are primarily synthetic chemicals manufactured for use in the agriculture, industrial and ornamental and turf industries. For many years, these products have been seen as toxins that poison plants and are equally harmful to the applicator.

The use of pesticides in modern agriculture has greatly improved yield through inhibition of disease causing organisms and by acting against pest in the fields and during storage of agricultural products (Taylor et al., 1997; Mackenzie et al., 1998). The mutagenic and carcinogenic action of herbicides, insecticides and fungicides on experimental animals is well known and several studies have shown that chronic exposure to low levels of pesticides can cause mutations and/ or carcinogenicity (IARC, 1990, 1991; Yu, 2005). Pesticide residues can be present in fruit and vegetables and represent a risk for human health. Several studies have shown that chronic exposure to low levels of pesticides can cause birth defects and that prenatal exposure is associated with carcinogenicity (FAO/WHO 2004; Feretti et al., 2007). Pesticides residues are known to persist in soil, water and food and have posed problems all over the world (Subbarao, 1999).

Bentazon (Basagran) was initially registered in 1975. It is a post emergence herbicide used for selective control of broadleaf weeds and sedges in beans, rice, corn, peanuts and mint. Bentazon is a contact herbicide, it causes injury only to the parts of the plant to which it is applied. It interferes with the ability of susceptible plants to use sunlight in the production of energy for survival (Hartley and Kidd, 1987).

Glyphosate (Glalica) is a non-selective, systemic herbicide that can control most annual and perennial plants. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants (Vigfusson et al., 1980; Dykstra and Ghali, 1991; Chan and Joel, 1992; Akcha et al., 2012). However, there are a confusing data about the genotoxicity of Glyphosate and Bentazon, so the aim of this work is to assess the genotoxicity of the acute and chronic doses of these two herbicides on mice.

2. MATERIALS AND METHODS

2.1. Animals. A total number of 65 adult Swiss albino mice of four month's old, weighting 20-25 g were used in this study. Mice were housed in plastic cages with wire cover and fed on a commercial basal diet and water ad libitum for one week before the experiment for acclimatization and to ensure normal growth and behavior.

2.2. Chemicals. Acute dose of two herbicides (1/5 LD_{50}, 1/10 LD_{50}) and the chronic dose 1/20 LD_{50}) were used. The herbicides are; Bentazon (Basagran) with 1100 mg/kg body weight oral LD_{50} (Hartely and kidd 1987) and 505 mg/kg body weight intraperitoneal LD_{50} (Toyoshima et al., 1978b). Glyphosate (Glalica) with 1568 mg/kg body weight oral LD50 and intraperitoneal 130 mg/kg body weight (Anne and Fan 1999).

2.3. Experimental design. For acute treatment, mice were divided into five groups for each herbicide (Bentazon and Glyphosate) each group was consists of five mice. Group 1: were fed basal diet only and serve as negative control group. Group 2: were injected intraperitoneal with 1/5 LD_{50} concentration of herbicide and were killed after 24 hours after administration of herbicide. Group 3: were injected intraperitoneal with 1/10 LD_{50} concentration of herbicide, were killed after 24 hours after administration of herbicide. Group 4: were injected intraperitoneal with 1/5 LD_{50} concentration of herbicides killed after 96 hours after administration of herbicide. Group 5: were injected intraperitoneal with 1/10 LD_{50} concentration killed after 96 hours. Chronic treatment for two months, one group received Bentazon orally 1/20 LD_{50} (20 mg/kg B.W), a second group received Glyphosate orally 1/20 LD_{50} (1.98/kg B.W) were added in drinking water and a control group without any treatment.

2.4. Cytogenetic analysis.

2.4.a. Analysis of chromosomal aberrations was done on one femur bone marrow. The animals were sacrificed two hours after intraperitoneal injection of 4 mg/kg body weight colchicine. Bone marrow preparation was made according to Giri et al. (1986). Analysis of chromosomal aberrations in 50 well spread metaphase per animal including fragments, deletions, ring chromosome, end to end associations and polyploidy were performed. The mitotic index were calculated from 1000 cells per animal.

2.4.b. Micronucleus preparations were prepared from the other femur following the procedures of (Schmid, 976; Essa 2012). Bone marrow was flushed out, with 1–1.5 ml of saline and centrifuged at 1000 rpm for 5 min. The sediment cells were then smeared onto clean slide (three for each animal). After drying, the slides were fixed in absolute methyl alcohol for 10 min. and then stained with 10% Giemsa stain. The incidence of micronucleated cells per 500 polychromatic erythrocytes was demonstrated for each animal. The micronuclei represent condensed or chromosome fragments that remain after the nucleus expelled.

2.5. Statistical analysis. Data were submitted to analysis of variance, $p \leq 0.05$, to compare the treated groups with the control group. Calculation were done using the SAS system (SAS 1989).

3. RESULTS

3.1. Bentazon in acute treatment

All acute doses of Bentazon (1/5, 1/10 of LD_{50} after 24 and 96 hours) induced genotoxic effect through significant increase in aberrant cells, budding and micronucleus when compared with the control (table 1). However, Bentazon 1/5, LD_{50} concentration at 96 induced the highest genotoxic effect through increased level of aberrant cells and the micronucleus. Bentazon 1/5 LD_{50} at 24 hours and Bentazon 1/5, 1/10 LD_{50} concentration at 96 hours induced higher level of mitotic index.

Compared to the control, Bentazon (1/5, 1/10 of LD_{50} after 24 and 96 hours) induced significant increase in different types of chromosomal aberration; fragment, deletion, ring chromosome, end to end association, break, gap and polyploidy (Table 2, Fig. 1). Bentazon 1/10 LD_{50} after 96 h. induced more chromosomal aberration especial in fragment and deletion. While, Bentazon 1/5 LD_{50} induced higher level in ring chromosome, break and polyploidy. Although end to end association were higher in Bentazon 1/5 LD_{50} concentration at 24 hours but gap were higher in Bentazon 1/10 LD_{50} concentration at 24 hours.

Table 1. Effect of acute dose of Bentazon on mitotic index, aberrant cell, budding and micronucleus cell

Time	Treatment	Mitotic index	Aberrant cell	Budding	MN
Acute experiment (At 24 hrs of experiment)	Bentazon 1/5 LD_{50}	5.48±0.41 [a]	31.60±1.29 [d]	6.40±0.51 [b]	6.00±0.95 [a]
	Bentazon 1/10 LD_{50}	3.50±0.26 [b]	32.67±2.03 [c]	11.00±5.03 [a]	5.67±1.20 [b]
	Control	2.00±0.20 [c]	16.00±2.00 [f]	2.50±0.50 [d]	4.00±0.20 [c]
Acute experiment (At 96 hrs of experiment)	Bentazon 1/5 LD_{50}	5.20±0.53 [a]	37.00±3.14 [a]	4.25±0.75 [c]	6.50±2.84 [a]
	Bentazon 1/10 LD_{50}	5.10±0.40 [a]	35.50±2.06 [b]	4.50±0.96 [c]	3.25±0.85 [d]
	Control	1.77±0.12 [c]	14.67±2.03 [g]	1.50±0.29 [e]	1.67±0.67 [f]

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE.

Table 2. Effect of acute doses of Bentazon on different types of chromosomal aberrations

Time	Treatment	N	Fragment	Deletion	Ring chromosome	End to End	Break	Gap	Polyploidy
Acute experiment (At 24 hrs)	Bentazon 1/5 LD_{50}	5	15.40±0.93[c]	6.40±0.81 [b]	8.20±0.97[b]	11.80±0.9[a]	6.80±1.16[a]	4.80±0.49 [b]	3.80±0.58 [b]
	Bentazon 1/10 LD_{50}	3	15.33±0.33[c]	2.00±0.00 [d]	4.00±1.53 [c]	3.00±0.58[e]	3.67±0.67[b]	10.33±0.88[a]	1.33±0.33 [c]
	Control	2	8.50±1.50[d]	1.00±0.00 [e]	00.0 [d]	4.50±1.5[c]	1.50±0.50[c]	1.00±0.00[c]	00.0[d]
Acute experiment (At 96 hrs)	Bentazon 1/5 LD_{50}	4	16.25±0.48[b]	5.50±0.96[c]	9.75±2.06 [a]	8.50±0.87[b]	7.50±1.50[a]	4.75±1.03[b]	5.25±0.85[a]
	Bentazon 1/10 LD_{50}	4	20.25±1.75[a]	7.25±1.18[a]	6.75±1.75[b]	8.25±1.38[b]	4.25±1.32[b]	4.00±0.82[b]	3.50±0.65[b]
	Control	3	7.00±1.00[e]	2.50±0.50[e]	1.00±0.00[d]	4.00±1.00[c]	2.67±0.33[c]	1.00±0.[c]	2.00±0.01[c]

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE

Table 3. Effect of acute doses of Glyphosate on mitotic index, aberrant cell, budding and micronucleus cell

Time	Treatment	Mitotic index	Aberrant cell	Budding	MN
Acute experiment (At 24 hrs of experiment)	Glyphosate 1/5 LD_{50}	3.70±0.31[b]	29.67±3.38[a]	2.67±0.33 [d]	1.33±0.33 [d]
	Glyphosate 1/10 LD_{50}	3.17±0.48[c]	24.67±6.69[b]	3.33±1.86[c]	2.00±0.58[c]
	Control	2.00±0.20[d]	16.00±2.00[d]	2.50±0.50 [d]	2.50±0.29 [c]
Acute experiment (At 96 hrs of experiment)	Glyphosate 1/5 LD_{50}	2.70±0.21[d]	26.25±2.95[b]	5.00±0.71 [b]	4.00±0.20[a]
	Glyphosate 1/10 LD_{50}	4.75±0.56[a]	35.75±3.07[a]	9.33±2.19[a]	3.00±2.00 [b]
	Control	1.77±0.12 [e]	14.67±2.03 [e]	1.50±0.29 [e]	1.67±0.67 [d]

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE

3.2. Glyphosate in acute treatment

Acute doses of Glyphosate (1/5, 1/10 of LD_{50} after 24 and 96 hours) induced genotoxic effect through significant increase in aberrant cells, budding (table 3). Glyphosate 1/10 LD_{50} concentration at 96 hours induced higher level in mitotic index. While, aberrant cells were significantly induced by Glyphosate 1/5 LD_{50} concentration at 24 hours and 1/10 LD_{50} concentration at 96 hours.

Data illustrated in table (4) showed significant effects of Glyphosate on induction of different types of chromosomal aberrations compared with the control. Glyphosate 1/10 LD_{50} concentration at 96 hours induced more chromosomal aberration than other treated groups.

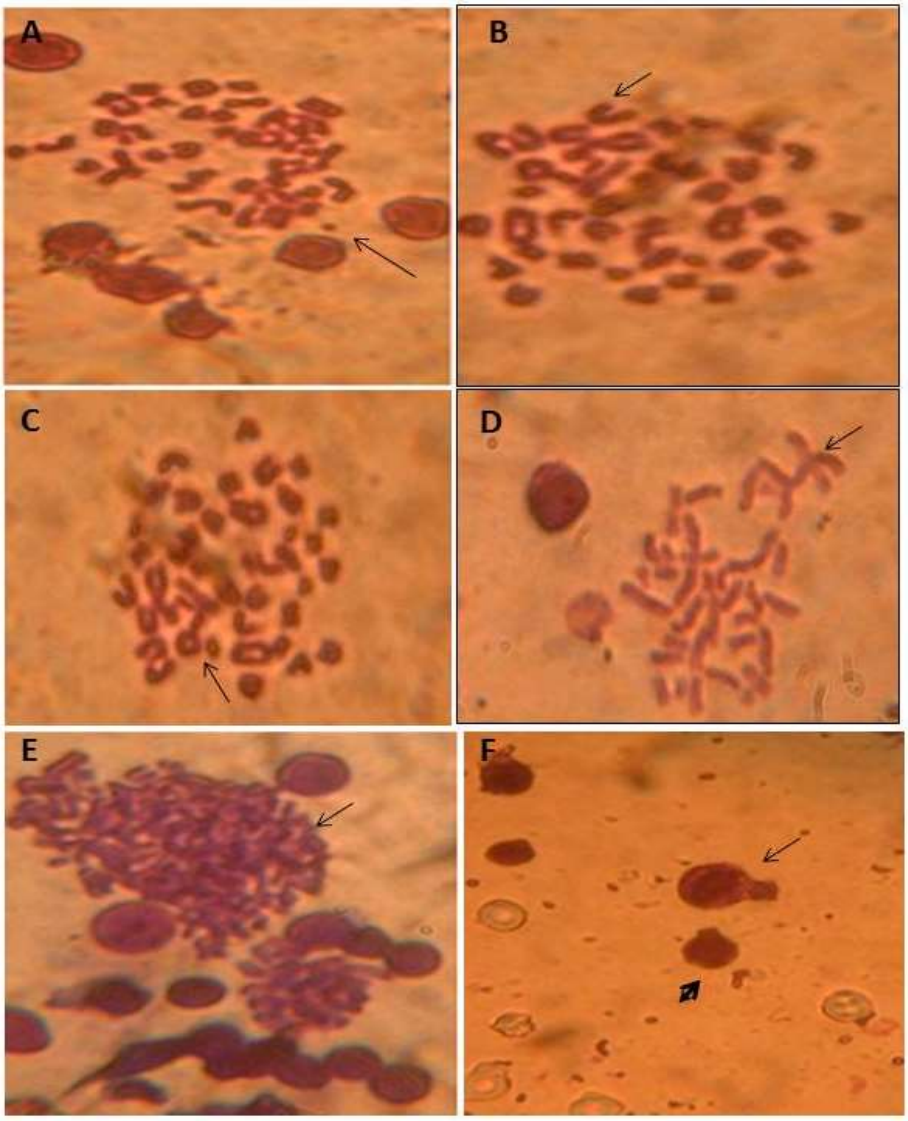

Fig.1. Photomicrographs showing different abnormalities in mice treated with herbicides. The arrow indicate fragment (A), deletion (B), ring chromosome (C), end to end association (D), Polyploidy (E) and (F) the arrow indicate Micronucleus, head arrow indicate the budding.

Table 4. Effect of acute doses of Glyphosate on different types of chromosomal aberrations.

Time	Treatment	N	Fragment	Deletion	Ring chromosome	End to End	Break	Gap	Polyploidy
Acute experiment (At 24 hrs)	Glyphosate 1/5 LD_{50}	3	7.33 ± 0.8^{b}	4.00 ± 1.1^{a}	3.67 ± 0.88^{b}	3.67 ± 0.8^{e}	7.33 ± 2.03^{b}	7.67 ± 0.8^{a}	3.33 ± 0.33^{b}
	Glyphosate 1/10 LD_{50}	3	8.50 ± 1.5^{a}	2.67 ± 0.8^{b}	3.67 ± 1.20^{b}	3.67 ± 0.3^{e}	7.67 ± 2.19^{b}	6.00 ± 1.5^{b}	2.33 ± 1.33^{c}
	Control	2	5.00 ± 1.1^{d}	1.00 ± 0.0^{c}	00.0^{d}	4.50 ± 1.5^{c}	1.50 ± 0.50^{d}	1.00 ± 0.0^{d}	00.0^{f}
Acute experiment (At 96 hrs)	Glyphosate 1/5 LD_{50}		7.00 ± 1.0^{b}	2.50 ± 0.9^{b}	3.50 ± 1.04^{b}	6.25 ± 0.7^{b}	6.00 ± 1.22^{d}	3.50 ± 0.8^{c}	1.33 ± 0.33^{e}
	Glyphosate 1/10 LD_{50}	4	8.75 ± 1.1^{a}	4.50 ± 0.8^{a}	6.00 ± 0.71^{a}	8.25 ± 1.9^{a}	10.75 ± 1.6^{a}	6.75 ± 1.2^{b}	4.00 ± 1.35^{a}
	Control	3	5.75 ± 0.8^{c}	2.50 ± 0.5^{b}	1.00 ± 0.00^{c}	$4.00\pm1.00^{d,e}$	2.67 ± 0.33^{c}	1.00 ± 0.0^{d}	2.00 ± 0.0^{d}

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE

Table 5. Effect of Bentazon and Glyphosate after 2 months on mitotic index, aberrant cells, budding and micronucleus

Treatment	Mitotic index	Aberrant cell	Budding	MN
Bentazon	4.70±0.72[a]	32.75±3.28[a]	4.75±1.44 [a]	2.50±0.29 [a]
Glyphosate	3.48±0.31 [b]	31.25±1.44 [a]	2.75±0.85 [b]	2.25±0.63 [a]
Control	1.85±0.18[c]	17.25±1.03 [b]	2.75±0.48 [b]	1.25±0.25 [b]

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE,

Table 6. Chronic effects of Bentazon and Glyphosate on chromosomal aberration for two months.

Treatment	Fragment	Deletion	Ring chromosome	End to End	Break	Gap	Polyploidy
Bentazon	18.00±4.69[a]	7.00±2.65[a]	9.25±4.33[a]	8.50±2.60[a]	8.75±5.11[a]	6.00±1.41[a]	4.75±2.10[a]
Glyphosate	9.25±1.11[b]	3.75±0.75[b]	5.00±0.82[b]	4.75±0.85[b]	7.50±1.85[b]	4.50±0.65[b]	4.00±0.58[a]
Control	8.00±0.91[c]	1.75±0.48[c]	1.25±0.25[c]	3.75±0.48[c]	2.25±0.25[c]	2.50±0.50[c]	1.50±0.50[b]

Means within the same column of different litters are significantly different at ($P < 0.05$). Each value represents mean ± SE,

3.3. Chronic treatment

Table (5) showed that Bentazon induced higher level in mitotic index and budding cells. While, Bentazon and Glyphosate induced same level in aberrant cells and micronucleus.

The data illustrated in table (6) showed that Bentazon induced higher different types of chromosomal aberrations (fragment, deletion, ring chromosome, end to end association, gap and break) compared with Glyphosate.

4. DISCUSSION

The data in these studies indicate presence of significant difference between mice treated with Bentazon with different concentration and different period of study. Bentazon 1/5 LD_{50} concentration at 96 hours were higher in aberrant cell and micronucleus. While, chronic treatment of bentazon induced higher level of chromosomal aberration.

Toxicological review of Bentazon (1998) has evaluated in animal studies involving both rats and mice for chronic periods of exposure. Though the data in the studies exhibited apparent increases in several tumor types, it was subsequently found that the tumor incidences were at rates that were normally found in the testing laboratory as historical control values (Tajima et al., 1984 and Cifone, 1985). Heres-Pulido et al., 2008 found that genotoxicity of Bentazon was demonstrated in the wing spot test of Drosophila melanogaster.

On the other hand Moriya et al., 1983 found that, Bentazon was not mutagenic to the onion root tip cells. All the cells observed were in late telophase which was indicative of the high toxicity of Bentazon. Louis and True (1995) found that Bentazon is slightly acutely toxic by all routes (Toxicity Category III) and is a skin sensitizer. It is classified as a "Group E" carcinogen--a chemical showing evidence of non-carcinogenicity to humans.

There were significant difference in Glyphosate treated group at different concentration and different period of study. Glyphosate 1/10 LD50 concentration at 96 hours showed higher level in mitotic index, budding and chromosomal aberration. While, chronic treatment induced significantly high levels of chromosomal aberration.

Monsanto, 1988 demonstrated that Roundup and Pondmaster (an aquatic herbicide consisting of glyphosate and a trade secret surfactant) both increased the frequency of sex-linked, recessive lethal mutations in fruit flies (These are mutations that are usually visible only in males because two damaged genes are required in order to be expressed in females). Vigfusson and Vyse, 1980 made a

laboratory study on human lymphocytes which showed an increase in the frequency of sister chromatid exchanges following exposure to high doses of Roundup (Sister chromatid exchanges are exchanges of genetic material during cell division between members of a chromosome pair). Rank, 1993 found that Roundup was weakly mutagenic at high concentrations in salmonella. In onion root cells, Roundup caused an increase in chromosome aberrations (Williams et al., 2000)

On other hand Dykstra and Ghali (1991) showed that no carcinogenic effects were observed in tests of Glyphosate. EPA, 1993 classified glyphosate as a "Group E" carcinogen or a chemical that has not shown evidence of carcinogenicity in humans. The genotoxic potential of glyphosate has been extensively tested in a wide range of assays both in vitro and in vivo, including end-points for gene mutation, chromosomal damage and DNA repair (Rudolf and Lars-Niemann 2004). Negative results were obtained in studies performed in compliance with these test guidelines.

We conclude that acute and chronic doses of Bentazon and Glyphosate herbicides induced genotoxicity in mice

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