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Hematocytological Evaluation of Hydroxyurea-Induced Toxicity in Male Rats

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ABSTRACT

The toxicity of hydroxyurea (HU), a treatment for specific neoplasms, sickle-cell disease, polycythemia vera and thrombocytosis was assessed in rats. Forty rats were divided equally into four groups. The first group was kept as control and received only purified water, the second group (HU_{250}) orally administered hydroxyurea 250 mg/kg b.wt/day, the third group (HU_{500}) administered hydroxyurea 500 mg/kg b.wt/day and the fourth group (HU_{750}) administered hydroxyurea 750 mg/kg b.wt/day. The treatment was continued for 20 days. The evaluated parameters were assessed after 10 and 20 days of the experiment. Results revealed decrease in body weight in all treated groups. In addition, decreased circulating leukocytes, erythrocytes and platelets with decreased bone marrow cellularity were evident in all treated groups with different degree of severity. Moreover, aplastic anemia associated with marked severe hypocellularity of bone marrow was detected in HU_{500} and HU_{750} groups on the second period of the experiment. It could be concluded that hydroxyurea administration induced hematopoietic and bone marrow toxicity in rats.

1. INTRODUCTION

Hydroxyurea, a hydroxylated molecule of urea, was first synthesized in 1869 by Dresler and Stein (Yarbro, 1968). Hydroxyurea is an antineoplastic drug which has been successfully used in treatment of several neoplasms, polycythemia vera and thrombocytosis in humans and dogs (Liebelt et al., 2007). Also, hydroxyurea is approved for the treatment of melanoma, chronic myelogenous leukemia, ovarian cancer and squamous neoplasia of the head and neck, in addition to using in treatment of renal cell, urinary bladder, prostatic and uterine cervix carcinoma (Liebelt et al., 2007). Hydroxyurea partially eliminated by liver while converted into urea, other part is eliminated by kidney; moreover, it may be secreted unchanged in the urine (Adamson et al., 1965). In both rats and humans, hydroxyurea renal clearance has been estimated to be 75% of the glomerular filtration rate (GFR) (Tracewell et al., 1995). The most common adverse finding of HU in human patients is leukopenia, anemia and thrombocytopenia (Rassnick et al., 2010; FDA 2010, 2012). In 1928, animals study was conducted and concluded leukopenia, macrocythemia, anemia, and deaths after exposure to hydroxyurea (Gwilt and Tracewell 1998). In addition, genotoxicity (FDA, 2012) and teratogenic (Wilson et al., 1975) effects of hydroxyurea was reported. Hydroxyurea produced methemoglobinemia, prostration, and death in dogs after a single dose of ≥250 mg/kg/day. As well, rats received HU at a dose of 500 mg/kg/day revealed decreased circulating leukocytes, erythrocytes, platelets and decreased cellularity of bone marrow (Morton et al., 2014). Additionally, severe methaemoglobinaemia in dogs (Wray, 2007) and cats (Plumb, 2005) associated with HU toxicity was reported. Hypocellularity in bone marrow and increased M/E ratio in relation to usage of HU was previously reported in mice (Meiler et al., 2011). Moreover, Card et al. (1968) concluded depression of erythropoiesis and hypoplasia of bone marrow smear in rabbits treated with hydroxyurea. Toxicological studies on hydroxyurea over dose are limited. Hence, our study was designed to evaluate the hematocytological effect of hydroxyurea in male albino rats.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Forty apparently healthy male albino rats (140 – 160 g b.wt), were purchased from a closed random bred colony at the Medical Research Institute of Alexandria University, Egypt. Rats were housed in separated clean and disinfected metal

cages (10 rats/ cage) at experimental laboratory of pathology department, Faculty of Veterinary Medicine, Alexandria University. Rats were kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water which was supplied *ad-libitum*. All rats were acclimatized for two weeks prior to the beginning of study for adaptation and ensure normal growth and behavior. Rats were divided equally into four groups. The first group was kept as control and received only purified water, the second group (HU_{250}) was orally administered hydroxyurea 250 mg/kg b.wt/day, the third group (HU_{500}) was given hydroxyurea 500 mg/kg b.wt/day and the fourth group (HU_{750}) received hydroxyurea 750 mg/kg b.wt/day. The treatment was continued for 20 days. The evaluated parameters were assessed after 10 and 20 days of the experiment.

2.2. Calculation of hydroxyurea dose

The lower-dose level (250 mg/kg/day) was chosen according to the major therapeutic dose (40 mg/kg b.wt) in human being. The dose was converted to the rat dose according to the following equation:

*AED (mg/kg) =Human does (mg/kg) × 6.2 (USFDA, 2005)

*denotes animal equivalent dose.

2.3. Samples for hematological study

Every ten days, five rats from each group were anesthetized. Samples for hematological examination were collected on dipotassium salt of ethylene diamine tetra-acetic acid (EDTA) as anticoagulant and used for evaluation of hemogram. The samples were collected on day 10 and 20 days and analyzed by hematology analyzer (CELL-DYN®3700, Abbott Diagnostics, U.S.A). The reticulocyte count also was made by using Brilliant Cresyl blue stain (100 microlitres of EDTA blood +100 microlitres of the stain).

2.4. Bone marrow smears

Rats were euthanized and necropsied. At necropsy, bone marrow was collected from the proximal end of the femur bone. Bone marrow smears were placed on clean dry slides. Dried bone marrow smears were stained by using leishman stain.

2.5.Statistical analysis

Data were collected and analysis of variance (ANOVA) was performed using the statistical analysis system software (SAS, 2011).

3. RESULTS:

3.1. Clinical signs

No abnormal clinical signs were observed in HU_{250} group till the end of experiment. But in HU_{500} group, rats showed decrease in appetite, depression, diarrhea with pale feces and rough hair coat compared to control rats. Also, rats of HU_{750} showed the same clinical signs but in more severe degree.

3.2. Hematological parameters:

During the first ten days, as shown in Table (1), significant increase in M/E ratio was detected in HU_{500}, HU_{750} groups as compared to control group, while rats in HU_{250} group showed non-significant change compared to the control rats. Moreover, RBCs count, hemoglobin and hematocrit showed significant decrease in HU_{750} group as compared to the control groups, but they did not show significant change in other treated groups. The erythrocytic indices (MCV, MCH, and MCHC) showed non-significant change in all treated groups as compared to the control ones but indicating normocytic normochromic anemia in HU_{750} group. Reticulocyte and platelet count showed significant decrease in all treated groups as compared to control ones. There was significant decrease in circulating white blood cells; neutrophils, lymphocytes and eosinophils in all treated groups compared to the control groups, while basophils showed significant decrease in HU_{750} group, but they did not show significant change in other treated groups**.** Monocytes showed non-significant change in all treated groups.

During the second ten days, as shown in Table (2), M/E ratio showed significant increase in HU_{500}, HU_{750} groups as compared to control group, while HU_{250} showed non-significant change compared to the control group. As well, RBCs count, hemoglobin and hematocrit revealed significant decrease in all treated groups as compared to the control ones, while the erythrocytic indices (MCV, MCH, and MCHC) showed non-significant change in all treated groups compared to the control groups indicating normocytic normochromic anemia. Reticulocyte and platelets count showed significant severe decrease in all treated groups compared to the control ones. There was significant decrease in circulating white blood cells; neutrophils, lymphocytes and eosinophils in all treated groups compared to the control ones, while basophils showed significant decrease in HU_{500} and HU_{750} groups, but they did not show significant change in other treated group. Monocytes showed non-significant change in all treated groups.

3.3. Examination of bone marrow smears

Bone marrow smears from control rats showed normal cellularity with normal erythroid and myeloid precursors and no abnormality in megakaryocytes (Fig.1a). In HU_{250} group (Fig.1b), examination of bone marrow smears showed slight hypocellularity, slight decrease in megakaryocytes, slight reduction with normal morphology of myeloid and erythroid series, in addition to formation of fat cells. In HU_{500} group (Fig.1c), bone marrow smears examination showed moderate hypocellularity and decreased number of megakaryocytes with normal morphology. Moreover, myeloid and erythroid series showed hypoplasia and there was an increase in fat cells with formation of fat globules. In HU_{750} group (Fig.1d), bone marrow smears examination revealed severe hypocellularity, marked depression of megakaryocytes and severe hypoplasia of myeloid and erythroid series. Additionally, increase in fat cells and formation of fat globules were more prominent in HU_{750} than in HU_{500} group. After 20 days of experiment, smears from HU_{250} group (Fig.2a) showed moderate hypocellularity, megakaryocytes were moderately decreased, myeloid and erythroid series showed hypoplasia and smears showed increased formation of fat cells more than HU_{250} group during the first period of experiment. In HU_{500} group (Fig.2b), bone marrow smears examination showed marked hypocellularity, megakaryocytes were marked decreased, myeloid and erythroid series showed aplasia, smears showed increase in fat cells and formation of fat globules more than HU_{500} group in the previous period and these features described a case of aplastic anemia. In HU_{750} group (Fig.2c), bone marrow smears examination showed marked severe hypocellularity, megakaryocytes were markedly depressed, myeloid and erythroid series showed marked aplasia, smears showed almost fat cells and formation of large fat globules and these features described a case of aplastic anemia.

Table (1): Data for the effect of hydroxyurea on the hematocytological parameters, 10 days post-administration in male albino rats.

	Data /Groups			
Parameters	control	HU_{250}	HU_{500}	HU_{750}
RBCs (10^6/ul)	6.87±0.40a	6.28±0.45ab	6.24±0.42ab	5.37±0.45b
Hb (g/dl)	12.73±0.84a	11.87±1.02ab	11.33±0.33ab	9.83±0.82b
HCT (%)	39.47±1.75a	36.93±2.93ab	34.67±1.69ab	29.43±2.67b
MCV (fl)	57.43±0.86ab	58.77±0.64a	55.63±1.15b	54.60±0.56b
MCH (pg)	18.40±0.25a	18.83±0.38a	18.57±0.62a	18.20±0.21a
MCHC (g/dl)	32.20±0.71a	32.00±0.32a	33.37±0.44a	33.37±0.33a
Reticulocyte (%)	5.0 ± 0.0a	2.33 ± 0.58b	1.33 ± 0.58c	0.43 ± 0.12d
Platelet (10^3/ul)	1107.67±92.34a	693.33±32.95b	318.00±57.66c	287.00±42.24c
M/E Ratio	2.10 ± 0.26c	2.40 ± 0.10c	4.17 ± 1.04b	6.33 ± 0.58a
WBCs (10^3/ul)	13.46±2.88a	4.54±0.43b	1.80±0.31b	2.46±0.53b
Neutrophil(10^3/ul)	2.90±0.80a	0.23±0.23b	0.07±0.03b	0.20±0.10b
Lymphocyte(10^3/ul)	9.97±2.09a	3.90±0.42b	1.47±0.18b	2.17±0.43b
Monocyte (10^3/ul)	0.07±0.03a	0.17±0.03a	0.20±0.15a	0.03±0.03a
Eosinophil (10^3/ul)	0.23±0.07a	0.03±0.03b	0.03±0.03b	0.00±0.00b
Basophil (10^3/ul)	0.30±0.06a	0.17±0.12ab	0.10±0.06ab	0.00±0.00b

Values are means ± standard errors

Means without a common letter within the same row differ significantly ($P < 0.05$).

Table (2): Data for the effect of hydroxyurea on the hematocytological parameters, 20 days post-administration in male albino rats.

	Data /Groups			
Parameters	control	HU_{250}	HU_{500}	HU_{750}
RBCs (10^6/ul)	7.26±0.14a	4.79±0.44b	4.04±0.25b	3.71±1.03b
Hb (g/dl)	13.17±0.26a	9.03±1.07b	6.97±0.17b	6.80±1.96b
HCT (%)	42.10±1.21a	29.10±3.20b	22.67±0.37b	21.73±6.50b
MCV (fl)	58.17±0.66a	60.50±1.48a	58.00±1.97a	56.43±1.76a
MCH (pg)	18.17±0.03a	18.73±0.59a	17.40±0.76a	18.27±0.20a
MCHC (g/dl)	31.17±0.32a	30.93±0.27a	30.87±0.22a	31.80±0.93a
Reticulocyte (%)	5.0 ± 0.0a	1.33 ± 0.58b	0.70 ± 0.17c	0.13 ± 0.15c
Platelet (10^3/ul)	962.67±36.08a	808.67±7.69b	48.33±9.35c	6.00±0.57c
M/E Ratio	2.10±0.26b	3.43±0.60b	8.0±2.0a	10.67±2.08 a
WBCs (10^3/ul)	14.87±0.43a	5.22±0.44b	2.05±0.50c	0.24±0.05d
Neutrophil(10^3/ul)	2.40±0.12a	0.37±0.19b	0.10±0.10b	0.00±0.00b
Lymphocyte(10^3/ul)	11.80±0.35a	4.50±0.32b	1.80±0.45c	0.20±0.06d
Monocyte(10^3/ul)	0.10±0.00a	0.03±0.03a	0.10±0.06a	0.03±0.03a
Eosinophil(10^3/ul)	0.30±0.00a	0.07±0.03b	0.00±0.00c	0.00±0.00c
Basophil(10^3/ul)	0.35±0.03a	0.20±0.10ab	0.07±0.03bc	0.00±0.00c

Values are means ± standard errors

Means without a common letter within the same row differ significantly ($P < 0.05$).

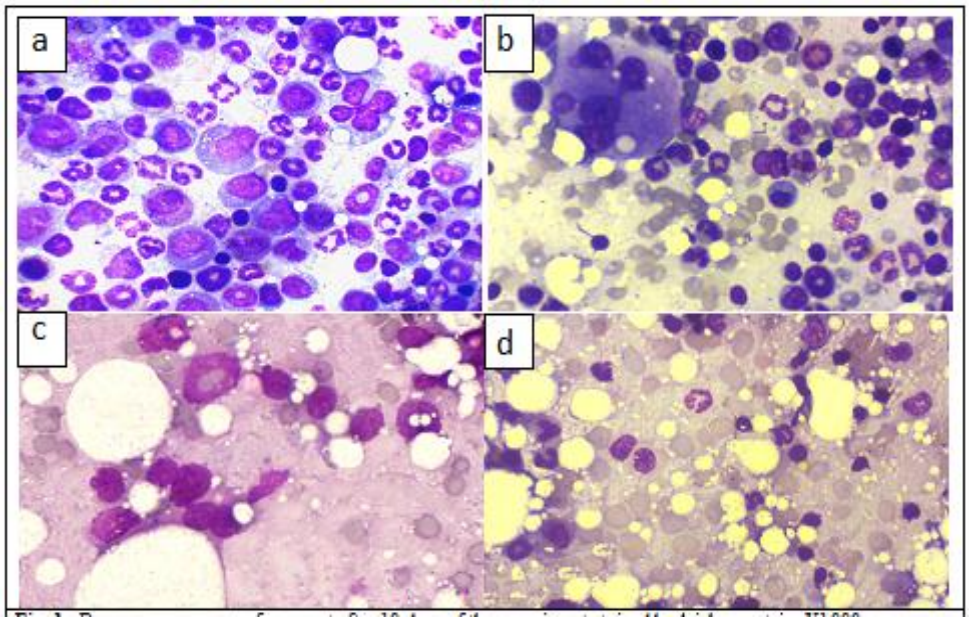

Fig. 1: Bone marrow smears from a rat after 10 days of the experiment stained by leishman stain. X1000
a) Control group. Numerous erythroid precursors and myeloid precursors.
b) HU_{250} group. Slight hypocellularity with appearance of some fat cells.
c) HU_{500} group. Moderate hypocellularity and increase in fat cells with formation fat globules.
d) HU_{750} group. Severe reduction in cellularity and increase in fat cells forming fat globules.

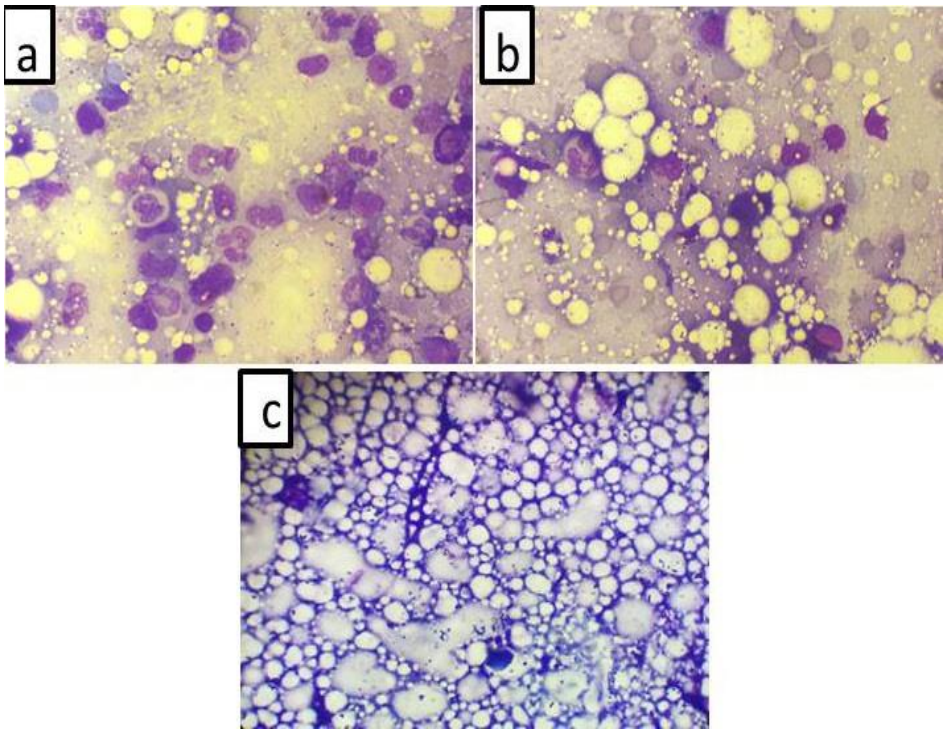

Fig. 2: Bone marrow smears from a rat after 20 days of the experiment stained by leishman stain.
a) HU_{250} group. Moderate decrease in cellularity and increase in fat cells. X1000.
b) HU_{500} group. Marked decrease in cellularity and increase fat cells inducing aplastic anemia. X1000.
c) HU_{750} group. Marked severe hypocellularity inducing aplastic anemia. X100.

4. DISCUSSION

Bone marrow is one of the target organs in toxicity studies induced by chemical exposure in the body (Lund, 2000). Evaluation of the blood and bone marrow has become routine procedure in the investigation of hematologic disorders in toxicity studies. Therefore, evaluations of whole blood samples and bone marrow aspirates are needed to understand the alterations in hematopoiesis that may occur in toxicity studies (Travlos, 2006). Hydroxyurea (HU) was selected in our study as one of the antineoplastic drugs to evaluate the hematocytological parameters associated with its toxicity. Hydroxyurea is an S-phase specific chemotherapeutic agent (Yarbro, 1968) which used in treatment of melanoma, resistant chronic myeloid leukemia, and recurrent metastatic or ovarian carcinoma (FDA, 1998; Bristol-Myers-Squibb, 2005), sickle cell anemia (McGann and Ware 2011), HIV (Lisziewicz et al., 2003), polycythemia vera (Dingli and Tefferi 2006), and psoriasis (Boyd and Neldner 1991). Hydroxyurea interferes with the synthesis of DNA with little or no effect on RNA or protein synthesis. Hydroxyurea inhibits the conversion of DNA bases by blocking ribonucleotide reductase, thereby preventing conversion of ribonucleotides to deoxyribonucleotides. Also, it inhibits the incorporation of thymidine into DNA, and may directly damage DNA (McEvoy 2006; Bristol-Myers Squibb Canada, 2006). Hydroxyurea is rapidly metabolized to nitroxide compounds including nitric oxide that are responsible for physiologic and toxic effects (Yarbro, 1992; Koviac, 2011). These nitroxides act as free radicals induce formation of reactive oxygen causing oxidative stress, interacting with nucleic acids and proteins to impair cellular functions (Koviac, 2011). Regarding to the present hematological results, significant decrease in RBCs count, Hb concentration and HCT values with normocytic normochromic anemia were evident during the second period of the experiment, while at the first 10 days, there was significant decrease in these parameter in HU_{750} group only. Moreover, there were reticulocytopenia and severe thrombocytopenia in all treated groups during the period of experiment. As well as, it triggered significant decrease in leukogram (WBCs, granulocytes, lymphocytes) in all treated groups during experiment, but there was no significant effect on monocytes. Our results come in agreement with Rassnick et al. (2010); FDA (2010, 2012) and Morton et al. (2014). The decrease in circulating white blood cells, neutrophils, lymphocytes, eosinophils, basophils, erythrocytes, reticulocytes, and platelets were consistent with decreased cellularity of the bone marrow at 500 mg/kg/day (Lerner et al., 1966). On the other hand, Oyewole et al. (2009) reported that oral administration of hydroxyurea in albino rats at dose (15 mg/kg/day) for a period of 28 days significantly increased packed cell volume (PCV), hemoglobin concentration (Hb) and mean corpuscular volume (MCV) in the blood of the rats. With regarding to bone marrow smears, there was significant increase in M/E ratio in all treated groups along the period of experiment except HU_{250} group which did not show any significant change in the first and second period of experiment. This result agree with Meiler et al. (2011) who reported increase in M/E ratio induced by hydroxyurea in mice injected 100 mg/kg intraperitoneally daily for 8 weeks. Moreover, all the bone marrow smears showed hypocellularity, reduction of myeloid and erythroid series and decrease in megakaryocytes. These findings came in accordance with Card et al. (1968) who reported depression of erythropoiesis and hypoplasia of the bone marrow in rabbits injected intraperitoneally by hydroxyurea 100 or 200 mg/kg/day for 8 days. Also, our findings agreed with Meiler et al. (2011). These results could be attributed to formation of nitric oxide that metabolized from HU (Yarbro, 1992; Koviac, 2011) and Hydroxyurea also inhibits the incorporation of thymidine into DNA and may directly damage DNA causing diminution in cellular proliferation in the marrow (Krakoff et al., 1968; Yarbro, 1992). In conclusion, hydroxyurea produced hematopoietic and bone marrow toxicity in rats after treatment with different doses for 20 days, but severe damaging effects occurred at high doses 500 and 750 mg/kg/day to lesser extent the lower dose 250 mg/kg/day.

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