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Hematological toxic effect and the frequency of micronucleus formation of different doses of cyproheptadine on albino male mice blood picture

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Abstract:

BACKGROUND: Synthetic drugs are created using chemicals rather than natural ingredients. One reason that synthetic drugs are extremely dangerous is that buyers do not know what chemicals they are ingesting. Histamine in the body can produce symptoms of sneezing, itching, watery eyes, and runny nose. Cyproheptadine (periactin) an antihistamine is used to treat all these symptoms of allergies by reduces the natural chemical histamine, but it has different side effects which include: confusion, hallucinations, unusual thoughts or behavior, and seizure (convulsions).

OBJECTIVE: The aim of this study is to prove the scope of danger from cyproheptadine abuse on lymphocyte cells of male mice treated with child dose, maximum dose, and adult dose that may affect blood picture and their cytotoxic effect by determination of micronucleus (MN) formation in the cells of mice bone marrow.

MATERIALS AND METHODS: In the current study, a method was used for determination of total and absolute counts of white blood cells and find the frequency of MN formation in three groups of albino male mice treated with three equivalent doses of cyproheptadine: child dose, adult dose, and maximum adult dose (0.065, 0.092, and 0.12 mg/kg) respectively.

RESULTS: The study showed that cyproheptadine decreases total leukocyte count in adult and child dose in comparison to mice blank group that had not get the drug; also, the frequency of MN increases significantly after treated mice with cyproheptadine drug in comparison with negative control.

CONCLUSION: More studies are necessary to elucidate the relationship between cytotoxic, genotoxic, and apoptotic effects and to make a possible risk assessment in patients receiving therapy with this drug.

Key words:

Absolute count of leukocytes, cyproheptadine, micronucleus, total count of leukocytes

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Synthetic drugs, as opposed to natural drugs, are chemically produced in a laboratory. Their chemical structure can be either identical to or different from naturally occurring drugs, and their effects are designed to mimic or even enhance those of natural drugs. The reported harmful effects of synthetic substances range from nausea to drug-induced psychosis. Due to the unpredictable nature of synthetic drugs and of human consumption of these drugs, the true effects of many of these drugs are unknown.[1] For over three decades, there has been national-level attention on the use and abuse of synthetic drugs.[2]

Establishing the safety of a drug before use in humans begins early in the development process as lead compounds go through a series of tests to provide a preliminary assessment of safety. Scientists assess how the body processes the investigational compound; also, they also evaluate the impact of the investigational compound has on various functions within the body or the pharmacodynamics.[3] Many new approaches for clinical trials are using novel drug development tools, such as biomarkers, to identify patients that may respond to a therapy. Basket studies in oncology, for example, identify a common genetic mutation across a variety of cancer types and enroll patients whose tumors have that mutation, regardless of the type of cancer they have, to test the effect of a single medicine.[4] One of the drug uses in our life is cyproheptadine. Cyproheptadine

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(periactin) is a first-generation antihistamine with additional anticholinergic, antiserotonergic, and local anesthetic properties which is indicated for the treatment of rhinitis, conjunctivitis, and urticaria and has been reported as a treatment for serotonin syndrome with alpha-blocking activity and central sedative effect.[5] Cyproheptadine has been found to be effective in stimulating appetite, but its direct effects on the intestine have not been documented.[5] This drug has adverse effects which may include: Sedation and sleepiness (often transient), dizziness, disturbed coordination, confusion, restlessness, excitation, nervousness, tremor, and weight gain.[6]

Cyproheptadine is well absorbed following oral ingestion, with peak plasma levels occurring after 1–3 h.[7,8] Its half-life when taken orally is approximately 8 h. Cyproheptadine is used in cats as an appetite stimulant and as an adjunct in the treatment of asthma.[5]

The elimination half-life of cyproheptadine in cats is 12 h.[9] However, cyproheptadine has not been used clinically in malnourished children who are otherwise normal. Poor appetite leading to poor weight gain is a characteristic finding among underweight children without underlying pathological conditions.[10]

In the present study, there will be highlight on the irrational and drug abuse in our society, especially for school-age children and those suffering from apatite problems.

The aim of this study is to prove the scope of danger from cyproheptadine abuse on lymphocytes cells of male mice treated with child dose, maximum dose, and adult dose that may affect blood picture and their cytotoxic effect by determination of micronucleus (MN) formation in the cells of mice bone marrow (BM).

Materials and Methods

Cyproheptadine doses and experimental design

Three groups of albino male mice were treated separately with three doses of the cyproheptadine drug according to the British National Formulary 2010 calculated on the base of mice/human differences in body weight: These are:

- Child dose = 8 mg/day equivalent to 0.065 mg/mice/day
- Adult dose = 12 mg/day equivalent to 0.092 mg/mice/day
- Maximum dose = 20 mg/day equivalent to 0.120 mg/mice/day.

A fourth group of untreated mice with the drug consider as control group.

Preparation of drug

Pure powder was supplied from Samarra Drug Industry/Iraq and dissolved in distilled water to prepare the require doses.

Laboratory animals

The laboratory animals, albino male mice (*Mus musculus*), their ages at the start of experiments were 8–10 weeks, and their weight was 23–27 g, were distributed into groups, and each group was kept in a separate plastic cage. The animals were maintained at room temperature and had free excess to food (standard pellets) and water (*ad libitum*).

Experimental design

To assess the cytogenetic and immunological effects of these three doses, the mice were distributed into four groups, each of four animals:

- Group I: Mice were administrated physiological saline (blank control)
- Group II: Mice were administrated the child dose (0.065 mg/mice/day)
- Group III: Mice were administrated the adult dose (0.092 mg/mice/day)
- Group IV: Mice were administrated the maximum dose (0.12 mg/mice/day).

The tested drug was injected intraperitoneally as a single dose (0.1 ml) per a day and for 10 days and then the mice were sacrificed in day 11 for laboratory assessments.

Total leukocyte count

Blood samples were collected by heart puncture using a disposable insulin syringe (1 ml) precoated with heparin. The method [11] was followed, in which an aliquot of 0.02 ml blood was mixed with 0.38 ml of leukocyte diluent in a test tube and left at room temperature for 5 min. A drop of the mixture was applied to the surface of Neubauer chamber under the cover slip, and the chamber was left for 3 min to settle the cells. The leukocytes were counted in four large squares (each with 16 small squares), and the total count of leukocytes was obtained using the following equation:

$$\text{Total count (cell / cu.mm.blood)} = \left(\frac{\text{Number of cells counted}}{4}\right) \times 20 \times 10$$

Differential count of leukocytes

One drop of blood was smeared on a clean slide using another slide and left to dry at room temperature. The smear was stained with Leishman stain for 5 min and buffered for 10 min and then washed with tap water. The slide was air-dried and then examined under oil immersion lens (×100).[11] At least 100 leukocytes were examined, and the percentage of each type was recorded while the total count of each type was obtained using the following equation:

$$\text{Total count (cell / cu.mm.blood)} = \left(\frac{\text{Percentage of cells} \times \text{total count}}{100}\right)$$

Micronucleus formation assay

To carry out the assessment of MN formation, the procedure [12] was followed; the mouse was sacrificed by cervical dislocation and then dissected to obtain the femur bone. After cutting both ends of the bone, it was grasped from the middle with a forceps in a vertical position over the edge of a test tube and then the cellular content was collected with a heat-inactivated (56°C for 30 min) human AB plasma (2 ml) using a disposable insulin syringe. Then, the test tube was centrifuged (1000 rpm) for 10 min, and the supernatant was discarded after that the cellular deposit was gently mixed, and a thin smear was made on a clean slide and air-dried at room temperature. Then, the smear was fixed with absolute methanol for 5 min and then air-

dried at room temperature. Finally, the smear was stained with Giemsa stain for 15 min and rinsed with distilled water, and the slides were examined under oil immersion lens (×100), and at least 1000 polychromatic erythrocytes (PCE) were examined for the presence of MN formation. The MN index was obtained using the following equation:

$$\text{MN index (MN / cell)} = \left(\frac{\text{Number of micronuclei}}{\text{Total count of PCE}}\right) \times 100$$

Statistical analysis

The values of the investigated parameters were given in terms of mean ± standard deviation, and differences between means were assessed by analysis of variance followed by least significant difference or Duncan test, using the computer program SPSS version 13.0 (SPSS Inc., Chicago). The difference was considered significant when the probability value was equal or <0.05.[13]

Results

Total count of leukocytes

The child dose (0.065 mg/mice/day) was able to increase the count significantly to 10525 ± 1311 cells/mm^3, in comparison to the blank control, but such count was significantly decreased to 5450 ± 412, 4750 ± 597, and 6000 ± 661 (cells/mm^3) in adult dose and maximum dose in relative to blank control count, respectively [Table 1].

Absolute count of lymphocytes

Mice treated with adult and maximum dose of cyproheptadine showed a decrease in absolute count of lymphocytes compared to blank controls (3623 ± 479, 3398 ± 455 vs. 4000 ± 469 cells/mm^3), respectively, whereas in the child dose, there was an increasing in the absolute lymphocyte count significantly to 7014 ± 790 cells/mm^3, and the differences was significant in comparison with blank control [Table 2].

Absolute count of neutrophils

The absolute count of neutrophils in blank control mice was 1800 ± 237 cells/mm^3, and when mice treated with cyproheptadine, the reduction was observed in adult and maximum dose, which was significant. Child dose was able to increase the percentage of neutrophil in comparison with blank control (3225 ± 610 vs. 1800 ± 237, respectively), and the differences were significant [Table 3].

Absolute count of monocytes

Treatment mice with child and adult cyproheptadine dose caused a significant increase in monocyte count in comparison with maximum dose and blank control (303 ± 123 and 556 ± 55 vs. 215 ± 74 and 200 ± 194 cells/mm^3), respectively [Table 4].

Micronucleus formation

The spontaneous frequency of MN in blank controls and child was 0.0317 ± 0.0028 and 0.0316 ± 0.0025 MN/cell, respectively, while in adult and maximum dose, the frequency increased to 0.0643 ± 0.0020 and 0.0686 ± 0.0023 MN/cell, respectively, and the differences was not significant between doses of cyproheptadine except in child dose [Table 5].

Discussion

Analysis of blood parameters is relevant to risk evaluation, and the changes in the hematological system have a higher predictive value for human toxicity when the data are translated from

Table 1: Effect of cyproheptadine on total count of leukocytes in treated albino male mice

Groups	Dose (mg/mice)	Mean±SD (cells/mm^3)
I (child dose)	0.065	10525±1311^a
II (adult dose)	0.092	5450±412^b
III (maximum dose)	0.12	4750±597^c
IIII (blank controls)	-	6000±661^b

Different letters: Significant difference ($P \leq 0.05$) between means of columns. SD = Standard deviation

Table 2: Effect of cyproheptadine on absolute count of lymphocytes in treated albino male mice

Groups	Dose (mg/mice)	Mean±SD (cells/mm^3)
I (child dose)	0.065	7014±790^a
II (adult dose)	0.092	3623±479^b
III (maximum dose)	0.12	3398±455^b
IIII (blank controls)	-	4000±469^b

Different letters: Significant difference ($P \leq 0.05$) between means of columns. SD = Standard deviation

Table 3: Effect of cyproheptadine on absolute count of neutrophil in treated albino male mice

Groups	Dose (mg/mice)	Mean±SD (cells/mm^3)
I (child dose)	0.065	3225±610^a
II (adult dose)	0.092	1270±175^c
III (maximum dose)	0.12	1137±103^c
IIII (blank controls)	-	1800±237^b

Different letters: Significant difference ($P \leq 0.05$) between means of columns. SD = Standard deviation

Table 4: Effect of different doses of cyproheptadine on absolute count of monocyte in treated albino male mice

Groups	Dose (mg/mice)	Mean±SD (cells/mm^3)
I (child dose)	0.065	303±123^b
II (adult dose)	0.092	556±55^a
III (maximum dose)	0.12	215±74^b
IIII (blank controls)	-	200±194^b

Different letters: Significant difference ($P \leq 0.05$) between means of columns. SD = Standard deviation

Table 5: Effect of cyproheptadine on micronucleus in treated albino male mice

Groups	Dose (mg/mice)	Mean±SD (micronucleus/cell)
I (child dose)	0.065	0.0316±0.0025^b
II (adult dose)	0.092	0.0643±0.0020^a
III (maximum dose)	0.12	0.0686±0.0023^a
IIII (blank controls)	-	0.0317±0.0028^b

Different letters: Significant difference ($P \leq 0.05$) between means of columns. SD = Standard deviation

animal studies.[14] The assessment of hematological parameters could be used to reveal the protective or deleterious effects of foreign compounds including plant extract, drug, or other compound on the blood cellular constituents of animals.[15] It is generally accepted that monocytes and granulocytes and humoral elements such as lysozyme-, agglutinin-, and metalloid-binding proteins are the main components of the nonspecific immune system, therefore, any foreign compound can affect the immune system.[16]

Like other drugs, cyproheptadine can cause hematologic side effects including hemolytic anemia, thrombocytopenia, and agranulocytosis,[17] in addition to other serious side effects but are not limited to low platelet count (noted by bruising easily and slow clotting), heat stroke, and inability of the BM to make certain white blood cells called neutrophils (agranulocytosis) so that our results are in agreement with the results of Kontaş *et al*.[18] who found that loratadine, which is a new-generation antihistamine used in the treatment of allergic disorders, may be a genotoxic drug and cyproheptadine may have the same effect according to antihistaminic uses of this drug. In addition, antihistaminic drugs cause decrease in the mitotic index significantly at the higher concentrations (15 and 25 μg/ml) compared with negative control, while these drugs increase the frequencies of sister chromatid exchange, chromosomal aberration, and MN in all lymphocyte cultures because of cytotoxic and genotoxic effects on human peripheral blood lymphocyte cultures.

Another study[19] demonstrated that the antiserotonin drug inhibits the growth of the connective tissue in the lungs and attenuates the course inflammatory process primarily due to inhibition of the granulocytic lineage, which was related to suppression of hematopoietic stem cells. Reduced content of the stromal precursor cells in BM and spleen was noted also. In addition to that, drugs of the 1st generation inhibited oxidative burst of human phagocytes in whole blood and in isolated neutrophils.[20]

The function of immune system is also genetically determined, and alternations (mutations) in the genetic makeup of animals do have their effect on such function.[21] Genetic damage induced in the dividing cells of root tips by fast neutrons and X-ray in the presence and absence of oxygen. It was found that all chromatid and chromosome breaks will give rise to acentric fragments at mitosis, and these fragments are excluded from the daughter nuclei and appear in following interphase as micronuclei. The use of BM smears to detect *in vivo* genetic damage induced by chemical mutagens was recommended in 1966, 1970 by determine the occurrence of micronuclei in BM cells in connection with cytogenetic damages.[22,23] Reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage can lead to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species increases the risk of DNA damage, and this will lead to mutations and can increase the exposure of DNA to mutagens.[24,25]

In 1973, a study conducted by von Ledebur and Schmid[26] reached the conclusion that the incidence of micronucleated PCE was particularly useful index of an *in vivo* BM cytogenetic damage, and such finding formed the basis to develop a simple *in vivo* assay based on an identification of micronuclei in PCE of mouse BM. Since then, many researchers have employed this assay for the assessment of mutagenic effects induced by different mutagens.[27,28]

Conclusion

The results of this study found that treatment of mice with cyproheptadine increased the frequency of MN, and the reason may be due to one of these effects indicated above because many antihistaminic drugs are genotoxic and increase the MN frequency.

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Conflicts of interest
There are no conflicts of interest.

References

1. Meetei PA, Rathore RS, Prabhu NP, Vindal V. Modeling of babesipain-1 and identification of natural and synthetic leads for bovine babesiosis drug development. J Mol Model 2016;22:71.
2. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000;33:179-89.
3. Sant'Anna AM, Hammes PS, Porporino M, Martel C, Zygmuntowicz C, Ramsay M. Use of cyproheptadine in young children with feeding difficulties and poor growth in a pediatric feeding program. J Pediatr Gastroenterol Nutr 2014;59:674-8.
4. Meddah B, Limas-Nzouzi N, Mamadou G, Miantezila J, Soudy ID, Eto B. Antisecretory effect of prescribed appetite stimulator drug cyproheptadine in rat intestine. Fundam Clin Pharmacol 2014;28:303-9.
5. Paoluzzi L, Scotto L, Marchi E, Seshan VE, O'Connor OA. The anti-histaminic cyproheptadine synergizes the antineoplastic activity of bortezomib in mantle cell lymphoma through its effects as a histone deacetylase inhibitor. Br J Haematol 2009;146:656-9.
6. Homnick DN, Marks JH, Hare KL, Bonnema SK. Long-term trial of cyproheptadine as an appetite stimulant in cystic fibrosis. Pediatr Pulmonol 2005;40:251-6.
7. He YL, Zhang CL, Gao XF, Yao JJ, Hu CL, Mei YA. Cyproheptadine enhances the I(K) of mouse cortical neurons through sigma-1 receptor-mediated intracellular signal pathway. PLoS One 2012;7:e41303.
8. Mao X, Liang SB, Hurren R, Gronda M, Chow S, Xu GW, *et al.* Cyproheptadine displays preclinical activity in myeloma and leukemia. Blood 2008;112:760-9.
9. Singh D, Goel RK. Proconvulsant potential of cyproheptadine in experimental animal models. Fundam Clin Pharmacol 2010;24:451-5.
10. Epifanio M, Marostica PC, Mattiello R, Feix L, Nejedlo R, Fischer GB, *et al.* A randomized, double-blind, placebo-controlled trial of cyproheptadine for appetite stimulation in cystic fibrosis. J Pediatr (Rio J) 2012;88:155-60.
11. Haen PJ. Principles of Hematology.McGraw-Hill publishing house. London.1995. p. 310-25.
12. Schmid W. The cell micronucleus test for cytogenes analysis. In: Hollaender A, editor. Chemical Mutagens Principles and Methods for Their Detection. Vol. 4. New York, London: Plenum Press; 1976. p. 31-53.

13. Pérez-Serrano J, Denegri G, Casado N, Rodríguez-Caabeiro F. *In vivo* effect of oral albendazole and albendazole sulphoxide on development of secondary echinococcosis in mice. Int J Parasitol 1997;27:1341-5.
14. John OR, Yahaya AA, Arch AE. Aqueous ethanolic extract of mangifera indica stem bark effect on the biochemical and haematological parameters of albino rats. Appl Sci Res 2012;4:1618-22.
15. Li Y, Hu Y, Shi S, Jiang L. Evaluation of antioxidant and immuno-enhancing activities of Purslane polysaccharides in gastric cancer rats. Int J Biol Macromol 2014;68:113-6.
16. Ardo L, Yin G, Xu P, Varadi L, Szigeti G, Jeney Z, *et al*. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture 2008;275:26-33.
17. Watemberg NM, Roth KS, Alehan FK, Epstein CE. Central anticholinergic syndrome on therapeutic doses of cyproheptadine. Pediatrics 1999;103:158-60.
18. Kontaş S, Şekeroğlu AZ. Investigation of cytotoxic and genotoxic effects of the antihistaminic drug, loratadine, on human lymphocytes. Drug Chem Toxicol 2015;38:57-62.
19. Skurikhin EG, Andreeva TV, Khmelevskaya ES, Ermolaeva LA, Pershina OV, Krupin VA, *et al*. Effect of antiserotonin drug on the development of lung fibrosis and blood system reactions after intratracheal administration of bleomycin. Bull Exp Biol Med 2012;152:519-23.
20. Nosál' R, Drábiková K, Jancinová V, Macicková T, Pecivová J, Holománová D. On the pharmacology and toxicology of neutrophils. Neuro Endocrinol Lett 2006;27 Suppl 2:148-51.
21. Abbas AK, Lichtman AH. Cellular and Molecular Immunology. 5th ed. Philadelphia: Elsevier Science (U.S.A.); 2008. p. 1-16.
22. Schroeder TM. Cytogenetische und cytologische Befunde bei enzymopenischen Panmyelopathien und Pancytopenien. Hum Genet 1966;2:287.
23. Schroder TM. Chemical Mutagenesis in Mammals and Man. Heidelberg: Springer; 1970. p. 214-9.
24. Barreto G, Madureira D, Capani F, Aon-Bertolino L, Saraceno E, Alvarez-Giraldez LD. The role of catechols and free radicals in benzene toxicity: an oxidative DNA damage pathway. Environ Mol Mutagen 2009;50:771-80.
25. Szczesny B, Olah G, Walker DK, Volpi E, Rasmussen BB, Szabo C, *et al.* Deficiency in repair of the mitochondrial genome sensitizes proliferating myoblasts to oxidative damage. PLoS One 2013;8:e75201.
26. von Ledebur M, Schmid W. The micronucleus test. Methodological aspects. Mutat Res 1973;19:109-17.
27. Martino-Roth MG, Viégas J, Roth DM. Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test. Genet Mol Res 2003;2:410-7.
28. Ad'hiah AH, Sulaiman GM, Al-Zaidy MS. Some immunological evaluations of propolis in albino male mice. J Fac Med 2007;49:121-5.