

Access this article online

Quick Response Code:



Website:
www.ijhonline.org

DOI:
10.4103/ijh.ijh_23_17

Immunomodulation of cyproheptadine hydrochloride on albino mice blood lymphocytes

Zainab Yaseen Mohammed Hasan, Ruqaya M. Al-Ezzy¹,
Jasim Mohammed Abdullah², Bashar Sabri AL-Hamdi³

Abstract:

BACKGROUND: Cyproheptadine hydrochloride (HCl) is antihistaminic drug which used widely in our country as an appetizer and for weight gain especially for low weight children.

OBJECTIVE: The aims of this study were to assess the effects of cyproheptadine HCl on blood picture including white blood cells count, lymphocytes count, and its effect on the level of different cytokines.

MATERIALS AND METHODS: This is an experimental study conducted at Biotechnology Research Center Laboratory in Alnahrain University from November 2016 to April 2017. It included five groups of albino male mice, these groups were treated separately with four doses of the cyproheptadine drug and fifth as control group; the doses were selected according to the British National Formulary (BNF) 2010 calculated on the base of mice/human deference's in body weight: These are (0.065, 0.092, 0.120, 0.24) mg/mice/day, respectively, with fifth untreated mice group considered as control.

RESULTS: The untreated mice (control) lymphocyte count was (4000) cell/ml and the total lymphocytes count increased up to (7014) cell/ml than the normal leading changes in interleukins (ILs) level in different manner, such as decreasing in both IL-6 and IL-10 levels with increasing in IL-12 level and no change in IL-4 level.

CONCLUSION: The current study showed that cyproheptadine HCl may have unwanted side effects, and its abuse may lead to disturbances in white blood cell especially lymphocyte which in turn will affect the immunity of persons taking it, especially in children, since the immune system modulates ILs secretion to face the irritability of drug behavior, that will lead abnormalities in human immune system.

Keywords:

Cyproheptadine hydrochloride, interleukins level, lymphocyte count

Introduction

Cyproheptadine is a type of drug group called antihistamine (a first-generation antihistamine) which are used to relieve allergy symptoms with additional anticholinergic,^[1] besides; they possess: anti-serotonergic, local anesthetic properties,^[2] and they have been used in treatment cyclical vomiting syndrome^[3,4] with drugs-induced hyperhidrosis.^[5] It can also be used as a preventive against migraine in children

and adolescents which may stimulate appetite.^[6] Prescription of cyproheptadine can be an alternative approach for patients who need nutritional support for a short period such as in cystic fibrosis patient and somatoform disorder.^[7-9] It has been found to improve sleep, calmness, and negative symptoms in chronic schizophrenics who do not respond to other therapies^[10,11]

Cyproheptadine overdose occurs when someone takes more than the normal or recommended amount of this medicine.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Mohammed Hasan ZY, Al-Ezzy RM, Abdullah JM, AL-Hamdi BS. Immunomodulation of cyproheptadine hydrochloride on albino mice blood lymphocytes. *Iraqi J Hematol* 2017;6:74-7.

Department of Plant Biotechnology, Biotechnology Research Center, Al-Nahrain University, ¹Department of Medical and Molecular Biotechnology, College of Biotechnology, Al-Nahrain University, ²Department of Animal House, Biotechnology Research Center, Al-Nahrain University, ³Department of Pharmaceutical Chemistry, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq

Address for correspondence:

Dr. Zainab Yaseen Mohammed Hasan, Department of Plant Biotechnology, Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq.
E-mail: zainaby2003@yahoo.com

Submission: 19-06-2017
Accepted: 31-07-2017

This can be by accident or on purpose. Moreover, the cyproheptadine was initially prescribed to promote weight gain, but its continued use seemed related to other psychological and/or pharmacological effects.^[12] On the other hand, cyproheptadine appetizer acts as a 5-hydroxy tryptamine receptor antagonist, act to block calcium channels with anticholinergic activity; all these effects can cause smooth muscle relaxation. Constipation is a common side effect that associated with drug administration.^[13] It is common adverse effects include drowsiness, dizziness, nausea, blurred vision, dry mouth, constipation, excitability, nervousness, and restlessness.^[14] Obesity, on the other hand, is one of the main risk factors of noncommunicable diseases worldwide, especially in Iraq.^[15] The use of cyproheptadine to increase body weight may lead to the risk of becoming obese.^[16] The present study highlights the need for educating health-care providers about the abuse potential of cyproheptadine and aiming to determine the prevalence of cyproheptadine misuse in Iraqi population and to describe its characteristics on adult and child immune effects emphasizing on interleukins (ILs) changing level in corresponding to control levels.

Materials and Methods

This is an experimental study conducted at Biotechnology Research Center Laboratory in Alnahrain University from November 2016 to April 2017. All institutional and national guidelines for the care and use of laboratory animals were followed.

Drug preparation

To prepare the required doses that were used in work; a pure powder (supplied from Samarra Drug Industry/Iraq) was dissolved in distilled water.

The laboratory animals groups used in the study

Albino male mice (*Mus musculus*), their weight between 23–27 g and 8–10-week-old at the start of experiments, were distributed into five groups and kept in a separate plastic cage.^[17]

Design of the experiment and cyproheptadine doses

Albino male mice (five groups), were treated separately with four doses of the cyproheptadine drug and fifth as control group. The selected doses were according to the British National Formulary (BNF) 2010 calculated on the base of mice/human deference's in body weight: these are,

- Group one: 0.065 mg/mice/day which equivalent to human child dose of 8 mg/day
- Group two: 0.092 mg/mice/day which equivalent to

human adult dose of 12 mg/day

- Group three: 0.120 mg/mice/day which equivalent to human max dose of 20 mg/day
- Group four: 0.24 mg/mice/day which equivalent to human toxic dose of 40 mg/day
- Group five: Untreated mice group considered as control.

Each dose was injected intraperitoneally as a single dose of 0.1 ml/mice/day for 10 days. At the end of the experiment blood were pooled from animal heart, and in day eleventh day, the mice were sacrificed for laboratory assessments.

Cyproheptadine effects on lymphocyte count and interleukins levels

Drug effects on lymphocyte count

With the aid of disposable insulin syringe (1 ml) pre-coated with heparin, blood samples were collected from mice heart by puncture. Aliquot of 0.02 ml from the collected blood was mixed with 0.38 ml of leukocyte diluent, in five separated glass test tubes, left for 5 min at 25°C. To the surface of Neubauer chamber, 10 µl of the mixture was applied under the cover slip. The chamber was left for 3 min to settle the cells. The lymphocytes were counted in the four large squares (each with 16 small squares), and calculate the average number of lymphocytes by applying the following equation^[8]:

$$\text{Lymphocytes count (cell / ml blood)} = \left[\frac{\text{Number of cells counted}}{4} \right] \times 20 \times 10$$

Drug effects on interleukins level

The work was done according to RayBio Kit (UK) protocol for IL-4, IL-6, IL-10, and IL-12. Aliquot of 100 µl from Standard and sample solutions per well were placed in duplicate to four types of IL plates each is conjugated in its specific micro titer-plate (IL-4, IL-6, IL-10, IL-12). The covered plate was incubated for 2 h at 25°C. A biotin antibody (100 µl) of each IL was added. The covered plate was incubated for another 2 h at 25°C then washed with washing buffer. Aliquot of 200 µl substrate solution to each well was added, then, incubated for 1 h at 25°C. Finally, 50 µl of Stop Solution was added, and the absorbance of each well was read on an ELISA reader at 450 nm wave length.

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. The difference was considered statistically significant when $P \leq 0.05$

Results

Effects on lymphocyte count

Figure 1 shows the effect of each cyproheptadine dose on the total count of lymphocytes. The group treated with toxic dose was excluded in this test due to rapid mice mortality before ending of experiment period.

Effects on Interleukins level

Figure 2 (a-d) shows the effect of cyproheptadine different doses on the level of IL-6, IL-12, IL-4, and IL-12, respectively.

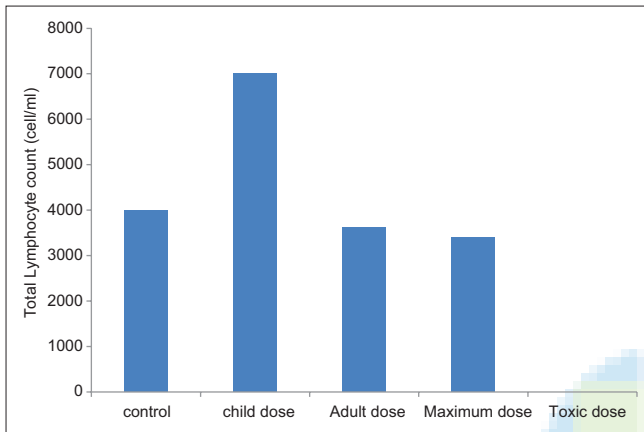


Figure 1: Effect of different cyproheptadine doses on total lymphocyte count

Discussion

From the above results that showed changing in lymphocyte count at different Cyproheptadine doses which leading in ILs' profile changes, one can imagine how the danger for drug misuse is?

As shown in Figures 1 and 2, the untreated mice (control) lymphocyte count was (6000) cell/ml and the normal ILs level for IL-4, IL-6, IL-10, and IL-12 reach (0, 104.33, 33.34, and 1019.75) pg/ml, respectively. When the mice treated with the equivalence of a child dose, the total lymphocytes count increased up to (7014) cell/ml than the normal leading changes in a different manner, such as decreasing in both IL-6 and IL-10 levels with increasing in IL-12 level and no change in IL-4 level.

On the other hand, adult and maximum doses cause a little decrease in lymphocyte count with increasing level of IL-12, decreasing in both IL-6 and IL-10 levels. IL-4 level does not affect the maximum dose but increased with adult dose. Toxic dose represent the suicide dose which excluded in lymphocyte count in this study while included in different ILs level that cause a different picture; three times increase in IL-6 level, six times increasing in level of IL-10, moderate increasing in IL-12 level with no change in IL-4 level in corresponding to control.

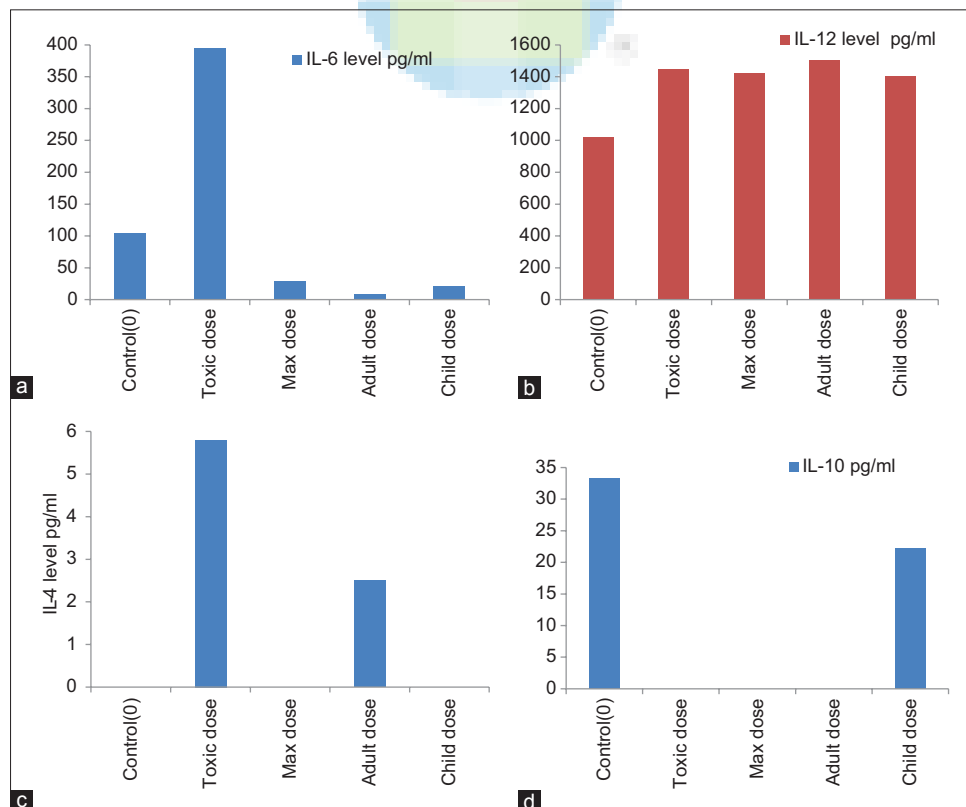


Figure 2: (a) Effect of cyproheptadine hydrochloride different doses on (a) interleukin-6, (b) interleukin-12, (c) interleukin-4 (d) interleukin-10 levels

Alternatively, all doses affect IL-10 and IL-12 levels by decrease the level of the first with increasing level of the later one when starting from child dose in an order design. The immune suppressive IL-10 that secreted from T and B cells acted to regulate growth and differentiation of T and mast cells,^[18] was affected by different doses of this drug by minimizing its effect in all doses in corresponding to control sample. IL-12 the T cells stimulating factor secreted from macrophage and B-lymphoblast cells, possessed elevation in its level after treated the lymphocytes with different cyproheptadine doses.^[19] IL-6 source is T cells, macrophages, and endothelium had target on liver, beside T and B cells acted to proliferate and produce of acute phase proteins.^[20] This effect not appeared with all cyproheptadine doses except the toxic dose. IL-6 also showed a range manner decreasing with relation to dose elevation except in toxic dose, its level increased 3 times than control level, while IL-4 level stays in constant level in all doses except a little increase in adult dose and six times increasing than control level in responding to toxic dose. IL-4 released from T cells.^[21] Its target was T and B cells and the macrophages, caused proliferation, differentiation, and activation of them.^[22] Results showed that animals treated for 10 day with cyproheptadine as follows: the child dose causes lymphocyte count to be doubled combined with increasing in IL-12 level, decreasing in both IL-6 & IL-10 levels and no obvious effect on IL-4 level. While in adult and maximum doses there was small decrease in lymphocyte count causes decreasing in IL-6 and IL-10 levels with increasing of IL-12 level, while toxic dose is extremely differ.

Conclusion

The current study showed that cyproheptadine hydrochloride may have unwanted side effects, and its abuse may lead to disturbances in white blood cell, especially lymphocyte which in turn will affect the immunity of persons taking it, especially in children, since the immune system modulates ILs secretion to face the irritability of drug behavior, that will lead abnormalities in human immune system.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Laurence LB, John SL, Keith LP. Goodman and Gilman's: The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill; 2002. p. 313-4.
2. Blaustein BS, Gaeta TJ, Balentine JR. Gindi M.

3. Cyproheptadine-induced central anticholinergic syndrome in a child: A case report. *Pediatr Emerg Care* 1995;11:235-7.
4. Waternberg NM, Roth KS, Alehan FK, Epstein CE. Central anticholinergic syndrome on therapeutic doses of cyproheptadine. *Pediatrics* 1999;103:158-60.
5. Andersen JM, Sugeran KS, Lockhart JR, Weinberg WA. Effective prophylactic therapy for cyclic vomiting syndrome in children using amitriptyline or cyproheptadine. *Pediatrics* 1997;100:977-81.
6. Ashton AK, Weinstein WL. Cyproheptadine for drug-induced sweating. *Am J Psychiatry* 2002;159:874-5.
7. Klimek A. Cyproheptadine (Peritol) in the treatment of migraine and related headache. *Ther Hung* 1979;27:93-4.
8. Homnick DN, Homnick BD, Reeves AJ, Marks JH, Pimentel RS, Bonnema SK, *et al.* Cyproheptadine is an effective appetite stimulant in cystic fibrosis. *Pediatr Pulmonol* 2004;38:129-34.
9. 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.
10. Craven JL, Rodin GM. Cyproheptadine dependence associated with an atypical somatoform disorder. *Can J Psychiatry* 1987;32:143-5.
11. Karia S, Dave N, De Sousa A, Shah N, Sonavane S. Cyproheptadine and dexamethasone abuse. *Natl J Med Res* 2013;3:88-9.
12. Goldberg SC, Halmi KA, Eckert ED, Casper RC, Davis JM. Cyproheptadine in anorexia nervosa. *Br J Psychiatry* 1979;134:67-70.
13. Akhondzadeh S, Mohammadi MR, Amini-Nooshabadi H, Davari-Ashtiani R. Cyproheptadine in treatment of chronic schizophrenia: A double-blind, placebo-controlled study. *J Clin Pharm Ther* 1999;24:49-52.
14. Hussein MA, Ibraheem NK, Abdalwahid AB. *In vitro* study of the effects of some pediatric tonics and appetizers on the male rabbits intestinal (jejunal) smooth muscle motility. *J Basrah Res Sci* 2013;39:22-7.
15. von Mühlendahl KE, Krienke EG. Toxicity of cyproheptadine. side effects and accidental overdosage (author's transl). *Monatsschr Kinderheilkd* 1978;126:123-6.
16. Hussein A, Maysaloon M. Prevalence and risk factors associated with overweight and obesity among under than 5 years children. *Iraqi J Med Sci* 2013;11:54.
17. Mirmiran P, Sherafat-Kazemzadeh R, Jalali-Farahani S, Azizi F. Childhood obesity in the Middle East: A review. *East Mediterr Health J* 2010;16:1009-17.
18. Al-Ezzy RM, Mohammed Hassan ZY, Farah TO. Hematological toxic effect and the frequency of micronucleus formation of different doses of cyproheptadine on albino male mice blood picture. *Iraqi J Hematol* 2017;12:152-75.
19. Zizzo G, Cohen PL. IL-17 stimulates differentiation of human anti-inflammatory macrophages and phagocytosis of apoptotic neutrophils in response to IL-10 and glucocorticoids. *J Immunol* 2013;190:5237-46.
20. Lasek W, Zagożdżon R, Jakobisiak M. Interleukin 12: Still a promising candidate for tumor immunotherapy? *Cancer Immunol Immunother* 2014;63:419-35.
21. Hodes GE, Ménard C, Russo SJ. Integrating interleukin-6 into depression diagnosis and treatment. *Neurobiol Stress* 2016;4:15-22.
22. Mendonça MS, Peraçolli TS, Silva-Vergara ML, Ribeiro SC, Oliveira RF, Mendes RP, *et al.* High interleukin-4 expression and interleukin-4 gene polymorphisms are associated with susceptibility to human paracoccidiodomycosis. *Mem Inst Oswaldo Cruz* 2015;110:781-5.
23. Zhao X, Wang H, Sun G, Zhang J, Edwards NJ, Aronowski J, *et al.* Neuronal interleukin-4 as a modulator of microglial pathways and ischemic brain damage. *J Neurosci* 2015;35:11281-91.