Iraqi Journal of Cancer and Medical Genetics

Studying of genotoxicity of Cyproheptadine hydrochloride on albino mice bone marrow cell

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Abstract:

Cyproheptadine hydrochloride is an antiserotonergic and antihis-taminic agent that can indicate allergic diseases there for it is used for treating symptoms of allergic reactions in addition to its sedative and anti cholinergic effects, Cyproheptadine is also used to stimulate appetite and weight gain in human and veterinary medicine Considering its potentially dangerous for human health. therefore, this study aimed to assess the effect of (25µg/kg) and (50µg/kg) of cyproheptadine, given to mice by "gavage" and observe cytogenetic effects (DNA damage) on mice bone marrow cells (in vivo) by using comet assay. The statistical analysis shows that cyproheptadine hydrochloride cause significant increase ($P \leq 0.0001$) of DNA damage of bone marrow cells in mice in comparing with the negative controls. The DNA damages are detected by the comet assay test in mice treated with Cyproheptadine hydrochloride. The prolonged use of Cyproheptadine hydrochloride led to increase the probability of DNA damage.

Keyword: *Cyproheptadine, DNA damage, Cytogenetic effect, Cometassay*

Introduction:

Periactin is the trade name of cyproheptadine hydrochloride which is a serotonin and histamine antagonist cyproheptadine is a histamine-1 receptor antagonist with nonspecific 5-hydroxytryptamine 5-HT-1A and 5-HT2A antagonistic properties. It is the recommended antidote for the treatment of serotonin syndrome in the setting of incomplete response with supportive therapy including aggressive cooling and benzodiazepines. (1) While it exerts weak anticholinergic actions, cyproheptadine's antidotal properties are attributed to the blockade and competition of 5-HT receptor sites receptors. A dose of 12–32 mg will bind 85%–95% of serotonin receptors. Cyproheptadine also with anticholinergic , sedative effects and blocks calcium channels. (2)

It is used for treating symptoms of allergic reactions (eg, caused by seasonal allergies, food, blood or plasma), treat mild uncomplicated hives, for Perennial and seasonal allergic rhinitis (3) Vasomotor rhinitis, Allergic conjunctivitis due to inhalant allergens and foods, Mild uncomplicated allergic, skin manifestations of urticaria and angioedema. ,Amelioration of allergic reactions to blood or plasma ,Cold urticaria , Dermatographism and also used to stimulate the appetite and may lead to weight gain. (4).

Side effects of the drug is include sedation , drowsiness , hypotention , dry mouth, diarrhea, constipation, nausea, tachycardia , palpitations, dry nose and throat, thickening of bronchial secretions, chest tightness, and wheezing, blurred vision, urinary retention (5) , Over dosage of antihistamines, particularly in infants and young children, may produce hallucinations, central nervous system depression, convulsions, respiratory and cardiac arrest, and death. hemolytic anemia and blood dyscrasias (6) .

Cyproheptadine has been included in "List of prohibited drugs used in feed and drinking water of animal" in China, and cannot be authorized as a veterinary medication intended for use in food-producing animals and included on the list of prohibited ingredients in cosmetics by the Japanese Pharmaceutical Affairs Act. (7) . Some researches Confirmed that cyproheptadine has cytotoxic and genotoxic effects on human peripheral blood lymphocyte cultures. (8)

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Materials and Methods:

Laboratory Animals

Experiments were performed on 30 albino mice (male and female), their ages ranged between 8-12 weeks with a body weight 25g. mice were obtained from animal house of National center for drug control and researches, housed in the animal house of Al-Nahrain University. They were kept in a

room supplied with air conditioner to keep the temperature between18-24 ˚C, the air of the room was changed continuously by using ventilating fan and light was controlled with range of 12 hours of light and 12 hours of darkness.

The animals were housed in plastic cages (2 mice\cage) with a wire grid covers, supported on ventilated racks (9) . The bedding material used was fine sawdust and wood shaving which was changed every other day to prevent accumulation of urinary pheromones (10) . The cages was washed regularly once a week with hot water, then 70% alcohol as disinfectant , mice were fed with standard balanced pellet that contains special dietary supplement to keep normals activity and growth . Before experimentation , all mice were left for at least two weeks for adaptation , during this period . absingleand sick mice were excluded from the experiment .

The used drug

The used drug was cyproheptadine in the form of tablets manufactured by kontam pharmaceuticals china each tablet contain 4mg of cyproheptadine that obtained from Baghdad pharmacies The drug was dissolved in (16ml) distilled water. The solution concentration became (250 µg/ml) this solution kept at (40c) and regarded as astock solution and the required dilution was prepared from this solution. for oraly giving, 1 ml from the stock solution was taken and mixed with 9 ml of D.W. to prepare a (25µg/ml) . To give a single dose (25 µg/kg b.wt.) for the mice in all experiments (0.1ml) taken from the last soultion and for the double dose (50 µg/kg b.wt.), (0.2ml) was taken from the last prepeared solution. (11)

Animals Groups

The experiment was achieved as following: First group included 10 mice were given only water and was considered as negative control animals . Second group included 10 mice were administered the single dose of cyproheptadine hydrochloride (25 µg/kg b.wt.) . Third group included 10 mice were administered the double dose of cyproheptadine hydrochloride (50 µg/kg b.wt.) . All these groups were treated seven days before week from examination.

Comet Assay Methods

The animals were sacrificed by cervical dislocation after the end of the experiments and the thigh bone was taken and cleaned from tissue and muscles, then catched from the middle with a forceps in vertical position over the edge of test tube. By a sterile syringes, 5 ml of warm Phosphate buffer saline (PBS) 37ºC was injected to wash and drop the bone marrow in the test tube to formation of cell suspension. Cells suspension was centrifuged at 1500 rpm for 2 min. The supernatant was discarded and the pellet washed once with ice-cold PBS (without Mg2+ and Ca2+) and centrifuged at 1500 rpm for 2 min. then supernatant was discarded.

The comet assay was performed under alkaline condition. Essentially according to (12) with slight modification.

Agarose preparation

1% low-gelling-temperature agarose (Cambrex /bioscience USA) was prepared by mixing powdered agarose (0.5gm) with distilled water (50ml) in a glass beaker or bottle. Bottle was placed in the 100 °C water bath for several minutes. Bottle was placed into a 40 °C water bath.

Preparation of Samples and Slides

Agarose slides were prepared by dipping the slides into singlemolten 1% (w/v) agarose. It was allowed to dry to a thin film. Cells sample was combined with low melting point agarose at 0.5 (w/v) and the mixture (75µl/ slid) immediately was added into slide comet by pipette. The slides were hold horizontally then transferred to 4ºC in a dark container for 30 min. The slide was transferred to a small basin overnight (18−20 h) at 4 °C in the dark. After overnight, the slide was immersed with electrophoresis buffer solution for 20 min. The slides were hold horizontally, then transferred to a horizontal electrophoresis chamber filled with a cold electrophoresis solution, 24volt and 300mA was applied to the chamber for 18 min. TBE electrophoresis solution was aspirated from chamber and replaced with 0.4 M of Tris-HCl solution (pH 7.5) for 5 min in order to neutralize of cells (The step repeated twice). Diluted vista green DNA dye (Sigma/ USA) 100 µl was added to each well comet assay slide and incubated at room temperature for 10 min. The slides were rinsed with distilled water to remove excess stain. The slides were examined by fluorescence microscope (Meiji/ Japan) . three pa-rameters were used. They were tail length {distance from the head center to the end of the tail}, mean tail moment {product of tail DNA\total DNA by the tail center of gravity} and tail DNA%=100X Tail DNA Intensity\ Cell DNA Intensity . Results analyzed with Comet Assay IV software (Perceptive/ England)

Results and Discussion :

Results of the comet assay, summarized in the table (1) show the percent of DNA tail was significantly higher in the singleand double dose groups with a mean (Mean ± SD) values 31.012± 2.606% and 5.523±0.630% respectively as compared with controls (1.693±0.387%). There was a significant difference (P≤0.0001) between the two groups single dose and double dose and its controls, in table (1) the mean of tail length (Mean ± SD) of control group and animals treated with drug single and double dose are 0.8 ± 0.106px, 5.3 ± 0.384px, 6.4 ± 0.357px, respectively (px. Pixel is length unit; one pixel is equal to one dot on the computer) There was a significant increase between experimental animals and controls for DNA Mean in tail (P ≤0.0001). DNA damage is statistically significantly (P ≤ 0.0001) in case of experimental animals for tail moment (Mean ±SD) and was 1.623±0.108, 0.210± 0.023, respectively than in control group 0.017±0.003. Tail length and mean tail moment were significantly higher in the single and double dose groups compared with controls.

Table (1): The results of comet assay (Mean ± SD) for bone marrow of mice treated with cyprohetadine hydrochloride drug and control groups as mean value of the measurements of 100 comets per subject.

Group	Mean ±S.D.		
	Tail length	Tail DNA	Tail mean moment
Control	0.8 ± 0.106 ^{C}	1.693± 0.387 ^{C}	0.017±0.003 ^{C}
cyrohetadine (25 µg/kg b.wt)	5.3 ± 0.384 ^{B}	31.012± 2.606 ^{A}	1.623±0.108 ^{A}
cyrohetadine (50 µg/ kg b.wt)	6.4 ± 0.357 ^{A}	5.523± 0.630 ^{B}	0.210± 0.023 ^{B}

Different letters: Significant difference (P≤0.0001) between means

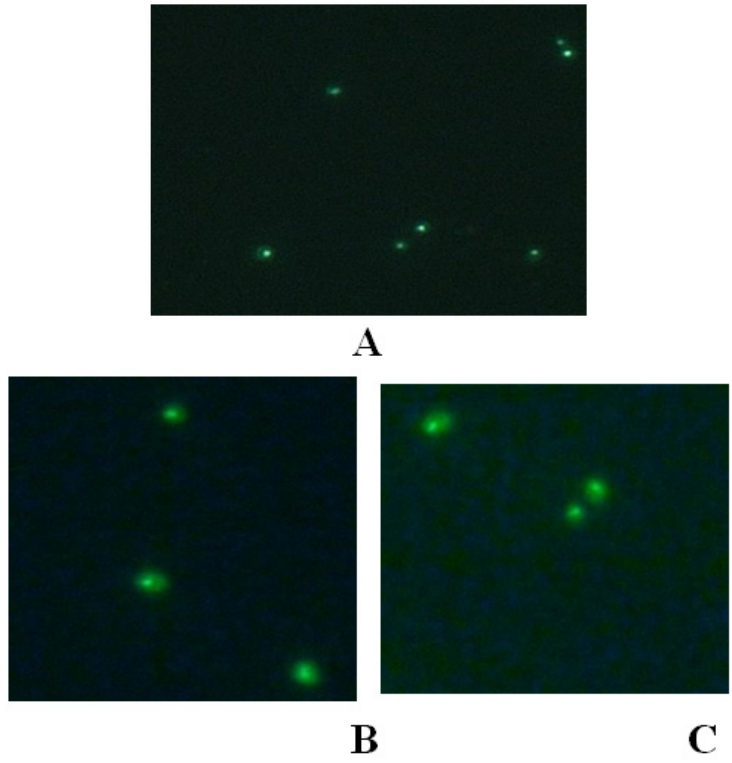

Figure (1): Comet assay in mice bone marrow cells examined by flores-cent microscope (400X); (A) of the control group showing fluorescent spheres without DNA damage (no tail) , (B) Group treated with a single dose (25 µg\kg) of cyrohetadine , (C) Group treated with adouble dose (50 µg\kg) of cyrohetadine .

Control sample showed no comets because there was no DNA damage However treated samples showed comets repre-sent in damaged appearance with increased doses (Fig. 1). The tail length increased depending on the drug dose. The tail end was wider and thicker than the head of comet 50µg\kg dose (Fig. 1).

Garaj-Vrhovac and Kopjar (13) used the alkaline comet assay to evaluate the genotoxicity towards peripheral lymphocytes of medical personnel regularly handling various drugs. The nature of the genotoxic agent as well as the scatter in tail length, % mi-grated DNA and tail moment depend on the target cell popula-tion (14). DNA damage occurs may be because cyproheptadine decreased expression of cyclins D1, D2, and D3 and arrested these cells in the G0/G1 phase , D-cyclins are regulators of cell

division that act in a complex with cyclin-dependent kinases to commit cells to a program of DNA replication.

These findings are in contradiction to the observations by (15) they approved there are No variations or aberrations were seen in the chromosomes of human lymphocytes that could be definitely related to treatment with cyproheptadine.

The result above agreed with (16) that approved that Cyproheptadine decreased D-cyclin levels and induced cell death in myeloma and leukemia cell lines and patient samples preferentially over singlehematopoietic cells.

Mao et al . (17) also agreed with the results above they approved that cyproheptadine inhibits D-cyclin expression and induces cell death in proliferative diseases involving increased expression of D-cyclins. It has also been shown that cyproheptadine and its analogs induce cell death in hematological malignant cells, including a wide variety of cell lines and patient cancer cells. Further it has been demonstrated that cyproheptadine abolishes formation of malignant as cites in an in vivo mouse model and decreases tumor volume of established tumors in mice.

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دراسة تأثير السمية الوراثية لعقار سيبروهبتادين هيدروكلوريد على خلايا نقي العظم في الفئران البيضاء

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الخلاصه:

سيبروهيبتادين هيدروكلوريد هو من العوامل المضادة للسيروتونين والهستامين و التي يمكن أن تشير إلى أمراض الحساسية لذا فهو يستخدم لعلاج أعراض الحساسية بالإضافة إلى اثاره المسكنة و المضادة للكولين،لذا يستخدم سيبروهيبتادين أيضا لتحفيز الشهية وزيادة الوزن في الطب البشري والطب البيطري والتي تصنف على انها خطرة على صحة الإنسان لذلك، تهدف هذه الدراسة إلى تقييم تأثير عقار سيبروهيبتادين باستخدام التركيزين (25 ميكروغرام / كغم من وزن الجسم) و (50 ميكروغرام / كغم من وزن الجسم) ،اعطيت هذه التراكيز للفئران بواسطة التجريع الفموي وملاحظة التأثيرات الوراثية الخلوية (تلف الحامض النووي) على خلايا نقي العظم للفئران (داخل الجسم الحي) وذلك باستخدام تقنية المذنب القاعدي . وقد اظهر التحليل الإحصائي أن السيبروهبتادين تسبب زيادة معنوية ($P\leq0.0001$) لتلف الحامض النووي في خلايا نقي العظم للفئران بالمقارنة مع السيطرات السالبة.