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Assessments of Cytotoxic and Genotoxic Effects of Prednisolone Drug in Male Mice

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Abstract: Prednisolone is a corticosteroids drug widely used for treatment of numerous conditions such as inflammation, asthma, allergies, cancer and immune diseases. The cytotoxic and mutagenic impact of this drug was evaluated in male mice by using mitotic index and sperm head abnormality assays. The study was carried out with thirty six adult male albino mice (*Mus musculs*).They were divided into the following groups: Treated group which orally given 0.1 mg/Kg of Prednisolone for 30days, the positive control group which take a single dose of cyclophosphamide drug (5mg/kg body weight) for 7 days other group considers as negative control received distilled water only for 30days. The drug did not induce a significant difference in the mean count of mitotic index of bone marrow and germ cells. As well as it doesn't induce a significant increase in sperm head abnormality. It can be concluded that tested dose of Prednisolone failed to induce genotoxic and cytotoxic impact in bone marrow and germ cells.

Key words:-Prednisolone, mitotic index, sperm head abnormality, cytotoxic**.**

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Introduction

Corticosteroids are naturally created in the adrenal cortex of vertebrates after cytokine stimulation of the hypothalamus-pituitary-adrenal. It's having an important role in the regulation of many biological processes, containing, metabolism, immune responses, cell growth and proliferation and reproduction(1). In 1949 corticosteroids were introduced as drug subsequently it used in wide in medicine for their anti-inflammatory immunosuppressive and cytotoxic effects (2).

Many studies indicate that corticosteroids induced programmed cell death in hematological cells. The researcher demonstrates two possible mechanisms by which corticosteroids mediated programmed cell death, through inhibiting the transcription of

growth, survival genes or by activation of the death inducing genes (3).

Previous study demonstrates that corticosteroids medicine (Dexmethasone) has genotoxic effect. Singh *et al*. (4) reported that *in vivo* treatment of mice with hydrocortisone induce sister chromatids exchange and micronuclei in bone marrow cells. In addition Fahmay *et al.* (5) demonstrate that repeated treatment with large dose of hydrocortisone repressed protein synthesis and induce cytotoxic effects in the germ and somatic cell of mice.

DNA damage induced by physical and / or chemical agents in male germ cells at any phase of the spermatogenesis process can be transmitted to the subsequent generation. These kinds of genomic damage have considered the main causes of implantation failure, embryonic death and embryonic malformations (6). Investigation of genomic damage in germ cells can identify by a number of methods. One simple, quick and economical method is sperm head abnormality assay(7). Prednisolone synthetic preparation of cortisol it is widely used for treatment of numerous conditions such as inflammation, asthma, allergies, cancer and immune diseases(8,9) data concerning the genotoxic effects of Prednisolone in mice are scarce. The aim of the present study is to assess the genotoxic and cytotoxic effects of Prednisolone in male mice using short term *in vivo* mutagenicity assay sperm head abnormalities and mitotic index of bone marrow and germ cells. Cyclophosphamide drug is an alkylating agent induced genotoxic damage in germ and somatic cells of mice (10). Cyclophosphamide was used as a positive control in the present study.

Materials and Methods

The study was carried out with thirty six adult male albino mice (*Mus musculs*), about(8-12) weeks old and weighing (25± 2) g, The mice were housed in polycarbonate boxes and maintained under standard laboratory conditions in the animal house of the Department of Biology, University of Al-Mustansiriyah . They were kept on a standard laboratory diet and water *ad libitum*. They were divided into the following groups: Treated group which orally given 0.1 mg/Kg of Prednisolone (AL-Hokamaa Company, Iraq) for 30days positive control group which take a single dose of Cyclophosphamide drug (Finland) (5mg / kg body weight for 7 days) (11) another group considers as negative control received distilled water only for 30days.

Preparation of Bone Marrow Cells

At the end of treatment time (30 days) all males were injected intraperitoneally with colchicines (2.5 mg\kg of body weight) after two hours animals were sacrificed by decapitation cells were isolated from bone marrow, according to the procedure mentioned by Agarwal, *et al.* (12). The mitotic index was determined by counting a range of 1000 cells from each animal, using the formula below:

Mitotic index (MI) = (Number of divided cells/Total number of cells) * 100.

Mitotic Index in Germ Cells

Testis were obtained from the same animals(treated and control groups). To assess the effect of Prednisolone on

germinal cells in different stages of spermatogenesis, Germ cells were isolated from testis according to methodology of Brewen and Preston (13). The mitotic index was counting from 1000 cells per animals.

Sperm Head Abnormality Assay

To analysis any morphological abnormality of sperm head the epididymis removed from treated males and crushed in 5 Ml of pre warmed (37C) physiological saline to prepare the sperm suspension after staining in 0.1% eosin at least 1000 sperm for each animal were scored and sperm head abnormality were classified following to the criteria of Wyrobeck and Bruce (7).

Statistical Analysis

To analysis the difference in the mean count among the treated groups one way analysis of variance (ANOVA) was utilized followed by Least significant difference –LSD test. Data are expressed as the mean ± standard error of the mean (SEM). The level of significance was considered $P< 0.05$ (14).

Results and Discussion

Analysis of data of the mean count of mitotic index (MI) of bone marrow cell shown that there was no significant difference ($P> 0.05$) were observed when compare treated males with negative and positive control group. Male mice receiving cyclophosphamide revealed a decrease in (MI) of bone marrow cells, mean count of (MI) was 2.17±0.36 compared to 2.71±0.21 as for the negative control group. The difference statistically insignificant ($P>0.05$) (Figure 1).

The mitotic index parameter contributes to identifying the cytotoxic impact of numerous chemicals and drugs (15). It is important to know that both the decrease and increase in mitotic index mediated by reduce protein synthesis(5). are meaningful indicators in the assessment cytotoxic compound, when the mitotic index value calculated in exposed animals significantly lower than the mitotic index of negative control this can reflect modifications, in the development and growth of exposed animals obtaining from the agents action. In a different way, when mitotic indices greater than the negative control are the results of an elevated in cell proliferation, which can be lead to creation of tumor tissues by disrupt cell division (16). The result of present work shows that Prednisolone failed to induce genotoxic and cytotoxic impact in bone marrow cells. This finding lack of concordance with previous studies Loeb *et al.*, (17) indicate that hydrocortisone constraining hepatoma cells multiplying through inhibiting DNA synthesis in addition, another study suggest that the cytotoxic effects of hydrocortisone can be mediated by reduce protein synthesis (5).

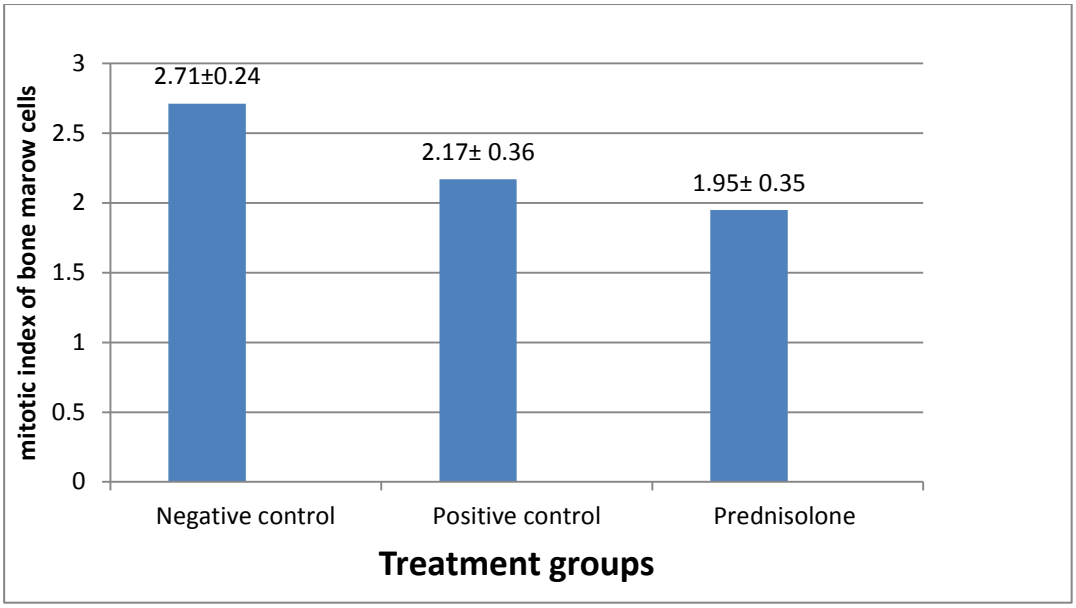

Figure 1 : Means of mitotic index of bone marrow cells of male mice (positive ,negative and treated groups)

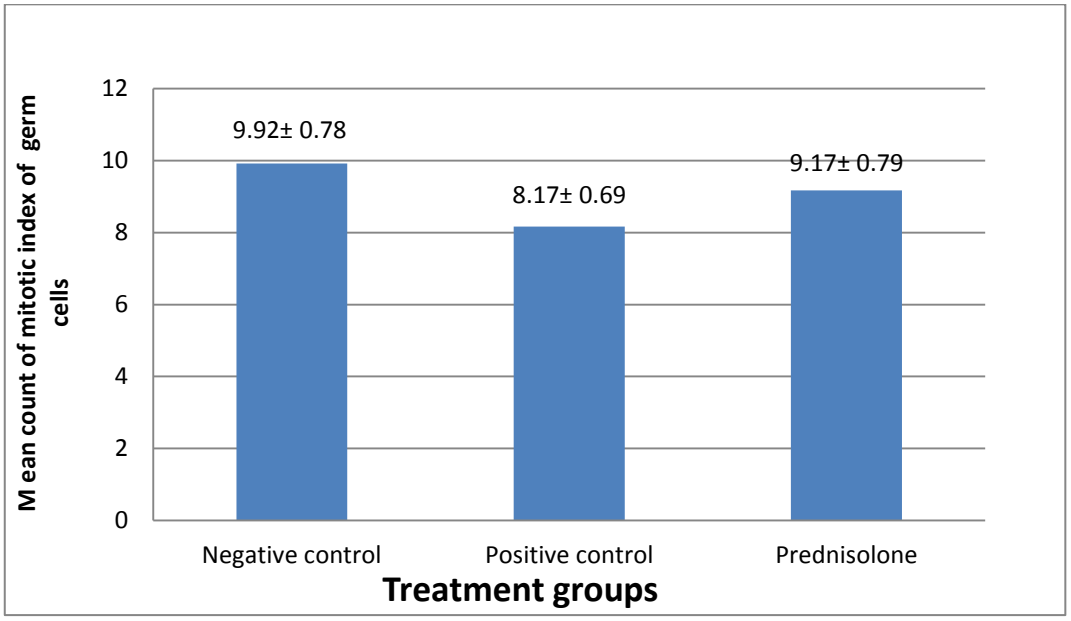

Figure 2 : Means of mitotic index of germ cells in male mice(positive ,negative and treated groups)

As shown in figure (2) the (MI) of spermatocytes cells in treating male with prednisolone was (9.17± 0.79)which was slightly lower than MI of spermatocytes of the negative control group (9.92±o.78) and it's higher than the mitotic index of spermatocytes cells in the positive control group(8.17 ± 0.69) the difference was statistically insignificant (P> 0.05).

Table (1): Percentage of total sperm head abnormality in male mice

Treatments groups	Total sperm head abnormality mean± S.E
Negative control	5.97± 1.12[a]
Positive control	6.28± 1.11[a]
Prednisolone	5.22±1.34[a]

(P > 0.05).

Table (1) shows the mean count of sperm head abnormality of male mice. The data displayed that treated mice with cyclophosphamide for 7 days led to increase mean count of sperm head defect (6.28 ± 1.11) but this increase was insignificant when compared with a negative control group (P> 0.05).

As well as treated male with Prednisolone for the 30 days didn't introduce any significant change in the percentage of sperm head abnormality (P>0.05). The sperm abnormality test identified as those agents which disturbs the differentiation of spermatocytes through inducing DNA damage in germ cells (point mutation or deletions or a mixture of both) which lead to defects in sperm morphology (7). It is known that Spermatogenesis takes around 5 weeks in male mice. Synthesis of genetic materials (DNA) takes place prior to pre-meiotic phase and no additional synthesis occur (18,19). Lower frequency of abnormal sperm after 30 days of treatment accompanied by no significant difference in mitotic index of spermatocytes when parallel with negative control or positive control groups. The present results reveled that tested dose used of Prednisolone not enough to generate genotoxic and cytotoxic effects in germ cells of male mice . Or in other words, it can be concluded that repair of induced DNA damage has occurred these result disagreement with previous studies by Vaisheva *et al.* (20) demonstrate that chemotherapy of Non-Hodgkin lymphoma included Prednisolone induced programmed cell death of germ cell in male mice and it was accompanied by an increased incidence of germ cell apoptosis

Cytotoxic and genotxic potential of synthetic glucocorticoidal can be mediated via free radical production as well as accumulation products of lipid peroxidation (21). In the present study, we can conclude that Prednisolone

failed to induce genotoxic and cytotoxic impact in bone marrow cells and germ cells may be due to the inability of synthetic glucocorticoidal at the treated dose (0.1mg\kg) to produce free radicals. The result of present work appears to be concordant with the findings of Fahmay *et al*., (5) who reported that genotoxic effect of hydrocortisone occur with repeated treatment of the higher concentration (52 mg/kg body weight). Further studies will be necessary to detectgenotoxic and cytotoxic impacts of Predoslone on somatic and germ cells in a wide range of concentrations using another cytotoxic and genotoxic end points.

References

1- Christaki, E.; Anyfanti, P. and Opal, S.M. (2011). Immunomodulatory therapy for sepsis: an update. Expert Rev Anti. Infect. Ther., 9 (11): 1013- 1033.

2- Watterberg, K. (2012). Evidence- based neonatal pharmacotherapy: postnatal corcorticosteroids. Clin Perinatol, 39 (1): 47-59.

3- Stephanie, G.; Kulsoom, G.; Nancy; L. Krett andSteven T. R. (2002). Mechanisms of Glucocorticoid-mediated Apoptosis in Hematological Malignancies.Clin Cancer Res 8 (6): 1681-1694.

4- Harbhajan, S., H.; jai Rup, S.; Varinderpal, S. D.; Deeksha, B. and Hari P. (1994). In vitro and in vivo genetoxicity evaluation of hormonal drugs. II. Dexamethasone Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesis, 308 (1):89–97.

5- Fahmy, M. A.; Farghaly,A.A.; Ali Hassan,N.H.and Diab, K. A. E. (2015). Molecular and cytogenetic evaluation for potential genotoxicity of hydrocortisone Asian Pac. J. Trop. Dis., 5(9): 726-731.

6- Salimi, M. ; Mozdarani, H. and Elmina Nazari, E.(2014). Cytogenetic Alterations in Preimplantation Mice Embryos Following Male Mouse Gonadal Gamma-irradiation: Comparison of Two Methods for Reproductive Toxicity Screening. Avicenna J Med Biotechnol. , 6(3): 130–139.

7- Wyrobek, A. J.and Bruce, W. R. (1975). Chemical induction of sperm abnormalities in mice. Proceedings of the National Academy of Sciences USA, 72: 4425–4429.

8- Spenerovaa,M. ; Dzubaka, P. ; Srovnala, j.; Radovaa,L.; Burianovaa,R.; Konecnya,P. and Salkovaa,S.(2014). Combination of prednisolone and low dosed dexamethasone exhibits greater *in vitro* antileukemic activity than equiactive dose of prednisolone and overcomes prednisolone drug resistance in acute childhood lymphoblastic leukemia. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 158(3):422-427.

9- Sweetman, S. C. (2009). Martindale: The Complete Drug Reference. 36th edition, Pharmaceutical Press, London, UK.1898 p.

10- Ray, B. and Potu, B. K. (2010).Ovarian folliculogenesis : Detrimental effect of prenatal exposure to cyclophosphomide : a preliminary study. Bratisal lek. Listy,111(7) :369-372.

11- Gurbuz, N.; Ozkul, A.and Burgaz, S.(2009). Effects of vitamin C and N-acetylcysteine against cyclophosphamide-induced genotoxicity in exfoliated bladder cells of mice in vivo. J Buon. 14(4):647-652.

12- Agarwal, K.; Mukherjee, A. and Chakrabarti, J. (1994). In vivo cytogenetic studies on mice exposed to natural food colourings. Food Chem. Toxicol. 32. (9): 937.

13- Brewen J.G and Preston R.J. (1978). Analysis of chromosome aberrations in mammalian germ cells, in A. Hollaender and F. J. de. Serres (Eds), chemical mutagens, Vol.5, plenum, New York: 127-150 pp.

14- SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

15- Amorim, M. I.; Mergler, D.; Bahia, M. O.; Dubeau, H. M.; Miranda, D. C.; Level, J.and Burbano, R.R.(2000). Lucotte MCytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. Ann. Acad. Br*as. Cienc., 72(4):487–507.*

16- Hoshina, M.M. (2002). Avaliac¸a˜o da possi´vel contaminac¸a˜o das a´guas do Ribeira˜o Claro - munici´pio de Rio Claro,

pertencente a` bacia do rio Corumbatai´, por meio de testes de mutagenicidade em Allium cepa, Trabalho de conclusa˜o (Bacharel e Licenciatura - Cieˆncias Biolo´ gicas), Universidade Estadual Paulista, Rio Claro/SP, 52 p.(Abstract)

17- Loeb, J. N.; Carmia,B. and Yeung,L.L.(1973). Suppression of DNA synthesis in hepatoma cells exposed to glucocorticoid hormone in vitro. Proc Natl. Acad. Sci. USA, 70(12): 3852-3856 .

18- Chauhan, L.K.S.; Pant, N.; Gupta, S.K. and Srivastava, S.P.(2000). Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbofuran exposure. Mutat. Res., 465(1-2): 123–129.

19- Giri, S. and Sharma, G.D. (2002). Genotoxic effects of malathion an organophospho insecticide using three mammalian bioassays in vivo. Mutat. Res., 514(1-2):222-231.

20- Vaisheva,F.;Delbes,G.;Hales,B.F.;Robairs, B.(2007) Effects of the chemotherapeutic agents for non- Hodgkin lymphoma cyclophosphamide,doxorubicin,Vincristine and Predensoneon the male rat reproductive system and progeny outcome journal of Andrology, 28(4):578-587.

21- Koshaeva, E.S. and Semenov, V.V. (2013). Clastogenic and anti clastogenic activity of glucocorticoide hormone hydrocortisone and its synthetics analysis predensolone and deoxmethasone.Bull.Exp.Med.,155(1)78-80.