Original article　　　　doi: 10.1016/S2222-1808(15)60921-5　　　　

Molecular and cytogenetic evaluation for potential genotoxicity of hydrocortisone

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ARTICLE INFO

ABSTRACT

Objective: To assess the risk of hydrocortisone sodium succinate through different end points of genotoxicity.
Methods: The study examined the induction of chromosomal aberrations in bone marrow cells, morphological sperm abnormalities, the effect on dominant lethal gene and protein synthesis. Hydrocortisone was given intraperitoneally at three dose levels 26, 39 and 52 mg/kg body weight which was equivalent to the therapeutic doses in man.
Results: The results showed that single dose treatment with different doses had no effect on chromosomal aberrations. The dose of 52 mg/kg body weight induced significant percentage of chromosomal aberrations in bone marrow cells after repeated treatment for 7 and 14 days. Significant effect of morphological sperm abnormalities was demonstrated only after treatment with the dose of 52 mg/kg body weight. For examining the dominant lethal mutation, male mice were injected with dose of 39 mg/kg body weight for 5 consecutive days. Mating between treated males and virgin untreated females were performed at different time intervals. The results showed that the percentage of fertile mating at 1–7 and 8–14 days reduced to 50% and 60% respectively compared with control group while no effect was recorded at 15–21 days. The percentage of dominant lethal mutation reached 0.32%, 4.4% and 0% in mating intervals respectively indicating pronounced effect of hydrocortisone at the interval 8–14 days which represented by the late spermatids. The results also showed that the repeated treatment with the dose of 52 mg/kg body weight inhibited protein synthesis which contributed to the cytotoxic effect of the drug.
Conclusions: It is concluded that long term treatment with large doses of hydrocortisone may have genotoxic effect.

1. Introduction

　　Corticosteroids are a class of chemicals that includes steroid hormones naturally produced in the adrenal cortex of vertebrates. They have many important functions in the body, including the control of inflammatory responses. Corticosteroid medicines are man-made derivatives of the natural hormones. Food and Drug Administration approves hydrocortisone as a drug in 1951. It works by acting within cells to prevent the release of certain chemicals that are important in the immune system. These chemicals are normally involved in producing immune and allergic responses, resulting in inflammation. By decreasing the release of these chemicals in a particular area, inflammation is reduced. This can control a wide variety of diseases[1,2]. Hydrocortisone sodium succinate (Figure 1) has the same metabolic and inflammatory control action as hydrocortisone.
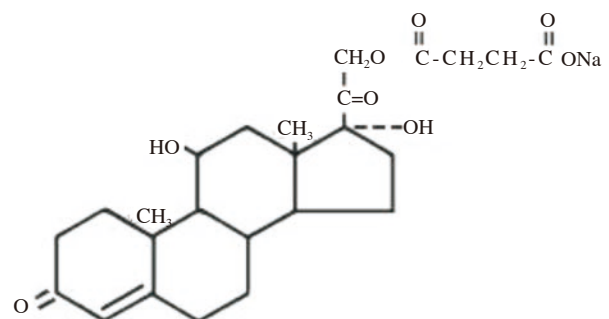

Figure 1. Structural formula of hydrocortisone sodium succinate.

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The excess use of corticosteroids is associated with various toxicities. Corticosteroids result in growth retardation in children when given systemically and affect various aspects of reproduction and fertility, including degeneration of epididymides, sperm density and morphometric measurements; impairment of metabolic∕secretion activity of epididymal cells; induction of apoptosis in leydig cells of the testis and inhibition of testosterone biosynthesis[3-5]. The exposure of pregnant rats to hydrocortisone in late stages of pregnancy might have a long-term effect on the fertility and the reproductive physiology in both male and female offsprings[6,7]. Azoospermia was reported in a 23 years old patient with testicular adrenal rest tumor received hydrocortisone[8].

Few researches show that synthetic steroids including hydrocortisone have genotoxic potential. Hydrocortisone was reported to induce micronuclei and sister chromatid exchange in mouse bone marrow *in vivo*[9] and chromosomal aberrations, micronuclei and sister chromatid exchange in cultured human lymphocytes *in vitro* with or without metabolic activation[10,11]. In addition, hydrocortisone at the dose level of 12.5 mg/mL increased chromosomal aberrations in *Crepis capillaris* test system in comparison with spontaneous level[12].

However, the screening of new drugs for potential genotoxicity is an important step during research and development. Therefore, the present study was designed to assess the risk of hydrocortisone sodium succinate through different end points of genotoxicity.

2. Materials and methods

2.1. Animals

Male white Swiss mice (*Mus musculus*), aged 9–12 weeks were used in all experiments. Except for dominant lethal mutation, both male and female mice were used. The animals were obtained from the animal house of the National Research Centre, Cairo, Egypt. The mice used for any one experiment were selected from mice of similar age (± 1 week) and weight (± 2 g). Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals/cage) and bedded with wood shavings. Ambient temperature was controlled at (22 ± 3) ℃ with a relative humidity of (50±15)% and a 12 h light/dark photoperiod. Food and water were provided *ad libitum*.

2.2. Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals did not suffer at any stage of the experiment.

2.3. Chemicals

Solu-Cotef™ (hydrocortisone sodium succinate) was manufactured by Egyptian Int. Pharmaceutical Industries Co., under the license of Upjohn s.a. Puuurs-Belgium.

2.4. Dosage

The doses used in the present work were 26, 39 and 52 mg/kg body weight which was equivalent to the therapeutic doses in man (200, 300, 400 mg/adult respectively).

2.5. Treatment and cytological preparations

2.5.1. Chromosomal aberrations in mouse bone marrow

In single-dose treatment, the three tested doses of hydrocortisone were *i.p.* injected to three groups of mice for 24 h. In repeated dose treatment, mice received a daily *i.p.* treatment with one of the doses of 26, 52 mg∕kg body weight for 4, 7 and 14 days.

For slide preparation and scoring, mice were *i.p.* injected with colchicine 2–3 h before sacrificed. Chromosomal preparations from bone marrow were made according to the technique developed by Doherty *et al.*[13]. A group of five mice was used for each treatment and 100 well spread metaphases were analyzed in each animal for scoring different kinds of abnormalities. Scoring was performed under 2 500× magnification with a light microscope (Litz, Germany).

2.5.2. Sperm shape abnormalities

Groups of five mice were *i.p.* treated with hydrocortisone for 5 consecutive days at dose levels of 26, 39 and 52 mg/kg body weight. Animals were sacrificed at the 35th day after the first treatment by cervical dislocation. Sperm were prepared according to the recommended method of Aziz El-Sayed Diab KA, Hassan Elshafey and smears were stained with 1% eosin Y[14]. A total of 1 000 sperm were counted per animal, scoring different types of sperm abnormalities. Sperm preparations were examined by light microscopy at 1 000× magnification.

2.6. Dominant lethal mutation assay

Dominant lethal assay was conducted on a daily time 5 dosing regimen, a widely accepted approach in the scientific community, according to standard procedures[15].

Five mature male mice were *i.p.* injected with a daily dose of 39 mg/kg body weight hydrocortisone for 5 consecutive days. After receiving the doses of hydrocortisone, each treated male was caged with 10 virgin females for a period of 7 days. At the end of the 7 days mating period, the females were removed from the male's cages and replaced with other females. This cycle of mating continued weekly until a total of 3 weeks period was completed. The presence or absence of vaginal plugs as indicator of successful coupling was checked every day between 8 and 9 h a.m. during mating cycle. In each group, females were taken and sacrificed at the 15th day of cohabitation. Uteruses were examined for the presence of living and dead fetuses. Frequency of dominant lethal mutation was calculated according to Attia as follows[16]:

$$\text{Frequency}_{\text{DLM}} = 1 - \frac{\text{Ae}}{\text{Ac}}$$

$$\text{Percent of frequency}_{\text{DLM}} = \left[1 - \frac{\text{Ae}}{\text{Ac}} \right] \times 100$$

where Frequency$_{\text{DLM}}$ is frequency of dominant lethal mutation; Ae is living fetus per pregnant female in experimental group; Ac is Living fetus per pregnant female in control group.

2.7. Treatment and molecular preparations

2.7.1. Electrophoretic pattern of protein assay

Male mice were *i.p.* injected with hydrocortisone at doses of 13, 26 and 52 mg/kg body weight for successive 7 days. Five mice were taken from each treatment. Electrophoretic pattern of liver protein was done by one dimensional polyacrylamide gel electrophoresis (PAGE) (sodium dodecyl sulfate-PAGE) according to Petushkova and Lisitsa[17]. Bands of the liver proteins were defined according to the methods of Kurien and Scofield[18]. The concentration of protein bands (g/dL) was determined according to Noble as follows[19]:

$$\text{Absolute value of protein in each band (g/dL)} = \left[\frac{\text{Area percent of the band}}{100} \right] \times \text{Total protein}$$

2.7.2. Determination of total liver protein

The concentration of total protein of the mice groups was determined according to biuret method using Bioadwic kit. Biuret method was based on the reaction of peptide bonds with copper ions (Cu^{+2}) in alkaline solution, forming a pink to violet complex that was measured spectrophotometrically at 546 nm. The intensity of the color was proportional to the protein concentration[20].

In all experiments, negative (non-treated) and positive (endoxan) control groups were run alongside the test material.

2.8. Statistical analysis

For statistical analysis, the difference between treated groups and controls was tested with the *t*-test.

3. Results

The frequency of chromosomal aberrations induced in bone marrow cells after single and repeated *i.p.* treatment with different doses of hydrocortisone was summarized in Table 1. The percentage of the induced aberrations was not statistically significant for 24 h after single treatment with the doses of 26, 39 and 52 mg/kg body weight. The results also demonstrated that repeated treatment with the dose of 52 mg/kg body weight induced statistically significant percentage of chromosomal aberrations in bone marrow cells 7 and 14 days ($P < 0.05$, $P < 0.01$ respectively), while the dose of 26 mg/kg body weight had no effect even after repeated treatment.

All the tested doses of hydrocortisone induced an increase in the percentage of morphological sperm abnormalities in male mice compared with the negative control. Such percentage was found to be statistically significant only with the dose 52 mg/kg body weight of hydrocortisone (Table 2).

With respect to dominant lethal mutation, the results showed

Table 1

Effect of hydrocortisone in chromosomal aberrations in mice bone marrow cells.

Treatment and doses (mg/kg body weight)		Period of treatment (days)	Percent of metaphases with different types of chromosomal aberrations (%)							Abnormal metaphases			
			Gap	Break and/or fragment	Deletion	M.A	R.T	Endo-mitosis	Poly	Including gaps [(n) %]		Excluding gaps [(n) %]	
Control		–	1.60	0.40	–	–	–	0.60	0.60	16	3.20 ± 0.73	8	1.60 ± 0.24
Cyclophosphamide		1	8.00	14.60	1.60	6.00	1.20	–	–	157	31.40 ± 1.50**	117	23.40 ± 1.08**
Single treatment	26	1	1.80	0.40	0.80	–	–	1.00	0.20	21	4.20 ± 0.58	12	2.40 ± 0.40
	39	1	2.20	0.40	0.80	–	–	0.60	0.60	23	4.60 ± 0.60	12	2.40 ± 0.75
	52	1	2.20	1.00	0.40	0.20	–	0.60	0.60	25	5.00 ± 0.55	14	2.80 ± 0.49
Repeated treatment	26	4	2.20	0.60	0.40	0.20	–	0.80	0.20	22	4.40 ± 0.98	11	2.20 ± 0.98
		7	1.80	1.20	0.20	0.40	–	0.60	0.20	22	4.40 ± 0.68	13	2.60 ± 0.51
		14	1.80	1.40	0.20	0.60	–	0.40	0.20	23	4.60 ± 0.75	14	2.80 ± 0.73
	52	4	1.80	1.20	0.60	0.80	–	0.40	0.20	25	5.00 ± 0.89	16	3.20 ± 0.86
		7	2.40	1.40	0.60	1.00	–	0.60	0.20	31	6.20 ± 0.66*	19	3.80 ± 0.73*
		14	0.60	3.40	0.80	–	–	1.00	0.60	32	6.40 ± 0.73*	29	5.80 ± 0.49**

A total of 500 metaphases were examined in 5 mice per each experimental group (100 metaphases/mouse). Including gaps and excluding gaps were expressed as No. and mean ± SE. M.A: More than one type of aberration; R.T: Robertsonian translocation; Poly: Polyploidy; *: $P < 0.05$; **: $P < 0.01$ (*t*-test).

Table 2

Percentage of the different types of sperm abnormalities induced in male mice after *i.p.* treatment with different doses of hydrocortisone.

Treatment and doses (mg/kg body weight)		Head abnormalities							Tail abnormalities	Abnormal sperm [(n) %]	
		Amorphous	Without hook	Triangle	Banana	Small	Big	Forked	Coiled tail		
Control		0.80	0.28	1.14	0.04	0.02	0.02	0.02	0.36	134	2.68 ± 0.68
Cyclophosphamide		1.06	0.62	0.94	0.18	0.06	0.16	0.26	0.62	195	3.90 ± 0.37*
Hydrocortisone											
	26	1.00	0.34	1.24	0.16	0.04	–	0.06	0.62	173	3.46 ± 0.59
	39	0.88	0.58	1.18	0.54	0.04	0.04	0.04	0.54	192	3.84 ± 0.62
	52	1.26	0.64	1.30	0.12	0.12	–	0.10	0.78	216	4.32 ± 0.63*

A total of 5 000 sperm were examined in 5 mice per each experimental group. *: $P < 0.05$ (*t*-test). Abnormal sperm was expressed as No. and mean ± SE.

that the percentage of fertile mating in female mated at 1–7 and 8–14 days reduced to 50% and 60% respectively compared with 90% and 100% in control mice (Figure 2). The percentage of fertile mating in female mated at 15–21 days reached 100% in both treated and control groups. Postmortem examination of female mated with treated males at 15 days after pregnancy (the mid gestational period) revealed that the pregnant female mice usually had average of 5–11 live fetuses in both control and treated groups. The mean number of live implants per pregnant female through mating intervals of 1–7, 8–14 and 15–21 days reached 8.20, 7.83 and 8.80 respectively compared with 8.22, 8.20 and 8.80 for concurrent controls respectively (Table 3).
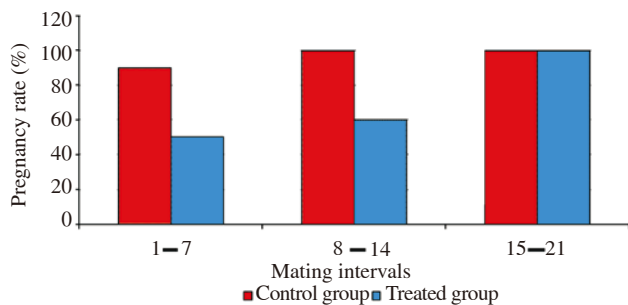

Figure 2. The percentage of pregnancy rate in different mating intervals after *i.p.* treatment of male mice with 39 mg/kg body weight of hydrocortisone.

Table 3
Induction of dominant lethal mutation in male mice after repeated *i.p.* treatment with 39 mg/kg body weight of hydrocortisone.

Groups	Mating intervals after treatment (days)	Pregnant females No.	Pregnant females %	Live implants/ pregnant female No.	Live implants/ pregnant female Mean	Dead implant	Dominant lethal Frequency	Dominant lethal %
Control	1–7	9	90	74	8.22	0.00	0.003	0.23
Treated		5	50	41	8.20	0.00		
Control	8–14	10	100	82	8.20	0.00	0.045	4.47
Treated		6	60	47	7.83	0.00		
Control	15–21	10	100	88	8.80	0.00	0.000	0.00
Treated		10	100	88	8.80	0.00		

Number of mated female in each group was 10.

These results also demonstrated insignificant decrease in the mean value of implantations per fertile female at the three time intervals. A variation in the frequency of dominant lethal between the mating intervals 1–7, 8–14 and 15–21 days post *i.p.* treatment with 39 mg/kg body weight of hydrocortisone was seen in Table 3. The percentage of dominant lethal mutations in the mating intervals 1–7 and 8–14 days reached 0.23% and 4.47% respectively. Such percentage was associated with the excess of unfertilized eggs rather than the presence of dead. While such percentage in the mating interval 15–21 days was zero value which indicated insignificant effect of hydrocortisone at this period.

The results of scanning the sodium dodecyl sulfate-PAGE of liver proteins showed that the mean number of bands reached (17.00 ± 1.09), (16.00 ± 0.96) and (16.00 ± 1.32) bands after treatment with the three tested doses of hydrocortisone respectively (Table 4). Such decrease in the total number of bands was found to

be insignificant compared with (18.00 ± 0.96) for the control. Concerning the number of bands within protein regions, the results showed insignificant difference in the number of bands for α, β, γ albumin and pre-albumin and for α-globulin after treatment with different doses of hydrocortisone compared with the control.

Table 4
Number of bands for different electrophoretic liver protein regions induced in male mice after repeated *i.p.* treatment with different doses of hydrocortisone for 7 days.

Protein groups	Control	Cyclophosphamide	Hydrocortisone doses (mg/kg body weight) 26	39	52
γ-Globulin	3.00 ± 0.32	3.00 ± 0.32	3.00 ± 0.32	2.00 ± 0.32	3.00 ± 0.45
β-Globulin	3.00 ± 0.32	2.00 ± 0.45	2.00 ± 0.32	2.00 ± 0.32	2.00 ± 0.32
Albumin	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Pre-albumin	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00
α-Globulin	10.00 ± 0.32	9.00 ± 0.32	10.00 ± 0.45	9.00 ± 0.32	9.00 ± 0.55
Total	18.00 ± 0.96	16.00 ± 1.09	17.00 ± 1.09	16.00 ± 0.96	16.00 ± 1.32

All the values were expressed as mean ± SE.

The results also showed the relative area percentage of the separated protein regions. γ-Globulin region increased with increasing the dose of hydrocortisone. Significant effect was found with the doses of 26 and 52 mg kg/body weight ($P < 0.05$ and $P < 0.01$ respectively). β-Globulin region decreased with increasing the dose of hydrocortisone and showed insignificant result with the lowest tested dose only. The results also showed that hydrocortisone had no effect on the area of albumin while significant increase was observed in the pre-albumin region.

Hydrocortisone induced a decrease in value of total liver protein. It reached (1.79 ± 0.21), (1.72 ± 0.21) and (1.66 ± 0.12) after treatment with the three tested doses respectively compared with (2.44 ± 0.26) for the control. Such percentage was found to be statistically significant with the dose 52 mg/kg body weight (Table 5 and Figure 3).
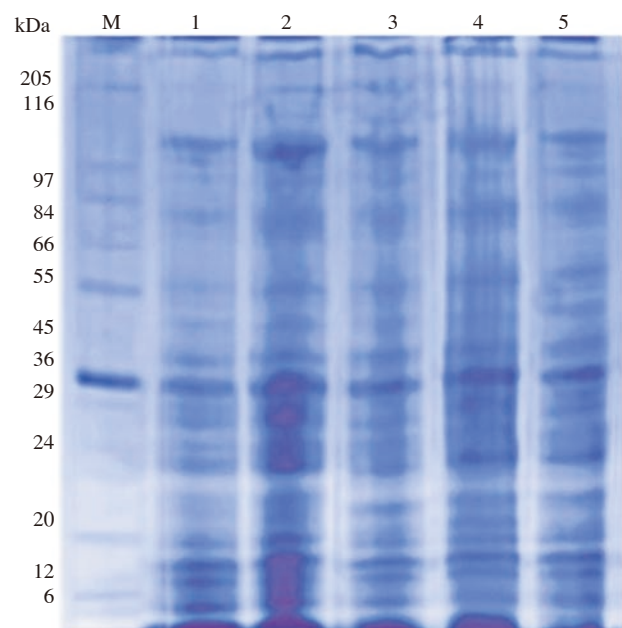

Figure 3. Electrophoretic patterns of liver proteins induced in male mice after repeated *i.p.* treatment with different doses of hydrocortisone for 7 days.
M: Marker; Lane 1: Control (non-treated); Lane 2: Positive control; Lanes 3, 4, 5: Representing the doses of 26, 39, 52 mg/kg body weight of hydrocortisone respectively.

Table 5

Area percentage and their protein concentration for different electrophoretic liver protein regions induced in male mice after repeated *i.p.* treatment with different doses of hydrocortisone for 7 days.

Protein groups	Area of protein regions (%)					Concentration of protein (g/dL)				
	Control	Cyclophosphamide	Hydrocortisone doses (mg/kg body weight)			Control	Cyclophosphamide	Hydrocortisone doses (mg/kg body weight)		
			26	39	52			26	39	52
γ-Globulin	13.01 ± 0.49	10.34 ± 0.64**	13.38 ± 0.26	15.71 ± 0.43*	17.97 ± 0.74**	0.32 ± 0.01	0.14 ± 0.01**	0.24 ± 0.02	0.27 ± 0.02	0.30 ± 0.01
β-Globulin	17.10 ± 0.49	10.12 ± 0.64**	16.24 ± 0.24	14.48 ± 0.56**	13.72 ± 0.67**	0.42 ± 0.03	0.15 ± 0.01**	0.29 ± 0.04**	0.25 ± 0.02**	0.23 ± 0.02**
Albumin	5.18 ± 0.60	4.81 ± 0.44	6.24 ± 0.58	6.12 ± 0.74	6.61 ± 0.52	0.13 ± 0.02	0.06 ± 0.04	0.11 ± 0.02	0.11 ± 0.01	0.10 ± 0.02
Pre-albumin	4.62 ± 0.33	3.98 ± 0.44	6.50 ± 0.50**	8.21 ± 0.33**	9.61 ± 0.34**	0.11 ± 0.03	0.05 ± 0.03	0.12 ± 0.01	0.14 ± 0.03	0.16 ± 0.03
α-Globulin	60.09 ± 1.28	70.21 ± 1.93**	57.58 ± 1.01**	55.47 ± 0.70**	52.00 ± 1.34**	1.47 ± 0.04	0.93 ± 0.21*	1.03 ± 0.15	0.95 ± 0.08	0.87 ± 0.07
Total protein						2.44 ± 0.26	1.33 ± 0.14**	1.79 ± 0.21	1.72 ± 0.21	1.66 ± 0.12*

*: $P < 0.05$; **: $P < 0.01$ (*t*-test).

4. Discussion

Hydrocortisone is a potent steroidal drug, used therapeutically for ameliorating sever inflammation, allergic reaction, in shock syndrome neoplasia and adrenal insufficiency[21]. For *in vivo* studies, bone marrow cytogenetic test is a useful short-term technique for elucidating the mechanism as well as to identify the substances for their clastogenic activity. The DNA is organized in chromosomes and the induced aberrations of these chromosomes can lead to cellular lethality and mutation. Our results showed insignificant percentage of chromosomal aberrations in bone marrow cells after single treatment with the tested doses. The dose of 52 mg/kg body weight induced its effect after repeated treatment for 7 and 14 days. Our data are in line with the findings of other authors who reported that certain related synthetic steroids 17α-estradiol was negative in chromosomal aberration test in Syrian hamster renal cells *in vivo*[22]. In addition, ethinyloestradiol and cyproterone were negative in mouse bone marrow cells[23]. On the contrary, hydrocortisone induced high incidence of chromosomal aberrations in cultured human lymphocytes and in mouse spermatocytes and in sister chromatid exchange in mouse bone marrow cells with a dose dependent manner[10,11,24].

The results indicated that hydrocortisone induced significant percentage of sperm abnormalities after treatment with the highest tested dose 52 mg/kg body weight ($P < 0.05$) compared with control. The effect is insignificant with the other doses 26 and 39 mg/kg body weight. These results are in agreement with other authors who reported that the administration of glucocorticoids in excess leads to cellular degeneration of epididymides, sperm density and morphometric measurements[4]. In addition, the activity of alkaline phosphatase reduced significantly while acid phosphatase, arylsulphatase and lactate dehydrogenase increased. Cornwall reported that epididymal secretions play a vital role in protecting the sperm plasma membrane, DNA and chromatin and increase the efficiency of energy utilization[25]. Therefore, some abnormalities may result from unsuitable epididymal secretions. There are two mechanisms that chemicals might indirectly affect sperm cell function and morphology: (i) exposure to chemicals could produce pituitary-hypothalamic or sex hormonal effects that in turn could affect spermatogenesis; (ii) exposure to chemicals could cause abnormalities in seminal fluid, resulting in functional or structural impairment of sperm. The manipulation of hypothalamus-pituitary-adrenal by the hormonal drug (hydrocortisone) support our results which indicat that the highest tested dose of hydrocortisone have an effect on morphological sperm abnormalities.

The present study was undertaken to validate the effect of hydrocortisone on dominant lethal mutation. Dominant lethal mutation assay is useful in identifying agents that present a risk of transmissible genetic damage. Dominant lethal mutation is genetic changes induced in the parent germ cells resulting in pre-implantation and post-implantation loss of zygotes[26]. Dominant lethal mutation test in male mice can demonstrate the response of different stages of development of male germ cells to the suspected drug. Different stages of gametogenesis may be scored for mutations depending upon the interval between treatment and fertilization.

The time schedule employed in mating scheme is similar to that reported previously for mice where 1–7 days post-injection sperm are regarded as spermatozoa of epididymis, 8–14 days post-injection sperm are testicular sperm and late spermatids and 15–21 days post-injection are regarded as sperm and early spermatids[27]. Regarding to the reprotoxicity of the tested drug, hydrocortisone treatment resulted in depression in the reproductive performance of male mice by decreasing the pregnancy rate in the mating intervals 1–7 and 8–14 days to 50% and 60%. The reduction of fertilization rate may be due to the presence of hydrocortisone in semen, which impair sperm motility. These results are consistent with Lerman *et al.* who suggested that mating capability of males is affected by the corticosteroid treatment, possibly due to reduced accessory sex organ function[28]. Marchini *et al.* reported that a 23 years old patient with testicular adrenal rest tumor received hydrocortisone was azoospermia[8]. However, azoospermia and infertility may be reversible by replacing hydrocortisone with short courses of equivalent dosage of dexamethasone.

In our results, the correlation between the induction of dominant lethal mutation and mating intervals that represent post-meiotic of spermatogenesis showed that the percentage of the induced dominant lethal mutation by hydrocortisone reached 0.23% and 4.47% in the intervals 1–7 and 8–14 days, respectively. However, such percentage reached zero in the intervals 15–21 days. Such frequency is due to equal number of live fetus in both control and treated groups. These results suggested that different stages of germ cells maturation respond differently and the effect of hydrocortisone is probably more pronounced in the interval 8–14 days, which represent late spermatide. Concerning the electrophoretic pattern of liver proteins, liver proteins are often classified on the basis of their electrophoretic mobility on gel into two major parts: albumin and globulin (α, β, γ). Albumin is simple protein soluble in water while, globulins are larger in size than albumin, comprised heterogeneous series of families of proteins with similar mobility and less soluble in pure water than albumin[29].

Our results showed that hydrocortisone is binding with proteins, and these bindings induce various alterations of different types of proteins by increasing, decreasing or fractionating protein molecules as compared with the control groups. This view is supported

by Perogamvros *et al.* who reported that about 90%–95% of hydrocortisone is bound to plasma proteins (10% to albumin and 80% to globulin)[30]. Only 5%–10% is unbound and biologically active.

Hydrocortisone induced also has significant decrease in the total protein concentration after repeated *i.p.* treatment with the dose 52 mg/kg body weight. These results indicated that hydrocortisone inhibits protein synthesis which may contribute to the cytotoxic effect of the drug. Our results also showed that all the tested doses of hydrocortisone induced significant decrease in the concentration of protein for β-globulin region. This finding considered a potential early predictor that hydrocortisone may affect liver. O'Connell *et al.* reported that the decreased levels of transferrin[31], which is a member of β-globulins family attributed to chronic liver disease and protein-losing disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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