Biochemistry and Biotechnology Research
Vol. 2(4), pp. 44-49, October 2014
ISSN: 2354-2136
Full Length Research Paper

Impact of Alnagma (dexamethasone) abused by Sudanese women as cosmetic on steroidogenesis in female Wistar rats

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Accepted 15 October, 2014

ABSTRACT

Alnagma (dexamethasone) is locally abused by Sudanese women as cosmetics for gaining weight and whiten skin. The current study aims to evaluate the impact of Alnagma on steroid hormones, steroidogenesis and thus fertility in Female Wistar Rats. Eighteen female Wistar rats weighing (106.7 ± 6.2 g) were randomly divided into three groups, group I: received high dose dexamethasone (45 µg/kg/day), group II: received low dose (15 µg/kg/day) for one month and group III received placebo as control group, body weight was measured before treatment, day 14 and at day 30, serum estradiol, estrone and testosterone were measured using competitive ELISA technique, and the ovaries were examined using microscopic histopathological assay. Alnagma treated significantly decrease body weight, and increase mean estrone, testosterone and estradiol level (p-value < 0.05) in both low and high dose treated groups. Microscopic analysis showed increase luminal steroid hormones with hyperplasia of theca and granulosa cells. The study concludes that the abuse of Alnagma increased testosterone which leads to infertility and estradiol prolonged luteal phase affecting menstrual cycle, while estrone increased susceptibility for breast, ovarian and endometrial cancers.

Keywords: Dexamethasone, Alnagma, steroids, infertility, drugs abuse, Sudan.

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INTRODUCTION

Dexamethasone is a synthetic glucocorticoid, is a potent anti-inflammatory drug with 25 to 50 times the potency of hydrocortisone and is up to 16 times as potent as prednisolone (Kara et al., 2007). It circulates mainly in the unbound form or bound to albumin and it is minimally bound to transcortin (corticosteroid-binding globulin, CBG) (Magiakou and Chrousos, 1994; Pugeat et al., 1981).

Dexamethasone used as treatment for certain types of cancers, autoimmune diseases as well as anti-inflammatory drug (Gonzalez et al., 2010). In Sudan, Alnagma (dexamethasone) is abused as cosmetic for gaining weight and whitening skin, without care of its side effects, doses, and recommended storage instructions, which sells as illegal trade (Amar et al., 2013).

Previous studies reported dexamethasone effect on the level of steroids hormones mainly estrone, estradiol and testosterone and lead to increase their levels in body, which cause the induction of aromatase enzyme (Iida et al., 1991), and also increases the estradiol, testosterone and androstenedione level in both plasma and ovaries cells (Illera et al., 2005). However, glucocorticoid receptors have been identified in granulosa cells, and glucocorticoids have been shown to exert direct effects on ovarian steroidogenesis, both *in vivo* and *in vitro* (Van Merris et al., 2007). Another mechanism by which the hypothalamic-pituitary-adrenal axis may influence reproductive function is by a direct effect of glucocorticoids on the target tissues of sex steroid production (Tsurng et al., 2001).

Increase level result from dexamethasone abuse or administration of testosterone in female induces morphological features of polycystic ovarian disease (Futterweit and Deligdisch, 1986; Spinder et al., 1989). However, these alterations have only been produced when serum levels of testosterone are elevated, higher than the levels encountered in normal females with virilizing tumors (Spinder et al., 1989). Most of the patients presenting with hyperandrogenism will be found to have polycystic ovary syndrome (PCOS) as the underlying diagnosis (Franks, 1995; Barnes, 1997). PCOS is a very heterogeneous syndrome which characterized by menstrual irregularities, usually oligomenorrhea or amenorrhea, associated with clinical and/or biochemical evidence of hyperandrogenism and hirsituism (Adams et al., 2004).

It should be noted that previous study examined the effect of dexamethasone on gonadal function which stated that, obtained results showed clear evidence that a single dose of dexamethasone may disrupt gonadal function in rats, and that possibly leads to infertility (Illera et al., 2005).

MATERIALS AND METHODS

Drugs

Drug used is dexamethasone 0.5 mg with molecular formula $C_{22}H_{29}FO_5$, molecular weight 392.5 and others ingredients (lactose monohydrate, microcrystalline cellulose, sodium starch glycolate (Type A) colloidal hydrated silica and magnesium stearate (E470b). It has shelf-life of 24 months, when stored in special conditions (store in the original package in order to protect from light), whereas the drugs used in our study were purchased from supermarket in Khartoum State are actually repackaged and the expiry date was crashed.

Experimental animals

Eighteen 2-month old female Wistar rats with average weight of 109.9 ± 5.5 g for group one, which treated with low dose (15 µg/kg/day), (104.2 ± 8.1 g) for group two treated with high dose (45 µg/kg/day) and (105.6 ± 4.9 g) for control group which received placebo. The rats were clinically healthy and housed under standard husbandry condition (30 ± 2°C, 60 to 70% relative humidity 12 h: 12 h day night cycle) and fed on rats diet. Animal experiments were designed in accordance of institutional animal ethical committee. Average body weight and weight gained were measured at day 14^{th} and 30^{th}. After 30 days of Alnagma administration blood samples were collected after rats scarified under mild chloroform anesthesia, then serum was obtained by centrifuged at 4000 rpm and stored at -20°C till used, the rats were dissected and the ovaries were collected and preserved in formalin, finally ovaries were embedded in paraffin.

Preparation of dexamethasone

Tablet was crashed (0.5 mg) into powder then mixed with 1 ml of distilled water and used as stock drug. Finally the stock suspension was homogenized by using Sonicator and diluted to appropriate doses concentration.

Measurement of estrone

The estrone level was measured by using ELISA technique briefly according to manufacturer's instruction. Solid phase competitive enzyme immunoassay was used to determine estrone by Germen Company, Reference DB52051. Samples were added to biotin microplate wells coated with specific monoclonal antibody to each hormone. After incubation antigen antibody reaction occurred, then unbounded hormones were removed by washing step, and 100 µl of streptavidin-peroxidase was added for the detection of bond biotin labeled hormone; lastly 100 µl tetramethylbenzidine substrate was added, showing a color intensity which is inversely proportional to the concentration of specific hormone in the sample. concentrations were calculated using plotted standards curve in Sunrise-TECAN (Kim et al., 1974; Speight, 1979).

Estimation of estradiol and testosterone

Estradiol and testosterone were estimated by using competitive ELISA fully automated Roche/Hitachi Cobas C-311 Systems, which automatically calculated the concentration of hormones from plotted standard carve (Kim et al., 1974; Speight, 1979).

Histological methods

The ovarian tissue was fixed in 4% formaldehyde for sample preservative, then the tissue was dehydrated by passing it through increasing concentrations of ethyl alcohol (from 0 to 100%) this step called (processing), after replacement occurs, the alcohol was replaced with xylene, which is miscible with alcohol. This step is called clearing. Then the tissue was embedded in paraffin wax which becomes harden, after which sections of 5 µm diameter were obtained by using rotary microtome. The sections were rehydrated by passing through xylene, and then decreasing strengths of alcohol (100 to 0%) and finally water, and stained with Heamoxlyin and Eosin (H and E) and then dehydrated again using xylene, finally mounted on the microscope slide then cover slip was placed on top to protect the sample and examined under microscope X40 (Fischer et al., 2008).

Statistical methods

Analysis of variance (ANOVA) with *post hoc* analysis and student t-test were employed to test whether the effect of Alnagma in the body weight and mean concentration of estradiol, estrone and testosterone were significantly difference in study groups. Also, the data obtains were analyzed with multiple comparison test (LSD) to compare between groups. All results are presented as Mean ± SD, with the level of significance set at P-value < 0.05.

RESULTS

Effect of Alnagma on body weight

In order to investigate the effect of various dose of Alnagma on body weight, the body weight was measured at day 0, 14, 30 and weight gain was calculated. Our observations showed significant decrease in body after administration of Alnagma for 30 days in both low and high doses (138 ± 36.8 and 139.7 ± 29.3 g) respectively versus control group (153.4 ± 42.3 g), with no significant difference at day 14 (Table 1). Therefore, there was

Table 1. Effect of abuse of Alnagma on body weight.

Days of measured weight/g	At day 0	At days 14	At days 30
Group 1 (high dose)	104.2 ± 8.1	128.1 ± 27.9(23.9)	139.7 ± 29.3 (11.6)**
Group 2 (low dose)	109.9 ± 5.5	131.5 ± 33.9(21.6)	138.2 ± 36.8 (6.7)**
Group 3 (control)	105.6 ± 4.9	132.6 ± 35.8(27.0)	153.4 ± 42.3 (20.8)

Results are expressed as mean ± SD, values between brackets show gained weight/g and statistical significant in comparison with control group were shown as (*) indicating p-valve ≤ 0.05 and (**) indicating P-value ≤ 0.01.

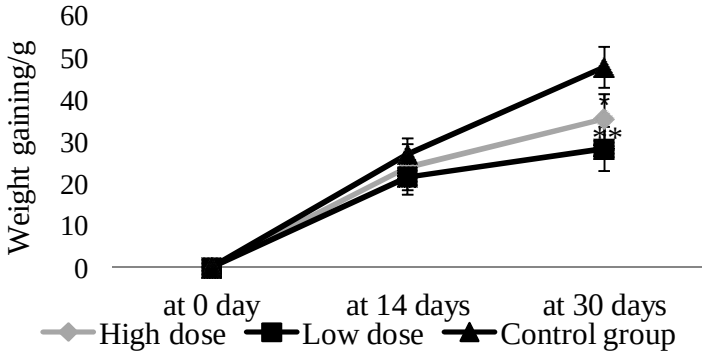

Figure 1. Plot of body weight gaining/gram in the time of study. Results are expressed as mean ± SD and statistical significant in comparison to control group were shown as (*) indicating p-value ≤ 0.05 and (**) indicating P-value ≤ 0.01.

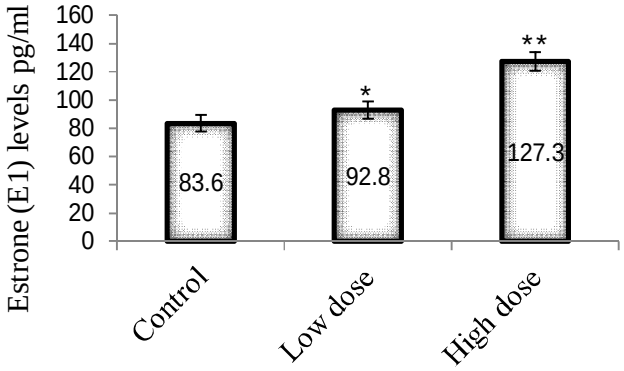

Figure 2. Mean concentration of E1 level in study groups after administration of Alnagma for one month. Results are expressed as Mean ± SD and statistical significant in comparison to control group were shown as (*) indicating p-value ≤ 0.05 and (**) indicating P-value ≤ 0.01.

significant decrease in weight gain after receiving Alnagma for one month for low and high doses (23.9 and 35.5 g) respectively, compared with control group which gained 47.8 g, also there is no significant difference at day 14 (Figure 1).

Alnagma increases estrone level

Significant increase in mean estrone levels were observed in both treated groups (low and high doses), (92.8 ± 6.1 and 127.3 ± 6.7 pg/ml) versus (83.6 ± 5.9 pg/ml) with *p*-value < 0.05 and < 0.01, respectively. Preliminary concentration of Alnagma was fixed at 15 µg/kg/day for low dose and 45 µg/kg/day for high dose (Figure 2).

Alnagma enhances the androgenesis in Wistar rats

Exposed to Alnagma for one month significantly increase

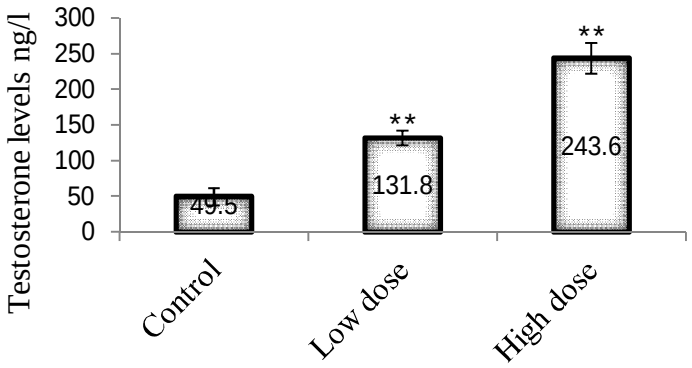

Figure 3. Mean concentration of testosterone level in study groups (low and high doses) versus control group, after administration of Alnagma for one month. Results are expressed as mean ± SD and statistical significant in comparison to control group were shown as (*) indicating p-value ≤ 0.05 and (**) indicating P-value ≤ 0.01.

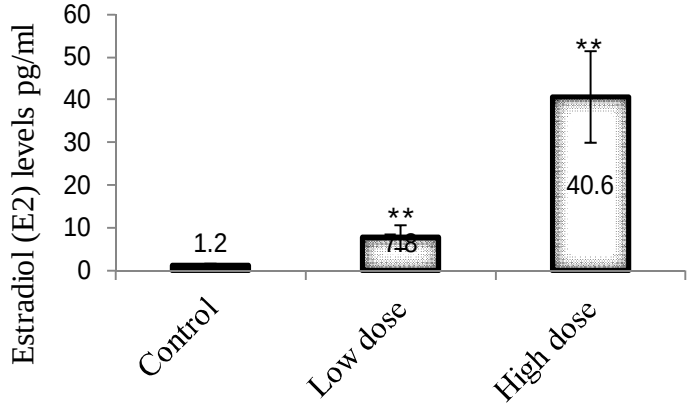

Figure 4. Mean concentration of estradiol level in study groups after administration of Alnagma for one month. Results are expressed as Mean ± SD and statistical significant in comparison to control group were shown as (*) indicating p-value ≤ 0.05 and (**) indicating P-value ≤ 0.01.

mean concentration of testosterone of both treated group (low and high doses), (131.8 ± 10.5 and 243.6 ± 21.4 ng/L) versus control group (49.5 ± 12.2 ng/L) with (p-value <0.01 and <0.01) respectively (Figure 3).

Alnagma increases estradiol level

Administration of Alnagma for one month significantly increase mean estradiol concentration of both treated groups with (high and low doses), (7.8 ± 2.8 and 40.6 ± 10.7 pg/ml) versus control group (1.2 ± 0.4 pg/ml) with (p-value <0.01 and <0.01) respectively (Figure 4).

Histological results

Treatment with low and high doses of Alnagma for 30 days showed the presence of fatty luminal (steroid precursors and hormones), and hyperplasia of theca and granulosa cells of ovarian treated groups sections, when compared with control group which show normal histological features (Figure 5).

DISCUSSIONS

Interestingly, the present study demonstrates the abuse of Alnagma (dexamethasone) by Sudanese women as cosmetic, regardless of recipes, doses and storage instructions recommended by the manufacturer. In contrast, previous studies evaluated the side effects of dexamethasone when used as normal treatment.

In this study, abuse of Alnagma for one month was shown to significantly decrease body weight in both rats treated with low and high doses. Since dexamethasone

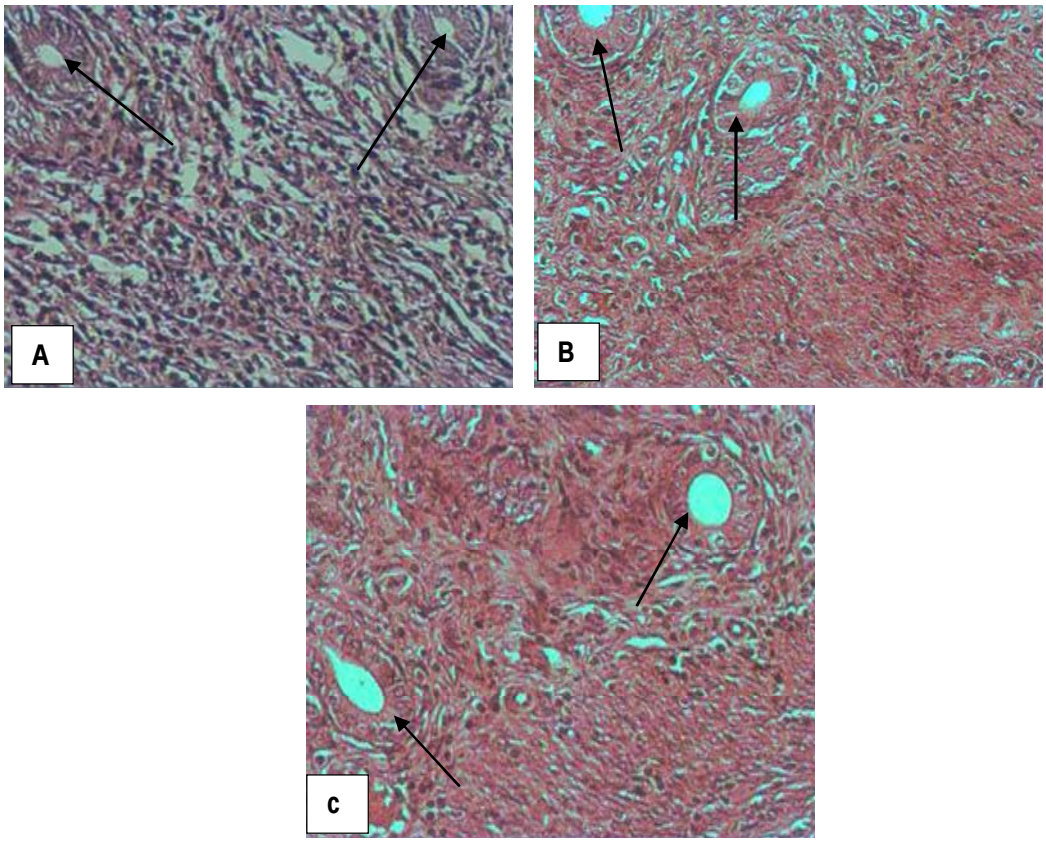

Figure 5. Histological sections of ovaries stained with (H&E), examined under microscope (X40). (A) Representative untreated normal section considered as control group compared with section (B) which treated with low dose (15 µg/kg/day), shows moderate increased luminal fats (steroid hormones), and hyperplasia in both granulosa and theca cells followed by section (C) which received high dose (45 µg/kg/day), shows marked increased luminal fats (steroid hormones), and hyperplasia in both granulosa and theca cells seen by top of the arrows.

affect lipids metabolism and carbohydrate, especially peripheral lipolysis and gluconeogenesis, in addition to fact that administration of dexamethasone increase leptin secretion from adipose tissues and thus causes sensation of satiety and increase energy expenditure, all these may explain weight loss in subject who was administrating Alnagma for long time (De Vos et al., 1995; Amar et al., 2013). The appearance of moon face, one of the consequences of dexamethasone abused, which is first observed, may explain falsely assumed gaining of weight for those females abusing this drugs (Amar et al., 2013). Treatment with Alnagma for one month significantly increased testosterone level in both low and high dose groups, which suggested that increased testosterone concentration in women (Hyperandrogenism) is an indicator of the presence of gradual symptoms like hirsutism, virlization, oligomenorrhea and even amenorrhea which may lead to infertility in female as a result of abuse of Alnagma for long time. In fact that, the treatment with dexamethasone for 10 days in rats does not affect the fertility and the development of the lungs, liver and kidneys of neonates, while the administration during 15 days leads to a high maternal mortality (Moraes et al., 2008), therefore administration effect of Alnagma depend on variations of duration and doses used. Since in Sudan, most people that abused Alnagma are teenagers, this may exaggerate the effect of this drug.

This study shows a differential effect of Alnagma abuse as cosmetic on E1 and E2. Overall one month exposure to Alnagma significantly increase both E1 and E2 in treated rats with low and high doses, thus confirming by previous findings report, dexamethasone effect on the level of steroids hormones mainly estrone, estradiol and testosterone and lead to increase of their levels in body by the induction of aromatase enzyme (Iida et al., 1991). As E1 is a risk factor for breast, ovarian and endometrial cancers and E2 prolonged the luteal phase and affecting menstrual cycle, our study suggests that the abuse of Alnagma increases the risk of cancers and infertility among Sudanese women. Dexamethasone acts to repress the LH-induced expression of StAR protein and progesterone production by a glucocorticoid receptor-mediated mechanism. These observations raise the

possibility that glucocorticoids *in vivo* may act directly on the ovary to modulate follicular steroidogenesis (Huang and Shirley Li, 2001; Tsurng et al., 2001; Van Merris et al., 2007). The finding of our study further observes that administration of dexamethasone induces the synthesis of all steroids hormones (E1, E2 and testosterone) both *in vitro* and *in vivo*.

The histological analysis reinforced our finding by previous fact that glucocorticoid receptors have been identified in granulosa cells and glucocorticoids have been shown to exert direct effects on ovarian steroidogenesis, both *in vivo* and *in vitro* (Tsurng et al., 2001). Our results show hyperplasia of both granulosa and theca cells with fatty luminal (with steroids precursors and hormones), confirming increase steroidogenesis in both theca and granulosa of ovarian cells. In fact, the presenting features of women with high glucocorticoids (Cushing's syndrome) is similar to those observed in patients with the polycystic ovarian syndrome (PCOS): obesity, oligomenorrhea or amenorrhea, hirsutism, low serum SHBG levels, increased circulating androgen levels, and an exaggerated gonadotropin response to GnRH (McKenna and Cunningham, 1995). This powered our finding that abuse of Alnagma lead to infertility in female administered this drug for long time.

Conclusion

The study concludes that abuse of Alnagma by Sudanese women for long time significantly increase testosterone and estradiol levels, which subsequently leads to hirsutism, amenorrhea, prolonged luteal phase and affects menstrual cycle, thus possibly causing infertility. While elevation in estrone level may increase risk of breast, ovarian and endometrial cancers, since paracrine function of estrone enhance proliferation and repress apoptosis mechanisms.

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Citation: Ismail AM, Ahmed AA, Mohammed HS, Alsamany EB, 2014. Impact of Alnagma (dexamethasone) abused by Sudanese women as cosmetic on steroidogenesis in female Wistar rats. Biochem Biotechnol Res, 2(4): 44-49.