Effects of Various Levels of Oral Doses Dexamethasone (Al-nagma) Abused as Cosmetic by Sudanese Women on Wistar Rats

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Dexamethasone (locally known as Al-Nagma) is used as an anti-inflammatory drug for different types of inflammatory diseases. Sudanese women abuse this drug as cosmetic for gaining weight and whiten skin, without care of the side effects. The present study aims to evaluate the toxicity of low (60 μg/kg/day) and high doses (180 μg/kg/day) of Dexamethasone on kidney and liver of Wistar rats and to estimate the effect of abuse in normal individual. The rats were randomly classified into three groups, each group contain six rats. Group one (control) received distilled water. Group two treated with low dose (60 μg/kg/day) and group three treated with high dose (180 μg/kg/day). To achieve the objective of this study, the body weight was measured before and after treatment, sodium, potassium, glyceded hemoglobin and other Haematological parameters were also measured. Dexamethasone significantly decreased the body weight of Wistar rats treated with low and high dose (22.8±7.4, 19.9±8.3), respectively compared with control group (32.7±9). In addition, there is a significant difference in sodium and HbA1c levels in both groups treated with the dexamethasone. In contrast potassium significantly decreased in both groups when compared with control group. Also pathological changes in fatal organs demonstrated as lesions in liver and kidneys, fatty cytoplasmic vaculation and necrosis of the hepatocytes, glomerular alteration, packing, dilatation, fatty change and necrosis. This study concluded that, Dexamethasone abuse decreased the body weight, rather than believed by Sudanese women. It causes hyperglycemia and hypertension affecting renal function.

Key words: Dexamethasone, alnagma, cosmetic, body weight, histopathology, hypertension
INTRODUCTION

Cosmetics considered being a branch of personal care products including skin lotions, topical creams and powders; it believed to have therapeutic activity in enhancing body appearance, but the excessive use of it or judge on it as dietary supplement may not exert their designed action (Knight, 2005).

The manufacture of cosmetics is fast growing sector due to the rapid demand by consumer. This industry were originated in the early 20th century in restricted areas but the distribution and sale of cosmetics now is extend among a wide range of different businesses (Reed, 2007; Knight, 2005).

The natural cosmetics common in Sudan used traditionally include Alkare oil (mama oil) has a great benefit because it contains animal extracts and extracts of bees (wax). Plant extracts is pink, it is beneficial for lengthening hair and smoothing skin texture. Other natural oils used in the Sudan for example sesame oil, for skin softens and prevent damage of cells and almond oil help lighten color and parsley oil significance for the skin and give ronq, freshness and black seed oil dries fat. Prevent the emergence of grain oily on skin, olive oil sun visor and prevent oxidative stress, prevent access to harmful x-rays of the skin, another examples are Aldokhan and Aldelka (personal observation).

Cosmetics could be well thought-out as products that clean, beautifying and promoting attractiveness with no effects on structure of body excluding the soaps as it stated by FDA (Lewis, 1998).

Chemical cosmetic used in form of creams or tablets such as Dexamethasone (Fig. 1) (De Wasch et al., 2001) locally known as Al-Nagma- is a corticosteroid, similar to a natural hormone produced by adrenal glands. It is used to substitute the low amount or the complete absence of the hormone. Corticosteroid used to treat certain types of inflammatory diseases such as arthritis, thyroid, colitis, severe allergies, and asthma. It is also documented to be used as treatment of certain types of cancer (Bloom et al., 2001), although it is believed that dexamethasone play a role in causing malignances (Siddiqui and Qazi, 2012).

It may be given to women at risk at delivering prematurely in order to promote maturation of the fetus lungs, this has been associated with low birth weight, although not with increased rate at neonatal death (Bloom et al., 2001).

Periactin and cortisol are used by Sudanese women as cosmetics that increase the weight. Periactin is used to relieve the allergic symptoms, itch and skin problems, it is also used to treat anaphylactic reactions migraines and other headaches, the action of periactin is to acts as an antihistamine by blocking the action of histamine, serotonin and other inflammatory products which causes symptoms of allergy or itchiness caused by unwanted effect of histamine (Welsh and Ede, 2013; Rossi, 2007). Cortisol is used to treat diseases such as Addison's disease, inflammatory and rheumatoid diseases and allergies. Low-potency hydrocortisone is now available in different concentrations under several commercial names; it used to treat dermatitis, rashes, eczema and skin irritations resulting from different causes. It believe to be acting by discontinue the secretion of inflammatory agents by the body, it also can activates anti stress and other diseases related to glucocorticoid deficiency (Scott, 2011; De Wasch et al., 2001; Pray, 2009).

**Sodium and potassium**: These minerals are responsible for development of electrical potentials at the cell membranes and for the maintenance of proper osmotic equilibrium between the extracellular and intracellular fluids (Murray et al., 2006).

This is the first trial done in the Sudan to study the effect of dexamethasone (Alnagma) as cosmetic and complication result from abuse of this drug by Sudanese women on Wistar rats. Emphasis was placed on comparative changes in growth, clinical abnormalities, lesion and alterations in haematological, sodium, potassium and HbA1c (%) of treated rats.

Therefore, the current article aimed to investigate the effect of mishandling of Dexamethasone as cosmetics by Sudanese women and its actual effect(s) on their bodies.

**MATERIALS AND METHODS**

**Drug**: Dexamethasone (Alnagma) tablets were obtained from super market in Khartoum, Sudan (April, 2012).

**Animals**: Eighteen-2-month old female Wistar rats with average body weight of 160±5 g were used.

The rats were clinically healthy and housed within the premises of Faculty of Science and Technology, Al-Neelain University, Khartoum. Animal house under standard husbandry conditions (30±2°C, 60-70% relative
humidity and 12 h: 12 h day-night cycle) and fed on the rat diet (flour 55.6%, meat 35%, edible oil 7.5%, sodium chloride 1.2% and vitamins and minerals 0.7) and water provided ad libitum. Animal experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee.

Experimental design: The rats were allotted at random into three groups; each of six rats. Group 1 (control) received distilled water. Groups 2 and 3 were given Dexamethasone drug at 60 and 180 µg/kg/day via oral route, respectively. All rats were dosed their designated experimental oral dose through catheter tube for one month. Clinical sings, average body weight and body weight gain for each group were recorded on day 0, 10th, 20th and day 30th of the treatment. Blood samples for hematological and serobiochemical parameters and tissue samples for histopathology were taken at day 30th after sacrificing six animals from each group under mild chloroform anesthesia. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidneys, immediately fixed in 10% neutral buffered formalin and processed for histopathology.

Methods
Preparation of the cosmetic dose: From Dexamethasone tablet, 0.5 mg was weighed accurately and crushed into powder, then dissolved in 3 mL of distilled water and used as stock drug.

Haematological methods: These techniques were performed according to an Automated Haematology Analyzer (Human GmbH, max-planck-Ring 21, D-65205 wiesbaden, Germany). The parameters measured were hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), platelets count, White Blood Cells (WBCs), differential WBCs counts and erythrocytes indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelets (PLT).

Sodium and potassium estimation: Internal standard (lithium chloride) used as diluents for estimation of sodium and potassium, then 5 mL was added in 3 tubes for sample, standard and blank, 50 µL of sample and standard added to the specific tube, blank used for adjustment of zero and standard for adjustment of sodium 140 mmol L⁻¹ and potassium 5 mmol L⁻¹, samples were measured directly on the flame photometer.

Glycated hemoglobin (HbA1c): Glycated HbA1C was detected using fast Ion-Exchange Resin Separation High performance liquid chromatography method (Hoelzel, 2004).

Pathological methods: Necropsy was conducted to identify gross lesion, after anesthesia, the rats were dissected. Specimens of the liver and kidneys were collected and immediately fixed in 10% neutral buffered formalin. The organs were embedded in paraffin wax, sectioned at 5 µm, diameter and stained routinely with hematoxylin and eosin (H and E) (Andrew et al., 2008).

Statistical analysis: The values have been analyzed by one way analysis of variance (ANOVA) followed by Duncan's simple T-test. The significance of differences between means (Mean±SE) was compared at each for all groups. p<0.05 was considered statistically significant (Snedecor and Cochran, 1989).

RESULTS

The effects of treatments of Dexamethasone on body weight of treated rats were presented in Table 1. After 30 days in all groups compared to the control (Group 1) there was a significant decrease in the weight of the treated groups (2 and 3). No death among the rats occurred.

Hematological changes: Hematological changes for rats given daily oral doses of Dexamethasone at 60 µg/kg/day (group 2) and 180 µg/kg/day (group 3) for one month are presented in Table 2. After 30 days, the value of Hb in group 3 was higher (p<0.05) than control and the other groups. PCV in groups 2 and 3 and PLT in group 3 were higher (p<0.05) than control. WBCs in group 2 and MCHC in group 3 were lower (p<0.05) than control.

Sodium, Potassium and HbA1c: Sodium, Potassium and HbA1c changes of rats treated with 60 and 180 µg/kg/day of Dexamethasone were shown in Fig. 2-4. After 30 days

| Table 1: Body weight changed in Wistar rats given dexamethasone orally for 30 days |
|-----------------|----------------|----------------|----------------|----------------|----------------|
| Groups          | Body weight (g) 0 day | Body weight (g) 10 days | Body weight (g) 20 days | Body weight (g) 30 days |
| Control         | 159.4±6.8           | 173.0±4.6 (13.6) | 203.0±7.2 (30.4) | 235.0±9.6 (32.4) |
| Dexamethasone (60 µg/kg/day) | 162.0±6.0       | 174.0±7.1 (12.6) | 186.6±5.3 (12.6) | 206.4±7.4 (22.8) |
| Dexamethasone (180 µg/kg/day) | 160.4±5.8       | 170.4±6.8(10.4)  | 182.9±5.2 (12.5) | 202.8±8.3 (19.9) |

Values are expressed as Mean±SED, Values in brackets show the increase in weight.
Table 2: Haematological parameters of rats given dexamethasone orally for 30 days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (normal diet)</th>
<th>Dexamethasone (60 µg/kg/day)</th>
<th>Dexamethasone (180 µg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g dL⁻¹)</td>
<td>12.4±0.9</td>
<td>12.5±0.85*</td>
<td>14.1±1.30*</td>
</tr>
<tr>
<td>RBCs (X10⁶ mm⁻³)</td>
<td>6.7±0.6</td>
<td>7.2±0.57*</td>
<td>7.6±0.70*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>38.4±2.04</td>
<td>42.8±2.90*</td>
<td>44.8±4.50*</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>56.1±6.1</td>
<td>59.8±1.00*</td>
<td>58.9±2.40*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.7±0.8</td>
<td>18.9±0.30*</td>
<td>18.5±2.50*</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.6±12.7</td>
<td>41.7±4.40*</td>
<td>31.4±0.60*</td>
</tr>
<tr>
<td>WBCs (X10³ mm⁻³)</td>
<td>7.6±2.06</td>
<td>5.3±1.00*</td>
<td>8.5±5.90*</td>
</tr>
<tr>
<td>PLT (X10³ mm⁻³)</td>
<td>980±426.9</td>
<td>895.2±20.5*</td>
<td>1044.2±12.8*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SE; ns: Not significant; *Significant at p<0.05

Fig. 2: Effects of various levels of oral doses of dexamethasone on sodium level, showed the relationship between the concentration of sodium mmol/L in control group, low dose (60 µg kg⁻¹) and high dose (180 µg kg⁻¹) group.

Fig. 3: Effects of various levels of oral doses of dexamethasone on potassium level, showed the relationship between the concentration of potassium mmol/L in control group, low dose (60 µg kg⁻¹) and high dose (180 µg kg⁻¹) groups of the treatment. Sodium mmol/L concentration were higher (p<0.05) in groups 2 and 3 (Fig. 2) than control (group 1). The concentration of potassium mmol/L in group 3 was lower (p<0.05) (Fig. 3) and concentration of HbA1c % in group 3 was higher (p<0.05) (Fig. 4) than control and group 2.

Fig. 4: Effects of various levels of oral doses of dexamethasone on HbA1c, showed the relationship between the concentration of HbA1c% in control, 60 and 180 µg kg⁻¹ groups.

Fig. 5: Liver of the rats received daily oral doses of dexamethasone at 180 µg/kg/day for 30 days, showing cytoplasmic fatty vacuolation and necrosis of the hepatocyte (H and E X100).

**Pathological changes:** After 30 days of treatment of the daily oral doses of dexamethasone there were lesions in liver and kidney in all treated groups, in Group 3 there was a cytoplasmatic fatty vacuolation and necrosis of the hepatocytes (Fig. 5), in Group 2, there was Glomerular alteration, packing, fatty change and necrosis (Fig. 6), severe fatty change and dilation in cortex (Fig. 7).
both protocols for 30 days induced less body weight gain in treated rats without affecting mean daily food intake. Since such an effect may be explained by an increase in caloric expenditure, possibly due to activation of the sympathetic nervous system by sucrose ingestion (Franco-Colin et al., 2006).

According to results represented in Table 2, there is a decrease in WBCs count in group 2 (p<0.05), compared with control. Richard and Denise stated; corticosteroids inhibit cytokine production by macrophages and T cell and thus decrease the normal proliferation of B cell. They may also act directly on B cell to inhibit antibody synthesis and in high concentration may even kill B cell. Dexamethasone suppresses cellular immunity by killing T cell (Goodman, 1993). The anti-inflammatory properties of increasing the relative percentages of circulating polymorphonuclear leucocytes, although lymphocyte levels are decreased (Laker, 1996).

This study revealed that, there is an increase in RBCs count when low dose and high dose were compared with control group. Increased RBCs count contributed to increased viscosity of blood result in hypertension.

Figure 2 showed that, there is significant increase in sodium concentration (mmol/l) when low dose was compared with control group and there is no significant change when the control was compared with the high dose group.

Graph 3 showed that, there is decrease in potassium (mmol L^{-1}), in low dose and high dose when compared with control. According to the previous study which showed that, Corticosteroids have weak mineralo-corticoid properties, increasing renal tubular sodium re-absorption and increasing potassium excretion. They antagonize the effect of vasopressin on the renal tubule, enhancing water excretion (Laker, 1996).

Side effects like moon face and truncal obesity with stretch marks, severe acne due to high concentration of cortisol prevent melanin synthesis, muscle weakness because breakdown of lactate, protein and lipolysis. Imbalance electrolyte, make Na high in extracellular and move from high concentration to low concentration that make water motion to high concentration that made edema, hypertension. The present study showed that Corticosteroids act on the immune system by blocking the production of substances that trigger allergic and inflammatory actions, such as prostaglandins. However, they also impede the function of white blood cells which destroy foreign bodies and help keep the immune system functioning properly. The interference with white blood cell function yields a side effect of increased susceptibility to infection (Sobel and Klein, 1992).
Other study found that, Gluco-corticoid hypertension was induced by oral administration of Dexamethasone (DX) in male Wistar rats. The mechanism of hypertension was studied by observing the changes in plasma renin activity, urinary excretion of Prostaglandin E2 (PGE2) and the pressor response to norepinephrine. Following administration of DX (0.1 mg day⁻¹), the blood pressure began to rise within 3 days and reached a plateau on the 5th day (from 108±2 to 162±7 mmHg, Mean±SE) (Handa et al., 1984).

Graph 3, obviously, showed that after administration of Dexamethasone for one month, HbA1c significantly elevated when the low dose was compared with high dose and both with control this have been confirmed by a study showed that, administration of Dexamethasone led to reversible insulin resistance and impaired glucose tolerance in healthy individuals. It has even been proposed that a low increase in insulin secretion after a Dexamethasone challenge may allow identification of individuals at risk of subsequently developing type 2 diabetes. Dexamethasone-induced impaired glucose tolerance is also known to involve both extrahepatic insulin resistance and increased endogenous glucose production. Glucose-induced insulin secretion, insulin-mediated glucose disposal and glucose production were measured during a two-step hyperglycemic clamp procedure. This allowed assessment of the extent to which hyper-insulinemia secondary to Dexamethasone (Nicolet al., 2003).

According to the liver and kidney histopathology results, the result of the present study has revealed experimental evidence that, use of Dexamethasone causes slight fatty liver cells and necrosis when used by high dose and with no changes in liver cells when used with low dose. In contrast with severe fatty change, dilatation and packing of glomerulus in kidney section, when compared with control group and the severity of the changes proportional to the dose of Dexamethasone (low in low dose and high in high dose). As HalilEken and others stated that, the low-dose corticosteroid provides an obstruction of bile flow through the extra-hepatic biliary system and results in development of oxidant injury, hepato-fibrosis, biliary cirrhosis and portal hypertension (Eken et al., 2006).

Other study found that high levels of maternal Glucocorticoid impair renal development and has led to arterial hypertension in offspring. Even though renal mass eventually normalizes glomerular damage as well as sodium retention occurs and these factors may contribute to the development of hypertension (Celsi et al., 1998).

These potent direct effects on podocyte illustrate a novel mode of action of Glucocorticoid and suggest potential new therapeutic strategies for glomerular disease (Xing et al., 2006).

CONCLUSION

This study provided experimental evidence that Dexamethasone decreases body weight rather than believed by Sudanese women whom they think it causes weight gain and whitening skin. The appearance of moon face resulted from use of this drug which is first observed, falsely though as gain of weight. Using of Alnagm for long time causes purple striae which occur on abdomen and skin as well as thin skin.

Dexamethasone prevents melanin synthesis which is essential for the protection of skin from UV light in which it increases susceptibility of skin cancer. As dexamethasone act as antagonist of insulin and have Aldosterone functions, it causes, hyperglycemia and hypertension.

REFERENCES


