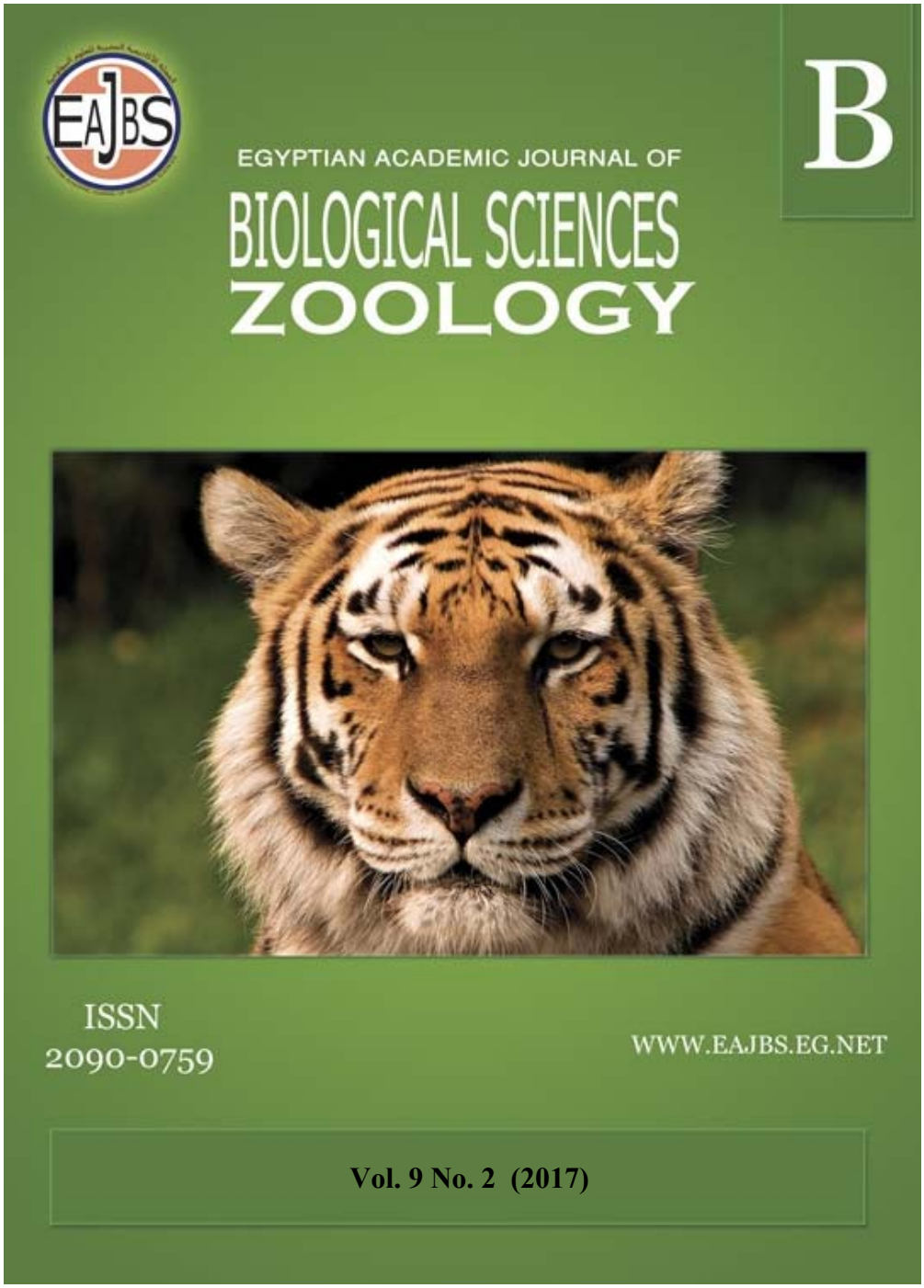

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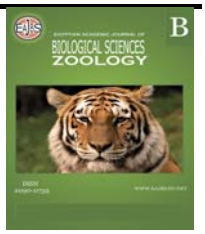

Effects of Dehydroepiandrosterone on Gross Morphology and Behavior of Perimenopausal Rats

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ABSTRACT

The androgenic adrenal steroid dehydroepiandrosterone (DHEA) has many biological activities. It decreases with age, so exogenous DHEA supplementation is being commonly used especially in the perimenopausal women. The objective of the study was to determine the side effects of DHEA on perimenopausal rat's. DHEA was administered at 3 doses: 50, 75, and 150 mg/kg for two consecutive months. The results showed that DHEA administration decreases the body weight gain, causes dorsal fur loss, with many behavioral changes. The observations increased in a dose-dependent manner. Based on the current data, high doses of DHEA have adverse effects on body weight, fur density, and behavior of perimenopausal rats.

INTRODUCTION

DHEA is the most abundant steroids in humans. It is produced from cholesterol mainly in the adrenals but also by the testis, ovaries, skin, and brain (Zouboulis et al., 2007; Panjari & Davis, 2010; Davis et al., 2011). The serum levels of DHEA decline markedly with advancing age (Krysiak et al., 2008; Goel & Cappola, 2011; Jayaprakasan et al., 2014). Recently, DHEA has been marketed as "the drug of youth", with reported benefits to physical and physiological well-being (Arlt, 2004; Olech & Merrill, 2005; Panjari & Davis, 2007; Ellington et al., 2011).

However, various side effects, including acne, hair loss, hirsutism and deepening of voice, have been reported with the use of physiological doses of DHEA in women (Legrain et al., 2000; van Vollenhoven, 2002; Sadock & Sadock, 2008; Gleicher and Barad, 2011; Traish et al., 2011; Yakin and Urman, 2011). The current study is designed to detect the potential side effects of therapeutic doses of DHEA among perimenopausal rats

MATERIALS AND METHODS

Drugs and chemicals:

Dehydroepiandrosterone (DHEA®) capsules were purchased from Puritan's Pride Company, Inc. (USA). Each DHEA capsule is made from active 100 mg of DHEA powder encapsulated in white capsules, authorised by the Medicines and Healthcare

products Regulatory Agency (MHRA). The capsules were opened and the powder was suspended in sesame oil purchased from El Captin Pharmaceutical Company (Cairo, Egypt). All other chemicals were obtained from El-Nasr pharmaceutical chemicals co. (Cairo, Egypt).

Experimental animals:

Healthy young adult female albino rats (4 to 5-months-old) weighing 120-160 g, and perimenopausal rats (10 to12-months-old) weighing 200-250 g, were obtained from the Medical Research Center at the Faculty of Medicine, Ain Shams University (Cairo, Egypt). The animals were given free access to water and standard rat chow, and were allowed 2 weeks to acclimatize before the start of any experimental procedure.

Experimental protocol:

Rats were divided into five groups, each of 5 rats, as follows:

- Group I: Control young adult rats (4 to 5-month-old).
- Group II: Control perimenopause rats (10 to12-month-old).
- Group III: Perimenopause rats orally treated with DHEA at a dose of 50 mg/kg b.wt.
- Group IV perimenopause rats orally treated with DHEA at a dose of 75 mg/kg b.wt.
- Group V perimenopause rats orally treated with DHEA at a dose of 150 mg/kg b.wt.

DHEA was given daily for 2 months. Control animals were given the vehicle in a similar manner.

Statistical analysis:

Numerical data are reported as mean values and standard deviation. GraphPad Prism (version 5.0, GraphPad software, San Diego, CA, USA) was used to conduct all statistical analysis. Data were analyzed statistically using One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups. $P<0{\cdot}05$ was regarded as statistically significant.

RESULTS

Fur examination:

At the end of the experiment, the fur of perimenopausal rats was lighter than those of the young adult rats. Treating perimenopausal rats with DHEA resulted in fur loss in a dose-related manner. In rats treated with the low dose of DHEA, the fur density was almost similar to the corresponding perimenopausal control rats. On the other hand, perimenopausal rats treated with the moderate or high dose of DHEA showed moderately hair loss compared to the perimenopausal control rats (Fig. 1).

Body weight change:

At the end the experiment, there was increase in the body weight in young and perimenopausal control groups. The increase in the final body weight of perimenopausal rats was significantly more than noted in that of young adult rats. The difference between final and initial body weights was decreased in all perimenopausal-treated rats in comparison with that of perimenopausal control rats. Moreover, body weight gain of DHEA-treated rats was decreased in a positive relation to the dose concentrations as shown in Table 1.

Table 1: Effect of DHEA on the body weight of perimenopausal rats

Body weight change (gm)	Young adult rats		Perimenopausal rats							
			Control		L. D. DHEA		M. D. DHEA		H. D. DHEA	
	Initial wt.	Final wt.	Initial wt.	Final wt.	Initial wt.	Final wt.	Initial wt.	Final wt.	Initial wt.	Final wt.
No. of rats (5)	191	210	316	341	320	346	323	343	313	327
	175	190	302	329	253	270	345	360	302	318
	173	189	293	320	241	263	325	345	377	400
	197	218	253	283	304	319	316	335	295	305
	204	232	301	329	218	235	314	327	316	327
Mean of initial & final weights	188	207.8	293	320.4	267	286.6	324.6	342	320.6	335.4
Mean of weight change	19.8±2.3		27.4±0.8**		19.6±2.1††		17.4±1.4†††		14.8±2.3†††	

Values are expressed as means ± SEM.

* indicates the significant difference of perimenopausal control group vs. the corresponding young control group, **P<0.01.

† indicates the significant difference of DHEA-treated groups *vs.* the perimenopausal control group, ††p<0.01, †††p<0.001.

Behavioral changes

There was no treatment-related death in any of treated rats along the experiment duration. In the beginning of the experiment, there was decrease in the food intake in perimenopausal-treated rats compared to their corresponding control ones, which was slightly improved by time. The activity and locomotion were obvious in young adult rats, but these behaviors in perimenopausal rats were relatively less than observed in the young rats (Table 2).

Young and perimenopausal rats can walk and interact freely in the cage. While, perimenopausal treated rats with different doses of DHEA had latency and frequency of the agonistic behaviors biting, sideway threats, upright postures, and aggressive grooming till the end of the first month of the experiment, then by time these behavioral changes were decreased leading to depression-like behavior, which was represented by decrease in food intake or body gain, locomotion, and activity. The decrease in locomotion, activity, and depression was more obvious in the end of the experiment and especially in high dose-treated rats compared to other doses-treated and control rats (Table 2).

Table 2: Effect of DHEA on behavioral parameters of perimenopausal rats

Behavioral parameters	Young adult rats	Perimenopausal rats			
		Control	L. D. DHEA	M. D. DHEA	H. D. DHEA
Food intake	3±0.0	3±0.0	2.8±0.18	2.6±0.22	2.2±0.18††
Locomotion	3±0.0	2.8±0.18	2.6±0.22	2.2±0.18	1.8±0.18††
Activity	3±0.0	2.8±0.18	2.4±0.22	2.2±0.18	1.6±0.22††
Depression	0±0.0	0.2±0.0	0.4±0.22	0.6±0.22†	1.2±0.18†††

Values are expressed as means ± SEM.
† indicates significant difference of DHEA-treated groups *vs.* the perimenopausal control group, †p<0.05, ††p<0.01, †††p<0.001.

DISCUSSION

The present experiment was designed to assess the potential side effects of therapeutic doses of DHEA in perimenopausal rats. The present results showed that all rats gained weight during the course of the experiment. The body weight gain of perimenopausal rats was significantly more than that noted in young adult rats. However, Yin *et al*. (2015) reported that the change in body weight between the young and old rats is insignificant. On the other hand, DHEA-treated perimenopausal rats gained less body weight than observed in non-treated perimenopausal rats. The decrease in body weight gain was dose-dependent. This observation in in line with previous studies, which reported that DHEA administration reduce body weight gain (Shepherd and Cleary, 1984; Cleary and Zisk, 1986; Cleary, 1991; Svec *et al*., 1995; Abadie *et al*., 2000; Zhao *et al*., 2007; Ma *et al*., 2008; Hakkak *et al*., 2010 & 2017). The decrease in body weight gain may be related to the decrease in food intake (Weindruch *et al*., 1984), or related to the decrease the abdominal or body fats (Cleary and Zisk, 1986; Villareal and Holloszy, 2004; Ma *et al*., 2008). However, other studies reported that DHEA treatment causes a decrease in the body weight (Tagliaferro *et al*., 1986; Mohan *et al*., 1990; Yamada *et al*., 1991; Han *et al*., 1998; Ng *et al*., 1999; Richards *et al*., 2000; Kopplow *et al*., 2005; de Heredia *et al.*, 2007; Caldwell *et al*., 2014; Chen *et al*., 2015), and other studies found no effect for DHEA on body weight (Lea-Currie *et al*., 1997 a, b; Aragno *et al*., 2004; Sander *et al*., 2006; Miyazaki *et al*., 2016). The cause for the observed difference in body weight could be attributted to different DHEA regimes (de Heredia, *et al*., 2007), or due to different routes of DHEA administration that might lead to differences in circulating DHEA levels and differences in body weight (Miyazaki *et al*., 2016).

There are many studies reported various side effects for DHEA, including acne, hair loss, hirsutism and deepening of voice, have been reported with the use of physiological doses of DHEA in women (Legrain *et al*., 2000; van Vollenhoven, 2002; Sadock & Sadock, 2008; Gleicher and Barad, 2011; Traish *et al*., 2011; Yakin and Urman, 2011). Facial hair growth and voice changes may be irreversible (Sadock & Sadock, 2008).

In the present study, DHEA administration exhibited hair loss as a side effect. This side effect was in a positive relation to the studied doses. This observation is in agreement with other clinical studies (Barad *et al*., 2007; (Sadock and Sadock, 2008; Gleiccher *et al*., 2009 & 2010; Gleicher and Barad, 2011; Yakin and Urman, 2011).

The hair loss effect of DHEA is primarily related to androgen effects as reported by Gleiccher et al. (2009) and Gleicher and Barad (2011).

The present experiment also assessed the effect of DHEA administration on behavioral changes in perimenopausal rats. DHEA-treated animals showed depression-like behavior, manifested by decreased activity and food intake. DHEA is known to influence a variety of behaviors, including cognition and mood (Maninger *et al*., 2009). The decrease in food intake is in line with the result of Weindruch *et al*. (1984); while Sato *et al*. (2012) reported that DHEA administration do not decrease the food intake. The study of Minkin *et al*. (1993) reported that anabolic steroids decrease locomotor activity in rats. On the other hands, other studies reported that anabolic steroids may increase aggressive behavior in laboratory animals (Scholtens *et al*., 1988) and in humans (Hannan *et al*., 1991). These contradictory findings could be attributted to different experimental designs, animal species, drug dose, duration, and mode of administration.

Since DHEA is a biosynthetic precursor of all steroid hormones, including E2 (Traish *et al.*, 2011), so its administration would definitely lead to the increase in serum E2 level. Therefore, the current results explanation may be related to the effect of E2. This is in consistent with Tsutsui and lshii (1981), who showed that injection of a sufficient amount of E2 is effective in inducing aggressive behavior in birds. While Wada (1982) reported that E2 did not fully enhance locomotor activity, nor did it fully improve sexual behavior. On the other hand, Taylor *et al*. (2012) hypothesized that DHEA has a direct influence on behaviors and this effect is not simply destined to an indirect manner by conversion to E2 or testosterone.

Based on the current data, high doses of DHEA have adverse effects on body weight, fur density, and behavior of perimenopausal rats.

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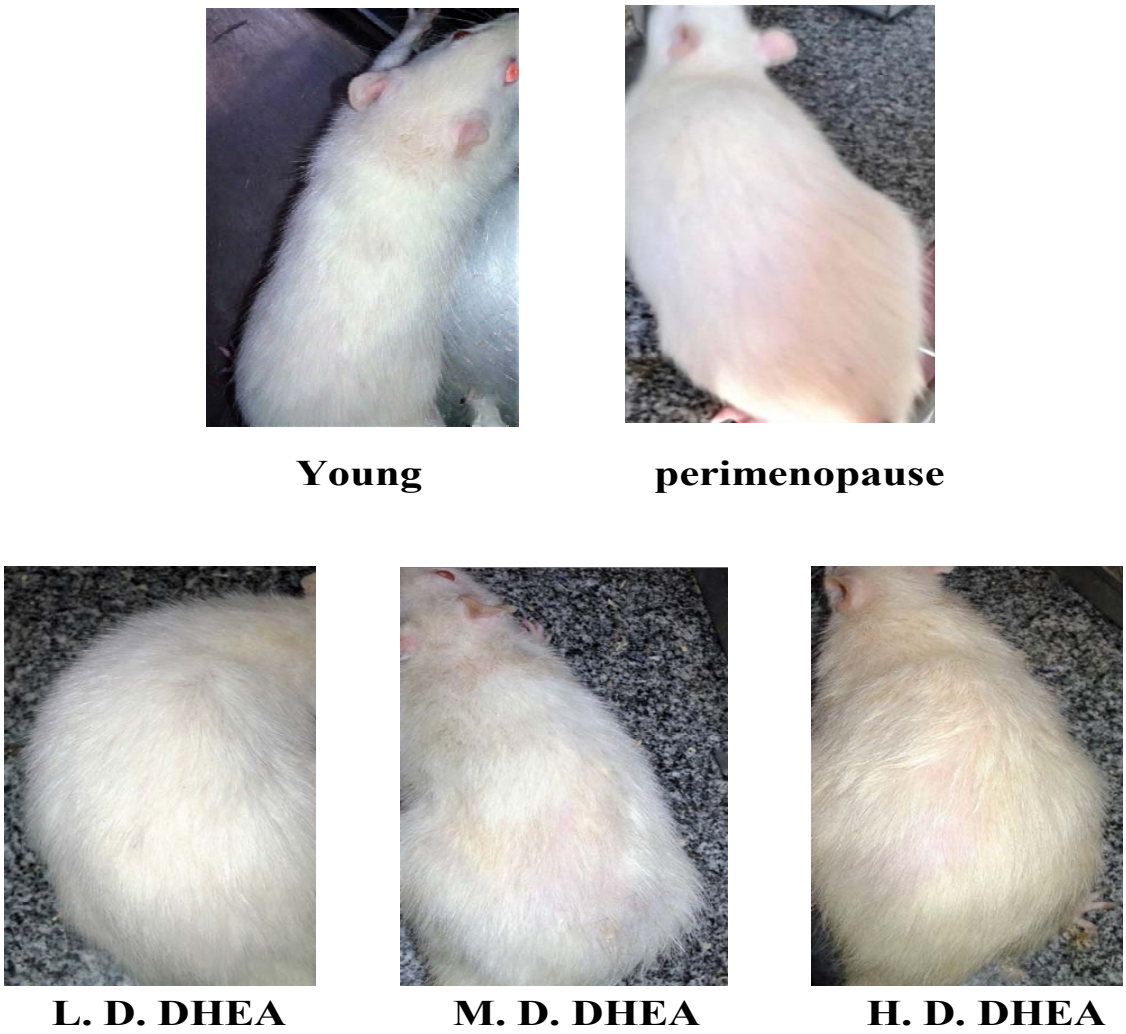

Fig. 1: The effect of DHEA on the fur of perimenopausal rats

ARABIC SUMMARY

تأثير الديهيدروإبي أندروستيرون على الشكل الظاهرى وسلوك إناث الجرذان فيما قبل سن اليأس

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قسم علم الحيوان ـ كلية العلوم ـ جامعة عين شمس

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تهدف الدراسة الحالية إلى تحديد أثر الديهيدروإيبى أندروستيرون (دهيا) على الشكل الظاهرى ونمو وسلوك الجرذان في مرحلة ما قبل سن اليأس. تم إعطاء الدهيا في ٣ جرعات: ٥٠، ٧٥ و ١٥٠ ملغ / كغ لمدة شهرين متتاليين. وقد وجد أن إعطاء الدهيا يقلل من اكتساب الوزن، ويسبب انخفاض كثافة الفراء الظهرية، كما أنه يؤثر سلباً على سلوك الجرذان. ولوحظ أن هذه التأثيرات تتناسب طردياً مع تركيزات الدهيا. وبناء على النتائج الحالية، ينصح بعدم استخدام جرعات عالية من دهيا إكلينيكيا لأنها تُحدث تأثيرات على وزن الجسم، وكثافة الفراء، والخصائص السلوكية.