Effect of Dietary Intake Ashwagandha Roots Powder on the Levels of Sex Hormones in the Diabetic and Non-Diabetic Male Rats

Nehal M. Belal, Eman M. El-Metwally and Ibrahim S. Salem

Abstract: This study was carried out to throw the light on the tested sexual hormones; estrogen, progesterone, testosterone, FSH and LH levels in diabetic and non-diabetic rats, as well to determine the impact of the dietary intake Ashwagandha roots powder for four weeks on the former sexual hormones and on serum glucose, cholesterol and triglyceride levels in diabetic and non-diabetic rats. The obtained Results illustrated that the dietary intake Ashwagandha roots powder was effective in lowering serum FSH level in Ashwagandha-treated animals compared to controls (p<0.05) in both diabetic and non-diabetic rat groups, whereas progesterone (p<0.05), testosterone (p<0.05) and the LH levels (p<0.001) were significantly higher in non-diabetic Ashwagandha treated animals. The orally intake Ashwagandha roots powder was also able to reverse the reductive effect of diabetes on the progesterone. The estrogen level did not show any significant difference between Ashwagandha treated and untreated rats of either the diabetic or non-diabetic groups. Therefore, the present investigation is showed that Ashwagandha can prevent the diabetes-induced hormonal dysfunction by inhibiting hyperglycemia and hyperlipidemia. It is suggested that Ashwagandha may have a regulatory effect on diabetes-induced change of the levels of gonadal-hormones, especially; progesterone, in male rats. Nevertheless, Ashwagandha is apparently only able to diminish the FSH serum level in intact animals.

Key words: Ashwagandha • Diabetes • Sex hormones • Glucose level • Lipid profile • Cholesterol • Triglyceride

INTRODUCTION

Ashwagandha, also known as Withania somnifera and winter cherry, has been an important traditional herbal medicine for over 3000 years [1]. Ashwagandha is a densely pubescent shrub up to 1-m tall belonging to the family of Solanaceae. The roots of this plant include some alkaloids and vitanolids [2]. This plant has been used for libido, anxiety, inflammation, Parkinson’s disease, cognitive and neurological disorders and also has been used as sedative [3]. Diabetes mellitus is one of the most common endocrine diseases in the world [4]. The prevalence of diabetes for all age-groups worldwide was estimated and found to be 2.8% in 2000 and 4.4% in 2030 [5]. Diabetes mellitus-induced hyperglycemia causes acute or chronic side effects that can affect all systems and organs such as sexual glands [6, 7]. It has been reported that about 90% of diabetic patients suffer from deficiency in sexual function including libido and fertility [8]. Diabetes mellitus produced reproductive dysfunction, but did not compromise sperm fertilizing ability in the cauda epididymis in an experimental model [9]. In addition, it has been shown that the administration of aqueous extract of Ashwagandha is able to decrease the serum level of the FSH and to increase the LH level in rats [10]. Furthermore, it has been reported that the administration of Ashwagandha also significantly increased serum testosterone and LH levels and also reduced the levels of the FSH in men [11]. Also, some studies were carried out in this concern and found that aqueous extract of Ashwagandha induces some changes in hypophysial gonadotropines accompanied by an increase of sperms in male rats and foliclegenesis in immature female rats [12, 13]. Diabetes mellitus is accompanied by hyperglycemia and hyperlipidemia. One of the probable mechanisms by which diabetes mellitus is involved in hyperglycemia and hypercholesterolemia is oxidative stress exhibiting effects which leads to tissue destruction and dysfunction [14]. On the other hand, it was observed that Ashwagandha inhibited lipid peroxidation and reduced oxidative stress in men, mice and rats [11, 15, 16].
Therefore, we have performed the present study to examine the sexual hormones; estrogen, progesterone, testosterone, FSH and LH levels in diabetic and non-diabetic rats, as well to determine the impact of the dietary intake Ashwagandha roots powder for four weeks on the former sexual hormones and on serum glucose, cholesterol and triglyceride levels in diabetic and non-diabetic rats.

**MATERIALS AND METHODS**

**Materials:** Ashwagandha (*Withania somnifera*) roots were obtained from the local market in Cairo city, Egypt and were scientifically identified by Institute of Food Technology, Giza, Egypt. The chemistry of Ashwagandha root has been extensively studied and found to be over 19 active chemical compounds have been identified by Al-Qarawi *et al.* [12] as shown in Table 1. Kits used for determination of tested serum sexual hormones (estrogen, progesterone, testosterone, FSH and LH), glucose, cholesterol and triglyceride were obtained from Biosub® (Biocon, Germany). The experimental animals; adult male Albino rats (Sprague Dawley strain) weighting about 195-220 g, were obtained from Helwan Experimental Animals Station, Ministry of Public Healthy, Egypt.

<table>
<thead>
<tr>
<th>No</th>
<th>Active Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Withaferin A</td>
</tr>
<tr>
<td>2</td>
<td>Diacetylwitaferin A</td>
</tr>
<tr>
<td>3</td>
<td>2,3-Dihydrowithaferin A</td>
</tr>
<tr>
<td>4</td>
<td>Withanolide viscoslastcetone B</td>
</tr>
<tr>
<td>5</td>
<td>27-o-b-D-Glucopyranosyl viscoslastcetone B</td>
</tr>
<tr>
<td>6</td>
<td>Dihydrowithaferin A</td>
</tr>
<tr>
<td>7</td>
<td>27-o-Glucopyranosylwitaferin A</td>
</tr>
<tr>
<td>8</td>
<td>Withanoside I-VII</td>
</tr>
<tr>
<td>9</td>
<td>24,25-Dihydro-27-deoxywitaferin A</td>
</tr>
<tr>
<td>10</td>
<td>Physagulin D(1®6)-b-D-glucopyranosyl-(1®4)-b-D-glucopyranoside</td>
</tr>
<tr>
<td>11</td>
<td>Sitoidosides VII-X</td>
</tr>
<tr>
<td>12</td>
<td>27-o-b-D-Glucopyranosylphysagulin D</td>
</tr>
<tr>
<td>13</td>
<td>5-Dehydroxywithanolide R</td>
</tr>
<tr>
<td>14</td>
<td>Withasomniferin A</td>
</tr>
<tr>
<td>15</td>
<td>1-Oxo-5b,6b-epoxy-witha-2-ene-27-ethoxy-olide</td>
</tr>
<tr>
<td>16</td>
<td>4-(1-Hydroxy-2,2-dimethylcyclopanone)-2,3-dihydrowithaferin A</td>
</tr>
<tr>
<td>17</td>
<td>D-Glucopyranosyl</td>
</tr>
<tr>
<td>18</td>
<td>D-Glucopyranoside</td>
</tr>
<tr>
<td>19</td>
<td>4,16-Dihydroxy-5b,6b-epoxyphysagulin D</td>
</tr>
</tbody>
</table>

* Identified by Al-Qarawi *et al.* [12]

**Methods:**

**Preparation of Ashwagandha Roots Power:** The roots were sorted from strange materials, cleaned, washed with distilled water, dried under vacuum at 55±5°C until the constant weight, ground by an electric grinder into a fine powder to pass through 20 mesh sieve, packaged in polyethylene bags and stored in a refrigerator at 4±1°C until they analyzed and used in biological experiments.

**Preparation of Ashwagandha Roots Diet:** Ashwagandha roots powder was mixed carefully using an electric mixer with basal diet at ratio of 6.25 % according to the procedure of Swanston-Flatt *et al.* [17].

**Biological Experiments:** They were performed according to the procedures described in AOAC [18] to estimate the sexual hormones; estrogen, progesterone, testosterone, FSH and LH levels in diabetic and non-diabetic rats, as well to determine the impact of the dietary intake Ashwagandha roots powder for four weeks on the former sexual hormones and on serum glucose cholesterol and triglyceride levels in diabetic and non-diabetic rats. In this experimental study, a total of 40 adult male Albino rats weighing 195-220g were kept in individual stainless steel cages under hygienic conditions and fed two weeks on basal diet for adaptation *ad libitum* in the animal house in Ophthalmology Research Institute, Cairo, Egypt. Then the rats were divided into four equal groups composed of ten rats for each as the following: the first group fed on basal diet and kept as a negative control, The second group was fed on Ashwagandha roots diet (the non-diabetic Ashwagandha-treated group) and left as a positive control, the third group (diabetic rats) was fed on basal diet only and the fourth group was fed on Ashwagandha roots diet (diabetic group treated with Ashwagandha roots powder). Four rats were housed in each cage at temperature 21±2°C and 12 h light-dark cycling with food and water provided for four weeks *ad libitum*. To induce diabetes mellitus rats, they were injected subcutaneously with 150 mg of alloxan/kg body weight after fasting overnight to induce hyperglycemia, according to the procedure of Buko *et al.* [19]. Diabetes was verified by a serum glucose level (using blood sample from ocular vein) higher than 250 mg/dl (glucose oxidase Kit); after alloxan injection, Ashwagandha-treated rat groups (2 and 4 groups) started to receive the oral administration of Ashwagandha roots diet for four weeks as reported by Buko *et al.* [19].
At the beginning (after the adaptation period) and at the end of the experiment (after four weeks), the blood sample was withdrawn from eye vein of each rat by means of fine capillary glass tubes according to the procedure of Zlao et al. [20]. Each blood sample was placed in a dry clean centrifuge tube and allowed to clot for 1-2 hr at about 37 °C. The blood serum was then separated by centrifugation at 1500 xg for 20 min. The clean individual nonhaemolysed supernatant serum samples were then pipetted into a Wassermann tube individually and kept frozen at-18°C until they analyzed according to the procedure of Zlao et al. [20].

Analytical Methods: Blood serum samples of all rats individually were collected from their hearts at the beginning and the end of the four weeks period of the biological experiment to determine serum estrogen, progesterone, testosterone, FSH and LH levels by radioimmuno-method according to the procedures of AOAC [21] provided with the kits. The serum triglyceride, cholesterol and glucose levels were also measured at the initial and after four weeks in all rats' groups according to the procedures of AOAC [21].

Statistical Analysis: The obtained data of tested parameter was expressed as means ±S.E. The comparisons were carried out using one way analysis of variance (ANOVA) followed by Post-hoc Tukey test and p values less than 0.05 were considered as significant differences.

RESULTS

A comparison of sexual hormone levels between the control and Ashwagandha-treated animals group samples indicated that there was no significant difference in blood estrogen level between treated and untreated of both diabetic and non-diabetic rats' groups (Fig. 1). In addition, the non-diabetic Ashwagandha-treated group, serum progesterone (p<0.05), testosterone (p<0.05) and the LH (p<0.001) levels had increased significantly when compared to the control group (Figures 2, 3 and 4). The serum level of the FSH level has been significantly decreased (p<0.05) in the Ashwagandha-treated group.

Furthermore, in Ashwagandha-treated rats diabetic group, serum progesterone, testosterone and the LH levels had increased significantly (p<0.05) (Figures 2, 3 and 4) when compared to non-diabetic rats group. On the other hand, as it is shown in Fig. 5, in Ashwagandha-treated experimental animals group, there was a significant

Fig. 1: Effect of the dietary intake Ashwagandha roots powder on estrogen level in control and diabetic groups.

Fig. 2: Effect of the dietary intake Ashwagandha roots powder on progesterone level in control and diabetic groups.

Fig. 3: Effect of the dietary intake Ashwagandha roots powder on testosterone level in control and diabetic groups.

* p<0.05 as treated groups are compared to control and diabetic groups and # p<0.05 as compared to control group.

* p<0.05 as treated groups are compared to control and diabetic groups and ### p<0.001 as is compared to control group.
Fig. 4: Effect of the dietary intake Ashwagandha roots powder on the LH level in control and diabetic groups.
*p < 0.05 (as compared to control group), ***p < 0.001 as compared to diabetic group and ## p < 0.01 as compared to control group.

Fig. 5: Effect of the dietary intake Ashwagandha roots powder on the FSH level in control and diabetic groups.
*p<0.05 as treated groups are compared to control and diabetic groups and # p<0.05 as compared to control group.

Table 2: Serum glucose, cholesterol and triglyceride levels (mg/dl) of control, diabetic and Ashwagandha-treated rats

<table>
<thead>
<tr>
<th>Rats groups</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130.6 ± 27.6</td>
<td>73.9 ± 5.9</td>
<td>123.7 ± 17.1</td>
</tr>
<tr>
<td>Control and Ashwagandha</td>
<td>123.1 ± 13.1</td>
<td>78.9 ± 3.4</td>
<td>124.4 ± 25.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>341.3 ± 73.4 *S</td>
<td>70.4 ± 4.3</td>
<td>154.9 ± 8.8</td>
</tr>
<tr>
<td>Diabetes and Ashwagandha</td>
<td>392.6 ± 40.2 **SS</td>
<td>66.4 ± 3.0</td>
<td>122 ± 16 #</td>
</tr>
</tbody>
</table>

M±SE: Mean ± Standard error; *p<0.05, **p<0.01 and ***p<0.001 (as compared to control group); $p<0.05 and $$p<0.01 (as compared to control and Ashwagandha group); #p<0.05 (as compared to diabetic group). All data represent Mean±S.E.M.

The obtained results also showed that the serum gonadal hormones levels in the control and diabetic control rats groups were totally different. In this regard, progesterone (Fig. 2) and the FSH (Fig. 5) levels in diabetic groups were significantly decreased (p<0.05), compared to control group. However, testosterone level in diabetic group (Fig. 3) showed a significant increment (p<0.001). In addition, the level of the LH in the diabetic group (Fig. 4) was markedly higher than that of the control group (p<0.01).

Untreated (p<0.05) and Ashwagandha-treated (p<0.01) diabetic rats also had elevated serum glucose level over those of control rats. However, in both control and diabetic groups, Ashwagandha did not induce any significant change in serum glucose levels (Table 2). On the other hand, cholesterol level of all rats' groups the feeding on Ashwagandha roots powder diet did not cause any significant alteration in serum cholesterol level of all rats' groups, while dietary intake Ashwagandha roots powder diet made a significant reduction in triglyceride level in diabetic rats (p<0.05); as evident in the obtained results in Table 2.

**DISCUSSION**

The results of present study in the effect of Ashwagandha on reducing the FSH and increasing the LH plasma level is in agreement with those obtained by Abdel-Magied *et al.* [10] that have shown administration of aqueous extract of Ashwagandha is able to decrease the serum level of the FSH and to increase the LH level in male rats. The effect of the dietary intake Ashwagandha roots powder on the FSH and the LH levels shows that possibly Ashwagandha has inhibitory and exhibitory effects on the FSH and the LH gonadotrophs;
respectively. Regarding the fact that ovulation and gestation, at least in early phases in women, are controlled by the LH/progesterone system [19], one may conclude that Ashwagandha has a positive effect on reproduction by increasing the LH and progesterone. The effect of dietary intake Ashwagandha roots powder on testosterone level in this research is not in accordance with the obtained results of previous study performed by Abdel-Magied et al. [10] in which a reduction had been shown in the testosterone level of Ashwagandha-treated rats. This discrepancy might be related to the type of Ashwagandha administration (in food in our experiment and in water and stomach tube in their experiment) and to the variance in Ashwagandha treatment period as we had treated the rats for four weeks, whereas in the previous study rats had been treated for six days. It is notable that Ashwagandha roots contain a steroidal lactone (withaferin A) [20]. The presence of steroidal compound in Ashwagandha, it appears that Ashwagandha is mimicking the steroidal hormones.

Generally, the present study indicates that diabetes can increase the testosterone and the LH levels and also decreases serum levels of progesterone and the FSH. Therefore, it is in accordance with the previous study that alloxan-induced diabetes could reduce the serum level of the FSH and progesterone in female rats [21]. Impaired action of the LH on the gonadal organ is a suggested mechanism for decreasing the reproductive hormone levels mainly progesterone from luteal cells [19]. Regarding this fact, present results in the increasing of the LH level can not be justified. Since the LH level in male and ovariectomized female diabetic rats did not change despite the decrease in GnRH level [22, 23], it seems that this subject requires more investigation. In contrary to this experiment, it was reported that testosterone level in diabetes cases in male rats was decreased [23]. Therefore, it could be related to change in the LH level which impairs gonadal hormone synthesis. However, Ashwagandha is only able to reverse the diabetic effect on progesterone level in the blood, i.e. Ashwagandha increases progesterone level in both control and diabetic groups. Since there is no report about the effect of Ashwagandha on progesterone level, we can conclude that Ashwagandha has excitatory effect on luteal cells. Regarding the antioxidant effect of Ashwagandha [11, 15, 16] and involvement of oxidative stress in reproductive dysfunction [14], the effect of this plant on gonadal hormone levels of diabetic rats as a reproductive function is probably performed via inhibition of oxidative stress.

Measuring the glucose level in blood samples of diabetic and non-diabetic rats shows that Ashwagandha treatment does not affect glucose level in the serum. Our results are in accordance with previous study by results which indicated that Ashwagandha did not reverse the hyperglycemic effect of diabetes in the male rats [24]. However, Andallu and Radhika [25] reported that administration of Ashwagandha root was able to induce hypoglycemia in non-insulin-dependent diabetes mellitus (NIDDM) known as type 2 diabetes mellitus in human subjects [26]. In our study, the type of diabetes was insulin-dependent and it is suggested that effective constituent of Ashwagandha in NIDDM patients acts by a mechanism in which insulin is released [14]. In our diabetic model, the STZ was injected in single dose and possibly because of cytotoxic effect of the STZ, where as the insulin secretion was not available. Therefore, regarding the different mechanisms of the two types of diabetes, the anti-diabetic effect of Ashwagandha is not exactly repeatable in our study or as reported by Andallu and Radhika [25] and Roghani et al. [27].

The effect of Ashwagandha on serum cholesterol level is also similar to its effect on glucose and no significant change in blood cholesterol has been seen after Ashwagandha treatment. Nevertheless, a small reduction in cholesterol level of blood in diabetic Ashwagandha-treated group relative to diabetic control group has been observed. However, it has been seen that diabetes enhances triglyceride level in the serum and Ashwagandha was able to reverse this increment significantly. Much of Ashwagandha’s pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D [28]. Ashwagandha is thought to be amphoteric and can help to regulate important physiologic processes. When there is an excess of a certain hormone, the plant-based hormone precursor occupies cell membrane receptor sites in such a way that the actual hormone cannot attach and exert its effect [3]. From this information, probably the withanolides as hormone precursor induces some chemical substrates that can affect metabolic activity; especially in liver and adipose tissue. Insulin-dependent diabetes is accompanied by increased oxidative stress and some of the biochemical changes in this type of diabetes are attributed to this activity [14]. In addition, Ashwagandha has the antioxidant property that can reduce free radicals-induced oxidative stress [15, 16, 29]. Thus, some of the useful effects of dietary intake Ashwagandha roots on triglyceride level in this experiment are attributable to the reduction of stress oxidative and lipid peroxidation.
CONCLUSION

Our study has indicated that long time oral administration of Ashwagandha root in experimental model of diabetes, could be used as a good candidate in the treatment of reproductive hormones deficiency. In addition, the plant has shown to have hypolipidemic effect, although it is not considered to have a significant hypoglycemic effect.

REFERENCES


