Iranian Journal of Reproductive Medicine Vol.7. No.4. pp: 163-168, Autumn 2009

Effect of withania somnifera on levels of sex hormones in the diabetic male rats

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Received: 20 April 2009; accepted: 27 October 2009

Abstract

Background: There are evidences regarding the prevalence of dysfunction in sexual function and behavior in diabetic people. Experimental studies revealed a positive effect of withania somnifera on sexual function and behaviors.

Objective: In this research, the effect of withania somnifera on sexual function in diabetic male Wistar rats was assessed by measuring the serum levels of testosterone, progesterone, estrogen, FSH and LH.

Materials and Methods: Experimental diabetes mellitus type I was induced by intraperitoneal injection of a single dose (60 mg/kg) of streptozotocin (STZ) in Wistar male rats. Oral withania somnifera root was given in pelleted food at ratio of 6.25% for 4 weeks. The levels of gonadadotropic hormones (LH, FSH), progesterone, estrogen and testosterone in animals' serum were determined after 4 weeks in all groups.

Results: Withania somnifera root was effective in lowering FSH serum level in somnifera-treated animals compared to controls ($p<0.05$) in both diabetic and non-diabetic groups, whereas progesterone ($p<0.05$), testosterone ($p<0.05$) and LH levels ($p<0.001$) were significantly higher in non-diabetic treated animals. Oral somnifera root was also able to reverse the reductive effect of diabetes on the progesterone. The estrogen level did not show any significant difference in any of the groups.

Conclusion: It is suggested that withania somnifera may have a regulatory effect on diabetes-induced change of the levels of gonadal-hormones, especially progesterone, in male rats. Nevertheless, somnifera is apparently only able to diminish FSH serum level in intact animals.

***Key words**: Withania somnifera, Diabetes, Sex hormones, Rat.*

Introduction

Withania somnifera, also known as ashwagandha and winter cherry, has been an important traditional herbal medicine for over 3000 years (1). Withania somnifera is a densely pubescent shrub up to 1-m tall belonging to the family of Solanaceae. The root of this plant includes 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 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). Data shows 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). Experimental studies express evidences about the effect of withania somnifera on the gonadal hormones and sexual behaviors. Abdel-Magied and colleagues have shown that administration of aqueous extract of withania somnifera is able to decrease the serum level of FSH and to increase

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the LH level in rats (10). In addition, administration of somnifera also significantly increased serum testosterone and LH levels, and also reduced the levels of FSH in men (11). Studies indicate that aqueous extract of withania somnifera 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 hyperlipidemy. 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 withania somnifera 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 effect of diabetes on the sexual hormone levels and to consider the effect of withania somnifera on diabetes-induced changes of gonadal hormones, glucose, cholesterol and triglyceride serum levels in male rats. This study is aimed at finding out whether somnifera can prevent the diabetes-induced hormonal dysfunction by inhibiting hyperglycemia and hyperlipidemy.

Materials and methods

Animals

In this experimental research, a total of 39 adult male Wistar rats weighing 195-220g (Razi Institue, Iran) were randomly divided into four groups including; 1- control group treated with withania somnifera for four weeks (n=8), 2- sham (n=11), 3- diabetic (n=9) and 4-diabetic group treated with withania somnifera for four weeks (n=11). Four rats were housed in each cage at temperature 21±2ºC and 12 h light-dark cycling with food and water provided *ad libitum*.

Induction of diabetes

To induce diabetes mellitus type I, a single dose of stereptozotocin (STZ, Sigma U.K.) 60 mg/kg (immediately before use was dissolved in cold 0.9% saline) was injected intraperitonealy in groups 3 and 4 (17). After 10 days, diabetes was verified by a serum glucose level (using blood sample from ocular vein) higher than 250 mg/dl (glucose oxidase Kit, Zistchimie, Tehran).

Plant administration

Withania somnifera root was provided from the local market and was scientifically identified by the Department of Botany of Shaheed Beheshti University. The plant was powdered and was mixed in pelleted food at ratio of 6.25%. Ten days after STZ injection in group 4, treatment groups (2 and 4) started to receive oral administration of plant-mixed pelleted food for four weeks (18).

Hormonal assay

To assess the sexual hormone levels, blood samples of all rats were collected from their hearts at the end of the four-week period. The serum levels of estrogen, progesterone, FSH, LH and testosterone were determined by radioimmuno-method according to the procedures provided in the kits (North Biochemistry Kit Center, Beijing). In addition, the serum levels of triglyceride, cholesterol and glucose were also measured four weeks in all groups according to the procedures provided in kits (Zistchimie, Tehran) after.

Statistical analysis

Data from hormone levels assessment were expressed as means ± S.E.M. 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 control and somnifera-treated group in animal samples indicated that there was no significant difference in the estrogen level between treated and non-treated groups (figure 1). In addition, in a similar comparison, in the non-diabetic somnifera-treated group (sham), serum levels of progesterone ($p<0.05$), testosterone ($p<0.05$) and LH ($p<0.001$) have significantly increased compared to the control group (as it is shown in figures 2, 3, 4). On the other hand, in the somnifera-treated group, serum level of FSH has been significantly decreased ($p<0.05$). Statistical analysis of sexual hormone levels in diabetic animals' blood samples indicated that there was no significant difference in the estrogen level between treated and non-treated diabetic groups (figure 1). Furthermore, in somnifera-treated diabetic group, compared to diabetic group, serum levels of progesterone, testosterone and LH have significantly increased ($p<0.05$) (figures 2, 3, 4). On the other hand, as it is shown in figure 5, in somnifera-treated group compared to non-treated group, serum level of FSH has been significantly decreased ($p<0.05$). Data also showed that the

serum levels of gonadal hormones in control and diabetic control groups were totally different. In this regard, progesterone (figure 2) and FSH (figure 5) levels in diabetic groups compared to control group were significantly decreased ($p<0.05$). However, testosterone level in diabetic group (figure 3) showed significant increment ($p<0.001$). In addition, the level of LH in the diabetic group (figure 4) was markedly higher than that of the control group ($p<0.01$). There was no significant difference in the estrogen level between control and diabetic groups.

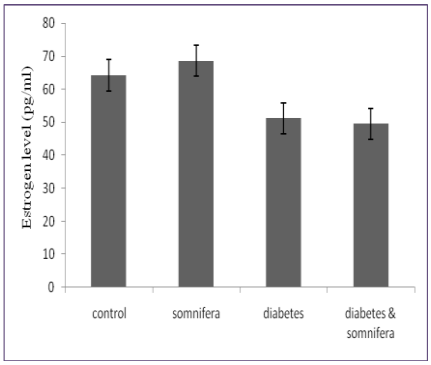

Figure 1. Effect of somnifera on estrogen level in control and diabetic groups.

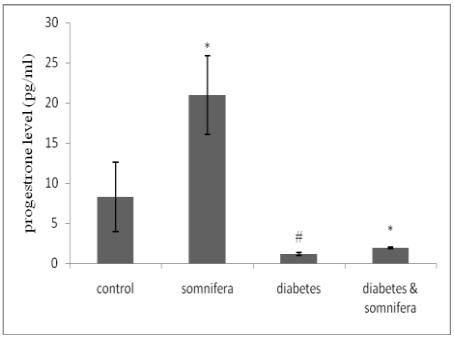

Figure 2. Effect of somnifera on progesterone level in control and diabetic groups. *p<0.05 as treated groups are compared to control and diabetic groups and # p<0.05 as compared to control group.

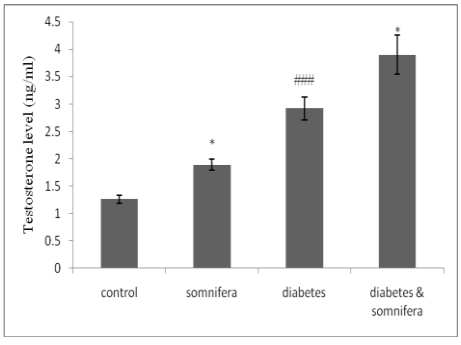

Figure 3. Effect of somnifera on testosterone level in control and diabetic groups. *p<0.05 as treated groups are compared to control and diabetic groups and ### p<0.001 as is compared to control group.

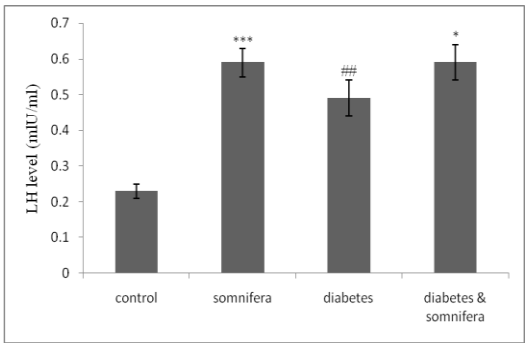

Figure 4. Effect of somnifera on LH level in control and diabetic groups. *p < 0.05 (as compared to control group). ***p < 0.001 as compared to diabetic group and ## p < 0.01 as compared to control group.

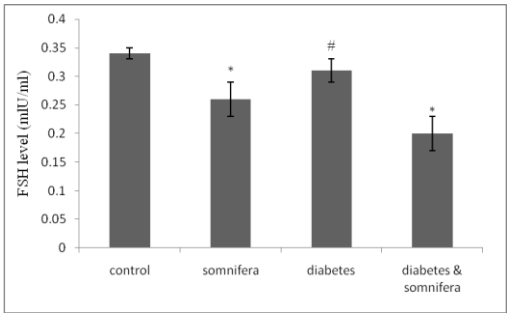

Figure 5. Effect of somnifera on FSH level in control and diabetic groups. *p<0.05 as treated groups are compared to control and diabetic groups and # p<0.05 as compared to control group.

Untreated ($p<0.05$) and somnifera-treated ($p<0.01$) diabetic rats also had elevated serum glucose level over those of control rats. However, in both control and diabetic groups, somnifera did not induce any significant change in serum glucose levels (Table I). Cholesterol level of serum in four groups also did not show any significant difference. Treatment of normal rats with somnifera did not cause a significant change (Table I). In this respect, in diabetic rats somnifera made a significant reduction in triglyceride level ($p<0.05$).

Table I. Serum glucose, cholesterol and triglyceride levels of control, diabetic, and somnifera-treated rats.

Groups	Glucose (mg/dl)	Cholesterol	Triglyceride
Control	130.6 ± 27.6	73.9 ± 5.9	123.7 ± 17.1
Control & Somnifera	123.1 ± 13.1	78.3 ± 3.4	124.4 ± 25.7
Diabetes	341.3 ± 73.4*$	70.4 ± 4.3	154.9 ± 8.8
Diabetes & Somnifera	392.6 ± 40.2**$$	66.4 ± 3	122 ± 16#

*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 & somnifera group); #p<0.05 (as compared to diabetic group). All data represent Mean±S.E.M.

Discussion

The results of present study in the effect of somnifera on reducing FSH and increasing LH plasma level is in agreement with previous work from Abdel-Magied and colleagues that have shown administration of aqueous extract of withania somnifera is able to decrease the serum level of FSH and to increase the LH level in male rats (10). The effect of somnifera on FSH and LH levels shows that possibly somnifera has inhibitory and exhibitory effects on the FSH and 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 somnifera has a positive effect on reproduction by increasing LH and progesterone. The effect of somnifera on testosterone level in this research is not in accordance with previous study by Abdel-Magied in which a reduction has been shown in the testosterone level of somnifera-treated rats. This discrepancy might be related to the type of somnifera administration (in food in our experiment and in water and stomach tube in their experiment). On the other hand, we have treated the rats for four weeks, whereas in the previous study rats have been treated for six days. It is notable that somnifera contains a steroidal lactone (withaferin A) (20). From this study and the presence of steroidal compound in somnifera, it appears that somnifera is mimicking the steroidal hormones.

Generally, the present study indicates that streptozotocin-induced diabetes can increase the testosterone and LH levels and also decreases serum levels of progesterone and FSH. Therefore, it is in accordance with the previous study that streptozotocin-induced diabetes could reduce the serum level of FSH and progesterone in female rats (21).

Impaired action of 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 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 LH level which impairs gonadal hormone synthesis.

However, somnifera is only able to reverse the diabetic effect on progesterone level in the blood, i.e. somnifera increases progesterone level in both control and diabetic groups. Since there is no report about the effect of somnifera on progesterone level, we can only conclude that somnifera has excitatory effect on luteal cells. Regarding the antioxidant effect of withania (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 somnifera treatment does not affect glucose level in the serum. Our result is in accordance with previous study by Roghani and colleagues which has indicated that somnifera does not reverse the hyperglycemic effect of diabetes in the male rats (24). However, Andallu and coworker have seen that administration of somnifera root is able to induce hypoglycemia in non-insulin-dependent diabetes mellitus (NIDDM) known as type 2 diabetes mellitus in human subjects (25). The type of diabetes in our research was insulin-dependent. It is suggested that effective constituent of somnifera in NIDDM patients, acts by a mechanism in which insulin is released (14). In our diabetic model, STZ was injected in single dose and possibly because of cytotoxic effect of STZ, the insulin secretion was not available. Therefore, regarding the different mechanisms of the two types of diabetes, the anti-diabetic effect of somnifera in Andallu's study is not exactly repeatable in our and Roghani's investigations.

The effect of somnifera on cholesterol is also similar to its effect on glucose and no significant change in blood cholesterol has been seen after somnifera treatment. Nevertheless, a small reduction in cholesterol level of blood in diabetic somnifera-treated group relative to diabetic control group has been observed.

However, it has been seen that streptozotocin-induced diabetes enhances triglyceride level in the serum, and somnifera was able to reverse this increment significantly. Much of somnifera's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D (26).

Somnifera 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, somnifera has the antioxidant property that can reduce free radicals-induced oxidative stress (15, 16). Thus, some of the useful effects of somnifera on triglyceride level in this experiment are attributable to the reduction of stress oxidative and lipidic peroxidation.

Conclusion

Our study has indicated that long time oral administration of somnifera root in experimental model of streptozotocin-inducd 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.

Acknowledgement

The authors would like to thank the Medical Research Center of Shahed University as the financial sponsor of this research.

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