

# Effects of Hydro-alcoholic Extract from *Arctium lappa* L. (Burdock) Root on Gonadotropins, Testosterone, and Sperm Count and Viability in Male Mice with Nicotinamide/ Streptozotocin-Induced Type 2 Diabetes

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## Abstract

**Background:** Reproductive dysfunction is a complication of diabetes. *Arctium lappa* (burdock) root has hypoglycemic and antioxidative properties, which are traditionally used for treatment of impotence and sterility. Therefore, the aim of this study is to investigate the effects of its hydro alcoholic extract on gonadotropin, testosterone, and sperm parameters in nicotinamide/streptozotocin-induced diabetic mice.

**Methods:** In this experimental study, 56 adult male Naval Medical Research Institute (NMRI) mice (30–35 g) were randomly divided into seven groups: control, diabetes, diabetes + glibenclamide (0.25 mg/kg), diabetes + extract (200 or 300 mg/kg), and extract (200 or 300 mg/kg). Diabetes was induced with intraperitoneal injection of nicotinamide (NA) and streptozotocin (STZ). Twenty-four hours after the last extract and drug administration, serum samples, testes, and cauda epididymis were removed immediately for experimental assessment.

**Results:** Body weight, serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels, and sperm count ( $P < 0.05$ ) and viability ( $P < 0.01$ ) decreased in diabetic mice. Administration of glibenclamide significantly improved these reductions in diabetic animals ( $P < 0.05$ ). However, the hydro alcoholic extract (300 mg/kg) enhanced sperm viability only in diabetic mice ( $P < 0.01$ ). In addition, this dose of extract increased sperm count, LH, FSH, and testosterone in nondiabetic animals compared with the control group ( $P < 0.05$ ).

**Conclusion:** The results indicate that applied burdock root extract has anti-infertility effects in nondiabetic mice. Hence, this part of the *A. lappa* plant has an effect on the health of the reproductive system in order to improve diabetic conditions.

**Keywords:** *Arctium lappa*, diabetes, gonadotropin, testosterone, sperm

## Introduction

Diabetes mellitus is a heterogeneous endocrine metabolic disorder, and is differentiated with hyperglycemia, glycosuria,

polydipsia, and polyuria due to absolute or relative defective insulin secretion and sensitivity (1,2). In addition, this disease is accompanied with multiple medical, psychological, and sexual dysfunctions (3). The prevalence rate of diabetes

is immense, approximately 194 million patients worldwide, and predictions suggest that the number of diabetic patients will reach at 333 million in 2025 (4). There are two main types of diabetes: type 1 and type 2. Approximately about 90% to 95% of all diabetic cases suffer from type 2 (5). Approximately 90% of diabetic patients have a sexual function disorder (2), and the main symptoms of sexual disturbances include decrease in testicular weight and, sperm count and motility, which are documented in diabetic rats (6). Furthermore, several studies revealed that alterations in the hypothalamic-pituitary-gonadal axis can decrease serum luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone levels in men with type 2 diabetes (7).

Growing evidence indicates that excessive production of free radicals, reactive oxygen species (ROS), and impaired antioxidative defenses occur in diabetes. Moreover, oxidative stress and free radicals show adverse effects on sperm motility and fertility via lipids and DNA oxidative damage of spermatozoa associated with weakened sperm fertility (2).

Despite advances in medicines for male sexual dysfunction, drug administration is affected by its efficacy, safety, and price. Therefore, utilizing plants as a safe source of medicines for treating sexual disorders has been considered by scientists (8). Recently, many investigations have emphasized that consumption of natural antioxidants, particularly fruits and vegetables, may reduce oxidative damage and chronic disorders in diabetic patients. Interest has increased in finding both hypoglycemic and antioxidative properties of natural antioxidants for improvement of diabetes complications (9).

*Arctium lappa* L., commonly known as burdock, is an edible perennial herb in traditional Chinese medicine that belongs to the *Compositae* family. It has been used therapeutically in Europe, North America, and Asia. This herb is rich in antioxidative agents, such as tannin, gallic acid, quercetin, and caffeoylquinic acid. Some experiments indicate that this herb has hypoglycemic properties in diabetic rats. The root of this plant has also been traditionally used for treatment of impotence and sterility (9). Therefore, because of the presence of antioxidative components in the roots of *A. lappa* and its traditionally use as an aphrodisiac agent, the aim of this study is to investigate the effects of the hydro alcoholic extract from *A. lappa* roots on gonadotropins (LH, and FSH), testosterone, and sperm count and viability in nicotinamide/

Streptozotocin (NA/STZ)-induced diabetic mice.

## Materials and Methods

### Plant extraction

Fresh roots of *A. lappa* (burdock) were obtained from Isfahan city in Iran and scientifically approved by the Department of Pharmacognosy of Ahvaz Jundishapur University of Medical Sciences (AJUMS). *A. lappa* root was powdered with a grinder after desiccation in the shade. Furthermore 300 g of this powder was soaked in 1200 mL of an ethanol and distilled water mixture (70:30) and stored for 72 h at room temperature. The combination was then filtered using Whatman No. 1 filter paper and centrifuged for 20 min in 3500 rpm. Finally, supernatant was dried at 37 °C, and the acquired semi solid mass was stored at 4 °C until use (10).

### Animal preparation

In this experimental study, 56 adult male NMRI mice (30–35 g) were purchased from the animal house of AJUMS. Animals were housed in standard laboratory condition (temperature 20 ± 4 °C with a 12 h light/ 12 h dark cycle) with access to commercial chow and tap water. All experimental protocols were performed according to standard laboratory animal care approved by the National Research Council of the National Academic.

After one week of acclimatisation, mice were divided into seven groups (eight mice in each group): control, diabetes group, diabetic mice receiving glibenclamide (0.25 mg/kg) (11) as a standard diabetes drug, diabetic mice receiving *A. lappa* root hydro alcoholic extract (200 or, 300 mg/kg), nondiabetic mice receiving the same doses of extract (200 or, 300 mg/kg) (9). Both drug and extract were orally administered for a period of 28 consecutive days (12,13).

### Experimental protocols

#### Induction of type 2 diabetes model

In our model, experimental type 2 diabetes was induced by intraperitoneal (IP) injection of a single dose of STZ (65 mg/kg b.w; dissolved in citrate buffer, pH 4.5) (Sigma-Aldrich, USA) 15 min after an IP administration of NA (120 mg/kg b.w; dissolved in normal saline) (Sigma-Aldrich, USA). The animals were then assessed for improvements in the induced diabetes by assaying blood glucose levels at three days after NA/STZ injection. Finally, mice with blood glucose levels higher than 250 mg/dL were used in the following experiments (14).

### Hormonal measurements

Twenty-four hours after the last drug administration, blood samples were obtained from the mice by cardiac puncture under deep anesthesia. Furthermore, serum samples were collected after blood centrifugation for 20 min at 3500 rpm. Ultimately, all samples were kept at  $-80^{\circ}\text{C}$  until hormonal assessments were performed.

Serum hormonal assessment was performed for LH, FSH, and testosterone by using the enzyme-linked immunosorbent assay (ELISA) method described in the instructions of commercial assay kits (DRG Instruments GmbH, Germany). Hormonal detection sensitivity for serum LH, FSH, and testosterone levels of each kits were 1.27, 0.856 mIU/mL, 0.083 ng/mL respectively (per assay tube).

### Testicular morphology assessment

After blood collection, the testis of each mouse was removed immediately for testicular weight, width (D), length (L), and volume assessments. In addition, testicular volume was measured by the following formula:  $\text{volume} = (D^2/4 \times \pi) L \times K$  where  $K = 0.9$ ,  $\pi = 3.14$  (15).

### Sperm counts and viability assessment

Mice cauda epididymis were excised and minced into small pieces in 1.5 mL of normal saline 0.9% for sperm counting. Then one drop of this sperm-containing solution was transferred into chambers of a Neubauer hemocytometer lam (HBG, Germany) (Tiefe depth profondeur 0.100

mm and  $0.0025\text{ mm}^2$  area), and sperm counting was manually monitored and counted by light microscopy (Olympus, Japan) in a white blood cell (WBC) chamber. At the end, the results were expressed as the count of sperm/mL (16).

Also, sperm viability was assessed by using eosin 1% staining (Merk Chemicals, Germany) for separated unstained (live) and red-stained (dead) sperm. Thirty seconds after maintaining eosin 1% in a WBC grid of the Neubauer hemocytometer, sperm viability was manually counted within 2 min and data were expressed as percentage (17).

### Statistical analysis

The results were analyzed statistically by SPSS software and presented as mean with standard error of mean (SEM). Also, differences between all groups were made by one-way ANOVA followed by post hoc Least Significant Difference (LSD) test, and  $P < 0.05$  was considered statistically significant.

## Results

### Effect of *A. lappa* root extract on animal body weight and testis morphology

Our results showed that NA/STZ-induced diabetes significantly decreased body weight ( $P < 0.01$ ). Administration of glibenclamide ( $P < 0.05$ ) and *A. lappa* root extract improved this weight reduction in all animals ( $P < 0.01$ ). In addition, there were no significant differences in testicular weight and morphology parameters among the groups (Table 1).

**Table 1:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on body weight and testis morphology in normal and NA/STZ induced diabetic mice. (n = 8)

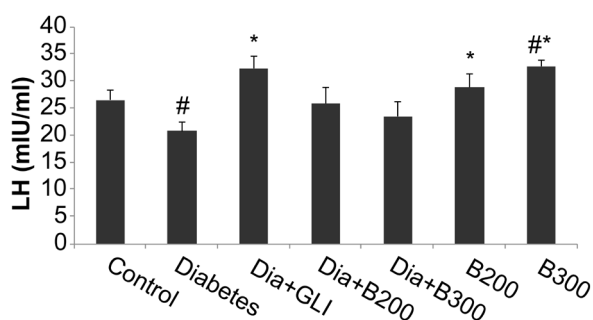
Groups	Factors				
	Animal Weight after (g)	Testicular weight (mg)	Testicular length (mm)	Testicular width (mm)	Testicular volume ( $\text{mm}^3$ )
Control	36.14 (1.06)	85.16 (6.25)	5.69 (0.14)	3.25 (0.17)	42.49 (6.21)
Diabetes	30.12 (1.31) <sup>##</sup>	83.16 (6.47)	5.71 (0.18)	3.28 (0.09)	43.42 (5.82)
Diabetes + GLI	34.16 (1.14) <sup>*</sup>	85.61 (6.04)	5.98 (0.24)	3.55 (0.07)	53.25 (7.23)
Diabetes + B200 mg/kg	37.14 (1.29) <sup>**</sup>	89.33 (5.48)	5.65 (0.09)	3.21 (0.13)	41.15 (4.84)
Diabetes + B300 mg/kg	37.92 (0.92) <sup>**</sup>	83.25 (7.51)	5.59 (0.21)	3.16 (0.06)	39.45 (5.26)
B200 mg/kg	38.82 (0.89) <sup>**</sup>	88.97 (6.98)	5.78 (0.15)	3.38 (0.08)	46.65 (6.43)
B300 mg/kg	38.96 (1.45) <sup>**</sup>	90.27 (7.09)	5.84 (0.26)	3.42 (0.07)	48.25 (4.97)

Mean (SEM), one-way ANOVA and post-hoc LSD test. <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$  versus diabetes group, <sup>##</sup> $P < 0.01$  versus control group.

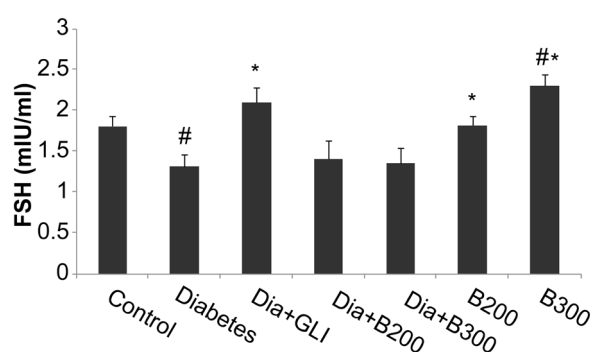
Abbreviation: GLI = Glibenclamide; B = Burdock.

*Effect of A. lappa root extract on serum levels of gonadotropins and testosterone*

Diabetes induced by NA/STZ significantly reduced serum LH, FSH, and testosterone levels in mice ( $P < 0.05$ ), and administration of *A. lappa* root extract did not improve this reduction in diabetic animals. However, this extract in doses of 200 and 300 mg/kg induced a significant difference in serum levels of LH, FSH, and testosterone in comparison with the diabetic group. *A. lappa* root extract (300 mg/



**Figure 1:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on serum LH levels in normal and NA/STZ induced diabetic mice. (n = 8), Mean (SEM), one-way ANOVA and post-hoc LSD test. Dia: diabetes, GLI: Glibenclamide, B: Burdock. \* $P < 0.05$  versus diabetes group, # $P < 0.05$  versus control group.



**Figure 2:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on serum FSH levels in normal and NA/STZ induced diabetic mice. (n = 8), Mean (SEM), one-way ANOVA and post hoc LSD test. Dia: diabetes, GLI: Glibenclamide, B: Burdock. \* $P < 0.05$  versus diabetes group, # $P < 0.05$  versus control group.

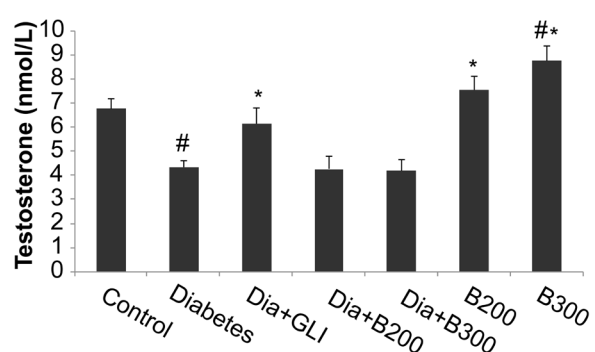
kg) increased serum levels of these hormones in nondiabetic mice when compared with the control group. Finally, administration of glibenclamide improved the effects of hormonal depletion in diabetic mice ( $P < 0.05$ ) (Figure 1, 2, and 3).

*Effect of A. lappa root extract on sperm count and viability*

Sperm count and viability were significantly decreased in the diabetic group ( $P < 0.05$  and,  $P < 0.01$ , respectively); glibenclamide treatment recovered these reductions in diabetic mice ( $P < 0.05$  and,  $P < 0.01$ , respectively). In addition, administration of *A. lappa* root extracts (200 and 300 mg/kg) in non-diabetic mice induced a significant increase in sperm count and viability compared with the diabetic group ( $P < 0.05$ , and  $P < 0.01$ , respectively). Therefore, the 300 mg/kg dose of this extract improved sperm viability in diabetic mice ( $P < 0.05$ ) and increased sperm count in nondiabetic mice when compared with the control group ( $P < 0.05$ ) (Figure 4 and 5).

**Discussion**

Nicotinamide protects  $\beta$ -cells from STZ-induced severe cytotoxic damages. Hence, NA/STZ co-administration leads to chronic diabetes mellitus by producing moderate hyperglycemia (18). The results of this study show that NA/STZ-induced diabetes decreased the body weight of mice. This result is in agreement with previous studies, which indicated body weight



**Figure 3:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on serum testosterone level in normal and NA/STZ induced diabetic mice. (n = 8), Mean (SEM), one-way ANOVA and post-hoc LSD test. Dia: diabetes, GLI: Glibenclamide, B: Burdock. \* $P < 0.05$  versus diabetes group, # $P < 0.05$  versus control group.

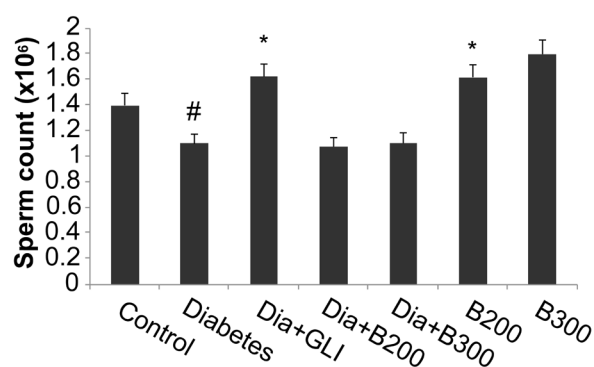


loss in diabetic mice. Failure to use glucose for producing energy leads to decreased storage of protein and fat. Therefore, untreated diabetes mellitus can cause severe wasting of the body tissues and asthenia (lack of energy) (19). Therefore, weight reduction of diabetic animals in the present study may be a consequence of this mechanism. In addition, *A. lappa* root extract and glibenclamide recovered this weight depletion in diabetic mice. Therefore, this extract may amend weight reduction via improved glucose utilization to produce energy instead of proteins (19).

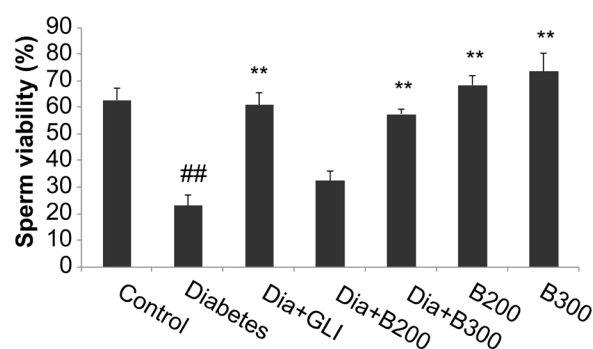
Diabetes mellitus is one of the main causes of male infertility and hypogonadism associated with a central origin. In addition, it was shown that LH and FSH hormone concentrations were decreased in diabetes (7). Hence, serum levels of testosterone were lower in patients with type 2 in those with type 1 diabetes (20); the present results of NA/STZ-induced type 2 diabetes revealing a significant decrease in serum LH, FSH, and testosterone levels are consistent with those of previous studies. Type 2 diabetes can result in hypogonadism through several mechanisms such as prevention of gonadotropin (LH and FSH) release, testosterone production by Leydig cells, and inhibition of steroidogenesis by increased cytokine levels and inflammatory factors, and reduction of the activities of antioxidative enzymes (7). Therefore, NA/STZ-induced type 2 diabetes may lead to hypogonadism via those mechanisms in the present study; however, more investigations are required to clarify the main mechanism by

which this occurs. In addition, administration of *A. lappa* root extract did not improve the reduction of these hormones in diabetic animals. Hence, this extract could not eliminate diabetes-induced hypogonadism; however, administration of high-dose extract increased serum levels of LH, FSH, and testosterone in nondiabetic mice. In some studies, *A. lappa* root extract enhanced gonadal hormones, testosterone, and sexual behavior in healthy animals; this is in agreement with our present results (8).

NA/STZ-induced diabetes resulted in decreased sperm count and viability in previous studies (21). The present results of sperm assessment revealed sperm count and viability depletion in diabetic mice. Administration of *A. lappa* root extract recovered sperm viability reduction towards control levels and increased sperm count in non diabetic animals. There is some evidence indicating that oxidative stress is associated with abnormalities in diabetes-induced male reproductive function; these abnormalities influence type 1 and type 2 models of diabetes (22). Diabetes mellitus can cause damage of sperm nuclear DNA by increasing reactive oxygen species generation and accelerating formation of advanced glycation end products (AGE) (23). ROS overproduction results in germ cell apoptosis, testicular oxidative damage, and sperm count and viability reduction (24,25). In addition, LH receptors are modulated by pituitary LH and are reduced in the testes of diabetic rats; hence, it may lead to Leydig cell dysfunction



**Figure 4:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on sperm count in normal and NA/STZ induced diabetic mice. (n = 8), Mean (SEM), one-way ANOVA and post-hoc LSD test. Dia: diabetes, GLI: Glibenclamide, B: Burdock. \* $P < 0.05$  versus diabetes group, # $P < 0.05$  versus control group.



**Figure 5:** Effect of hydro-alcoholic extract of *Arctium Lappa* (Burdock) root on sperm viability in normal and NA/STZ induced diabetic mice. (n = 8), Mean (SEM), one-way ANOVA and post-hoc LSD test. Dia: diabetes, GLI: Glibenclamide, B: Burdock. \*\* $P < 0.01$  versus diabetes group, ## $P < 0.01$  versus control group.

(22). Thus, NA/STZ-induced diabetes may decrease sperm count and viability via excessive generation of ROS, oxidative stress, and Leydig cell dysfunction. The membrane of spermatozoa is full of polyunsaturated fatty acid (PUFA), which is very sensitive to  $H_2O_2$  (26). This free radical can diffuse into the cells and cause to depletion of sperm viability through attenuation of activities of antioxidative enzymes (27). Thus, according to the sperm viability results of *A. lappa* root extract, this extract may improve antioxidative defense into the sperm cells and enhance sperm viability in diabetic animals.

Phytochemical studies reveal that *A. lappa* root contains saponins, flavonoids, tannin, and vitamins (28). Saponins and flavonoids are bioactive agents, responsible for elevated sexual behavior and, testosterone and androgen bioavailability (29,30). Thus, the enhancing effects of *A. lappa* root extract on serum gonadotropin, testosterone levels, and sperm viability in nondiabetic mice may have been caused by the presence of these components. However, there were no significant changes in hormonal assessment and sperm count of our diabetic mice model; hence, the bioactive agents of *A. lappa* root were not strong enough to remove the effects of NA/STZ-induced diabetes on gonadal hormone and Leydig cells.

Sulfonylurea derivatives such as glibenclamide, are commonly used in type 2 diabetes mellitus to promote insulin secretion through ATP-dependent potassium channel blockage on  $\beta$ -cell plasma membranes (31). In a study by Rabbani et al., glibenclamide increased serum gonadotropin, testosterone levels, and sperm generation in diabetic animals via improved activities antioxidative enzymes (18). Therefore, administration of glibenclamide in the present study is in accordance with previous studies, and this drug may eliminate reproductive system dysfunctions in diabetic animals through its antioxidative activities and properties.

## Conclusion

According to our results, the hydro alcoholic extract of the *A. lappa* root may have anti-infertility effects by increasing sperm count and, serum gonadotropin and testosterone levels of nondiabetic mice. Hence this part of *A. lappa* may have more effects on the health of the reproductive system with respect to diabetes. Moreover, further research is required to reveal the exact mechanisms of the administered extract on reproductive organs.

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## Conflict of Interest

None.

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## Authors' Contributions

Conception and design, analysis and interpretation of the data, critical revision of the article for the important intellectual content, provision of study materials or patient, statistical expertise and obtaining of funding: AA, AAO

Drafting of the article, administrative, technical or logistic support collection and assembly of data: AA, AAO, HH, EG, RT

Final approval of the article: AA, AAO

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