

Effect of *Azadirachta excelsa* leaves extract on uterine histology and testosterone level in alloxan induced diabetic rats

S.Nurdiana*, M.S.Nur Zulaikha, M.I.Syamimi Wahida, J.Elizabeth Jega, S.Nurul 'Izzati
Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, (MALAYSIA)
E-mail: nurdiana7251@salam.utim.edu.my

ABSTRACT

The protective effect of *Azadirachta excelsa* leaves extract against infertility in male and female diabetic rats was studied. The fertility experiment was done on twenty four Sprague Dawley male and female rats respectively. The male and female rats were divided into four groups each which one of them was kept as normal control, while the others were rendered diabetic by intraperitoneal injection of alloxan (130 mg/kg) for 28 consecutive days. On day 29 of treatment, after 8 hours of fast, blood were withdrawn for blood glucose and testosterone analysis, uterine were harvested for histological study and semen samples were collected to determine the characters of epididymal sperm. Administration of *A.excelsa* was significantly reduced the level of blood glucose in both male and female diabetic rats. Examination on semen was found that the plant extract was increased the number of sperm for 37.32% while the sperm cell abnormalities was revealed to be decreased on the end of experimental period. Microscopic observation was showed that *A.excelsa* able to reduce atrophy and increase the proliferation of stromal cells in the uterine of female diabetic rats. These results suggest that *A.excelsa* can potentially be developed as an alternative agent to restore the characters of epididymal sperm and to normalize distribution of stromal cells is an indicator towards improving the fertility of men and women with diabetes.

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KEYWORDS

Azadirachta excelsa;
Diabetes;
Sperm;
Testosterone;
Uterus.

INTRODUCTION

Diabetes mellitus is a degenerative disease that has deleterious effects on male and female reproductive function, possibly through an increase in oxidative stress. The oxidative stress is increased in diabetes due to the overproduction of reactive oxygen species (ROS), and decreased efficiency of antioxidant defences. In female, the excess of ROS has been found to affect the blood flow to the sites of implantation in the uterus^[1]. This alteration reduces the strength of uterine, thus increas-

ing the risk of miscarriage and emergency caesarean births. Numerous studies were also revealed that excess amounts of ROS reduced male fertility by altering the spermatogenesis and sperm quality including maturation and mitochondrial function^[2,3].

Azadirachta excelsa is a wild plant found in Malaysia has been recognized as an herbal medicine for improving fertility and related disorders. Our laboratory study revealed that the *A.excelsa* leaf extract possess a great antioxidant and hypoglycemic activities. However, analysis on male and female fertility under

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patho-physiological conditions such as diabetes was not reported. Therefore, the present study was designed to address these issues to lend support to the existing information pertaining to the beneficial effect of this plant in treating diabetes and fertility problems.

MATERIALS AND METHODS

Preparation of *A. excelsa* leaves extract

The powdered leaves of *A. excelsa* were soaked in 70% ethanol for two days at room temperature (37°C). The suspension was then filtered to obtain the extract. The extract was then evaporated using the rotary evaporator at 40°C until it was completely dried. A dark semi-solid material was obtained and stored at 4°C to be used later on.

Induction of diabetes

Rats weighing between 160 to 300g were fasted overnight and injected by intraperitoneal administration of freshly prepared alloxan monohydrate (130 mg/kg) in saline solution of pH 7.4. On day seventh, the rats showing stable glycemic values above 7.0 mmol/L were considered diabetic and selected for further experimentation^[4]. Blood glucose levels of the rats were estimated by collecting the blood samples from the tail vein by using Glucometer Accu-check Active.

Experimental design

The male and female rats were divided into four groups respectively with six rats in each group and treated orally twice a day as follows:

Group A : normal rats received saline.

Group B : diabetic rats received saline.

Group C : diabetic rats received metformin.

Group D : diabetic rats received ethanolic extract of *A. excelsa* at dose of 500 mg/kg.

On day 29 of treatment, after 8 hours of fast, all animals were scarified by decapitation. Blood were withdrawn for blood glucose and testosterone analysis. The uterine were harvested for histological study and semen samples were collected to determine the sperm cell count, motility and sperm abnormalities.

Epididymal sperm characters

The epididymal contents of treated rats were ob-

tained after cutting the tail of epididymis, squeezing it gently on clean slide and the progressive motility and sperm cell count were examined microscopically. Microscopical examination of seminal smears was also performed to determine the epididymal sperm abnormalities.

Testosterone determination

Testosterone levels were determined using radio-immunoassay method (RIA) which is intended for the quantitative determination of total testosterone in the serum. This method is based upon the competitive binding principal. The unknown or standard samples were incubated with radioactive iodine 125 (I125) labeled testosterone in antibody-coated tubes. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter. Serum total testosterone concentrations were then calculated.

Uterine histology

The uterine horn of the rats was fixed in 10% formalin for 24-48 hours. Then, the tissues were dehydrated by a graded series of ethanol concentrations, xylene:ethanol (1:1), xylene (100%) and finally paraffin. Then, the tissues are serially sectioned at 5 µm thickness, mounted onto glass slides, and some of them stained with hematoxylin and eosin (H&E) according to standard protocols and analyzed using a Zeiss Axiophot light microscope.

Statistical analysis

Data were expressed as mean ± SEM. The variance of the data was statistically evaluated by ANOVA. Value of $p < 0.05$ were considered statistically significant.

RESULTS

Fasting blood glucose

Blood glucose levels were significantly higher in male and female alloxan induced diabetic rats when compared to respective controls ($p < 0.001$). However, the rats treated with *A. excelsa* presented mild hyperglycemias (8-10 mmol/L), while metformin-treated rats presented severe hyperglycemias (>13

mmol/L) for both male and female diabetic rats.

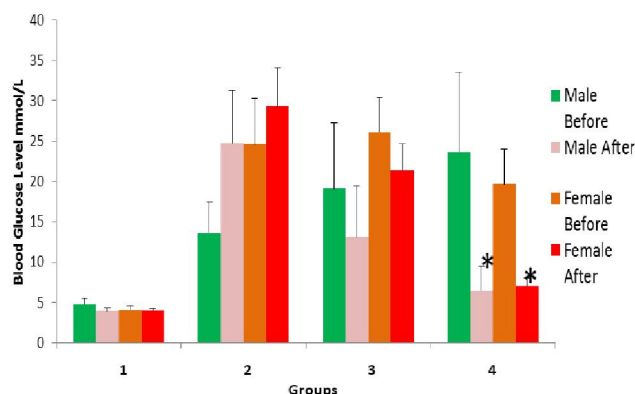


Figure 1 : The concentration of blood glucose in male and female diabetic rats. Data were expressed as means ± SEM; n=4; * statistical significant versus diabetic control (p<0.05)

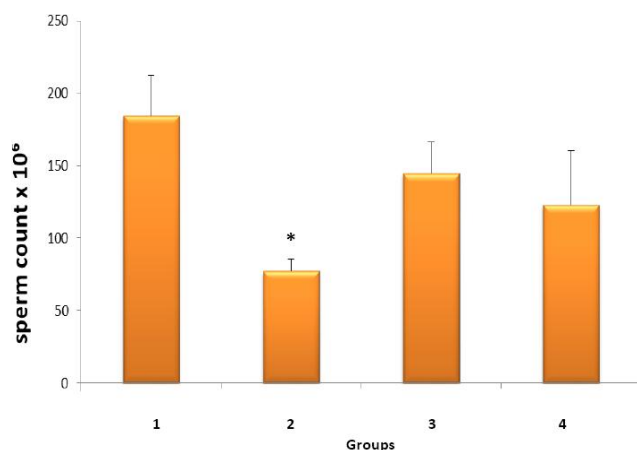


Figure 2 : Sperm count in normal and diabetic rats. Data were expressed as means ± SEM; n=4; * statistical significant versus normal control (p<0.05)

Epididymal sperm characters

Examination of semen parameters of rats given orally with *A.excelsa* leaf extract revealed significant increases in progressive sperm motility and sperm cell count associated with a decrease in sperm cell abnormalities as compared to the diabetic control group. The adminis-

tration of *A.excelsa* and metformin on diabetic rats for 28 days was increased the number of sperm for 37.32% and 46.63% respectively.

Level of serum testosterone

Serum testosterone levels in diabetic rats given orally ethanolic extract of *A.excelsa* leaf and metformin for 28 consecutive days were 778.39 ± 61.6 and 888.46 ± 94.1 pg/dL, respectively, versus to 655.85 ± 324.4 pg/dL in the diabetic control group. Serum testosterone level of the normal control rats was 908.60 ± 283.7 pg/dL as recorded in Figure 3.

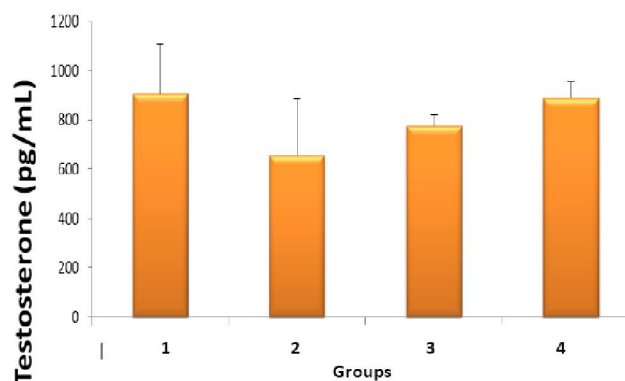


Figure 3 : Effect of oral administration of *A.excelsa* leaf for 28 successive days on serum testosterone level in male diabetic rats. Data are expressed as means ± S.E.M; n=4

Histology of the uterine wall

The histological examination showed a large number stromal cells and absence of atrophy in uterus of normal rats. However, the stromal cells appeared to decrease and atrophy easy to occur in uterus of diabetic rats. However, administration of *A.excelsa* leaf extract appears to improve the number of stromal cells. The normal distribution of stromal cell is crucial for pregnancy to occur. According to histological patterns, administration of metformin in diabetic rats possesses a

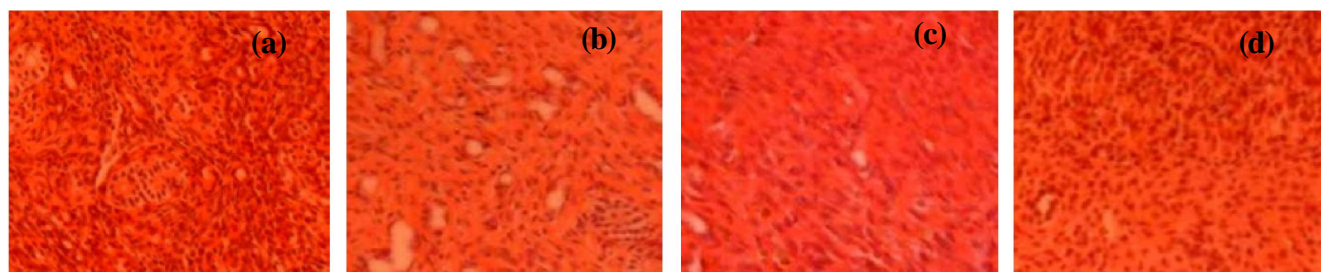


Figure 4 : Histological sections of uterine after treatment with the different formulations. (a) normal rats received saline; (b) diabetic rats received saline; (c) diabetic rats received metformin; (d) diabetic rats received ethanolic extract of *A.excelsa* at dose of 500 mg/kg; (d) diabetic rats received metformin

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lower number of stromal cells than diabetic control.

DISCUSSION

In this study, we examine how hyperglycemia-induced infertility in both male and female. Novel findings aimed at reducing hyperglycemia-induced infertility of male and female by *A. excelsa* leaves extract may be a useful adjuvant to antihyperglycemic therapies in the restoration of fertility and the prevention of diabetic.

Sustained hyperglycemia is considered as a major cause of sexual dysfunction in human population^[2]. The findings from our laboratory study revealed that a fall in blood glucose mediated by *A. excelsa* leaves extract provokes free radicals reduction and release insulin antagonist in diabetic rats. Aligned with other work, male rats with short-term diabetes show a greater than 30% reduction of serum testosterone^[5]. However, administration of *A. excelsa* leaves extract found to ameliorate testosterone level better than metformin. Much evidence supports the increasing of testosterone secretion is an indicator towards improving the semen parameters

In female, the normal distribution of stromal cell is crucial for pregnancy to occur. According to histological patterns, administration of metformin in diabetic rats clearly failed to repair the damage. This finding aligned with previous study that reported the metformin only able to improve hyperglycemia primarily by suppressing glucose production of the liver but did not have any antioxidant properties^[6].

CONCLUSION

It was observed that *A. excelsa* leaves extract gave promising result in enhancing hypoglycemic activity of alloxan induced male and female diabetic rat. The plant extract was able to reduce the blood glucose in diabetic rats better than metformin. Plus, the administration of *A. excelsa* had effectively increased the concentration of testosterone than untreated diabetic rats. Ameliorating the characters of epididymal sperm and distribution of stromal cells is an indicator towards curing diabetes-induced sexual dysfunction and compromised sexual potency.

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