



EFFECT OF *FICUS DELTOIDEA* LEAVES EXTRACTS ON BLOOD CLOTTING, SPERM QUALITY AND TESTOSTERONE LEVEL IN ALLOXAN-INDUCED MALE DIABETIC RATS

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ABSTRACT

Ficus deltoidea is believed to have a reputation for reducing the risk of diabetes and heart diseases. Diabetes mellitus has been known for many decades to be often associated with heart attack, infertility and sexual dysfunction in men. Therefore, the current study was designed to evaluate the antithrombotic activity of aqueous and ethanolic extract of *Ficus deltoidea* leaves and to investigate their effects have on the sperm quality and testosterone level of diabetic rats. The study was performed in five different groups for a period of four week. Two groups of diabetic rats were orally administered by aqueous and ethanolic extract of *Ficus deltoidea* leaves. The injection of alloxan monohydrate significantly decreased the testosterone level, sperm count and motility but significantly increased the blood glucose levels, blood clotting rate and sperm abnormalities. However, rats given aqueous and ethanolic extract of *Ficus deltoidea* leaves had significant improved the testosterone level, sperm count and motility. These treatments also significantly reduced the blood clotting rate, blood glucose levels and sperm abnormalities. In conclusion, effects of alloxan monohydrate induced on the blood clotting, sperm quality and testosterone level in male rats might be reversed by oral administration of the aqueous and ethanolic extract of *Ficus deltoidea* leaves.

Keywords: *Ficus deltoidea*, Diabetes, Sperm quality, Testosterone, Thrombin.

INTRODUCTION

Diabetes mellitus is a heterogenous metabolic disease and often considered as a contributing factor to impotence, infertility and sexual dysfunction in men. Infertility is one of the major health problems in life and approximately 30% of this problem is due to male factors¹. Previous studies have revealed that diabetic men have 60% increases in sperm DNA fragmentation compared to non-diabetic men due to oxidative stress. This might be responsible for the poor embryonic development and pregnancy outcome in these individuals' partners². Growing evidence also indicates platelet and clotting abnormalities in diabetes³, but little is known about their relationship to antidiabetic activity. This has lead to several research attempts to find plants with potential hypoglycemic activities to ameliorate male fertility and blood clotting process.

Ficus deltoidea, locally known as mas cotek, serapat angin and telinga beruk is becoming accepted amongst Malaysian community as food supplement. It has been used traditionally for many decades to take care of diabetes and cardiovascular disease. However, till now there is little scientific report regarding to these claim. The present study was aimed at determining the ameliorative effects of aqueous and ethanolic extract of *Ficus deltoidea* on the blood clotting rate, sperm quality parameter and serum testosterone level in alloxan induced male *Sprague dawley* rats. Since alloxan toxicity has been associated with infertility, increased abnormal sperm and motility, decrease serum testosterone level which are complications of diabetes mellitus, the

ameliorative effects of this plant on these complications of diabetes therefore need to be evaluated.

MATERIALS AND METHODS

Plant Material

The dried plant sample obtained from Sri Ain D' Herbs Sdn. Bhd. A voucher number (SK1510/2007) is housed in the Phytomedicinal Herbarium, Institute of Biosciences, University Putra Malaysia, Serdang, Selangor. The leaves were chopped into small pieces.

Preparation of plant extract

Aqueous extract of *Ficus deltoidea* leaves

Ficus deltoidea leaves (200 g) were extracted with 1000 ml distilled water by continuous hot extraction at 60 °C for 6 hours. The mixture was then filtered to obtain the extract. The extract was completely lyophilized by continuous freeze drying operation for 54 hours, yielding 20 g of crude extract. A dark semi-solid material was stored at 4°C until usage.

Ethanolic extract of *Ficus deltoidea* leaves

Ficus deltoidea leaves (500 g) were extracted with 80% ethanol (2.5L) for 3 days at room temperature (27°C). The mixture was then filtered to obtain the extract. The extract was concentrated at 40°C to yield 50 g of crude extract. A dark semi-solid material was stored at 4°C. The crude extracts was first dissolved in water and used in this study⁴.



Experimental design

Fifty male *Sprague dawley* rats weighing approximately 200 g were divided into five groups, which were: Group I (normal control), Group II (diabetic control), Group III (diabetic treated with metformin), Group IV (diabetic treated with aqueous extract) and Group V (diabetic treated with ethanolic extract). The animals were kept in the animal room, Faculty of Applied Sciences under standard laboratory conditions of humidity, temperature and light. The rats were fed on a standard pellet diet and water *ad libitum*.

Induction of diabetic condition using alloxan

The rats were intravenously injected with alloxan monohydrate dissolved in sterile normal saline at dose of 150 mg/kg body weight. Since alloxan is capable of producing total hypoglycemic as a result of massive pancreatic insulin release, rats were then treated with 20% glucose solution (15-20 mL) intraperitoneally after 6 hours. The rats were fed for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia⁵. After two weeks, rats with moderate diabetes having hyperglycemia with blood glucose range of 200-260 mg/100 mL were used for the experiment. Blood glucose concentration was determined using Accu-check Advantages II glucometer, based on the glucose oxidase method.

CaCl₂-induced clotting time assay

This assay allows for the determination of a 50% clotting time and effect on fibrin formation. The extracts were screened at 1 and 10 mg/ml saline, using 2% DMSO (v/v) to solubilise the plant extracts. The assay was performed by adding plant extract (40 µl) to rat plasma (100 µl). The reaction was mixed and left to incubate for 5 min at room temperature. Clotting was induced by the addition of 20 µl 0.16 M CaCl₂, and the reaction was followed at 412 nm with a microtitre plate reader for 2 hours at 3 minutes intervals.

Sperm count

The right testes was cut into very fine pieces using a scalpel and homogenized for 20 minutes in 50 mL of STM solution containing 0.9 NaCl, 0.05% Triton X-100 and 0.01% merthiolate. Four separate hemocytometer slides were prepared and the testes sperms were counted under the light microscopy with the use of a manual counter⁶.

Sperm motility

The left testes were chopped by a sharp blade and was immersed in 1 mL of physiological saline and the solution was keeping in 37°C. After gentle mixing, a dropped of the solution took on Neubaur chambers counting and then each was assessed for sperm motility. This was done by counting motile and non-motile sperm in different fields and was expressed as percentage. All the solutions and instruments that were used in this experiment were kept in an incubator at 37°C⁶.

Determination of testosterone level

This hormone assay was performed using Cayman's testosterone enzyme immunoassay kit provided with 96-wells microtiter plate. The plate was coated with mouse anti-rabbit IgG and blocked with a proprietary formulation of proteins. The plate was observed at a wavelength of between 405 and 420 nm with an increment of 5nm.

Statistical analysis

Data was represented as mean ± S.E.M. Student t-test and ANOVA analysis were applied to test the significance of differences between the results of the treated, untreated and control group⁶.

RESULTS AND DISCUSSION

CaCl₂-induced clotting time assay

Figure 1 shows that untreated diabetic rats have the highest activity of CaCl₂ induced clotting time activity compared to normal diabetic rats which is 62.8%. However, the administration of metformin, aqueous and ethanolic extract among diabetic rats significantly inhibited the clotting time by 34.44%, 37.4% and 47.7% respectively. This might be due to antidiabetic activity possess in all treatments. Previous researches indicate a few plants have high antidiabetic activity which prolonged blood clotting time^{7,8}.

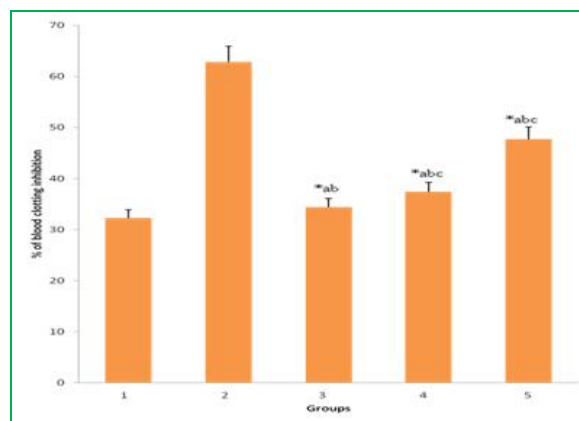


Figure 1: Effect of aqueous and ethanolic extract of *Ficus deltoidea* on CaCl₂-induced clotting time assay in normal and alloxan-induced diabetic male *Sprague dawley* rats. Data are expressed as means ± S.E.M; n = 3; *a = statistical significance versus normal control (p< 0.05); *b, statistical significance versus diabetic control (p<0.05); *c statistical significance versus diabetic treated with metformin.

Sperm count

Data in Figure 2 show the sperm count of untreated diabetic group decreased by 55.7% as compared with the normal control group. The significant reduction of sperm count seen in the diabetic rats may be derived from a hormonal imbalance including serum levels of testosterone. However, the oral administration of aqueous and ethanolic extract of *Ficus deltoidea* leaves among diabetic rats successfully increased the number of sperm but these treatments failed to normalized the



sperm count. Previous research indicate that *Ficus deltoidea* has high antioxidant compounds such as flavonoids, tetrapenoids, tannins and phenols which might enhance the number of sperms in diabetic rats^{9,10}.

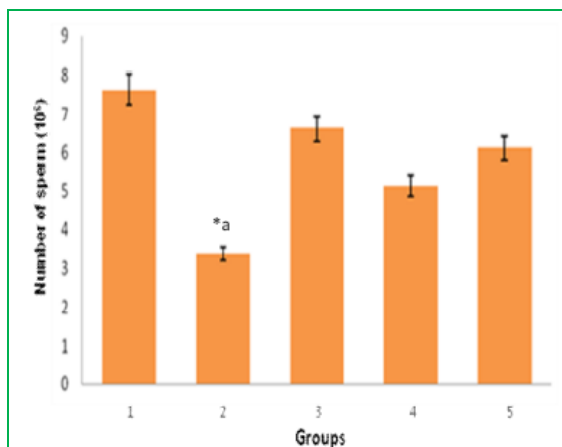


Figure 2: Effect of aqueous and ethanolic extract of *Ficus deltoidea* on the sperm count in normal and alloxan-induced diabetic male *Sprague dawley* rats. Data are expressed as means \pm S.E.M (n=5); *a, statistical significance versus normal control (p<0.05); *b, statistical significance versus diabetic control (p<0.05).

Sperm motility and abnormalities

Figure 3 shows the percentage of motile and abnormal sperm in the diabetic male rats. There was significant reduction in sperm motility of untreated diabetic group when compared with normal control rats. Lower motility of the sperms in diabetic rats might due to the lower glucose oxidation and utilization which is associated with the lack of insulin production⁶, reported that glucose oxidation and utilization are important means by which spermatozoa derives energy for their motility.¹¹ demonstrated that seminal plasma contain immune reactive insulin in higher concentration than serum, and suggested a role for insulin in the regulation of sperm metabolism and motility¹².

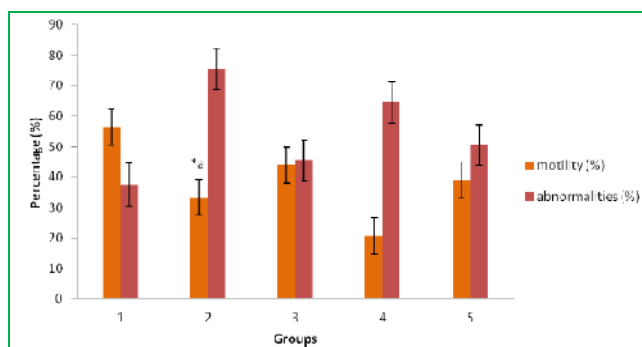


Figure 3: Effect of aqueous and ethanolic extract of *Ficus deltoidea* leaf on sperm motility, morphology and maturity in normal and alloxan-induced diabetic male *Sprague dawley* rats. Data are expressed as means \pm S.E.M (n=5); *a, statistical significance versus normal control (p<0.05); *b, statistical significance versus diabetic control (p<0.05).

Serum testosterone level

Testosterone is one of the requirements to maintain normal spermatogenesis. Figure 4 show that intravenous

injection of alloxan monohydrate at dose of 150 mg/kg body weight to normal rats induced a significant decrease in serum testosterone to 7.5 pg/ml versus to 15.5 pg/ml in normal rats. This is in line with an earlier report in humans¹⁶ and in rats¹⁷. However, oral administration of aqueous and ethanolic extract of *Ficus deltoidea* at a dose of 800 mg/kg body weight for four weeks causes significant increase by 53.3% and 32.4% respectively.

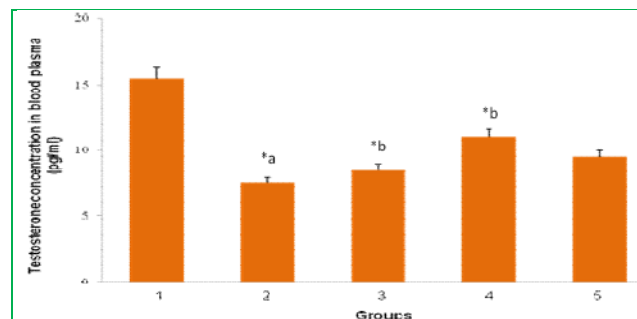


Figure 4: Effect of aqueous and ethanolic extract of *Ficus deltoidea* on testosterone concentration in normal and alloxan-induced diabetic male *Sprague dawley* rats. Data are expressed as mean \pm SEM, (n = 3); *a, statistical significance versus normal control (p<0.05); *b, statistical significance versus diabetic control (p<0.05).

CONCLUSION

The present results clearly indicated that the *Ficus deltoidea* extracts could be presume as a popular food supplement lead to ameliorative testosterone level, inhibit the clotting time and counteract of the number of sperm in male alloxan-induced diabetic rats.

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