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Cytotoxicity Activity and Reproductive Profiles of Male Rats Treated with Methanolic extracts of *Ficus deltoidea*

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ABSTRACT

Ficus deltoidea is a shrub belongs to Moraceae family. The Malaysian traditional medicinal practitioners use the *F. deltoidea* leaves to treat poor circulation after giving birth and to reduce blood glucose level and blood pressure. In this study, methanolic extract of *F. deltoidea* fruit and leaf were tested on human leukemic HL 60 cell line for their cytotoxicity activity and its effects on reproductive system of male rats. The methanol extracts was employed to assess its effects on body weight, testes, epididymis, sperm count, sperm viability and sperm morphology. Both methanolic leaf and fruit extracts showed significant cytotoxic activity against HL-60 cell line with IC₅₀ value of 11.4 and 13.6 µg mL⁻¹, respectively. The administration of the *F. deltoidea* leaf extract did not significantly alter the body weight of male rats. Testes and epididymis weight, sperm count, sperm viability and the number of normal sperm morphology were significantly decreased when treated with leaf extract. Meanwhile, rats treated with extract from stem showed significant increase in epididymis weight, sperm count and sperm viability. However, there is a reduction in testes weight and the number of normal sperm morphology of male rats. Cytotoxic activity against HL 60 cell line of the leaf methanolic extract was more potent than fruit methanolic extract. There was a significant effect on testes and epididymis weight, sperm count, sperm viability and the number of normal sperm morphology. However, the mechanism of action of *F. deltoidea* extracts still remains unclear and an intensive research should be needed in the future.

Key words: Cytotoxicity, HL-60 cell lines, *Ficus deltoidea*, sperm analysis, reproductive system

INTRODUCTION

In Malaysia, *F. deltoidea* family Moraceae is commonly known as mas cotek, serapat angina, telinga beruk, ara terbang and kangkalibang. The aerial parts of *F. deltoidea* are highly commercialized. The traditional medicinal practitioners used the decoction of the leaves to treat various diseases. Various parts of the plant can be used to treat poor blood circulation after giving birth (Zaki, 2005). Medicinally, the fruit and leaf can be used to recover arthritis and to reduce blood glucose and blood pressure (Aris *et al.*, 2009). There were few studies have been done on the identification of phytochemicals and biological activities of this plant. It was reported that petroleum ether, chloroform, methanol and water extracts of *F. deltoidea* leaves contain saponin,

amino acid, flavonoids and terpenoids (Shafaei *et al.*, 2011). It was also found that extracts of the plant showed more than 90% inhibition when tested with Ferric Thiocyanate Method (FTC) and thiobarbituric acid method (TBA) and good radical scavenging activity with DPPH method. Previous finding has reported the blood glucose lowering effect of *F. deltoidea* (Aminudin *et al.*, 2007; Adam *et al.*, 2007). Another study on the antidiabetic mechanism of *F. deltoidea* has revealed that methanolic extract of this plant stimulated insulin secretion from BRIN BD11 cell lines by 1-enhance both basal and insulin-stimulated glucose 3 folds as compared to 1.1 mM glucose alone (Hamid *et al.*, 2008). According to Adam *et al.* (2009) *F. deltoidea* extracts have the ability to enhance the basal and insulin-stimulated glucose uptake into liver cell and possess either insulin-mimetic activity or insulin-sensitizing activity or combination of both activities. It was also found that extracts from *F. deltoidea* possess α -glucosidase inhibitory activity *in vitro* (Adam *et al.*, 2010). Post-treatment of the aqueous extracts of the leaf and fruits of *F. deltoidea* (50 mg kg⁻¹) reduced significantly the external glucose load. The leaf and fruit aqueous extracts showed non-toxic effect in the brine shrimp toxicity test.

Until now, cytotoxicity study on *F. deltoidea* plant extracts was very limited. The cytotoxicity of compounds and crude plant extracts can be tested using various bioassay such as brine shrimp lethality bioassay (Uddin *et al.*, 2003; Ahmad *et al.*, 2003), XTT assay (Aisha *et al.*, 2011) and MTT assay (Endrini *et al.*, 2007). The present study has been done to evaluate the cytotoxicity effect of *F. deltoidea* extract on human leukemic HL 60 cell lines. In addition, the methanol extract of *F. deltoidea* leaf and stem was employed to assess its effects on male rat reproductive system.

MATERIALS AND METHODS

Plant materials: Leaf, fruit and stem of *F. deltoidea* were collected in 2010 from forest near Lata Kinjang, Tapah Perak, West Malaysia. The herbarium voucher specimen was identified by Shamsul Khamis, Institute of Bioscience, Universiti Putra Malaysia.

Preparation of methanolic extract: The leaf, stem and fruit were air dried and then ground to powdery form. The ground leaf, stem and fruit of *F. deltoidea* were extracted with methanol. The extracts were evaporated to dryness under reduced pressure. The process was repeated three times to give dark brown crude methanolic extracts.

Culture of cells: The human leukemic HL-60 cell lines were obtained from Institute of Bioscience, Universiti Putra Malaysia. Cells (from monolayer subcultures) were trypsinized and resuspended in complete growth medium (CGM) (10 mL). Cells were maintained in 25 cm² flasks (Nunc, Denmark) with 10 mL of CGM in a CO₂ incubator at 37°C until confluence was achieved. Medium supplemented with 10% (w/v) FBS (Fetal bovine serum) and 1% penicillin/streptomycin mixture was aspirated from MCF-7 cells grown to about 90% confluence. Cells were washed with PBS (Phosphate buffer solution), trypsinized, counted with a haemocytometer and diluted with RPMI-1640 to 5×10⁵ cells mL⁻¹.

MTT assay: Cytotoxicity or antiproliferative activity was determined using MTT assay (Mosmann, 1983).

Animals: Sprague-Dawley male rats (180-250 g) were obtained from the Animals House, Institute for Medical Research (IMR). The animals were kept in wire mesh cages, acclimated to laboratory

conditions (12 D: 12 L; 25+1°C) and had access to food and water *ad libitum*. Each animal was certified fertile by isolated mating technique before inclusion in the study.

Experimental design: A total of 12 male rats divided into three equal groups and treated as follow: group 1 (n = 12) were administered with 0.1 mL distilled water and served as control meanwhile group 2 and 3 administered with 50 mg kg⁻¹ b.w day⁻¹ of the *F. deltoidea* leaf and stem methanol extracts, respectively for three weeks. During the experiment, body weight was recorded every week. At the end of the treatment period, rats were sacrificed by cervical dislocation. The testis and epididymis were excised from the animals, cleared of adherent tissues and weight.

Sperm analysis: The sperm analysis consisted in the determination of sperm concentration, progressive motility and morphology. The sperm count was performed as previously described Raji *et al.* (2006).

Statistical analysis: All data were expressed as Mean±SEM. Differences between control and treated animals were analyzed by Student's t-test using (SPSS) for Windows. The p values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In cytotoxicity study, MTT assay was used to analyze the cytotoxic effect of methanolic *F. deltoidea* extract on human leukemic HL-60 cell lines. Yellow MTT enters the cells and it will be reduced to a colored, insoluble formazon in the mitochondria of the living cells. The cells were then solubilised with an organic solvent DMSO (Mosmann, 1983). The results of cytotoxic activity of leaf and fruits of *F. deltoidea* methanolic extracts against HL-60 cell line are summarized in Table 1. The IC₅₀ value of the positive control, goniiothalamine against HL-60 cell was 2.4 20 µg mL⁻¹. Fruit extract produced a dose-dependent decline in cell viability with IC₅₀ (inhibitor concentration required for 50% inhibition of cell viability) of 13.6 µg mL⁻¹ as determined by MTT assay after 4 h exposure. The leaf extract moderately inhibited the growth of human leukemic HL-60 cell lines with an IC₅₀ value of 11.4 µg mL⁻¹. The leaf extract showed better inhibition compared to the fruit extract. The crude extract of a plant is considered cytotoxic against the treated cells if IC₅₀ value obtained is less than 20 µg mL⁻¹ (Geran *et al.*, 1972). Thus, the *F. deltoidea* fruit and leaf extracts were shown to possess the bioactive compounds that need to be studied further. It was found that microscopic evaluation of the dead HL-60 treated with *F. deltoidea* fruit and leaf extracts exhibited cellular DNA fragmentation. This suggested that the mechanism of action responsible for the death of HL-60 cell lines may be caused by apoptosis. However, further evaluation on apoptotic cell death is necessary which can be done by TUNEL method (terminal deoxynucleotidyl transferase (TdT)-biotin nick end-labeling) (Khorshid *et al.*, 2011; Ali *et al.*, 2011).

Table 1: *In vitro* antiproliferative activity (IC₅₀ µg mL⁻¹) of leaf and fruits of *Ficus deltoidea* methanolic extracts against HL-60 cell line

Extract	IC ₅₀ (µg mL ⁻¹)
Positive control (Goniiothalamine)	2.4
<i>Ficus deltoidea</i> fruit	13.6
<i>Ficus deltoidea</i> leaf	11.4

Table 2: Effect of *Ficus deltoidea* on male rat body weight after 3 weeks

Treatment	Initial (g)	Final (g)
Control	204.50±6.19	217.00±6.65
Leaf	216.50±8.22	201.80±7.18
Stem	196.83±7.19	186.75±7.56

Values are presented as Mean±SEM, n = 12

Table 3: Effects of *Ficus deltoidea* on sperm count, viability and normal morphology

Treatment	Sperm count (10^6 mL^{-1})	Sperm viability (%)	Normal morphology (%)
Control	86.92±6.55	83.80±0.95	86.44±3.54
Leaf	41.40±3.71	43.13±0.68	54.71±6.97
Stem	152.75±8.13	97.60±0.81	84.29±5.03

Values are presented as Mean±SEM, n = 12

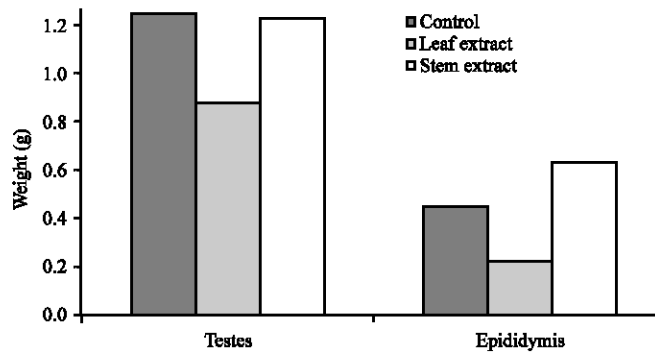


Fig. 1: Weight of testes and epididymis of male rats treated with leaf and stem extract of *Ficus deltoidea*

The body weight did not change significantly during 3 weeks of treatment (Table 2). All control rats recorded about 5% weight gain within the period of the experiment. The body weight of the male rats was reduced to 14.7 and 10.08 g when treated with methanolic leaf and stem extract of *F. deltoidea*, respectively.

There was a significant ($p < 0.05$) decrease in the weight of testes and epididymis in rats treated with methanolic leaf extract of *F. deltoidea* compared to control (Fig. 1). A significant increase ($p < 0.05$) in epididymis weight was however, recorded in the rats treated with methanolic stem extract of *F. deltoidea*. Rats from the same group showed insignificant ($p < 0.05$) reduction in testes weight.

A significant ($p < 0.05$) reduction in sperm count was recorded in rats treated with methanolic leaf extract of *F. deltoidea*. However, a significant ($p < 0.05$) increase in sperm count was recorded in rats treated with methanolic stem extract of *F. deltoidea* when compared with the control group (Table 3). Percentage live spermatozoa also decreased significantly ($p < 0.05$) in methanolic leaf treated group but significantly increased ($p < 0.05$) in methanolic stem treated group compared to control. The results of sperm morphology showed significant ($p < 0.05$) reduction of normal morphology in methanolic leaf treated group. Similarly, there was a reduction in normal morphology for rats treated with methanolic leaf extract of *F. deltoidea* but the reduction was not significant ($p > 0.05$).

The decrease in sperm count, sperm viability and normal sperm morphology in rats treated with methanolic leaf extract of *F. deltoidea* indicates interference with testicular spermatogenesis. The decreased in testes and epididymis weight, the presence of detached head and the impaired sperm motility in rats treated with *F. deltoidea* leaf extract suggest that the reproductive organ also maybe the target for *F. deltoidea* toxicity. In other research, *Quassia amara*'s toxicity on male reproductive system leads to the presence of fragile tails and detached heads, the impaired sperm motility and the decrease in tissue α -glucosidase (Parveen *et al.*, 2003).

The increase in sperm count indicates that *F. deltoidea* stem extract promote the process of spermatogenesis in testes. An increase number of sperm count may be associated with the increase in serum levels of testosterone. Testosterone was necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands (Prins *et al.*, 1991). The increase in weight of epididymis may also indicate the increase number of sperm produced by testes as reported earlier by Espinoza-Navarro and Bustos-Obregon (2005). The number of sperm viability also increased up to 14.13%, it may be associated with the improvement of epididymal function of maturation (Sadik *et al.*, 2001).

The results of this study demonstrate that the methanolic leaf extract of *F. deltoidea* decreased the sperm quality. Thus, it can be suggested that there is the possible exploitation of *F. deltoidea* extract as an antifertility agent. Interestingly, in contrast, *F. deltoidea* stem extract improved sperm quality.

CONCLUSION

In conclusion, the present study demonstrated the cytotoxic activity against human leukemic, HL-60 cell line of the leaf methanolic extract to be more potent than that of the fruit methanolic extract of *F. deltoidea*. Further study will be conducted to identify the principle and the mechanism by which the *F. deltoidea* extracts affects reproductive function in rats.

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