Phytochemical Characteristic and Uterotonic Effect of Aqueous Extract of Ficus deltoidea Leaves in Rats Uterus

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Abstract: *Ficus deltoidea* is traditionally consumed by Malay woman to augment labour and hastening parturition. This study was to investigate the phytochemical present and uterotonic activity of *F. deltoidea* var. Deltoidea (FDD) and *F. deltoidea* var. Angustifolia (FDA) leaves aqueous extract. FDD and FDA were qualitatively analysed. In uterine contraction activity, adult female Sprague Dawley rats were pretreated with 0.2 mg kg⁻¹ diethylstilbestrol 24 h to induce oestrus phase. The rats then killed and uterine horns were taken out, cut into two centimetres length and put into organ bath that connected to Powerlab instrument. The uterus separately tested with cumulative concentrations of FDD (10-1280 µg mL⁻¹), FDA (10-1280 µg mL⁻¹), oxytocin (0.02-0.64 µg mL⁻¹) and combination of oxytocin (0.08 µg mL⁻¹) with FDD and FDA (10-1280 µg mL⁻¹). FDD showed presence of flavonoid, saponin and tannin meanwhile FDA consist of flavonoid, tannin and terpenoid. Result showed FDD, FDA and oxytocin induced a dose-related increase in force of contraction of isolated rat uterus. The maximum uterine contraction (Emax) produced by FDD, FDA and oxytocin were at the concentration 640 µg mL⁻¹ (EC50, 5.903±0.529 µg mL), 20 µg mL⁻¹ (EC50, 290.5±0.18 µg mL⁻¹) and 0.4 µg mL⁻¹ (EC50, 0.060±0.011 µg mL⁻¹) respectively. Combination effects of oxytocin with FDD and FDA produced Emax at the concentration 80 µg mL⁻¹ (EC50, 270.3±0.643 µg mL⁻¹) and 1280 µg mL⁻¹ (EC50, 26.83±0.727 µg mL⁻¹), respectively. Study indicated *F. deltoidea* possess contractile effect on uterine contraction. This plant has great potential to develop as natural uterotonic agent in inducing labour and treatment for post-partum haemorrhage.

Key words: *Ficus deltoidea*, uterine contraction, uterotonic activity, oxytocin

INTRODUCTION

Traditionally, old folks rely on natural products especially plants to aid labour, to treat menstrual problems, health beneficial during pregnancy and for management of post-partum haemorrhage. In certain countries such as Africa, more than 90% of the populations consume plants as their major source of natural uterotonics agents. Study showed that there are at least 56 species of herb plants consumed by pregnant woman in Africa as uterotonics agents (Veale et al., 1992). *Ficus deltoidea* or mistletoe fig is a traditional herb that belong in the division of Magnoliophyta, class Magnoliopsida, order of Rosales and family Moraceae (Starr et al., 2003). This plant is native in peninsular Malaysia and distributed elsewhere such as Thailand, Indonesia and Philippines (Mat et al., 2012). Locally, it is known as *Mas cotek*. There are also some other name for *F. deltoidea* such as *serapat* and *semjit-sempit*. In Indonesia, *F. deltoidea* known as *tabat barito* meanwhile in Philippine it is called *angulora*. Based on its taxonomy, this plant consists of seven varieties which are known as var. Deltoidea, var. Angustifolia, var. Trenggamenensis, var. Bilobata, var. Intermedia, var. Kursileri and var. Motleyana (Mat et al., 2012). Among these varieties, *F. deltoidea* var. Angustifolia (FDA) and Deltoidea (FDD) are most commonly used by Malay populations. The differences of these two variants based on its leaf’s shape and size. Leaf of FDA is small-size, brood-soften shaped and has 1 red dot at the back of the leaf meanwhile for FDD is big, round leaf and has a few red spots at the back of the leaf (Musa et al., 2004).

*F. deltoidea* is gaining popularity among traditional practitioners because it is recognised for health benefits and medical value. All parts of this plant can be used to treat many ailments (Wahid et al., 2010). Fruits of *F. deltoidea* chewed to relief toothache (Sulaiman et al., 2008). Meanwhile the roots of *F. deltoidea* use to treat...
headache and fever (Mat-Salleh and Latiff, 2002). Traditionally, this plant used by local folks as treatment of diabetic, hypertension, gout, inflammation and pneumonia. Decoction of *F. deltoidea* leaves specially consumed by woman to augment labour, strengthen uterus after parturition and improved menstrual flow (Musa et al., 2004). Previous study showed aqueous extract of *F. deltoidea* possess antidiabetic activity (Farsi et al., 2011) and has ability to enhance wound healing (Abdulla et al., 2010). Based on sub-acute toxicity study, *F. deltoidea* tea has no toxicity signs in rats (Hadjial et al., 2004). FDD also contain higher antioxidant activities compared with FDA. However, there is still paucity of information of uterotonic activity by *F. deltoidea*. Hence, the present study was to characterize the phytochemical constituents and assess the uteroton effect of FDD and FDA leaves aqueous extract on isolated rat uterus.

**MATERIALS AND METHODS**

**Plant material:** *F. deltoidea* leaves were obtained from Juaseh Tengah in Negeri Sembilan, Malaysia. The plant samples were taxonomically identified and authenticated by the Herbarium, Faculty of Sciences and Technology, Malaysia National University with voucher number UKMB 29780 (FDA) and UKMB 29781 (FDD).

**Preparation of the extract:** After the leaves were cut into smaller pieces, 250 g of the leaves was extracted with distilled water for 16 hours by using Soxhlet apparatus. The extract was filtered and freeze dried to give 6.40 g (12.8% w/w) of the FDD and FDA dried aqueous extract. The extract material was kept in an air tight container and preserved in the refrigerator at 4°C until needed.

**Phytochemical screening:** FDD and FDA were qualitatively analysed for the presence of flavonoids, saponin, alkaloid, pholabatannins, resins, terpenoids, sterol, lipids, anthraquinones, tannins, cardiac glycosides and acidic compounds (Sofowora, 1993; Adetuyi and Popoola, 2001; Trease and Evans, 2002).

**Flavonoids (Shinoda test):** A 0.5 g of each portion of extracts was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

**Saponins:** A 0.2 g of the extracts was shaken with 5 mL of distilled water and then heated to boil. Frothing persisted on warming indicated the presence of saponins.

**Alkaloid (Mayer’s reagent):** One gram of extracts was treated with 5mL of 1% HCl in test tube. Reacted in water bath at 40°C for 10 min and filtered. 1mL of filtrate added with few drops of Mayer’s reagent. Presence of but colour precipitate indicated presence of alkaldid.

**Pholabatannins:** A 0.5 g of extracts was dissolved in distilled water and then filtered. The filtrate was boiled with 2% HCl solution. Red precipitate indicated presence of pholabatannins.

**Resins:** Five millimetres of distilled water was added into the extracts. Turbidity observed for the presence of resins.

**Terpenoids (Salkowsky method):** A 0.5 g of extracts was added into 2 millimetres chloroform. A few drops of concentrated H$_2$SO$_4$ carefully added to form a layer. A reddish colouration of the interface was form indicated positive result for terpenoids.

**Sterols (Liebermann buchard test):** A 0.2 g of extracts was dissolved in chloroform. A few drops of acetic anhydride were added along with a few drops of concentrated sulphuric acid from the sides of test tube and observed for the formation of blue to blood red colour.

**Lipids:** A small quantity of extracts was rubbed on a filter study. Presence of permanent translucent strain showed positive result for lipids.

**Anthraquinones (Borntrager’s test):** A 0.2 g of extracts was shaken with 10ml of benzene. The solution then filtered. 5 millimetres of 10% ammonia solution added into the filtrate and shaked. Appearance of pink, red, or violet in ammonia layer indicated positive result for anthraquinones.

**Tannins (Ferric chloride test):** A 0.2 g of extracts mixed with water and heated on water bath. Mixture was filtrated and ferric chloride was added into it. Dark green solutions showed positive result for tannins.

**Cardiac glycoside (Keller-Killiani test):** A 0.5 g of extracts was added with 0.4 mL glacial acetic acid containing a trace amount of ferric chloride. The solution transferred into small test tube. A 0.5 mL concentrated H$_2$SO$_4$ was carefully added by side of tube. Blue colour appears in acetic acid layer show positive result.

**Animals:** Virgin female Sprague-Dawley rats (weight 200-250 g) from Animal House Unit, Malaysia.
National University (UKM) were used. The animals were kept in the animal laboratory, Health Science Faculty, UKM. They were fed with standard laboratory pellet diet and water supplied ad libitum. All animal procedures were approved by Animal Ethics Committee, Malaysia National University (UKMAEC No: FSK/BIOMED2011/NIHAYAH/403-JUN/403-JUN-2011-JUN-2014).

**Uterine tissue experiment:** Virgin female *Sprague-dawley* rats were injected with 0.2 mg kg⁻¹ diethylstilbestrol 24 h prior to the start of the experiments in order to induce oestrous in the uterus. Once the vagina smear confirmed the rats in oestrus phase, the rats were killed and the uterine horn was taken out. Uterine strips cut approximately two centimetres, threaded and mounted in a 60 mL organ bath containing Tyrode solution which aerated with 95% O₂, 5% CO₂ and maintained at 37°C. Organ bath then connected to a Powerlab system (ADInstruments Pty. Ltd.). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection with a one gram corresponding weight. The uterine tissue was allowed to stabilize for 30 min before application of extracts or drugs.

**Effect of Ficus deltoidea leaves aqueous extract on uterus contraction:** Uterus contraction was tested with distilled water for 5 min as negative control. Uterus then washed three times with Tyrode solution. The effect of FDD, FDA (10-1280 µg mL⁻¹) and oxytocin (0.02-0.64 µg mL⁻¹) were tested alone for 5 min each concentration. Combination effects of oxytocin (0.08 µg mL⁻¹) with FDD and FDA (10-1280 µg mL⁻¹) were also tested.

**Statistical analysis:** Data are presented as Mean±SEM, with data obtained each 1 from different animal. 1 way analysis of variance (ANOVA) followed by post hoc Dunnett were use to analyse statistical different among groups. In all experiments, the contractile responses were expressed as a percentage of the maximal contractile response to a reference drug. EC₅₀ or IC₅₀ values were calculated using Graphpad Prism version 6.0 for windows, Graphpad Software, San Diego, Ca, USA. A probability of p<0.05 was accepted as significant.

**RESULTS**

**Phytochemical screening:** The result of the phytochemical screening of FDD and FDA leaves aqueous extract are presented in Table 1. When the FDD and FDA tested in Shinoda’s test, there were presences

| Table 1: Phytochemical screening of FDD and FDA leaves aqueous extract |
|---------------------------|-----------------|-----------------|
| Variables                 | FDD             | FDA             |
| Flavonoids                | +               | +               |
| Saponins                  | +               | -               |
| Alkaloid                   | -               | -               |
| Phenolic flavonoids        | -               | -               |
| Resins                     | -               | -               |
| Terpenoids                 | -               | +               |
| Sterol                     | -               | -               |
| Lipids                     | -               | -               |
| Anthraquinones             | -               | -               |
| Tannins                    | +               | +               |
| Cardiac glycosides         | -               | -               |

Phytochemical screening showed the presence of flavonoids, saponin and tannins for FDD and flavonoids, terpenoids and tannin for FDA. +: Positive, -: Negative

![Fig. 1: Effect of aqueous extract of FDD on uterine contraction. The aqueous extract of FDD produced uterine contraction in dose-dependent manner. The value represent Mean±SEM, n = 6 strips of red to purple colouration. Meanwhile, mixture of FDD and FDA with ferric chloride produced dark green solution. This proved that both FDD and FDA contain flavonoid and tannin. However, for saponin test, frothing persisted on warming presence for FDD only. This indicated FDD contain saponin. For FDA, this extract contains terpenoids due to formation of reddish colouration of the interface between chloroform and concentrated H₂SO₄ solution.](image)

**Effect of aqueous extract of FDD, FDA and oxytocin on uterus contraction:** Aqueous extract of FDD (Fig. 1), FDA (Fig. 2) and oxytocin (Fig. 3) induced a dose-related increase in force of contraction of isolated rat uterus. Oxytocin (EC₅₀, 0.06±0.011 µg mL⁻¹) showed the lowest EC₅₀ followed by FDD (EC₅₀, 5.90±0.529 µg mL⁻¹) and the highest was FDA (EC₅₀, 290.5±0.158 µg mL⁻¹). The highest uterine contraction (Emax) produced by FDD, FDA and oxytocin were at the concentration 640 µg mL⁻¹ (2.14±0.053 g), 20 µg mL⁻¹ (1.493±0.157 g) and 0.4 µg mL⁻¹ (2.546±0.112 g), respectively. However,
Fig. 2: Effect of aqueous extract of FDA on uterine contraction. The aqueous extract of FDA produced uterine contraction in dose-dependent manner. The value represent Means±SEM, n = 6 strips.

Fig. 3: Effect of oxytocin on uterine contraction. Oxytocin produce uterine contraction in dose-dependent manner at lower concentration compared to extract. The value represent Means±SEM, n = 6 strips.

Effect of combination of oxytocin with F. detoidea leaves aqueous extract on uterus contraction: Figure 4 and 5 showed the graph for the combination effect of oxytocin with aqueous extract of FDD and FDA on uterine contraction respectively. Addition of cumulative concentration of FDD and FDA in the presence of 0.08 μg mL⁻¹ oxytocin produced the Emax at the concentration of 80 μg mL⁻¹ (1.96±0.271 g) and 1280 μg mL⁻¹ (1.07±0.297 g), respectively. The presence of oxytocin (0.08 μg mL⁻¹) on FDD-induced uterine contraction resulted in the shifted to the left of the dose respond curve. There were no significant different between the EC50 of FDD (270.3±0.643 μg mL⁻¹) in the absence and presence of oxytocin 0.08 μg mL⁻¹. However, EC50 for FDA (26.83±0.727 μg mL⁻¹) showed significant different in the absence and presence of oxytocin 0.08 μg mL⁻¹.

Fig. 4: Effect of combination of oxytocin and FDA leaves aqueous extract. Combination of oxytocin (0.08 μg mL⁻¹) and FDA not significantly elicted increase in uterine contraction. The dose-response curve shifted to the left. The value represent Means±SEM, n = 6 strips.

Fig. 5: Effect of combination of oxytocin and FDD leaves aqueous extract. Combination of oxytocin (0.08 μg mL⁻¹) and FDD not significantly elicted increase in uterine contraction. The value represent Means±SEM, n = 6 strips.

DISCUSSION

The effect of FDD and FDA aqueous extract on isolated uterine strip able to produce rhythmic and spontaneous contraction with uterotonic-like activity. FDD showed the most effective in stimulate uterine contraction compared to FDA. This suggested that the
FDD contain more or different bioactive compounds compared to FDA which able to activate the pathway/s that involved in stimulation of uterine contraction. Studies showed that FDD consist high phenolic acid, phospholipid and flavonoids compared to FDA (Hakiman and Maziah, 2009). However, from the result, oxytocin is the most potent agent in induced uterine contraction compared to both extracts. This is because oxytocin is a pure compound meanwhile the extracts are crude extracts (Je and Zam, 2008).

Oxytocin binds to the oxytocin receptor (OTR), a classical membrane receptor with seven transmembrane domains linked through a G protein complex to a phospholipase C–protein kinase C signal transduction system (Phaneuf et al., 1993). After oxytocin stimulation, there are markedly increase intracellular concentrations of inositol trisphosphate and calcium ions (Ca²⁺). The higher Ca²⁺ and calmodulin increase the myosin light chain kinase which catalyzes the contraction response. Oxytocin has dual action in the uterus. Oxytocin binds to myometrium oxytocin receptor (OT1a) to produce uterine contraction. It is also act on oxytocin receptor (OT1b) at the endometrium to stimulate prostaglandin release (Dawood, 1995). The ability of F. deltoidea to produce uterine contraction is still unclear. Previous study showed that Musanga ceceropoides stem bark aqueous extract produce oxytocin-like effect towards uterine contraction through activation of muscarinic receptor (Ayinde et al., 2006). Moreover, aqueous extract of Ficus exasperate leaves stimulate uterine contraction via calcium channel, histamine (H1) receptor and also activation of α 1-adrenergic (Bafor et al., 2010). Different pathways involved in stimulation of uterine contraction by uterotonics plants are depend on active compounds presence in those plants. The dose response curve of FDD-induced uterine contraction in the presence of oxytocin shifted to the left proved that FDD has the potentiating effect. The FDD leaves aqueous extract probably enhance the binding of the oxytocin to OTR on myometrium tissues hence, producing the greater response of uterine contraction. Previous study showed that the aqueous extract of Globubetula braunii (Loranthaceae) has potentiating effects (Je and Zam, 2008). Addition of increasing concentrations of oxytocin in the presence of 80 mg mL⁻¹ aqueous extract of Globubetula braunii produced significant highest uterine contraction response compared to oxytocin and extract alone.

Previous phytochemical studies on the leaves of FDD and FDA revealed the presence of polyphenol, flavonoid and phenolic acid (Hakiman and Maziah, 2009). In this study, flavonoid was found to be present in both FDD and FDA. Common function of flavonoid includes antibacterial, antitumor, antiviral and platelet aggregation (Okwu and Omodamiro, 2005). Moreover, it is also inhibit lysosomal enzyme secretion and arachidonic acid release. This would explain the antiinflammatory activity produced by F. deltoidea. In present study, flavonoid is the most common secondary active compound in plant extracts and has been reported to have pharmacological actions of its own by acting on estrogen receptor and give effect towards uterine contraction (Revuelta et al., 1997). Others uterotonic plants such as Ficus exasperate aqueous extracts contain saponin and tannin (Bafor et al., 2010). In this study, both FDD and FDA contain tannin. Tannin is proven to have uterotonc effect through affecting calcium availability for uterine tissue and cardiac muscle contraction (Calixto et al., 1986; Polya et al., 1995). Other functions of tannin are anti-diarrhea, hemostatic and antihemorrhoidal compounds (Vattem et al., 2005). Thus, flavonoid and tannin may have a role in stimulating uterine contraction in this study. However, the exact chemical constituent in FDD and FDA that responsible for uterotonic properties is still remains speculative. Further studies are undergone to identify the active compounds presence in the extract using HPLC and NMR.

In conclusion, F. deltoidea leaves aqueous extract possess contractile effect towards rat's isolated uterine tissues. The uterotonic effect produce by these two extracts would explain the use of F. deltoidea plant in augment labour and as treatment of post-partum haemorrhage by Malay woman in Malaysia. The determination of uterotonc activity of crude extract of F. deltoidea provides starting point towards determination of F. deltoidea potential use in human as natural source uterotonc agent. Further study will be carried out to elucidate the mechanism of the F. deltoidea in stimulating uterine contraction and isolation of active compounds responsible for its uterotonc effect.

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