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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (9): 1046-1051, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.1046.1051

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±0.158 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 uterotonic agents. Study showed that there are at least 56 species of herb plants consumed by pregnant woman in Africa as uterotonic 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 *sempit-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. Trengganuensis, var. Bilobata, var. Intermedia, var. Kunstleri 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, broad-spoon 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

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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 (Hadijah *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 uterotonic 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 butt colour precipitate indicated presence of alkaloid.

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 (Salkowsi method): A 0.5 g of extracts was added into 2 millimetres chloroform. A few drops of concentrated H_2SO_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_2SO_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-NOV/403-NOV-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 oestrus 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% O_2, 5% CO_2 and maintained at 37°C. Organ bath then connected to a Powerlab system (ADIntruments 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. EC_{50} or IC_{50} 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 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 H_2SO_4 solution.

Table 1: Phytochemical screening of FDD and FDA leaves aqueous extract

Variables	FDD	FDA
Flavonoids	+	+
Saponin	+	-
Alkaloid	-	-
Pholabatannins	-	-
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

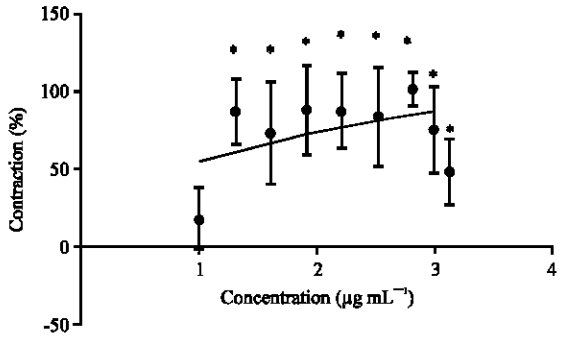

Fig. 1: Effect of aqueous extract of FDD on uterine contraction. The aqueous extract of FDD produced uterine contraction in dose-dependent manner. The value represent Means±SEM, n = 6 strips

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 (EC_{50}, 0.060±0.011 μg mL^{-1}) showed the lowest EC_{50} followed by FDD (EC50, 5.903±0.529 μg mL^{-1}) and the highest was FDA (EC_{50}, 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} (2.143±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,

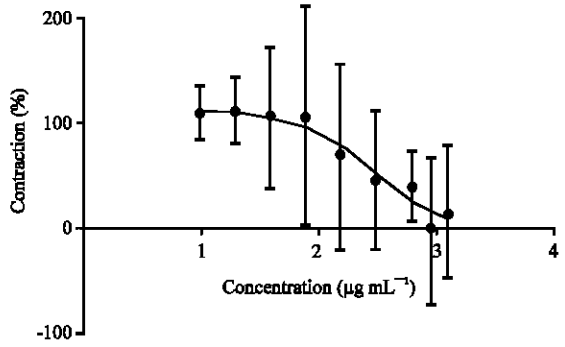

Fig. 2: Effect of aqueous extract of FDA on uterine contraction. The aqueous extract of FDA produced uterine contraction in dose-dependent manner. The value represent Means±SEM, n = 6 strips

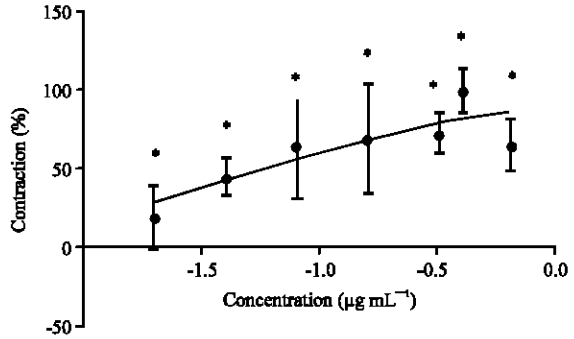

Fig. 3: Effect of oxytocin on uterine contraction. Oxytocin produce uterine contraction in dose-dependent manner at lower concentration compared to extract. The value represent Means±SEM, n = 6 strips

addition of the higher concentration of FDD, FDA and oxytocin after the concentration of Emax caused the decreased in uterine contraction.

Effect of combination of oxytocin with *F. deltoidea* 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} (1.964±0.271 g) and 1280 µg mL^{-1} (1.075±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,

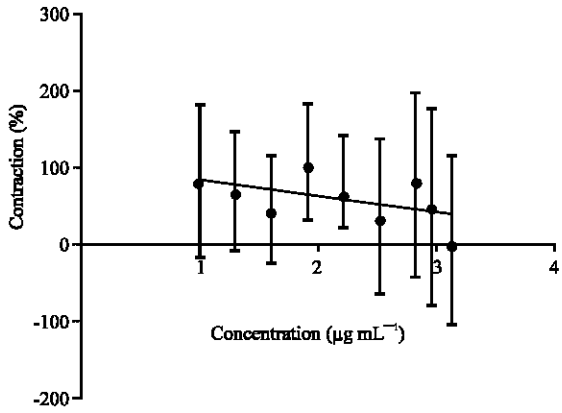

Fig. 4: Effect of combination of oxytocin and FDD leaves aqueous extract. Combination of oxytocin (0.08 µg mL^{-1}) and FDD not significantly elicited increase in uterine contraction. The dose-response curve shifted to the left. The value represent Means±SEM, n = 6 strips

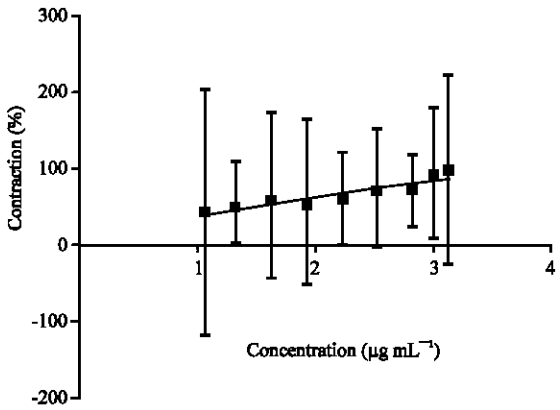

Fig. 5: Effect of combination of oxytocin and FDA leaves aqueous extract. Combination of oxytocin (0.08 µg mL^{-1}) and FDA not significantly elicited increase in uterine contraction. The value represent Means±SEM, n = 6 strips

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}.

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, pholiphenol 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 (Ie and Zam, 2008).

Oxytocin binds to the oxytocin receptor (OTR), is 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 (Ca^{2+}). The higher Ca^{2+} and calmodulin increase the myosin light chain kinase which catalyses 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 cecropioides* 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 uterotonic 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 *Globimetula braunii* (Loranthaceae) has potentiating effects (Ie and Zam, 2008). Addition of increasing concentrations of oxytocin in the presence of 80 mg mL^{-1} aqueous extract of *Globimetula 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 antiinflamatory 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 uterotonic 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 uterotonic activity of crude extract of *F. deltoidea* provides starting point towards determination of *F. deltoidea* potential use in human as natural source uterotonic 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 uterotonic effect.

ACKNOWLEDGEMENT

Research fund FRGS/1/2011/SKK/UKM/02/22.

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