Malays. Appl. Biol. (2017) 46(1): 147–152

Ficus deltoidea ETHANOLIC LEAVES EXTRACT IMPROVES HORMONAL BALANCE AMONG LETROZOLE INDUCED POLYCYSTIC OVARIAN SYNDROME (PCOS) RATS

NUR AJEERAH SUHAIMI*, NOORAAIN HASHIM and NURDIANA SAMSULRIZAL

School of Biological Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
**E-mail: nurajeerah0910@yahoo.com*

Accepted 31 January 2017, Published online 31 March 2017

ABSTRACT

The global prevalence of polycystic ovarian syndrome (PCOS) has reached epidemic proportion contributing to female infertility. The two phenotypes of PCOS, hyperandrogemia and hyperinsulinemia have gained much attention even the exact etiology of the syndrome remained unclear. In the present study, *Ficus deltoidea* was evaluated to elucidate the effects of its ethanolic leaves extract on several PCOS key hormones of female Sprague Dawley rats induced with letrozole (1.0 mg/kg bwt.). Experimental animals were divided into six groups (A, B, C, D, E and F). Five groups (B, C, D, E and F) were induced into PCOS using 1.0 mg/kg letrozole and the sixth group, Group A was the non PCOS normal control (NC). Groups of PCOS induced rats were treated with: saline (B, PC), 10 mg/kg bwt. clomiphene citrate (C, PCC), and Groups D, E and F were treated with 25, 125 and 250 mg/kg bwt. of *F. deltoidea* ethanolic leaves extracts, respectively, (PFD25, PFD125 and PFD250). Absence of estrus phase from rat reproductive cycle throughout the induction period (21 days) with significantly higher (p<0.05) testosterone level than NC rats were observed among PCOS induced groups manifested PCOS development in the studied animal model. Hormonal profiling at the end of treatment period revealed significant reduction (p<0.05) in testosterone, FSH and LH levels, but significant increase (p<0.05) in both progesterone and estrogen levels in extract treated groups compared with PC group. Meanwhile, insulin level was significantly reduced (p<0.05) with value 0.265±0.046 ng/mL in PFD125 group compared with that of PC rats (0.801±0.025 ng/mL) suggesting improved insulin sensitivity in the extract treated PCOS rats. In summary, these results indicate the potential use of *F. deltoidea* leaves in ameliorating PCOS associated risks, since it is capable of alleviating excess androgen and enhancing insulin sensitivity in letrozole-induced PCOS rats.

Key words: Serum hormones, estrous cycle, letrozole, polycystic ovarian syndrome, *F. deltoidea,* ethanolic leaves extract, infertility

INTRODUCTION

Nowadays, reproductive failure is a significant concern worldwide in which infertility affects approximately 8-12% couples globally, involving 50-80 million people (Hajshafiha *et al*., 2013). World Health Organization (WHO) has classified infertility as a disease that is defined as inability to conceive after 12 months of unprotected sexual intercourse (Cooper *et al.,* 2010). This has contributed to declining total fertility rate (TFR) in many developing countries over the past decades and it is projected that the trend will continue if immediate effective measure is not taken.

Polycystic ovarian syndrome (PCOS) is the most common cause of infertility among females. It is a multifaceted heterogeneous disorder of clinical and public health importance affecting almost 20% of women in their child bearing age. PCOS is often associated with significant and diverse implications including reproductive, endocrine, metabolic and also psychological features (Teede *et al.,* 2010). Being the most common cause of an ovulatory, that accounts for 75% of overall infertility cases, PCOS patients usually have elevated circulating luteinizing hormone (LH), androgens and insulin often manifested by hirsutism, obesity and enlarged ovary with multiple cysts, leading to oligomenorrhea or amenorrhea (Saad, 2009).

Ficus deltoidea or locally known as 'Mas Cotek' in Malaysia is a high medicinal value plant commonly used by oldfolks for multiple purposes in daily life. All parts of the plant which include leaves,

* To whom correspondence should be addressed.

fruits, stems and roots are very useful especially for women wellbeing. Traditionally, decoction of the plant part is prepared and taken to alleviate various illnesses. These include; to serve as postpartum medicine, helping in the contraction of uterine and vagina muscles, to improve blood circulation, to regain body strength and to relieve period pain during days of menstruation (Sulaiman *et al.,* 2008). As the plant is getting more attention worldwide, various scientific studies have been performed to ascertain its pharmacological activities. Extensive research revealed discoveries of voluminous pharmacological properties of the plant. Among those are antioxidant and anti-diabetic activities (Adam *et al.,* 2012), anti-inflammation and anti-nociceptive (Abdullah *et al.,* 2009), wound healing (Abdulla *et al.,* 2010), antiulcerogenic (Zahra *et al.,* 2009), antibacterial (Suryati *et al.,* 2011), anticancer (Mat-Akhir *et al.,* 2011), antimelanogenic (Oh *et al.,* 2011) and natural uterotonic agent in inducing labor (Umi *et al.,* 2015). However, best to our knowledge, there is limited information pertaining to the effects of *F. deltoidea* leaves extract on PCOS model rats. Thus, the aim of the present study was to provide scientific information emphasizing on effects of *F. deltoidea* leaves on hormonal status of letrozole-induced PCOS in Sprague Dawley rats. We hypothesized that *F. deltoidea* leaves ethanolic extract may rectify hormonal imbalances in letrozole induced PCOS animal model.

MATERIALS AND METHODS

Preparation of *F. deltoidea* leaves ethanolic extract

Fresh leaves of *F. deltoidea* were collected from Forest Research Institute of Malaysia (FRIM), Kuala Lumpur. The sample was deposited at the Herbarium Unit, Universiti Kebangsaan Malaysia for identification of the plant species with voucher number UKMB40315. The ethanolic extraction of the leaves began by washing the samples thoroughly and oven-dried at 37±5°C. Then it was ground to form fine powder and soaked in 80% ethanol inside covered conical flasks for three days at room temperature. The mixture later was filtered and evaporation of the ethanolic residues from the filtrate was conducted using rotary evaporator at 40°C (Shafie *et al.,* 2015). The yield appeared as dark brown semi solid paste, stored at 4°C until use.

Animals

A total of 36 female Sprague Dawley rats procured from Chennur Supplier, Seri Kembangan were used with age of six weeks, weighing between 180 to 220 g. The selected females exhibited normal four to five-days of estrus cycle. The animals were acclimatized at room temperature (25±2°C) with controlled light/dark cycle to be switched on and off at 0800 and 2000, respectively. They were kept in the Animal Holding Facility, Faculty of Health Sciences, UiTM Selangor, Puncak Alam Campus. The animals were given free access to food and water *ad libitum*. All procedures were carried out in accordance to the guide for the care and use of laboratory animals as approved by institutional Committee on Animal Research and Ethics (UiTM CARE) with reference no: 59/2014.

Initial screening on procured female rats

The ovarian cycle of the procured female rats were closely observed following acclimatization period. Cytology of vaginal lavage was examined. The air-dried vaginal lavages were prepared and stained using crystal violet and observed under light microscopy at total magnification of 100*x*. Stages of estrous cycle were identified based on relative proportion of three distinctive cells observed in the smears. Animals with four to five days estrous cycle were determined and used in the experiment.

Induction of PCOS among experimental rats

Selected females were randomly divided into six groups with six rats each (n=6). Five groups were administered with 1.0 mg/kg body weight (bwt). letrozole dissolved in 2.0 mL/kg bwt. of 0.5% carboxymethylcellulose (CMC). Induction was given to the rats for 21 consecutive days orally, once daily. The sixth group was tagged as normal control (NC) and given the vehicle throughout the induction period.

Testosterone level signified excess androgen in letrozole-induced PCOS model

Serum testosterone level was tested among females after 21 days of PCOS induction period. Rats exhibited higher titer of testosterone level (Mamata *et al.,* 2013; Walters *et al.,* 2012) than that of the normal rats (Table 2) with pronounced absence of estrus stage in their estrous cycle were selected to be used in the experimental design for testing the effect of ethanolic extract of *F. deltoidea* leaves on serum hormones of letrozole-induced PCOS female rats.

Experimental design involving PCOS rats treated with ethanolic extract of *F. deltoida* leaves

Three doses of *F. deltoidea* leaves extract which were 25, 125 and 250 mg/kg bwt were administered to three different groups of PCOS females. Meanwhile, a positive control PCOS group (PCC) was given 10 mg/kg bwt. clomiphene citrate while another group of PCOS and a group of non-PCOS females were given saline and tagged as PCOS control (PC) and normal control (NC), respectively.

Table 1. Experimental groups with their respective six weeks of treatments

Group	Treatment	Remark
NC	Normal (Non PCOS) + saline	Normal control
PC	PCOS + saline	Negative control
PCC	PCOS rats + 10mg/kg bwt. clomiphene	Positive control
FD 25	PCOS + 25mg/kg bwt. plant extract	
PFD125	PCOS + 125 mg/kg bwt. plant extract	Test
PFD250	PCOS + 250 mg/kg bwt. plant extract	

The treatments were given for a period of six weeks, once daily via oral gavage. The leaves extract and clomiphene were suspended in normal saline (vehicle) at a volume of 1 mL/100 g bwt. The normal and PCOS control rats received the vehicle throughout the treatment period. The experimental design is summarized in Table 1.

Blood sampling

Twenty four hours after the last dose of each treatment, rats were checked for their reproductive cycle and sacrificed at the stage of estrus. Those exhibited proestrus, metestrus and diestrus were kept until the next estrus for euthanasia. Prior to euthanasia, rats were anesthetized using combination of ketamine and xylazine (8.0:0.8 mL). Approximately 5.0 mL of blood sample was collected into serum tube from each rat. Blood samples were centrifuged at 5000 rpm for 15 minutes. The serum was stored in vials at -80°C until use.

Assessments on hormones among experimental animals

Hormones included testosterone, LH, FSH and insulin were analyzed using enzyme immunoassay (EIA) kits while progesterone and estrogen levels were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Statistical Analysis

The collected data were analyzed using the Statistical Package for Social Science (SPSS). A significance difference between means of the test and control groups was established using Oneway Analysis of Variance (ANOVA) followed by post-hoc Duncan test for multiple group comparison. A value of $p<0.05$ was used to denote statistical significance. All results were expressed as mean±SEM.

RESULTS

Table 2 shows mean concentrations of testosterone at the onset of PCOS in the experimental female rats. A significant increase in testosterone levels was observed in all groups of rats receiving continuous administration of letrozole. In addition, their reproductive cycle had pronounced absence of estrus validated the development of PCOS among the experimental animals.

Mean serum testosterone level in NC group was 266.51±20.36 pg/mL. Upon the end of induction period, mean testosterone level had significantly elevated to 748.51±52.33 pg/mL, 631.66±21.71 pg/mL, 558.38±81.93 pg/mL, 633.97±25.00 pg/mL, 709.83±23.03 pg/mL in PC, PCC, PFD25, PFD125 and PFD250 groups respectively, than that observed in NC rats.

***F. deltoidea* leaves extract reduces serum testosterone levels among PCOS rats**

The non PCOS females maintained low testosterone at the end of the study period in which the level was 259.52±8.03 pg/mL. This value was not significantly different with that of serum testosterone at the onset of the study. PCOS rats treated with clomiphene citrate showed significant reduction in mean testosterone level from 631.69±21.71 pg/mL to 232.87±9.93 pg/mL, which exhibited approximately 63% reduction. Surprisingly, significant reduction in the means of testosterone levels were also recorded in the three groups of PCOS females administered with the three concentrations of *F. deltoidea* leaves extract. The

Table 2. Mean concentrations of serum testosterone at the end of letrozole induction period

Group	Mean serum testosterone ± SEM of PCOS females (pg/mL)
NC	266.48±20.36^{a}
PC	748.22±52.30^{c}
PCC	631.66±21.71^{b}
PFD25	558.38±81.93^{b}
PFD125	633.97±25.00^{b}
PFD250	709.83±23.03^{c}

Different superscripts within a column indicated significant difference at $p<0.05$.

Table 3. Mean concentrations of serum hormones in experimental animals after 42 days post-treatment

Groups	Mean concentrations of hormones					
	Testosterone (pg/mL)±SEM	*Progesterone (pg/mL)±SEM*	*Estrogen (pg/mL)±SEM*	*FSH (mIU/mL)±SEM*	*LH (mIU/mL)±SEM*	*Insulin (ng/mL)±SEM*
NC	259.52±8.030^{a}	52.78±3.66^{d}	46.32±1.52^{d}	5.083±0.127^{a}	3.700±0.05^{a}	0.300±0.007^{b}
PC	748.22±52.30^{b}	16.25±0.32 ^{a}	29.18±2.07^{a}	5.426±0.033^{b}	4.281±0.13^{b}	0.801±0.025^{d}
PCC	232.85±9.93^{a}	34.05±1.13^{b}	37.40±1.29^{b}	5.146±0.043^{a}	3.648±0.05^{a}	0.583±0.037^{c}
PFD25	251.12±32.54^{a}	30.32±2.47^{b}	36.29±1.59^{b}	5.198±0.128^{b}	3.562±0.17^{a}	0.332±0.040^{b}
PFD125	226.83±31.34^{a}	43.62±1.94^{c}	40.95±1.41^{c}	5.194±0.069^{b}	3.574±0.05^{a}	0.265±0.046^{b}
PFD250	219.43±38.73^{a}	47.18±2.37^{d}	43.68±1.55^{d}	5.174±0.054^{a}	3.655±0.05^{a}	0.196±0.028^{a}

Different superscripts within a column indicated significant difference at $p<0.05$

reductions were 55%, 64% and 70% for the extract concentrations of 25, 125 and 250 mg/kg bwt. respectively, suggesting the reduction was dose dependent manner. The end values for the means of testosterone levels for PFD25, PFD125 and PFD250 were 251.12±32.54 pg/mL, 226.83±31.34 pg/mL, and 219.43±38.73 pg/mL compared with those of the initial values which were 558.38±81.93 pg/mL, 633.97±25.00 pg/mL, and 709.83±23.03 pg/mL, respectively (Table 3).

F. deltoidea ethanolic leaves extract increases progesterone level

Progesterone level was assayed and the results revealed that letrozole-induced PCOS rats (PC) had significantly reduced progesterone level (16.25±0.32 pg/mL) compared with that of NC rats (52.78±3.66 pg/mL). Treatment of PCOS rats with 10 mg/kg bwt. of clomiphene citrate had significantly elevated ($p<0.05$) serum progesterone level to 34.06±1.13 pg/mL. Interestingly, significant increase ($p<0.05$) in progesterone level was also observed in PCOS rats treated with all the three doses of *F. deltoidea* ethanolic leaves extract. The values in the dose dependent manner were 30.32±2.47 pg/mL, 43.62±1.94 pg/mL and 47.18±2.37 pg/mL for the respective extract concentrations. Moreover, the mean progesterone value among PCOS rats treated with 250 mg/kg bwt. of the extract showed no significant difference compared with that of the NC group (52.78±3.66 pg/mL), indicating the hormone level reverted from high value to normalcy at the end of the extract treatment period (Table 3).

F. deltoidea ethanolic leaves extract increases estrogen level

Estrogen level was found to be significantly reduced ($p<0.05$) in letrozole induced PCOS rats (PC), in which the mean value was 29.18±2.07 pg/mL compared with the NC group which was 46.32±1.52 pg/mL. After six weeks of treatment with clomiphene citrate, mean estrogen level had significantly increase ($p<0.05$) to 37.40±1.29 pg/mL. The value was comparable with that assayed from serum of PCOS rats treated with 25 mg/kg bwt. of *F. deltoidea* leaves extract (36.29±1.59 pg/mL). Higher concentrations of the extract resulted in significantly higher oestrogen levels which were 40.95±1.41 pg/mL and 43.68±1.55 pg/mL, respectively, for PCOS groups treated with 125 and 250 mg/kg bwt. of the extract (Table 3).

F. deltoidea ethanolic leaves extract reduces LH and FSH levels

LH was significantly higher in letrozole-induced rats (PC) as compared with NC rats in which the value was 4.28±0.13 mIU/mLand 3.70±0.05 mIU/mL, respectively. Treatment with clomiphene in PCC group had reduced the LH level to 3.65±0.05 mIU/mL while treatments with 25, 125 and 250 mg/kg bwt. of *F. deltoidea* ethanolic leaves extract reduced LH levels to 3.56±0.17 mIU/mL, 3.57±0.05 mIU/mL and 3.66±0.05 mIU/mL, respectively. The values obtained were significantly ($p<0.05$) different from PC, however, there was no significant difference of LH values among NC, PCC and all the extract treated groups.

Assay of FSH, on the other hand revealed the mean level was significantly increased in PCOS induced rats (PC) in which the value was 5.426±0.033 mIU/mL. Treatment with clomiphene citrate had reduced the FSH level to 5.146±0.043 mIU/ml, while treatments with 25, 125 and 250 mg/kg bwt. *F. deltoidea* ethanolic leaves extract had significantly ($p<0.05$) decreased the FSH levels to 5.198±0.128 mIU/mL, 5.194±0.069 mIU/mL and 5.174±0.054 mIU/mL, respectively, which was comparable with the mean FSH level of NC rats (5.083±0.127 mIU/mL).

F. deltoidea ethanolic leaves extract increases insulin sensitivity

Circulating insulin level was found to be the highest in letrozole-induced PCOS rats (PC), with the mean value of 0.801±0.025 ng/mL. Treatment

with clomiphene had slightly reduced the mean level to 0.583±0.037 ng/mL, a value which was above the normal insulin level observed in NC rats (0.300±0.007 ng/mL).

Treatments with *F. deltoidea* leaves extract had significantly (p<0.05) decreased insulin levels lower than those observed in PC and PCC groups with values 0.332±0.040 ng/mL, 0.265±0.046 ng/mL and 0.196±0.028 ng/mL, respectively, for 25, 125 and 250 mg/kg bwt. of the extract. In addition, the values in PFD125 and PFD 250 groups were significantly (p<0.05) lower than that of the NC group (Table 3).

DISCUSSION

Polycystic ovaries can be induced by androgen exposure either via exogenous androgen administration or as a result of secondary endogenous androgen excess (Abbott *et al.,* 2005) Letrozole is an aromatase inhibitor that could block the conversion of testosterone to estradiol, creating androgen excess condition (Kafali *et al.,* 2004). Letrozole treatment of adult rats resulted in ovarian morphologic and endocrine disturbances similar to those observed in human polycystic ovarian syndrome (Baravalle *et al.,* 2006). Decrease in aromatase activity of P450 cytochrome which is normally expressed in ovary could increase the intraovarian production of androgen which simultaneously reduces estrogen level leading to development of polycystic ovaries. PCOS was confirmed among the females induced with 1 mg/kg bwt. of letrozole as evidenced in their estrous cycle pattern and testosterone profile.

Enzyme immunoassays of various PCOS key hormones revealed elevated testosterone, FSH and LH levels with reduced estrogen and progesterone levels in letrozole-induced PCOS rat model. Present research work showed treatment of PCOS-induced rats with *F. deltoidea* leaves extract had significantly reduced serum testosterone, FSH and LH levels. Additionally, significant increased in serum estrogen and progesterone levels were observed. The changes in these hormonal levels might suggest anti androgenic and phyto-estrogenic properties of the plant. Low progesterone level might be associated with anovulation, reflected by the minimal estrus frequencies. Underway histopathology studies might reveal fewer or no corpus luteum.

Elevated serum FSH concentration, with decreased estrogen level as observed in the PCOS animal model, however, was not a typical diagnostic found in human PCOS. It appears to be the main limitation of this model. Increased LH concentration, meanwhile, is the hallmark of the syndrome (Allahbadia & Merchant, 2011).

Insulin resistant plays a key role in pathogenesis of the PCOS syndrome. Increased insulin levels contributed to PCOS-associated hyperandrogenism (Panidis *et al.,* 2006). There are immense reports of increased insulin secretion observed in PCOS women. In this experiment, serum insulin level was found to be elevated in PCOS-induced rats, suggesting reduced insulin sensitivity in the experimental model, which might be due to irresponsiveness of insulin action (Cerf, 2013), or increased insulin secretion (Panidis *et al.,* 2006). However, treatment with *F. deltoidea* ethanolic leaves extract had significantly reduced the circulating insulin levels which were comparable to that observed in the normal control rats. Besides, *F. deltoidea* has been known for its insulin sensitizing property in normal and diabetic model (Farsi *et al*., 2014). A complete insulin indices however need to be reviewed to confirm its effectiveness in managing hyperinsulinemia in PCOS model.

CONCLUSION

Treatment of PCOS-induced rats with *F. deltoidea* ethanolic leaves extract exhibited improvement in serum hormonal profiles. Findings from the study may provide baseline data for designing further investigations on therapeutic benefits of *F. deltoidea* as one of possible adjunct therapy in managing the PCOS syndrome among women.

ACKNOWLEDGEMENTS

This work was funded by Exploratory Research Grant Scheme (600-RMI/ERGS 5/3 (6/2013)). Research facilities were provided by Faculty of Applied Sciences and Faculty of Health Sciences of Universiti Teknologi MARA Selangor (UiTM).

REFERENCES

Abbott, D.H., Barnett, D.K., Bruns, C.M. & Dumesic, D.A. 2005. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Human Reproduction Update*, **11(4):** 357–74.

Abdulla, M.A., Abdul-Aziz Ahmed, K., Abu-Luhoom, F.M. & Muhanid, M. 2010. Role of Ficus deltoidea extract in the enhancement of wound healing in experimental rats. *Biomedical Research*, **21(3):** 241–245.

Abdullah, Z., Hussain, K., Ismail, Z. & Ali, R.M. 2009. Anti-inflammatory Activity of Standardised Extracts of Leaves of Three Varieties of *Ficus deltoidea*, *International Journal of Pharmaseutical and Clinical Research*, **1(3):** 100–105.

Adam, Z., Khamis, S., Ismail, A. & Hamid, M. 2012. *Ficus deltoidea*: A Potential Alternative Medicine for Diabetes Mellitus. *Evidence-Based Complementary and Alternative Medicine/: eCAM*, **2012:** 632763.

Allahbadia, G.N. & Merchant, R. 2011. Polycystic ovary syndrome and impact on health. *Middle East Fertility Society Journal*, **16(1):** 19–37.

Mat-Akhir, N.A., Lee, S.C., Fadzilah Adibah, Abdul-Majid, F.A. & Sarmidi, M.R. 2011. Cytotoxicity of aqueous and ethanolic extracts of *Ficus deltoidea* on human ovarian carcinoma cell line. *British Journal of Medicine & Medical Research*, **1(4):** 397–409.

Baravalle, C., Salvetti, N.R., Mira, G.A, Pezzone, N. & Ortega, H.H. 2006. Microscopic characterization of follicular structures in letrozole-induced polycystic ovarian syndrome in the rat. *Archives of Medical Research*, **37(7):** 830–839.

Cerf, M.E. 2013. Beta Cell Dysfunction and Insulin Resistance. *Frontiers in Endocrinology*, **4:** 1–12.

Cooper, T.G., Noonan, E., von Eckardstein, S., Auger, J., Baker, H.W.G., Behre, H.M., Haugen, T.B., Kruger, T., Wang, C., Mbizvo, M.T. & Vogelsong, K.M. 2010. World Health Organization reference values for human semen characteristics. *Human Reproduction Update*, **16(3):** 231–245.

Farsi, E., Ahmad, M., Hor, S., Ahamed, M.B., Yam, M. & Asmawi, M. 2014. Standardized extract of *Ficus deltoidea* stimulates insulin secretion and blocks hepatic glucose production by regulating the expression of glucose-metabolic genes in streptozitocin-induced diabetic rats. *BMC Complementary and Alternative Medicine*, **14:** 220(1-13).

Hajshafiha, M., Ghareaghaji, R., Salemi, S., Sadegh-Asadi, N. & Sadeghi-Bazargani, H. 2013. Association of body mass index with some fertility markers among male partners of infertile couples. *International Journal of General Medicine*, **6:** 447–451.

Kafali, H., Iriadam, M., Ozardali, I. & Demir, N. 2004. Letrozole-induced polycystic ovaries in the rat: A new model for cystic ovarian disease. *Archives of Medical Research*, **35:** 103–108.

Mamata, J., Sasikumar, M. & Sunita, S. 2013. Anti-androgenic effect of *Symplocos racemosa* Roxb. against letrozole induced polycystic ovary using rat model. *Journal of Coastal Life Medicine*, **1(4):** 309–314.

Oh, M.J., Abdul Hamid, M., Ngadiran, S., Seo, Y.K., Sarmidi, M.R. & Park, C.S. 2011. *Ficus deltoidea* (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity *in vitro* and by suppressing tyrosinase gene expression in B16F1 melanoma cells. *Archives of Dermatological Research*, **303(3):** 161–170.

Panidis, D., Macut, D., Farmakiotis, D., Rousso, D., Kourtis, A., Katsikis, I., Spanos, N., Petakov, M., Bjekic, J. & Damjanovic, S. 2006. Indices of insulin sensitivity, beta cell function and serum proinsulin levels in the polycystic ovary syndrome. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, **127(1):** 99–105.

Umi Romaizatul Amiera, Z., Nihayah, M., Farah Wahida, I. & Rajab, N.F. 2015. Phytochemical characteristic and uterotonic effect of aqueous extract of *Ficus deltoidea* leaves in rats uterus. *Pakisthan Journal of Biological Science*, **17(9):** 1046–1051.

Saad, A.K.A. 2009. Polycystic ovarian syndrome: diagnosis and management of related infertility. *Obstetrics, Gynaecology and Reproductive Medicine*, **19(10):** 263–270.

Shafie, N.I., Samsulrizal, N., Ahmad-Sopian, N.F., Rajion, M.A., Goh, Y.M. & Ahmad, H. 2015. Qualitative phytochemical screening and GC-MS profiling of Azadirachta excelsa leaf extract. *Malaysian Applied Biology*, **44(3):** 87–92.

Sulaiman, M.R., Hussain, M.K., Zakaria, Z.A., Somchit, M.N., Moin, S., Mohamad, A.S. & Israf, D.A. 2008. Evaluation of the antinociceptive activity of *Ficus deltoidea* aqueous extract. *Fitoterapia*, **79(7-8):** 557–561.

Suryati, Nurdin, H., Dachriyanus. & Lajis, M.N. 2011. Structure elucidation of antibacterial compound from *Ficus deltoidea* Jack leaves. *Indonesian Journal of Phytochemistry*, **11(1):** 67–70.

Teede, H., Deeks, A. & Moran, L. (2010). Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Medicine*, **8:** 41.

Walters, K.A., Allan, C.M. & Handelsman, D.J. 2012. Rodent Models for Human Polycystic Ovary Syndrome. *Biology of Reproduction*, **8:** 149–149.

Zahra, M.A.S.F., Mahmood, A.A., Hapipah, M.A., Suzita, M.N. & Salmah, I. 2009. Anti-ulcerogenic activity of aqueous extract of *Ficus deltoidea* against ethanol-induced gastric mucosal injury in rats. *Research Journal of Medical Sciences*, **3(2):** 42–46.