A novel method of extraction of bamboo seed oil (*Bambusa bambos* Druce) and its promising effect on metabolic symptoms of experimentally induced polycystic ovarian disease

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**ABSTRACT**

**Objective:** To evaluate the potential effect of bamboo seed oil in decreasing the major metabolic symptoms associated with letrozole-induced polycystic ovarian disease using female rat model.

**Materials and Methods:** A new method of microwave-assisted extraction was developed. Female rats were grouped into four with six animals each. All rats were daily administered with letrozole (1 mg/kg b.wt.) for 21 days except control, and during this period, changes in estrous cycle were observed. After letrozole treatment, Group 2 was considered negative control, Groups 3 and 4 were treated orally with bamboo oil, 0.5 ml/kg b.wt. and 1 ml/kg b.wt., respectively, for 3 weeks (five consecutive estrus cycles). Various parameters such as estrus cycle, blood sugar level, lipid profile, and weights of reproductive system were determined. The characteristics of cystic ovaries were evaluated by histopathological studies.

**Results:** The isolated bamboo oil restored estrus cyclicity showed hypoglycemic and hypolipidemic effects. 1 ml/kg b.wt. of bamboo oil showed a marked glucose reduction from 254.04 ± 2.08 to 92.6 ± 1.63, and levels of total cholesterol, very low-density lipoprotein, triglyceride were reduced from 186.45 ± 2.28, 30.07 ± 2.36, 100.36 ± 2.35 to 152.14 ± 2.63, 25.94 ± 1.66, 93.32 ± 1.09, respectively. Histopathological results showed the presence of ovulation and recovery from cystic ovaries.

**Conclusion:** A novel and promising drug was isolated in the treatment and maintenance of various metabolic symptoms associated with polycystic ovary disease.

**KEYWORDS:** Bamboo seed oil, *Bambusa bambos* Druce, cystic ovary, estrus cycle, lipid profile, polycystic ovary disease, polycystic ovary syndrome
ovaries, with the exception of other etiologies. Features of PCOD may manifest at any age, ranging from childhood (premature puberty), teenage years (hirsutism, menstrual abnormalities), early adulthood, and middle life (infertility, glucose intolerance) to later life (diabetes mellitus and cardiovascular diseases). An effective treatment therapy for PCOD is with less side effect is still a challenge and evaluation of new strategies to treat this disease is of great priority.

*Bambusa bambos* (Druce) is commonly known as Indian thorny bamboo belongs to the family of gramineae or poaceae. They distinct from ordinary grasses in their perennial tree-like growth habits, and seed only once at the end of a long vegetative growth phase followed by its death. *B. bambos* has been widely used as Indian folk medicines. The entire plant is used as laxative, diuretic, and in inflammatory conditions. Shoots are used to prepare various culinary preparations and in the treatment of ulcer. Decoction prepared from leaves helps in easy discharge of menses and helps in treatment of amenorrhea as well used as an antispasmodic agent in dysmenorrhea. Bamboo seeds are commonly known as bamboo rice, a main source of food for tribal people throughout India and the overall nutritive value of these grains excel both rice and wheat. Bamboo seeds are traditionally used as an aphrodisiac, astringent, hypolipidemic, and in urinary discharges. The Kani tribe of Kanyakumari district of South India believes that the seeds enhance fertility.

It was reported that when rats fed on bamboo seeds (*Dendrocalamus hamiltonii*), they become sexually active into such an extent that each female rat gives birth to as many as 800 offsprings during the season of bamboo flowering. This phenomenon was well-explained by researchers that the bamboo seeds cause changes in genetic material of chromosomes in rats. The bamboo seeds were reported to contain carbohydrates and proteins contents higher than that of rice and wheat. The seeds also contain calcium (5.0 mg %), phosphorus (18.0 mg %), iron 9.2 (mg %), Vitamin B1 (0.1 mg %), nicotinic acid (0.03 mg %), riboflavin 36.3 (g %), carotene (12.0 mg %) as well as all essential amino acids. Our previous research on isolated bamboo oil support that it is a potent antioxidant and antimicrobial agent, its gas chromatography-mass spectrometry characterization support the presence of 38.37% of linoleic acid and 27.36% of palmitic acid.

The indigenous people (tribes) of South India make the use of bamboo seeds as a primary source of food and also believed to have fertility enhancement and hypolipidemic properties. As bamboo seeds lack scientific studies, this information’s were collected orally from tribal medical practitioners. Bamboo oil is found to be a powerful source of antioxidants in our previous work, this study focuses on the beneficial effect of bamboo oil isolated from bamboo seeds on experimentally induced PCOS using female Wistar rats, and a microwave technique was newly developed to improve the yield of oil and reduce the duration of extraction.

**Materials and Methods**

**Chemicals and Equipment**

Seventy-five percent aqueous ethanol (Merck Sps. Pvt. Ltd., Mumbai, Maharashtra, India), letrozole (Novartis India Ltd., Mumbai, Maharashtra, India), catalyst scientific microwave (Catalyst scientific, Pune, Maharashtra, India), glucometer (Accu chek-active, Roche Diagnostics India Pvt. Ltd., Mumbai, Maharashtra, India), carboxymethyl cellulose (CMC) (Merck Sps. Pvt. Ltd., Mumbai, Maharashtra, India), Giemsa stain (Merck Sps. Pvt. Ltd., Mumbai, Maharashtra, India), and Ecoline-kits (E. Merck Ltd., M.I.D.C., Taloja, Mumbai, Maharashtra, India).

**Collection and Identification of Bamboo Seeds**

The dried seeds of *Bambusa bambos* (Druce) was collected from the local market of Wayanad, Kerala, India. The seeds were identified and authenticated in the Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, and a specimen (crccoq/455/2014) was preserved.

**Microwave-Assisted Extraction of Oil**

Dried seeds were coarsely powdered and extracted with aqueous ethanol (75% v/v) under microwave irradiation at 245 W intensity for 5–15 min separately. When the irradiation period was complete, samples were removed from the microwave cavity and allowed to cool to room temperature before opening. The aqueous ethanolic extract was concentrated under vacuum which resulted in separation of an oily layer at the surface of the extract. The oil was further isolated by employing petroleum ether as a solvent and concentrated.

**Pharmacological Screening Methods**

Female Wistar rats of body weight 150–200 g with a regular 4-day estrus cycles were selected for the experiment. Animals were preserved and treated as per the guidelines of the Animal Ethical Committee Approval no-CRRCP/AEC/PH.D/PH.CHEM/06/2012. Toxicity studies of the bamboo oil were carried out, and it was found that there was no toxic effect up to the dose level of 5 ml/kg b.wt. in Wistar rats. Hence, the lowest dose levels of 0.5 ml/kg b.wt. and 1 ml/kg b.wt. were selected for the study.

Animals were grouped into four by keeping 6 animals in each. Group 1 was served as control which received only 1% CMC throughout the study. To induce PCOD rats were administered orally with letrozole (1 mg/kg b.wt.) dissolved in 1% CMC once daily for 21 days. After 21 days, all the letrozole-induced rats showed a disturbed or absence of estrus cycle. The induced rats were randomized into three groups containing six animals each. Group 2 (negative control) which receives letrozole 1 mg/kg b.wt. dissolved in 1% CMC daily and Groups 3 and 4 were administered orally with bamboo oil 0.5 ml/kg b.wt. and 1 ml/kg b.wt., respectively, for 3 weeks. During the study, the estrus cycles were monitored regularly by vaginal smear method. After the conclusion of the study, rats were sacrificed by decapitation, and blood samples were collected. The reproductive system was separated and preserved.

**Estrus Cycle**

During the study, vaginal smears were observed microscopically using Giemsa stain for determination of estrus cyclicity. A cotton bud dipped in normal saline was inserted gently in the vaginal opening of the female rats, and a swab was obtained. The cotton bud was rolled on a clean grease-free slide to make a smear and allowed to air dry. The cells in the smear are fixed with few drops of methanol. Giemsa stain...
was added to the slide to cover the smear. The slide was kept covered in petridish for 5 min. Distilled water was added to the Giemsa stain and gently rocked. A green scum appeared on top of the slide. The slide was stained for 10 min in dilute Giemsa. The stained slide was dried and then washed in tap water. The washed slide was air dried and observed under the microscope in 40× objective.[12]

**Determination of Blood Glucose Level**

The blood sugar levels were monitored for all the groups at the end of the study. Blood samples were withdrawn from each rat to determine the glucose level by electronic glucometer (Accu chek-active, Roche Diagnostics India Pvt. Ltd., Mumbai, Maharashtra, India).

**Preparation of Blood Serum**

After decapitation of animals, the blood samples of each animal were collected and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation for 15 min with 3000 rpm and analyzed for various biochemical parameters.

**Lipid Profile**

Lipid profile which includes total cholesterol (TC), low-density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) were estimated by autoanalyzer microlab 200 using Ecoline-kits.

**Evaluation of Reproductive System**

Uterus and ovaries were dissected out from each animal and weighed by a digital electronic weighing balance to evaluate the effect of the extract.[13] Three samples of ovaries in each group were selected for histopathological evaluation.

**Statistical Analysis**

Values are reported as mean ± standard error of the mean. The effect of extract on different parameters such as blood glucose levels, lipid profile, and weights of reproductive system was compared with negative control by one-way analysis of variance using turkey’s multiple comparison test.

**Results**

The bamboo oil was isolated newly by microwave assisted extraction method. A good yield of 40.2% was obtained at 11 min. The yield and extraction time of oil at 245 W are shown in Figure 1.

The control rats showed a normal 4-day estrus cycle [Figure 2a-d]. The PCOD-induced rats showed irregular and prolonged estrus cycle. The PCOS-induced rats which have received a high dose of bamboo oil (1 ml/kg b.wt.) showed estrus cyclicity after 2 weeks, whereas the low dose (0.5 ml/kg b.wt.) treated rats showed estrus cyclicity only after 3 weeks of the study.

The isolated oil was tested for its hypoglycemic activity at the end of the study, and the results are shown in Table 1. The low dose of bamboo oil 0.5 ml/kg b.wt. could not show a significant reduction in blood glucose levels in PCOD-induced groups. The high dose of 1 ml/kg b.wt. of bamboo oil showed a marked reduction from 254.04 ± 2.08 to 92.6 ± 1.63.

Hypolipidemic activities of isolated bamboo oil were tested, and a significant reduction in lipid profile was obtained [Figure 3]. The levels of TC, very-LDL, and TG were reduced from 186.45 ± 2.28, 30.07 ± 2.36, and 100.36 ± 2.35 to 152.14 ± 2.63, 25.94 ± 1.66, and 93.32 ± 1.09, respectively, for PCOD-induced rats which have received 1 ml/kg b.wt. of bamboo oil. The low dose (0.5 ml/kg b.wt.) treated groups showed moderate hypolipidemic activity.

The uterine weights which decreased from 73.76 ± 1.32 mg to 55.73 ± 2.04 in PCOS-induced rats were increased to 59.15 ± 1.24 and 65.64 ± 2, respectively, with different doses of bamboo oil. The ovary weight is increased from 65.06 ± 1.08 to 94.8 ± 1.53 in PCOD-induced groups. The bamboo oil treated groups significantly decreased the ovarian weights when compared to control. The results of weights of uterus and ovaries are shown in Table 2.

Control rats showed the presence of a matured secondary follicle with oocyte. The follicle has a well differentiable theca and granulosa cell layer. It also showed the presence of developing corpus luteum [Figure 4a]. In PCOD-induced rats, many cystic follicles were found with the absence of corpus luteum. Theca cell layer was delineating, and the cell debris was shown in the antrum of follicle. The cystic follicles showed the presence of thin granulose cell layer [Figure 4b]. Section of ovary treated with bamboo oil at a concentration of 0.5 ml/kg b.wt. showed the presence of developing antral primary follicle as well as cystic follicle [Figure 4c]. The rats which received

**Figure 1:** The time and yield of bamboo oil by microwave at 245 W

**Figure 2:** Microscopical view of normal 4 day estrus cycle of control rats. (a) Estrus stage shows the presence of nucleated epithelial round cells, (b) metaestrus stage-Section with low cell number, (c) diestrus stage section with many lymphocytes, (d) proestrus section with many round-shaped cells
1 ml/kg b.wt. of bamboo oil showed normal ovarian architecture with the presence of primary follicle and oocyte [Figure 4d].

Discussion

The microwave-assisted extraction method results in good yield at a short period of time when compared with conventional Soxhlet extraction method in our previous work.[12] Satisfactory results were obtained at 9 and 10 min, and a highest yield of 40.2% was obtained at the 245 W intensity for 11 min, thus saving 5 h 49 min.

The nature of cell types in the vaginal smear determines the stage of the estrus cycle.[14] Letrozole-treated rats showed irregularity in its estrus cycle determination and which changed to normal sequence of estrous cycle after treating the rats with the bamboo oil. The restored estrus cycle indicating the recovery of anovulation to normal ovary functions. A considerable amount of evidence suggests that plants such as Matricaria chamomilla[15] and Aloe barbadensis[16] showed potential effects to bring down estrus cyclicity of PCOD-induced animal models.

The hypoglycemic activity of bamboo oil contributes its beneficial effect in lowering down one of the major metabolic symptom associated with PCOD. Insulin resistance and compensatory hyperinsulinemia are present in 50–70% of the women with PCOS and may be as high as 95% in overweight women.[16] The hypoglycemic activity may be due to the presence of linoleic acid (omega 6-fatty acid) present in the bamboo oil. Research work[17] support the presence of linoleic acid in a concentration of 38.37% and which can decreases insulin resistance thereby making insulin more sensitive to glucose.

The recommended diet in the treatment of PCOS has focused on weight reduction since half of women with PCOS are obese, and weight loss is helpful in normalizing hormonal levels and clinical symptoms. The isolated bamboo oil showed a good concentration of linoleic acid (omega 6-fatty acid)[9] and it reported to possess antioxidant activity, increases insulin resistance, reduces blood pressure, reduces blood cholesterol, reduces anti-inflammatory markers levels, and decrease the risk of coronary heart disease.[17] It was reported that a modest weight loss of 5% body weight has been shown to result in significant improvements in both symptoms of hyperandrogenism and ovulatory function in women with PCOS.[18,19] The uterus weight is gradually increased in both the treated groups which indicate the ovulation. The isolated bamboo oil also could decrease the ovarian weight to normalcy. The bamboo oil at a dose level of 1 ml/kg b.wt. showed significant recovery from cystic ovaries which is further confirmed by its histopathological studies. It has been reported that plants Cocos nucifera,[11] A. barbadensis,[12] and Labisia pumila[20] showed a similar effect in PCOD-induced rats.

The histopathological results of control ovary show normal ovarian architecture with matured secondary follicles and oocyte. The fresh corpus luteum indicates the presence of previous ovulation. The results of PCOD-induced groups clearly discuss that cysts are developed in the ovaries which attributes the elevated androgen levels. The follicle was found to be atretic with dying oocyte and lacked interplay of theca and granulose cell layer. In PCOD groups, the number of corpus luteum was diminished indicates the absence of ovulation and regular estrus cycle. Androgen-induced follicular atresia occurs by the entry of androgens into the granulose cell layer where they bind to the cell receptors and causes cell death.[21] In rats received with 0.5 ml/kg b.wt. of bamboo oil showed the presence of both atretic and developing primary follicles. However, the groups which have received 1 ml/kg b.wt. of bamboo oil showed marked recovery that many secondary follicles with oocyte were visible in the histopathological results. It also showed a fresh and thick corpus luteum indicates ovulation. These beneficial activities of bamboo oil may achieve by its potent antioxidant activity.[20] It has been reported that free radicals set up a chain of biochemical reactions which lead to the formation of many highly reactive...
intermediates and that affects the process of fertilization and implantation in uterus.\textsuperscript{11} In the ovaries, this increased oxidative stress may lead to the loss of gonadotropin receptors and normal ovarian functions. This study could not compare the results with a standard drug because there is no single drug available for the treatment of PCOD as it involves multidrug therapy. Further studies should be carried out to check the effect of bamboo oil in decreasing the androgen level and on insulin sensitivity.

**Conclusion**

In conclusion, the present work scientifically explores the traditional use of bamboo seed on its hypoglycemic and hypolipidemic effects in rats. These beneficial effects along with its potent antioxidant activity contribute to the treatment of major metabolic symptoms of PCOD such as irregular estrus cycle and poly cystic ovaries to normal level. Hence, a novel drug from a plant kingdom was isolated, which can restores ovulation thereby improves the anovulation related fertility problems and serve as a novel promising agent in treatment of PCOD.

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**Conflicts of Interest**

There are no conflicts of interest.

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