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EFFECT OF AQUEOUS SOLUBLE PROANTHOCYANIDINS FROM *COCOS NUCIFERA* L. INFLORESCENCE ON PROGESTERONE AND OESTROGEN LEVELS IN FEMALE RATS

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ABSTRACT

The immature inflorescence of *Cocos nucifera* L. variety aurantiaca is used by Ayurvedic and traditional medical practitioners for the treatment of menorrhargia in Sri Lanka. We have previously reported the effect of ethyl acetate soluble proanthocyanidins (EASPA) of the inflorescence of *Cocos nucifera* L. on reproductive hormone levels of female rats in relation to its ethno medical usage. AQSPA obtained from immature *Cocos nucifera* L. (var. aurantiaca) inflorescence was evaluated for its effect on the reproductive hormonal levels of female rats. AQSPA (2.8 mg/day) dissolved in water was administered orally to female rats for 28 consecutive days. At the end of the study period, oestrogen and progesterone levels were measured and compared with the control group (water). Statistical analysis was performed with one-way ANOVA, followed by student T test using Minitab 17.0 software. The length of the reproductive cycle was 4.89 ± 0.21 days and 4.37 ± 0.16 days for the control and test group rats, respectively. No significant changes were noticed in the length of the cycle nor were there any difference in vaginal cytology in test and control group rats. There were no significant difference in both estrogen and progesterone levels between control and test group animals. This may be as a result of low bioavailability of AQSPA due to its high molecular weight profile. In addition, this may also be due to an inadequacy of the dose or time duration that AQSPA was administered.

KEY WORDS

Cocos nucifera inflorescence, Menorrhagia, Proanthocyanidins and Progesterone.

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INTRODUCTION

Proanthocyanidins are secondary metabolites that belong to a class of polyphenolic compounds called flavonoids. The occurrence of proanthocyanidins in nature is extremely diverse. That is they exist as dimers, trimers, higher oligomers or polymers consisting of flavan-3-ol units. The most common flavan-3-ol units are (+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin[1] (Figure

No. 1). There are other flavan-3-ols that have been found in plants but rarely such as (+)-afzelechin and (-)-epiafzelechin[1]. These monomers may carry acyl/glycosyl substituents linked to the C-3 or the C-5 position[1]. There are two types of linkages between successive units in proanthocyanidins. When the linkage is between the C-4 and the C-8 or C-6, proanthocyanidins are named as B-type proanthocyanidins. A-type proanthocyanidins possess an additional ether linkage between C-2 and C-7 and has two hydrogen atoms less compared to the B-type[1]. Proanthocyanidins have recently attracted a considerable amount of attention in the fields of medicine, health and nutrition. They have been reported to exhibit antioxidant[2], anti-inflammatory[2], bacterial anti-adhesion[3], anticancer[4], and cardioprotective[5] activities. Today, nutritional supplements containing proanthocyanidin extracts from various plant sources are available, alone or in combination with other nutrients, as herbal extracts, capsules, or tablets[6,7].

The coconut palm is botanically known as *Cocos nucifera* L. And is a member of the monocotyledonous family Arecaceae and is the only species of the genus. It is found over the Asian continent, Pacific islands, Africa and in Central and South America[8]. *Cocos nucifera* L. is classified in to three varieties in Sri Lanka: Typica, Nana and Aurantiaca[9]. The orange coloured variety aurantiaca, is intermediate in stature and is popularly known as "thembili" in Sinhala in Sri Lanka. The immature inflorescence of *Cocos nucifera* L. (var. aurantiaca) is used by Ayurvedic and traditional medical practitioners for the treatment of menorrhagia in Sri Lanka. Our previous studies have shown that the inflorescence of *Cocos nucifera* L. Predominantly contains proanthocyanidins[10].

We have previously reported the effect of ethyl acetate soluble proanthocyanidins (EASPA) of the inflorescence of *Cocos nucifera* L. on reproductive hormone levels of female rats in relation to its ethnomedical usage[11]. In allopathic medicine, synthetic progestogens are used to treat menorrhagia[12]. It is significant that proanthocyanidins, are chemically different to progestogens used in allopathic medicine. Our finding suggested a possible mode of action, to explain the use of coconut inflorescence in controlling menorrhagia in Ayurveda and traditional medicine in Sri Lanka. Since our previous study has been shown that the EASPA mediate progestrogenic activity in female rats, it was interesting to find out whether the extracted aqueous soluble proanthocyanidins also possess progestrogenic activity in female rats. Here we report, effect of aqueous soluble proanthocyanidins of the inflorescence of *Cocos nucifera* L. on reproductive hormone levels of female rats.

MATERIALS AND METHODS

Materials

Light microscope (Meiji MT5000) with x10 and x40 objective lenses were used for vaginal cytology observations. Oestrogen and progesterone levels were assessed using AxSYMO Oestradiol and AxSYM Progesterone test kits respectively.

Plant material

Inflorescences were collected from healthy adult *Cocos nucifera* L. (var. aurantiaca) palms situated in the University of Sri Jayewardenepura premises, Sri Lanka from May 2012 to April 2014. Immature inflorescence (the inflorescence which was situated just above the freshly opened inflorescence in the palm) was plucked and the spa the was removed. The inflorescence was botanically authenticated by Mr. I. U. Kariyawasam at Department of Botany and voucher specimen (Assess. No. A3 S13, 001) was deposited in the herbarium of the Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka.

Extraction and Purification

Extraction and purification of aqueous soluble proanthocyanidin (AQSPA) fraction in the immature inflorescence of *Cocos nucifera* L. was carried out according to a previously published method[10,13,14]. AQSPA fraction of an acetone/water (7:3) extract of *Cocos nucifera* L. inflorescence was purified on Sephadex LH-20 to yield purified AQSPA as an off white powder in 0.26 % by

weight of the fresh inflorescence and was used for the study.

Experimental Animals

Female Wistar albino rats (origin- Wistar Institute of Biology, USA, *Rattusnorvegicus*), approximately 14-16 weeks old, weighing 200-250 g obtained from Animal Centre, Medical Research Institute, Colombo 8, Sri Lanka, were used for the study. The animals were housed in standard cages, total of 12 rats, 3 in each cage with sawdust as bedding. They were fed pelleted standard rat feed twice daily and watered *ad libitum*. Rats were exposed to a 12 hours light/dark cycle at room temperature. They were identified by colour markings on their body. This study was approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. The rats were handled in accordance with the CPCSEA guidelines for the care and use of laboratory animals.

Test method

Rats were checked for regular reproductive cycles by observing vaginal cytology for two weeks prior to the study period. Rats exhibiting regular reproductive cycles were used for the study. Rats were weighed and divided in to two groups with each containing six rats. Group I animals were administered orally using a sondi needle with 2.8 mg of purified AQSPA dissolved in 2 mL of tap water for 28 consecutive days. Available knowledge on usage of immature inflorescence of *Cocos nucifera* L. in Ayurveda was used to calculate the dose. Human dose in grams was extrapolated to rat dose according to the standard chart given in literature[15]. The resulting value was multiplied by the yield of purified AQSPA to obtain the final dose. The group II animals received 2 mL of tap water in the same way for 28 days and served as control group. The animals were observed daily for behavioral activities. Body weight was recorded every day during the study period.

Vaginal cytology

Every afternoon during the 28-day study period, between 2.00 and 3.00 pm each animal cage was carried to the experimental room. The vaginal cell samples of the female rats were obtained as previously reported[11] and examined under a light microscope (with x10 and x40 objective lenses) to determine the cytology of the vaginal epithelium in order to identify the different phases of the reproductive cycle as described in literature[16].

Serum oestrogen and progesterone levels

Blood samples were collected from rats after 28 days of study period at proestrous phase and oestrous phase and analyzed as previously reported[11].

Statistical analysis

The results are represented as the mean ± SEM. Every statistical analysis was performed with one-way ANOVA, followed by student T test using Minitab 17.0 software. Differences were accepted as statistically significant at $P \leq 0.05$ and $P \leq 0.001$.

RESULTS AND DISCUSSION

The reproductive cycle of female rats is characterized by proestrous, oestrous, metestrous and diestrous phases[16]. Ovulation occurs from the beginning of the proestrous phase up to the end of the oestrous phase. The mean cycle length in a female rat is 4 to 5 days[16]. Vaginal smear cytology was performed during the 28-day study period to determine the phase and the length of the reproductive cycle as previously published[11]. The characterization of each phase is based on the proportion among three types of cells observed in the vaginal smear: epithelial cells, cornified cells and leukocytes[16]. Proestrous phase smears were characterized by rounded, nucleated, epithelial cells generally in low to moderate (occasionally high) numbers. Oestrous phase smears consisted entirely of cornified cells, in high numbers. Metoestrous phase consisted of large numbers of leucocytes and smaller numbers of large, non-granular and non-nucleated epithelial cells. Dioestrous phase smears predominantly consisted of leucocytes. During the reproductive cycle, oestrogen and progesterone levels vary depending on the stage of the cycle[17]. Oestrogen levels begin to increase at late met estrous, reaching peak levels during proestrous and returning to baseline at oestrous phase.

Progesterone value rises in diestrous to reach its peak towards the end of proestrous and early oestrous phase with a decrease afterwards. The oestrogen and progesterone levels were measured in their peak phases, proestrous phase and oestrous phase, respectively. Mean body weight changes of test group (group I) and control group (group II) rats during the study period are shown in Figure No. 2. There is no significant difference in the weight gained of the female rats after 28 days of oral administration with AQSPA when compared with respective controls ($P \leq 0.05$). Treatment related changes in the behavioral pattern of rats in test group compared to control group were not observed during the study period. Rats belonging to both control and test groups appeared healthy and alert. This suggests that AQSPA is safe to be used on female rats at the dose level employed. Results of length of the reproductive cycle and hormonal assay for both control and test group rats are given in Table No. 1. The length of the reproductive cycle was 4.89 ± 0.21 days and 4.37 ± 0.16 days for the control and test group rats, respectively. No significant changes were noticed in the length of the cycle nor was there any difference in vaginal cytology in test and control group rats. The levels of oestrogen and progesterone for the control group rats were within the normal range. There were no significant difference in both estrogen and progesterone levels between control and test group animals ($P \leq 0.05$). The administration of AQSPA into Wistar rats at the dose of 2.8 mg/day for a period of 28 days did not result in any changes of the reproductive hormone levels compared to control group, although changes in serum progesterone level were observed for EASPA administered rats in our previous study[11]. According to literature and our previously reported results, EASPA is comprised of low molecular weight proanthocyanidins, whereas AQSPA is composed of higher molecular weight proanythocyanidins[10,14]. The high molecular weight polymeric proanthocyanidins are poorly absorbed by the body leading to low bioavailability compared to low molecular weight proanthocyanidins. Thus, this may be the main reason that the changes were not observed in progesterone levels of AQSPA administered rats compared to control group. In addition, this may also be as a result of an inadequacy of the dose or time duration that AQSPA was administered.

Table No. 1: Effect of AQSPA on length of reproductive cycle and reproductive hormone levels of group I and group II female rats

S.No	Parameter	Group I(AQSPA administered)	Group II (Control)
1	Duration of estrous cycle (days)	4.37 ± 0.16	4.89 ± 0.21
2	Progesterone level (ng/mL)	35.92 ± 0.93	32.79 ± 1.60
3	Oestrogen level (pg/mL)	52.63 ± 1.10	53.13 ± 4.06

n = 6, all values are presented as Mean ± SEM.

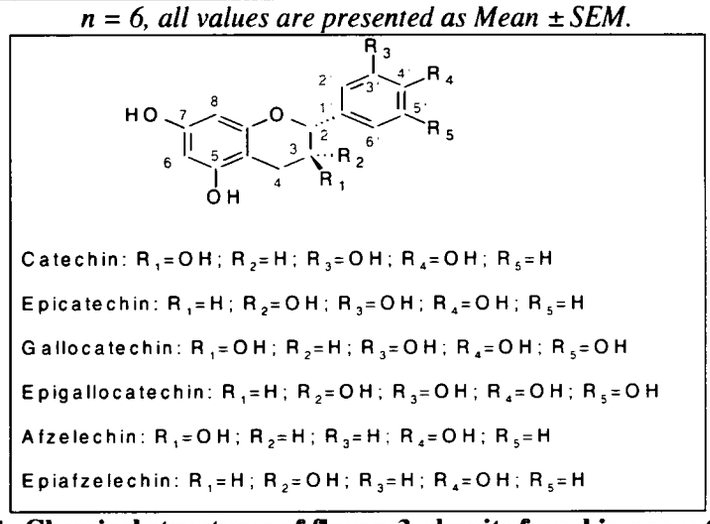

Figure No. 1: Chemical structures of flavan-3-ol units found in proanthocyanidins

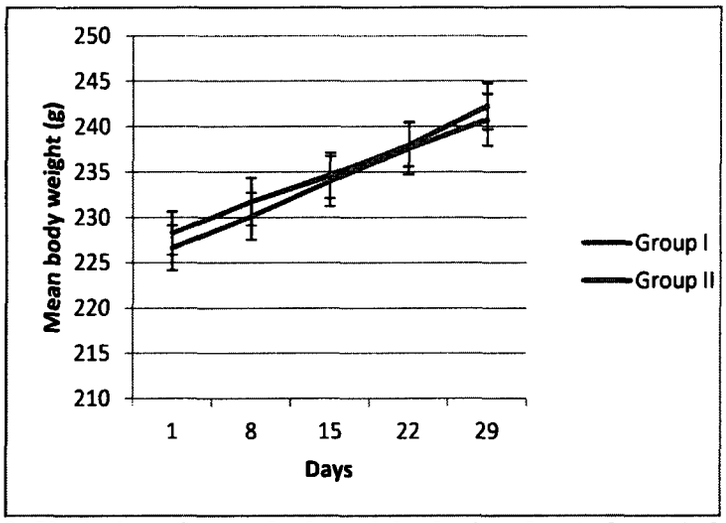

Figure No. 2: Variation of mean body weight during the study period of AQSPA administered and control group female rats

CONCLUSION

The administration of AQSPA into Wistar rats at the dose of 2.8 mg/day for a period of 28 days did not result in changes of the reproductive hormone levels compared to control group, although changes in serum progesterone level were observed for EASPA administered rats in our previous study.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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