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The Effects of Coconut Sprout Administration during Pregnancy in Rats

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Abstract

An experiment was conducted to study the effects of coconut sprouts administration during pregnancy on reproductive performances of *Sprague-Dawley* rats. Eighteen virgin female rats were assigned into 3 treatments and each treatment consisted of 6 experimental rats. The treatments were doses of coconut sprout administration i.e., 0, 100, and 200 mg/rat/day. The experimental rats were administered with coconut sprout for 19 days since day 1 of pregnancy. Parameters measured were body weights, uterine weights, fetal number, total fetal weights, fetal weight/fetus, MDA, SOD, katalase, maternal serum concentrations of estrogen, progesterone, and thyroxin, hematological parameters, and histology of the uterus, placenta, and mammary glands. The results showed that the administration of coconut sprout at doses of 100 and 200 mg/rat/day decreased body weight, uterine weights, fetal number, and total fetal weights ($P<0.05$). Histological observation showed that the uterus, placenta, and mammary gland of the experimental rats administered with coconut sprout during pregnancy showed a normal and a better growth and development compared to control rats. The decreased litter size in rats administered with coconut sprout caused the increased weight of individual fetus. Administration of coconut sprout at dosages of 100 and 200 mg/rat/day decreased serum estradiol concentrations ($P<0.05$) without significant effects on progesterone and thyroxin hormones concentrations.

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In addition, administrations of coconut sprout at dosages of 100 and 200 mg/rat/day decreased oxidative stress conditions as was indicated by the increased SOD ($P<0.05$) without significant effects on MDA and katalase ($P>0.05$). Administrations of coconut sprouts at doses of 100 and 200 mg/rat/day did not affect hematological and leucocyte differential counts parameters ($P>0.05$) of the experimental rats. However, at the week 3 of pregnancy, the lymphocytes of the experimental rats administered with coconut sprouts at a dose of 200 mg/kg BW decreased compared to those of administered with 0 and 100 mg/kg BW ($P<0.05$), even though it was still in the normal range. It was concluded that the administration of coconut sprout at dosages of 100 and 200 mg/kg BW decreased fetal number and increased uterine weight.

Key words: coconut sprout; litter size; fetal weight; estrogen; progesterone; reproductive parameters; rats.

1. Introduction

The optimum uterine environment during pregnancy will support the optimum availabilities of nutrients and compounds required by developing embryos and fetuses. Birth weight of the offspring is the final effects of complexes processes in the uterus [1, 2] that eventually affects postnatal growth until maturity. Improved uterine and placental environments will support the optimum growth and development of the embryos and fetuses. The whole process of pregnancy is controlled and regulated by the pregnant hormones, especially estrogen and progesterone. Higher maternal serum progesterone concentration improved birth weight [3] and improved endogenous secretions of pregnant hormones by injection of the mother with gonadotropin prior to mating improved uterine and placental environment that support the optimum embryos and fetal growth and development [4, 5, 6] that eventually improved birth weight [7, 8, 9].

The growth and development of the embryos and fetuses during pregnancy are supported by the optimum growth and development of the uterus and placenta [10, 11, 12]. Two main factors affecting the growth and development of embryos and fetuses are the reproductive hormones and maternal effects [13]. The success of pregnancy is determined by the improved uterine and placental environments to support the optimum growth of embryos and fetuses [14]. In addition, the optimum vascularization of the uterus and placenta will support the transport of nutrients and oxygen and other compounds required by the developing fetus that eventually support the optimum growth of the embryos and fetus until parturition [11, 15].

Coconut sprout is a sprout as a candidate of the coconut tree where the embryo of the coconut plant attaches to the endosperm has a position near the germination pores or wholes of the coconut plants [16]. Coconut water is reported to contain phytoestrogen that functions to improve reproductive functions [17]. Previous studies reported that coconut sprout had antioxidant contents and cardio-protective effects [18]. Those effects were reported as a result of the flavonoid content that works as a phytoestrogen [19]. This experiment was conducted to study the effects of coconut sprout on reproduction performances of female rats during 20 days of pregnancy by using *Sprague-Dawley* rats as a model. The limitations of the present experiment were the non-observed data on birth weights of the offspring rats in each group of coconut sprout administration. In addition, there was no observation on the fetal mortality and the profiles of estrogen and progesterone at certain ages of pregnancy in experimental rats administered with coconut sprout.

2. Materials and Methods

The experiment was conducted in November to December 2015, in the Unit of Laboratory Animal Management, Faculty of Veterinary Medicine, Bogor Agricultural University. The blood serum estrogen concentrations were measured in the Unit of Reproduction Rehabilitation, Division of Reproduction and Maternity, while maternal serum progesterone concentrations were measured at the Center of Primate Study of Bogor Agricultural University. The uterine, mammary glands, and placental histological preparations were prepared and observed in the laboratory of Indonesian Veterinary Research Institute, Bogor. The complete hematological analysis was conducted in the Laboratory of Physiology, Department of Anatomi, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University.

2.1. Experimental Animals and Maintenance

The experimental animals used in this experiment were mature female rats of *Sprague-Dawley* strain that were reproductively mature. The ages of the experimental animals ranged from 50 to 60 days with the average body weight of 200 g. The virgin female rats were maintained in plastics cages with 50x30x10 cm^3 in size and one experimental rat was raised individually in each cage. Before treatment, the experimental rats were acclimatized for one month to adapt the experimental rats to the cage and management conditions. During this adaptation period, the experimental rats were fed commercial diet. The experimental rats were mated naturally by mixing with a male rat with the ratio of 1:1. The pregnant experimental rats were fed 20 g commercial feed and supplemented with 0, 100, or 200 mg coconut sprout meal according to the treatment. The coconut sprout meal was added to 20 g of commercial diets and the mixtures were made in pellet. So every day the experimental rats were fed the same level (20 g) of commercial diet supplemented with 0, 100, or 200 mg coconut sprout according to the treatment. However, the drinking water was available *ad libitum*. The nutrient contents of the experimental diets were presented in Table 1.

Table 1: Nutrients compositions of experimental diets containing 0, 100, and 200 mg coconut sprout per 20 g commercial ration

	Dose of coconut sprout (mg)		
	0	100	200
Protein, g/100 g	23.40	22.43	20.61
Flavonoid, %/w/w	0	0.03	0.01
Dry matter (%)	93.12	91.06	90.50
Crude protein (%)	23.57	20.52	19.88
Crude fiber (%)	1.02	2.14	3.57
Crude fat (%)	7.65	7.62	8.06
NFE (%)	55.34	54.82	52.27
Gross energy (cal/g)	37.37	39.42	39.74

Nutrients and chemical analyses of coconut sprout were conducted in the Laboratory of Animal Nutrition and Feed Science, Faculty of Animal Husbandry, Bogor Agricultural University. NFE = Nitrogen-free extract.

2.2. Experimental Design

Eighteen non pregnant rats were divided into a completely randomized design with 3 treatments and 6 replications. The treatments were the dose of coconut sprout administration consisted of 0, 100, and 200 mg per 20 g commercial diet. The supplementations of coconut sprout were conducted 19 days during pregnancy from day 1 to day 19 of pregnancy. At the age of 2 and 3 weeks of pregnancy, blood samples were collected for measurements of hematological and leukocyte parameters. At the age of 19 days of pregnancy, the experimental rats were sacrificed for measuring fetal number, fetal weight, uterine weight, and uterine and the mammary gland histological observations. Blood samples were also collected for measurement of serum MDA, SOD, catalase, estrogen, progesterone, and thyroxin concentrations.

2.3. Sample Collection

Blood samples were collected to analyze red blood cells parameters (erythrocyte count, hematocrit, and hemoglobin) and leukocyte differentiation (leukocyte counts, netrophils, lymphocyte, monocytes, eosinophil, and basophils). Blood samples were also collected to obtain serum to analyze malondialdehyde (MDA), superoxide dismutase (SOD), catalase (cat), estrogen, progesterone, and thyroxine concentrations. The uterus, placenta, and mammary gland were sampled for histological observations.

2.4. Paramaters measured

Parameters measured in the experiment were body weight, uterine weight, fetal weight, fetal number, MDA, SOD, catalase, maternal serum estrogen, progesterone, thyroxin concentrations, and histology of uterus, mammary glands, and plascenta. In addition, the parameters of hematological profiles (red blood cells count, hematocrit, and hemoglobin concentration) and white blood cell differential counts (white blood cell count, netrophils, lymphocyte, monocyte, eosinophil, and basophil) were also measured by method of Sahli. Blood serum was collected for MDA, SOD, and catalase analyses were collected in week 4 by using spectrophotometry method. Analyses of serum estrogen, progesterone, and thyroxine concentrations were measured by using high performance liquid chromatography (HPLC) method. MDA was measured by following [21]. SOD was measured by following [22].

Katalase was measured by following method of Sinha [23]. Histological preparation of uterus, mammary glands, and placenta were conducted by using hematoksilin eosin (HE) staining method with the magnification of 200x.

2.5. Data Analysis

The collected data were analyzed with Analyses of Variance followed by Duncan test by using α=0.05 by using Minitab 16 program.

3. Results and Discussions

The experimental rats administered with coconut sprout both at doses of 100 and 200 mg/rat/day, had lower body weights compared to control rats without administration of coconut sprout ($P<0.05$). The order of body weights from the highest to the lowest was found in the experimental rats administered with coconut sprout at doses of 0, 200, and 100 mg/rat/day (Table 2). Based on the data presented in Table 2, it was clear that the experimental rats administered with coconut sprouts at doses of 100 and 200 mg/rat/day had decreased body weights ($P<0.05$) by 18.42 and 6.57%, respectively, compared to control rats without coconut sprout administration. In contrast, the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had higher uterine weights compared to control rats without coconut sprout administration ($P<0.05$). The order of uterine weights from the highest to the lowest was found in the experimental rats administered coconut sprout at doses of 200, 100, and 0 mg/rat/day. The experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had higher uterine weights by 50.57 and 139.87%, respectively, compared to control rats without coconut sprout administration (Table 2). Even though the experimental rats administered with coconut sprout had higher uterine weights, administration of coconut sprouts at doses of 100 and 200 mg/rat/day significantly decreased fetal number found in the placenta ($P<0.05$). The order of fetal number from the highest to the lowest was found in the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day. The experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had lower fetal number by 27.39 and 37.07%, respectively, compared to control rats without coconut sprout administration ($P<0.05$) (Table 2).

Table 2: Body weights, uterine weights, fetal number, fetal weights, malondialdehyde (MDA), superoxide dismutase (SOD), catalase, serum estrogen, progesterone, and thyroxin concentrations on days 20 of pregnancy in pregnant rats administered coconut sprout at doses of 0, 100, and 200 mg/rat/day.

Parameter	Doses of coconut sprout administration (mg/rat/day)		
	0 (n=6)	100 (n=6)	200 (n=6)
Body weight (g)	312.0 ± 20.6^{a}	254.5 ± 19.1^{b}	291.5 ± 16.2^{ab}
Uterine weight (g)	0.963 ± 0.458^{b}	1.450 ± 0.125^{ab}	2.310 ± 0.455^{a}
Fetal number	10.33 ± 1.15^{a}	7.50 ± 1.09^{ab}	6.50 ± 1.26^{b}
Total fetal weight (g/rat)	56.33 ± 3.68^{a}	37.5 ± 10.3^{ab}	32.17 ± 8.30^{b}
Fetal weight (g/fetus)	4.67 ± 0.33^{a}	5.67 ± 0.42^{a}	5.00 ± 0.52^{a}
MDA concentration (mmol/L)	1.089 ± 0.751^{a}	0.228 ± 0.000^{a}	0.231 ± 0.000^{a}
SOD concentration (mg/unit)	0.762 ± 0.191^{b}	1.238 ± 0.095^{a}	1.238 ± 0.095^{a}
Catalase concentration (M)	0.124 ± 0.003^{a}	0.125 ± 0.002^{a}	0.128 ± 0.010^{a}
Estrogen concentration (pg/mL)	44.97 ± 6.82^{ab}	22.03 ± 4.00^{c}	30.47 ± 3.12^{bc}
Progesterone concentration (ng/mL)	28.92 ± 7.93^{a}	35.24 ± 3.01^{a}	32.33 ± 4.37^{a}
Thyroxin concentration (T4) (nmol/L)	33.61 ± 3.38^{a}	38.95 ± 8.13^{a}	54.3 ± 13.6^{a}

Different superscripts in the same row indicate a significant difference ($P<0.05$).

Similar to fetal number, the experimental rats administered with coconut sprout had lower total fetal weights. Administration of coconut sprouts at doses of 100 and 200 mg/rat/day significantly decreased total fetal weight ($P<0.05$). The order of total fetal weights from the highest to the lowest was found in the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day. The experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had lower total fetal weight by 33.2 and 42.89%, respectively, compared to control rats without coconut sprout administration ($P<0.05$) (Table 2).

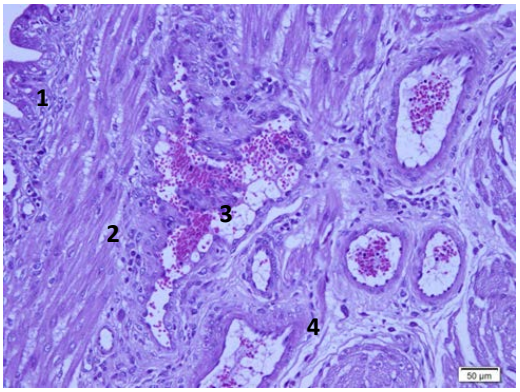
Total number of fetus in the placenta will affect the growth and development of the fetus. The higher the fetal number the higher the competition to obtain nutrients and oxygen required for metabolism to support the growth and development of the fetus. Even though it was not significant ($P>0.05$), the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had numerically higher individual fetal weights even though they had lower total fetal weights compared to control rats without coconut sprout administration ($P<0.05$). The order of average fetal weight in the experimental rats from the highest to the lowest was found in the experimental rats administered with coconut sprout at doses of 100, 200, and 0 mg/rat/day. The experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had higher average fetal weights by 21.41 and 7.07%, respectively, compared to control rats without coconut sprout administration (Table 2).

The MDA concentrations of the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day were similar to those of control rats without coconut sprout administration ($P>0.05$). However, the control experimental rats without coconut sprout administration had numerically higher MDA concentration compared to those administered with coconut sprout at doses of 100 and 200 mg/rat/day (Table 2).

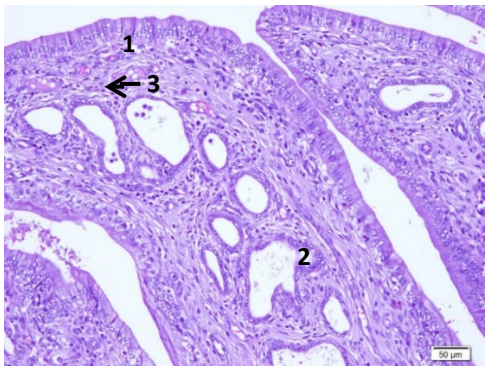
However, the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had similar SOD concentrations that were higher than those in control rats without coconut sprout administration ($P<0.05$). The experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had higher SOD concentrations by 62.46% compared to control rats without coconut sprout administration ($P<0.05$) (Table 2).

Administration of coconut sprout did not affect catalase values. The catalase values in the experimental rats administered with coconut sprouts at doses of 0, 100, and 200 mg/kg BW were similar ($P>0.05$) (Table 2).

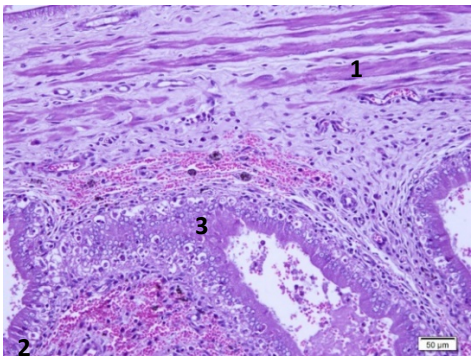
Administration of coconut sprout in the pregnant rats significantly decreased serum estrogen concentration ($P<0.05$). The control experimental rats without coconut sprout administration had higher ($P<0.05$) serum estrogen concentrations compared to those administered with coconut sprout at a dose of 100 mg/rat/day. However, the experimental rats administered with coconut sprout at a dose of 200 mg/rat/day had similar serum estrogen concentrations compared to control rats without coconut sprout administration and experimental rats administered with coconut sprout at a dose of 100 mg/rat/day ($P>0.05$) (Table 2). Administration of coconut sprout did not affect serum progesterone concentrations in the pregnant rats and the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day had similar serum progesterone concentrations ($P>0.05$) (Table 2).



The uterus of control rat without coconut sprout administration. 1. The uterine mucosa, 2. The endometrium, 3. Haemorrhage, and 4. The endometrium glands. HE. x200.



The endometrium of experimental rat administered with coconut sprout at dose of 100 mg/rat/day. 1. The endometrium mucosa, 2. The endometrium glands, and 3. Haemorrhage. HE. x200.



The uterus of experimental rat administered with coconut sprout at dose of 200 mg/rat/day. 1. The myometrium, 2. The uterine mucosa, and 3. Haemorrhage. HE. X200.

Figure 1: The histological conditions of uterus and endometrium of the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day with HE. $_{X}$200 staining.

Administration of coconut sprout did not affect serum thyroxin concentrations. Serum thyroxin concentrations in the experimental rats administered with coconut sprout at doses of 100, 200, and 0 mg/rat/day were similar ($P>0.05$). However, there was a tendency that the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had 15.89 and 61.56% higher serum progesterone concentrations compared to control rats without coconut sprout administration (Table 2).

Histological observation strongly confirmed that the administration of coconut sprout did not significantly affect the uterine growth and development. The experimental rats administered with coconut sprout at doses of 0, 100,

and 200 mg/rat/day, did not show any specific difference in uterine histological conditions (Figure 1) as was also found in the similar concentrations of progesterone (Table 2) that control uterine growth and development during pregnancy. The histological conditions of experimental rats without coconut sprout administration showed that the control rats were in the condition similar to anestrus status. The uterus of the experimental rats without coconut sprout administration (control) showed that the uterine or endometrium glands did not develop well as compared to those administered with coconut sprout at doses of 100 and 20 mg/rat/day. The endometrium glands of control experimental rats without coconut sprout administration were covered by the fiber of skeletal muscle, and the mucosa was covered by cuboidal epithelial cells. In addition, it was found blood vessels in the myometrium layer with the indication of thinning of endometrium layer (Figure 1).

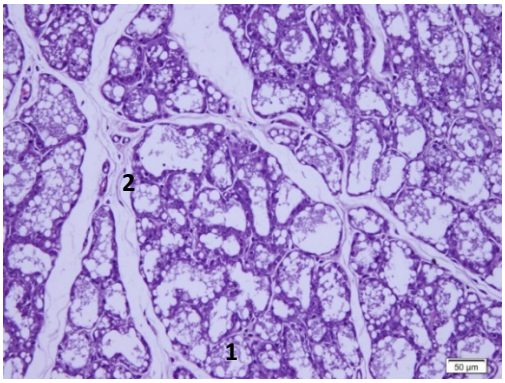
The experimental rats administered with coconut sprout at dose of 100 mg/rat/day showed the uterine condition in the active status (Figure 1). The experimental rats administered with 100 mg/kg BW showed that the endometrium mucosa of the uterus was covered by cuboidal epithelial cells. However, the number of endometrium glands in the experimental rats administered with coconut sprout at a dose of 100 mg/rat/day was very low and it was found some cytotrophoblast cells. The endometrium layers in the uterus of the experimental rats administered with coconut sprout at dose of 100 mg/rat/day were filled with hyaline and muscular skeletal fibers (connective tissues). The histological conditions of the experimental rats administered with coconut sprout at a dose of 200 mg/rat/day showed that the endometrium mucosa consisted of cuboidal epithelial cells, the endometrial glands, the cytotrophoblast cells, and mononuclear cell infiltration. The reproductive status of the experimental rats administered with coconut sprout at a dose of 200 mg/rat/day were in the status of diestrus and endometris.

The non-significant difference in serum progesterone and progesterone in the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day did not show significant differences in the histological conditions of the mammary gland (Figure 2). The histological observation of the mammary glands showed that the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day did not show any specific abnormal condition and they grew normally. The mammary glands of the experimental rats without coconut sprout administration were in active conditions as indicated by the alveoli filled with lipid or fat and amylacea globules. The alveoli of the mammary glands of the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day were covered with the cuboidal epithelial cells.

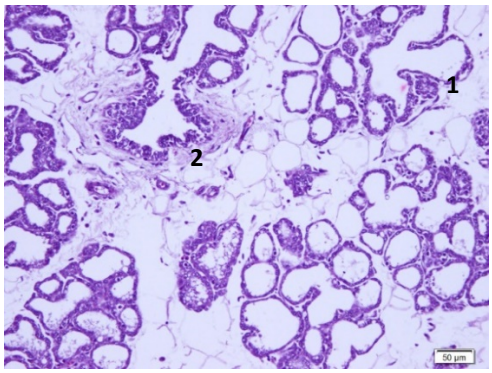
The mammary glands of the experimental rats administered with coconut sprout at a dose of 100 mg/rat/day showed active conditions and the alveoli grew well. In addition, the alveoli of the experimental rats administered with coconut sprout at a dose of 100 mg/rat/day were covered by the cuboidal epithelial cells and filled with globules. The mammary glands of the experimental rats administered with coconut sprout at a dose of 200 mg/rat/day were also active with alveoli grew well and filled with globules and covered by cuboidal epithelial cells (Figure 2).

The experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day had placental histological conditions that were in the early growth stadia (Figure 3). In addition, the histological observation of the placenta with HE staining in the experimental rats without administration of coconut sprout showed the

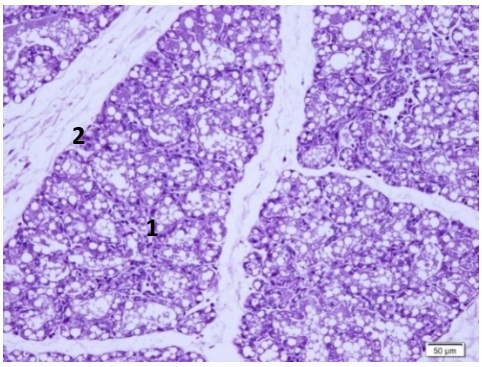
existences of blood vessels and allantois and some maternal blood vessels. Allantois layer consisted of cuboidal epithelial cells, and cytotrophoblast cells were also found in the allantois layer. The experimental rats administered with coconut sprout at a dose of 100 mg/rat/day showed the active stadium of the placenta. The experimental rats administered with coconut sprout at a dose of 100 mg/rat/day had allantois and maternal blood vessels that were developed well and filled with red blood cells (congestion).



The mammary glands of control experimental rat administered coconut sprout at a dose of 0 mg/rat/day. 1. The alveole of the mammary glands filled with fat globules, and 2. The mammary gland ducts. HE. x200.



The mammary glands of experimental rat administered with coconut sprout at a dose of 100 mg/rat/day. 1. The alveoli of the mammary glands, and 2. The mammary glands ducts. HE. x200.

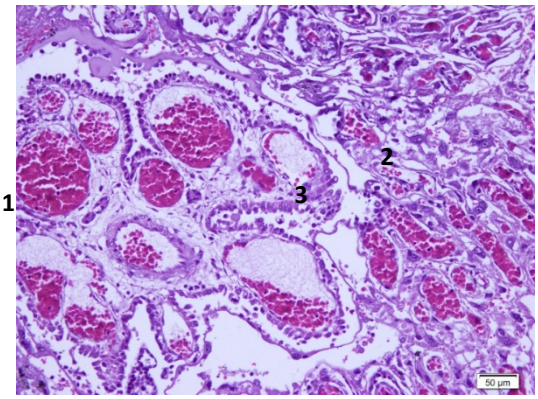


The mammary glands of experimental rat administered with coconut sprout at a dose of 200 mg/rat/day. 1. The alveoli filled with fat globules, and 2. The mammary gland ducts. HE. x200.

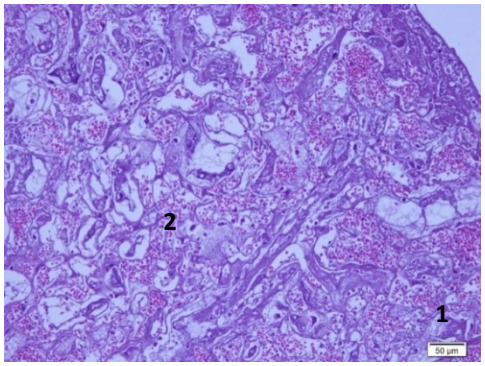
Figure 2: The histological condition of the mammary glands of experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day with HE. X200 staining.

The other observations in the histological conditions of the experimental rats administered with coconut sprout at a dose of 100 mg/rat/day were the occurrence of hyperemia and haemorrhage in the allantoic layer. The experimental rats administered with coconut sprout at a dose of 100 mg/rat/day had mucosal allantois layer consisted of cuboidal epithelial cells and some of them were in the desquamated conditions. In addition, the

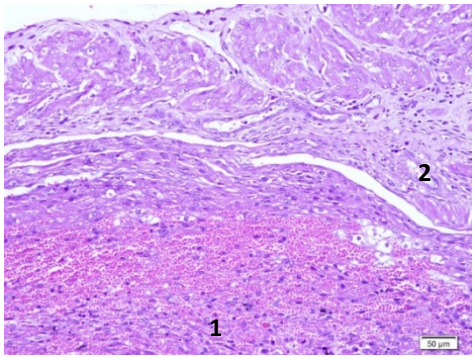
experimental rats administered with coconut sprout at a dose of 100 mg/rat/day did not have allantois glands and cytotrophoblast cells.



The placenta of control experimental rat without administration of coconut sprout. 1. Congestion, 2. Haemorrhage, and 3. The placental glands. HE. x200.



The placenta of experimental rat administered with coconut sprout at a dose of 100 mg/rat/day. 1. Haemorrhage, and 2. The placental glands. HE. x200.



The placenta of experimental rat administered with coconut sprout at a dose of 200 mg/rat/day. 1. Haemorrhage, and 2. The placental muscle fiber. HE. x200.

Figure 3: Histology of the placenta of experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day with HE. x200 staining.

The histology of the placenta in the experimental rats administered coconut sprout at a dose of 200 mg/rat/day showed that the condition or stadium was in the early stage of active development. The placenta of experimental rats administered with coconut sprout at a dose of 200 mg/rat/day had allantois and maternal blood vessels that grew and developed well and filled with red blood cells. In addition, the experimental rats administered with coconut sprout at a dose of 200 mg/rat/day had cytotrophoblastic cells and megacells (cyoblast), connective tissues, as well as hyperemia (Figure 3).

At weeks 2 and 3 of pregnancy, the experimental rats administered coconut sprout at doses of 0, 100, and 200 mg/rat/day had similar erythrocyte concentrations, hemoglobin concentrations, and hematocrit or PCV.

Administration of coconut sprouts did not affect erythrocyte concentrations, hemoglobin concentrations, and hematocrit or PCV (Table 3).

Table 3: The hematological profiles of the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day in the weeks 2 and 3 of pregnancy.

Age of pregnancy	Parameters	Doses of coconut sprout administration (mg/rat/day)		
		0 (n=6)	100 (n=6)	200 (n=6)
Week 2	RBC concentrations (10^6/mm^3)	6.75±0.52[a]	7.56±1.15[a]	8.33±0.88[a]
	Hemoglobin (g%)	14.15±1.18[a]	11.55±2.05[a]	12.68±1.40[a]
	PCV (%)	24.13±2.42[a]	25.50±4.79[a]	28.67±3.02[a]
Week 4	RBC concentrations (10^6/mm^3)	6.63±0.62[a]	5.96±0.80[a]	7.71±1.13[a]
	Hemoglobin (g%)	11.46±1.27[a]	9.02±1.62[a]	10.80±1.86[a]
	PCV (%)	24.75±2.33[a]	23.47±5.76[a]	25.20±4.68[a]

[ab]Different superscripts in the same row indicate a significant difference (P<0.05).
RBC = red blood cell; PCV = packed cell volume.

The experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day showed similar white blood cell counts, lymphocytes, neutrophil, monocytes, and eosinophil (P>0.05) and the basophils was not found in weeks 2 and 3 of pregnancy. However, in the week 3 of pregnancy, the monocytes of the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day were lower than that of control rats without administration of coconut sprout (P<0.05). The order of monocyte number in the week 3 of pregnancy from the highest to the lowest levels were found in the experimental rats administered with coconut sprout at doses of 0, 200, and 100 mg/rat/day. In the week 3 of pregnancy, the experimental rats administered with coconut sprout at doses of 100 and 200 mg/rat/day had decreased monocytes by 54.95 and 39.93%, respectively, compared to control rats without administration of coconut sprout (Table 4).

In summary, the experimental rats administered with coconut sprout had lower body weight even though they were fed with the same levels of feed (20 mg commercial feed per day) during the experiment. Therefore, the decreased body weight was not associated with the decreased feed intake. The most pronounce effects of coconut sprout administration in pregnant rats were the decreased number of fetus in the placenta with the increased uterine weight. The decreased number of fetus could not be related to the decreased uterine environment. Macroscopically, the experimental rats administered with coconut sprout had increased uterine weight with the increased dose of coconut sprout administration. Microscopic observation showed that the activities of the uterus and placenta were higher in the experimental rats administered with coconut sprout. The most dominant factors affecting pregnancy is progesterone. Even though statistically it was not significant, progesterone and thyroxin concentrations tended to increase with the increased dose of coconut sprout

administration. In the antioxidant status of the experimental rats, the MDA status tended to be low and SOD and catalase tended to be higher in the experimental rats administered with coconut sprout. Therefore, the most dominant effects of coconut sprout administration in decreasing fetal number could be associated with the compounds in the coconut sprout.

Table 4: The profiles of white blood cells (WBC) at the ages of weeks 2 and 3 of pregnancy in the experimental rats administered with coconut sprout at doses of 0, 100, and 200 mg/rat/day.

Age of pregnancy	Parameters	Doses of coconut sprout administration (mg/rat/day)		
		0 (n=6)	100 (n=6)	200 (n=6)
Week 2	WBC (10^3/mm^3)	12.64±2.41[a]	11.51±2.95[a]	14.17±1.08[a]
	Lymphocyte (%)	60.67±4.10[a]	61.67±9.29[a]	57.33±5.85[a]
	Netrophil (%)	34.50±4.49[a]	32.33±8.15[a]	36.67±6.25[a]
	Monocyte (%)	1.67±0.33[a]	3.33±0.56[a]	3.67±0.99[a]
	Eosinophil (%)	3.16±0.65[a]	2.67±0.75[a]	2.33±0.98[a]
	Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00
Week 3	WBC (10^3/mm^3)	14.73±1.89[a]	10.79±1.45[a]	11.27±2.36[a]
	Lymphocyte (%)	74.67±4.88[a]	77.83±4.44[a]	59.17±2.30[b]
	Netrophil (%)	21.67±5.58[a]	19.00±4.35[a]	34.50±3.20[a]
	Monocyte (%)	2.83±0.65[a]	2.17±0.17[a]	3.33±0.95[a]
	Eosinophil (%)	0.83±0.47[a]	1.00±0.26[a]	3.00±1.61[a]
	Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00

[ab]Different superscripts in the same row indicate a significant difference ($P<0.05$).

The decreased fetal number in the pregnant rats could be related to the decreased number of ovulating follicles, the decreased fertilization rate, implantation, and the maintenance of pregnancy. Since the administration of coconut sprout was conducted after fertilization, the processes that could be affected by the compounds in the coconut sprout were implantation and the maintenance of pregnancy. Since all of the experimental rats administered with coconut sprout were pregnant at the observation at the end of pregnancy, the effects could be in the limitation of implantation. Flavonoid found in the coconut sprout could be the main compound that limited the implantation. Flavonoids in the herbal preparations were reported to have estrogenic effects that are called phytoestrogen that could bind to estrogen receptor [19, 20]. The increased phytoestrogen stimulation during implantation could affect the success of implantation that eventually affects the fetal number. In the mid-1940, it was reported an infertility syndrome in sheep associated with the ingestion of phytoestrogen-rich clover [19]. However, administration of phytoestrogen during pregnancy in Sprague-Dawley rats did not affect implantation, live pups per litter, and pups birth weight [20]. Naturally, during implantation, the dominant hormone is progesterone secreted by the corpora lutea formed after ovulation of follicles and the synthesis of estrogen were decreased and directed to the synthesis of progesterone. The increased phytoestrogen availability in the experimental rats administered with coconut sprout during early pregnancy could probably limit the

implantation sites that eventually decreased fetal number. This preliminary result could be developed to be used as a strategy to limit the number of litter size in polytocus animals. The higher the number of fetus during pregnancy the higher the competition of the fetuses to obtain nutrients and compounds required for optimum growth and development with the final results in the lower birth weight that eventually reduced the growth and survival of the offspring during postnatal life. The decreased litter size in rats administered with coconut sprout in the present experiment improved the average of fetal weights.

It is required to study the use of higher doses of coconut sprout to proof the activities of the preparation in improving and increasing estrogen, progesterone, and thyroxin in pregnant rats. It is also required to study the effect of coconut sprout administration on reproductive performances of virgin non-pregnant female animals. It is also recommended to study the effects of coconut sprout administration on oocyte quality, fertilization rate, implantation rate and fetal survival in the experimental animals.

4. Conclusion and Recommendation

The administrations of coconut sprout at doses of 100 and 200 mg/rat/day decreased estrogen concentration and the number of fetus or litter size even though uterine weight and histological conditions were effectively improved. However, the decreased litter size with the improved uterine and placental weight improved fetal growth and development as was indicated by the improved fetal weight. Administration of coconut sprout did not affect red blood and white blood cells profiles but improves the SOD status of the experimental pregnant rats. There is an indication that coconut sprout could reduce the litter size in the polytocus animals. It is recommended that coconut sprout can be used to improve fetal growth and development in polytocus animals by reducing fetal number.

Conflict of Interest

This experiment and the article do not contain any conflict of interest.

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