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Effects of aqueous leaf extract of *Cajanus cajan* on litter size and serum progesterone in pregnant rats

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Aqueous leaf extract of *Cajanus cajan* is consumed by pregnant women in our locality. However, its effect on pregnancy has not been studied. *C. cajan* is known to contain genistein and diadzein which are potent phytoestrogens. We studied the effect of aqueous leaf extract of *C. cajan* on litter size and serum progesterone in pregnant rats. Oral administration of *C. cajan* on timed-pregnant rats increased litter size from 7.2 ± 1.1 in the control gorup to 10.1 ± 1.5 ($p < 0.01$) and 10.6 ± 0.8 ($p < 0.003$) in 100 and 200 mg/kg respectively. Serum progesterone increased from 98.6 ± 3.5 ng/ml in the control group to 112.4 ± 5.3 ng/ml ($p < 0.003$) and 114.2 ± 3.7 ng/ml ($p < 0.002$) in the 100 and 200 mg/kg treated groups respectively. There was reduction in litter weight from 6.93 ± 0.2 g in the control group to 4.60 ± 0.3 g ($p < 0.0002$) and 4.40 ± 0.1 g ($p < 0.0003$) in the 100 and 200 mg/kg treated groups respectively. There was no statistical significant difference in the litter weight among the treated groups. Aqueous extract of *C. cajan* caused reduced maternal weight gain in the treated group compared to the control. Maternal weight gain reduced from 62.4 ± 3.4 g in the control to 58.9 ± 2.8 g ($p = 0.053$) and 57.6 ± 3.1g ($p < 0.05$) in the 100 and 200 mg/kg treated groups respectively. There was no statistical significant difference in the litter weight among the treated groups. In conclusion, oral administration of aqueous leaf extract of *C. cajan* increases litter size and plasma progesterone in pregnant rats.

Key words: *Cajanus cajan,* litter size, serum progesterone.

INTRODUCTION

Plant-derived chemicals that influence endocrine activities in both human and animals have received a great deal of attention due to their possible beneficial as well as adverse effects (Gamache and Acworth, 1998). Many herbs either wholly or their extracts are consumed by pregnant women, effects of which are not known in the mother and the children. This is common in the developing countries of the world.

Cajanus cajan belongs to the botanic family Fabaceae. It is known as pigeon pea (English), Otili (Yoruba), and Waken turawa (Hausa). It is grown in the forest and savannah regions of the world. Sun-dried leaf of *C. cajan* contains 70.4% moisture, 7.0% crude protein, 10.7% crude fibre and 7.9% nitrogen-free extract, 1.6% fat and 2.3% ash. Phytoestrogen constituents of *C. cajan* include genistein and diadzein with lignan secoisolariciresinol (Mazur and Aldercreutz, 1998).

Some of the medicinal uses of *C. cajan* according to Morton (1976) and Duke (1981) are for the treatment of jaundice, bronchitis, cough, antihelminthic, sedative and child delivery. *C. cajan* leaf extract has also been shown to have dose-dependent reduction in uterine contraction in rats (Olatunji-Bello et al., 2002). *C. cajan* also has hypoglycaemic, antisickling and anti-plasmodial properties (Giri et al., 1987; Ogoda et al., 2002; Duker-Eshun et al., 2004). In view of the fact that *C. cajan* is consumed by pregnant women, this study was designed to provide information on

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Table 1. Effects of aqueous extract of *C. cajan* on litter size, litter weight, maternal weight gain and serum progesterone in rats.

	Group I (Control)	Group II (*C. cajan* 100 mg/kg)	Group II (*C. cajan* 200 mg/kg)
Litter Size	7.2 ± 1.1[a]	10.1 ± 1.5[b]	10.6 ± 0.8[b]
Litter Weight (g)	6.93 ± 0.2[a]	4.60 ± 0.3[b]	4.40 ± 0.1[b]
Maternal Weight Gain (g)	62.4 ± 3.4[a]	58.9 ± 2.8[ab]	57.6 ± 3.1[b]
Serum Progesterone (ng/ml)	98.6 ± 3.5[a]	112.4 ± 5.3[b]	114.2 ± 3.7[b]

Values are expressed as mean ± SD. Number of rats in each group = 7.
[abc] Different superscripts on means ± SD along the same row indicate $p < 0.05$.

the effect of aqueous extract of *C. cajan* leaves on plasma progesterone and litter size in rats.

MATERIALS AND METHODS

C. cajan leaves were purchased from a local herb store at Ijora, Lagos, Nigeria. The leaves were authenticated at the Pharmacognosy Department of the College of Medicine, and Department of Botany and Microbiology, University of Lagos, using a herbarium specimen. The leaves were washed and air-dried. The aqueous extract of *C. cajan* leaf was prepared using the method described by Farida et al. (1987), then filtered and the residue was discarded. The filtrate was subsequently evaporated to dryness. The resulting powder of the extract was stored in capped bottles until needed. The extract of *C. cajan* (5 g) was dissolved in 1000 ml distilled water to make a stock of 5 mg/ml.

Twenty one albino rats (*Rattus norvegicus*, Muridae) weighing between 150 - 200 g and obtained from Animal Breeding Unit, College of Medicine, University of Lagos, were used for this study.

All animals were housed in plastic cage with stainless steel mesh cover under standard laboratory conditions (light period 6.30 am - 7.00 pm), temperature 27 ± 2°C, relative humidity 55%. The animals were allowed free access to tap water and rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) with following composition: carbohydrate 67%, protein 21%, fat 3.5%, fibre 6%, calcium 0.8% and phosphorus and phosphate 0.8%. They were acclimatized for two weeks before the commencement of the experiment. They received humane care.

The rats were divided into three groups as follows:

Group I: Control, received 10 ml/kg body weight of distilled water (vehicle).
Group II: Received 100 mg/kg body weight of the extract.
Group III: Received 200 mg/kg body weight of the extract.

The various groups were orally administered with 1 ml each of distilled water (control) and the extract (100 and 200 mg/kg body weight) once daily (08:00 - 08:30 h) using plastic syringes attached to metal oropharyngeal cannula from gestation day 2 to gestation day 19 when the animals were sacrificed.

Female and male rats were caged together during the night. The morning that conception was verified by the presence of sperm in a vaginal smear was designated gestational day 0. The *C. cajan* groups were fed with 100 and 200 mg/kg aqueous extract of *C. cajan* respectively from gestation day 2 to gestation day 19 when the animals were sacrificed.

On the 19th day, the animals were sacrificed by means of cervical displacement. Blood was taken and the uteri removed allowing for examination of the foetuses. The weight and number of foetuses, as well as the external morphological examination were recorded (Beck, 1989).

With the help of a magnifying glass, the presence or absence of cleft palate was observed, as well as the position of eye and ear implantations, the tail (form and length). The extremities were examined for abnormalities such as polydactyl and syndactyl.

Immediately after the rats were sacrificed, blood was collected from the heart by syringe and transferred into serum bottle. The tubes were thereafter centrifuged at 33.5 g x 15 min using Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals and Essex, England). The sera were later aspirated with Pasteur pipettes into clean, dry, sample bottles and were then stored at -20°C. Serum progesterone concentration was determined by enzyme immunoassay technique (Progesterone Enzyme Immunoassay Test Kit, Catalog No. 2077Z, Diagnostic Automation Inc., Calabasas, CA, U.S.A.). The assay detection limit was 0.3ng/ml and cross-reactivity with other steroids (e.g. testosterone, cortisone, estradiol etc.) was less than 0.15%. The intra-assay coefficient of variation ranges from 2.4 - 7.1% and inter-assay coefficient of variation ranges from 2.6 - 12.6%.

All data are shown as means ± standard deviation (SD). Statistical analysis was performed with one-way ANOVA followed by a Student Newman-Keuls test using SPSS 13.0 for Windows. Values at $p < 0.05$ were considered statistically significant.

RESULTS

Effects of aqueous extract of *C. cajan* on litter size, litter weight, maternal weight gain and serum progesterone in rats were shown in Table 1.

C. cajan caused increased litter size from 7.2 ± 1.1 in the control group to 10.1 ± 1.5 ($p < 0.01$) and 10.6 ± 0.8 ($p < 0.003$) in 100 and 200 mg/kg *C. cajan* treated group respectively. There was no statistical significant difference in the litter size among the treated groups.

There was reduction in litter weight from 6.93 ± 0.2 g in the control group to 4.60 ± 0.3 g ($p < 0.0002$) and 4.40 ± 0.1g ($p < 0.0003$) in the 100 and 200 mg/kg treated groups respectively. There was no statistically significant difference in the litter weight among the treated groups. Aqueous extract of *C. cajan* caused reduced maternal weight gain in the treated group compared to the control. Maternal weight gain reduced from 62.4 ± 3.4 g in the control to 58.9 ± 2.8 g ($p = 0.053$) and 57.6 ± 3.1g ($p < 0.05$) in the 100 and 200 mg/kg treated groups respectively. There was no statistically significant difference in the litter weight among the treated groups.

There was increase in serum progesterone in *C. cajan* treated group compared to the control group. Serum pro-

gesterone increased from 98.6 ± 3.5 ng/ml in the control group to 112.4 ± 5.3 ng/ml ($p < 0.003$) and 114.2 ± 3.7 ng/ml ($p < 0.002$) in the 100 and 200 mg/kg treated groups respectively.

None of the litter has any external morphological abnormality.

DISCUSSION

The results of our study indicate that aqueous extract of *C. cajan* increased litter size and serum progesterone in rats and reduced litter weight and maternal weight gain during pregnancy.

Studies have shown that *C. cajan* contains phytoestrogen genistein, diadzein and secoisolariciresinol (Shinde et al., 2008; Mazur and Aldercrentz, 1998). Even though, phytoestrogens are group of plants derived compounds that structurally and functionally mimic mammalian oestrogen, studies have shown that their administration increases progesterone synthesis and production. Kaplanski et al. (1981) showed that genistein increases progesterone production, though at a higher concentration, there is a decrease in the production of progesterone. Williams et al. (1997) also showed that *in vitro* administration of genistein in rabbit and bovine granulosa cells stimulates progesterone secretion. However, studies by Haynes-Johnson et al. (1999) and Nynca and Ciereszko (2006) showed that genistein inhibit the production of progesterone in rats and porcine granulosa cells respectively. However, Cotroneo et al. (2001) reported an increase in progesterone receptors in the uterus of rats that have prepubertal or *in utero*/lactational exposure to genistein while Picherit et al. (2000) observed decrease contractility of *in vitro* rat uterine muscle following exposure to diadzein and genistein due to blockade of estrogen receptor. A study by Olatunji-Bello et al. (2000) showed that aqueous extract of the leaves of *C. cajan* caused a significant reduction in the force and frequency of contraction of rat uterus.

The increased concentration of progesterone may be due to effect of genistein on the rats' granulosa cells. Genistein is the most potent phytoestrogen and is known to stimulate the production of progesterone. However, study by Richter et al. (2009), showed that phytoestrogens (genistein and daidzein) reduced progesterone production in human term trophoblast cells. In rats, studies by Haynes-Johnson et al. (1999) showed that genistein reduced plasma progesterone in rats, but studies by researchers such as Picherit et al. (2000) and Cotroneo et al. (2001), have shown that phytoestrogens increase progesterone receptors in the uterus of rats.

The increase in progesterone detected in our study may be due to several differences in the study designs, including the strain of rats used for the experiment (Sprague-Dawley) and timing of serum collection. The serum progesterone was higher in the treated groups compared to the control, though, all the values for treated and control were within normal physiological value during the third trimester of pregnancy (65 - 229 ng/ml). The timing of our serum collection was at the point of highest plasma progesterone concentration during pregnancy in humans and rats (Guyton and Hall, 2000; Puri and Garfield, 1982).

Progesterone has an antiestrogenic effect on the myometrial cell, decreasing their excitability, their sensitivity to oxytocin, and their spontaneous electrical activity while increasing their membrane potential (Ganong, 2003). The increased litter size could be due to the ability of progesterone to reduce myometrial cells excitability, thereby increasing the number of implantation and subsequent number of life fetuses. The reduced litter weight in the treated groups could be due to increased number of fetuses which could have resulted into inadequate nutrients, poor placental perfusion, fetal crowding and unknown factors (Chitkara and Berkowitz, 2002). However, because of the accompanied reduction in maternal weight, there might be associated antinutrient agents in the *C. cajan* extract. There is need for further study in order to isolate the active compound that is responsible for the increased progesterone and those that are responsible for the reduced maternal weight and fetal weight.

In conclusion, oral administration of aqueous leaf extract of *C. cajan* increases litter size and plasma progesterone in pregnant rats.

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