Vol. 12 · March 2013
Print ISSN 2012-3981 • Online ISSN 2244-0445
doi: http://dx.doi.org/10.7719/jpair.v12i1.209

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by AJA Registrars, Inc.

Soybean-Genistein as Endocrine Disruptor on Estrous Cyclicity and Ovarian Follicular Development in Albino Rats (*Rattus norvegicus*)

GERALDINE C. SANCHEZ
ORCID NO.: 0000-0003-4628-227X
gengsanchez923@gmail.com

RONALDO D. DIZON

ARIS F. MICLAT
ORCID NO.: 0000-0003-3749-8570
aris.miclat@gmail.com
Institute of Veterinary Medicine and Zootechnics
Pampanga Agricultural College
Magalang, Pampanga

MARIA FE S. BULAO
ORCID NO.: 0000-0002-6049-3885
mariafesimbulanbulao88@gmail.com

JULIA A. EGGERT
ORCID NO.: 0000-0003-1350-8420
Jaegger@clemson.edu
College of Health, Education and Human Development,
Edwards Hall, Clemson University, Clemson,
South Carolina U.S.A. 29631

ABSTRACT

Endocrine disruptors are chemicals that interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife. One example of endocrine disruptor is phytoestrogen which is a group of naturally occurring compounds that have been reported to cause fertility problems in animals. The major phytoestrogen in soy products is genistein, which has potent estrogenic activity both in vitro and in vivo. Previous findings have demonstrated that the control of primordial follicle development and subsequent folliculogenesis appears to be mediated by local production and action of specific paracrine factors. Preliminary studies also have shown that steroid hormones like estrogen play a critical role in the onset of primordial follicle assembly. These findings led us to further look into the effects of genistein on estrous cyclicity and ovarian folliculogenesis specifically on pre-antral and antral follicular development including their possible effects on ovarian morphometry of sexually matured female albino rats. The objectives of the study was to determine the effects of genistein on estrous cyclicity and ovarian folliculogenesis specifically on pre-antral and antral follicular development including their possible effects on ovarian and uterine morphometry of sexually matured female albino rats. Furthermore, the study elucidated its effect on the apoptosis of granulose and theca cells resulting to follicular atresia. A total of 24 female albino rats approximately 2-3 months of age of almost the same size were used in this study. The treatments were: Control (T0) distilled water, 8 mg/kg body weight genistein (T1), 12.5 mg/kg body weight genistein (T2) and 16 mg/kg body weight genistein (T3). Estrous cyclicity was determined using vaginal cytology. The experimental animals were sacrificed after five weeks and their ovaries and uterus were collected. Ovarian tissues were subjected to Paraffin technique for tne microscopic examination. All data gathered were subjected to One-Way Analysis of Variance (ANOVA) and significant differences among treatments were analyzed using Least Significant Difference (LSD). Results showed an increased length of proestrus and estrus period in treated rats, metestrus on the first week of treatment and diestrus on the second week of treatment period. In terms of antral and preantral follicles, rats treated with genistein have greater mean number compared with the control and the mean number of non-atretic follicles was high in the control group and $T_{4.}$ Genistein treated rats at 12.6 and 16 mg/kg body weight have greater mean number of pre-antral and antral follicles as compared with those treated at 8 mg/kg body weight and the control. Genistein in soybean has endocrine disruption effect by altering estrous cyclicity and ovarian folliculogenesis but it has no adverse effect on heart and respiratory rates as well as on body temperature.

Keywords - Antral Follicle, Estrous Cycle, Genistein, Ovary and Soybean

INTRODUCTION

There is a considerable concern on endocrine disruptors which are chemicals that interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife (www.niehs.nih.gov,2000)[2]. One example of endocrine disruptors is phytoestrogen which is a group of naturally occurring compounds that have been reported to cause fertility problems in animals. Of particular concern is genistein, the major phytoestrogen in soy products, which has potent estrogenic activity both in vitro and in vivo (Diel, 2001). It can bind to the estrogen receptor to induce estrogen-like effects in animals, humans and cultured cells (Liu, 2006).

The possibility that some chemicals may disrupt the endocrine systems in humans and animals has received considerable attention in the scientific and public community. Endocrine disruption is on the agenda of many experts' groups, steering committees and panels of governmental organizations, industry, and academia throughout the world. Because the disturbance of the endocrine system is a very sensitive topic, scientific findings or observations are often controversially discussed among scientists, environmentalists, and authorities (Lintelmann, 2003).

Soy and products derived from soy, such as soya milk, tofu, tempeh, soy flour, soy sauce, taho and isoflavone supplements, are being consumed in increasing quantities by humans. Similarly, high quantities are used as a feed ingredient for laboratory, companion and food animals (Brown, 2001).

Although enormous progress has been made in understanding the events and regulation of the later stages of ovarian follicular development, the early stages of development, to a large extent and particularly in large mammals, remain a mystery. Mechanisms that regulate the initiation of follicular growth and the ensuing growth and differentiation of preantral follicles are of considerable interest, since their elucidation is a prerequisite to use of the primordial pool to enhance reproductive efficiency in domestic animals, humans, and endangered species (Fortune [15]2003).

The study of Kouki (2003) on the effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function obtain findings in genistein treated groups that ovaries were smaller and contained no corpora lutea. Ovaries from daidzein treated females were also small.

Moreover, the study of Flyn (2000) on the effect of genistein in sexually dimorphic behavior of rats revealed findings of decreased in average weight per live pup at birth.

High amounts of isoflavones given to newborn rats resulted in disturbance of estrous cyclicity at a very early stage of life (Delclos, 2009). Experimental data from cell cultures and whole animal studies showed that such concentrations had strong estrogenic effects (Mueller, 2004).

Previous research and preliminary studies have demonstrated that the control of primordial follicle development and subsequent folliculogenesis appears to be mediated by local production and action of specific paracrine factors involving theca cells, granulose cells, and the oocyte. Preliminary studies also have shown that steroid hormones like estrogen play a critical role in the onset of primordial follicle assembly (Skinner[30] 2008).

These findings led us to further look into the effects of genistein on estrous cyclicity and ovarian folliculogenesis specifically on pre-antral and antral follicular development including their possible effects on ovarian morphometry of sexually matured female albino rats. Also, the effect of genistein on cellular changes particularly on apoptosis of granulose and theca cells resulting to follicular atresia was investigated.

OBJECTIVES OF THE STUDY

The general objective of the study was to elucidate the effects of genistein on estrous cyclicity and ovarian follicular development using rats as the animal model.

Specifically, it aimed to determine genistein's effects on:

1. different stages of the estrous cycle;
2. body temperature, heart and respiratory rates;
3. gross changes in the heart, liver, lungs and kidney;
4. body weights;
5. uterine morphometry (horn length and width);
6. ovarian morphometry (length, width, weight)
7. antral and pre-antral follicles
8. atretic and non-atretic follicles

MATERIALS AND METHODS

Experimental Animals

A total of 24 female albino rats approximately 2-3 months of age of almost the same size were used in this study. They were caged individually in 6 x 6 x 8 inches cage and were given one-week acclimatization period prior to the conduct of the study. The experimental rats were housed at the Animal Research Laboratory of the Institute of Veterinary Medicine and Zootechnics at room temperature with a relative humidity of at least 30 % and not exceeding 70 % measured with the use of a digital thermometer (CDR- KING, 2010). The heart rate, respiratory rate and rectal temperature were taken every other day to avoid additional stress on the rats. Body weights were taken using a triple beam balance (OHAUS).

Preparation and administration of genistein

Genistein was purchased from Xi'an Feida Bio-Tech Co., Ltd, China with a purity of 95 %. In preparing dosages of genistein powder, the manufacturer's prescription was used. Moreover, the dosage used by Zhou (2008) which is 12.5 mg/kg body weight were adopted in the study to serve as baseline for dosage determination. Genistein powder was dissolved in normal saline solution and was administered to rats subcutaneously using a glass syringe.

Monitoring of Estrous Cycle

In determining the estrous cyclicity of rats, the protocol of Reyes (2006) was adopted. Data on estrous cycle was taken two weeks before the start of the study (D_0) until the rats were sacrificed. Daily monitoring of estrous cycle was done through microscopic examination of sample vaginal smear. The estrus stage was determined by the presence of abundant cornified cells in the smear. The rats were sacrificed through cervical dislocation a week after the two (2) weeks treatment period when they were at the diestrus stage.

Collection, gross examination and processing of ovaries, uterus and other visceral organs

The experimental animals were sacrificed after five weeks and their ovaries and uterus were collected. The ovaries, uterus, heart, lungs, liver and kidney were observed

for gross changes. With the use of a digital weighing scale, the ovaries were weighed and the length and width were measured. The collected ovaries, uterus and lungs with gross lesions were then preserved in ten percent (10%) formalin solution and were brought to Histopathology Laboratory of University of the Philippines, Los Baños, Laguna for processing using the Paraffin Technique. The thickness of the ovarian section used in this study was 5 microns (Sanchez, 2005) and the processed tissues were stained using hematoxylin and eosin (H & E).

Microscopic examination of processed tissues

Processed ovarian, uterine and lung tissues were examined using a microscope at 100 x magnification. Pre-antral, antral, atretic, and non-atretic follicles were identified and quantified on the ovaries. The uterine and lung tissues were observed for histopathological lesions.

Experimental design and treatments

A Complete Randomized Design was used in this study. The experimental animals were randomly distributed in four treatments with six replications per treatment by means of draw lots. The treatments were as follows:

T1 – distilled water (control)
T2 – 8 mg/kg body weight genistein
T3 – 12.5 mg/kg body weight genistein
T4 – 16 mg/kg body weight genistein

RESULTS AND DISCUSSION

Length of proestrus period

The proestrus stage is composed of nucleated epithelial cells and can be seen for a period of 12 - 14 hours in a 5-day estrous cycle. This could be increased or decreased depending on several factors such as stress and hormonal problems. And at this stage the female rat make acceptance to the male at the end of the phase (www.lssu.edu)[3]

As reflected in Table 1, the proestrus stage was observed on the average, for a period of once a week for the control. Those treated with genistein showed an increased in their proestrus stage especially during the second week of treatment period. It also appears that although T2 received a dose of 8mg/kg body weight of

genistein only, it had an increase of 0.17 days proestrus stage during the first week of treatment period. On the second week of treatment, T_2 maintained its proestrus stage of 1.17 days however, for T_3 which have received a dose of 12.5 mg/kg body weight of genistein and T_4 with 16 mg/kg body weight of genistein, showed an increase of 0.6 and 0.67 days, respectively. On the withdrawal of genistein, all of the treatments resume on their once a week average proestrus stage.

The increased in the proestrus stage during the second week of treatment best describes that genistein is highly absorbed and elicited its effect to prolong the estrous cycle by increasing this stage on T_2, T_3 and T_4.

Since the monitoring of estrous cycle was done only on a per day basis, the exact proestrus length in terms of hours (hr) was not observed by the researcher.

Table 1. Length of proestrus during pre-treatment, first week of treatment, second week of treatment and post-treatment period (days)^{ns}

Treatment	Pre-treatment	1st week	2nd week	Post-Treatment
T1	1.00	1.00	1.00	1.00
T2	1.00	1.17	1.17	1.00
T3	1.00	1.00	1.60	1.00
T4	1.00	1.00	1.67	1.00

ns- not significantly different (P>0.05)

Length of estrus period

Estrus stage is characterized by the presence of 75% nucleated epithelial cells and 25 % cornified cells on the vaginal smear. This could be observed for a period of 12 – 27 hours in a 5-day estrous cycle. Estrus can be detected when the vulva becomes slightly swollen and the vagina becomes dry in contrast to the usual moist pink. Female rats in heat are hyperactive and brace themselves when touched. The ears quiver when they are stroked on the head or back, and touching the pelvic region induces a posture termed lordosis, in which the head and rump are raised and the back is arched downward.

As shown in Table 2, there were differences in the length of estrus period of rats treated with genistein compared with the control (T_1). On post treatment, all treatments showed significant difference showing longer estrus for T3. Treatment 1 showed longer estrus in the first week only but not on the second week. T2 exhibited an increase in estrus length during the first and second week of treatment with

genistein compared during the pre-treatment period .On the withdrawal of genistein, a slight increase in estrus length was also noted.

The effect on T3 exhibited more during the post treatment period which exhibited longer estrus than the other treatments. For the Control (T_1), it could be noted that a slight increase in estrus length from 1 at pre-treatment to 2 days on the first week of treatment period as shown in Table 2, was observed during the time that the other rats were treated with genistein. This could be explained by the presence of the vomeronasal organ present in rats which primarily detect pheromones that specialize in non-volatile chemicals found in the urine and other secretions. The introduction of genistein on the other rats by means of the vomeronasal organ of the Control rats shoot up a separate pathway to the *accessory olfactory bulbs*, and from there to the amygdala, then to both the preoptic area and the hypothalamus, which are areas known to be involved in reproductive behavior (Brennan, 2001).

Based on the findings of this study, an increased in estrus length as shown in Treatments 1, 2 and 3 during the first and second week of treatment is due to the action of genistein, which mimicks the hormone estrogen in the body. This finding is significant in prolonging the estrus period on the average by 1.5 days longer than the normal estrus length of 1.0 day only in female rats. Thus, soybean-genistein prolongs and enhances sexual receptivity and sexual activity. This breakthrough could help in addressing fertility problems both in animals and human beings. Women who are on their menopausal stage may have exogenous source of estrogen as in this case, the genistein present in soybeans which could help in the prevention of vaginal dryness thus improving sexual activity. Further study on this aspect is therefore necessary.

Table 2. Length of estrus during pre-treatment, first week of treatment, second week of treatment and post-treatment period (days)

Treatment	Pre-treatment	1st week	2nd week	Post-Treatment
T1	1.00	2.00	1.00	1.00[d]
T2	1.00	2.16	1.83	2.67[c]
T3	1.00	1.50	1.67	3.17[a]
T4	1.00	2.50	2.17	2.83[b]

Means having different superscripts are significantly different at ($P<0.05$)

Length of metestrus period

The metestrus stage is characterized by the presence of many leukocytes with nucleated and cornified cells in a vaginal smear. It can be observed for a period of 21 hours in a 5-day estrous cycle and the female rat is observed with no male acceptance.

As revealed in Table 3, there was an increased in the length of metestrus stage of treated rats. During the first week of treatment period, $T_{2,}$ T3 and T4 gained an increase of 0.83, 1.17 and 1, days, respectively. On the second week of treatment and post treatment periods, there was a general decrease in the length of metestrus compared during the first week.

The increase on the metestrus stage during the first week of treatment can be associated to the effect of genistein administration. However, the increase of 0.5 day metestrus stage on the control (T_1) during the second week of treatment period was just normal to meet the normal 5-7 days length of estrous cycle and can be compared to a slight decrease on the metestrus stage of treated rats as affected by genistein. Also, the long metestrus period during the post-treatment period of treated rats is correlated to the increase in average estrus length of treated rats on the same period.

Table 3. Length of metestrus during pre-treatment, first week of treatment, second week of treatment and post-treatment period (days)

Treatment	Pre-treatment	1st week	2nd week	Post-Treatment
T1	1.00	1.00[d]	1.00	1.00
T2	1.00	1.83[c]	1.17	1.00
T3	1.00	2.17[a]	1.60	1.00
T4	1.00	2.00[b]	1.67	1.00

Means with different superscript in the same column are significantly different ($P<0.05$)

Length of diestrus period

Diestrus stage is characterized by the presence of leukocytes in a vaginal smear and this can be observed for a period of 57 hours in a 5-day estrous cycle. During this stage, the female rat has no male acceptance.

As shown in Table 4, the length of diestrus period prior to genistein treatment (Pre-treatment period) revealed same length of all treatments with a mean of 4. However, on the 1st and second week of treatment, there was a marked decrease in diestrus

length of treated rats as compared with the control (T_1) and the whole pre-treatment period except for T3 which showed longer diestrus of 4.4 days. Furthermore, on the post-treatment period, an increase of 0.67 day was again observed in the control (T_1) while a decrease of 0.33 day on T_1, 1.73 on T_2 and 0.33 day on T_3. This could be explained by an increase in the proestrus, estrus and metestrus stage of rats will result to a decrease on the length of diestrus stage. A decrease on the proestrus, estrus and metestrus stage also result to an increase on the length of diestrus stage of rats and vice versa.

Table 4. Length of diestrus during pre-treatment, first week of treatment, second week of treatment and post-treatment period (days)

Treat-ment	Pre-treatment	1st week	2nd week	Post-Treatment
T1	4.00	3.00^{a}	3.33	4.00^{a}
T2	4.00	1.83^{ab}	3.00	2.67^{ab}
T3	4.00	2.17^{ac}	4.40	2.67^{ab}
T4	4.00	1.50^{ad}	2.83	2.5^{ad}

Means with different superscript are different at (>0.05).

Mean body temperature

As revealed in Table 5, the rectal temperature values obtained from Treatment 1, 2 and 3 were within the normal temperature of rats which indicate absence of hyperthermia post genistein treatment.

Table 5. Mean body temperature (°C)^{ns}

Treatment	1st week	2nd week
T1	36.7	36.9
T2	36.8	36.9
T3	36.7	36.9
T4	36.5	36.9

ns- not significantly different at (P>0.05).

Effects on heart rate and gross appearance

As shown in Table 5, there is a decreased in the heart rate of rats during the 2nd week of treatment although their values fall on the normal range. In the study of Al-Nakkash (2010), genistein had no effect on the weights of heart and heart-to-body ratio. Moreover, fat pad significantly decreased heart rate and pulse pressure. No pathological gross lesions were found in the heart.

Table 5. Mean heart rate (beat/minute)ns

Treatment	1st wk	2nd wk
T1	346	298
T2	346	296
T3	348	292
T4	349	280

ns- not significantly different at (P<0.05).

Effects on respiratory rates, gross and microscopic appearance of the lungs

As revealed in Table 6, there was no significant difference noted among treatments during the first and second week of treatment although it appears that during the second week, the respiratory rates fall until the termination of the study. However, the values obtained are still within the normal range. Pathological gross lesions were found on the lungs of T_1 and T_2 wherein they were pale, hard and granulomatous. Sneezing was also observed on rats during the second week of treatment period.

Microscopically, the lungs have numerous neutrophils, hemorrhage and congestion which is suggestive of pulmonary emphysema. The study of Yellayi (2002) determined that genistein treatment suppressed immune system function. Genistein-treated mice might have produced lower amounts of antibodies following administration and it might have contributed to the gross lesions found in the lungs.

Table 6. Mean respiratory rate (breaths/minute)[ns]

Treatment	1st Week	2nd Week
T1	91.0	89.2
T2	91.0	88.7
T3	91.0	89.4
T4	92.0	88.4

ns-not significantly different at (P>0.05)

Body weights

Shown in Table 8 is the mean body weight of mice. During the first week, there was no significant difference noted among treatments but on the second week, there was a reduction in the body weight of treated group and the untreated remained to have the heaviest weight. This suggests that genistein influence the body weight of rats.

Table 8. Mean body weights (grams)

Treatment	1st Week	2nd Week
T1	244	261[a]
T2	253	242[ab]
T3	222	209[bc]
T4	222	210[b]

Means with different superscripts in the same column are significantly different at (P<0.05) level.

Length of uterine horn, gross and microscopic appearance

Reflected in Table 9 is the length of the uterine horn. Statistical analysis revealed that T3 exhibited the longest left horn. Generally, the increased in the uterine length of treated rats as compared to the Control is suggestive of uterine hypertrophy. Also on microscopic examination , treated rats exhibited hemorrhages, congestion and hypercellularity of the myometrium and endometrium.

Table 9. Length of the uterine horn (mm)

Treatment	Right horn	Left horn
T1	27.5	23.7^{bd}
T2	30.7	26.0^{b}
T3	32.5	29.0^{a}
T4	29.0	25.5^{bc}

Means with different superscripts in the same column are significantly different at P (< 0.05) level.

Width of the uterine horns

As shown in Table 10, there is no difference on the width of the left and right uterine horns among treatments.

Table 10. Width of the uterine horns (mm)^{ns}

Treatment	1st week	2nd wk
T1	2.33	2.33
T2	2.67	2.67
T3	3.00	3.00
T4	2.67	2.67

ns-not significantly different at (P>0.05)

Mean ovarian length

Revealed in Table 11 are the length of the right and left ovaries showing no significant difference among the treatment groups.

Table 11. Mean ovarian length(mm)^{ns}

Treatment	Right ovary	Left ovary
T1	6.5	6.0
T2	5.8	5.5
T3	5.2	4.7
T4	5.5	4.8

ns-not significantly different at (P>0.05)

Mean ovarian width

Table 12 presents the mean width of the right and left ovaries. It appears that there was no significant difference among treatments in the right ovary but there was among the left showing a reduction in the width of the treated group especially the one that received the highest amount of genistein. At (P<0.01).

Table 12. Mean ovarian width (mm)[ns]

Treatment	Right ovary	Left ovary
T1	5.83	5.67[a]
T2	5.33	5.00[ab]
T3	4.67	4.17[b]
T4	5.00	3.67[c]

Means with different superscripts in the same column are significantly different at (P<0.05) level.

Mean ovarian weight

Shown in Table 13 is the mean ovarian weight. There was no significant difference among the treatments in the right ovary but there was in the left ovary at (P<0.01). All treated animals manifested much lower ovarian weight which implies that genistein affects ovarian weight.

Table 13. Mean ovarian weight (grams)

Treatment	Right ovary	Left ovary
T1	0.063	0.059[b]
T2	0.057	0.054[ab]
T3	0.050	0.045[a]
T4	0.054	0.035[a]

Means with different superscripts in the same column are significantly different at (P<0.05) level.

Mean preantral and antral follicles

Shown in Table 14 are the mean pre-antral and antral follicles showing significant difference ($P < 0.05$) in the mean number of pre-antral follicles of treated groups compared with the control. The highest number of pre-antral follicles was observed in T4 followed by T3, T2 and T1.In terms of antral follicles, there was no significant difference among treatments which implies that genistein affects only growing follicles.

Zhuang (2010) in their study in genistein treated rats showed a higher percentage of primordial follicles by 4 months of age and a greater number of surviving follicles at 15 months of age compared to a control group ($P < 0.05$). In addition, vaginal cytology showed that age-dependent cessation of regular estrus was delayed for 2 months in genistein-treated group than control group which suggest that genistein alters rat ovarian follicular development and increases the number of surviving follicles, which may prolong ovarian reproductive life.

Table 14. Mean pre-antral and antral follicles (grams)

Treatment	Pre-antral	Antral
T1	1.83^{cd}	11.5
T2	1.80^{c}	10.4
T3	6.17^{b}	12.0
T4	8.17^{a}	12.5

Means with different superscripts in the same column are significantly different at ($P<0.05$)

Mean Number of Atretic and Non-atretic follicles

As reflected in Table 15, untreated rats had the least number of pre-antral follicles that underwent apoptosis of granulose and theca cells. However, among the treated rats, findings revealed that T3 had the highest mean number of 1.83 followed by T_1 with mean number of 1.4 and T4 with mean number of 1.33. Furthermore, T3 had the highest mean ratio of atretic to antral follicles of 0.15 followed by T_2 with a mean ratio of 0.13 and T3 with mean ratio of 0.11. This indicates that for every 100 antrals, 15 became atretic in T3, 13 in T2 and 11 in T4. The Control (T_1) got the least with 10 atretic follicles for every 100 antrals. Statistical analysis revealed no significant at difference at ($P<0.05$). In terms of the mean number of non-atretic

antral follicles, there was also no difference noted among treatments showing T4 to contain the most number of non-atretic follicles.

The results of this study show that genistein alters the rat ovarian follicular development resulting to higher number of surviving follicles. Pubertal genistein treatment can possibly lead to follicular atresia. However, genistein treatment at old stages of rats increases the number of surviving follicles as obtained from the results of the study of Zhuang (2010) because at this stage, estrogen level is triggered by different factors.

Table 15. Mean number of atretic and non-atretic follicles

Treatment	Atretic	Non atretic
T1	1.00	10.50
T2	1.40	9.00
T3	1.83	10.20
T4	1.33	11.17

CONCLUSIONS

Based on the findings of the study, the following are concluded:

1. Genistein exerted effects even at the lowest dosage of 8 mg/kg body weight of rats.
2. Genistein altered the estrous cyclicity of rats. The length of estrus was increased in treated rats while that of diestrus decreased.
3. Genistein causes atrophy of the ovaries.
4. Genistein has weight reducing effects.
5. There are no adverse effects on the vital signs.
6. Rats treated with genistein have greater mean number of pre-antral and antral follicles as compared with the control.

RECOMMENDATIONS

a. Do hormonal assays specifically on the level of endogenous estrogen present per rats

b. Do clinical trial trials in livestock as well as human beings particularly those with fertility problems.

c. The effects of genistein on cholesterol level

d. The actual amount of genistein absorbed and metabolize by body tissues
e. The frequency of vaginal discharge collection in order to evaluate the exact length of estrus stage in terms of hours
f. The effects of genistein on different ages of rats
g. The effects of genistein in male reproductive tract.

LITERATURE CITED

Al-Nakkash L, Markus B, Batia L, Prozialeck W and Broderick L.
2010 *"Genistein Induces Estrogen-Like Effects in Ovariectomized Rats but Fails to Increase Cardiac GLUT4 and Oxidative Stress."* Jmed food (6):1369-75

BP
2000 "*Endocrine Disruptors*" Human Reproduction.Yahoo Appl [Internet] National Institute of Environmental Health Sciences – National Institute of Health. [cited 2010 August 21].Available from: http://www.niehs.nih.gov/health/topics/agents/endocrine/index.cfm

BP
2000 *"The Laboratory Rat"*. Google Appl [Internet]. Iowa State University. Available from: http://www.lssu.edu/faculty/jroese/AnimalCare/Rat/OccHealth.htm

BP
2000 *"The Ovarian Process"*. Google Appl [Internet].Available from: http://www.siumed.edu.html, cited August 17

Brennan P.
2001 "*The vomeronasal system*', Google Appl [Internet]. [cited 2010 December 28].Cell Mol Life Sci. 58(4):546-55,2001

Brown N and Setchell K.
2001 *"Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones."*,2001.Google Appl [Internet]. [cited 2010 July 23],

Delclos K, Weis C, Bucci T.
2009 "*Overlapping but distinct effects of genistein and ethinyl estradiol (EE(2)) in female Sprague-Dawley rats in multigenerational reproductive and chronic toxicity studies*", Reprod Toxicol 27: 117-132.

Diel P, Smolnikar K, Schulz T, Laudenbach-Leschowski U, Michna H, Vollmer G.
2001 "*Phytoestrogens and carcinogenesis-differential effects of genistein in experimental models of normal and malignant rat endometrium*."Hum Reprod 16:997-1006.

Flynn K, Ferguson S, Delclos B, Newbold R.
2000 "*Effects of genistein exposure on sexually dimorphic behaviors in rats*". 55: 311-319.

Fortune J.
2003 *"The early stages of follicular development: activation of primordial follicles and growth of preantral follicles."* Anim Reprod Sci. 78(3-4):135-63.

Kezele P and Skinner M.
2003 "*Regulation of ovarian primordial follicle assembly and development by estrogen and progesterone: endocrine model of follicle assembly*",Google Appl [Internet]. [Cited 2010 August 11].Endocrinology144, 3329–3337. Available from:http://endo.endojournals.org/cgi/content/abstract/144/8/3329.

Kouki T, Kishitake M, Okamoto M, Oosuka I, Takebe M, Yamanouchi K.
2003 "*Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis*"Horm Behav 44:*140*-145.

Lintelmann J, Katayama A, Kurihara N, Shore L, Wenzel A.
2003 "Endocrine Disruptors in the Environment**",** IUPAC, Pure and Applied Chemistry 75, 631–681.

Liu H, Zhang C, Ge C, Liu *J*
2007 *"Effects of daidzein on mRNA expression of gonadotropin receptors and P450 aromatase in ovarian follicles of white silky fowls. Asian-australias*" J. Anim. Sci. 20:1827–1831.

Mueller S, Simon S, Chae K, Metzler M Korach K
2004 ‘’*Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ER alpha) and ER beta in human cells”* Toxicol Sci 80: 14-25.

Reyes B, Bautista N, Tanquilut N, Leung A, Sanchez G, Tsukamura H, Maeda K.
2006 ‘’*Anti-diabetic Potentials of Momordica charantia and Andrographis paniculata and their Effects on Estrus Cyclicity of Alloxan-Induced Diabetic Rats”* Elsevier Journal of Ethnopharmacology 105: 196-200.

Sanchez G, Palabasan C, Acosta J, Leung A, Reyes
2005 “The Effects of Sinta (*Andrograhis paniculata*), Neem (*Azadirachta indica*), Makabuhay (*Tinospora rumphii*) and Mill (*Aloe barbadensis*) on Ovarian Follicular Development in Rats (*Rattus norvegicus*)”, PSAS proceedings: Recent Development in Animal Production.

Setchell K, Clerici C, Lephart E, Cole S, Heenan C, Castellani D.
2005 “*S-equol, a potent ligand for estrogen receptor, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora*”Am J Clin Nutr.; 81:1072–1079.

Skinner MK,.
2008 “*Glial derived neurotrophic factor promotes the ovarian primordial to primary transition*”Reproduction 135: 671-682.

Yellayi S, Naaz A, Szewczykowski M, Sato T, Woods J, Chang J, Segre M, Allred CD, Helferich W, Cooke P
2002 ”*The phytoestrogen genistein induces thymic and immune changes: a human health concern?*” Google Appl [Internet]. [cited 2010 August 15]Society for Endocrinology, Great Britain. PNAS 99 7616–7621. Online version via http://www.endocrinology.org.

Zhou S, Hu Y, Zhang B, Teng Z, Gan H, Yang Z, Wang Q, Huan M, Mei Q.
2008 “*Dose-Dependent Absorption, Metabolism, and Excretion of Genistein in Rats”* J. Agric. Food Chem., 56 (18): 8354–8359.

Zhuang X, Fu Y, Xu J, Kong X, Chen Z and Luo L.
2001 "*Effects of Genistein on Ovarian Follicular Development and Ovarian Life Span in Rats*'Google Appl [Internet]. [cited 2011 March 2] Elsevier B.V. 10: 1016. Online version via http://www.scie ncedirect.com,2001.

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