Reproductive activities of female albino rats treated with quassin, a bioactive triterpenoid from stem bark extract of *Quassia amara*

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Summary: To evaluate the effect of quassin on female reproductive functions, 42 albino rats (35 females and 7 males) were used. The female albino rats were divided into seven groups of five rats each. Group I served as the control group and received distilled water while Groups II, III and IV rats were treatedorally with 0.1mg/kg, 1.0 mg/kg and 2.0 mg/kg body weight of quassin for 60 days respectively. Groups V, VI and VII rats were also treated orally with 0.1 mg/kg, 1.0mg/kg and 2.0 mg/kg body weight of quassin for 60 days but were left untreated for another 30 days, to serve as the recovery groups. At the end of each experimental period, blood samples were collected from each rat. Fertility study was done by cohabiting one untreated male with the five female rats in each group for 10 days. Quassin did not adversely affect the weight of the kidney, heart, liver and the body of the rats. However there was a significant decrease($P < 0.05$) in the weight of the ovary and uterus in all the groups relative to the control. There was also a significant decrease ($P <0.05$) in serum estrogen levels in quassin treated rats. The quassin treated rats had a significantly decreased ($P < 0.05$) mean litter number and weight. Histological studies show a disorganization and degeneration in the ovary while the uterus showed signs of vacuolation and disorganization. However, these effects were ameliorated after quassin was withdrawn from the rats. The results suggest that quassin has female anti-fertility properties, possibly acting via inhibition of estrogen secretion.

Keywords*:* Quassin, Female rat, Reproduction, Estrogen

INTRODUCTION

Plants all over the world have been used for the treatment of various human diseases since antiquity. They are still in use up till now in developing countries in form of folkloric or traditional medicines. Through experience, a lot of discoveries were made that tropical forest can provide many product apart from drugs, but also narcotics, hallucinogens and stimulants among many other products that make life more meaningful in remote areas (Soedigdo, 1980). Many plant species, half of them from tropical forest have been found to contain anti fertility compounds. About 370 have been shown to provide assurance for safer and effective contraceptives suitable for both males and females (Maurga *et al.*, 2004). Also, more than 600 medicinal plant species are believed to have potential abortifacient property; a good number of them from tropical forests (WHO, 1981)

Many of the herbs that are used medically have a traditional reputation for the purpose they are used for, but there is little or no scientific or medical documentation in respect of their active constituents, pharmacological actions or clinical efficacy. Relatively few herbal ingredients have been subjected to rigorous scientific study with their pharmacological activities and active principles successfully investigated (Newell *et al.*, 1996).

Many plant extracts have been reported to affect fertility in rodents. Gebri *et al.,* [2005] reported that methanolic extract of *Rumex steudelii* decreased the number of implantation sites significantly. It was also showed that the extract of this plant did not affect the serum estrogen- progesterone ratio (Gebri *et al.*, 2005). Nivsarkar *et al.* (2005) showed that *Hibiscus*

rosa – sinensis flowers has anti fertility, abortifacient activity and exhibits anti-estrogenic activity as judged by increase in uterine weight. Kularni *et al.,* (2005) reported that the alcoholic extract of lemon seeds exerted reversible anti-fertility effect in female mice by virtue of its anti-zygotic action. Maca (lepodiummeyeni) root had been acknowledged by Peruvians to improve sexuality and fertility (Gonzales *et al.*, 2004). The methanol extract of *Ricinus communis* seed was also found to prevent implantation and when implantation occurred, it induced abortion in female guinea pigs (Makonnel *et al.*, 1999).

Quassia amara belongs to the plant family of Simaroubaceae and is naturally distributed in several tropical countries. The ethnobotany database lists Amargo, Bitter wood, Quassia, Cuassia, Guabo, Hombre grande, Jamaica bark, palomuneco, pauamarelo, pauquassia, Quassia de caiena, Quassia amarga, Quassia wood, Surinam wood, and Wewegifi as other common names for this plant. Traditionally the bark and leaves are used in herbal remedies since they are rich in biologically active principles. Quassia amara also has antileukemic, antineoplastic, and anti-tumorous property (Kupchan and Streelman, 1976; Considine *et al.*, 1983). The herb also has a prominent effect on the digestive system as an aperitif, astringent, antiulcerogenic, stomachic, antihelmintic, and laxative agent (Grieve, 1992). It has also been used as insecticide, larvicide, pediculicide, and vermifuge (Jenson, 1979; Park *et al.*, 1987). Antimalarial activity of the plant extract has been carried out in mice *in vivo* (Ajaiyeoba *et al.,* 1999). Several phytochemicals have been isolated from the bark of *Quassia amara* that can be broadly classified as quassinoids (Dou *et al.*, 1996; Kitagawa *et al.*, 1996).

Njar *et al.*, (1995) studied the effect of *Quassia amara L* on the steriodogenesis in rat Leydig cells in an *in vitro* system and the result showed that the extract inhibited both basal and luteinizing hormone (LH) stimulated testosterone secretion from rat Leydig cells. Quassin and the alkaloid, 2-methoxycanthin- 6- one was isolated from the *Quassia* extract according to Njar *et al.*, (1993). Raji and Bolarinwa, (1997) have also studied the anti-fertility activity of *Quassia amara L* in male rats. The crude methanol extract, quassin and the alkaloid were used and it was concluded that quassin appears to be the anti-fertility principle of *Quassia amara L.* Although, there were many reports on the effects of quassin on the male reproductive functions; its effects on the female reproductive functions have not been reported. The present study was therefore designed to investigate the impact of quassin on the female

reproductive functions such as fertility, serum estrogen levels and histology of the ovaries and uterus.

MATERIALS AND METHODS

Extraction and purification of plant material: Stem bark of *Quassia amara* was collected at the botanical garden, University of Ibadan, Nigeria. A voucher specimen was deposited at the Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. The stem bark was air-dried and pulverized with blender to obtain 1kg of the plant materials. This was carried out as described by Njar *et al.*, 1993. The pulverized stem bark (1kg) was exhaustively extracted with methanol by means of Soxhlet apparatus and the extract evaporated *in vacuo*. Water was added to the residue and the mixture extracted with hexane and then with $CHCl_3$. The $CHCl_3$ extract was dried using (anhydrous magnesium sulphate ($MgSO_4$) and evaporated to give a residue (3.5g) called quassionoid. The residue (3.3g) was chromatographed on a silica gel column as previously described (Njar *et al*., 1993) to yield quassin.

Animals and treatments: Experiments were performed on 42 rats (35 females and 7 males) whose initial average weight ranged between 150g and 170g. They were obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. The animals were housed in wire mesh cages in the Central Animal House, College of Medicine, University of Ibadan and maintained in a well-ventilated room with a 12:12-hour light-dark at room temperature. Food and water were provided *ad libitum*. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The rats were treated with varying doses of quassin dissolved in distilled water for 60 days (i.e. 0.1, 1.0 and 2.0mg/kg body weight of quassin (Raji and Bolarinwa, 1997).The female rats were divided into seven groups of five rats each. Group I served as the control group and were given 0.2mL of distilled water as solvent (vehicle) for dissolving the quassin. Groups II, III and IV rats were treatedorally with 0.1mg/kg (low dose), 1.0 mg/kg (medium dose) and 2.0 mg/kg (high dose) body weight of quassin for 60 days respectively. Groups V, VI and VII rats were also treated orally with 0.1 mg/kg, 1.0mg/kg and 2.0 mg/kg body weight of quassin for 60 days but were left untreated for another 30 days.

Fertility studies: The experimental female rats were cohabited with untreated male in ratio 5:1 for a

minimum of 10 days (Long and Evans, 1922). The presence of vagina plug was taken as indicator for positive mating. It was taken as the first day of pregnancy. Fertility test was calculated with the following formula as earlier reported (Raji *et al.,* 2006).

% Fertility Success = <u>No. of Pregnant Female X 100</u>
                              No. of Mated female

The rats were allowed to give birth after pregnancy and the number and weight of the litter of each rat were recorded using a sensitive electronic balance made in England, the model is DT- 1000 A with capacity of 0.1-1000g.

Blood sample collection: Blood (2ml) was collected from each animal via the retro-orbital sinus with 70μl heparinized capillary tube (Ezzai, 1995) and put into plain sample bottle for estrogen analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyze the level of estrogen.

Organ collection: After parturition, the animals were killed by cervical dislocation. The ovaries, uterus heart, kidney and liver were removed and cleared of adherent tissues before they were weighed immediately with an electronic weighing balance, model DT 1000 England with a capacity of 0.1 to 1000g.

Estrogen assay procedure: Blood samples were collected from the animals at their estrous phase and an enzyme –based immunoassay (EIA) system was used to measure estrogen level in serum samples collected. The EIA kit was obtained from Immunometrics (London, UK) and contained an estrogen EIA enzyme label, estrogen EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation.

Histological study: Ovary and uterus of the control and treated rats were fixed in Bouin's fluid for 6 hours before they were transferred into 10% formalin for histological evaluation. The tissues were routinely processed and examined under the light microscope. Photomicrograph of the slide was then taken.

Statistical analysis: The results are presented as Means ± SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA). $P<0.05$ was accepted as significant.

RESULTS

Effect of quassin on body weight of female albino rats

There was no significant difference in the mean body weight of all the groups, before and throughout the treatment period when compared with the control group. However, there was a weight gain of 7.4%, 8.5%, 9.6%, 9.7%, 8.9%, 8.9%, 8.3% and 7.2% in the control, low dose, medium dose, high dose, and their respective recovery groups, when the weight after treatment (8th week) was compared with the weight before treatment.

Effect of quassin on organ weight of female albino rats

There was significant decrease (P< 0.05) in the mean weight of ovary of rats treated with 0.1, 1.0 and 2.0 mg/kg body weight of quassin when compared with the control group but there was insignificant decrease in the mean weight of ovary in their respective recovery groups when compared with the control as shown in table 2. There was significant decrease (P < 0.05) in the mean weight of uterus in 0.1, 1.0, 2.0 mg /kg treated and 2.0 mg /kg recovery group. There was insignificant increase in the mean weight of the uterus in the recovery group for 0.1 and 1.0 mg/kg body weight of quassin when compared with the control group as shown in table 2.

Table 1:
Mean body weight of female rats treated with quassin.

Treatment Groups	Before treatment (g)	1st Week (g)	6th Week (g)	7th Week	8th Week
I. Control (Vehicle)	168.2 ± 2.43	172.6 ± 2.52	179.8 ± 3.53	180.2 ± 3.57	180.7 ± 3.43
II. Low dose(0.1mg/kg)	153.2 ± 3.84	156.6 ± 4.29	163.0 ± 5.12	164.8 ± 5.38	166.2 ± 4.76
III. Medium dose (1mg/kg)	`160.2 ± 3.09	162.6 ± 3.44	173.0 ± 4.42	174.4 ± 4.42	175.6 ± 4.32
IV. High dose(2mg/kg)	161.3 ± 3.04	163.4 ± 3.22	173.6 ± 2.94	175.2 ± 3.02	177.0 ± 3.15
V. Low dose recovery	151.5 ± 4.61	154.8 ± 4.92	162.4 ± 4.63	164.0 ± 4.85	165.0 ± 5.19
VI. Medium dose recovery	152.3 ± 5.65	154.6 ± 6.59	161.2 ± 7.56	162.6 ± 7.58	165.0 + 7.40
VII. High dose. Recovery	155.2 ± 2.65	157.2 ± 2.78	164.4 ± 2.69	164.8 ± 2.35	166.4 ± 2.48.

Table 2:
Mean organ weight of female albino rats treated with quassin

Treatment groups	Ovary (g)	Uterus (g)	Heart (g)	Kidney (g)	Liver (g)
I. Control(Vehicle)	0.124 ± 0.008^a	0.52 ± 0.04^a	0.62 ± 0.02^a	0.67 ± 0.04^a	6.68 ± 0.15^a
II. Low dose(0.1mg/kg)	0.082 ± 0.002^b	0.42 ± 0.02^b	0.56 ± 0.02^b	0.59 ± 0.02^b	5.52 ± 0.23^b
III. Medium dose(1mg/kg)	0.060 ± 0.002^b	0.44 ± 0.02^b	0.50 ± 0.04^b	0.70 ± 0.03^c	5.92 ± 0.51^b
IV. High dose(2mg/kg)	0.060 ± 0.002^b	0.44 ± 0.02^b	0.58 ± 0.04^b	0.65 ± 0.05^a	5.74 ± 0.19^b
V. Low dose recovery	0.102 ± 0.004^a	0.55 ± 0.04^c	0.66 ± 0.02^c	0.57 ± 0.04^b	7.52 ± 0.63^c
VI. Medium dose recovery	0.104 ± 0.004^a	0.54 ± 0.03^c	0.70 ± 0.04^c	0.70 ± 0.02^c	9.65 ± 0.62^c
VII. High dose recovery	0.122 ± 0.001^a	0.41 ± 0.03^c	0.78 ± 0.02^c	0.59 ± 0.03^b	7.64 ± 0.08^c.

Values are expressed as Mean ±S.E.M of 5 rats per group. Means in rows showing different superscript letters [a,b,c] are significantly different; $p < 0.05$.

Table 3:
Mean value of fertility test of female rats treated with quassin.

Treatment groups	Litter size	Litter weight	Positive mating %	Fertility%
I. Control(Vehicle)	6.0 ± 1.05^a	5.64 ± 0.26^a	100^a	**100^a**
II. Low dose(0.1mg/kg)	4.4 ± 0.81^b	4.52 ± 0.22^b	100^a	**100^a**
III. Medium dose(1mg/kg)	3.5 ± 1.01^b	4.61 ± 0.29^b	100^a	**100^a**
IV. High dose(2mg/kg)	4.0 ± 0.24^b	4.55 ± 0.26^b	100^a	**20^a**
V. Low dose recovery	5.8 ± 0.38^a	5.60 ± 0.40^a	100^a	**100^a**
VI. Medium dose recovery	5.8 ± 0.52^a	5.53 ± 0.23^a	100^a	**100^a**
VII. High dose recovery	5.0 ± 0.49^a	5.00 ± 0.14^a	100^a	**100^a**

Values are expressed as Mean ±S.E.M of 5 rats per group. Means in rows showing different superscript letters [a,b] are significantly different; $p < 0.05$.

There was significant increase ($P < 0.05$) in the mean heart weight for 1.0 and 2.0 mg /kg body weight of quassin treated rats and there was also significant increase in their respective recovery groups when compared with the control. The 0.1 mg/kg body weight of quassin treated group showed a significant decrease ($P < 0.05$) and a significant increase ($P < 0.05$) in its recovery group when compared with the control as shown in table 2.

There was a significant decrease ($P < 0.05$) in the mean kidney weight for 0.1mg /kg body weight of quassin treated rats and its corresponding recovery group. There was significant increase ($P < 0.05$) in the mean kidney weight for 1.0mg /kg body weight of quassin treated rats as well as their recovery group. There was no significant change in the kidney weight of 2.0mg/kg body weight of quassin treated rats but its recovery group showed a significant decrease ($P < 0.05$) when compared with the control as shown in table 2.

There was significant decrease ($P < 0.05$) in all the treated groups when compared with the control but their respective recovery group showed significant increase ($P<0.05$) when compared with the control as shown in table 2.

Effect of quassin on fertility of female albino rats
There was 100% positive mating in all the groups. This was confirmed by the presence of vaginal plug of the female rats, a day after cohabitation. There was significant decrease ($P < 0.05$) in the mean litter size in all the treated groups when compared with the control while their respective recovery group showed an insignificant reduction ($P > 0.05$) when compared with the control as shown in table 3.

There was significant decrease ($P < 0.05$) in the mean litter weight in all the treated groups when compared with the control while their respective recovery group showed an insignificant reduction ($P > 0.05$) when compared with the control as shown in table 3. There was 100% fertility for the control and treated rats except for 2.0mg/kg body weight that had a significant decrease ($p < 0.05$) as shown in table 3.

Table 4:
Mean value of serum level of estrogen of female albino rats treated with quassin.

Treatment groups N = 5	Estrogen (Pg/ml) Mean ± S. E. M.
I. Control(Vehicle)	38.1 ± 3.39[a]
II. Low dose(0.1mg/kg)	23.3 ± 1.23[b]
III. Medium dose(1mg/kg)	21.8 ± 3.47[b]
IV. High dose(2mg/kg)	21.6 ± 2.13[b]
V. Low dose recovery	35.2 ± 1.23[a]
VI. Medium dose recovery	32.5 ± 1.66[a]
VII. High dose recovery	25.2± 1.28[b]

Values are expressed as Mean ±S.E.M of 5 rats per group. Means in rows showing different superscript letters [a, b] are significantly different; p<0.05.

Table 5:
Effects of quassin on the histology of the ovary and uterus in rats

Treatment groups	Ovarian architecture	Uterine architecture
I. Control(vehicle)	No lesions observed	No lesions observed
II. 0.1 mg/kg treated	Numerous developing follicles seen and corpora present	Stratified squamous epithelium seen (dysplasia)
III. 1.0 mg/kg treated	Prominent stroma. No developing follicle seen.	Swollen squamous epithelia cells seen.
IV. 2.0mg/kg treated	Few follicles seen	Swollen squamous epithelia cells seen
V. 0.1 mg/kg recovery	No lesions observed	No lesions observed. Mucosa layer present
VI. 1.0 mg/kg recovery	No lesions observed	No lesions observed
VII. 2.0 mg/kg recovery	Large corporaalbican completely filled with parenchyma	No lesions observed

Effect of quassin on mean value of serum level of estrogen in female albino rats
The result showed a significant decrease (P<0.05) in all the treated groups and their corresponding recovery group when compared with the control group as shown in table 4.

Effects of quassin on the histology of the ovary and the uterus of female albino rats.
The effects of quassin on the histology of the ovary (Fig.1) and uterus (Fig. 2) in female albino rats are summarized in table 5.

DISCUSSION

The results obtained from this study showed that quassin caused adverse effects on fertility in female rats, sufficient to cause reversible infertility. Female reproduction is functionally controlled by normal estrogen level which usually peaks during the estrous phase of the cycle. This gives an indication that quassin may act on the ovary (the source of estrogen) through altered endocrine functions associated with decreased estrogen level. The result of this study showed that the serum estrogen levels in all treated rats decreased significantly relative to the control.

The present study reports for the first time the effect of quassin on estrogen secretion. Previous studies have focused on the effect of quassin in the male. Evidence in support of the present findings could therefore be inferred only from the male studies. Njar *et al.*, (1995) reported that quassin inhibited testosterone secretion in male rats, thereby causing infertility. The work of Raji and Bolarinwa, (1997) also confirmed this finding, suggesting that quassin inhibited testosterone in the Leydig cell. Thus, impairment in the production of testosterone which is a pre-hormone for estrogen is probably an indirect impairment on the production of estrogen.

The significant structural alterations in the histological sections of the ovary and uterus in quassin treated rats compared with the control further support the possible deleterious impact of quassin on female reproduction. Quassin induced degeneration of the follicular wall, may be responsible in part for the significant decrease in serum estrogen levels. This probably led to the anovulatory cycles and the consequent decrease in litter number and litter weight. The fetal morphology was not adversely affected which indicate that quassin might not affect fetal development if administered before conception (Garcia *et al.*, 1997). Lucidi *et al.*, (2003) suggested that steroidogenesis could be influenced by active development of the oocyte. It follows then that the atretic follicles in the histological sections could be due to a reduction in estrogen level. The hormonal and histological changes could lead to the significant reduction in the ovarian and uterine weights. Noteworthy was a slight increase in the serum level of estrogen in the recovery groups, following the withdrawal of quassin.
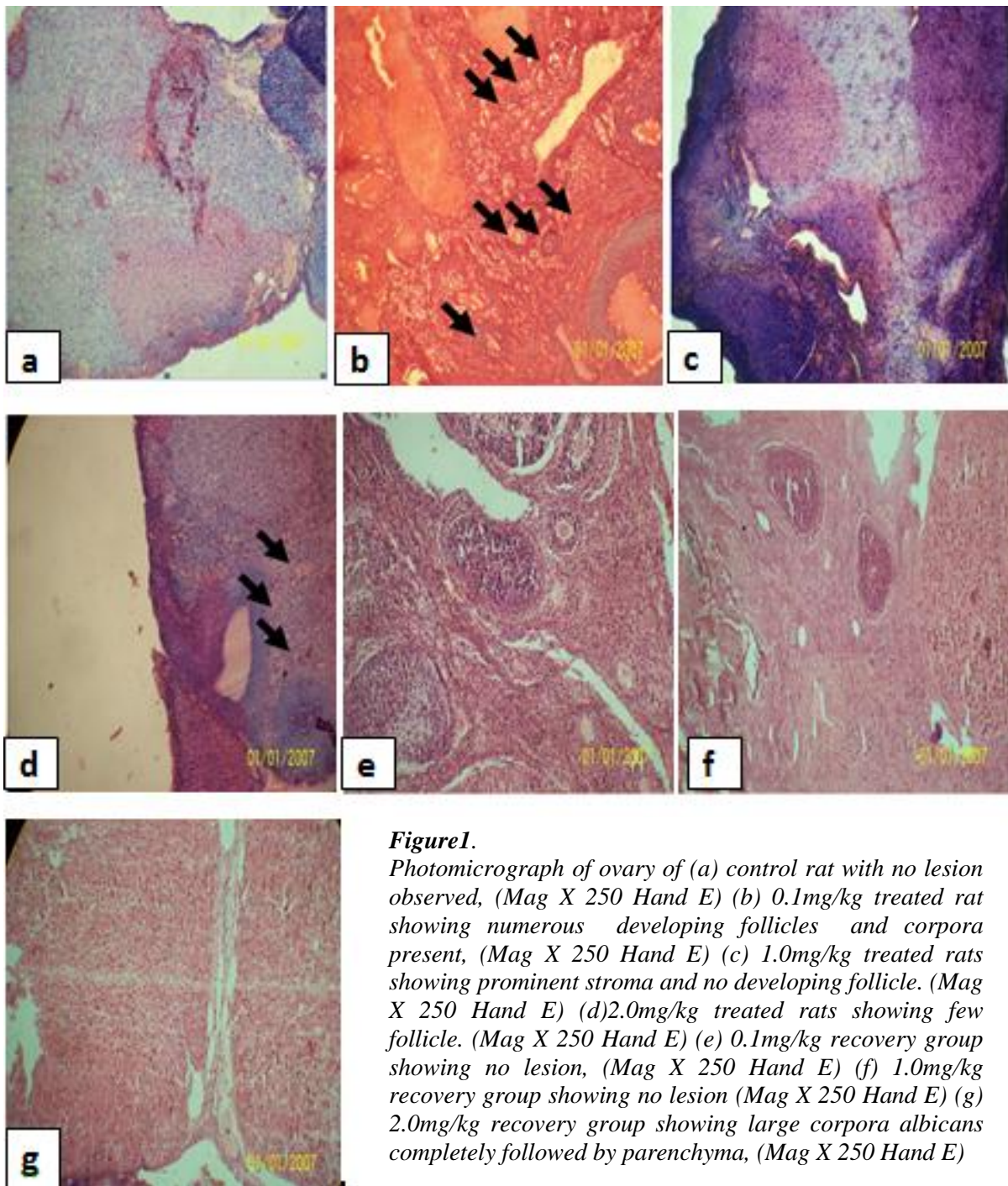
Figure1.
Photomicrograph of ovary of (a) control rat with no lesion observed, (Mag X 250 Hand E) (b) 0.1mg/kg treated rat showing numerous developing follicles and corpora present, (Mag X 250 Hand E) (c) 1.0mg/kg treated rats showing prominent stroma and no developing follicle. (Mag X 250 Hand E) (d)2.0mg/kg treated rats showing few follicle. (Mag X 250 Hand E) (e) 0.1mg/kg recovery group showing no lesion, (Mag X 250 Hand E) (f) 1.0mg/kg recovery group showing no lesion (Mag X 250 Hand E) (g) 2.0mg/kg recovery group showing large corpora albicans completely followed by parenchyma, (Mag X 250 Hand E)

This could probably be due to release of the inhibitory impact of quassin on the pituitary gonadotrophin secretion and release as the rats recover from effects of the quassin. After withdrawal of quassin for a period of 30 days, the mean weight of the ovary, uterus, litter number, litter weight, serum estrogen levels and the cytoarchitecture of the ovary and uterus were almost the same as those of the vehicle-treated control group which suggest that the impact of quassin on female reproductive functions were reversible.

At the doses employed, there was no adverse effect on the mean weight of liver, kidney, heart which was consistent with previous studies (Isichei *et al..,* 2000). Also, there was no treatment related abnormalities in the body weight throughout the period of treatment. Aqueous solution of quassin had earlier been reported not to show any untoward effect on body weight despite its anti-fertility effects in male rats (Margaria, 1963).

Body weight is well known to play an important role in the regulation of gonadotrophin secretion and

its crucial role for regular cyclic function is well documented (Knuth *et al.*, 1977 and Schwartz, 1969). Thus the adverse response of quassin on endocrine functions and fertility was not primarily a function of impact on general body weight.

In conclusion, the results of the present study suggested that quassin possesses reversible anti-fertility and anti-estrogenic properties, which was attributed to reduction in litter number, litter weight, ovarian and uterine weight. This was probably a consequence of reduced serum levels of estrogen. This effect was ameliorated after withdrawal of quassin from the rats.
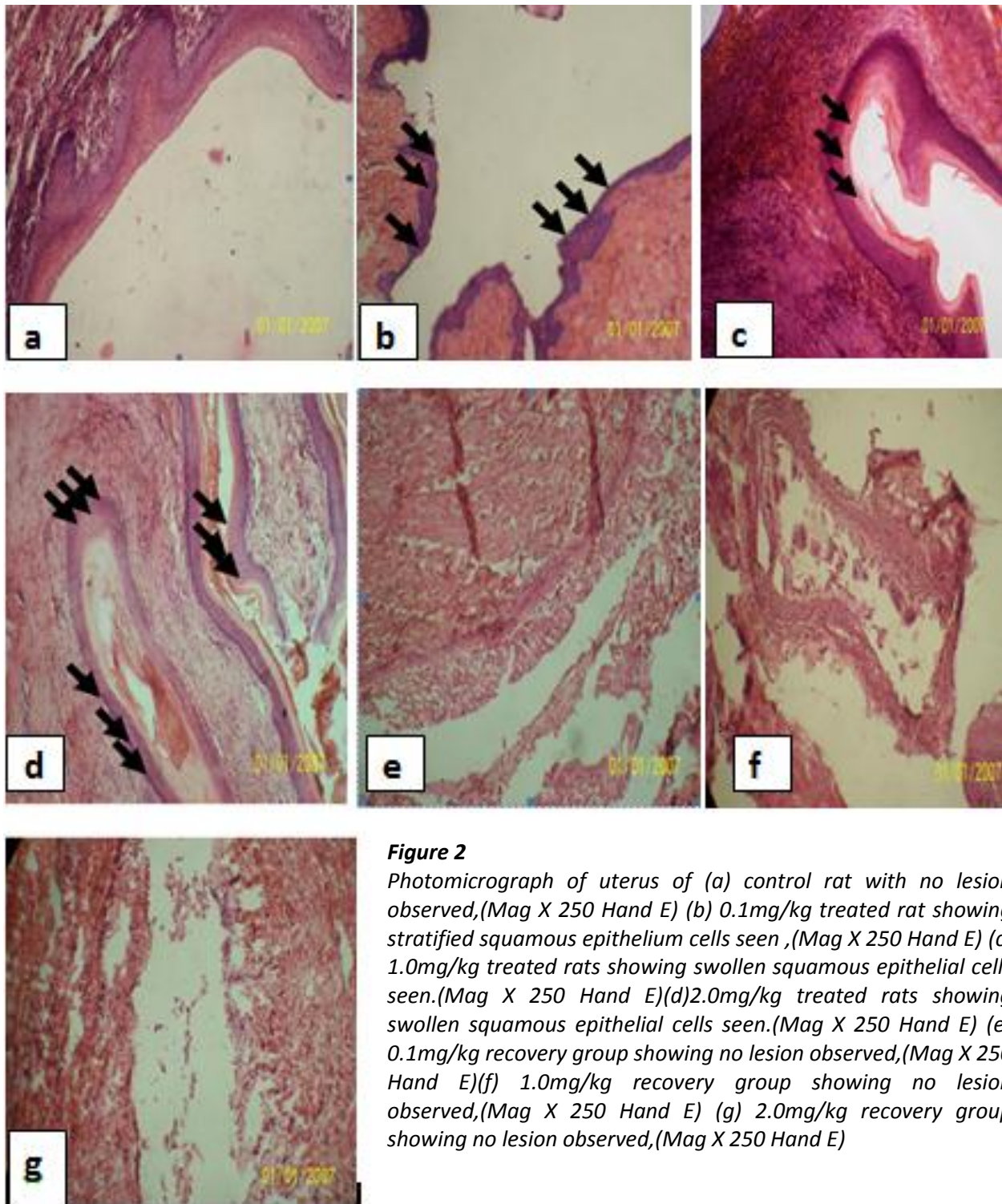

Figure 2

Photomicrograph of uterus of (a) control rat with no lesion observed,(Mag X 250 Hand E) (b) 0.1mg/kg treated rat showing stratified squamous epithelium cells seen ,(Mag X 250 Hand E) (c) 1.0mg/kg treated rats showing swollen squamous epithelial cells seen.(Mag X 250 Hand E)(d)2.0mg/kg treated rats showing swollen squamous epithelial cells seen.(Mag X 250 Hand E) (e) 0.1mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E)(f) 1.0mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E) (g) 2.0mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E)

REFERENCES

Ajaiyeoba, E.O.Abalogu, U.I Krebs, H.C, Oduola, A.M. 1999: In vivo anti-malaria activities of Quassia amara and Quassia undulata plant extracts in mice. *J. Ethnopharmacol ; 67: 321-5 .*

Considine R.T, Willingham Jr W, Chaney S.G, WyrickS, Hall, I.H. 1983.Structure – activity relationships for binding and inactivation of rabbit reticulocyte ribosomes by quassinoids antineoplastic agents. *Eur J Biochem; 132: 157-63.*

Dou J, Mcchesney; J.D, Sindelar, R.D,Goins, D.K, Khan IA, Walker LA .1996 :Anew quassinoid from crude quassin-extract of Quassia amara.*Int J Pharamacognosy; 34:349-54.*

Ezzai, S. El-Daly. 1995. Effect of intrathecal morphine on blood glucose, glucagon and tissue glycogen in rat, comparison with the effect of Xanthan gum on blood glucose. *J. Isl. Aca. Of Sc. 8: 1, 3-8.*

Garcia, G.M., Gonzalezi, S.M.C., Pazos, L.S. 1997. [Pharmacologic activity of the aqeuous wood extract from Quassia amara (Simurabaceae) on albino rats and mice]. *Rev. Biol. Trop., 44-45, 47-50.*

Gebri, E; Makonnen E; Zerihun L; Debella A. 2005: The possible mechanisms for the anti-fertility action of methanolic root extract of rumex studelii. *Afr. Health Sci; 5:119-125.*

Gonzales G; Cordora A; Vega K; Chung A and Vellena A. 2004: Effect of Lepidium meyeni (maca) on Serum Reproductive Hormone Levels in Adult Healthy Men. *Journal of Endocrinology. 176: 163-168.*

Grieve M. 1992: A modern herbal. London: Tiger books international.

Henshaw,S.K. 1998: Unintended Pregnancies in United States.Fam. Plan. Prespect; 30:24-29.

Isichei C.O; Das S.C; Ogunkeye O.O; Okwuasaba F.K; Uguru V.E, Onoruvwe O 2000: Preliminary clinical investigation of the contraceptive efficacy and chemical pathology effects of RICOM-1013-J of Ricinuscommunisvar minor on women volunteers. *Phytother Res; 14:40-2.*

Jenson O. 1979: Treatment of head lice with quassia tincture. UgeskrLaeger; 141: 225 – 6.

Kitagawa I, Mahmud T, Yotota K, Nakagawa S, Mayumi T, Kobayashi M, *et al..* 1996 : Indonesia medicinal plants. XVII. Characterization of quassinoids from the stems of Quassiaindica. *Chem pharm Bull (Tokyo); 44:2009-14 .*

Knuth, U.A., Hull, M. G. and Jacobs, H.S. 1977: Amenorrhea and loss of weight. *Br. J. Obstetrics Gynecol. 84: 801-807.*

Kupchan S.M., Streelman D.R. Quassimarin 1976.A new antileukemic quasssinoid from Quassia amara *J. Org. chem. 41: 3481 – 2*

LaiP.k 2004 : Antimicrobial and chemopreventive properties of herbs and spices". *Curr Med Chem.: 1451 – 60 PMID 15180577.*

Long, J.A. and Evans, H.M. 1922: The esrous cycle in rat and its associated phenomena. Memories of University of California, 6:1-148.

Lucidi, P., Bernabo, N., Turriani, M., Mattioli and Barboni, B. 2003. Cumulus Steroidogenesis is influenced by the degree of oocyte maturation. *Reprod. Biol. Endocrinolol. 1: 45-55.*

Makonnel, E; Zerihun L; Assefa, G, Rostom A.A. 1999: Antifertility activity of Ricinuscommunis seed in female guinea pigs. *East Afr Med. J. 76:335-7.*

Margaria, R. 1963. Analisi dei gruppi lattinici una quassina greggia. Communication et relation au comite por l' Etude des Bossions Al cooliques Aromatisees de la Federvini. Milan, Institut de physiologie de l' universite,.1-10.

Maurga R: Scrivstara S; Kulshresh D.K. and Gupta C.M. 2004: Traditional remedies for fertility regulation. Current Medical Chemistry.11: 1431 -1450.

Newell C.A; Anderson L.A; Philipson J.D. 1996: Herbal Medicine- a Guide for Health Care Professionals. Pharmaceutical Press. 9-11.

Nivsarkar M; Patel, M; Padh H; Bapu C; Shrivastava N. 2005: Blastocyst implantation failure in mice due to non-receptive endometrium: Endometrial alterations by Hibiscus rosa-sinensis leaf extract.contraception; 71: 227-230.

Njar, V.C.O., Alao, T.O., Okugun, J.I., Holland, H.L. 1993: 2 – Methoxycanthin – 6 – one: A new alkaloid from the stem wood of *Quassia amara: PlantaMed. 59: 259-261.*

Njar, V.C.O; Alao, T.O, Okugun, J.I., Raji, Y., Bolarinwa, A.F., Nduka, E.U. 1995: Antifertility activity of *Quassia amara*: Quassin inhibits the steroidogenesis in rat Leydig cells *in vitro. Planta Med. 61:180-182.*

Park, M.H; Maeda M; komura H, Nakanishi K, Komoto K. 1987: Acute insecticidal activity of quassin and its congeners against the American cockroach. *Chem. Pharm. Bull (Tokyo); 35: 3082 – 5.*

Raji, Y., Bolarinwa, A.F. 1997. Antifertility activity of *Quassia amara* in male rats – *in vivo* study. *Life Sci., 61: 1067-1074.*

Raji, Y., Oloyo A.K Morakinyo A.O (2006) Effect of methanol extract of *Ricinuscommunis* seed on reproduction of male rats *Asian Jour. Andrology* 8:115 – 121.

Schwartz, N.B. 1969: A modelfor the regulation of ovulation in the rat. *Recent Progress in Hormone Research, 25:1-55.*

Soedigdo S. 1980: Studies on the chemistry and pharmacology of Indonesia medicinal plants. *Proceedings of fourth Asian symposium on medicinal plants and spices; 112 – 119*

World Health Organization. 1981: Report of Task Force on Anti-fertility plants Especially from Tropical Forests. WHO Geneva, Switzerland.