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Effect of Administration of Aqueous Suspension of *Ricinus communis* Seeds on Serum Estrogen and Progesterone in Female Albino Rats

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Abstract
In this study, twenty seven (27) female albino rats were used in the determination of contraceptive effect of oral administration of aqueous suspension of *Ricinus communis* seed. Estrogen and progesterone levels of these rats were analysed using electrochemiluminescent method. The result revealed estrogenic property of the aqueous suspension of *R. communis* seed, which is partly attributed to the anticonceptive effect of the seeds. The estrogenic activity was high with 107×10^{-2}mg/kg dose administered after 48hours and 7 days respectively, indicating some degree of dose-dependency. In addition, alteration of estrogen/progesterone balance as indicated by this study may be responsible for the contraceptive effect of aqueous suspension of *R. communis* seed.
Keywords: Contraceptive effect, Estrogen, Estrogenic activity, Progesterone, *Ricinus* communis

1. Introduction

Population explosion is one of the major problems of present era in the world. The increment of population raises so many sufferings like lack of food, water, energy and raw material supply. In view of the above, scientists have started to tackle this serious problem by developing effective contraceptives, among which are hormone containing pills. The use of hormonal contraceptives is the most common practice worldwide (WHO, 2009). These hormonal contraceptives contain a small amount of synthetic estrogen and progestin hormones (Romero, 2007). These hormones work to inhibit the body's natural cyclical hormones to prevent pregnancy. The hormonal contraceptive usually stops the body from releasing an egg from the ovary or changes the cervical mucus to make it difficult for the sperm to find an egg (Massai *et al.,* 2005). However, the use of hormonal contraceptives, which is the most common practice, is associated with a number of side effects such as irregular bleeding, high risk of synthetic estrogen sensitivity, dry eyes, hypertension, carcinogenicity, teratogenicity, pain, phobia, miscarriage (after the expected period of efficacy) and failure to conceive when the need arises (WHO, 2009).

Due to the above stated problems, there is actually a need to carry out a research on the possible employment of herbal contraceptives as an alternative to the use of modern contraceptives in the prevention of unwanted pregnancy. The use of herbs for disease management could be traced to early man who probably acquired the skill of healing through deliberate or accidental selection of plants and their parts (Sofowora, 1993).

The castor oil plant (*Ricinus communis)* is a species of flowering plant in the spurge family, *Euphorbiaceae*. It belongs to a monotypic genus, *Ricinus*, and sub tribe, *Ricininae* (Everitt *et al.,* 2007). Although monotypic, the castor oil plant can vary greatly in its growth habit and appearance (Korwar *et al.,* 2006). It is a fast-growing, suckering perennial shrub, which can reach the size of a small tree (around 12 metres / 39 feet) (Christopher, 1996). The plant has many common names such as castor plant, castor oil plant, castor bean plant and wonder boom (Van Rheenen, 1976). Locally the plant is known in Nigeria by such names as “Zurman” (Hausa), “Laraa” (Yoruba). “Ogilisi” (Igbo), “Kpamfini gulu” (Nupe), “Jongo” (Tiv), and “Era ogi” (Bini) (Van Rheenen, 1976). Its seed is the castor bean which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant) (Phillips *et al.,* 1999).

Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein (Anonymous, 2008). It is considered antidote, bactericide, expectorant, insecticide, larvicidal, laxative and tonic. Castor seed oil is also an ingredient in folk remedies for abscess, arthritis, asthma, boils, burns, cancer, catarrh, cholera, cold, colic, convulsions, dermatitis, headache, inflammation, osteomyelitis, palsy, parturition, rash, rheumatism, scald, skin, sores, stomachache, swellings, toothaches, tuberculosis, tumors, uteritis, warts, whitlows, and wounds (Anonymous, 2005, Anonymous, 2009). Leaves are applied to the head to relieve headache and as a poultice for boils (Duke and Wain, 1981). However, the seed contains a toxic substance called ricin. The presence of ricin is attributed to the toxicity of the seed and hence the oil (Wedin *et al.,* 1986). With regard to contraceptive uses of castor beans, it has been reported that some women in India and Korea took castor beans to prevent subsequent pregnancy (Malhi and Trivedi, 1972).

In order to assess the contraceptive effect of *R. communis* seed, serum estrogen and progesterone were

analysed in female albino rats orally administered with aqueous suspension of the seed.

2. Materials and Methods

2.1 Collection and Preparation of Samples

The sample (*Ricinus communis* seed) was collected from Kanya village in Babura Local Government, Jigawa State, Nigeria. It was authenticated at the Department of Plant Science, Bayero University, Kano, Nigeria. Two (2) *R. communis* seeds weighing 0.3g were crushed using mortar and pestle, the pulverized particle was added into distilled water and shaken gently for five minutes to make a homogenous suspension. The suspension was made to contain 5.36×10^{-1}mg/kg on the basis of dose taken by human subject, by adjusting the volume to 500cm^3.

2.2 Experimental Animals

Twenty seven (27) non pregnant female white albino rats (weighing 120-130g) were obtained from the Animal House of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria. They were housed in animal house, Department of Biological Sciences, Bayero University, Kano and allowed to acclimatise for two weeks and fed with their normal starter feed and water *ad libitum.*
Instat Graphad 3.0 statistical software was used to analyze the data obtained.

2.3 Experimental Design

The rats were divided into three (3) groups; Group I contained nine (9) rats that served as control; they were not administered with *Ricinus communis* seed but allowed free access to water and food. Groups II and III also contained nine (9) rats each. Group II was orally administered with 0.9cm^3(reference dose) of the suspension (reference dose is equivalent to 5.36×10^{-1}mg/kg based on the dose taken by human subject). Animals in Group III were given 107×10^{-2}mg/kg (twice reference dose). The hormonal assay was carried out in three phases i.e. after 48 hours of seed administration; three (3) rats from each group were sacrificed. The second phase was after 7 days and the last phase after 14 days.

2.4 Hormonal assay

Method of electrochemiluminescent developed by Roche diagnostic elecsys (2010) was employed for serum estrogen and progesterone determination.

3. Result and Discussion

Estrogen level in control rats presented in Table 1 was found to be 23.56 ± 4.50 (pg/cm^3). The estrogen level of rats after 48 hours of oral administration of 5.36×10^{-1} mg/kg (reference dose) *R. communis* seed aqueous suspension was not significantly different the control value. The level of estrogen after 48 hours of oral administration of 107×10^{-2} mg/kg (twice reference dose) was significantly higher compared to both the control and the reference dose ($P<0.05$). The serum progesterone levels after 48 hours of oral administration of 5.36×10^{-1} mg/kg and 107×10 *R. communis* seed aqueous suspension respectively show no statistical difference compared to control value (Table 1).

Table1: Serum estrogen and progesterone levels in albino rats after 48 hours of oral administration of aqueous suspension of *R. communis* seed.

Dose of *R. communis* aqueous Suspension (mg/kg) administered	**Estrogen (pg/cm^3)**	**Progesterone (ng/cm^3)**
Group I 0.00 (Control)	23.56 ± 4.50^{a}	2.23 ± 0.30
Group II 5.36×10^{-1} (reference dose)	18.49 ± 3.94^{b}	2.57 ± 0.30
Group III 107×10^{-2} (twice reference dose)	$52.92\pm13.90^{a,b}$	6.93 ± 4.16

The values are expressed as mean and standard deviation (mean±SD) of triplicate determinations. Values within the column, bearing the same superscript (a,b) are statistically different at p<0.05

Estrogen levels of rats orally administered with 5.36×10^{-1} mg/kg of the suspension after 7 days (presented in Table 2) was significantly lower compared to estrogen value of the control rats (23.56 ± 4.50pg/cm^3) at $P<0.05$. However, estrogen level of rats orally administered with 107×10^{-2} mg/kg of the aqueous suspension after 7 days was found to be statistically higher compared to the control and the reference dose ($P<0.05$) respectively. The estrogenic activity of *R. communis* seed is more pronounced with 107×10^{-2} mg/kg dose after 48hours and 7 days of administration, indicating a partial dose-dependency as previously observed by Okwasaba *et al.* (1997) that the estrogenic activities exhibited by the *R. communis* extract was dose-dependent and the anticonceptive effect may be due at least in part to such estrogenic action. This statement also is in conformity with previous findings by Salhab *et al.* (1997) that injection of 3ng of estrogen was enough to prolong uterine receptivity in the mice for up to four days. Increasing the estrogen dose to 10 ng rapidly closed the window of receptivity within 24 hours,

implantation no longer occurred. A second dose of estrogen, whether low or high, did not rescue receptivity after an initial high dose. High level of estrogen is believed to result in severe health disorders like breast cancer, infertility and auto-immune diseases (Woo *et al.,* 1981). High estrogen levels as also reported by Dafallah and Al-Mutairy (1994) may cause difficulty in conceiving, in many cases because of problems with egg development and maturity. Common symptoms of too much of this hormone include a much lower rate of successful conception, poor ovulation which can mean few or no eggs ready for impregnation, and a lowered response to fertility drugs.

Table2: Serum estrogen and progesterone levels in albino rats after 7 days of oral administration of aqueous suspension of *R. communis* seed.

Dose of *R. communis* aqueous Suspension (mg/kg) administered	**Estrogen (pg/cm^3)**	**Progesterone (ng/cm^3)**
Group I 0.00 (Control)	23.56±4.50^{a}	2.23±0.30^{a}
Group II 5.36×10^{-1} (reference dose)	13.41±2.93^{a,b}	3.17±0.54^{a,b}
Group III 107×10^{-2} (twice reference dose)	62.23±15.7^{a,b}	7.79±1.04^{a,b}

The values are expressed as mean and standard deviation (mean±SD) of triplicate determinations. Values within the column, bearing the same superscript (a,b) are statistically different at p<0.05

The progesterone level in rats after 7 days of oral administration of 5.36×10^{-1} mg/kg and 107×10^{-2} mg/kg *R. communis* seed suspension (Table 2) were significantly higher than that of control value (P<0.05). Although a slight progestational activity was observed, it may not be attributed to the antifertility effect of *R. communis* as stated previously by Okwasaba *et al.* (1991) that high antifertility efficacy of *R. communis* is unlikely to be due to any direct progestational activity and may be due partly to alteration in estrogen/progesterone balance as well as to a direct effect on the uterus and fallopian tube. However, sex hormones are known to exert both a positive and negative feedback effects on the release of gonadotrophins from the pituitary gland. In the luteal phase of the menstrual cycle, the combined effect of estrogen and progesterone will be to block the release of luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) from the pituitary. This situation however as suggested by Kholkute *et al.*(1976) helps inhibit maturation of the follicle in the ovary and prevent ovulation. Since *R. communis* seed extracts gave positive tests for steroids, and sex hormones being steroidal compounds, the plants' sterols (phytosterols) may be suspected to be responsible for the anti-fertility effects of the seeds.

The estrogen values of rats orally administered with 5.36×10^{-1} mg/kg and $107\text{x}10^{-2}$ mg/kg *R. communis* aqueous suspension after 14 days were not significantly different from the control value (P>0.05) (Table 3). However serum progesterone level in rats after 14 days of oral administration of 5.36×10^{-1} mg/kg of *R. communis* suspension was significantly higher than that of control rats (P<0.05). The drop in serum estrogen levels after 14days of oral administration of 5.36×10^{-1} mg/kg and $107\text{x}10^{-2}$ mg/kg respectively may be attributed to the increase in serum progesterone level as indicated in Table 3. This suggests alteration in estrogen/progesterone level as reported earlier by Okwasaba *et al.* (1991), and could be a probable reason behind its antifertility effect.

Table3: Serum estrogen and progesterone levels in albino rats after 14 days of oral administration of aqueous suspension of *R. communis* seed.

Dose of *R. communis* aqueous Suspension (mg/kg) administered	**Estrogen (pg/cm^3)**	**Progesterone (ng/cm^3)**
Group I 0.00 (Control)	23.56±4.50	2.23±0.30^{a}
Group II 5.36×10^{-1} (reference dose)	47.05±15.0	6.62±2.36^{a}
Group III 107×10^{-2} (twice reference dose)	61.71±42.6	7.77±5.20

The values are expressed as mean and standard deviation (mean±SD) of triplicate determinations. Values within the column, bearing the same superscript (a) are statistically different at p<0.05

4. Conclusion

Although a slight progestational activity was observed, it may not be attributed to the antifertility effect of *R. communis* seeds as stated previously by Okwasaba *et al.* (1991). However, the result of this research also reveals estrogenic property of the aqueous suspension of *R. communis* seed, which is partly attributed to the anticonceptive effect of the seed. The estrogenic activity was higher with 107×10^{-2}mg/kg dose administered after 48hours and 7 days respectively, indicating some degree of dose-dependency. In addition, alteration of estrogen/progesterone balance as indicated by this study may also be attributed to the contraceptive effect of aqueous suspension of *R. communis* seed. The effect of the aqueous suspension of *R. communis* seed on serum progesterone level, is however not consistent as in serum estrogen level.

5. Recommendation

Alteration of estrogen/progesterone balance as well as estrogenic property of the aqueous suspension of *R. communis* seed has been discovered. Therefore, it is usage as herbal anticonceptive seed is recommended. However, the toxic effect of the seed should be taken into consideration, since a highly toxic substance, ricin, has been previously reported to be present in the seed.

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