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Effects of *Croton menyharthii* and *Uvariodendron kirkii* extracts on ovarian corpora lutea and reproductive hormones

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ABSTRACT

Uvariodendron kirkii and *Croton menyharthii* are traditionally used as fertility regulators in Kenya. The rapidly increasing population has put a strain on the limited resources and poses serious challenge to national planning. The aim of the study was to validate the traditional claims by investigating the effect of root bark extract of both plants on reproductive hormones and ovarian structures. Twenty five mature normocyclic female winstar rats were used. Group 1 consisted of 5 animals that acted as control. Group 2 and 3 with 5 animals each; received 500 and 800mg/Kg *Croton menyharthii* respectively on alternative days for 28 days through intra- abdominal gavage. Group 4 and 5 were treated in a similar manner but received *Uvariodendron kirkii* aqueous extract. Serum was harvested from all animals on 28th day and hormone levels determined. Left ovaries were harvested and processed for histomorphology. Both *Croton menyharthii* and *Uvariodendron kirkii* caused a significant increase of progesterone in a dose dependent manner. *Croton menyharthii* extracts caused a degeneration of corpora lutea. At 800mg/kg *Croton menyharthii* caused a significant increase in corpora lutea numbers but a decline in size. *Uvariodendron kirkii* caused hypertrophy and a significant increase in corpora lutea numbers. Enhanced/ hypertrophied corpora lutea possibly led to high levels of progesterone seen, interfered with the implantation window due to disrupted hormonal milieu thereby leading to compromised fertility and implantation index. The study validates the traditional use of the plant in fertility regulation. We suggest further investigation on these potential plants to address the call for novel contraceptive drugs.

Keywords: *Croton menyharthii, Uvariodendron kirkii,* ovary, anti-fertility, reproductive hormones

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INTRODUCTION

Fertility regulation is an important economic and social determinant of quality of life of women. Unfortunately not all women have access to contraceptive method of choice especially in rural parts of Kenya. Single women and adolescents rarely have access and are often excluded from contraceptive services. Twenty million unsafe abortions are carried out yearly; 97% of these in developing countries. Conventional contraceptive though potent are not devoid of side effects. Tana River is rural with in ill-equipped and in adequate health facilities. Majority of women in Tana River drink medicinal plants concoctions as a means of fertility regulation.[1] The aim of the study was to validate the traditional claims by investigating the effect of root bark extract of *Croton menyharthii* and *Uvariodendron kirkii* plants as anti-fertility agents.

MATERIAL AND METHODS

Plant collection, identification and extract preparation

Medicinal plants used as fertility regulators in Tana River County were harvested and brought to the University of Nairobi, School of Biological Sciences for botanical identification. Voucher specimens were preserved for future reference. *Croton menyharthii* (CK021) and *Uvariodendron kirkii* (CK008) fresh roots were cut into small pieces using a knife. The roots were then kept under shade and dried at room temperature for a period of two weeks. The roots were ground into powder in a fume chamber using a Cunningham grinder.[2] The plant powder was packed in 300g satchets and stored in cool and airy cupboards away from direct sunlight.

Extract preparation

300g of *Croton menyharthii* root bark powder was weighed using (Lark digital weighing balance LP502A, 500G/0.01g). The root bark powder was macerated in distilled water at a ratio of 1 to 6 (w/v) in a volumetric flask. The suspension was rotated on a shaker for 24 hours at room temperatures and left to soak for 48 hours. Filtration was carried out using whatman filter paper (number 4). The filtrate was freeze dried for 48 hours and the extract weighed to determine yield. *Uvariodendron kirkii* aqueous extract was prepared in a similar manner. The aqueous extract yield for *Croton menyharthii* and

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Uvariodendron kirkii was 83.89 and 118.93 grams respectively.

Animal husbandry

Twenty five mature female winstar rats weighing between 170-210g were used for the study. The animals were purchased from the Department of Biochemistry and kept in the animal house, Anatomy and Physiology Department, of the University of Nairobi, Kenya. They were caged in groups of five and were maintained under standard environmental conditions of 12 hours light and 12 hours darkness at 24-25 °C. The rats were fed on commercially obtained diet pellets and tap water was provided *ad libitum*. They were monitored daily for the first 10 days using vaginal smears to ascertain cyclicity. Only those with regular 4-5 day estrous cycles were used for the experiment. The rats were divided into five groups (5 rats each). Group one to four received 500 and 800 mg/Kg body weight of an aqueous extract of Croton menyharthii and Uvariodendron kirkii respectively through intra-abdominal gavage on alternative days for four weeks. The control group received the vehicle (distilled water) at 0.5ml through intra-abdominal gavage on alternative days for four weeks. After 28 days all rats were anaesthetized using diethyl ether and sacrificed. Whole blood was collected via cardiac puncture using sterile needles and syringes into plain tubes and allowed to clot for two hours. The clotted blood was centrifuged at 3000rpm for 10 minutes for serum collection meant for hormonal profiling. After centrifugation the serum samples were stored at -20°C. Luteinizing and progesterone hormone in the serum were determined by the Enzyme- Linked Immunoabsorbent Assay (ELISA) method using Microwell's kits. Physiological saline was used to flush the body of all rats and immediately thereafter left ovaries were harvested and processed for histology, Ovaries were fixed, cut in sections of 8 micron thickness and stained with Hematoxylin and Eosin and observed under a microscope.

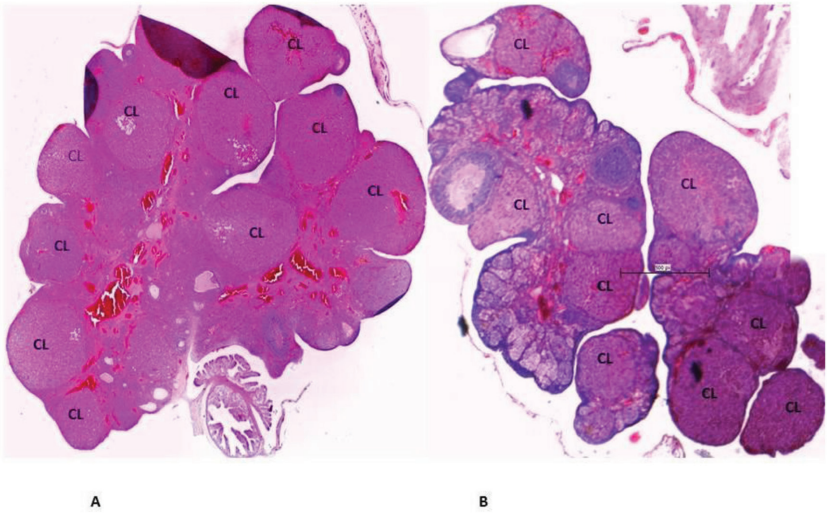

Figure 1 The effect of *Croton menyharthii* on corpus luteum

Statistical analysis

Luteinizing hormone and progesterone hormone measurements are presented as mean ± SEM. One way Analysis of Variance (ANOVA) was used to analyze the data ($P<0.05$).

RESULTS

Effect of *Croton menyharthii* and *Uvariodendron kirkii* on reproductive hormones

Progesterone levels significantly increased (Table 1) in treated groups compared to the control in a dose dependent manner. Similarly, *Uvariodendron kirkii* aqueous extract caused a significant increase in serum progesterone levels when compared to the negative control (Table 1). Luteinizing hormone levels (Table 1) were significantly increased by *Croton menyharthii* at both doses compared to the negative control. *Uvariodendron kirkii* aqueous extract on the other hand, caused a reduction in the levels of luteinizing hormone (Table 1). The reduction was however not significant when compared to the negative control.

Effect of *Croton menyharthii* and *Uvariodendron kirkii* on corpora lutea

Figures 1B to 5B are representative photomicrographs showing the effect of *Croton menyharthii* extracts on corpus luteum. The ovaries were harvested at metestrus (1A and 2A) and Diestrus (3A to 5A). Fig 1B shows degenerating corpora lutea though the numbers were not significantly different from control 1A. Fig. 2B shows significant increase in corpora lutea numbers and a decline in size compared to the control 2A. At 800mg/ kg

Table 1 **Effects of *Croton menyharthii* and *Uvariodendron kirkii* on reproductive hormones**

	Control	CM 500 mg/kg	CM 800 mg/kg	UK 500 mg/kg	UK 800 mg/kg
Progesterone hormoneng/ml	0.886 ± 0.03	0.906 ± 0.02**	0.911 ± 0.02**	0.931 ± 0.04***	0.899 ± 0.01*
Luteinizing hormone IU/ml	0.053 ± 0.01	0.069 ± 0.04**	0.059 ± 0.03*	0.0443 ± 0.01	0.048 ± 0.02

CM-Croton menyharthii UK-Uvariodendron kirkii

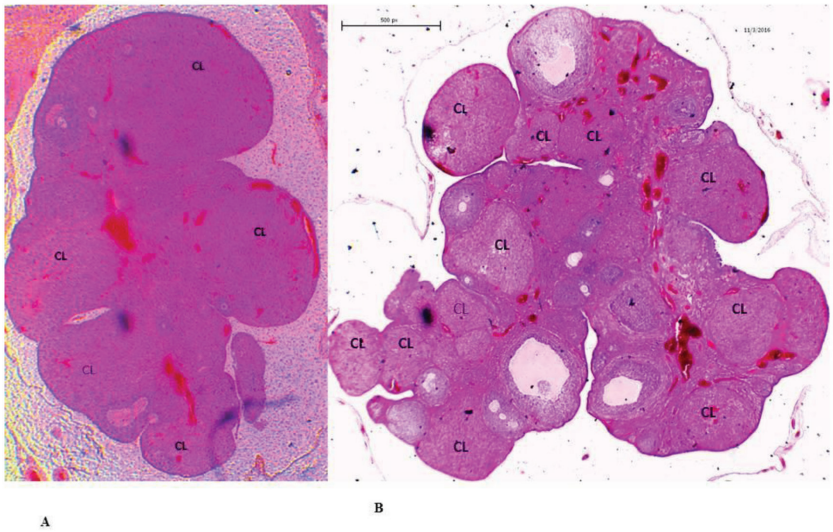

Figure 2 The effect of *Croton menyharthii* on corpus luteum

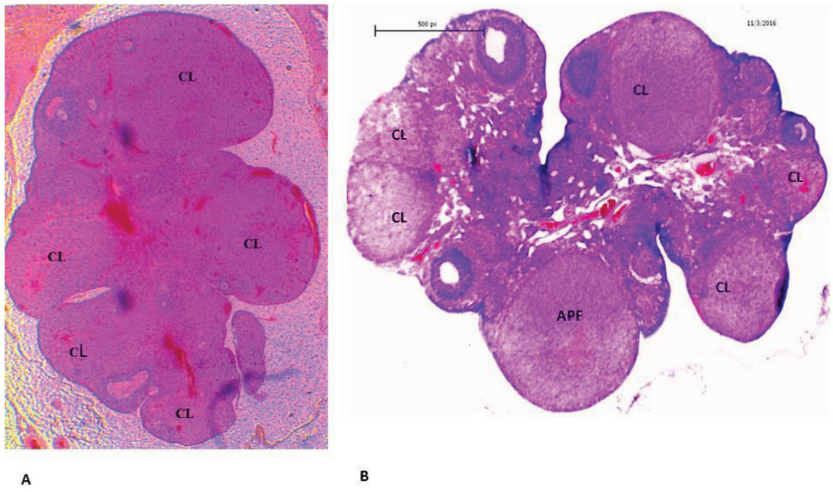

Figure 3 The effect of *Croton menyharthii* on corpus luteum

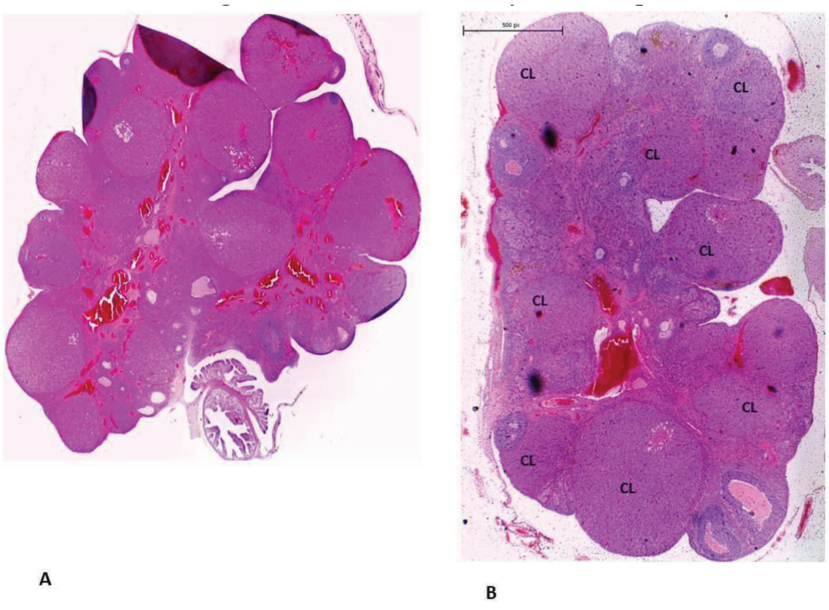

Figure 4 The effect of *Uvariodendron kirkii* on corpus luteum

Croton menyharthii extract (Fig. 2B and 3B) compromised the structural intergrity of the corpora lutea compared to the control 2A. Fig. 3B shows a disruption of the corpora lutea structural intergrity, condensed cytoplasm and presence of pyknotic cells in corpora lutea compared to the control 3A. The number of corpora lutea are however not significantly different from the control 3A. Figures 4B to 5B are representative photomicrographs showing the effect of *Uvariodendron kirkii* extracts on corpus luteum. 4B shows a few of the corpora lutea undergoing hypertrophy, condensed cytoplasm and presence of pyknotic cells in corpora lutea compared to control 4A. There was a significant increase in number of corpora lutea compared to the control 4A. Fig. 5B shows a significant increase in number of corpus lutea compared to the control 5A. The ovarian stroma is mostly occupied by corpora lutea and some atretic ovarian follicles compared to the control 5A. At 800mg/Kg *Uvariodendron kirkii* extracts caused a disruption of corpora lutea structure (Fig. 5B), condensed the cytoplasm and there was presence of pyknotic cells. One of the corpus luteum on the right side of the figure had undergone hypertrophy. The ovarian stroma structure is disrupted and calcified. Uvariodendron kirkii caused a significant reduction in corpora lutea number compared to the control 5A.

The ovary was harvested at diestrus phase of the cycle. 1A: Control photomicrograph shows intact structural intergrity of the corpora lutea. 1B: photomicrograph shows a disruption of the corpora lutea structural intergrity. Condensed cytoplasm and presence of pyknotic cells in corpora lutea. The number of corpora lutea were not significantly different from the control 1A. Magnification × 100.

Key- CL-corpus luteum

2A: control- intact structural intergrity of corpus luteum, no signs of vacuoles. 2B: At 800mg/kg the photomicrograph shows the extract caused a significant increase of corpora lutea numbers but a decline in corpora lutea sizes compared to the control 2A. Structural intergrity of the corpora lutea was compromised compared to the control 2A. Magnification × 100. Key- CL-corpus luteum

3A: control- intact structural intergrity of corpus luteum, no signs of vacuoles. 3B: the photomicrograph shows the presence of various stages of ovarian follicles. The number and size of corpus luteum is not significantly different from control 3A. However the corpus luteum on the left of photomicrograph were atretic showing signs of vacuolation. Magnification × 100. Key- CL-corpus luteum

4A: Control photomicrograph shows intact structural intergrity of the corpus lutea. 4B: Some of the corpus lutea have undergone hypertrophy,

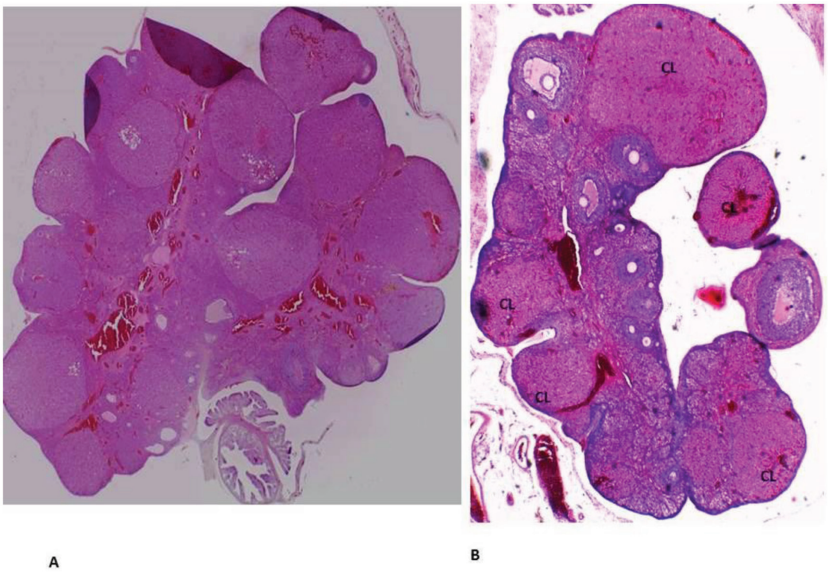

Figure 5 The effect of *Uvariodendron kirkii* on corpus luteum

condensed cytoplasm and presence of pyknotic cells. There is a significant increase in number of corpus lutea compared to the control 4A. The ovarian stroma is mostly occupied by corpus lutea and some atretic ovarian follicles.

Magnification × 100 Key- CL-corpus luteum

The ovary was harvested at diestrus phase of the cycle. 5A: control intact structural intergrity of the corpora lutea and ovarian stroma. 5B: Disrupted corpora lutea structure, condensed cytoplasm and presence of pyknotic cells. One of the corpus lutum on the right sie of photomicrograph has undergone hypertrophy. The ovarian stroma structure is disrupted and calcified. The number of corpora lutea is significantly reduced compared to the control 5A.

DISCUSSION

Croton menyharthii extracts caused a degeneration of corpora lutea (Fig. 1B) but the numbers were not significantly affected compared to control 1A. At 800mg/kg *Croton menyharthii* caused a significant increase in corpora lutea numbers (Fig. 2B) but a decline in size compared to the control 2A. *Uvariodendron kirkii* caused a significant increase in number of corpus lutea (Fig. 4B) compared to the control (Fig. 4A). In Fig. 5B a few of the corpora lutea were undergoing hypertrophy. *Croton menyharthii* caused a significant increase of progesterone in a dose dependent manner compared to the negative control. Similarly, *Uvariodendron kirkii* aqueous extract also caused a significant increase in serum progesterone levels compared to the negative control. Our results are corroborated by Monsefi et al., 2015. He reported that *Anethum graveolens* caused elevated progesterone levels and increased numbers and size of corpora lutea.[3] *Alchornea cordifolia* caused a significant increase in the level of progesterone but no effect on corpora lutea.[4] Our results are in contrast to Attia et al., 2016. He reported that; *Quebracho* tannins caused a reduction of corpora lutea numbers and progesterone level decline in dairy cows.[5] Palm Pollen extract caused a reduction in corpora lutea numbers in rat.[6] Frankincense's hydro alcoholic extract decreased corpora lutea numbers significantly but increased levels of progesterone.[7] *Millettia aboensis* leaf extract caused a reduction of progesterone levels.[8] Methanolic extract of unripe *Carica Papaya* fruit caused a dose dependent reduction of corpora lutea numbers.[9] *Ficus deltoidea* ethanolic leaf extract reduced the number of corpora lutea.[10] The corpus luteum secretes progesterone hormone. Progesterone in turn plays a crucial role in preparing the endometrium for implantation. An increase in number and /or hypertrophied corpora lutea possibly led to disrupted estradiol progesterone ratio therefore suggesting a compromised window of implantation. Progesterone is key in pregnancy maintenance. Near term; levels of progesterone reduce triggering other hormonal and molecular changes that terminates gestation. An elevation of progesterone hormone level will possibly lead to an extended gestation.

Androgen production by theca cells is dependent on luteinizing hormone levels. Granulosa cells convert the androgens to produce estradiol. A dominantly estrogenic environment is correlated with oocyte competence. Higher or lower LH secretion interferes with androgen production by theca cells. This in turn compromises estradiol secretion by granulosa cells leading to incompetent folliculogenesis and oogenesis thereby causing infertility. In this study *Croton menyharthii* significantly enhanced luteinizing hormone production (Table 1) while *Uvariodendron kirkii* caused a non-significant reduction of luteinizing hormone.

CONCLUSION

Both plants have potential as antifertility agents. More studies need to be undertaken to further elucidate effects of plant extracts on estradiol, follicle stimulating hormone, oogenesis and on folliculogenesis.

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