Full Length Research Paper

Toxicology, phytochemistry, hormonal and ovarian disruption properties of *Uvariodendron kirkii* and *Croton menyharthii*, medicinal plants from Tana River County, Kenya

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**Croton menyharthii** and *Uvariodendron kirkii* are used as traditional contraceptives in Kenya. This study aims at documenting the safety, efficacy, phytochemical composition, hormonal and ovum disruptive properties of both plants extracts to validate continuous usage in indigenous communities. Methods: Phytochemical screening was done to determine phyto-constituents of *Croton menyharthii* and *Uvariodendron kirkii* extracts. Acute oral toxicity was carried out as per OECD test guideline 423. Estradiol hormone and ovarian histology was also evaluated. *Croton menyharthii* and *Uvariodendron kirkii* aqueous extract revealed high concentrations of flavonoids, quinones and terpenoids with mild concentrations of sterols, phenols, saponins and alkaloids. While *Uvariodendron kirkii* had some levels of tannins, *Croton menyharthii* had none. In acute toxicity, the LD50 of both plants was greater than 2000 mg/Kg. As levels of estradiol reduced there was a correlating percentage loss of ovum in ovarian follicles.

**Keywords:** *croton menyharthii*, *Uvariodendron kirkii*, toxicity, phytochemistry, estradiol levels, ovum loss.

**INTRODUCTION**

Plants are an important source of novel compounds as evidenced by the fact that 25% of current antibacterial, anti-malarial and anti-tumor prescriptions contain active principles of plant origin (Dinesh et al., 2012). Bioactive compounds from plants have been associated with chronic disease management in humans. Saponins exhibit abortifacients, anti-zygotic and anti-implantation properties. Some have caused sterility in mice (Francis et al., 2002). Alkaloids from *Mussaenda pubescens* extract terminated pregnancy in rats. Alkaloids are used to manage many illnesses including cancer, malaria, as analgesics and to control hypertensive disorder among others. Among plant derived constituents; abridine (*Abrus precatorius*), saponins (*Achyranthes bidentata*), Butin (*Butea monosperma*), Embelin (*Embelia ribs Burm*) momorcharins, β sitosterol (*Ananas comosus*), Vicorides among others have shown potential antifertility effect (Dinesh et al., 2012). Nitisinone derived from *Callistemon citrinus* is used as an anti tyrosinaemia; galantamine derived from *Galanthus nivalis* is used for the management of Alzheimers disease; apomorphine from *Papaver somniferum* for the management of Parkinson disease and capsaicin from *Capsicum annuum* as a pain reliever (Veeresham, 2012). Oxymethyl anthraquinone
from Polygonum hydropiper Linn caused 60% inhibition of ovulation. Glycosides and cardenolides from Calotropis gigantea had significant anti-implantation activity. Spondias mombin flavonoids cause significant anti-conceptive activity (Chukwuza and Uchendu, 2008). Plants therefore, hold a great promise for the discovery of new and effective antifertility agents. Croton menyharthii and Uvariodendron kirkii are traditional used as fertility regulators in rural parts of Kenya and should be evaluated as potential novel contraceptives.

MATERIAL AND METHODS

Phytochemistry

Phytochemical analysis was carried out as per the method used by (Ashokkumar et al., 2010) to decipher the presence and quantities of saponins, steroids, tannins, alkaloids, phenols, flavanoids, quinones and terpenoids.

Acute oral toxicity

The acute oral toxicity study protocol was carried out as per the OECD guidelines number 423 using 3 female rats (weighing between 170-200 grams) per step at any of the defined dose levels. Depending on the mortality rate 3 but never more than 6 rats were used per dose level. The result of each step determined if further testing was needed for 3 additional animals at same dose level or 3 additional at the next lower dose level. Food was withheld overnight but water was provided ad libitum. The animals were weighed just before extract was administered through intra-abdominal lavage. Food was withheld for a further 3-4 hours after extract administration. The extract dose range was 2000 to 5 mg/Kg starting at 300 mg/Kg. The observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic nervous system, central nervous systems, behavior pattern and death. Other observations included tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and death.

Effect of Croton menyharthii and Uvariodendron kirkii extracts on ovarian histology and estradiol hormone

The rats were divided into five groups (5 rats per group). Group 1 and 2 received 500 and 800 mg/Kg Croton menyharthii while group 3 and 4 received 500 and 800 mg/Kg Uvariodendron kirkii aqueous extract respectively daily for 28 days through intra-abdominal lavage. Five control animals received 0.5 ml physiological saline for 28 days. All rats were humanely sacrificed after the 28th day using carbon dioxide. 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 3000 rpm for 10 minutes for serum collection meant for hormonal profiling. After centrifugation the serum samples were stored at -20°C. 17β estradiol hormone in the serum was determined by the Enzyme- Linked Immunoabsorbent Assay (ELISA) method using Microwell’s kits. Physiological saline was then 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 & Eosin and observed under a light microscope as per protocol described.

Statistical Analysis

17β estradiol measurements are presented as mean ± SEM. One way Analysis of Variance (ANOVA) was used to analyze the data (P<0.05).

RESULTS

Phytochemistry

Croton menyharthii and Uvariodendron kirkii aqueous extract have high concentrations of flavonoids, quinones and terpenoids with mild concentrations of sterols, phenols, saponins and alkaloids. While Uvariodendron kirkii had some levels of tannins, Croton menyharthii had none.

Acute oral toxicity

Croton menyharthii and Uvariodendron kirkii aqueous extract did not cause any mortality even at the highest dose of 2000 mg/Kg. The rats showed tremors and lethargy within 30 minutes of treatment, followed by minimal activity for about 3 hours. The rats recovered thereafter. None of the animals died in 24 hours.

Effect of Croton menyharthii and Uvariodendron kirkii extract on 17β Estradiol hormone level and ovarian structures.

Croton menyharthii at 500 mg/kg body weight caused a reduction of 6.6 pg/ml estradiol with a corresponding 18% loss of ovum within ovarian follicles compared to the negative control (Table 1). Croton menyharthii at 800 mg/kg body weight caused a reduction of 7.1 pg/ml estradiol with a corresponding 48% loss of ovum within ovarian follicles; Uvariodendron kirkii at 500 mg/kg body weight caused a reduction of 9.19 pg/ml estradiol with a corresponding 39% loss of ovum within ovarian follicles (Table 1); Uvariodendron kirkii at 800 mg/kg body weight
caused a reduction of 11.30 pg/ml estradiol with a corresponding 67% loss of ovum within ovarian follicles compared to the negative control.

**DISCUSSION**

_Croton menyharthii_ and _Uvariodendron kirkii_ extracts are traditionally used as fertility regulators in Kenya. Acute toxicity studies did not cause any mortality even at the highest dose of 2000 mg/kg body weight. Preliminary phyto-chemistry established the presence of alkaloids, flavonoids, quinones, terpenoids, saponins, sterols and phenols. Several studies have reported the effect of alkaloids as fertility regulators. _Abrus precatorius_ indole alkaloid completely blocked ovulation and disrupted the estrus cycle in female rats (Okoko et al., 2010). _Acrelypha indica_ pyranoquinoline alkaloid had a post coital antifertility effect. Several authors (Elumalai et al., 2009; Sasmal et al., 2012; Balakrishnan et al., 2011; Vijayalakshmi et al., 2011; Musa and Bimbo, 2009; McNeil et al., 2003; Bhargava et al., 2012) reported on abortive effect of alkaloids from: _Achyranthes aspera_, _Aerva lanata_, _Alangium salvifolium_, _Amaranthus spinosus_, _Annona squamosal_, _Bambusa vulgaris_, _Gloriosa superba_, _Ricus communis_ and _Zingiber officinalis_. Several other studies (Saravanan and Renuka, 2012; Jyoti et al., 2010; Ibrahim and Fulya, 2013; Circosta et al., 2001) also reported on anti-oestral properties of alkaloids from _Ailanthus excella_, _Areca catechu_, _Curcuma longa_, _Papaver sominiferum_ and _Calotropis procera_ plants. Endocrine disruption effect of alkaloids from _Citrus bergamia_, _Cuscuta reflexa_ _Roxb_, _Datura metei Linn_, _Derris brevipes_, _Dioscorea pentaphylla_ Linn, _Duckesia verrucosa_, _Ehretia cymosa Thonn_, _Eriosema crinitum_, _Ficus religosa_, _Ficus wassa_, _Huperzia Linn_, _Indigofera linnaei_, _Justicia simplex_, _Mentha arvensis_, _Mentha longifolia Linn_, _Mouriri pusa_, _nardostachys grandiflora Persea Americana_, _Petroselinum crispum_, _Pirus communis_, _Phoradendron macrophyllum_, _Pouzolzia hypoleuca_, _Senecto aureus Linn_, _Solanum incanum_, _Trichosanthes tricuspidata_ has also been reported (Saravanan and Renuka, 2012; Panda et al., 2011; Soni et al., 2012; Ankush et al., 2011). _Tinospora cordifolia_, _Sesbania sesban_, _Mentha arvensis_, _Hibiscus rosasinensis_, _Daucus carota_, _Crataeva nurvala_, _Cassia fistula_, _Carum carvi_, _Azardichtha indica_, _Antiaris toxicaria_, _Allium cepa_ had antifertility, anti-implantation and caused a resorption of embryos. They also disrupted estrus cycle due to the presence of alkaloids (Dinesh et al., 2012). In this study alkaloids, along with other compounds were present in both plant extracts. It is possible that the antifertility effect in female rats seen in this study could partially be attributed to the presence of the alkaloids, as seen in studies mentioned above. Studies by Francis et al., 2002 indicated anti fertility effect in female rats as possibly being due to the presence of saponins and flavonoids. They further reported on the saponins as having abortifacients, anti-zygotic and anti-implantation effects. These findings are closely corroborated by the findings of this present study. The results of this study are also consistent with those of Londonkar et al., 2009 who showed similar results in rats following treatment with crude _sida acuta_ extract. Their report attributed the antifertility effect as being due to the presence of flavonoids which had antizygotic, blastocytotoxic and anti-implantation activity. Sasmita, 2014 working on _Piper betel_, suggested that possibly flavonoids and saponins from this plant were responsible for the significant disruption of the estrus cycle leading to infertility in their study. Abrin and abridin compounds from _Abrus precatorius_ have been suggested by several authors (Pillai et al., 1982; Bhargava, 1984; Hiremath and Rao, 1990; Yuan et al., 1991; Alam et al., 1992) as being responsible for anti-implantation activity in female rats. This is corroborated by Modaresi et al., 2012 who reported on phytochemical compounds exhibiting antifertility activity. (Zhu et al., 1987; Hu et al., 1984; Bhargava and Dixit, 1985) reported on saponins, vicolide D; embelin, methyl aristolote, yuanhuacine, yuanhuatine, momorcochin and plumbagin causing abortion in female rats. On the other hand studies done by (Mats et al., 1982;  

<table>
<thead>
<tr>
<th>Estradiol (pg/ml)</th>
<th>Negative control</th>
<th>CM 500 mg/ kg</th>
<th>UK 500 mg/ kg</th>
<th>UK 800 mg/ kg</th>
</tr>
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<tr>
<td>44.80 ± 0.23</td>
<td>38.20 ± 0.11*</td>
<td>37.70 ± 0.12*</td>
<td>35.61 ± 0.1**</td>
<td>33.50 ± 0.2***</td>
</tr>
<tr>
<td>Percentage (%) ovum loss</td>
<td>0</td>
<td>18</td>
<td>48</td>
<td>39</td>
</tr>
</tbody>
</table>

The values are mean ± SEM. *** P<0.001 ** P<0.01 *P<0.05

CM: Croton menyharthii  UK: Uvariodendron kirkii
Figure 1. Phytochemical compounds in Croton menyharthii. The figure shows preliminary phytochemical compounds in Uvariodendron kirkii and Croton menyharthii aqueous extract.

Figure 2. shows a correlation between 17β Estradiol concentration levels and percentage loss of ovum at varied doses of Croton menyharthii and Uvariodendron kirkii extracts. As levels of estradiol decreased there was a correlating increase in percentage loss of ovum compared to the control (0% loss of ovum).

Mats et al., 1984; Prakash et al., 1991) suggested that contraceptive effect in rats were due to vicilide D, triterpene glycoside, lithospermic acid, cirantine and ferujol phytochemical compounds. In this study Croton menyharthii and Uvariodendron kirkii phytochemical analysis showed the presence of several phytochemical compounds (Figure 1) in the aqueous extract. Probably the anti-fertility effect of both plants as claimed by the TMPs could be due to the presence of these compounds especially flavonoids, saponins and alkaloids as has been reported in other studies. The indole alkaloid, trans-\(N\)-(p-coumaroyl) serotonin (4) present in Croton menyharthii has shown promising fertility inhibitory effect at a very low dose. Croton menyharthii and Uvariodendron kirkii aqueous extracts at 500 and 800 mg/ kg body weight caused a significant reduction in serum estradiol levels (Table 1; Figure 2). These findings together with a significant reduction in number of ovum probably indicate a direct effect of Croton menyharthii and Uvariodendron kirkii on the ovary structure in the rats. Female fertility is driven by the developmental competence of the oocyte. In its ability to undergo meiosis, be fertilized and give rise to a viable embryo. The embryo has to successfully implant and establish itself within the uterus up to the end of gestation. Estradiol is responsible for multiple functions in the female; one of
them causing the secretory cells lining the lumen of the oviduct to synthesize and secrete glycoproteins that play a key role in the nourishment of the embryo. Differentiation and ciliation of the oviduct inner most oviduct layer are induced by estradiol. A reduction of estradiol levels possibly compromised oviduct integrity and might have interfered with fertilization, early embryonic growth and possibly oviduct contraction and hence impeded movement of the fertilized embryo towards the uterus. Estrogen acts in a feedback mechanism, influencing the production of follicle stimulating hormone (FSH) from the pituitary gland. It is known that the FSH in turn promotes the development of the antral ovarian follicles, which increases the production of estrogen from the ovary. A disruption of estrogen progesterone ratio can interfere with implantation and establishment of the pregnancy. The maturation of pre ovulatory follicles and ovulation is under the combined and balanced influences of ovarian and anterior pituitary hormones. Imbalances or alterations in these hormones might have led to irregularity in ovarian function and duration of estrus cycle.

CONCLUSION

The presence of alkaloids, saponins and flavonoids might be responsible for the anti-fertility activity of Croton menyharthii and Uvariodendron kirkii aqueous extract. Further anti fertility, anti-ovulatory and anti-implantation studies on the indole alkaloid, trans-N-(p-coumaroyl) serotonin (4) isolated from Croton menyharthii should be explored.

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Conflict of interest

The authors declare that there was no conflict of interest

REFERENCES


