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Phytochemical screening and pharmacological evaluations for the antifertility effect of the methanolic root extract of *Rumex steudelii*

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Abstract

The practice of traditional medicine for the control of fertility in most parts of Ethiopia is based on the uses of plant medicines for many years. The fact that herbal medicines have been employed for such a long time does not guarantee their efficacy and safety. The aim of the present study was, therefore, to carry out phytochemical screening, efficacy and safety studies on one of the traditionally used antifertility plants: *Rumex steudelii*. The secondary metabolites of the root of this plant were determined. The methanolic extract of the roots of this plant were investigated for their antifertility activity in female rats and oral LD_{50} was determined in mice. The identification of the secondary metabolites showed that the roots of the plant contained phytosterols and polyphenols. It was found that the extract reduced significantly ($p < 0.01$) the number of litters. It also produced antifertility effect in a dose dependent manner and the contraceptive effect was manifested for a definite period of time. Furthermore, the extract prolonged significantly the estrus cycle ($p < 0.05$) and the diestrous phase ($p < 0.01$) of the rats. The wet weights of the ovaries and uterus were shown to be reduced significantly ($p < 0.01$) and ($p < 0.05$), respectively. The oral LD_{50} of the extract was found to be 5 g/kg in mice. All these observations suggest that the extract has antifertility effect and is safe at the effective antifertility doses employed in this study.

Keywords: Antifertility; Estrus cycle; Female rats; LD_{50}; *Rumex steudelii* extract; Secondary metabolites

1. Introduction

Global search for antifertility agents is continued to tackle the problem of population explosion that may lead to economic and health impact on the family in particular and the society in general especially in developing countries like Ethiopia where the population growth is very high (Ministry of Health, 2003). Although contraceptives containing estrogen and progesterone are effective and popular, the risks associated to the drugs have triggered the need to develop alternative methods from medicinal plants. Hence, there is a need for a suitable product search from indigenous medicinal plants that could effectively be used in the place of pills (Pal, 1990).

A number of investigations have been carried out on traditionally claimed antifertility plants to validate the claim. Recent literature review revealed that 48 out of 72 traditionally employed medical plants for fertility control had antifertility potential (Maurya et al., 2004).

Rumex steudelii Hochst (Polygonaceae), locally known as "Tult" or "Yeberemelas" is one of the traditionally used antifertility plants in Ethiopia. It is an erect, perennial herb, and grows up to 1 m tall. The plant is distributed in the North and Central part of Ethiopia, at an altitudinal range of 1200–3900 m (Edwards et al., 2000).

The roots of this plant in combination with other medicinal plants are traditionally used in the treatment of rectal

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proplase, haemorrhoid, wounds, eczema, swelling, leprosy, toncillitis, abdominal colic and tinea nigra. It is also used as hemostatic and oxytocic agents (Abebe and Ayehu, 1993). The preliminary studies on the root extract of the plant showed anti-implantation effect in rats (Desta, 1994). The present study was, therefore, carried out to identify the chemical constituents of the roots of *Rumex steudelii*, evaluate further its claimed antifertility effect using different models, and determine its oral LD_{50}.

2. Materials and methods

2.1. Plant material

2.1.1. Collection and identification

The roots of *Rumex steudelii* were collected around Addis Ababa in December 2002. The plant was identified by a taxonomist and a voucher sample representing, Herbarium No. RS-2084 was deposited in the Herbarium of Medicinal Plants of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia.

2.1.2. Processing and extraction

Grabbing (removal of extraneous matter such as dirt and adulterants) besides the plant part intended to be studied was performed following collection before drying and processing of the roots. The drying operation was carried out under room temperature with out exposure to sunlight. Air dried and powdered roots of *Rumex steudelii* (300 g) were then extracted by percolation at room temperature using methanol. The methanolic extract was concentrated under a vacuum in a rotary evaporator to yield dark brown semi-solid mass which is further dried under a vacuum oven drier (at approximately 25 °C for 3 days) to give (as a percentage of dried powdered plant materials) 8.5% solid residue. This extract was reconstituted in distilled water to get the desired concentration for all pharmacological tests.

2.2. Animals

All antifertility experiments were performed on in-bred adult, cyclic virgin female albino rats (2-months-old and weighing 190–230 g body weight). Male and female albino mice weighing 25–30 g were also used for the acute toxicity study. All the animals used for this experiment were bred in a standard animal house. The animals were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 h light and dark cycle for each 24 h period at a temperature of approximately 25 °C. They were fed on pellets and tap water ad libitum. The animals were allowed to acclimatize to the laboratory environment for 1 h before being subject to the experiments. All experiments were carried out in a quiet laboratory setting with ambient illumination and temperature close to those of the animal house.

2.2.1. Test material administration

Administration of the extract was done with intragastric tube on the basis of the animal's body weight. The dose for each animal was calculated considering the human dose (dry weight equivalent approximately 4 g/kg aqueous macerate employed as vaginal douche in divided doses) based on ethnomedical use of the plant part and the anti-implantation study of the alcoholic extract of the same part of the plant in a dose of 1.61 g/kg in rats (Desta, 1994).

2.3. Phytochemical screening

Identification of the chemical constituents were carried out on the powdered roots and on the methanol extract using chemical methods and TLC according to the methodology proposed by Fransworth (1966), Marini-Bettolo et al. (1981) and Harborne (1984).

2.4. Pharmacological screening

2.4.1. Preliminary screening for the antifertility activity

Five groups of matured female rats (5–6 rats per group) were selected for this experiment. One group served as a control and received the vehicle intragastrically for 7 days. The other four groups received 2.2, 2.5, 2.8 and 3.0 g/kg animal body weight of the crude extract daily by the same route of administration for the same period. All the experimental animals were then allowed to mate with matured proven fertility male rats (one male for two females) and administration of the vehicle and the extract continued for 21 days (Makonnen et al., 1997). The number of litters was determined after the completion of one gestation period in both the control and test groups as described by Gara (1975) on chromatographic fractions of *Daucus carota*. The litters of the extract treated rats were then allowed to grow in order to check for postnatal growth and congenital anomalies.

The reversibility of the antifertility effect of the extract was also studied according to the modified method of Salhad et al. (1997) on *Ricinus communis* seeds. In our study, four rats were treated with the extract 2.2 g/kg of body weight for 21 days. After another 21 days of drug free period, the animals were allowed to mate with males of proven fertility in the ratio of 1 male to 2 females. After the completion of one gestation period (21 days) the number of litters was determined.

2.4.2. Effect of the extract on the estrous cycle

Five matured female Wistar albino rats (200–210 g) were employed. Vaginal smear from each animal was examined under a microscope every morning at 9.00 a.m. for 21 days (about 5 cycles). The smears were evaluated as described by Vogel (1997). The duration of the estrous cycle together with that of the various phases was determined as described by Makonnen et al. (1997, 1999) for 21 days.

All rats then received 2.2 g/kg of body weight of the extract every day by intragastric route for another 21 days and the same parameters were determined. The vaginal smears from