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Screening for *in vivo* (anti)estrogenic and (anti)androgenic activities of *Tropaeolum majus* L. and its effect on uterine contractility

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ABSTRACT

Ethnopharmacological relevance: Tropaeolum majus L. (Tropaeolaceae) is a medicinal herb popularly used in Brazil for treatment of inflammatory and cardiovascular diseases. Despite some published data on its efficacy, there are still few toxicological data describing the safety of this plant. The aim of this study was to evaluate the (anti)estrogenic and (anti)androgenic activity of the hydroethanolic extract obtained from *Tropaeolum majus* L. (HETM), as well as its possible effects on uterine contractility.

Materials and methods: Three experimental protocols were performed, (a) uterotrophic assay, (b) Hershberger assay and (c) an *ex vivo* test to investigate the effects of maternal administration of HETM on uterine contractility at the end of pregnancy. In all protocols three doses of the HETM were administered to Wistar rats: 3, 30 and 300 mg/kg.

Results: In vivo tests for detection of (anti)androgenic and (anti)estrogenic activities did not show any significant alterations. Similarly, no alterations were observed on uterine contractility induced by oxytocin and arachidonic acid.

Conclusions: HETM was unable to produce (anti)estrogenic or (anti)androgenic activities in the short-term *in vivo* screening assays performed. In addition, there was no evidence that HETM can affect uterine contractility following gestational exposure of rats.

1. Introduction

Medicinal herbs are widely used for treatment of several diseases, as well as for research and development of new drugs. There are over 20,000 species being used in traditional medicine and these are potential sources for discovery of new therapeutic compounds (Gupta et al., 2008). Historically, in folk medicine, different ethnic groups have used alternative therapies for treatment of diseases or as alimentary supplement. The widespread use of medicinal plants is partly due to the low toxicity attributed to these natural products (Ling et al., 2008). However, medicinal plants may cause a series of toxic effects, including metabolic disorders, alterations in immune and endocrine system, hepatic toxicity and behavioral effects (Buttar and Jones, 2003; Jurgens, 2003; Gadano et al., 2006). Due to this evidence, the FDA (Food and Drug Administration) describes that when a plant is used to heal, treat or prevent some human disease, this will be classified as a drug, and the suppliers must provide scientific evidence that the product is effective and safe for human use (Wu et al., 2008).

Tropaeolum majus L. (Tropaeolaceae) is a native plant of the Andes in South America and it is widely distributed around the world. In Brazil, it is popularly known as "chaguinha", "capuchinha" and "nastúrcio" (Ferreira et al., 2004; Ferro, 2006). It has been used by the population in form of tea made from its leaves for treatment of several conditions, including inflammatory processes, high blood pressure, edema and genitourinary tract infections (Corrêa et al., 2001; Lorenzi and Matos, 2002).

Phytochemical studies detected the presence of fatty acids, benzyl isothiocyanate and flavonoids in seeds and leaves of *Tropaeolum majus* (De Medeiros et al., 2000; Mietkiewska et al., 2004; Zanetti et al., 2004). Glucosinolates were isolated from leaves of this plant, as well as tetracyclic triterpenes (Lykkesfeldt and Moller, 1993; De Medeiros et al., 2000; Griffiths et al., 2001; Kleinwachter et al., 2008). Several studies in experimental pharmacology have been performed with *Tropaeolum majus* L or compounds isolated from it. Binet (1964) demonstrated that benzyl isothiocyanate possesses antimicrobial activity in genitourinary infections. Pintão

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et al. (1995) showed that benzyl glucosinolate isolated from *Tropaeolum majus* have *in vitro* anticancer activity in several human tumor cell lineages. Likewise, Picciarelli et al. (1984) and Picciarelli and Alpi (1987) demonstrated that the triterpene curcubitacin has antineoplastic activity. De Medeiros et al. (2000) described antithrombotic effect of some extracts of *Tropaeolum majus* L. leaves. More recently, studies from Gasparotto et al. (2009, 2011a,b) demonstrated diuretic and antihypertensive activity of the ethanolic extract of *Tropaeolum majus* (HETM), purified fraction and a flavonoid, isoquercitrin, from the leaves of *Tropaeolum majus* L. Nevertheless, despite many studies on the pharmacological properties of *Tropaeolum majus* L., several aspects of its safety have not yet been thoroughly studied (Wielanek and Urbanek, 2006). Recently, another species of the same family, *Tropaeolum tuberosum*, was reported as being able to reduce testicular function in rats following treatment with extracts prepared from the roots (Cardenas-Valencia et al., 2008). In addition, it has been reported that some flavonoids, which are major components of the hydroethanolic extract of *Tropaeolum majus*, are potential endocrine active compounds (Le Bail et al., 1998).

In the last decades, there has been growing concern over the effects of either synthetic or natural products on the endocrine systems of humans and wildlife. The so-called endocrine disruptors can affect development, reproduction, metabolism, immunity and several other hormonally dependent processes. Identification of potential endocrine disruptive properties of medicinal herbs has become significantly important, particularly because of the wide acceptance and use of such products by sensitive populations, including children and pregnant women. The aim of the present study was to investigate the possible (anti)estrogenic and (anti)androgenic activities of the hydroethanolic extract from leaves of *Tropaeolum majus* (HETM) in short-term *in vivo* screening tests, as well as the effects of the extract in an *ex vivo* assay of uterine contractility.

2. Materials and methods

2.1. Animals

Wistar rats were obtained from the Federal University of Parana and maintained in controlled conditions at 22 ± 2 °C and a constant 12 h light/dark cycle. Standard pellet food (Nuvital, Curitiba, PR, Brazil) and tap water were available *ad libitum*. All animal studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Federal University of Parana (Protocol number: 383).

2.2. Plant Material

Tropaeolum majus L. leaves were collected in June 2009 from the botanical garden of University Paranaense (UNIPAR), at 430 m altitude above sea level (S23°47′55–W53°18′48). The plant was identified and voucher specimens were deposited at the Herbarium of this University under number 2230.

2.3. Preparation of the hydroethanolic extracts of Tropaeolum majus (HETM)

Tropaeolum majus L. leaves were air-dried in an oven at 40 °C for 4 days and the resulting dry plant was cut and pulverized. This plant material was macerated for 7 days using 90% ethanol as solvent. The solvent was then eliminated by a rotary vacuum evaporator under reduced pressure and lyophilized, representing a yield of 15.5% of the dry material extracted.

2.4. Drugs

17-α-Ethynylestradiol (95% pure; $C_{20}H_{24}O_2$) and testosterone propionate (97% pure; $C_{22}H_{32}O_3$) was obtained from Sigma–Aldrich (Steiheim, Germany). Tamoxifen (tamoxifen citrate, 99.5% pure; $C_{26}H_{29}NO$) was obtained from Galena Laboratory (Curitiba, PR, Brazil) and Flutamide was purchased from Galena (São Paulo, SP, Brazil).

2.5. Uterotrophic assay

The uterotrophic assay have been routinely used to investigate possible (anti)estrogenic activities of different compounds (Ashby et al., 1997; Odum et al., 1997). Immature female rats, aged 21 ± 1 day, were randomly assigned to different experimental groups and were treated daily for three consecutive days with HETM (3, 30 and 300 mg/kg/day). In addition, one group was treated with distilled water to serve as negative control, while another group received 17-α-ethynylestradiol (dose of 0.4 mg/kg/day by gavage) and was used as a positive control for estrogenicity (Andrade et al., 2002). The possible antiestrogenic activity was tested by administration of the same three doses of HETM (3, 30 and 300 mg/kg/day) to female rats previously treated with 17-α-ethynylestradiol. The last group received tamoxifen (dose of 10 mg/kg/day by gavage) after 17-α-ethynylestradiol and served as positive control for antiestrogenicity. Twenty-four hours after the last treatment, animals were weighed and sacrificed by cervical dislocation (AVMA, 2007). Uteri were excised, trimmed free of fat, pierced, and blotted to remove fluid. The body of each uterus was cut just above its junction with the cervix and at the junction of the uterine horns with the ovaries. Wet uterus weight was determined and expressed as relative weight (wet uterus weight × 100/body weight).

2.6. Hershberger assay

For the Hershberger assay, 7-week-old male rats were castrated *via* the scrotum (midline incision) under anesthesia (ketamine 75 mg/kg/and xylazine 1.5 mg/kg; i.p.). Chemical treatment was not commenced until 7 days after castration to allow for complete recovery. Seven-week-old rats (peripubertal) were chosen as this is the typical age used in several studies (Ashby and Lefevre, 2000; Yamada et al., 2001).

The HETM was given daily for 7 consecutive days by either oral gavage (p.o.) or subcutaneous injection (s.c.). For assessment of (anti)androgenicity, three doses of HETM (3, 30 and 300 mg/kg/day) were administered orally to castrated animals and to testosterone-treated males (testosterone propionate 0.25 mg/kg/day; s.c.). For detection of androgenicity, the same doses of the HETM were administered orally to castrated males treated with vehicle (canola oil 1.0 mL/kg/day; s.c.). Animals treated with the vehicle by either oral gavage (5.0 mL/kg/day) or subcutaneous injection (1.0 mL/kg/day) were used as negative controls for androgenicity, while castrated rats administered testosterone propionate (0.25 mg/kg/day; s.c.) and vehicle (5.0 mL/kg/day p.o.) were used as positive controls for androgenicity. Flutamide (10.0 mg/kg/day; s.c.) given to castrated, testosterone-treated males (testosterone propionate 0.25 mg/kg/day; s.c.), which also received the vehicle (canola oil 5.0 mL/kg/day; p.o.), was used as a positive control for antiandrogenicity. The dosing volume for all solutions was 5.0 mL/kg when using the oral route and 1.0 mL/kg when using subcutaneous injections. One day after the final administration, all rats were weighed and sacrificed by cervical dislocation after deep anesthesia (Ketamine). At necropsy, the prostate, seminal vesicle without fluid, levator ani/bulbocavernosus muscle (LABC) and glans penis were carefully dissected free of adhering fat and weighed.