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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.

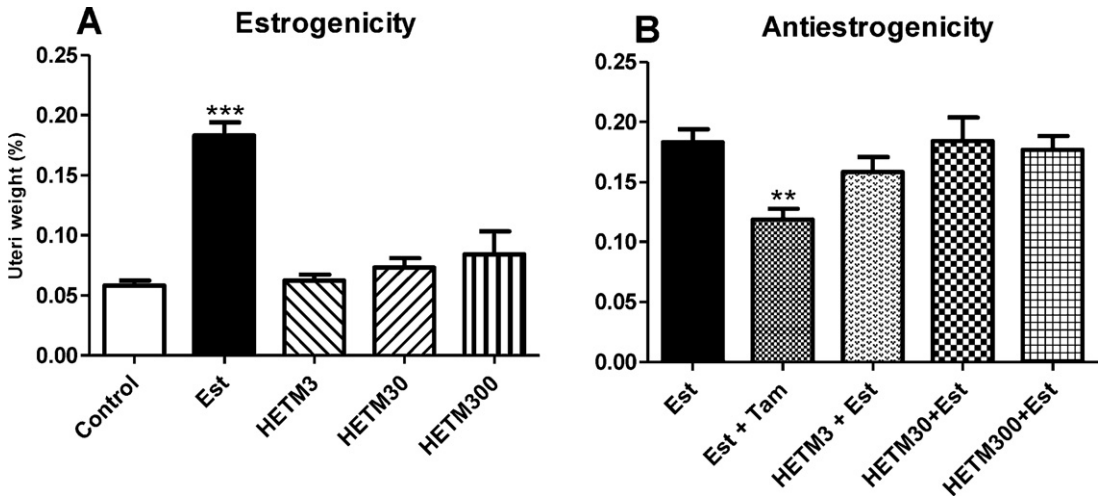

Fig. 1. Test for estrogenicity and antiestrogenicity (uterotrophic assay). Columns represent mean ± standard error of the relative uterus weight of immature females rats ($n = 11$ animals per group). $^{**}p < 0.01$ and $^{***}p < 0.001$ (one-way ANOVA followed by Bonferroni test). Est, estradiol (1 μg/kg); Tam, tamoxifen (10 mg/kg); HETM, hydroethanolic extract of *Tropaeolum majus*.

All organs were weighed and their masses expressed as relative weights (organ weight/body weight × 100).

2.7. Effect on the contractility of isolated pregnant rat uterus

Adult female rats were mated for three hours during the dark cycle at the proportion of three females to each male. Vaginal smears were collected daily and the day of sperm detection was considered as day 0 of gestation. Pregnant rats were treated daily from day 8 to 21 of pregnancy with HETM (3, 30 and 300 mg/kg/day) and sacrificed at day 21 of pregnancy by deep ketamine anesthesia. The uterus was immediately removed, placed in nutritional liquid Krebs–Henseleit, and cleaned of fat and excessive connective tissue. The uterus and the myometrium were transversally sectioned in strips of approximately 5 mm of length at the central portion of each uterine horn, and each of the two obtained strips was submitted to different experimental conditions. Uterine strips were placed in a 10 mL organ bath containing nutritional liquid Krebs-Henseleit maintained at 32 °C and continuously aerated with 95% O_2 and 5% CO_2. Uterine strips were subjected to an optimal resting force of 1 g and 60 min of stabilization. The uterine strips were initially submitted to contraction with KCl (80 mM) to verify the viability of the tissue, and the response was considered as 100%. After 30 min, tissues were directly stimulated with arachidonic acid (0.3 mM) or oxytocin (10 mUI/ml). In between curves, a time of 30 min was respected and the nutritional solution was renewed every 10 min. Contractile isotonic responses of uterine strips were recorded using a Kymograph and converted into percentage, compared with KCl response. This procedure was repeated twice to allow a medium value for the contraction induced by oxytocin and arachidonic acid. The nutritional liquid Krebs–Henseleit presents the following composition (concentrations in mM): NaCl (133), KCl (5.0), $CaCl_2$ (2.5), $MgSO_4$ (1.3) KH_2PO_4 (1.2), $NaHCO_3$ (20) and glucose (10).

3. Statistical analysis

Parametric data were analyzed by variance analysis (ANOVA) and differences between groups were assessed by Bonferroni's test. Nonparametric data were analyzed by Kruskals–Wallis followed by Dunn's test. Differences were considered to be statically significant at a probability level of 5% ($p < 0.05$). Preparation of graphs and statistical analysis was carried out using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

4. Results

4.1. Uterotrophic assay

Regarding the relative weight of the uterus there were no significant differences between the groups that received HETM and vehicle (Fig. 1A). Administration of 17-α-ethinyl estradiol (positive control) significantly increased uterine weight, but coadministration with HETM was unable to block this uterotrophic activity (Fig. 1A and B). On the other hand, coadministration of 17-α-ethinyl estradiol and tamoxifen resulted in a significant reduction in uterine weight when compared to the group receiving only 17-α-ethinyl estradiol.

4.2. Hershberger assay

The data in Fig. 2A–D illustrate the results of Hershberger test. The results demonstrate that the weight of androgen dependent organs (seminal vesicle, prostate, LABC and glans penis) of HETM groups did not differ from vehicle control group. HETM was also unable to block the testosterone-induced growth of these tissues. Anti-androgenic effects were observed only when testosterone-treated animals were co-treated with flutamide, a classic androgen receptor antagonist.

4.3. Effect on the contractility of isolated pregnant rat uterus

The treatments performed with HETM in pregnant female rats from gestational day 8 to 21 did not alter the response of uterine contractility induced by oxytocin or arachidonic acid in all tested doses, indicating lack of modulatory effects of HETM on rat uterus (Fig. 3A and B).

5. Discussion and conclusions

In the last decade, several studies have reported some important biological effects of *Tropaeolum majus* L. On the other hand, few studies have given emphasis on possible toxic effects caused by this species. In this regard, recently was demonstrated that oral administration of up to 5 g/kg of infusion or hydroethanolic extract obtained from the leaves of *Tropaeolum majus* did not cause signs of acute toxicity in normotensive rats (Zanetti et al., 2003; Gasparotto et al., 2009). Thus, the objective of the present work

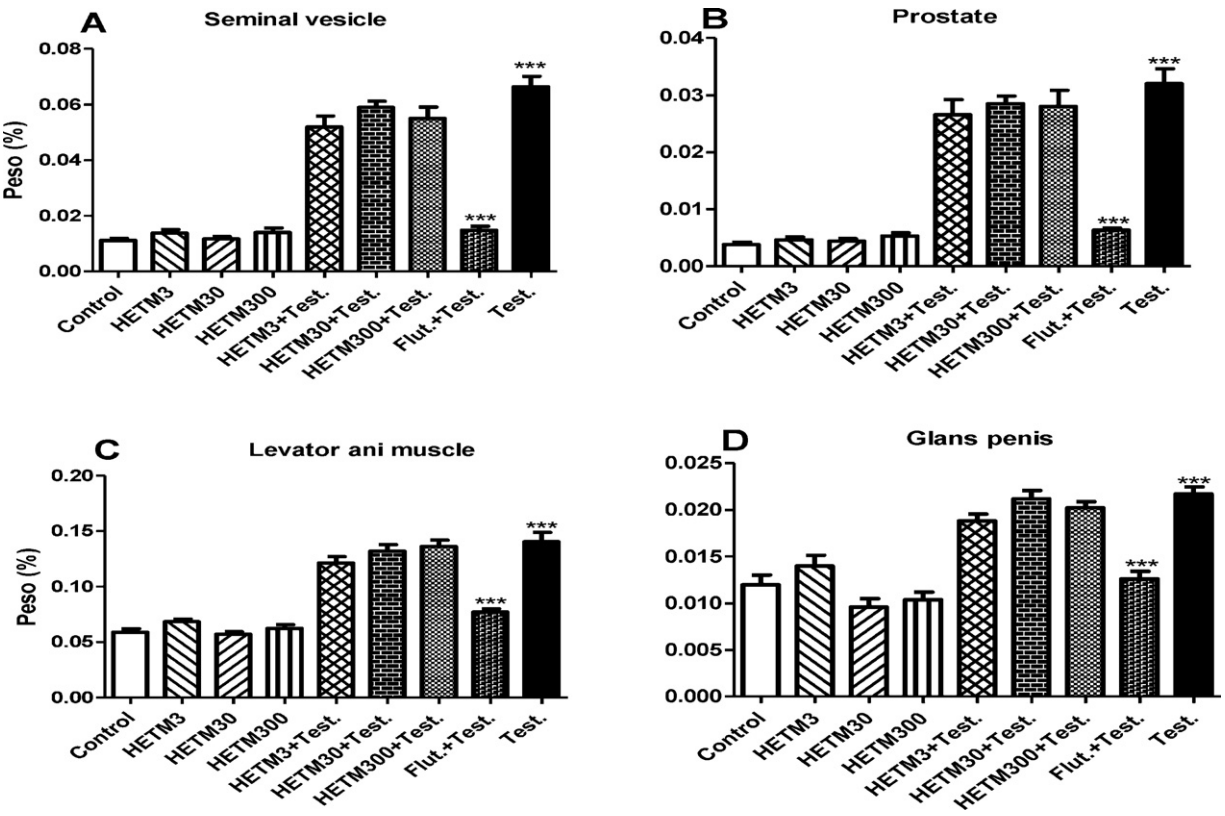

Fig. 2. Test for androgenecity and antiandrogenicity (Hershberger assay). Columns represent mean ± standard error of the seminal vesicle, ventral prostate, levator ani/bulbocavernosus muscle and glans penis relative weights (n = 8–11 animals per group). ***$p < 0.001$ (one-way ANOVA followed by Bonferroni test). Test, testosterone (0.25 mg/kg); Flut, flutamide (10 mg/kg).

was to extend the safety data of this species through investigation of possible (anti)estrogenic and (anti)androgenic activities of the hydroethanolic extract obtained from the leaves of *Tropaeolum majus* L. (HETM), as well as its effect on uterine contractility.

Some studies performed *in vivo* and *in vitro* with *Tropaeolum tuberosus*, a member of the Tropaeolaceae family, showed reductions in testosterone levels, as well as decreased binding of estradiol to its receptor, when the extract obtained from the roots of this species was tested (Johns et al., 1982; Cardenas-Valencia et al., 2008). In addition, root extracts of *Tropaeolum tuberosus* reduced the testicular function of adult rats after 12, 21 and 42 days of treatment (Cardenas-Valencia et al., 2008). On the other hand, benzyl isothiocyanate, a major component *Tropaeolum majus*, *Tropaeolum tuberosus* and cruciferous vegetables, did not change pregnancy outcomes following rat exposure during pre- and post-implantation periods (Adebiyi et al., 2004). Corroborating these findings, the results obtained here did not demonstrate any changes in tissues that are sensitive to the action of sex steroid hormones in males and females rats.

A recent pharmacological and phytochemical study performed with *Tropaeolum majus* L. revealed that flavonoids, particularly isoquercitrin, are major components of the hydroethanolic extract (Gasparotto et al., 2011a,b). Previously, *in vitro* studies performed with flavonoids in lineages of breast cancer MCF-7 cells (hormone

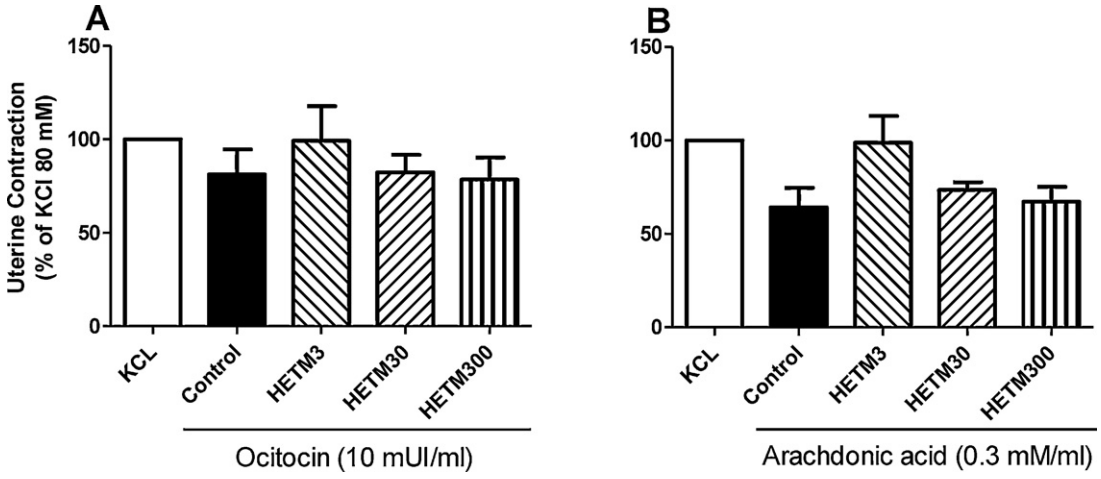

Fig. 3. Effect of the HETM on uterine contractility induced by arachidonic acid (0.3 mM) (A) and oxytocin (10 mUI/ml) (B). Female rats were exposed to HETM from day 8 to 21 of pregnancy. Columns represent mean ± standard error of the percentage relative to KCl response (n = 5 animals per group) (Kruskals–Wallis followed by Dunn's test).

dependent), in the absence or presence of estradiol, revealed estrogenic and antiestrogenic responses, depending on flavonoid and concentration tested (Le Bail et al., 1998). Isoquercitrin, the major flavonoid in the HETM, did not produce a clear dose-dependent curve, but reduced MCF-7 cell proliferation at the highest concentration tested (50 μM) either alone or in the presence of estradiol (Le Bail et al., 1998). More recently, a research performed with the same MCF-7 cells and isoquercitrin confirmed this antiproliferative effect (Yang and Liu, 2009).

In the past years, several *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory potential of isoquercitrin (Fernandez et al., 2005; Rogerio et al., 2007). These studies, associated with the popular use of *Tropaeolum majus* L. leaves to treatment of acute inflammation, make its use in pregnant women an appealing possibility (Rehecho et al., 2011). In the same way, Gasparotto et al. (2009) suggest that the effect of one or more active components of *Tropaeolum majus* partially facilitates the release of renal prostaglandins in male and female rats. This information led us to investigate the possible effects of the HETM on the contractility of isolated pregnant rat uterus. With demonstrated, the data indicate that treatment of pregnant rats with HEMT does not affect the *ex vivo* uterine contractility induced by oxitocin or arachidonic acid.

The uterotrophic assay did not reveal any change in uterine weight following treatment with HETM at 3, 30 and 300 mg/kg/day either isolated or in combination with estradiol, when compared to appropriate controls. These results indicate lack of *in vivo* estrogenic or anti-estrogenic activity of the HETM. At the end of pregnancy there is a rise in estrogen levels and a decline in the amount of progesterone, which ultimately result in upregulation of oxitocin receptors in the uterus, increased production of prostaglandins and increased uterine contractility (Muller et al., 2009). Taken together the results indicate that *Tropaeolum majus* does not modulate estrogen responses *in vivo*, as well as has no obvious influence on uterine contractility.

In addition to absence of effects in the uterotrophic assay, the results with castrated male rats (Hershberger assay) indicate that the HETM is unable to elicit androgenic activities at tested doses. Also, HETM cannot block the effects of testosterone on androgen-sensitive tissues such as prostate, seminal vesicle, glans penis and levator ani/bulbocavernosus muscle. However, it is important to mention that in addition to substances that disturb the normal interaction of hormones and their receptors, adverse effects in the reproductive tract can be elicit by compounds that induce alterations in synthesis, storage, release, transport or excretion of endogenous hormones (US EPA, 1996). Most *in vitro* and *in vivo* assays designed to screen endocrine disruptors, including the uterotrophic and Hershberger assays, are mainly suitable to detect classic receptor-mediated activities and may fail to recognize substances that change endogenous hormone bioavailability to responsive cells (Andrade et al., 2002).

Despite the presence of flavonoids, which have been reported as potential endocrine active compounds, HETM was unable to produce (anti)androgenic or (anti)estrogenic activities in the short-term *in vivo* screening assays performed in the present study. In addition, there was no evidence that HETM can affect uterine contractility following gestational exposure of rats. Additional studies should be performed with *Tropaeolum majus* L. and its major flavonoid, isoquercitrin, to fully determine the absence of reproductive and developmental toxicity.

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References

Adebiyi, A., Adaikan, P.G., Prasad, R.N.V., 2004. Pregnancy outcomes following pre- and post-implantation exposure of Sprague-Dawley rats to benzyl isothiocyanate. Food and Chemical Toxicology 42, 715–720.

Andrade, A.J., Araujo, S., Santana, G.M., Ohi, M., Dalsenter, P.R., 2002. Screening for *in vivo* (anti)estrogenic and (anti)androgenic activities of technical and formulated deltamethrin. Regulatory Toxicology and Pharmacology 35, 379–382.

Ashby, J., Lefevre, P.A., 2000. The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators. Journal of Applied Toxicology 20, 35–47.

Ashby, J., Lefevre, P.A., Odum, J., Tinwell, H., Kennedy, S.J., Beresford, N., Sumpter, J.P., 1997. Failure to confirm estrogenic activity for benzoic acid and clofibrate: implications for lists of endocrine-disrupting agents. Regulatory Toxicology and Pharmacology 26, 96–101.

AVMA (Association American Veterinary Medical), 2007. Guidelines on Euthanasia. Michigan, pp. 1–36.

Binet, L., 1964. A biologist physician in the country. Biology and Medicine Journal 53, 5–28.

Buttar, H.S., Jones, K.L., 2003. What do we know about the reproductive and developmental risks of herbal and alternate remedies? Birth Defects Research Part B: Developmental and Reproductive Toxicology 68, 492–493.

Cardenas-Valencia, I., Nieto, J., Gasco, M., Gonzales, C., Rubio, J., Portella, J., Gonzales, G.F., 2008. *Tropaeolum tuberosum* (Mashua) reduces testicular function: effect of different treatment times. Andrologia 40, 352–357.

Corrêa, A.D., Batista, R.S., Quintas, L.E.M., 2001. Plantas medicinais: do cultivo a terapêutica, 4th ed. Vozes, Petrópolis.

De Medeiros, J.M., Macedo, M., Contancia, J.P., Nguyen, C., Cunningham, G., Miles, D.H., 2000. Antithrombin activity of medicinal plants of the Azores. Journal of Ethnopharmacology 72, 157–165.

Fernandez, J., Reyes, R., Ponce, H., Oropeza, M., Vancalsteren, M.R., Jankowski, C., Campos, M.G., 2005. Isoquercitrin from *Argemone platyceras* inhibits carbachol and leukotriene D4-induced contraction in guinea-pig airways. European Journal Pharmacology 522, 108–115.

Ferreira, R.B.G., Vieira, M.C., Zárete, N.A.H., 2004. Análise de crescimento de *Tropaeolum majus* 'jewel' em função de espaçamentos entre plantas. Revista Brasileira de Plantas Medicinais 7, 57–66.

Ferro, D., 2006. Fitoterapia: conceitos clínicos. Atheneu, São Paulo.

Gadano, A.B., Gurni, A.A., Carballo, M.A., 2006. Argentine folk medicine: genotoxic effects of Chenopodiaceae family. Journal of Ethnopharmacology 103, 246–251.

Gasparotto Jr., A., Boffo, M.A., Lourenco, E.L., Stefanello, M.E., Kassuya, C.A., Marques, M.C., 2009. Natriuretic and diuretic effects of *Tropaeolum majus* (Tropaeolaceae) in rats. Journal of Ethnopharmacology 122, 517–522.

Gasparotto Jr., A., Gasparotto, F.M., Boffo, M.A., Lourenco, E.L., Stefanello, M.E., Salvador, M.J., da Silva-Santos, J.E., Marques, M.C., Kassuya, C.A., 2011a. Diuretic and potassium-sparing effect of isoquercitrin—an active flavonoid of *Tropaeolum majus* L. Journal of Ethnopharmacology 134, 210–215.

Gasparotto Jr., A., Gasparotto, F.M., Lourenco, E.L., Crestani, S., Stefanello, M.E., Salvador, M.J., da Silva-Santos, J.E., Marques, M.C., Kassuya, C.A., 2011b. Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.: evidence for the inhibition of angiotensin converting enzyme. Journal of Ethnopharmacology 134, 363–372.

Griffiths, D.W., Deighton, N., Birch, A.N., Patrian, B., Baur, R., Stadler, E., 2001. Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. Phytochemistry 57, 693–700.

Gupta, D., Bleakley, B., Gupta, R.K., 2008. Dragon's blood: botany, chemistry and therapeutic uses. Journal of Ethnopharmacology 115, 361–380.

Johns, T., Kitts, W.D., Newsome, F., Towers, G.H., 1982. Anti-reproductive and other medicinal effects of *Tropaeolum tuberosum*. Journal of Ethnopharmacology 5, 149–161.

Jurgens, T.M., 2003. Potential toxicities of herbal therapies in the developing fetus. Birth Defects Research Part B: Developmental and Reproductive Toxicology 68, 496–498.

Kleinwachter, M., Schnug, E., Selmar, D., 2008. The glucosinolate-myrosinase system in nasturtium (*Tropaeolum majus* L.): variability of biochemical parameters and screening for clones feasible for pharmaceutical utilization. Journal of Agricultural and Food Chemistry 56, 11165–11170.

Le Bail, J.C., Varnat, F., Nicolas, J.C., Habrioux, G., 1998. Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids. Cancer Letters 130, 209–216.

Ling, S., Nheu, L., Dai, A., Guo, Z., Komesaroff, P., 2008. Effects of four medicinal herbs on human vascular endothelial cells in culture. International Journal of Cardiology 128, 350–358.

Lorenzi, H., Matos, F.J.A., 2002. Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum, São Paulo.

Lykkesfeldt, J., Moller, B.L., 1993. Synthesis of benzylglucosinolate in *Tropaeolum majus* L. (isothiocyanates as potent enzyme inhibitors). Plant Physiology 102, 609–613.

Mietkiewska, E., Giblin, E.M., Wang, S., Barton, D.L., Dirpaul, J., Brost, J.M., Katavic, V., Taylor, D.C., 2004. Seed-specific heterologous expression of a nasturtium FAE gene in *Arabidopsis* results in a dramatic increase in the proportion of erucic acid. Plant Physiology 136, 2665–2675.

Muller, J.C., Botelho, G.G., Bufalo, A.C., Boareto, A.C., Rattmann, Y.D., Martins, E.S., Cabrini, D.A., Otuki, M.F., Dalsenter, P.R., 2009. *Morinda citrifolia* Linn (Noni): *in vivo* and *in vitro* reproductive toxicology. Journal of Ethnopharmacology 121, 229–233.

Odum, J., Lefevre, P.A., Tittensor, S., Paton, D., Routledge, E.J., Beresford, N.A., Sumpter, J.P., Ashby, J., 1997. The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Regulatory Toxicology and Pharmacology 25, 176–188.

Picciarelli, P., Alpi, A., 1987. Embryo-suspensor of *Tropaeolum majus*: identification of gibberellin A_{63}. Phytochemistry 26, 329–330.

Picciarelli, P., Alpi, A., Pistelli, L., Scalet, M., 1984. Gibberellin-like activity in suspensors of *Tropaeolum majus* L. and *Cytisus laburnum* L. Planta 162, 566–568.

Pintão, A.M., Pais, M.S., Coley, H., Kelland, L.R., Judson, I.R., 1995. In vitro and *in vivo* antitumor activity of benzyl isothiocyanate: a natural product from *Tropaeolum majus*. Planta Medica 61, 233–236.

Rehecho, S., Uriarte-Pueyo, I., Calvo, J., Vivas, L.A., Calvo, M.I., 2011. Ethnopharmacological survey of medicinal plants in Nor-Yauyos, a part of the Landscape Reserve Nor-Yauyos-Cochas, Peru. Journal of Ethnopharmacology 133, 75–85.

Rogerio, A.P., Kanashiro, A., Fontanari, C., da Silva, E.V., Lucisano-Valim, Y.M., Soares, E.G., Faccioli, L.H., 2007. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. Inflammation Research 56, 402–408.

US EPA (United States Environmental Protection Agency), 1996. Guidelines for reproductive toxicity risk assessment. EPA/630/R-96/009, Washington, pp. 1–163.

Wielanek, M., Urbanek, H., 2006. Enhanced glucotropaeolin production in hairy root cultures of *Tropaeolum majus* L. by combining elicitation and precursor feeding. Plant Cell, Tissue and Organ Culture 86, 177–186.

Wu, K.M., Ghantous, H., Birnkrant, D.B., 2008. Current regulatory toxicology perspectives on the development of herbal medicines to prescription drug products in the United States. Food and Chemical Toxicology 46, 2606–2610.

Yamada, T., Sunami, O., Kunimatsu, T., Kamita, Y., Okuno, Y., Seki, T., Nakatsuka, I., Matsuo, M., 2001. Dissection and weighing of accessory sex glands after formalin fixation, and a 5-day assay using young mature rats are reliable and feasible in the Hershberger assay. Toxicology 162, 103–119.

Yang, J., Liu, R.H., 2009. Synergistic effect of apple extracts and quercetin 3-beta-d-glucoside combination on antiproliferative activity in MCF-7 human breast cancer cells *in vitro*. Journal of Agricultural and Food Chemistry 57, 8581–8586.

Zanetti, G.D., Manfron, M.P., Hoelzel, S.C.S.M., Pagliarin, V.P., Morel, A.F., 2003. Toxicidade aguda e atividade antibacteriana dos extratos de *Tropaeolum majus* L. Acta Farmaceutica Bonaerense 22, 159–162.

Zanetti, G.D., Manfron, M.P., Hoelzel, S.C.S., 2004. Análise morfo-anatômica de *Tropaeolum majus* L. (Tropaeolaceae). IHERINGIA Série Botânica 59, 173–178.