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Herbs of interest to the Brazilian Federal Government: female reproductive and developmental toxicity studies

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Abstract: In 2009 the Brazilian Ministry of Health published a document named RENISUS that lists 71 herbs traditionally used in Brazil that could result in phytomedicines to be dispensed by the governmental health care program. This manuscript reviews female reproductive and/or developmental toxicity information of these herbs. More than half (35) of the herbs lack information regarding female reproductive and/or developmental effects. From the fourteen herbs used traditionally to disturb female reproduction, five present experimental data corroborating their actions as abortifacients (*Maytenus ilicifolia, Momordica charantia, Plectranthus barbatus, Ruta graveolens*) or labour facilitator (*Bidens pilosa*). For 23 of the herbs evaluated experimentally for any type of female reproductive endpoint, only a single study was retrieved and at least twelve of these studies were conducted with a single dose. This scenario suggests that the scientific power of the published information is very low and that a scientifically-based risk/benefit analysis about the use of these herbs during pregnancy is not possible. Considering the appeal that phytomedicines have for pregnant women, usually aware and afraid of the risks that synthetic drugs may have in their pregnancy and progeny, well designed studies evaluating reproductive and/or developmental toxicity of these herbs urge.

Review

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Introduction

In 2006, the Federal Government from Brazil announced the National Politics of Herbal Products and Phytomedicines that would result in actions to warranty a safe and rational use of herbal products by the Brazilian population (Brasil, 2006). Since then, documents have been published in order to accomplish the program goals. Among these documents is RENISUS, a list of 71 herbal species and/or genus traditionally used in Brazil and that the Ministry of Health considers that could result in phytomedicines to be prescribed and dispensed by the governmental health program (Sistema Único de Saúde-SUS) (Ministério da Saúde, 2009). Many of those herbs lack scientific pharmacological validation for their use as well as toxicological evaluation but the publication of RENISUS should trigger public and private research to fill this lack of data. Supplemental Table 1 presents the species and their taxonomy whereas the common names in English and Portuguese can be found in Supplemental Table 2.

Due to the widespread use of herbal medicinal products (HMP) worldwide, governments from different countries have been regulating their marketing. In Brazil, phytomedicines are regulated as drugs but the pre-marketing studies necessary for registration are different from conventional drugs. For instance, reproductive and carcinogenic studies are not mandatory for registration.

HMP use during pregnancy has been reported at 4-55% (Nordeng & Havnen, 2005; Refuerzo et al., 2005; Tiran, 2003) not considering abortifacient use. Besides abortifacient use, which should not be underestimated especially in countries where abortion is forbidden by law (*e.g.*, Brazil), there are two other situations in which pregnant women might be exposed to HMP: i) substitution of prescribed medications by HMP generally perceived as "natural and safe"; ii) unintended or accidental use since many pregnancies are unplanned and women of childbearing age use HMP for a variety of disorders, such as disphoric premenstrual disorder, depression, anxiety, sleep aid as well as weight loss. Despite the prevalent use of HMP by pregnant women, information regarding their safety on this population is sparse. In most of the international reference monographs for HMP as well as in the physician prescribing information of phytomedicines marketed in Brazil, HMP can be broadly categorized into two groups. In the first group are HMP contra-indicated during pregnancy based on reports of historical use of

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herbs as abortifacients or uterotonics. In the second group are HMP that have no data regarding safety of use during pregnancy and whose use are not recommended during these periods without medical advice. Advising without any scientific support certainly is not an easy task for physicians and other health professionals.

In this work we reviewed the scientific literature as well as some reference monographs in order to update and summarize the knowledge regarding female reproductive and/or developmental toxicity of 69 out of the 71 herbal species listed in RENISUS. *Glycine max* (soy) was not included due to the wide knowledge on its properties as well as *Copaifera* spp. because there are multiple species in Brazil and the species of interest have not been defined in RENISUS. For clarification purposes, in this study, the term "female reproductive toxicity" refers to any effect that would interfere with reproductive ability or capacity whereas the term "developmental toxicity" refers to adverse effects resulting from maternal exposure that can be manifested at any point in the life span of the litter. The readers should be aware that his review is intended to provide a general overview about the existing scientific data (or lack of it). It is beyond the scope to judge the adequacy of the retrieved studies for regulatory purposes. In this way, all the retrieved studies are being reported; independently of study desing weakness, plant material or type of extraction employed.

Methods

Literature search

We scanned articles through the website from Biblioteca Virtual em Saúde (Virtual Health Library, http://regional.bvsalud.org/php/index.php) using the Latin name of each herb listed in RENISUS. This website allows the simultaneous search in thirty electronic bibliographic databases including Medline, Lilacs, IBECS, Cochrane, SciELO, PAHO, WHOLIS as well as some databases of dissertations and thesis developed in Brazil. No language restriction was applied. Searching by hand for published data was also conducted through: 1) the reference lists of identified articles; 2) The European Medicines Agency (EMA) website (http://www.europa.ema.eu); 2) ESCOP Monographs (Escop, 2003); 3) The WHO monographs on selected medicinal plants (WHO, 1999; 2002; 2007; 2009). The final internet search of the literature was conducted in January 17th, 2011.

Evaluation of the data retrieved

To filter out toxicological studies, two independent observers (Moreira EG and Bacchi AD) reviewed the titles, abstracts and keywords of every record retrieved after the search by the Latin name of each herb. The toxicological studies that reported data regarding female reproductive and/or developmental endpoints were carefully evaluated and summary tables were constructed for each herb containing plant material, animal, exposure regimen (route, dose, period of treatment) and effects observed.

Results

Herbs lacking female reproductive and/or developmental toxicity studies

In the current review, studies or traditional information regarding female reproductive and/or developmental toxicity could not be retrieved for 35 out of 69 herbs listed in RENISUS. For fifteen out of these 35 herbs, no toxicological information was retrieved (*Arrabidaea chica, Costus* spp., *Eleutherine plicata, Erythrina mulungu, Lamium album, Malva sylvestris, Orbignya speciosa, Petroselinum sativum, Polygonum* spp., *Portulaca pilosa, Solidago microglossa, Syzygium cumini, Tagetes minuta, Trifolium pretense, Vernonia* spp.) whereas for twenty of them at least one study evaluating acute oral toxicity, genetic toxicity and/or suchronic toxicity was available (*Alpinia speciosa, Anacardium occidentale, Caesalpinia ferrea, Casearia sylvestris, Chenopodium ambrosioides, Croton spp., Cynara scolymus, Equisetum arvense, Eugenia uniflora, Jatropha gossypiifolia, Kalanchoe pinnata, Lippia sidoides, Mentha piperita, Mentha pulegium, Mikania glomerata, Morus* spp., *Persea americana, Psidium guajava, Schinus terebinthifolius, Solanum paniculatum*).

Despite the lack of toxicological studies for *T. pratenses*, WHO monograph contra-indicates its use during pregnancy and breastfeeding due to the potential estrogenic effects of the crude extract (WHO, 2009).

Herbs with traditional information or experimental evaluation of female reproductive and/or developmental toxicity

Table 1 lists fourteen herbs from RENISUS that presented published reports about their traditional use for female reproductive effects. These effects include: emmenagogue, contraceptive, abortifacient, and labour induction.

Experimental studies evaluating reproductive and/or developmental toxicity were retrieved for 28 herbs listed in RENISUS and they are summarized in Tables 2-4. Unfortunately we could not have full access to six out of 31 studies (indicated in Tables 2 and 4) and we are reporting information extracted from WHO and

EMA monographs, ESCOP, or paper abstracts.

From the fourteen herbs with traditional use (Table 1), five of them have experimental studies corroborating their actions. *Maytenus ilicifolia, Momordica charantia, Plectranthus barbatus* and *Ruta graveolens* are used as emmenagogues and abortifacients and there is at least one experimental study reporting pre-implantation loss in rodents treated with these plants or, in the case of *Mormodica charantia*, isolated proteins (references listed in Tables 2 and 3). *Bidens pilosa*, used traditionally to facilitate labour, has shown in vitro uterotonic effect (Table 2). Even though there is one experimental study conducted with *Phyllanthus amarus* (Rao & Alice, 2001), one with *Curcuma longa* (Garg, 1974), and one with *Achillea millefolium* (Boswell-Ruys et al., 2003), the reported endpoints are not directly related to their traditional use. Rao & Alice (2001) designed their study to evaluate estrous ciclicity and not embryo/fetal loss that is the *P. amarus* traditional use. *Curcuma longa*, used as contraceptive, did not present antiovulatory effect in rabbits but induced pre and postimplantation loss in rats (Garg, 1974). *Achillea millefolium*, used as emmenagogue and abortifacient did not induce pre or postimplantation loss in rats but decreased fetal weight (Boswell-Ruys et al., 2003). Noteworthy is the lack of experimental studies with four species that have traditional use for inducing female reproductive effects: *Ananas comosus, Foeniculum vulgare, Justicia pectoralis* and *Uncaria tomentosa*. Actually, for *Foeniculum vulgar*e there are some alerts in the literature. WHO monograph (2007) reports that it should not be used in pregnancy due to the potential estrogenic effects of essential oil from the seeds and ESCOP (2003) highlights that for aqueous infusions estrogenicity would not be of concern. However, a study published in 2008 reported the case of four patients with premature thelarche associated with long term use of *Foeniculum vulgare* tea to eliminate gas pain (Turkyilmaz et al., 2008). In this way, estrogenicity of *Foeniculum vulgare* is certainly an issue that deserves further investigation.

For 23 out of the 28 herbs that have been evaluated experimentally for any type of female reproductive and/or developmental endpoint only a single study was retrieved (Tables 2 and 4). Broadly, the results could be divided into two major groups: the herbs that have presented reproductive adverse effect and the herbs that have not. The first group (Tables 2 and 3) can be subdivided into herbs that induced maternal toxicity (*Baccharis trimera*), anti-implantation effect (*Artemisia absinthium, Curcuma longa, Maytenus ilicifolia, Mormodica charantia, Plectranthus barbatus, Punica granatum, Ruta graveolens*); embryo/fetotoxicity (*Achillea millefolium, Curcuma longa, Ruta graveolens, Stryphnodendron adstringens, Tabebuia avellanedae*); teratogenicity (*Momordica charantia, Tabebuia avellanedae*); uterotonic effect (*Bidens pilosa, Passiflora incarnata, Harpagophytum procumbens*) and altered estrous ciclicity (*Phyllanthus amarus*). In the second group (*i.e*., herbs that did not present reproductive adverse effect) are: *Aloe vera, Bauhinia forficata, Calendula officinalis, Carapa guianensis, Chamomilla recutita, Cordia verbenacea, Dalbergia subcymosa, Eucalyptus globulus, Ocimum suave* (synonym for *O. gratissimum*), *Rhamnus purshiana, Salix alba* and *Vernonia condensat*a (Table 4).

Table 1. Herbs listed in RENISUS with published traditional use for reproductive effects.

Traditional use	Herbs	Reference
Emmenagogue	*Achillea millefolium*	WHO, 2009
	Foeniculum vulgare (essential oil)	WHO, 2007
	Maytenus ilicifolia	Montanari & Bevilacqua, 2002
	Momordica charantia	Gover & Yadav, 2004; WHO, 2009
	Plectranthus barbatus	Almeida & Lemonica, 2000
	Ruta graveolens	De Lazlo & Henshaw, 1954
	Uncaria tomentosa	WHO, 2007
Contraceptive	*Curcuma longa*	Garg, 1974
	Maytenus ilicifolia	Montanari & Bevilacqua, 2002
	Momordica charantia	Gover & Yadav, 2004; WHO, 2009
	Plantago major	Samuelsen, 2000
Abortifacient	*Achillea millefolium*	Boswell-Ruys et al., 2003
	Ananas comosus	Pakrashi & Basak, 1976
	Justicia pectoralis	Lans, 2007
	Maytenus ilicifolia	Montanari & Bevilacqua, 2002
	Momordica charantia	Gover & Yadav, 2004; WHO, 2009
	Phyllantus amarus	Singh & Gray, 2001
	Plantago major	Samuelsen, 2000
	Plectranthus barbatus	Almeida & Lemonica, 2000
	Ruta graveolens	De Lazlo & Henshaw, 1954
Labour induction	*Bidens pilosa*	Frida et al., 2008
	Harpagophytum procumbens	Mahomed & Ojewole, 2009

It should be noted that the studies being reported for *Mormodica charantia, Rhamnus purshiana* and *Tabebuia avellanedae* were conducted with isolated compounds. Even though these studies would not be appropriate to make extrapolations to the traditional human use, they may serve as alerts on potential adverse effects. In fact, WHO monograph on *Momordica charantia* considered the studies conducted with momorcharins to describe a precautionary alert for teratogenicity (WHO, 2009).

Our review on *Zingiber officinale* resulted in puzzling data. Due to its use by pregnant women for morning sickness, there are at least two clinical trials that have tested ginger during pregnancy. Reproductive

endpoints were assessed and no adverse effect was detected (Fischer-Rasmussen et al., 1991; Portnoi et al., 2003). In experimental studies with rodents, maternal toxicity was not detected either but embryonic loss was observed (Wilkinson, 2000). Certainly human data should overweight rodents' data but there are concerns regarding the full adequacy of clinical trials with HMP since they are conducted with reduced number of individuals and adverse events are evaluated through self-reports. This discussion is beyond the scope of this paper but readers can have a general idea about different points of views regarding the safety of ginger in the literature (for example, Fugh-Berman et al., 2005; Marcus & Snodgrass, 2005).

Finally, for *Allium sativum* no reproductive and/or developmental study was retrieved but both ESCOP (2003) and WHO monograph (1999) report that there are no objections to use during pregnancy and lactation because neither long term nutritional experience nor any other important circumstances give reason for suspicion.

Discussion and Conclusions

In this work, studies and reference monographs were reviewed looking for information about female reproductive and/or developmental toxicity of herbs listed by the Brazilian government as potential phytomedicines to be prescribed and dispensed by the governamental health care program. Few of these herbs are already registered for marketing in Brazil and most of them are classified as category C for pregnancy risks by the Brazilian sanitary surveillance agency (Anvisa) following the classification previously adopted by the Food and Drug Administration (FDA) (Doering et al., 2002). Category C is assigned to drugs with positive or inadequate animal teratology studies that are lacking well-controlled human studies. In this way, the physician prescribing information of the marketed species attributes to the physician the decision of prescribing them to pregnant women which is certainly not an easy task with the available information. This work aimed to summarize female reproductive and/or developmental toxicity information available for all the herbs listed in RENISUS in order to sense the real scenario regarding this type of information.

In the present review, no information on reproductive and/or developmental effects have been retrieved for more than half of the herbs listed in RENISUS, *i.e.*, for 35 out of 69. Per se this result would already be disappointing but to make things worse, for the herbs with published studies, only for five more than one study was available and for at least twelve studies were conducted with single doses. Moreover, the majority of the studies were conducted with lower number of animals in each experimental group than the recommended by international guidelines such as OECD (Organization for Economics Cooperation and Development) and ICH (International Conference on Harmonisation of Technical Requirements for Registraion of Pharmaceuticals for Human Use). With this scenario, a deep analysis of study design and quality is certainly not necessary to assume that the scientific power of the published information is very low and that a scientifically-based risk/benefit analysis about the use of these herbs during pregnancy is not possible at this time. Based on the precautionary principle, these herbs should be avoided during pregnancy due to the lack of data. However, considering the appeal that "the natural and safe" HMP have for special population including pregnant women, studies evaluating reproductive and/or developmental toxicity urge in order to refine the risk evaluation and communication. Prioritization of species to be evaluated could take into account information on genetic toxicity as well as endocrine and pro-apoptosis actions, which are known to be involved in the disruption of pregnancy course and/or embryo/fetal development. Studies evaluating these endpoints are more usual in the literature. It seems reasonable to suggest that herbs with central nervous system action should also be prioritized due to their ability to cross barriers (blood-brain and blood-placental) and reach the embryo or fetus. Additionaly, developmental neurotoxicity endpoints should also be included when evaluating herbs that target the nervous system. The developing central nervous system is especially susceptible to toxic insults and it is known that functional changes can be induced at a lower exposure level than those resulting in structural teratogenicity (for one example, Francis et al., 1990).

After reviewing the literature, it seems that the only species that we could deposit more weight on the available information are *Maytenus ilicifolia, Momordica charantia, Plectranthus barbatus, Ruta graveolens* and *Bidens pilosa.* These species have reported traditional use as emmenagogues and abortifacients (*Maytenus ilicifolia, Momordica charantia, Plectranthus barbatus, Ruta graveolens*) or labour facilitator (*Bidens pilosa*) supported by experimental evidences of these actions. However, it should be noted that the weight of the experimental evidences is weak due to experimental design issues or lack of studies replication.

Two observations are noteworthy. The first one was the high number of experimental studies performed without taking into account any guideline for toxicological evaluation, such as those published by ICH or OECD. As a result, not only there is lack of harmonization among studies as well as there are some basic biases regarding experimental design (number of replicates, experimental unit, route of exposure and period of treatment) that could have been avoided if a guideline had been followed. It was surprising to realize

that most of the LD50 studies retrieved (references not included in this review) employed an unnecessary high number of animals, a situation that could be avoided if OECD guidelines (OECD, 2001a, 2001b, 2001c) had been followed. These guidelines are a scientific advance for LD50 estimation but it seems that few researchers from the HMP area are employing them. The second observation to be pointed out is the lack of information regarding extract standardization. It is common sense the variation in active principles that different extracts may have and efforts should be taken to present at least a qualitative chromatogram of the extract for which toxicological results are being reported.

In summary, the present review summarizes the available information regarding female reproductive and/or developmental toxicity of the herbs listed in RENISUS and indicates the paucity of studies in this area. Considering the appeal that phytomedicines have

Table 2. Herbs listed in RENISUS with only one experimental study of female reproductive and/or developmental toxicity retrieved and that presented positive results.

Herb	Material	Animal	Treatment	Findings	Reference
Achillea millefolium	Aerial parts but flowers extracted in 45% ethanol and diluted with water to 20% w/v	Rats	Oral; GD1-GD8 or GD8-GD15; 2.8 g/kg; n=5	Increased placental weight (GD1-GD8). Increased placental weight and decreased fetal weight (GD8-GD15). No pre or postimplantation loss, no teratogenicity for both treatment periods	Boswell-Ruys et al., 2003
Artemisia absinthium	50% dry ethanolic extract	Rats	Oral; 200 mg/kg for 7 days	66% reduction in the number of pregnancy	Escop, 2003a
Baccharis trimera	Aerial parts 70% dry ethanolic extract	Rats	Oral; GD1-GD19 (n=12) or GD6-GD15 (n=11); 8.4 mg/kg;	GD1-GD19: decreased corrected body weight; increased blood urea and kidney weight; histopathological alterations in kidneys and liver; no hematological effect GD6-GD15: no alteration detected	Grance et al., 2008
Bidens pilosa	Leaves aqueous and 95% dry ethanolic extracts	Mice Rats	Subcutaneous; 3 days; 0.25, 0,5; 1; 2; 4 or 8 mg/g; n=6 Incubation of primed-oestrogenized uterine horns from virgin rats with 0.03, 0.09, 0.22, 0.47, 0.97; 1.97 or 3.97 mg/mL; n=not informed	Estrogenic in the uterotrophic bioassay (ethanolic>aqueous) Uterotonic/oxytocic-like effect *in vitro* (aqueous>ethanolic)	Frida et al., 2008
Curcuma longa	Rhizoma dry petroleum ether, 95% dry alcoholic and aqueous extracts	Rats Rabbits	Gavage; GD1-GD7; 100 or 200 mg/kg; n=10 Gavage, 3 days, 100 or 200 mg/kg, n=5	Pre and post-implantation loss with both doses from all the extracts evaluated No anti-ovulatory activity	Garg, 1974
Harpagophytum procumbens	Roots aqueous extract	Wistar rats	Incubation of uterine horns from pregnant and non-pregnant rats with 10-800 µg/mL; n=6-8	Uterotonic dose-dependent effect	Mahomed & Ojewole, 2009
Passiflora incarnata	Active principle as a mercury derivative	Guinea pigs and rabbits	Incubation of uterine horns from virgin animals with 3% of an aqueous solution containing the mercury derivative; n=3	Stimulation of uterine contractions	Ruggy & Smith, 1940
Phyllanthus amarus	Whole plant 70% dry hydroalcoholic extract	Swiss mice	Oral (food); 100 mg/kg for 30 days; n=10-12	Irregular estrous cyclicity. Dominance of dioestrous phase	Rao & Alice, 2001
Plectranthus barbatus (Coleus barbatus)	Leaves 70% hydroalcoholic extract (alcohol evaporated)	Wistar rats	Gavage; GD0-5 or GD6-15; 220, 440 or 880 mg/kg; n=10-12	No maternal toxicity. The highest dose increased preimplantation loss, number of skeletal variations and reduced number of fetal ossification centers	Almeida & Lemonica, 2000
Punica granatum	Aqueous, methanolic extracts	Rats	Not available	Pregnancy inhibition in 70-90% of rats	Prakash et al., 1985[a]
Stryphnodendron adstringens	Seeds 2:1 hydroalcoholic extract (alcohol evaporated)	Wistar rats	Gavage; GD1-GD7; 1 mL/100 g; number of animals not informed	Decreased live fetuses and uterus weight without affecting implantation	Burger et al., 1999

[a]Incomplete information because the complete original study could not be retrieved; GD: gestational day; n: number of animals per group.

for pregnant women, usually aware and afraid of the risks that synthetic drugs may have in their pregnancy and progeny, well designed studies evaluating female reproductive and/or developmental toxicity of these herbs urge if they may become phytomedicines.

Table 3. Herbs listed in RENISUS with more than one experimental study evaluating female reproductive and/or developmental toxicity retrieved and that presented positive results.

Herb	Material	Animal	Treatment	Findings	Reference
Maytenus spp. (M.aquifolium or M. ilicifolia)	*M. ilicifolia* leaves dry 70% ethanolic extract	CF1 mice	Gavage; GD1-3 or GD4-6 or GD7-9; 1000 mg/kg; n=10	No maternal toxicity. Embryonic loss before implantation. No resorptions or malformations	Montanari & Bevilacqua, 2002
			Gavage; 3 days; 1000 mg/kg	Estrogenic in the uterotrophic bioassay	
	M. aquifolium and *M. ilicifolia* leaves mixture aqueous extract	Wistar rats	Oral; 30 days; 272 or 544 mg/kg; n=5	No effect on estrous ciclicity.	Oliveira et al., 1991
			Oral; 45 days prior to mating; 272 or 544 mg/kg; n=10	No effect on the course of pregnancy. No external malformations.	
			Oral; GD1 until delivery; 136 or 272 mg/kg; n=10	272 mg/kg decreased the weight of pups at birth but it recovered from PND7. No alterations in pups development (righthing reflex, ambulation, eye-opening) as well as general activity and learning when adults	
Momordica charantia	Protein extracted from the seeds (momorcharin)	Mice	Intraperitoneal; GD 4, 6, 8 or 12; 0.4, 0.8 or 2 mg/kg; n=15-30	Early and midterm abortions.	Chan et al., 1984
		Mice embryos	Incubation at the early organogenesis stage; 50 or 100 µg/mL; n=21-31	Morphological abnormalities observed in the head, trunk and limbs.	Chan et al., 1986
Ruta graveolens	Aerial parts dry 70% ethanolic extract	CF1 mice	Gavage; GD1-GD3 or GD4-GD6 or GD7-GD19; 1000 mg/kg; n=10	No maternal toxicity, preimplantation loss or resorptions. Fetal death in one treated female.	Freitas et al., 2005
			Gavage; 3 days; 1000 mg/kg; n=10	No estrogenicity in the uterotrophic bioassay	
	Aerial parts suspension or aqueous extract	Rats	Gavage; GD1-GD10; 4-8 g/kg; n=10	Dose-related maternal weight loss for suspensions but not for aqueous extract. Pre-implantation loss with all the doses evaluated	Gandhi et al., 1991
		Hamsters	Gavage; GD1-GD8; 8-20 g/kg; n=10		
	Leaves aqueous extract	Swiss mice	Oral (drinking water); GD0-GD4; 10 mL of 5%, 10% or 20% extract; n=5-6	Dose-dependent alteration of normal blastocyst formation	Gutierrez-Pajares et al., 2003
Tabebuia avellanedae	Lapachol, a naphthoquinone isolated from the bark	Wistar rats	Intraperitoneal; GD1-GD12; 40, 80 or 160 mg/kg; n=10	All doses decreased maternal weight gain which may be responsible for the embryotoxicity and teratogenicity observed	Almeida et al., 2009
		Wistar rats	Gavage; GD8-GD12; 20 mg/rat; n=9-14	No maternal toxicity but 100% resorptions	Guerra et al., 1999
Zingiber officinale	Standardized ethanolic extract	Wistar rats	Gavage; GD6-GD15; 100, 333 or 1000 mg/kg; n=22	No maternal toxicity. No pre and postimplantation losses. Ossification may have been altered	Weidner & Sigwart, 2001
	Ginger tea	Sprague-Dawley rats	Oral (drinking water); GD6-GD15; 20 or 50 g/L; n=13-16	No maternal toxicity. Increased proportion of resorption sites but no difference in the number of litters with resorptions. No external malformations. Increased fetal growth	Wilkinson, 2000

GD: gestational day; n: number of animals per group.

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Table 4. Herbs listed in RENISUS with negative results in studies of female reproductive and/or developmental toxicity evaluation.

Herb	Material	Animal	Treatment	Findings	Reference
Aloe vera or barbadensis	Aloe extract	Rats	Oral; 1000 mg/kg	No teratogenicity or fetotoxicity	WHO, 1999a, EMA, 2006
Bauhinia forficata	Leaves aqueous extract	Wistar rats	Gavage; GD0-GD20 with increasing doses (500-1000 mg/kg); n=13	No effect on implantation. No embryo/fetotoxicity or teratogenicity	Volpato et al., 2008
Calendula officinalis	Flowers dry 70% hydroalcoholic extract	Wistar rats	Gavage; GD1-GD6 (n=9-11) or GD7-GD14 (n=6-8) or GD15-19 (n=6-8); 0.25, 0.5, 1.0 g/kg	No maternal toxicity except for weight reduction after treatment with 1 g/kg from GD15-19. No pre or postimplantation loss. No external malformation	Silva et al., 2009
Carapa guianensis	Seeds dry hexanic extract	Wistar rats	Oral; GD7-GD14; 0.375, 0.75, 1.5 or 3.0 g/kg; n=5-9	No maternal toxicity. No embryo/fetotoxicity or external malformation	Costa-Silva et al., 2007
Chamomilla recutita (Matricaria chamomilla, Matricaria recutita)	Not available	Not available	Not available	No adverse effects reported *in vivo*	WHO, 1999a
Cordia verbenacea	Leaves dry 70% ethanolic extract	Wistar rats	Gavage: - 30 days; 1.24 or 7.44 mg/kg to evaluate estrous cycle; n=10 - 45 days prior to mating; 1.24 or 7.44 mg/kg to evaluate fertility impairment; n=8-11 - during pregnancy; 1.24 or 7.44 mg/kg to evaluate fetal development; n=8-11	No effects in estrous cycle. No maternal toxicity, embryo/fetotoxicity or teratogenicity. No influence on sexual maturation or fertility of the offspring	Sertié et al., 2005
Dalbergia subcymosa	Stem bark decoction	Wistar rats	Gavage; GD6-15; 40 mg twice a day; number of litters not informed	No maternal toxicity, embryo/fetotoxicity and no influence on pups physical development	Peters & Guerra, 1995
Eucalyptus globulus	Leaves essential oil	Mice	Subcutaneous; GD6-15; 135 mg/kg	No teratogenicity	WHO, 2002
Ocimum gratissimum	Leaves aqueous extract	Rats	Oral; GD1-GD14; 500 and 1000 mg/kg, n=5	No embryo/fetotoxicity and no teratogenicity	Tan et al., 2008
Rhamnus purshiana	Aloin A	Rats	200 mg/kg	No embryo/fetotoxicity or teratogenicity.	WHO, 2002a
Salix alba	Bark 40% ethanolic extract	Rats and rabbits	1.6 mL/kg	No disruption of estrous cycle, ovulation or fertility No teratogenicity or embryotoxicity	WHO, 2002a
Vernonia condensata	Leaves aqueous extract		Gavage; GD10-12; 500 or 2000 mg/kg , n=18-23	No maternal toxicity or teratogenicity. The highest dose slightly retarded fetal growth	Monteiro et al., 2001

[a] Incomplete information because the complete original study could not be retrieved; GD: gestational day; n: number of animals per group.

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