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Methanol and water extracts of *Withania somnifera* roots has no abortifacient effect in rats

I. Ilayperuma[1] W.D. Ratnasooriya[2] and T.R. Weerasooriya[1]
[1]Department of Anatomy, Faculty of Medicine, University of Ruhuna, Sri Lanka.
[2]Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka.

Correspondence to:

Prof W. D. Ratnasooriya
Department of Zoology, University of Colombo, Colombo-03, Sri Lanka.
Tel +94-1-503399
e-mail: wdrratna(@webmail.cmb.ac.lk

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Abstract

The aim of this study was to determine the effects of a methanolic and water extract of *Withania somnifera* (L) Dunal roots on early pregnancy of rats. Female rats (n= 10/group) were orally administered with either 500 or 3000 mg/kg/day of methanolic extract or 500 mg/kg/day of water extract of root consecutively from days 1-7 of pregnancy. On day 14 of pregnancy, animals were laparotomised under mild ether anesthesia under aseptic conditions, and the number of implantation sites, resorption sites, viable embryos and copora leutea were noted. *Withania somnifera* root extracts showed no abortifacient and antifertility effects when administered orally to female rats during early pregnancy. Further the extracts were well tolerated. The result obtained from this study is in contrast to its claimed effect as an abortifacient in traditional medicine in several countries.

Keywords: *Withania somnifera*, abortifacient; pregnency; preimplantation loss; postimplantation loss

1. Introduction

Withania somnifera (L.) Dunal (Family: Solanaceae; Amukkara in Sinhala and Amukkiray in Tamil) is an erect hoary perennial or semi-woody shrub which grows in sunny dry places in several tropical countries including Sri Lanka [(1)]. According to traditional medicine the root is used as an abortifacient and to produce criminal abortion[(1,2)]. It is also used to treat repeated

miscarriages and to remove retained conception products[2] However, to our knowledge, presence of this activity in the roots of *W. somnifera* is not scientifically proven.

On the other hand, in animal models, alcoholic extract of the root of this plant is shown to possess anticonvulsive, barbiturate potentiation, central nervous system depressant, membrane stabilizing, sedative, hypotensive and gamma amino butyric (GABA) activities [1] Phytochemically, the roots contain number of alkaloids, ergostane steroids and amino acids including tryptophan [1]

The aim of this study was to investigate the effects of *W. somnifera* roots on early pregnancy. This was done using laboratory rats and a methanolic and water extract of *W. somnifera* roots.

2. Materials and Methods

Animals

Cross-bred virgin albino rats (180-220g) with regular estrous cycles of 4-5 days and males of proven fertility (220-270g) were used. Rats were housed in wire mesh cages under standardized animal house conditions (temperature: 28-30^{0}C, humidity 65%, photoperiod: 12 h light and 12 h dark) with free access to food and water.

Plant material

Dried roots of *Withania somnifera* (L.) Dunal (Solanaceae) were bought from a local drug store and authenticated by Dr. H. M. A. Tissera, Department of Materia Medica, Institute of Indigenes Medicine, University of Colombo, Rajagiriya, Sri Lanka.

Preparation of root extract
Extraction in methanol

The roots were powdered and stored in (80%) methanol for 14 days. This was filtered and concentrated under negative pressure at 28 ± 2^{0}C to obtain the crude extract. Any remaining traces of the solvent were further removed by storing the crude extract under vacuum overnight (yield: 2.9%, w/w). The crude extract was dissolved in absolute ethanol and mixed with an equal volume of an ethanolic solution of polyvinylpyrolidone (PVP) (Sigma Chemical, St. Louis, MO, USA) (1:1.5, crude extract: PVP by weight). The resulting mixture was gradually evaporated under negative pressure at 28 ± 2^{0}C to dryness to obtain a co-precipitate. This co-precipitate was dissolved

in distilled water soas to obtain a 500 mg/kg/day and 3000 mg/kg/day dose in 1 ml aliquots. 3000 mg/kg/day dose is the maximum dose possible with this mode of preparation. This dose is equivalent to fifteen times the human dose[3].

Extraction in water

Dried and powdered *Withania somnifera* roots (250g) were soaked in distilled water (1 L) for 4 hours under constant stirring. This was filtered through 2 layers of gauze on a filter paper and used to feed the animals. An aliquant of this extract was subjected to freeze drying to determine the concentration (1.2g/ml).

Treatment and observations

Proestrous rats were selected by vaginal smearing and were individually paired (between 17.00 and 18.00 h) with a male rat of proven fertility. Positive matings were verified by the presence of sperm in the vagina on the following morning (8.00-9.00 h) and designated as day 1 of pregnancy. These pregnant rats were randomly divided into 4 groups and orally (through gastric intubation) fed either with 1.0 ml of crude extract (methanolic and water extracts), or (PVP co-precipitate in distilled water or water extract) daily for 7 consecutive days between 15.00-16.00 h from day 1 through day 7 of pregnancy as indicated below:

Group I (n= 10) 3000 mg/kg/day PVP in distilled water
Group 2 (n= 10) 750 mg/kg/day PVP in distilled water
Group 3 (n= 10) distilled water
Group 4 (n= 10) 3000 mg/kg/day methanolic extract
Group 5 (n= 10) 500 mg/kg/day methanolic extract
Group 6 (n= 10) 500 mg/kg/day water extract

The rats were closely observed twice daily for survival, changes in appearance or behaviour and signs of vaginal bleeding. Furthermore, food and water intake, texture of the faeces and colour of urine were also noted.

All animals were observed daily for mortality, overt signs of clinical toxicity (salivation, lachrymation, ptosis. wilting, convulsions, stupor, tremor, rapid rotational movement of head, yellowing of fir, loss of hair, postural changes), stress (erection of fur and exopthalmia) aversive behaviours (biting and scratching behaviours, licking at tail or paw, or vocalization) and non sexual behaviours (such as cleaning of face, self grooming, climbing in the cage, rearings).

On day 14 of pregnancy, animals were laparotomised under mild ether anesthesia under aseptic conditions, and the number of implantation sites, resorption sites, viable embryos and copora leutea were noted. After the examination of above parameters, the animals were sutured, treated locally and subcutaneously with antibiotics (teramycin), and allowed to recover and deliver.

The following reproductive indices were then calculated: preimplantation loss = [(total number of corpora leutea - total number of implants) / (total number of implants) x 100], postimplantation loss = [(total number of implants - total number of viable implants) / (total number of implants x 100)] and fertility index = (number pregnant / number paired).

Effects on liver function

Rats were orally treated with either 3000 mg/kg/day dose methanolic root extract (n=10) or PVP (n=10) for 7 consecutive days as described previously. On day 1 post treatment, these rats were anaesthetized with ether and about 2ml of blood collected from tail. Blood was allowed to clot at room temperature (28-30^{0}C) and subjected to centrifugation (at 3200xg). Serum was collected and SGOT, SGPT levels determined using Randox enzyme kits (Randox Laboratories Ltd., CoAntrim, UK) and a spectrophotometer (Jasco V500, Jasco Corporation, Tokyo, Japan).

Statistical analysis

Data are expressed as means ± SEM. Statistical evaluations were made using Mann-Whitney U test. Significance was set at $P<0.05$.

3. Results

Mortality, general health, side effects

There were no treatment related deaths. Food and water intake, and general health of the treated rats were essentially similar to that of controls. There were no signs of vaginal bleeding and/or fetal or placental discharges. Further, no overt signs of toxicity, stress or aversive behaviours were evident in the treated rats. Nonsexual behaviours of the treated rats appeared to be unaltered.

Effect on pregnancy

Results are shown in Table 1. At the doses tested both methanolic and water extracts of the root failed to significantly ($p>0.05$) alter any of the reproductive parameters tested.

Liver function

Subchronic methanolic root extract treatment had no significant effect ($P > 0.05$) on serum SGOT [(control vs treatment) *57.5 ± 5.6* vs 62.2 ± 4.6 U/L] or SGPT [(control vs treatment) 16.8 ± 0.80 vs 20.3 ± 1.2 U/L].

4. Discussion

In this study, we used rats as our experimental model because it is one of the species that has proven to be extremely useful in pharmacologic and toxicologic research as there are many similarities between rat and human metabolic pathways.[4] A water extract was used as decoctions are usually made with water. We also used a methanolic extract as most of the phytochemical work on *W. somnifera* roots are done on alcoholic extracts [1] and because our previous study with male rats was based on methanolic extracts [5]

The results showed that both water and methanolic extracts made from Sri Lankan variety of *W. Somnifera* roots were well tolerated in rats but failed to induce abortions (in terms of vaginal bleeding, pre- and post- implantation losses) and impair fertility (as judged by the number of uterine implants) when orally administered to rats during early pregnancy period (days 1 -7). Preliminary studies have also shown that these extracts were devoid of any anti-reproductive effects in rats when given orally to rats during mid (days 7-14) and late (days 14-21) gestation period (our unpublished data). Drugs can have harmful effects on the fetus at any time during pregnancy and the period of greatest risk is at the first trimester[6]. Therefore, results obtained were unexpected especially because *W. Somnifera* roots are claimed to be used as an abortifacient in the traditional medicine of South Africa, Tanzania, India, Nepal or Pakistan[1]. In this regard, it is of interest to note that, this methanolic extract of the root impaired sexual competence in rats although *W. Somnifera* roots are claimed to be an aphrodisiac and/or sexual stimulant in traditional folk medicine of several tropical countries.

The precise cause for the failure of the extracts made from roots of local variety of *W. Somnifera* to induce an abortifacient action remains to be established but several potential explanations may be given. Storage and drying of botanicals are known to alter chemical constituents[7]. Since we have used dried roots whose storage time is unknown alterations in chemical structure and composition of the constituents cannot be completely ruled out. Although the active principal/s responsible for the abortifacient activity of *W. Somnifera* roots are not yet been isolated and characterized, ergesterone steroids may be a potential candidate[1] Another possibility is that the

extraction procedures used were ineffective in extracting the ingredient/s inducing the abortifacient action. However, this is unlikely as the water extract of the root is claimed to be used as an abortifacient agent [1,2]. Further, in the methanolic extract, extraction of nonpolar compounds should be higher. Interspecies variations in chemical constituents and bioactivity among herbs exists due to geographical location[7]. For example, the leaves of the Indian variety of *Erythrina indica* (Family: Fabacece) possess analgesic activity whilst its Sri Lankan variety lacks any analgesic action[8]. A strong possibility exists for such a interspecies variation in bioactivity with *W.sonmifera* roots with respect to abortifacient action. The stage of development of plants also affects its bioactivity profile[7]. For example, water extracts of leaves and sterms of preflowering but not flowering plants of *Anisomeles indica* (Family: Lamiaceae) has anti-inflamatory activity in rats[9]. However, no information is available about the developmental stage of the roots used here. Growth conditions and time of harvest can also alter bioactivity[7] but we have no information to comment on these aspects.

In contrast, lack of abortifacient and antifertility effects with our extracts could be due to species specificity: for example, norethisterone is a weak progestogen in the dog and a potent one in women[10]. It should be noted that, regardless of tissue or organ system studied, data exist to demonstrate differences in responsiveness to chemicals between different species[4]. In the case of traditional, folk and Ayurveda medicine the role of food is taken into account during drug administration in humans. However, this factor was not considered in this study and rarely, if ever, in other studies (4). Enhanced hepatic and/or renal clearance of the bioactive constituent/s responsible for inducing abortifacient action is yet another possibility. In complete contrast, production of a metabolite in rats which overrides the abortifacient activity of these root extracts of *W. sonmifera* is possible. However, additional studies are needed to clarify these possible explanations.

5. References

1. Arambewala, L., Silva. R. (1999). *Withania somnifera.* Colombo: Ceylon Institute of Scientific and Industrial Research, pp 1-26.

2. Jayaweera, DMA. Medicinal plants used in Ceylon. National Science Council, Colombo, Sri Lanka.

3. Annonimus, The use of traditional medicine in primary health care: A manual for health workers in South-East Asia. SEARO regional health papers 1990, **19,** pp 96-7.

4. Kacew, S. (2001). Confounding factors in toxicity testing. *Toxicology,* **160,** 87-96.

5. Ilayperuma, I., Ratnasooriya, W.D. and Weerasooriya, T.R. (2002). Effect of *Withania somnifera* root extract on sexual behaviour of male rats. *Asian J Androl.*, **4**, 295-298.

6. Annonymous, British National Formulary 2000. The British Medical Association and the Royal Pharmaceutical Society of Great Britain, London, pp 644.

7. Percival, S.S. (2002). Use of Echinacea in medicine. *Biochem. Pharmacol.* **60,** 155-158.

8. Ratnasooriya, W.D. and Dharmasiri, M.G. (1999). Aqueous extract of Sri Lankan variety of *Erythrina indica* leaves has sedative but not analgesic activity. Fitotherapia, **70**, 311-313.

9. Dharmasiri, M G., Ratnasooriya, W.D. and Thabrew, MI. (2002). Anti-inflamatory activity of decoctions of leaves and stems of *Anisomeles indica* at preflowering and flowering stages. *Pharm. Biol.* **40,** 433-439.

10. Heywood. R. (1986). Animal models: their use and limitations in long-term safety evaluation of fertility-regulating agents. *Hum. Reprod.* **1,** 392-399.

Table 1

Effect of methanolic and water extracts of *Wilha,uia somnifera* root on some fertility parameters of female rats

Treatment	Dose[1]	n	Duration[2]	Preimplantation loss (%)	Postimplantation loss (%)	Fertility index	Number of uterine implants
PVP	*750*	10	Dl-D7	21 4712,9	2.08 ± 2.1	100	8.33 ± 0.8
Methanolic extract	500	10	Dl-D7	24.88±3.6	1 58± 1.6	100	8.57 ±0.3
PVP	3500	10	D1-D7	26.17±4.4	0	100	8.6 ± 0.7
Methanolic extract	3000	10	D1-D7	25,31 ±4.1	1.42±1.4	100	9.14±3.4
Distilled water	500	10	D1-D7	26 67 ± 3.7	0	100	8.83 ± 0.6
Water extract	500	10	D1-D7	24.70± 4.8	3.42 ± 2.7	100	9.14 ± 0.76

[1] As mg extract/kg body weight

[2] (D1-D7) — day 1 to day 7 of pregnancy; *n* = number of rats; values are mean ±SEM