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Adverse pregnancy outcome in rats following exposure to a *Salacia reticulata* (Celastraceae) root extract

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Abstract

The root extract of *Salacia reticulata* Wight (family: Celastraceae) is used in Sri Lanka by traditional practitioners as a herbal therapy for glycemic control even during pregnancy. It is recognized that some clinically used antidiabetic drugs have harmful effects on pregnancy but the effects of the *S. reticulata* root extract on reproductive outcome is unknown and deserves examination. We determined the effects of the *S. reticulata* root extract on the reproductive outcome of Wistar rats (250-260 g) when administered orally (10 g/kg) during early (days 1-7) and mid- (days 7-14) pregnancy. The root extract significantly ($P<0.05$) enhanced post-implantation losses (control *vs* treatment: early pregnancy, 4.7 ± 2.4 *vs* $49.3 \pm 13\%$; mid-pregnancy, 4.7 ± 2.4 *vs* $41.7 \pm 16.1\%$). Gestational length was unaltered but the pups born had a low birth weight ($P<0.05$) (early pregnancy, 6.8 ± 0.1 *vs* 5.3 ± 0.1 g; mid-pregnancy, 6.8 ± 0.1 *vs* 5.0 ± 0.1 g) and low birth index ($P<0.05$) (early pregnancy, 95.2 ± 2.4 *vs* $50.7 \pm 12.9\%$; mid-pregnancy, 95.2 ± 2.4 *vs* $58.3 \pm 16.1\%$), fetal survival ratio ($P<0.05$) (early pregnancy, 95.2 ± 2.4 *vs* 50.7 ± 12.9; mid-pregnancy, 95.2 ± 2.4 *vs* 58.3 ± 16.1), and viability index ($P<0.05$) (early pregnancy, 94.9 ± 2.6 *vs* $49.5 \pm 12.5\%$; mid-pregnancy, 94.9 ± 2.6 *vs* $57.1 \pm 16.1\%$). However, the root extract was non-teratogenic. We conclude that the *S. reticulata* root extract can be hazardous to successful pregnancy in women and should not be used in pregnancy complicated by diabetes.

Key words
- *Salacia reticulata*
- Hypoglycemia
- Pregnancy outcome
- Birth weight
- Postnatal development

Introduction

In Sri Lanka, many people including pregnant women use either a water extract of *Momordica charantia* L (Cucubitaceae) or roots of *Salacia reticulata* Wight (Celastraceae) as a herbal therapy for diabetes mellitus. Currently, even mugs made of *S. reticulata* wood are available to be used routinely by diabetic patients to drink water. Experimentally, the root extract of *S. reticulata* has been found to have potent hypoglycemic activity both in normal (1,2) and in streptozotocin-induced diabetic rats (1,3) and spermicidal activity against human spermatozoa (4). Furthermore, the decoction of *S. reticulata* roots is used in the treatment of rheumatism, gonorrhea, itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea (1). Phytochemically, the presence of a variety of chemical constituents such as 1,3-diketones, dulcitol and leucopelargonidin (a linear iso-

mer of natural rubber), iguesterin (quinonemethides), mangiferin and epicatechin (phenols), phlobatannin and glycosidal tannins, triterpenes, 30-hydroxy-20(30) dihydroisoiguesterin, salacinol and kotalanol (thiosugar) has been detected in the root of *S. reticulata* (1,4-7).

However, treatment with oral hypoglycemic agents in gestational diabetes mellitus remains a controversial topic since even a minor degree of hypoglycemia can adversely affect the reproductive outcome. Recently, we showed that water extracts of *M. charantia* fruit, if administered during mid-pregnancy (days 7-14) to rats, induced prenatal growth deficiencies (8) and mild teratogenesis (9), as reported with some clinically used antidiabetic drugs in Western medicine (10). Regrettably, the possible harmful effects on pregnancy outcome after *in utero* exposure to a water extract of *S. reticulata* have not been investigated.

Therefore, the aim of the present study was to determine the effect of an aqueous extract of *S. reticulata* roots on reproductive outcome and on prenatal and early postnatal development using the rat model at a dose equivalent to ten times the recommended human dose (1,11).

Material and Methods

Plant material

Dried *S. reticulata* roots were purchased from a herbal outlet and authenticated by Dr. Y.M.H.B. Yapabandara, Natural Products Development Group, Industrial Technology Institute, Colombo, Sri Lanka. A voucher specimen has been deposited in the museum of the Department of Zoology, University of Colombo (specimen number 1SR).

Extract preparation

Sixty grams of powdered root was boiled in 1920 ml distilled water for approximately 3 h until a final 24-ml volume of extract was obtained (yield: 40%, w/v).

Animals

Nulliparous pregnant (days 1 and 7) Wistar rats (250-260 g) were used. Pregnancy was induced in rats by individually pairing a proestrous female overnight with a sexually experienced male (between 17:00 and 18:00 h) and examining vaginal smears for the presence of sperm on the following day, which is designated as day 1 of pregnancy. They were placed individually in plastic cages under standardized animal house conditions (temperature: 28-31ºC; light: approximately 12 h natural light per day; humidity: 50-55%) with free access to pelleted food (Master Feeds Ltd., Colombo, Sri Lanka) and tap water.

Study protocol

Twenty-three pregnant rats were randomly assigned to four groups and treated (between 9:00-10:00 h for 7 consecutive days) in the following manner: group 1 (N = 4), 1 ml distilled water *po*; group 2 (N = 6), 1 ml root extract (10 g/kg) *po* from days 1 to 7 of pregnancy; group 3 (N = 6), 1 ml distilled water *po*; group 4 (N = 7), 1 ml root extract (10 g/kg) *po* from days 7 to 14 of pregnancy. Due to ethical reasons, the number of animals was minimized.

Cage-side examinations were performed daily to detect overt signs of toxicity (salivation, rhinorrhea, lacrimation, chewing jaw movements, ptosis, squinting, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur, vocalization and exophthalmia), behavioral abnormalities, and moribund or dead rats. Food and water intake was also determined by measuring the daily leftovers in the cages (12). On day 15 of pregnancy, the rats were laparotomized under ether anesthesia and aseptic conditions. The uteri were examined *in situ*

for the presence and location of resorption sites and for live and dead fetuses. The appearance and the number of corpora lutea in each ovary were also recorded.

The animals were sutured, treated with 190 mg/kg tetracycline *im*, and allowed to deliver, and the day of parturition was recorded. The number of viable or stillborn pups was recorded, and their body weights were determined. All pups were evaluated for gross external congenital abnormalities (open eyelids, tail anomalies, clubfoot, oligodactyly or syndactyly). Pup mortality up to 6 days, the day of eye opening, and the appearance of fur were recorded.

Based on these data, the following indices were computed (13): quantal pregnancy = (number of pregnant dams/number mated) x 100; implantation index = (total number of implants/number mated) x 100; pre-implantation loss = [(number of corpora lutea - number of implantations)/number of corpora lutea] x 100; post-implantation loss = [(number of implantations - number of viable implantations)/number of implantations] x 100; viability index = (number of viable pups on day 4 after delivery/number of liveborn pups) x 100; birth index = (number of pups born/number of implantations) x 100; fetal survival ratio = (number of surviving pups/number of implantations) x 100; live birth index = (number of liveborn pups/total number of pups born) x 100; gestation index = (number of live pups/number of pregnant dams) x 100.

Statistical analysis

Data are reported as means ± SEM. Statistical comparisons were made using the G-test (a modified chi-square test) and the Mann-Whitney U-test (14), with the level of significance set at $P<0.05$.

Results

No mortality or treatment-related overt signs of maternal toxicity, stress or abnormal behavioral changes were observed. None of the pregnant rats showed vaginal bleeding or expulsion of products of conception. The food and water intake of the treated rats was similar to that of controls. At laparotomy, all the extract-treated rats had normal numbers of apparently healthy looking corpora lutea, as observed in controls.

Since there was no significant difference between the two control treatments (groups 1 and 3) the data for these groups were pooled. Data concerning reproductive parameters are presented in Table 1. The root extract caused a marked and significant ($P<0.01$) increase in post-implantation losses when given during either early or late pregnancy. There was no significant difference in any other parameter monitored or computed.

The results obtained concerning the pups are presented in Table 2. Neither parturition-related maternal deaths nor stillbirths were observed. The root extract induced a signifi-

Table 1. Effect of an aqueous extract of *Salacia reticulata* roots on some reproductive parameters of female rats.

Parameter	Control (N = 10)	Early pregnancy (N = 6)	Mid-pregnancy (N = 7)
No. of uterine implants	6.7 ± 0.21 (6-8)	8.6 ± 0.8 (5-10)	8.8 ± 0.7 (6-12)
Quantal pregnancy (%)	100	100	100
Implantation index (%)	670	866.6	885.7
Pre-implantation loss (%)	18.4 ± 1.4 (12.5-25)	19.6 ± 3.6 (10-37.5)	15.4 ± 3.3 (9-33.5)
Post-implantation loss (%)	4.7 ± 2.4 (0-16.6)	49.3 ± 13* (12.5-100)	41.7 ± 16.1* (0-100)
Gestation index (%)	100	83.3	71.4
Mean gestational length (days)	21.3 ± 0.2 (21-23)	23.0 ± 0.6 (21-26)	21.6 ± 0.3 (21-24)

The rats received 10 g/kg, *po*, daily from day 1 to day 7 of pregnancy (early pregnancy group) or from day 7 to day 14 (mid-pregnancy group). Data are reported as means ± SEM (range within parentheses).
*$P<0.05$ compared to control (Mann-Whitney U-test and G-test). See Methods for definition of indices.

cant reduction (P<0.01) in the weight of the pups born (22% in early pregnancy and 27% in mid-pregnancy), in viability index at day 4 after delivery, and in birth index and fetal survival ratio.

Discussion

Oral administration of the *S. reticulata* root extract during early or mid-pregnancy had no effect on fertility in terms of uterine implants, implantation index or gestation index. However, it posed a considerable threat to successful pregnancy (as judged by a reduction of fetal survival ratio and birth index and enhancement of post-implantation losses). These deleterious effects on pregnancy resulted from enhanced embryonic deaths as evident at laparotomy possibly at an early stage of prenatal development since no signs of vaginal bleeding or expulsion of products of conception were evident. Since the root extract was non-toxic, and a correlation exists between maternal toxicity and developmental toxicity (15), the fetal deaths are unlikely to be due to a toxic action on the developing offspring. On the other hand, there could possibly be an indirect effect of maternal hypoglycemia. This is a matter of concern, especially for women who are diabetic and are at high risk of miscarriage.

Table 2. Effect of an aqueous extract of *Salacia reticulata* roots on some litter parameters of female rats.

Parameter	Control (N = 10)	Early pregnancy (N = 6)	Mid-pregnancy (N = 7)
Number of pups born	6.4 ± 0.3 (5-8)	4.6 ± 1.1 (0-7)	5.7 ± 1.7 (0-12)
Number of liveborn pups	6.4 ± 0.3 (5-8)	4.6 ± 1.1 (0-7)	5.7 ± 1.7 (0-12)
Pup weight (g)	6.8 ± 0.1 (5.9-7.2)	5.3 ± 0.1* (4.8-5.8)	5.0 ± 0.1* (4.8-5.2)
Number of malformed pups	0.0	0.0	0.0
Eye opening (days)	14.9 ± 0.2 (14-17)	14.8 ± 0.5 (14-17)	15.5 ± 0.5 (14-17)
Appearance of fur (days)	7.3 ± 0.21 (6-8)	7.2 ± 0.3 (6-8)	7.4 ± 0.3 (6-8)
Live birth index (%)	100	100	100
Viability index (%)	94.9 ± 2.6 (83.3-100)	49.5 ± 12.5* (0-85.71)	57.1 ± 16.1* (0-100)
Birth index (%)	95.2 ± 2.4 (83.3-100)	50.7 ± 12.9* (0-85.71)	58.3 ± 16.1* (0-100)
Fetal survival ratio	95.2 ± 2.4 (83.3-100)	50.7 ± 12.9* (0-85.7)	58.3 ± 16.1* (0-100)

The rats received 10 g/kg, *po*, daily from day 1 to day 7 of pregnancy (early pregnancy group) or from day 7 to day 14 (mid-pregnancy group). Data are reported as means ± SEM (range within parentheses).
*P<0.05 compared to control (Mann-Whitney U-test and G-test). See Methods for definition of indices.

The pups born to dams treated with the root extract had low mean birth weights. This indicates intrauterine growth retardation (IUGR). Some antidiabetic drugs induce IUGR (16). The degree of IUGR observed with the use of the root extract, however, was lower than that reported for *M. charantia* fruits (8). The IUGR in this study was not due to reduction of gestational length or preterm delivery, and was probably not due to intrinsic fetal factors such as chromosomal abnormalities or other malformations. However, it may be attributed to impaired glucose supply to the fetuses.

Extremely potent natural α-glucosidase inhibitors, salacinol (6) and kotalanol (5), have been isolated from *S. reticulata* roots, and several workers have shown that the root extracts of *S. reticulata* reduce blood glucose levels in normoglycemic and streptozotocin-induced diabetic rats (1,3). The root extract appeared to be non-teratogenic. On the other hand, allopathic antidiabetic agents like sulfonylureas and biguanides (10,17), and plant products such as *M. charantia* fruit extracts (9) induce teratogenesis in rodents. The viability index of pups born following fetal exposure to the root extract was low, although some parameters of postnatal development (day of eye opening and day of appearance of fur) remained unaltered. If these data can be applied to women, then consumption of the root extract during preg-

nancy can have serious implications in countries like Sri Lanka, India and Nepal where more than two thirds of all infants born are small for gestational age (18). Low birth weight has a major influence on neonatal morbidity, neurocognitive deficiencies, neurobehavioral effects and mortality (19). Furthermore, reduced growth *in utero* is reported to be linked to decreased glucose tolerance in adult life (20).

We conclude that the use of the *S. reticulata* extract should be avoided by women with pregnancy complicated by diabetes.

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