ANTI-FERTILITY EFFECTS OF THE METHANOLIC ROOT EXTRACT OF CAREYA ARBOREA ROXB IN ALBINO MICE

JOGEN CHANDRA KALITA*, ANSARUL HAQUE, EVARANI KALITA1, RONIM SHARMA2 AND M. MUKHLESUR RAHMAN3
Animal Physiology and Biochemistry Laboratory, Department of Zoology, Gauhati University, Guwahati - 781 014,
1Department of Zoology, Handique Girls’ College, Gauhati University, Guwahati - 781 001, Assam, INDIA
2Department of Haematological Medicine, Rayne Institute, King’s College London,
123 Cold Harbour Lane - SE5 9NU, UK
3Department of Pharmacy, University of Rajshahi, Rajshahi - 6205, BANGLADESH
E-mail: jogenck@yahoo.co.in

ABSTRACT
The antifertility effects of methanolic root extract of Careya arborea Roxb. has been investigated in albino mice. The methanolic root extracts of the plant did not show any sign of acute toxicity up to the dose level of 5000 mg/kg bodyweight (bw) in adult mice. A short term treatment of the methanolic root extract for a period of 14 days revealed strong antifertility effects in mice. It was found that the estrous cyclicity in normal adult cyclic mice was severely affected by the root extracts. At the dose level of 500 mg/kg bw, the root extracts showed strong pregnancy inhibitory effects. The duration of gestation period and litter size was significantly reduced in pregnant mice treated with the extracts. The antifertility effects exerted by the root extracts were found to be reversible. In ovariectomised mice treated with 500 mg/kg bw, root extract resulted in the occurrence of a persistent estrus phase, similar to the effects produced by 17β-oestradiol (E2). The GC-MS analysis on the methanolic extract showed the presence of some phenolic compounds- hydroquinone, resorcinol, syringic acid, vanillic acid, 4-hydroxy-3-(o-hydroxyphenyl)-5,7-dimethoxy-coumarin, 3′, 4, 4′, 7-tetra methoxy-, trans-2, 3, cis-2, 4′(+-)-3-flavanol and 2-methoxydibenzoferan. Presence of these phenolic compounds might be responsible for the antifertility activity of the plant.

INTRODUCTION
Careya arborea Roxb., commonly known as wild guava, belongs to the family Lecythidaceae. It is a medium sized deciduous tree, bark dark grey exfoliating in thin strip. It is a plant of tropical region widely distributed in the Indo-Malaysian region (Wadkar and Chandrakant, 2009). It is occasionally planted in gardens and on roadsides for its large conspicuous leaves and showy flowers and fruits. This plant has been extensively used in Indian traditional medicine for the treatment of different diseases. Stem bark of Careya arborea is traditionally used for the treatment of tumours, antihelmintic infections, bronchitis, epileptic fits, astringents and antidiotes, snake-venom and skin diseases (Kirtikar and Basu, 1975). The red decoction of the bark is reported to cure diarrhoea (Sikarwar et al., 1994), dysentery and also for ear pain (Bhandary et al., 1995). It is also used for washing eyes for any kind of eye complaints (Jain, 1965). Antipyretic, leech repellent and antivenom activities are also reported in literature (Talapatra et al., 1981; Selvanayaghgam et al., 1994). The aqueous extract of fresh root bark is used as fish poison (Gedeon and Kinel, 1956). In vitro antimicrobial and antioxidant activity of the stem bark of this plant is also reported. An important ayurvedic drug “Padmaka” is prepared from the flower for treatment of diseases like gastritis and anaemia (Shantha et al., 1987). A recent survey conducted by Haloi and his associates in the Nalbari District of Assam (India) revealed that the root of Careya arborea Roxb. has been used traditionally as an oral contraceptive agent largely by the members of native tribal people for several years (Haloi et al., 2010). The root portion of the plant was boiled with water for 5-6 hrs and then concentrated to make a semi-liquid substance. The women used this substance orally for 15 days starting from the 4th day of menstruation to prevent pregnancy. They used it at 3-4 months intervals until they would have a desire to conceive. We, therefore, conducted some experiments on the effects of the methanolic root extracts on pregnancy of adult cyclic mice as well as the in vivo estrogenic effects of the root extract using ovariectomised mice.

MATERIALS AND METHODS

Chemicals
All chemicals used in this study were obtained from Sigma (Sigma-Aldrich Corporation, St. Louis Missouri–63178, USA), Qualigen (Qualigens Fine Chemicals, Mumbai, India) and Merck (Merck Limited, Mumbai, India).

Animals
The experiments were conducted using healthy Swiss albino mice (C3H strain) approximately 3 months of age and weighing 20-25g. The animals were obtained from the Animal
House Facility of the Department of Zoology, Gauhati University, India. Animals were maintained under uniform conditions of natural photoperiod (12h light/dark cycle), humidity (60-94%) and temperature (24-32°C) and they had free access to food and water. Mice were housed individually in wire mesh plastic cages with a solid bottom containing sawdust. Body weight and clinical signs were recorded on a daily basis throughout the period of the experiments.

Ovariectomy

Mice were ovariectomized at least two weeks before the starting of experiments. Mice were subjected to bilateral ovariectomy under tribromoethanol anaesthesia. After wiping the mouse back with 90% ethanol, a small (1-1.5 cm long) dorsal midline incision was made in the skin just below the level of the last rib. The connective tissues between the skin and the body wall, in the area around the incision were cleared and the skin moved to one side so that a second incision could be made, through the skin incision into the peritoneal cavity in the body wall directly above the ovary. The ovary was removed by cutting the oviduct as close to the ovary as possible. The remaining oviduct and uterus was replaced into the body and the skin pulled over to the other side to repeat the procedure. The skin incision was closed using Michael clips.

Collection and preparation of plant materials

The roots of Careya arborea Roxb. were collected from Nalbari District of Assam in August, 2010 and was authenticated by Dr. Gajen Chandra Sarma, Curator, Department of Botany, Gauhati University, where a herbarium specimen (Accession No. 02523) of this collection was deposited. After collection and proper washing, the root portions were cut into small pieces, shade-dried and moderately powdered. Air-dried powdered roots (200g) were extracted with 1500 mL methanol in a Soxhlet apparatus for 18h and extract was concentrated in a rotary vacuum to get the semi-solid sticky mass and stored at 0°C. A fresh sample was prepared from this dried extract just before administration into the animal.

Preparation of 17β-estradiol

17β-estradiol was prepared in analytical grade of alcohol (100% ethanol) as 1 mM stock solution and diluted with normal saline containing <10% alcohol as per experimental requirements.

Test compounds administration

Test materials were orally administered via gastric intubations, using an orogastric tube comprising a 16G polyethylene catheter fitted to a hypodermic syringe following the method of Khokhote et al. in a volume of 0.5 mL (Khokhote et al., 1978). Treatments were carried out every morning at 24h intervals for 14 consecutive days. For oral administration the required amount of extracts were suspended in vehicle Tween-80 (Polyoxymethylene sorbitan Mono-oleate) in 100 μL/mL normal saline to 1g of extract to make the concentration of the solution 1g/mL.

Preparation of vaginal smears and counting of cells

Vaginal smears were prepared at each 12h intervals throughout the treatment period (Kalita, 1998). The smears were evaluated by scoring the number of cornified cells relative to the population of all cell types present (Terenius, 1971).

Toxicity study

For toxicity studies, a group of 10 adult healthy normal mice were treated with a dose of root extract ranging from 50 to 5000 mg/kg bw and mortality was observed after 96h and the LD50 was determined (Omkar, 1994). The root extract did not show any sign of acute toxicity or mortality over the observation period of 96h up to the dose level of 5000 mg/kg bw when administered orally to adult mice. Therefore, it was assumed that the extract was devoid of any acute toxic effect proving their wide margin of safety up to the dose level used in the present study.

Effects of vehicle control

In this experiment, Tween-80 was mixed with normal saline at the ratio 1:10 and was used as a vehicle for different experimental purposes. Vehicle control animals did not reveal any significant difference with normal control animals for all variables.

General experimental procedures

The adult normal cyclic mice and also ovariectomised mice were grouped with 6 animals in each group. To assess the antifertility effects of the extract in normal mice and oestrogenic effects in ovariectomised mice, the animals were treated with the different doses of the extracts for a short period of 14 consecutive days. A control group (with no treatment), a vehicle control group (Tween 80 mixed with normal saline) and a positive control group (10 μg/kg bw of E2) were also maintained. The extracts were administered orally at 24h interval.

Preliminary identification of metabolites of the crude methanolic extract

The crude methanol extract of Careya arborea was analyzed by GC-MS. In order to identify the different compounds using GC-MS analysis, the fragmentation pattern of the individual components from the total ion chromatogram (TIC).

For sample preparation, the crude extract was first dissolved in methanol then diluted with diethyl ether (3-4 drops of methanol for 2 mL of diethyl ether) and then injected in to the GC-MS.

RESULTS AND DISCUSSION

Effects of the methanolic root extract on the estrous cyclicity in adult mice

In order to assess the effects of the methanolic root extracts on estrous cycle of adult cyclic mice, the animals were grouped into 5 groups with 6 animals in each group. Two groups of animals were treated separately with two doses of plant extracts of 250 mg/kg bw and 500 mg/kg bw respectively for a period of 14 consecutive days. A control group, a vehicle control group (Tween 80 mixed with normal saline) and a positive control group (10 μg/kg bw of 17β-estradiol) were used. The extracts were administered orally at 24h interval. Results are summarized in Table 1.

The root extracts induced dose dependent effects on the estrous cyclicity of mice. It was observed that in animals treated with 250 mg MRE the duration of the diestrus phase was...
The present experiment was designed as per the method described by Sharpe et al. (1995). To assess the effects of the methanolic root extracts on pregnancy, the animals were grouped in 7 groups with 6 animals in each group. They were treated with different doses of extract: 250 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw for a short period of 14 consecutive days and allowed for mating and pregnancy determination. After confirming their pregnancies, they were kept for 14 days to ascertain the pregnancy. If they did not deliver by the end of the period of 22 days, it was once again observed for cycles and allowed them to mate again. For comparisons, animals of the control and vehicle control group were also subjected to the same procedures. The results are shown in Table 2.

The root extracts severely influenced the pregnancy in treated mice. The effects of the extract were expressed as percentage of inhibition of pregnancy. The minimum effective dose of the root extract to prevent pregnancy was found to be 500 mg/kg bw. The dose of the extract that could induce strong pregnancy inhibitory activity was 1000 mg/kg bw. The root extract had a significant dose-dependent pregnancy inhibitory effect. After 1st mating, animals receiving 250 mg/kg bw of root extracts showed 40% pregnancy inhibitory effects. On the other hand, animals receiving 500 mg, 750 mg and 1000 mg/kg bw showed 100% inhibition. By the end of the repeated 2nd mating (i.e., after 22 days of 1st mating), animals treated with 250 mg/kg bw of root extracts showed 100% pregnancy inhibitory activity even after the repeated 2nd mating. By the end of the repeated 3rd mating (i.e., after 22 days of 2nd mating), the animals treated with 500 mg and 750 mg dose became pregnant. But the animals treated with 1000 mg/kg bw exhibited 100% pregnancy inhibitory activity even after the repeated 2nd mating. By the end of the repeated 3rd mating (i.e., after 22 days of 2nd mating), the animals treated with 500 mg and 750 mg dose became pregnant. But the animals treated with 1000 mg/kg bw exhibited 80% pregnancy inhibitory activity even after the repeated 3rd mating. In this E₁₁ (10 μg/kg bw) exhibited 100% inhibitory activity after 1st mating. In this E₁₁ treated group, 60% animals did not conceive after 2nd mating. But, by the end of the repeated 3rd mating, the animals became pregnant. In contrast, Interestingly enough, all the animals of group I (untreated control) and II (vehicle control) became pregnant. In animals treated with 500 mg and 750 mg/kg bw, 40% and 50% animals did not conceive after 2nd mating respectively. On the other hand, animals treated with 1000 mg/kg bw exhibited 100% pregnancy inhibitory effect. After 1st mating, animals receiving 250 mg/kg bw of root extracts showed 40% pregnancy inhibitory effects. On the other hand, animals receiving 500 mg, 750 mg and 1000 mg/kg bw showed 100% inhibition. By the end of the repeated 2nd mating (i.e., after 22 days of 1st mating), animals treated with 250 mg/kg bw of root extracts showed 100% pregnancy inhibitory activity even after the repeated 2nd mating. By the end of the repeated 3rd mating (i.e., after 22 days of 2nd mating), the animals treated with 500 mg and 750 mg dose became pregnant. But the animals treated with 1000 mg/kg bw exhibited 80% pregnancy inhibitory activity even after the repeated 3rd mating. In this E₁₁ (10 μg/kg bw) exhibited 100% inhibitory activity after 1st mating. In this E₁₁ treated group, 60% animals did not conceive after 2nd mating. But, by the end of the repeated 3rd mating, the animals became pregnant. In contrast, Interestingly enough, all the animals of group I (untreated control) and II (vehicle control) became pregnant immediately by the end of the 1st mating.

### Effects of the methanolic root extract on gestation period, litter size and birth weight

Animals were grouped with six animals in each group and they were treated with the extract at 250 mg/kg bw and 1000 mg/kg bw for 14 consecutive days and allowed for mating and after confirming their pregnancies they were kept for determining the effects on duration of pregnancy period and litter size. Results are shown in Table 3. In the animals treated

---

### Table 1: Effects of different doses of the methanolic root extracts on pregnancy in mice

<table>
<thead>
<tr>
<th>Experimental groups (dose per kg bw)</th>
<th>Length of the cycle (days)</th>
<th>Duration of estrous phases (days / h)</th>
<th>Pregnancy inhibition (% after 1st mating)</th>
<th>Pregnancy inhibition (% after 2nd mating)</th>
<th>Pregnancy inhibition (% after 3rd mating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Untreated control)</td>
<td>5-Apr</td>
<td>12-15 h</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Group II (Vehicle control)</td>
<td>5-Apr</td>
<td>12-15 h</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Group III (10 μg E₁)</td>
<td>Persistent estrus</td>
<td>10-20 h</td>
<td>100</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>Group IV (250 mg extract)</td>
<td>7-Jun</td>
<td>4-5 h</td>
<td>40</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Group V (500 mg extract)</td>
<td>7-Jun</td>
<td>5-6 h</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Group VI (750 mg extract)</td>
<td>7-Jun</td>
<td>5-6 h</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Group VII (1000 mg extract)</td>
<td>7-Jun</td>
<td>5-6 h</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Single asterisk denote all pregnant; double asterisk denote rest are pregnant; NE, No effect.
Effects of the methanolic root extract in inducing estrus in ovariectomised mice

To determine the potential of the root extract to induce estrus in ovariectomised mice, the animals were grouped in indiffrent groups with 6 animals in each group. They were treated with 500 mg and 1000 mg/kg bw of the root extracts daily for 14 days at the interval of 24h. In control mice, the treated with 500 mg and 1000 mg/kg bw of the root extracts daily for 14 days at the interval of 24h. Results are shown in Fig. 2. Analysis of the uterine protein content revealed significant increase between any of the treated groups and the controls (p<0.05). Uterine protein content achieved the maximal value (513.58 ± 5.28 mg/gm tissue) in the animals treated with E2. In animals, uterine protein contents were increased with increasing concentration of the extracts (302.47 ± 4.53 mg and 386.91 ± 4.9 mg/g tissue for 250 mg and 500 mg of extract respectively). Control and vehicle control had the values 251.11 ± 1.57 mg and 250.59 ± 1.42 mg/g tissue respectively.

By GC-MS analysis the major components of the methanol extract were identified as hydroquinone, resorcinol, syringic acid, vanillic acid; 4-hydroxy-3-(p-hydroxyphenyl)-5,7-dimethoxy-coumarin; 3-(o-hydroxyphenyl) coumarin; 3′, 4, 4′, 7-tetra methoxy-, trans-2,3, cis-2, 4-(+)-3-flavanol and 2-methoxy dibenzofuran. All these compounds belong to different classes of phenol. The present study investigated the antifertility activity and nature of estrogenic effects of the methanolic root extracts of Careya arborea Roxb. in mice. The root extract of the Careya arborea Roxb. showed no acute toxicity up to the dose level of 5000 mg/kg bw. Therefore, it is suggested that the experimental doses of the root extracts in the present study were devoid of any acute toxic effect proving their wide margin of safety. This finding was strongly consistent with our earlier reports on crude extracts that were evaluated for their acute toxicity (Haloi et al., 2010). In this experiment, 10 μg/kg bw of E2 was used as a positive control for different experimental purposes. Uses of these doses of E2 were supported by earlier studies of different workers (Padilla-Banks et al., 2001). The report suggested that the dose levels ranging from 1 to 500 μg/kg bw of E2 were sufficient to cause uterotrophic responses in mice and rats.
This experiment suggested that the methanolic root extracts of Careya arborea Roxb. adversely affected the normal reproductive cycle in mice. Comparing the duration of every phase of the estrous cycle between treated and non-treated mice it was found that each phase of the cycle was influenced by the extracts. The average length of estrus phase was prolonged at higher dose (500 mg) of extract treated animal. But, in the animals exposed to the lower dose (250 mg) of extract the diestrus phase was prolonged. It might be due to the imbalance in hormonal status probably because of the effects of the root extract as suggested by different workers (Cassidy et al., 1994; Lu et al., 1996). In rodents, different phases of oestrous cycle are regulated by interaction of pituitary gonadotrophins and ovarian steroid hormones (Greenwald, 1978). It was observed that administration of the root extract increased the length of the diestrus phase in the animals exposed to lower dose, but increased the length of the estrus phase in the animals exposed to higher dose of the root extract. Animals exposed to E_2 exhibited the positive oestrogenic response by inducing persistent estrus.

In the present study, the crude methanolic root extract of Careya arborea Roxb. induced comparatively a strong antifertility activity (pregnancy inhibition) in 3 months old Swiss albino mice at the dose level 1000 mg/kg bw, when administered orally for a short period (14 days). The results of this study on the antifertility activity of the root extract were consistent with earlier published data on many crude extracts and the active principles from medicinal plants that were evaluated in different laboratories for their antifertility effects in different animal models. Kholkute et al. (1978) reported dose dependent antifertility activity of methanolic extract of dried berries of Embelia ribes Burm. in rats. They reported a graded dose related effect of methanolic extract. At 15 mg and 30 mg/kg bw, the extract showed 37% and 50% antifertility activity, while at 60 mg dose, inhibition of pregnancy was 75% of the treated animals. In our study though the plant induced 100% antifertility at 500 mg and 750 mg of extract by the end of the treated animals. In our study though the plant induced 100% while at 60 mg dose, inhibition of pregnancy was 75% of the bw, the extract showed 37% and 50% antifertility activity, related effect of methanolic extract. At 15 mg and 30 mg/kg

Embelia ribes Roxb. induced comparatively a strong antifertility activity of methanolic extract of dried berries of Careya arborea Roxb. on gestation period, number of young ones produced (litter size) and birth weight

<table>
<thead>
<tr>
<th>Experimental groups(dose per kg bw)</th>
<th>Gestation period (days)</th>
<th>Litter size</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Untreated control)</td>
<td>23.17 ± 0.31</td>
<td>8.00 ± 0.52</td>
<td>1.27 ± 0.01</td>
</tr>
<tr>
<td>Group II (Vehicle control)</td>
<td>23.00 ± 0.37</td>
<td>7.83 ± 0.48</td>
<td>1.33 ± 0.02</td>
</tr>
<tr>
<td>Group III (10 ìg E_2)</td>
<td>22.00 ± 0.41</td>
<td>6.75 ± 0.41</td>
<td>1.75 ± 0.19</td>
</tr>
<tr>
<td>Group IV (250 mg extract)</td>
<td>21.50 ± 0.43</td>
<td>5.67 ± 0.21</td>
<td>1.54 ± 0.01</td>
</tr>
<tr>
<td>Group V (500 mg extract)</td>
<td>21.83 ± 0.40</td>
<td>5.50 ± 0.22</td>
<td>1.54 ± 0.01</td>
</tr>
</tbody>
</table>

Note: Values are expressed as Mean ± SEM (n=6/group). Superscript ‘a’ and ‘b’ as significant difference with control groups (p<0.05).

ACKNOWLEDGEMENT

Authors are grateful to the Director and other Scientists of the Forensic Science Laboratory, Kasturipara, Guwahati (India) for providing facilities for GC-MS work. Authors are also grateful to local people of Nalbari District (Assam) who helped in collecting the plant-parts and also to Dr. Gajen Chandra Sarma, Department of Botany, Guwahati University for his timely help and co-operation in proper identification of the plant.

REFERENCES


