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Effects of Sri Lankan Black Tea (*Camellia sinensis* L.) on Pregnancy of Rats

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Abstract: The aim of this study was to demonstrate the potential effects of black tea brew of *Camellia sinensis* using Sri Lankan high grown dust grade no. 1 tea on pregnancy outcome of rats when exposed during early (days 1–7), mid (days 8–14) and late (days 15–21) pregnancy of rats. Different doses of black tea brew (mg/ml/day) was orally administered daily during this period to separate groups of rats ($n = 6/\text{group}$): 84 (equivalent to 1.5 cups), 167 (3 cups), 501 (9 cups), and 1336 (24 cups). The results showed that black tea brew did not significantly ($P > 0.05$) change the pregnancy outcome (in terms of quantal pregnancy, number of uterine implants, number of viable implants, implantation index, pre-implantation loss, post-implantation loss, gestation index, number of pups born, litter index, live birth index and viability index) and pre- (in terms of length of the implants/foetus, gestation length, cranial length, cranial diameter and tail length of pups) and postnatal (in terms of time taken to open eyes, eruption of incisors and appearance of fur) development. Furthermore, black tea brew did not induce gross morphological birth abnormalities. If the results are applicable to women, it is concluded that even heavy consumption of black tea brew during pregnancy may not be harmful for pregnancy outcome.

Tea, which is produced from tender shoots of *Camellia sinensis* (L.) O. Kuntze (family: Theaceae) plant, is the most consumed beverage in the world besides water [1]. It is consumed by both men and women including those who are pregnant. At present, globally, about 3–5 billion cups of tea are consumed daily [1] of which black tea accounts for about 80% of global tea consumption (mostly in Europe, North America and South East Asia) [2]; based on the manufacturing process, there are three major types of tea: black (fully aerated or fermented); green (un-aerated or unfermented) and oolong (partially aerated or semifermented) [1,2].

A typical black tea brew contains about 35% solids in water [1]. This includes a variety of water soluble phytochemicals constituents such as flavonols (polyphenols) and caffeine (alkaloids) [1,3]. Results from several animal and epidemiological studies suggest, although not conclusively, that maternal intake of caffeine is detrimental to pregnancy outcome (in terms of foetal growth, time of delivery, abortifaciant activity or still births) [1,4–7]. In view of the fairly high caffeine content is black tea [1], a possibility exists that consumption of tea during gestation may pose hazards to the outcome of pregnancy. However, information on this aspect of black tea brew on female reproductive health is scarce.

This study was thus initiated to investigate the effects of consumption of black tea brew during gestation on pregnancy outcome. This was tested in pregnant rats (at early, mid or late pregnancy stages) using dust grade no. 1 tea which is consumed widely by the Sri Lankans.

Materials and Methods

Animals. Healthy adult Wistar rats (males 200–250 g and females 200–225 g) purchased from Medical Research Institute, Boralla, Sri Lanka, were used. They were maintained singly in plastic cages at the Department of Zoology, University of Colombo under standardized animal house conditions (temperature: 28–30°C; photoperiod: approximately a 12-hr light:dark cycle; and relative humidity 50–55%) with free access to tap water and pelleted food (Master Feed Ltd., Colombo, Sri Lanka) containing 19.5% proteins, 7.5% oil, 4.5% fibre, 7.9% ash, 0.48% methylamine, 0.9% calcium and 0.7% phosphorus.

All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1999) and rules of the Department of Zoology, Faculty of Science, University of Colombo, for animal experimentations.

Manufacture of black tea samples. The black tea belonging to the grade of dust no. 1 was manufactured at St. Coombs estate tea factory of the Tea Research Institute, Talawakelle, Sri Lanka, with its own green leaves (1382 m above mean sea level) using the orthodox-rotorvane manufacture technique. Tea samples were packed in triple laminated, aluminium foil bags, (1 kg each) and stored at -20°C until use.

Preparation of black tea brew. Black tea brew was made according to the ISO standards [8]: adding 2 g of black tea to 100 ml of boiling water and brewing for 5 min. This contains 43.7% (w/w) tea solids in water as reported previously [9]. Based on these data, 1336 mg/ml (equivalent to 24 cups, 1 cup = 170 ml) of black tea brew in 2 ml was made by adding 8 g black tea to 15 ml boiling water and brewing for 5 min. Five hundred and one mg/ml (equivalent to 9 cups), 167 mg/ml (equivalent to 3 cups) and 84 mg/ml (equivalent to 1.5 cups) concentrations of black tea brew were then made by diluting appropriately with boiling water.

Determination of caffeine contented in black tea brew. Caffeine was determined in black tea sample using high performance liquid