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Evaluation of a lactogenic activity of an aqueous extract of *Cyperus rotundus* Linn

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ABSTRACT

Ethnopharmacological relevance: In Ayurveda, the rhizome of *Cyperus rotundus* Linn has been reported for wide spectrum of biological activities including lactational therapy for increasing milk quantity. However, not a single report is available on validation of its herbal galactagogue potentiality in literature. Thus, the present study is aimed to assess the lactogenic property of aqueous extract of *Cyperus rotundus* (CRE).

Materials and methods: The effect of aqueous extract of *Cyperus rotundus* rhizome was evaluated by measuring weight of the pups during suckling period. Quantitatively, total protein and carbohydrate contents of mammary tissue and serum prolactin and cortisol level were calculated. Histopathological analysis of mammary gland, pituitary gland, heart, liver, spleen, kidney, and ovary tissues was carried out. Acute toxicity of CRE against rat was assessed by the Hippocratic test and biochemical profile of blood serum.

Results: Oral administration of 300 and 600 mg of CRE induced about 23% and 40% more milk in experimental group of animals as compared to the control group of animals. Weight gain by pups and mother rats of treated groups were significantly higher following administration of CRE as compared to that of control group. Moreover protein and carbohydrate content of mammary gland tissue were also significantly more than control group of animals. The CRE was found to stimulate the synthesis of prolactin significantly. In addition, the mammary gland tissues of experimental group showed obvious lobulo-alveolar development with milk secretion. Administration of CRE did not cause any signs or symptoms of toxicity which implied that *Cyperus rotundus* is toxicologically safe.

Conclusion: This study demonstrates that the aqueous extract of *Cyperus rotundus* can stimulate milk production in the female rats which may be consequently effective in increasing the lactation of human too.

1. Introduction

The Cyperaceae form one of the larger and more diverse cosmopolitan monocotyledonous angiosperm family with 106 genera and 5387 species worldwide. The genus *Cyperus* occurs in the tropical, subtropical and temperate regions. *Cyperus rotundus* Linn is one of the most popular members of this genus. It is native to Asia, South Africa, Southern and Central Europe and is known by more than 70 tribal vernacular names (Supplementary Table 1) and popularly called “the world's worst weed”. *Cyperus rotundus* has been reported to have diuretic, digestive stimulant, anthelmintic, antisaturative and plasma purifier properties (Singh et al., 2012). The alcoholic extract of *Cyperus rotundus* exhibits significant anti-inflammatory activity against exudative and proliferative phases of inflammation due to the presence of triterpenoid as an active compound of extract (Gupta et al., 1971). The methanolic extract of its rhizome shows antidiarrhoeal activity (Daswani et al., 2011). The ethyl acetate extract of rhizome has definite hepatoprotective activity (Kumar and Mishra, 2005). An aqueous and alcoholic extract of *Cyperus rotundus* plays key role in reducing the obesity with decrease in serum cholesterol and triglycerides (Karnick, 1992). Its aqueous extract shows significant hypolipidaemic activity in Wistar rats (Nagulendran et al., 2007). An alcoholic extract of its tuberous part exhibits significant wound healing activity in three different types of wound models (Puratchikody et al., 2006). In addition to this, a number of pharmacological activities *viz.*, anticancer, cytoprotective, gastroprotective, antidiabetic, antimalarial, antimicrobial and antioxidant properties have been attributed to this plant (Singh et al., 2012). Benefits of *Cyperus rotundus* have been documented in *Charaka Samhita*. In Chinese Traditional Medicine it is credited with the ability to

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improve the circulation of 'qi'. In Ayurveda, its root and rhizome have been reported to possess multiple therapeutic actions. In addition, *Cyperus rotundus* is ethnobotanically well-known as herbal galactagogue, which induces secretion of milk (Ahmad, 2007). However, there is no scientific evaluation/validation on this assertion of traditional practices established earlier in scientific way. With this goal in mind, we aim to evaluate the lactogenic activity of aqueous extract of *Cyperus rotundus* rhizome (CRE) for increasing milk yield in lactating animal. Experiments were performed to determine whether CRE can stimulate milk production and mammary gland development in rat which may be useful in development of a potential herbal galactagogue agent.

2. Materials and methods

2.1. Plant specimen and preparation of plant extract (CRE)

Plant specimen sample of *Cyperus rotundus* Linn (Voucher specimen No.: BM-25) was identified by Prof. D. A. Patil, Taxonomist, North Maharashtra University, Jalgaon, India. Rhizome of *Cyperus rotundus* was air dried in shade, pulverized by a mechanical grinder and passed through a sieve of mesh size 0.25 mm. Then, resulting rhizome powder was boiled in water for 15 min (1:4 w/v). After filtration, the filtrate was freeze-dried and the dry material was weighed.

2.2. Effect of oral treatment with CRE on milk production

Twenty four lactating dams weighing 250–300 g at the beginning of lactation and suckling six pups were used for this experiment. The experimental protocol was approved by Institutional Animal Ethics Committee of National Institute for Research in Reproductive Health, Mumbai. The females were divided into four groups of six animals each. Control group i.e. Group I rats were treated with vehicle (1 ml of 0·9% NaCl) orally, Group II and Group III rats were orally administered 300 and 600 mg/kg BW, respectively, of CRE suspended in vehicle. Group IV animals were treated orally with 2.5 mg/kg BW of domperidone (reference galactagogue). The extracts were administered orally with a gavage syringe each day at 1600 h from second day of lactation. Milk production was measured from day 3 to day 15 of lactation by using method described by Hosseinzadeh et al. (2013).

2.3. Estimation of the glycogen and protein content of mammary gland tissue

On 16th day of parturition, the lactating mother rats were euthanized and whole mammary glands were excised. About 100 mg of mammary tissue was homogenized in equal volume of distilled water and 30% saturated potassium hydroxide. Then, the reaction mixture was incubated for 30 min at 65 °C. The resulted reaction homogenate was used for quantitative estimation of glycogen and protein (Dubois et al., 1956; Bradford, 1976).

2.4. Serum prolactin and cortisol level

On 16th day of parturition, mother rats were adjusted to blood sampling procedure through retro-orbital sinus. The blood samples were centrifuged and serum was stored at −70 °C. Hormonal level of serum was estimated by using enzyme immunoassay.

2.5. Histopathological examination

Histopathological analysis of mammary gland and vital organs were performed by way of optical microscopy on paraffin material. After termination of experiment, whole mammary glands and other vital organs *viz.*, pituitary gland, heart, liver, kidney, spleen, and ovaries with oviduct were excised from each experimental group of animal. Separated tissues of each organ were fixed in Bouin solution. The method of Slaoui and Fiette (2011) was used for processing and staining of tissues for histological studies.

2.6. Toxicity assay

Experimental group of rats received CRE at doses of 200, 400, 600, 800, 1000, 1500, 2000 mg/kg BW. Three animals were used for each dose of the CRE and one control animal received the vehicle (Goyal and Sasmal, 2014). Animals were observed individually in an open field at 10, 20, 30, 40, 50, 60, 120, 240, 360 min and 24 h for a period of 4 days for toxic symptoms. At the end of the experiment, blood samples of each animal were collected through retro orbital plexus/sinus and subjected for biochemical analysis. Commercial Erba Diagnostics Kits, Germany were used for biochemical analysis of serum samples of experimental animals. Examination was performed on fully automated biochemistry analyzer (Erba 200, Mannheim, Germany).

2.7. Statistical analysis

The mean, SEM and the level of significance for the difference between means was determined by Dunnett Test and was computed using GraphPad Prism 4.

3. Results

3.1. Effect of CRE on milk production and pups body weight

Milk production of both groups receiving 300 and 600 mg of CRE was higher than control and domperidone treated groups (Supplementary Fig. 1). Milk yield increased from 1.62, 1.09, 1.51 and 1.61 g/pup per day to 4.83, 4.21, 5.57 and 6.75 g/pup per day for the controls, domperidone treated and those receiving 300 and 600 mg of CRE respectively. The milk yield was 0.33, 0.37, 0.42 and 0.54 g/pup/5 h over the experimental period respectively (Supplementary Table 2).

All pups gained weight during the study period (Supplementary Fig. 2) and the rate of weight gain for the CRE-treated groups was significantly higher than that for the control and domperidone treated groups. Body weight increased from 7.82 to 26.98 g/pup per day for the controls, from 7.58 to 29.02 g/pup per day for the domperidone, from 7.93 to 32.46 g/pup per day and 8.82 to 35.78 g/pup per day for those receiving 300 and 600 mg of CRE respectively (Supplementary Fig. 2). The daily weight gain was 1.60, 1.79, 2.04 and 2.25 g/pup respectively (Supplementary Table 2).

3.2. Mother weight profile

CRE significantly increased mother rat's weight compared to the control animals (Supplementary Table 2). Interestingly, a noteworthy visual difference was noted in the external appearance of mammary glands of experimental animals. Control groups of mammary glands were small sized with small nipples where as CRE and domperidone treated group of animal's mammary gland exhibited a great enlargement in their size.

3.3. Total protein and glycogen content of mammary tissue

The level of milk protein and glycogen were significantly increased in mammary tissue of plant extract treated mother rats as compared with control group of animals. About 32.44%