



## EFFECT OF *COSTUS SPECIOSUS* KOEN ON REPRODUCTIVE ORGANS OF FEMALE ALBINO MICE

Choudhury Najma<sup>1\*</sup>, Kalita Jogen Chandra<sup>1</sup> and Haque Ansarul<sup>2</sup>

<sup>1</sup>Animal Physiology and Biochemistry Lab., Department of Zoology, Gauhati University, Guwahati-14, Assam, India

<sup>2</sup>Department of Zoology, Baosi Banikanta Kakati College, Nagaon, Barpeta, Assam, India

Article Received on: 11/02/12 Revised on: 19/03/12 Approved for publication: 20/04/12

\*E-mail: najmach@gmail.com

### ABSTRACT

*Costus speciosus* Koen. Retz. belongs to family Zingiberaceae. It is known as Crepe zinger in English and Jom lakhuti in Assamese. Traditionally, rhizome of this plant is used as ethno-medicine for curing different health ailments. This plant is widely used for fertility control in women by the rural people of Rangia Sub-division of Kamrup District, Assam. In ayurveda, the rhizomes are ascribed to be bitter, astringent, acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant and tonic. The methanolic rhizome extract was investigated for its effect on ovary and uterus of Gonado-intact female adult mice. The extract at two different doses (250 mg/kg, 500mg/kg body weight) for 10 days has showed significant decrease in ovarian weight and increase in uterine weight in comparison to normal control. The phytochemical screening revealed the presence of secondary metabolites i.e., alkaloids and flavonoids. The finding of the present study put some light showing the endocrine active effects of the *Costus speciosus* in animal model.

**Keywords:** *Costus speciosus*, phytochemical screening, ovarian weight, Rangia Sub-division

### INTRODUCTION

*Costus speciosus* Koen., belongs to family Zingiberaceae. It is known as Crepe zinger in English, Jom lakhuti in Assamese. It is a succulent perennial herb, growing up to 2.7 m high and having an erect stem. The plant possesses horizontal rhizomatous rootstock. It generally grows luxuriantly on clayey loam soil near inland forest under moderate shade. The plant propagates vegetatively through rhizomes or via seeds dispersed by birds. Rhizome of this plant is used as medicine. In ayurveda, the rhizomes were ascribed to be bitter, astringent, acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant and tonic. It is mentioned in the Kama Sutra as an ingredient in a cosmetic to be used on the eyelashes to increase sexual attractiveness. The rhizome of the plant is used as estrogenic, antibacterial, anti inflammatory, diuretic, antidiabetic and hepatoprotective. There are many reports on the antifertility action of *Costus speciosus*. The present investigation was carried out to elucidate the possible mechanisms for the endocrine active effect of phyto-constituents and endocrine active effect of methanolic rhizome extract of *Costus speciosus* on mice. As far our knowledge this study was not reported previously.

### MATERIALS AND METHODS

#### Collection and Identification of Plant Materials

The rhizome of *Costus speciosus* was collected from the rural area of Kamrup district of Assam, India during August, 2009 and the flowering plants were collected during July, 2009. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam. One voucher specimen was deposited in the same department for future reference.

#### Processing and Extraction

The collected rhizome was rinsed gently with tap water and then dried for one week in a shady well ventilated room. The rhizomes were grinded into fine powder by using stainless steel blender, the powder placed in glass bottle and stored at -20 °C until use. Then, 40 gm powder of the rhizomes was

extracted with 1L of methanol (Merck India Pvt. Ltd.) in a Soxhlet extractor for 72 h. The extract was concentrated and dried under reduced pressure and controlled temperature (50-60 °C) to yield a reddish solid (3.55 g). The yield of plant extract was 8.8% and thus prepared extract was stored in refrigerator for future use.

#### Preliminary Phytochemical Screening Test

Preliminary phytochemical screening of the plants was done using standard method for secondary metabolites like as tannins, phenols, alkaloids, steroids, terpenoids, flavonoids, saponins and volatile oils<sup>1,2,3</sup>. The types of compounds and their method of qualitative investigation were as follows-

**Tannin:** One gm grinded sample was boiled in 20 ml ethanol (70%) for 2 minutes in a hot plate. The mixture was filtered and a portion of filtrate diluted in water in a ratio of 1:4 and 3 drop of 10% ferric chloride (FeCl<sub>3</sub>) solution was added. Blue black precipitate indicates the presence of tannin.

**Phenol:** 2 ml of above extract was added to 2 ml of neutral ferric chloride (FeCl<sub>3</sub>) solution. A deep bluish green solution was formed with the presence of phenol.

**Alkaloid:** 5 gm ground plant material extracted with 10 ml ammonical chloroform and 5 ml chloroform. After filtration, the solution was shaken with 20 drops of 0.05M sulphuric acid, a creamish precipitate indicate the presence of alkaloids.

**Steroid and Terpenoid:** 200 gm plant material boiled in 10 ml chloroform and mixture was filtered. Out of this, 2 ml of filtrate was taken and into this filtrate, 2 ml acetic anhydride and concentrated sulphuric acid were added. A blue green ring and red colour of the solution indicated the presence of steroid and terpenoid respectively.

**Flavonoid:** Alcoholic extract (15 ml ethanol in 3 gm of plant material) was treated with few drops of concentrated HCl and Magnesium ribbon (0.5 gm); pink tomato red colour indicated the presence of flavonoid.

**Saponin:** Methanolic extract (0.5 gm) shaken with ethyl ether and 5 ml HCl, forming of precipitation indicated the presence of Saponin.

**Volatile Oil:** One gm fresh sample was boiled in 10 ml petroleum ether, filtered and then 2 ml of extract was shaken with 0.1 ml diluted sodium hydroxide and a small quantity of diluted HCl, white precipitate indicated the presence of volatile oil.

#### Experimental Animal

Adult female albino mice weighing 25-30 gm were used for the study of endocrine activity and adult albino mice of either sex were used for acute toxicity studies. All the mice were maintained under standard husbandry conditions at the animal house of the Department of Zoology, Gauhati University providing food and water ad libitum. Experiments on animals were approved by the Institutional Animal Ethics Committee (IAEC) and accepted according to veterinary medical practice.

#### Acute Toxicity Studies

The acute oral toxicity was carried out by using the Turner method<sup>4</sup>. The animals were fasted overnight and caged in five different groups containing three animals in each group. The plant extract suspension was prepared using 1% Tween-80 in normal saline. The plant extracts were administered orally at four different doses 500, 1000, 1500 and 2000 mg/kg body weight respectively to each group. The control group received the vehicle 1% Tween-80 only. After treatment, all the animals were observed for mortality and behavioural changes at least once daily for 14 days.

#### Effect of Plant Extract on Ovary and Uterus

A total 18 numbers of sexually matured, healthy virgin female albino mice weighing 20-25 mg were used for this experiment. The animals were divided into 3 groups consisting six animals in each group. Group A was treated as control received 0.2 ml Tween-80 (1%) while group B and group C were treated by plant extract at the doses of 250 and 500 mg/kg body weight in 0.2 ml 1% Tween-80 respectively. All the treatments were given orally by intragastric gavages. The treatments were started from estrous phase only as the ovarian activities change markedly from one phase to another phase of estrous cycle. The treatments were given orally everyday morning between 10 to 11h for 10 days<sup>5</sup>.

#### Determination of Ovarian and Uterine Weight

Body weights were determined just before the killing of each animal. After making an incision in the abdomen, the uteri along with ovaries were removed<sup>6</sup>. Fat and connective tissue if any was trimmed away and weighed immediately (wet weight) using a sensitive electronic balance. The relative ovarian and uterine wet weight to body weight ratios were calculated for each animal by dividing the organ wet weight by the body weight (bw) and multiplying by 100.

#### RESULTS

##### Preliminary Phytochemical Screening of *Costus speciosus*

The result of the various test methods of preliminary phytochemical screening of the plant showed positive response for alkaloids, flavonoids, saponins, steroids and tannins. However, the screening tests for phenol, terpenoid and volatile oil did not show encouraging results (Table 1)

##### Effects of Plant Extract on Ovary and Uterus

The results of the effects of at two different doses of methanolic extracts of the *Costus speciosus* on ovarian weight in adult female albino mice are shown in Table 2. After 10 days of oral exposure both the low and high doses (250 and 500 mg/kg bw/d respectively) caused a significant decrease in the ovarian weight in comparison to the untreated vehicle control mice (Table 2). On the other hand, in the case

of uterine wet weight, some reverse trends of results were documented while compared to ovarian weight. Both the plant extracts exerted an increasing effect in uterine wet weight in the experimental mice. The untreated control animal had a comparatively low uterine weight than that of the treated groups of the present study (Table 3).

#### Acute Toxicity Study

Acute toxicity study of the plant extract on albino mice of both sexes showed that up to dose 2000 mg/kg body weight no mortality and no any significant behavioural changes were found during the study.

#### DISCUSSION

In the study on preliminary phytochemical screening for *Costus speciosus* alkaloids, flavonoids, saponins, sterols, tannins were detected. Alkaloids and flavonoids from plant origin were tested for antifertility efficacy and found to be effective for including estrogenic response and thus having contraceptive activity<sup>7</sup>. In this study, the extracts of the plants exhibited marked uterotrophic activity in treated animals. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Treatment of methanolic extract of *Costus speciosus* at both high dose and low dose showed significant decrease in the ovarian weight, this might be associated with inhibition of release of tropic pituitary gonadotropins due to the negative feedback mechanism of the drug which is estrogenic in nature<sup>5</sup>. Treatment of the plant extract showed significant increase in the uterine wet weight and it indicated that the plant had endocrine active estrogenic property because typical estrogenic compound possesses ability to increase the uterine weight<sup>8</sup>. In the present study, the methanolic extract of *Costus speciosus* cause reduced in the ovarian weight, increased in uterine wet weight, hence it can be concluded that this plant have potential endocrine active agents.

#### ACKNOWLEDGEMENT

We are thankful to Dr. G. C. Sharma, Department of Botany, Gauhati University, India for providing necessary facilities in Herbarium and Library consultation. We also express our deep sense of gratitude to Prof. R. K. Bhola, Head of the Department of Zoology, Gauhati University for providing all facilities for experiment and laboratory animals. We are thankful to UGC and Ministry of Minority Affairs, Govt. of India for financial assistance.

#### REFERENCES

1. Sofowora A. Screening plants for bioactive agents, *In: Medicinal Plant and Traditional Medicine in Africa*, 2<sup>nd</sup> ed, Sptum Books Ltd., Ibadan, 1993; 289.
2. Evans WC. An overview of drugs having antihepatotoxic and oral hypoglycemic activities. *In: Trease and Evans, Pharmacognosy*, 14<sup>th</sup> ed. UK, Sanders Company Ltd.; 1996: 119-159.
3. Dahiru D, Onubiyi JA, Umaru HA. Phytochemical screening and anticarcinogenic effect of *Moringa Oleifera* aqueous leaf extract. *African J Tradition Comp Alt Med* 2006; 3:70-75.
4. Turner RA. *Screening Methods in Pharmacology*, New York: Academic Press.;1971; 2.
5. Kage ND, Malashetty BV, Seetharam YN, Suresh P, Patil SB. Effect of Ethanol extract of whole plant of *Trichosanthes cucumerina* var. *Cucumerina L.* on Gonadotropins, ovarian follicular kinetics and estrous cycle for screening of antifertility activity in albino Rats. *Int J Morphol* 2009; 27(1):173-182.
6. Padilla-Banks E, Jefferson WN, Newbold RR. The immature mouse is a suitable model for detection of estrogenicity in the uterotrophic assay. *Environ Health Perspect* 2001; 109: 821-826.
7. Thakur S, Bawara B, Dubey A, Chauhan NS, Saraf DK. Effect of *Carum carvi* and *Curcuma longa* on hormonal and reproductive parameter of female rats. *Int J Phytomed* 2009; 1: 31-38.

8. Sarangouda, Patil SB. Estrogenic activity of petroleum ether extract of seeds of
- Citrus medica*
- on immature albino rats. Int J Green Pharm 2008; 91-94.

**Table 1:** Preliminary phytochemical screening of *Costus speciosus* for secondary metabolites

Phytochemicals	Status of detection
Alkaloid	+
Flavonoid	+
Saponin	+
Steroid	+
Tannin	+
Phenol	-
Terpenoid	-
Volatile oil	-

'+' indicates the presence while '-' shows the absence of the chemical in the plant

**Table 2:** Effect of methanol extract of rhizome of *Costus speciosus* (MECS) on relative ovarian weight of 10 days exposed oral fed female adult albino mice. Values are shown as mean  $\pm$  SEM

Animal groups (n = 6 animal per group)	Treatment doses	Relative ovarian weight (mg/100 gm bw)
Control	1% Tween-80, 10 ml/kg	48 $\pm$ 0.003
MECS	250 mg/kg bw/d	29 $\pm$ 0.001*
MECS	500 mg/kg bw/d	26 $\pm$ 0.002*

Note: Asterisk denote the statistically significant difference with control ( $p < 0.05$  and  $0.05$ )

**Table 3:** Effect of methanol extract of rhizome of *Costus speciosus* (MECS) on uterine wet weight of uterus of 10 days exposed oral fed female adult albino mice. Values are shown as mean  $\pm$  SEM

Animal groups (n = 6 animal per group)	Treatment doses	Relative uterine weight (mg/100 gm bw)
Control	1% Tween-80, 10 ml/kg	58 $\pm$ 0.002
MECS	250 mg/kg bw/d	197 $\pm$ 0.034*
MECS	500 mg/kg bw/d	205 $\pm$ 0.033*

Note: Asterisk denote the statistically significant difference with control ( $p < 0.01$  and  $0.05$ )

Source of support: Nil, Conflict of interest: None Declared