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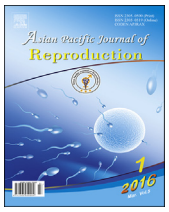

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Phytochemical screening and evaluation of anti-fertility activity of *Dactyloctenium aegyptium* in male albino rats

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ABSTRACT

Objective: To find out the effect of ethanol extract of *Dactyloctenium aegyptium* (*D. aegyptium*) on the fertility of male albino rats.
Methods: Experimental animals were divided in four groups of six each. Group first received normal saline and served as control. The second, third and fourth groups of animals were administered with ethanolic extract of *D. aegyptium* extract at a dose of 200, 400, and 600 mg/kg body weight, respectively for a period of 30 days.
Results: A non significant increase in the bodyweight and a significant decrease in weight of testes, accessory sex organs, and reduction in sperm count, increase motility and abnormality were observed. Some serum biochemical parameters showed significant variations and were as the serum hormonal levels are significantly decreased.
Conclusions: The decreased levels of sperm count, weight of reproductive organs, serum hormonal levels and number of implantations in female rats reveals the antifertility activity of *D. aegyptium* and it was with dose dependent manner.

1. Introduction

Population control is an issue of global and national public health concern. Birth control is an essential part of our life. A variety of synthetic contraceptive agents are available in the market only for women's and their use is associated with severe side effects, the progress and possibilities on male are still slow and limited [1]. The World Health Organization suggested that practice of usage of traditional medicine for the control of fertility, instead of synthetic drugs, as cost effective management for Birth control [2]. *Dactyloctenium aegyptium* (*D. aegyptium*) is a common coarse grass belonging to Poaceae family [3], found commonly throughout India, Nepal, Pakistan, Afghanistan, Israel, Lebanon, Turkey, Sri Lanka, Malaysia, Myanmar, Philippines, China, Japan, Singapore, Thailand, Vietnam, New Guinea, Algeria, Morocco, Egypt, Sudan, Tunisia and Libya. It is reported to have astringent, bitter tonic, anti-anthelmintic and used to treat gastrointestinal, biliary, urinary ailments, polyurea, fevers, smallpox, heart burn, immunodeficiency, urinary lithiasis, spasm of maternity, renal infections, gastric ulcers, wounds healing and the seeds are used by tribesmen to prepare liquor as well as famine food with unpleasant taste [4] and it is also used against cough [5]. And, it also found that plant also reported to have antimicrobial activity [5], diuretic, antipyretic, and anti-proliferative activity [6]. The main objective of the present study is to evaluate antifertility activity of ethanolic extract of *D. aegyptium*.

2. Materials and methods

2.1. Procurement and identification of plant materials

Plant of *D. aegyptium*, family Poaceae, was collected from Madanapalle, chittoor district of A.P. The plant was identified and authenticated by Dr. J. Raveendra Reddy, M.Pharm Ph.D., Raghavendra Institute of Pharmaceutical Education and

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2.2. Preparation of extract

The whole plants of *D. aegyptium* were dried in shade. Powdered using a grinder and sieved. Weighed quantity (1 000 g) of fresh, finely grounded powder was mixed with ethanol (95%) and subjected to maceration with intermittent shaking for seven days. The extract then obtained was collected by filtration using muslin cloth. Thus obtained filtrate was subjected to solvent evaporation to obtain solid extract which was weighed and stored in an air tight container.

2.3. Preliminary phytochemical screening

Freshly prepared ethanolic extract was subjected to quantitative analytical tests for the detection of various chemical constituents like carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, terpenoids and alkaloids [6].

2.4. Animals

Male Albino Wistar Rats weighing 200–250 g were used for the present study. The animals were collected from Central Animal Facilities, Indian Institute of Science, Bangalore. The animals were maintained under controlled conditions of temperature (22 ± 2 °C), humidity [(50 ± 5)%] and 12 h light–dark cycles. All the animals were acclimatized for seven days before the study. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Ananthapuramu. Approval No. 878/ac/05/CPCSEA/003/2015 dated 06/01/2015, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.5. Acute toxicity study of the extract

The test performed according to OECD guidelines 423. Swiss albino mice were administered with ethanolic extract up to 2 000 mg/kg. Animals were observed for gross behaviour changes as well as for motility for 14 days [7].

2.6. Treatment protocol

The animals were equally divided into four groups (6/group). Group I animal were given normal saline for 30 days and they served as control. Group II, III, and IV animals received ethanolic extract at dose levels of 200, 400, 600 mg/kg body weight daily per oral for 30 days.

2.7. Determination of body and reproductive organ weight

The initial and final body weight of animals was recorded. Blood samples were collected by retro orbital puncture, then the testes, epididymis, vas deferens, seminal vesicle and ventral prostate were dissected out, freed from adherent tissue and weighed.

2.8. Sperm concentration, motility and abnormality

The caput and cauda regions of epididymis was chopped separately in two petridishes and 1 mL of normal saline at temperature of 36 °C was added to the semen to enhance sperm survival *in-vitro* during the period of the study. The semen mixture was then sucked into a red blood pipette to the 0.5 mark, diluted with warm normal saline, sucked up to the 101 mark. The normal saline at the stem of the pipette was discarded and the content was mixed thoroughly. A drop of the semen mixture was placed on the neubauer counting chamber which spread under the cover slip by capillary action. The chamber was mounted on the stage of microscope, viewed under the magnification of ×40 and counted and expressed in million per mL [8]. A drop of the sperm saline mixture was taken in a separate glass slide. One slide was covered with a cover slip and examined under the microscope. Sperm motility at the caudal epididymis was then assessed by calculating the motility spermatozoa per unit area. A smear was made on another slide and total morphological abnormalities were observed [9].

2.9. Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and liver marker enzymes like SGPT, SGOT, and ALP was estimated by using standard procedure [10–13].

2.10. Estimation oxidative parameters in blood serum

Blood were collected from retro orbital method and serum was separated by using micro-centrifuge.

2.10.1. Catalase (CAT)

Two milliliter of serum diluted with 1 mL of H_2O_2 and take the absorbance at 240 nm for 3 min with 30 s intervals. (Add H_2O_2 just before taking O.D) [Aebi-1984].

2.10.2. Reduced glutathione (GSH)

The serum was precipitated with 20% trichloro acetic acid (TCA) and centrifuged. 0.25 mL supernatant was taken for GSH estimation using freshly prepared DTNB solution (2 mL) and volume up to 3 mL with phosphate buffer (pH 8). The intensity of the yellow colour formed was read at 412 nm against blank for each sample without reagent was run. The GSH content was calculated by using $\varepsilon = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$ expressed as nmol/g serum [14].

2.10.3. Superoxide dismutase (SOD)

0.5 mL of serum was diluted with 0.5 mL distilled water, to this add all chilled reagents, 0.25 mL ethanol, 0.5 mL of chloroform and shaken for 1 min and centrifuged at 2 000 rpm for 20 min supernatant enzymatic activity was determined to it 0.05 mL of carbonate buffer (0.05 M pH 10.2) and 0.5 mL EDTA (0.49 M) was added. The reaction was initiated by addition of 0.4 mL epinephrine and the change in optical density/mm was measured at 480 nm. SOD was expressed as U/mg protein [15].