

ANTIFERTILITY AND CONTRACEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF *SACCHARUM OFFICINARUM* LINN IN RATS

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ABSTRACT

In the present study, antifertility activity, contraceptive activity of methanolic extract of *Saccharum officinarum* Linn was evaluated. The methanolic extract of *Saccharum officinarum* showed significant antifertility activity and contraceptive activity. Pretreatment with methanolic extract showed significant inhibition of number of implant site at a dose of 500 mg/kg. There was no change in ovulation, hence the antifertility activity observed in the present study with *Saccharum officinarum* can be attributed largely to its anti-implantation, blastocidal, antizygotic & abortifacient activity.

KEYWORDS: *Saccharum officinarum*, antizygotic, blastocidal, anti-implantation, abortifacient, contraceptive activity.

1. INTRODUCTION

India, within few years of time span will be the leading country, as far as the population growth is concerned. Since the population rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effect produced by the use steroidal contraceptive and use of abortifacient drugs (Chopra, 1992). There is a need of drug which is effective with lesser side effects.

The use of natural products as fertility regulating agent have been known since times immemorial and are still practiced in rural areas. A large number of plants have been reported to exhibit anti-implantation and abortifacient activity but a few have been evaluated for such effects in laboratory animals (Chopra, 1992). Many of these plant products having inherent estrogenic or antiestrogenic effects possibly bring about alteration in tubal transport of blastocyst or hormonal milieu of the uterus making the uterine environment hostile for implantation or foetal development.

Saccharum officinarum (Family-Poaceae) commonly known as sugarcane, noblecane is widely cultivated throughout tropic and subtropic regions. It is used as a folk medicine and is used as an antidote, antiseptic, antivenomous, bactericide, cardiogenic, demulcent, diuretic, intoxicant, laxative, pectoral,

refrigerant and stomachic. It is a folk remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhoea, dysentery, eyes, fever, hiccups, inflammation, laryngitis, opacity, penis, skin, sores, sore throat, spleen, tumors and wounds (Duke and Atchley, 1998). Powdered sugar is used as a 'drawing' agent for granulations and "proud flesh" and in a 1:3 solution in water, for gonorrhoea and vaginal discharges (Hartwell, 1967; Watt and Breyer, 1962).

2. Materials and Methods

Plant material and extraction :

Leaves of *Saccharum officinarum* were collected, authenticated by the Department of Botany, Annamalai University, India. The collected leaves were dried under shade, segregated and pulverized by mechanical grinder and the powder was passed through sieve No: 22. The powdered materials were successfully extracted by hot continuous percolation method in Soxhlet apparatus for 10 hours. The residue obtained was then utilized for evaluating antifertility efficacy by suspending in tween 80(2%).

Animal:

Healthy, adult female albino rats (weighing 120-160 g) for antifertility studies and bilaterally ovariectomized immature female rats (8 weeks old) for bioassay studies were used as experimental animal model. All the animals were housed in standard laboratory conditions (temperature 22 ± 2°C and 12hr light/12hr dark cycle & 45-60% humidity) with standard pellet diet (Ashirwad Industries Ltd; India) and tap water

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ad libitum. All the experimental procedures were performed according to the guidelines for the care and use of experimental animals and approved by the Institutional Ethical Committee for Animals.

Experimental Design:

Albino rats are divided into 6 groups (n=6) in each group as per OECD guidelines and one group was administered with normal saline (1ml/kg/p.o) which serves as control (group-I) and the remaining groups (II,III,IV,V,VI) were administered with 500 mg/kg/ p.o of *Saccharum officinarum* extract. Different groups of female rats with vaginal smear from each rat were examined daily for 15 days and those rats exhibited three regular cycles were used. Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. The animal which showed thick clumps of spermatozoa in vaginal smear were separated from the male partner.

The anti-implantation and anti-fertility activities of each sample were calculated using the following formula.

Anti-implantation Activity =

$$\frac{\text{No. of implants in control} - \text{No. of implants in test group}}{\text{No. of implants in control group}} \times 100$$

Anti-fertility activity =

$$\frac{\text{No. of animals showing no implantation}}{\text{Total No. of animals}} \times 100$$

Acute toxicity study

Acute toxicity study of methanolic extract of *Saccharum officinarum* Linn were carried out in mice according to OECD-423 guidelines. Extract at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 14 days after acute toxicity dose. There was no toxic reaction or mortality and found to safe. Based on acute toxicity results it was selected 500 mg/kg/p.o were used for antifertility and contraceptive for further studies. All the results of biochemical parameters are expressed as mean \pm SEM and significance was analyzed statistically by students 't' test.

Antizygotic activity

Group II animals received methanolic extract of *Saccharum officinarum* at 500 mg/kg, respectively from day 1 to 3 after mating. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy.

Abortifacient activity:

Methanolic extract of *Saccharum officinarum* at 500 mg/kg was administered to group III animals

respectively from day 14th, 15th and 16th after mating. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy.

Contraceptive activity:

From 1st day to 21st day after mating (Bingel and Benoit, 1973), methanolic extract of *Saccharum officinarum* at 500 mg/kg were administered to group IV animals respectively.

All the three groups (II,III, IV) animals were checked with ultra sonic scanner and animals of all the groups were laprotomized on the 21st day of pregnancy and the numbers of implantation sites in both the horns of the uterus are to be recorded. The numbers of pups delivered after completion of term are recorded (table-1).

Blastocidal activity:

Methanolic extract of *Saccharum officinarum* at 500 mg/kg were administered to first half of the group respectively from day 4 to 5 of pregnancy. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy.

Anti-implantation activity:

Group II animals received methanolic extract of *Saccharum officinarum* at 500 mg/kg, respectively from day 1 to 7 after mating. The day when spermatozoa detected in vaginal smear was considered as day 1 of pregnancy.

First half of the animals present in the groups (V&VI) were checked with ultra sonic scanner, laprotomized on the 21st day of pregnancy and the numbers of implantation sites in both the horns of the uterus are to be recorded (table-1). The remaining half of the animals were sacrificed with mild ketamine anaesthesia for studying tissue biochemical parameters (table-2).

3.RESULTS

Table: 1. Results of Activities of *Saccharum officinarum* extract at 500 mg/kg, p.o administered

Groups	Activity	Drug Admin/Dose	No. of Rats Treated	No. of Rats pregnant	Percentage reduction of pregnancies	No. of implants in individual Rats	No of Rats delivered
I	Control	1 st day to 21 st day	6	6	0	5,7,8,6,8,4	4,6,7,5,7,4
II	Antizygotic activity	1-3 days after mating, 500mg/kg, p.o	6	0	100	0,0,0,0,0,0	0,0,0,0,0,0
III	Abortifacient activity	14 th , 15 th and 16 th after mating, 500mg/kg, p.o	6	0	100	0,0,0,0,0,0	0,0,0,0,0,0
IV	Contraceptive	1 st day to 21 st day, 500mg/kg, p.o	6	0	100	0,0,0,0,0,0	0,0,0,0,0,0
V	Blastocidal activity	4 th and 5 th days after mating, 500mg/kg, p.o	6	0	100	0,0,0,0,0,0	0,0,0,0,0,0
VI	Anti implantation activity	6 th and 7 th days after mating, 500mg/kg, p.o	6	0	100	0,0,0,0,0,0	0,0,0,0,0,0

Table-2 Comparison of tissue biochemical parameters of *Saccharum officinarum* extract with control treated rats

Parameters	Tissue	Control	<i>Saccharum officinarum</i> extract
Glycogen (mg/g)	Ovary	5.59 ± 0.21	3.76 ± 0.23*
	Uterus	7.07 ± 0.23	3.54 ± 0.21*
Sialic acid (mg/g)	Ovary	0.964 ± 0.01	0.744 ± 0.01**
	Uterus	0.963 ± 0.01	0.770 ± 0.01**
Cholesterol (mg/g)	Ovary	8.84 ± 0.55	10.91 ± 0.33**
	Uterus	4.15 ± 0.18	7.07 ± 0.19**

Values are mean ± S.E.M, Percentage inhibition when compared to control. Values are statistically significant at *=P < 0.001, **=P < 0.01.

DISCUSSION:

The results revealed that *Saccharum officinarum* extract 500mg/kg/p.o for 21 days has shown diminished circulating level of estrogen. Endometrial glycogen (Montgomery, 1957) is one of the most important factors for the development anti-implantation of blastocyst in early stages of gestation. An increase in glycogen mobilization provides nutritive support to the developing blastocyst for their survival. However, in the present study, a significant decline (p<0.001) in the uterine glycogen content in extract treated female rats indicate poor nutritive support to the developing blastocyst for their survival. These findings are correlated with many earlier reports which indicates that substances of plant origin besides exhibiting antifertility efficacy also causes a concomitant inhibition of the glycogen level of the mammalian uterus. Inhibition of glycogen content in the uterus is due to the antiestrogenic nature of the extract and may cause suppression in motor activity of the uterus.

Sialomucoprotein, a derivative of sialic acid (Warren, 1959), forms mucous in the uterus and vagina and sticks around the blastocyst fluid which ultimately helps in the attachment of the blastocyst. In addition, sialic acid also acts as an immuno-barrier between the mother and embryo, thereby, has a vital role in embryo implantation in uterus. In the present study, a significant change in the sialic acid content of uterus in extract treated female rats was observed, which account for blastocidal activity (Christie, 1966; Saldarine, 1968).

Cholesterol (Zlatkis, 1953) is the precursor of sex hormones and is utilized during steroidogenesis. During the investigation the cholesterol concentration of ovary and uterus increased after *Saccharum officinarum* extract treatment, indicating non utilization of cholesterol by the system. Hence reduced level of circulating estrogen contributes to altered physiology of

female reproductive system.

On the basis of the above observations it may be concluded that *Saccharum officinarum* methanolic extract owing to its potent anti-implantation, blastocidal, antizygotic & abortificant activity which lead to change the normal status of the reproduction in female reproductive tract of rat and thus produce significant antifertility effect.

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