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Antifertility activity of methanolic extract of *Saccharum officinarum Linn*. (Leaves) on Female Albino Rats

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Abstract: Normal cyclic female Wistar strain weighing between 120-160 gm were treated with *Saccharum officinarum Linn*. (Leaves) methanolic extract (500 mg/day/b.wt) for 21 days. The results revealed that *S.officinarum* treatment caused reduction in reproductive organ weights, circulating level of estrogen, fertility, number of litters, serum glucose concentration, enzyme activity of alkaline phosphatase, SGOT and SGPT as compared to control value. Whereas, the concentration of cholesterol increased by *S.officinarum* treatment, revealing non-utilization of cholesterol by the system. However the haematological parameters remained within normal range. The data suggests that the *S. officinarum* methanolic extract exerted antifertility and antiestrogenic effects in female rats. The effects brought by *S. officinarum* extract are non-toxic and transient. **Key words:** Antifertility, *Saccharum officinarum Linn*

Introduction

Population explosion is an imminent hurdle for a country's development as the natural resources are limited. The population of India is multiplying at an alarming rate and has crossed one billion. Fertility regulation has therefore become the major concern of people of all walks of life. In recent years, plants are persued over steroidal contraceptive drug because plants are easily available, economic and devoid of harmful side effects.

A large number of plants have been reported to exhibit anti-implantation & abortifacient activity but a few have been evaluated for such effects in laboratory animals¹. So far no single plant is available which can be developed further as a postcoital antifertility agent. Many of these plant products having inherent estrogenic or antiestrogenic effects possibly bring about alteration in tubal transport of blastocyte 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 also used as an antidote, antivenomous. bactericide. cardiotonic. antiseptic. 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 wounds². Powdered sugar is used as a *drawing* agent for granulations and *proud* $flesh^3$ and in a 1:3 solution in water, for gonorrhea and vaginal discharges⁴. A decoction of the root of the race of 'tebu lanjong' is used for whooping cough; and the cane juice is given for catarrh. It is used in elephant medicine; the juice is used to 'make an elephant sagacious', and in a poultice for sprains⁵. In India, the plant as well as its juices is used for abdominal tumors.

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 No 22 sieve. The powdered material was successfully extracted with methanol by hot continuous percolation method in soxhlet apparatus for 10 hours⁶. The residue obtained was then utilized for evaluating antifertility efficacy by suspending in tween 80(2%).

Experimental animal:

Healthy, adult Wistar rats (weighing 120-160 g) for antifertility studies and bilaterally ovarectomized 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 $22 \pm 2^{\circ}$ C and 12hr light/12hr dark cycle & 45-60% humidity) with standard pellet diet (Ashirwad

Industries Ltd; India) and tap water *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.

Dose and route of administration:

The animals of Group I received normal saline (1ml/Kg) only and served as control. Animals of Group II received crude methanolic extract of *S. officinarum* at 500 mg/kg b.wt./day (suspended with tween 80 [2%]) dose, administered orally using a curved needle and tuberculin syringe for 21 consecutive days respectively. The hematological and biochemical profile in rats was performed of 6 in a group and the results were analyzed statistically using student's 't' test.

Table – 1: Body weight (gm), organ weight (mg) of control and Saccharum officinarum treated

Treatm ent	Body weight (gm)		Organ weight(mg)						
	Initial	Final	Ovary	Uterus	Vagina	Adrenal	Liver	Kidney	Heart
Control	219.30± 0.71	244.00 ± 0.60	22.99± 0.03	76.95± 0.29	34.61± 0.10	10.31± 0.07	3751.55± 6.66	354.20± 1.06	374.65± 1.28
Extract (500mg/ kg/p.o)	186.36± 0.68	194.67 ± 1.25	17.01*± 0.18	52.9*± 1.78	24.06*± 0.53	13.02± 0.03	3454.42± 24.44	443.89± 2.13	346.05± 1.82

Values are mean \pm S.E.M, Percentage inhibition when compared to control.

*Values are statistically significant at P< 0.001.

Table – 2: Anti implantation activity of *Saccharum officinarum* in female rats

Treatment	No. of pregnancies / No. of treated	No. of implantation (mean ± S.E.)	Average litters delivered	Percentage (%) fertility
Control (Normal saline)	6/6	6.3±0.6	6.3	0
Extract (500 mg/kg/p.o)	0/6	0	0	100

Table - 3: Haematology of control and Saccharum officinarum treated rats

Tests/Groups	Normal values	Group-I (Control)	Group-II 500mg/kg/oral.
RBC(mill/mcl)	7-10	9.56±1.82	8.87±1.42
WBC(thous/mcl)	6-18	10.25±1.56	12.56±1.36
Hb(g/dl)	11-19.2	17.45±2.45	12.47±1.31
BT(sec)	60-120	70±2.11	76±1.17
CT(sec)	20-180	20±2.36	29±1.26
HT (%)	35-48	42.92±2.09	41.56±0.65
MCV(fl)	48-70	65.12±22.0	56.98±0.88
MCH(pg)	18-29	25.87±6.67	23.16±1.88
MCHC (%)	40-46	45.19±5.44	41.37±2.01
Lymphocytes (%)	40-80	42±3.1	46±2.13
Monocytes (%)	1-4	4±0.08	12±0.96
Neurtophils (%)	20-75	42±2.60	78±4.32

Values are mean \pm S.E.M, Percentage inhibition when compared to control.

Table – 4: Serum biochemistry of control and <i>Saccharum officinarum</i> on female rats						
	N	C	C II			

Biochemical Parameters	Normal values	Group-I	Group-II
/Groups		Control	500mg/kg/ p.o.
Glucose (mg/dl)	75-150	86.59±4.7	59.23±4.53*
Cholesterol (mg/dl)	20-80	55.11±1.14	92.32±1.35*
Creatinine (mg/dl)	0.5-1.6	0.5±0.04	0.6±0.04
Urea (mg/dl)	17-23.5	20.14±1.87	21.45±1.981
Uric acid (mg/dl)	2.5-7.5	5.44±0.57	5.11±1.08
Triglycerides (mg/dl)	124-156	134.72±4.4	152.23±4.1
Total Bilirubin (mg/dl)	0.25-0.74	0.5±0.07	0.58±0.07
Total Protein (g/dl)	5.3-7.5	6.3±2.3	6.4±2.5
Albumin (g/dl)	3.2-4.6	3.9±1.5	3.98±1.6
Alkaline Phosphates (U/l)	90-170	54.67±3.7	157.87±3.6*
Gamma Glutamyl Transferase (GGT) (U/l)	22-50	24.45±3.1	21.84±3.1
Serum Glutamic Oxaloacetic Transaminase (SGOT) (U/l)	42-98	66.45±2.14	50.89±3.00*
Serum Glutamic Pyruvic Transaminase (SGPT) (U/l)	12-67	57.29±2.8	40.45±3.0*
Lactate Dehydrogenate (LDH) (U/I)	120-220	142.69±5.8	153.54±6.2

#Merk manul & veterinary data; Values are mean \pm SEM; n=6 in each group. Percentage inhibition compared to control. *Values are statistically significant at P< 0.05.

Results and Discussions

The results revealed that S. officinarum methanolic extract (500mg/day/rat) for 21days did not change the body and vital organ weights, but the reproductive organ weights diminished significantly (p<0.001) as compared to control rats (Table 1). Following piper betel treatment to male rats decreased reproductive organ weights⁷. Administration of methanolic extract of S. officinarum extract at the dose 500mg/kg b.wt/day prevented pregnancy in all the treated female rats by virtue of antiimplantational property with antiestrogenic activity. Since the uterine biochemical milieu serves various functions, it enables the spermatozoa to ascend to the site of fertilization within the oviduct. It provides adequate nutrition for the embryo during its various developmental stages between its arrivals in the uterine lumen until it has achieved implantation and maintains an appropriate environment for the physical and biochemical integrity of the blastocyte structure. This meets specific immunological requirements, which becomes increasingly important during the pre implantation phase. There is possibility that estrogen agonistic or antagonistic activities of the plant substance may influence the uterine microenvironment making it hostile for implantation or for blastocyte to survive.

The haematological parameters WBCs, RBCs, haemoglobin content and haemotocrit values were all found within normal range ⁸(Table 3). This indicates that the methanolic extract of S. officinarum has no toxic effect on physiology of rat. Serum biochemistry revealed that glucose level was declined but cholesterol concentrations were elevated beyond control value; indicate non-utilization of cholesterol by the system, hence decrease in estrogen level9 However, nonsignificant change in protein concentration was observed as compared to control value. The enzyme activity of SGOT, SGPT diminished significantly (p<0.05) (Table 4), revealing enzyme inhibiting effects of plant extract. The alkaline phosphatase activity enhanced beyond control value¹⁰. The effects brought by *Saccharum* officinarum are antiestrogenic, leading to antifertility in female rats.

On the basis of the above observations it may be concluded that *Saccharum officinarum* methanolic extract owing to its potent antiestrogenic nature alters the biochemical milieu of the reproductive tract 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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