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Effect of methanolic extract of *Saccharum officinarum* on the biochemical milieu and histopathological profile of female albino rats

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ABSTRACT

The development of new fertility regulating drugs from medicinal plants is an attractive preposition. In the present study an attempt has been made to analyze the possible modulatory influence of methanolic extract of *Saccharum officinarum* on biochemical constituents (viz. glycogen, sialic acid, cholesterol, acid phosphatase and alkaline phosphatase profile which may play an important role in implantation and foetal development) and histopathology. Administration of methanolic extract of *Saccharum officinarum*(500mg/kg b.wt/p.o) from day 21st resulted in a statistically significant decline ($p<0.01$) in the acid phosphatase , alkaline phosphatase activity and glycogen content ($p<0.001$) when compared with control pregnant rats while the activity of cholesterol is increased as compared to the controls on day21st. Therefore, it can be suggested that the changes in the biochemical constituents of uterus may be responsible, for the anticontraceptive / anti-implantational effect of methanolic extract of *Saccharum officinarum* in the treated female rats.

Key words: *Saccharum officinarum*, fertility drugs, medicinal plants.

INTRODUCTION

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[1] but a few have been evaluated for such effects in laboratory animals. Many of the 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 originated in the South Pacific Islands and New Guinea, found throughout the tropics and subtropics. In the US it is cultivated from Florida to Texas. Sugarcane is cultivated as far as north as 36.7° (Spain) and as far south as 31° (South Africa)[2] (Irvine, 1981). Cane sugar, cane syrup, molasses, wax, and rum are products of sugarcane. Molasses is used as a sweetener, in industrial alcohol, for explosives, synthetic rubber, and in combustion engines. Sugar is used as a preservative for fruits and meats; cane is also made into a liquor. It is a folk remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhea, dysentery, eyes, fever, hiccups, inflammation, laryngitis, opacity, penis, skin, sores, sore throat, spleen, tumors, and wounds[3] (Duke and Wain, 1981). Powdered sugar is used as a 'drawing' agent for granulations and "proud flesh" (Hartwell, 1967–1971) and, in a 1:3 solution in water, for gonorrhea and vaginal

discharges[4,5] (Watt and Breyer-Brandwijk, 1962).

MATERIAL 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 material were successfully extracted with methanol 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%).

EXPERIMENTAL

Animal

Healthy, adult albino 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. This experiment was conducted on 6+6 (First 6 animals were subjected for histopathology and remaining animals for tissue biochemical parameters). All the animals were housed in standard laboratory conditions (temperature 22 ±2ºC 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 methanolic extract of *Saccharum officinarum* at 500 mg/kg b.wt./day (suspended with tween 80 [2%]) dose, administered orally by using a curved needle and a tuberculin syringe. for 21 consecutive days respectively.

Biochemical estimations

The female rats which were in proestrous or estrous phase were left over night with males of proven fertility (in the ratio 2:1) and next morning the vaginal smears were checked for the presence of spermatozoa. The day on which the spermatozoa were observed in the vaginal smear was considered as day "Zero" of pregnancy. These females were isolated and used for further experimentation. The antinidational property of the test substance was assessed by oral administration of 500 mg/kg b.wt./ day dose of methanolic extract of *Saccharum officinarum* to the mated female rats. On day 21st, autopsy was performed under light Ketamine anesthesia (50mg/Kg/i.p) and after recording their body weights, the two uterine horns were observed for the number of implantation sites, live and dead/ degenerated fetuses. The fetuses were removed from the uterine horns and suitable parts of the horns of rats treated with 500 mg/kg b.wt./day which showed 100% antifertility effect were frozen and used for tissue biochemical analysis. Quantitative biochemical estimations of glycogen[6],cholesterol[7], acid and alkaline phosphatases[8]and sialic acid[9] were made in the uterine tissue samples of control and treated female rats. All the results are expressed as mean ± SEM and significance was analyzed statistically by students 't' test.

Histopathological Analysis

After 10 days of acclimation, the animals were randomly assigned to either the experimental groups, 1st group was control treated with normal saline (1ml/kg) for 21 days, 2nd group was treated with 500 mg/kg, p.o daily for 21 days. Each group rats were housed individually in labeled cages with solid plastic sides and stainless-steel grid tops. The vital organs liver, heart, uterus, brain and kidney were dissected out the end of 24hrs.The isolated organs were sliced into 5 mm pieces and fixed in neutral formalin (10 %) solution for 3 days and washed under running water for about 12 hrs. This was followed by dehydration with alcohol of increasing strength (70%, 80%, and 90%) for 12 hrs each. Final dehydration was carried out using absolute alcohol with about 3 changes at 12 min interval. Cleaning was done by using xylin with changes at 15 – 20 min interval. After cleaning the pieces were subjected to paraffin infiltration in automatic tissue processing unit. The pieces were washed under running water to remove formalin completely. Then the pieces were embedded in paraffin, sectioned, stained, mounted & observed under light microscope.(Fig.1). Photograph of heart,

liver, kidney, brain and uterus histopathology of rats treated with 500 mg/kg,po. *Saccharum officinarum* extract (H &E × 400)

RESULTS

Table 1& 2 represents the values of the biochemical parameters of the control and extract treated female rats. Biochemical analysis of uterine horns of rats treated with the pregnancy interceptory dose (500 mg/kg b.wt./day) of methanolic extract of *Saccharum officinarum* showing cent percent antifertility effect, revealed a significant value ($p<0.01$) in the acid phosphatase activity and glycogen content($p<0.001$) when compared with control pregnant rats while the activity of alkaline phosphatase, cholesterol and sialic acid concentration is significantly changed in comparision with control.

DISCUSSION

It has been reported that oral administration of methanolic extract of *Saccharum officinarum* at the dose 500mg/kg b.wt./day from day 21st prevented pregnancy in all the treated female rats by virtue of antiimplantational property with antiestrogenic activity in presence of a strong estrogen. 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 arrival in the uterine lumen until it has achieved implantation and maintains an appropriate environment for the physical and biochemical integrity of the blastocyst structure and it meets specific immunological requirements, which becomes increasingly important during the preimplantation phase[10]. 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 blastocyst to survive[11].

It is also observed that the histopathological profile has no significant change on administering with *S. officinarum* extract,hence the extract has no toxic effect on vital organs. Endometrial glycogen is one of the most important

Table 1: Effect of methanolic extract of *Saccharum officinarum* leaves on the uterine biochemical parameters

Treatment Group	Glycogen (mg/g tissue)	Cholesterol (mg/g tissue)	Sialic Acid (mg/g tissue)
Control (Normal Saline)	7.07±0.23	4.15±0.18	0.963±0.01
Experimental *S.officinarum* extract	3.54±0.21*	7.07±0.19**	0.770±0.01**

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

Table 2: Effect of the methanolic extract of *Saccharum officinarum* in *albino rats* (wistar strain) on uterine Acid and Alkaline phosphatase activity of mated female rats

Treatment Group	Acid phosphatase (mg/g tissue)	Alkaline phosphatase (mg/g tissue)
Control (Normal Saline)	3.98±0.32	7.01±0.42
Experimental *S.officinarum* extract	2.13±0.28**	5.82±0.39**

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

**Values are statistically significant at P< 0.01.

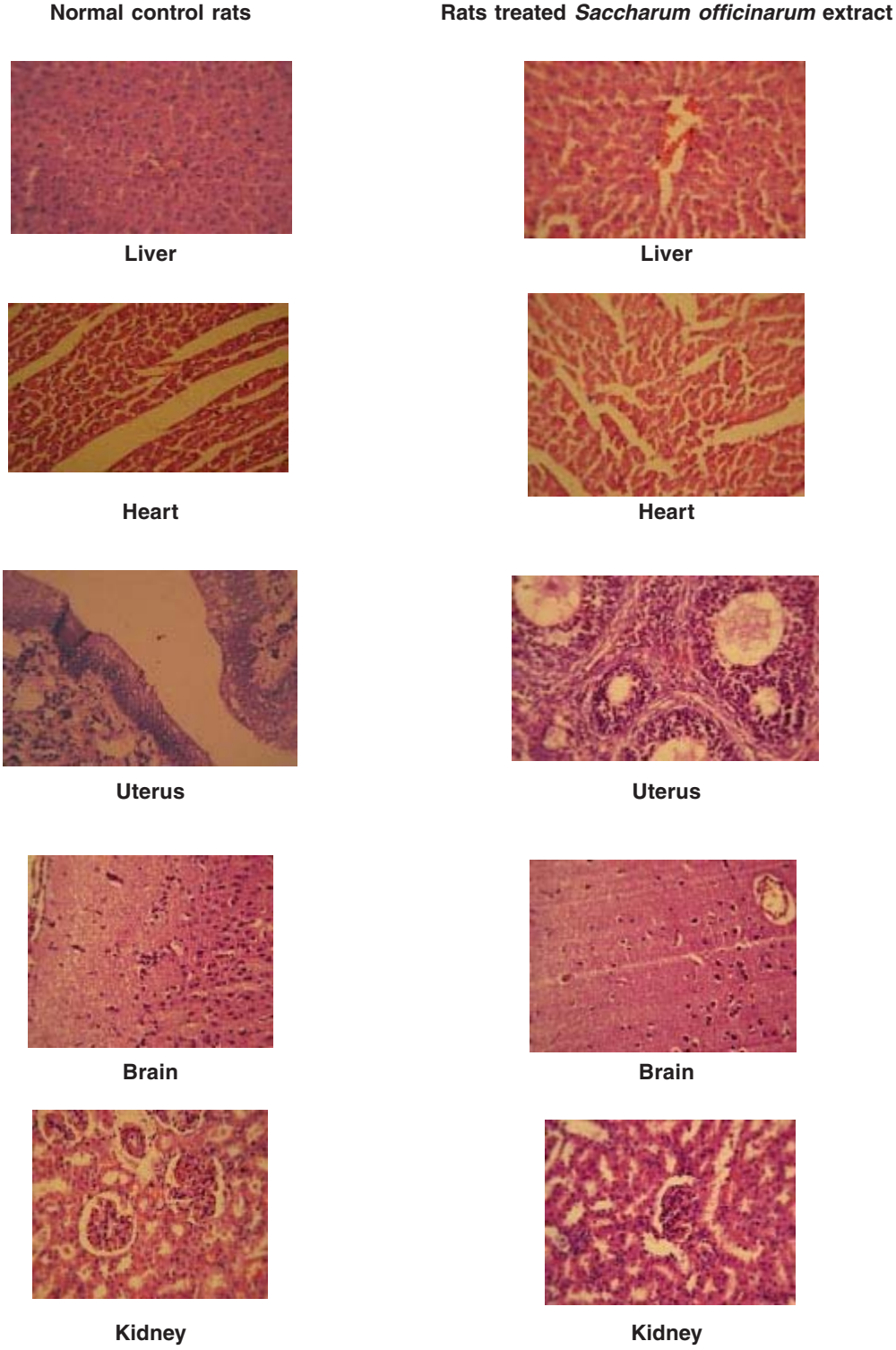

Fig. 1: Histopathological studies of female rats treated with normal saline and *S. officinarum* extract(500 mg/kg b.wt./day)

factor for the development and implantation of blastocyst in early stages of gestation[12]. 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. Inhibition of glycogen content in the uterus is due to the antiestrogenic nature of the extract and or activity of the uterus[13], which can account for their antifertility action. It is also possible that the ciliary action is decreased by the decrease in the glycogen level and the ova is not transferred to the uterus, thus, causes antifertility action[14].

Cholesterol 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. Thus, the present investigation suggests that *Saccharum officinarum* extract exerts antifertility and antiestrogenic activity in female rats[15].

It is well established that alkaline and acid phosphatases are associated with the decidual cell reaction and play important role in implantation[16]. A high acid phosphatase activity at the time of implantation is associated with its involvement in the preparation of the implantation chamber. A significant decline in uterine acid phosphatase activity in extract treated mated female rats indicate adverse effect on uterine milieu, making it unsuitable for implantation.

Therefore, it can be suggested that the pregnancy interceptory effect of methanolic extract of *Saccharum officinarum* might be due to the inhibition of circulating estrogen-progesterone balance which create a non receptive stage in the uterus by changing the reproductive biochemical milieu especially uterine environment which is directly involved in the implantation of eggs[17] and thus produce significant antifertility effect.

Although it would be premature to correlate the changes in the uterine biochemical constituents and the antinidational effect of the test substance but the changes in the activity of the uterine biochemical milieu could conjecturally be playing a role in the prevention of pregnancy. The antiestrogenic efficacy of the extract of the test plant in presence of a strong estrogen produced inhibitory effect which merely supports the contention that methanolic extract of *S. officinarum* offers itself as a very promising substance for further research in pregnancy interception.

On the basis of the above observations it may be concluded that methanolic extract of *Saccharum officinarum* 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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