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EFFECT OF BETLE LEAF STALK EXTRACTION ON TESTICULAR METABOLISM IN ALBINO RATS

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ABSTRACT

This study aimed to evaluate the betle leaf stalk extraction as an antispermatogenic in male albino rats. The oral administration of betle leaf stalk extraction resulted in significant increase in body weight and testes weight with no significant changes in TSI. The water content in testes does not show any significant changes while dry matter slightly reduced. These results indicate that there was not much effect on structural composition of testes. The biochemical studies indicate there was significant elevation in total proteins and significant reduction in carbohydrates & lipids. The reduction in total lipid content indicating impaired lipid metabolism in the testis. The elevated protein content suggests the accumulation of proteins, which were not utilized for spermatogenesis in the form of proteins or in the form of enzymes. The accumulation of testicular cholesterol by betle leaf stalk extraction indicates reduced steroidogenesis, which leads to impaired spermatogenesis, thus acting as antispermatogenic agent.

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INTRODUCTION

In recent years, plants are perused over synthetic contraceptive drug because plants are easily available, economic and devoid of harmful side effects [1]. The primary requisite of an antifertility agent for human is that it should be non-toxic, non-teratogenic and should not interfere with the normal metabolic and behavioral process. Further, the method should be reversible [2]. Several plant species have been described as antifertility agents [3]. The oral administration of seed extracts of Cuminum cyminum, fruit extracts of S. emarginatus, T. belerica and Allium cepa were lowered androgen dependent parameters revealing reduction in the circulating androgen [4&5].

The betel plant is an ever green and perennial creeper, with glossy heart shaped and white catkin. The piper betel leaves extract contains large number of bio active molecules like poly phenol, alkaloids, steroids, saponin and tannin. Piper betel has light yellow aromatic

essential oil, with sharp burning taste [6]. Its promising traditional applications have led to many chemical and biological studies. The P. betle treatment caused reduction in reproductive organ weights, as compared to control value. The data suggests that the P. betle ethanolic extract exerted antifertility and antiestrogenic effects in female rats. The effects brought by P. betle extract are non-toxic and transient [1].

A study to develop an orally effective male contraceptive agent was extensively carried out in male mice with various doses of the leaf-stalk extracts of Piper betel. The results show no toxicity in all metabolically active tissues of mice and interestingly, the contraceptive efficacy emphasised reversible fertility after withdrawal of treatment [7]. There was no biochemical studies has been carried out in male rats so far, hence the present investigation was undertaken to focus on antifertility efficacy of Betel leaf stalk ethanolic extract on male albino rats.

MATERIALS AND METHODS

Healthy adult male Wister strain albino rats (90 day old, weight 230±10g) were administered with 50 mg/kg body wt/day of alcoholic extract of betel leaf stalk through oral route for 15 days by Gavages method. The alcoholic extract was prepared according to [8] WHO (1983) protocol CG-04. The betel leaf stalk was dried, powdered and extracted with 95% ethanol (v/v) at 55-60ºC for 3 h. The solvent was distilled under reduced pressure; the resulting mass was dried under vacuum and kept at 24ºC until use. The control animals were given normal saline or sterile distilled water. Both control and experimental rats were maintained under standard animal house facilities, with a temperature of 25±2ºC, and 12h dark & 12 h day light, and fed on standard rat feed obtained from Hindustan Lever Ltd., Mumbai, India.

Twenty four hours after the last dose, the animals were autopsied and testes, epididymis, seminal vesicles, prostate gland and liver were isolated; blood serum was collected by puncturing the heart, chilled immediately and used for biochemical analysis. The TSI (Tissue Somatic Index) dry mater & water content were analyzed gravimetrically. The total proteins[9] , total carbohydrates[10], total lipids[11], total cholesterol[12], free fatty acids[13] and Glycerol[14] were estimated biochemically both in control and experimental rat tissues.

RESULTS AND DISCUSSION

The data represented in table-1to 3 indicates the gravimetric analysis, proximate analysis and lipid profiles in testes of control and betle leaf stalk extraction treated rats. The oral administration of betle leaf stalk extraction resulted in significant increase in body weight and organ weight with no significant changes in TSI[15]. The water content does not shows any significant changes while dry matter slightly reduced. These results indicate that there was not much effect on structural composition of testes. The biochemical studies indicate there was significant elevation in total proteins and significant reduction in carbohydrates& lipids.

The testis, a primary sex organ is the site for spermatogenesis. The leaf stalk extraction as an antispermatogenic, it shows its effect on spermatozoa with changes in seminal plasma composition.

The proteins, carbohydrates and lipids are the major fuel for mammalian organisms. The carbohydrates are the first fuel and lipids are the second major fuel. The testicular carbohydrates were decreased over control, indicates the impaired carbohydrates, lead to impaired spermatogenesis. Generally the reproductive tissues like testes largely depend on the carbohydrates for spermatogenesis [16&17]. The mature germ cell population depends on testicular carbohydrate reserves, suggesting impaired germ cell structure and function of spermatocytes, spermatids and spermatozoa [18&19]. Hence the reduced carbohydrates by leaf stalk extraction represents the reduced spermatogenesis thus shows its antispermatogenic effect.

Apart from carbohydrates, lipids are the second major fuel for mammalian organisms. Lipids are water-insoluble biomolecules and have a

variety of biological roles: as energy stores and fuel molecules and as signal molecules and structural components of membranes [20]. There was significant decrease in total lipid content indicating impaired lipid metabolism in the testis [21]. The elevated protein content suggests the accumulation of proteins, which were not utilized for spermatogenesis in the form of proteins or in the form of enzymes.

The cholesterol levels were significantly increased in testes .Cholesterol is the precursor of the steroid hormones [22], providing the backbone of the steroid molecule. The biosynthesis of testosterone directly from cholesterol can only occur in the Leydig cells [23]. Furthermore, it is the precursor molecule of steroid hormones, such as progesterone, testosterone and cortisol. The unsaponifiable fraction of the neutral lipid fraction of the rat testis represents primarily cholesterol and steroids [24]. Cholesterol is an important precursor for the steroid hormones. The testis and its metabolism are dependent on the plasma and endogenously synthesized cholesterol. Hence the cholesterol levels were estimated and found to be significantly increased in the experimental rat testis. This observation indicates either its increased uptake from the plasma or increased synthesis or decreased mobilization towards androgenesis or decreased catabolism.

The accumulation of testicular cholesterol by betle leaf stalk extraction indicates reduced steroidogenesis, which leads to impaired spermatogenesis, thus acting as antispermatogenic agent [25]. The treatment does not show any effect on free fatty acids. The free fatty acid (FFA) is primary fuel for skeletal muscle during sub maximal work [26]. Hence, there was no effect of betel leaf stalk extraction on skeletal muscle. However the glycerol concentration was elevated. Thus it is concluded that the betle leaf stalk extraction shows antispermatogenic effect.

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Table: 1 Effect of Betel leaf stalk extraction on **Body weight, Organ weight, TSI, Dry matter** & **Water content** in **testes** over Control rats.

S.No	Parameter	Control	Betel leaf stalk extract treated	% Change
1	Body weight(g)	236.21± 21.47	265.31±23.74	+12.29 ***
2	Organ Weight(g)	2.54±0.12	2.78±0.11	+9.44 **
3	TSI	1.07±0.10	1.04±0.12	-3.25 NS
4	Dry matter (mg/g wet wt.)	305.31 ± 28.43	272.73 ± 24.18	-10.66 ***
5	Water content (mg/g wet wt.)	732.56 ± 70.63	763.44± 75.45	+4.21 NS

Mean± SD of six individual observations. + And – indicates percent increase and decrease respectively over control. **indicates P<0.01, ***indicates P<0.05, NS indicates non significant changes.

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Table 2: Effect of Betel leaf stalk extract on **Total Proteins, Total Carbohydrates** and **Total Lipids** in **testis** over control rats.

S.N0	Parameter	Control	Betel leaf stalk extract treated	% Change
1	Total Proteins (mg/g wet weight)	164.21 ± 10.73	198.16 ± 12.43	+20.67*
2	Total Carbohydrate (mg/g wet weight)	35.34 ± 1.14	28.79 ± 1.32	-18.53*
3	Total Lipids (mg/g wet weight)	80.10 ± 6.32	65.81 ± 3.42	-17.84*

Mean± SD of six individual observations. + And – indicates percent increase and decrease respectively over control. * indicates P<0.001 the level of significance, NS indicates non significant changes.

Table: 3. Effect of Betel leaf stalk extraction on **Cholesterol, Free Fatty acids** and **Glycerol** in **Testes** over control rats.

S.N0	Parameter	Control	Betel leaf stalk extract treated	% Change
1	Cholesterol (mg/g wet wt.)	2.01 ±0.12	3.19 ±0.23	+59.71*
2	Free Fatty Acids (mg/g wet wt.)	21.60 ±1.82	22.16 ±1.74	+2.59 NS
3	Glycerol (mg/g wet wt.)	18.83±1.23	36.67±2.01	+94.74*

Mean± SD of six individual observations. + And – indicates percent increase and decrease respectively over control. * indicates P<0.001 the level of significance, NS indicates non significant changes

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