EFFECTS OF ALCOHOLIC EXTRACT OF *MALVAVISCUS CONZZATTI* (GREENUM) ON GERMINAL COMPONENTS AND SERTOLI CELLS OF TESTES OF MALE ALBINO RATS

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Alcoholic extract of leaves of *Malvaviscus conzatti* at the rate of 25 mg/100gm body weight caused variable degree of changes in the germinal components. In the treated group, after 10 days of extract feeding defragmentation of nuclei was observed at primary spermatocyte, secondary spermatocyte and spermatic stages. After 20 days of extract feeding similar changes occur in larger area. In some reasons karyorehexis was observed in the germinal epithelium. After 30 days of extract feeding, there was rupture of germinal epithelium, some cells completely dissolved with total loss of cytoplasm. Leydig cells were also few in number. Due to destruction of germinal components lumen of tubules was almost empty. Vacuoles were visible between germinal components in somniferous tubules, Sertoli cells appeared unaffected.

Although germ cell movement across the seminiferous epithelium is one of the most important and interesting biological phenomena during spermatogenesis, very few studies have been performed to examine the participating molecules and the mechanism by which this event is regulated. Our belief is that these studies are significant because not only can they expand our knowledge of spermatogenesis pertinent to junction restructuring, but a thorough understanding of the biology of germ cell movement should lead to the development of novel and safer male contraceptives. We have hypothesized that if germ cells are induced to translocation across the epithelium rapidly, even before they complete their development, germ cells found in the seminiferous tubular lumen will be immature and lack the ability to fertilize the ovum. This can also be achieved by perturbing cell adhesion in the testis prompting the depletion of germ cells from the seminiferous epithelium. Alternatively, if germ cell movement is hampered and germ cells are retained in the epithelium for a prolonged period of time, they will become "aged" and be removed by Sertoli cells via phagocytosis. In both instances, infertility will result. A disruption of fertility by this approach is likely to induce minimal side effects since the hypothalamus-pituitary-testicular axis is not disrupted. However, a model to study the events of germ cell movement is lacking. If such a model was available, it could be used to study the cascade of events leading to germ cell movement. This, in turn, could be used to identify target genes and/or proteins that perturb cell movement and/or cell adhesion. Once the technique to obtain staged tubules for in vitro studies became available studies were performed to identify target genes that associated with germ cell movement, such as spermiation¹.

**MATERIALS AND METHODS**

**Plant material used**

For the purpose of this study fresh samples of the desired components of the plants were collected. Plant was authenticated by the department of Botany, Meerut College, Meerut. Bark and fresh green leaves of *Malvaviscus conzatti* were procured, dried in shade, powdered and alcoholic extract was prepared, in absolute alcohol.
powder of bark and leaves were soxhalated in double distilled water and filtered through whatman paper number 40 and dried in oven at 80°C.

**Experimental animals:** For the purpose of present study only male albino rats wistar strain (*Rattus rattus*) of controlled breeding, weighing between 100 to 150 gm were used in this study. Before the commencement of experiment the animals were acclimatized in laboratory for a week on normal diet and water ad libitum. After this period experimental animals were randomly divided in different groups.

**Experimental protocol**

The experimental animals were weighed and randomly divided into three groups of 24 animals each. In each group four sub groups of were formed. For each extract group one served as control group, group two received 25 mg /100 gm body weight dose of the extract and the fourth group received 50 mg/100 gm dose of the extract. Each group

i) Received normal diet or extract for 10 days,

ii) Received normal diet for extract for 20 days and

iii) The experimental animals received normal diet of extract for 30 days.

iv) The animals of (iv) subgroup of control group was given control diet for 60 days. The animals of subgroup IV of Group II and III received respective doses of the extract for 30 days followed with normal diet for 30 days (in a total experimental period of 60 days), to assas the effect of any of the extract is permanent or reversible.

**Dose:** The dose of the extract at the rate of 25 mg/100 gm body weight was given for consecutive 10, 20 and 30 days duration to rats of first, second and third groups, respectively. After 30 days of extract feeding, fourth group was kept on normal diet for 30 days more to see the reversibility effect of extract.

The animals of control group and treated group were weighed before and after the commencement of experiment and were dissected after the duration of 10, 20, 30, 60 (30 days on dose + 30 days control diets) days to see the responses of doses.

**RESULTS AND DISCUSSION**

**Normal Histology**

At the time of tubule is about 1 m in length and 0.5 mm in diameter. Figure -1 shows the cross-section of a typical seminiferous tubule from an adult rat testis. The close morphological association between Sertoli cells and germ cells at different stages of their development (such as spermatogonia, spermatocytes, round spermatids, and elongated spermatids) is clearly visible in the seminiferous epithelium (Fig. 1). As a result of such morphological intimacy between Sertoli and germ cells, it is conceivable that extensive interactions and communications take place between these cells throughout spermatogenesis, both at the biochemical and molecular level. Morphometric analysis of the adult rat testis has shown that each Sertoli cell is associated with about 30-50 germ cells at each stage of the spermatogenic cycle in the epithelium, illustrating not only that germ cell development relies heavily on the sertoli cell but that extensive communications take place to coordinate the various events of spermatogenesis. Studies from the past two decades have demonstrated that germ cells largely rely on sertoli cells for structural and nutritional support. For instance, in the rat, the entire process of germ cell development, except for the early phase
of spermatogenesis from type B spermatogonia up to preleptotene and leptotene spermatocytes, is segregated from the systemic circulation because of the blood-testis barrier (BTB) created by tight junctions (TJ) between sertoli cells near the basal lamina. As such, germ cells and sertoli cells develop an intimate and elaborate cellular network for cell-cell communications via paracrine factors and signaling molecules, so that sertoli cells can provide developing germ cells with the needed nutrients and biological factors. In vitro studies have shown that there is bidirectional trafficking between sertoli and germ cells and that each cell type regulates the function of the other.

Throughout spermatogenesis, different biochemical, cellular, and molecular events take place in the seminiferous epithelium leading to the formation of eight spermatids (haploid) from a single type B spermatogonium (diploid). Furthermore, preleptotene and leptotene spermatocytes must migrate progressively from the basal to the adluminal compartment of the seminiferous epithelium traversing the BTB, while differentiating into haploid spermatids (Fig.1,2). Without this timely movement of developing germ cells across the seminiferous epithelium, spermatogenesis cannot go to completion, and infertility will result. Moreover, this event of cell movement is accompanied by extensive restructuring of cell-cell actin-based adherens junctions (AJs) between sertoli and germ cells, such as ectoplasmic specializations (ES). Although the subject of spermatogenesis, in particular its morphological changes and hormonal regulation, has been extensively studied, the subject of cell junction restructuring pertinent to spermatogenesis from a biochemical and molecular standpoint has largely been neglected. In 1991 Chakraborty et al. studied the antifertility effect of chronically administered M. conzattii flower extract on fertility of male rats and reported the extract have an inhibitory influence on gonadotropin release which may be held responsible for the decline in testosterone production leading to change in spermatogenesis caused by the M. conzattii extract. Similar observations were observed during the present study of M. conzattii leaves extracts. Rao studied the effect of alcoholic extract of Solanum xanthocarpum at 2% concentration, and reported it possess good spermicidal activity and antigonadotropic effect. The results showed elevated testicular cholesterol and reduced circulating testosterone levels in 60 days. The impact of feeding ethanolic extracts of Achyranthes aspera Linn. on reproductive functions in male rats are reported to decrease the serum testosterone level after feeding 50% ethanolic extract of Achyranthes aspera. In the present study Malvaviscus alcoholic extracts caused noticeable changes in the germinal components which became more prominent when time period was increased. The changes observed were not histological but also biochemical and hormonal. The treatment with alcoholic extract of Malvaviscus leaves caused slight changes in germinal components in case of 10 and 20 days dose. But all stages of spermatogenesis were disturbed after 30 days treatment and only germinal epithelial layer was intact, However, the connections of cells was normal in case of 10 days treatment, but due to destruction of the tissue, heavy cellular connection destruction was observed. The normal secretion of the Leydig cell was also disturbed. In case of reversibility group, very little recovery was observed. The present result shows the possible antifertility activity of the plant.
Effect of feeding of 25 mg/100gm dose of alchohalic extract of *Malvaviscus conzatti* leaves on testes of male albino rats

Fig 1 and 2 showing section of testis showing seminiferous tubule and all stage of spermatogenis like spermatogonia (SG), spermatocytes (SPC), spermatide (SP) and leydig cells. (X10, X 40). Fig 3 and 4 testis showing large number of normal seminiferous tubules with different stages of spermatogenesis after 10 days of extract feeding (X40). Fig 5 and 6 showing degenerative changes in some seminiferous tubules and hamorhage after 20 days of extract feeding. (X40). Fig 7 and 8 showing many tubules with shrinkage and degenerative changes in seminiferous tubules after 30 days of extract feeding. (X40). Fig 9 showing empty tubules and absence of sperms in lumen after 30 days of extract feeding. (X10).
REFERENCES