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Histomorphological Evaluation of Reproductive Organs Following *Piper betel* (Linn.) Leaf Stalk Extract Administration in Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To evaluate the histological effects of *Piper betel* leaf stalk extract on reproductive organs and the liver in male rats
Study Design: Aqueous *P. betel* leaf stalk extract was administered to rats at the dose of 50 mg/Kg/day by oral gavage for 15 days. Twenty four hours after the last dose, the animals were autopsied. Testis, epididymis, seminal vesicles, prostate and the liver were isolated and fixed immediately in Bouin's fluid for 24 hours. The tissues were dehydrated in various grades of alcohol, cleaned in xylene and after embedding in paraffin, blocks were prepared. Paraffin sections of 5µ thickness were cut with rotary microtome and processed for staining.
Place and Duration of Study: Place of study was S.V. University, Department of Zoology, Tirupati, India and experiment lasted for 6 months.
Results: *P. betel* leaf stalk extract administration caused seminiferous tubular derangement and sloughing of germ cells in testes. The histological changes were more pronounced in caput epididymis, reduction in lumen size due to creeping in of mucosal folds and lowering of secretion.

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Hepatic cell proliferation and Kupffer cell activation were also observed in liver of extract treated rats.
Conclusion: *Piper betel* leaf stalk extract is deleterious to histology or architecture of reproductive organs, which may affect spermatogenic function of the testis.

Keywords: Piper betel leaf stalk; histoarchitecture; spermatids; spermatogenesis.

1. INTRODUCTION

In spite of great advances observed in modern medicine in recent decades, plants still make an important contribution to healthcare. Much interest, however, has been shown in recent years, to control male fertility by using plants [1,2]. Numerous plants have been used historically to reduce fertility and modern scientific research has confirmed anti-fertility effects in at least some of the herbs tested, *Eurycoma longifolia*, *Ruta graveolons* [3,4]. Although, the toxicity profiles of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived *Tinospora cordifolia* from plant products are safer than their synthetic counterparts [5,6]. The plant products which are very commonly used in daily life, *Piper betel* also known to have antifertility effect. *Piper betel* leaf extract mainly contains amide alkaloids, Caryophyllene, ß- sitosterol, Hydroxychavicol, Safrole and Piperine. Piperin is the major active component [7,8].

Adhikary and Madhusnata [9] studied the effect of oral administration of extract of *Piper betel* stems on the reproductive function of female and male rats. In male rats, significant reduction in fertility and in the number and motility of sperm was observed at higher doses, as well as a reduction in the relative weight of the testis and accessory sex organs of administered animals. Another study revealed the anti-gonadal property of extracts of *Piper betel* stems in rats. Following subcutaneous injection for 21 days with either extract or vehicle, males and females were allowed to mate; *Piper betel* stem extract caused a 100% reduction in male fertility and a 63% reduction in female fertility when mated with fertile partners [10]. A reduction in the weight of gonads and other reproductive organs was also observed. Sarkar et al. [11] studied the antifertility effect of an alcoholic extract of air-dried leaf stem of *Piper betel* in male mice. The weights of reproductive organs (testes, epididymis, Seminal vesicles and prostate) of the administered animals decreased, and a decrease in the sperm count and sperm motility was also observed. Recovery experiments revealed that these effects were reversible. The exact mechanism of action of extract is not studied so far. However, there has been no previous histological evaluation. Hence the present study was undertaken.

2. MATERIALS AND METHODS

In the present study, healthy adult (3-4 months old, weight 215±10 g) male wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. The albino rats were divided into two groups, each group contains 6 rats. First group rats were control rats administered with 1 ml of distilled water. The second group (experimental) rats were administered with *P. betel* leaf stalk extract at the dose of 50 mg/Kg/day by oral gavage for 15 days [12]. The ethanol extract was prepared according to WHO [13] protocol CG-04. Stalks were shed-dried, powdered and extracted with 95% ethanol (v/v) at 55-60ºC for 3h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24ºC until use. Animals were housed in a clean polypropylene cage under hygienic conditions in well ventilated clean air conditioned room, with photoperiod of 12 hours light and 12 hours dark cycle, at 25±2ºC with a relative humidity of 50±5%. The rats were fed with standard laboratory feed (Hindustan Lever Ltd, Mumbai) and water *ad libitum*. Twenty four hours after the last dose, the animals were autopsied. For histological and histometric studies, the reproductive organs like testis, epididymis, seminal vesicles, prostate and liver were fixed immediately after isolation in Bouin's fluid for 24 hours. The tissues were dehydrated in various grades of alcohol, cleaned in xylene and after embedding in paraffin, blocks were prepared. Paraffin sections of 5µ thickness were cut with rotary microtome and processed for staining. Sections were stained with hematoxyline and the water staining was made with eosin. 10 sections were taken for each tissue. The light microscope with digital camera was used for photography.

3. RESULTS AND DISCUSSION

Histology is the study of the cellular organization of body tissues (cells) and extra-cellular material

into organs. The light microscope is the tool used most widely for clinical applications of histology. It also forms the structural basis for understanding function (Physiology) and is the preparation for the study of abnormal structure and function (Pathology).The principle aim of the study is to provide knowledge of tissue structure which is sufficient for the understanding of physiology. The *P. betel* leaf stalk extract at dose level of 50mg/kg b. w/day for 15 days showed some alterations in the histo-architecture of the reproductive tissues and liver.

3.1 Histology of Normal Testes

The rat testicular tissue showed number of seminiferous tubules with narrow lumen occupied by spermatozoa (Figs. 1A & 1B). The seminiferous tubules were surrounded by intertubular connecting tissues, interstitial cells of Leydig and blood vessels. Surrounding the germinal epithelium of the seminiferous tubules is a basement membrane on which rests actively dividing spermatogonia and Sertoli cells. The primary spermatocytes, secondary spermatocytes, early and late spermatid were arranged in a concentric manner from the basal layer of the spermatogonia towards the lumen of the seminiferous tubules [14].

3.2 Effect of *P. betel* Leaf Stalk Extract on Histology of Testes

P. betel leaf stalk extract administration (Figs. 1C & 1D) caused seminiferous tubular derangement, sloughing of germ cells from germinal epithelium with disintegration of spermatocytes and spermatids resulting in disruption of spermatogenesis. Regressive changes were seen in the tubular epithelia that affected both the germinal and Sertoli cells. Basement membranes of most of the tubules were distorted and ruptured. The interstitium was highly reduced and packed with atrophied Leydig cells. There was marked widening of interstitial space with area of necrosis and less Leydig cells. The Leydig cells are the most important components of the interstitial compartment and their main function is the production of steroid hormones [14]. Decreased number of Leydig cells leads to decreased production of steroid hormones [15] may and to impaired fertility caused by *Betel* leaf stalk administration [16].

3.3 Histology of Normal Caput and Cauda Epididymis

The transverse sections of caput (Figs. 2A & 2B) and caudal epididymis (Figs. 3A & 3B) showed normal histology. The epithelial cells of the caput were tall, columnar with nuclei arranged in a row near the thin basement membrane. The epithelium of the cauda contained few cuboidal cells. The lumen of the ductile was larger in the cauda and smaller in the caput. Segments of sterocilia were more profuse in the caput region than in the cauda. Both portions of the epididymis were full of spermatozoa. Inter tubular connective tissue and vascularity was observed to be normal in both caput and cauda epididymis.

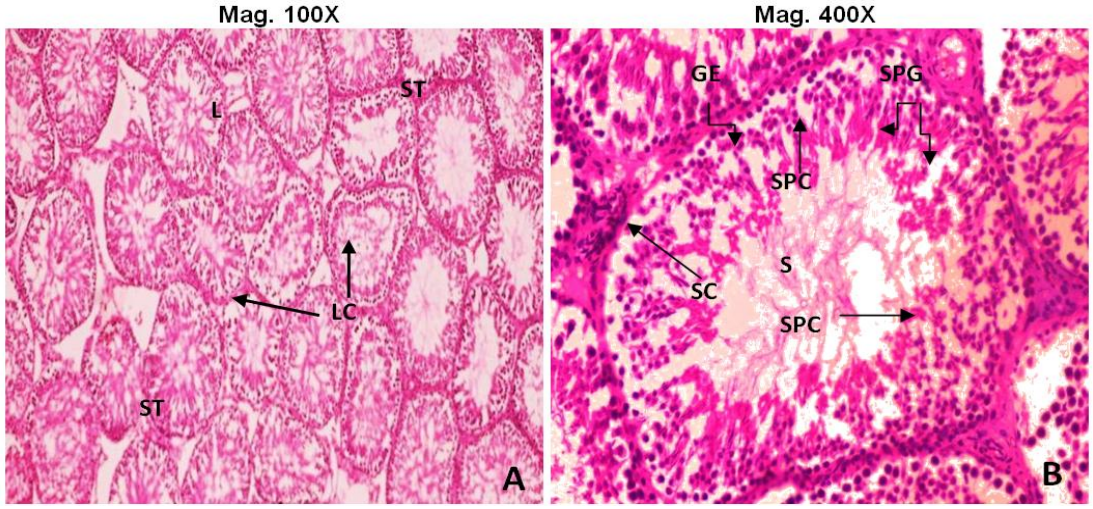
3.4 Effect of *P. betel* Leaf Stalk Extract on Histology of Caput and Cauda Epididymis

The caput and cauda epididymis showed marked histological alterations in the epithelium of the ductules. The histological changes (Figs. 2C & 2D) were more pronounced in caput epididymis, there was reduction in lumen size due to creeping in of mucosal folds and lowering of secretion. The patterns of epithelial layer were highly distorted and nuclear pyknosis appeared among the epithelial cells. The destruction of the basement membrane and the lumen contained less spermatozoa and cellular debris. The interstitial spaces were filled with loose connective tissue.

In caudal epididymis (Figs. 3C & 3D), there was a discontinuous layer of basal cells in its inner surface. In cauda there were very little spermatozoa which had clumped. The interstitial spaces were filled with loose connective tissue. Hence, the histometric studies further confirmed the androgenic effect. This showed that a significant decrease in the sperm motility observed in the caudal epididymis was due to the effect of *betel* leaf stalk extract [17].

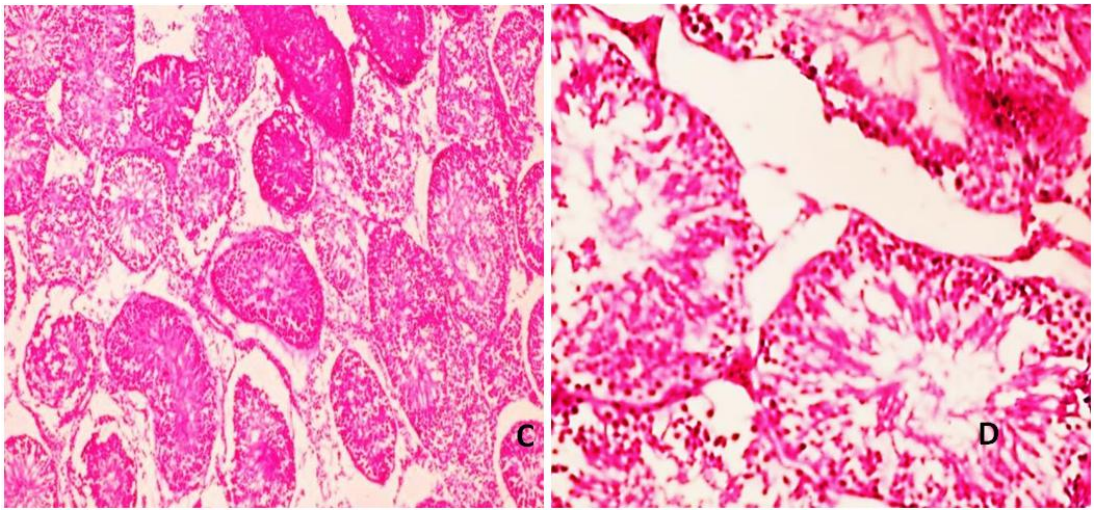
3.5 Histology of Normal Seminal Vesicle

The seminal vesicle of the control rats contained numerous ducts, which were thrown into folds. The epithelial lining of mucosa consist of a single layer of tall columnar cells with basal oval nuclei and the lumen contained thick secretions, numerous tubules lined with folds of epithelia (Figs. 4A & 4B) [18].

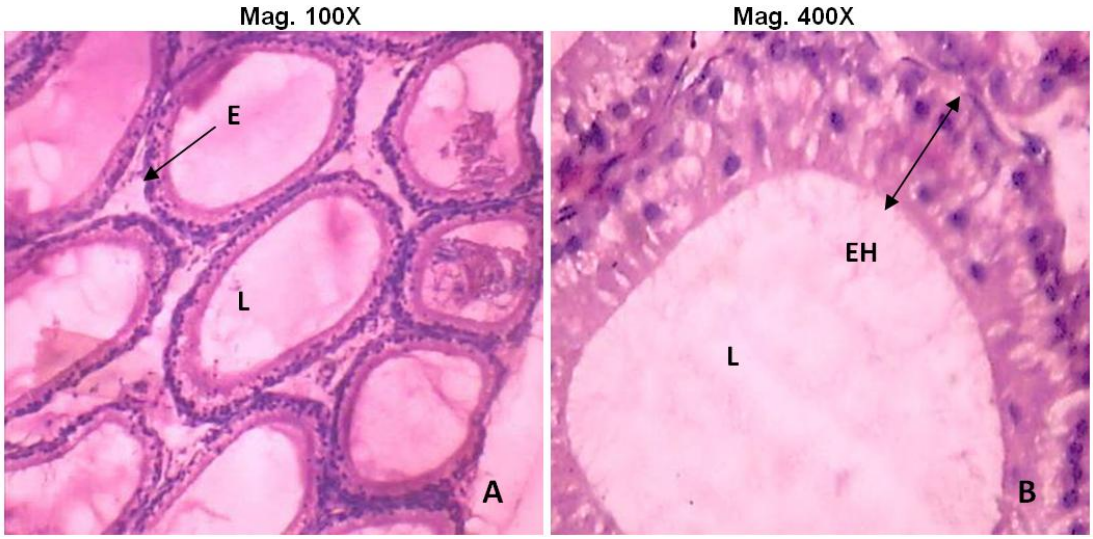


Figs. 1A&B. Transverse section [TS] of testes in control rat showing seminiferous tubules with an obliterated narrow lumen occupied by spermatozoa. Germinal epithelium has been good. Spermatids were seen 4-5 layers thick in the different stages of spermatogenesis. The Leydig cells were polyhedral in shape

LC- Leydig cells
GE- Germinal epithelium
ST - Seminiferous tubule
L - Lumen
S – Sperms
SC - Sertoli cells
Spg- Spermatogonia
Spc- Spermatocytes
Spt- Spermatids

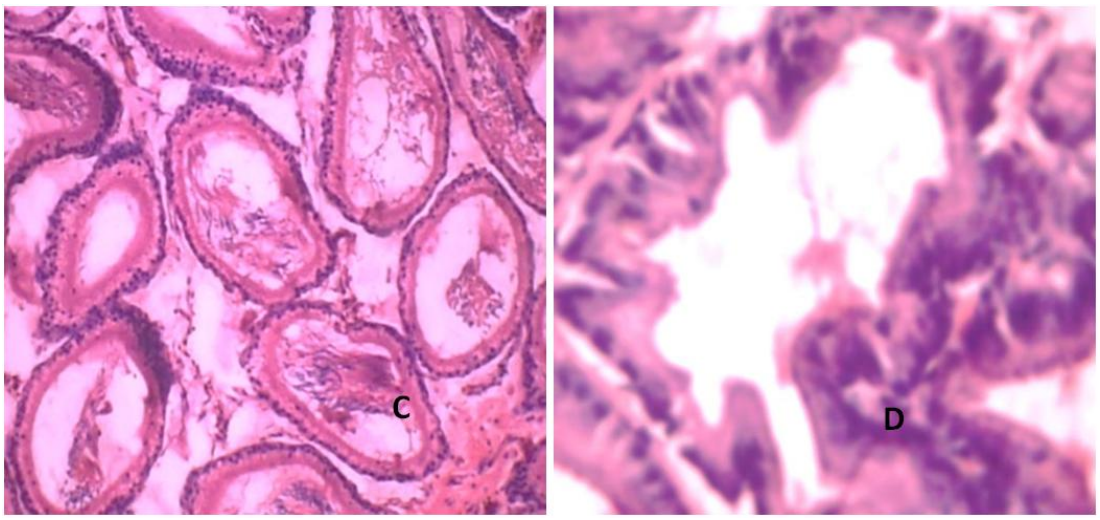


Figs. 1C&D. Transverse section [TS] of testes in *P. betel* leaf stalk extract administered rats, showing the damage of the germinal epithelium and degeneration of spermatozoa, spermatogonia, spermatocytes and spermatids. The interstitium was highly reduced and packed with atrophied Leydig cells



Figs. 2A&B. Transverse section [TS] of caput epididymis in control rat appearing normal lumen, The epithelial cells of the caput were tall, columnar with nuclei arranged in a row near the thin basement membrane

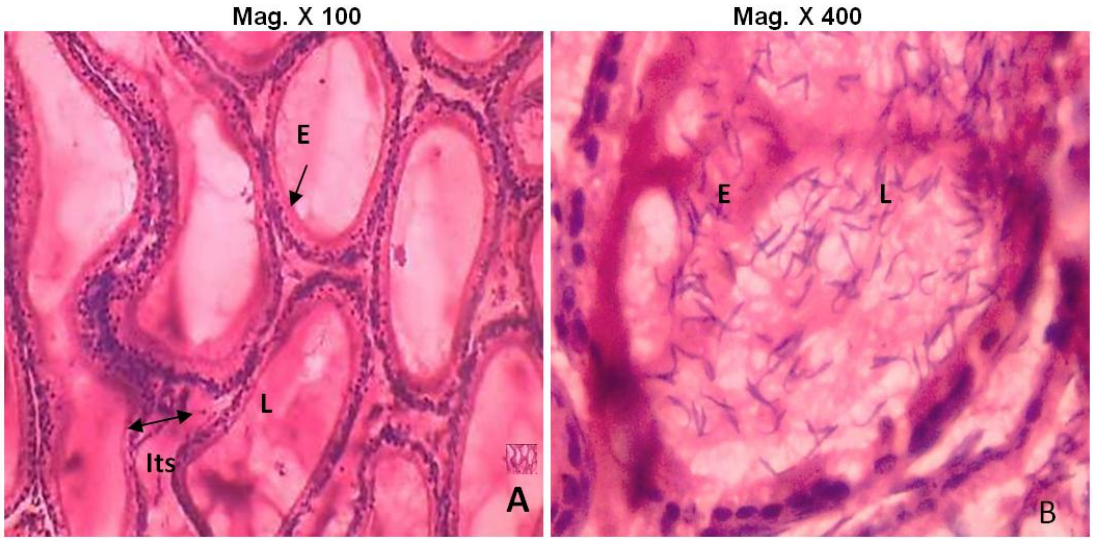
E- Epithelium *N- Nuclei*
L- Lumen *EH-Epididymal epithelial height*



Figs. 2C&D. Transverse section [TS] of caput epididymis in *P. betel* leaf stalk extract administered rats showing the reduction in lumen size due to creeping of mucosal folds and lowering of secretion. The patterns of epithelial layer were highly distorted and nuclear pyknosis appeared among the epithelial cells

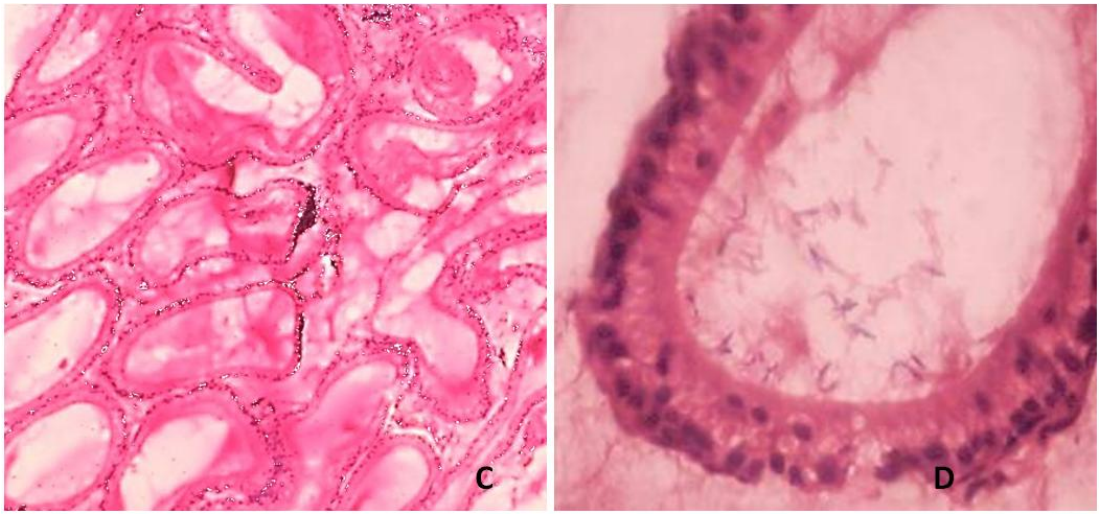
3.6 Effect of *P. betel* Leaf Stalk Extract on Histology of Seminal Vesicles

The seminal vesicles were affected with little secretions, damaged epithelial cells and poor vascularity in lamina propria. The lamina propria and connective tissue were in poor condition. Desquamations of some necrotic tubule alveolar glandular epithelial cells were observed. In addition, testosterone was reported to be essential for the maintenance of height of the mucosal epithelium cells important for seminal vesicle and prostate functions. This steroid was also found to affect the function of smooth muscle in the seminal vesicles [18,19].



Figs. 3A&B. Transverse section [TS] of caudal epididymis in control rat showing normal with basement membrane. The lumen contained more spermatozoa

E- Epithelium
Its-Inter tubular space
L- Lumen



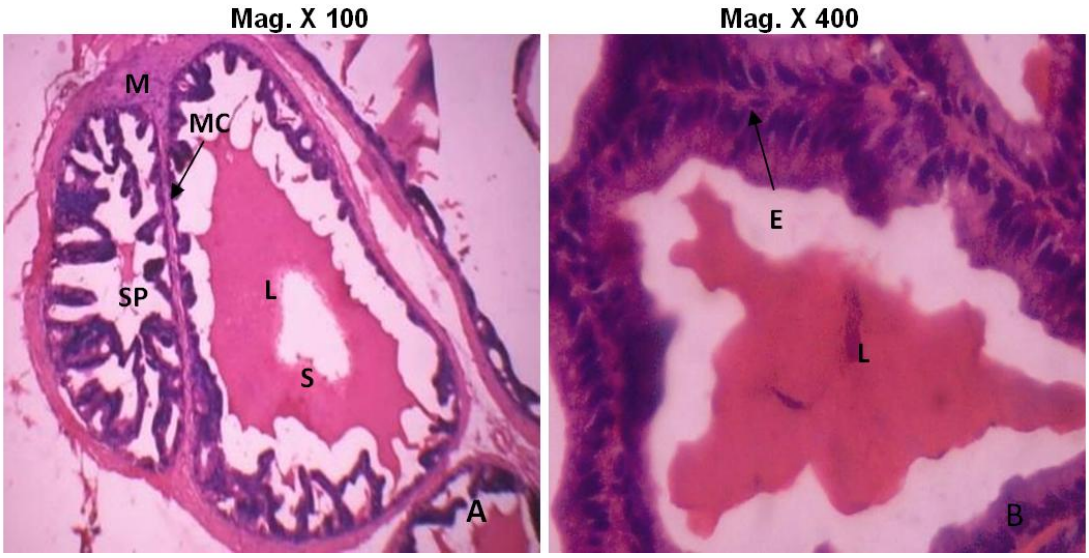
Figs. 3C&D. Transverse section [TS] of Caudal epididymis in *P. betel* leaf stalk extract administered rats showing the destruction of the basement membrane and the lumen contained less spermatozoa

3.7 Histology of Normal Prostate Gland

The histology of prostate gland in control rats showed normal prostatic acini surrounded by fibro muscular stroma (Sm), alveoli lined by the low columnar glandular epithelium (Figs. 5A & 5B). There were numerous glandular structures lined by tall to low columnar epithelium. The follicular lumen was full of secretions. There was an intervening fibre muscular stoma. The epithelium had proliferated into the crypts having invaded the lumen. Folding of the mucosal lining was observed in smaller tubules but distended tubules had no mucosal folds [20].

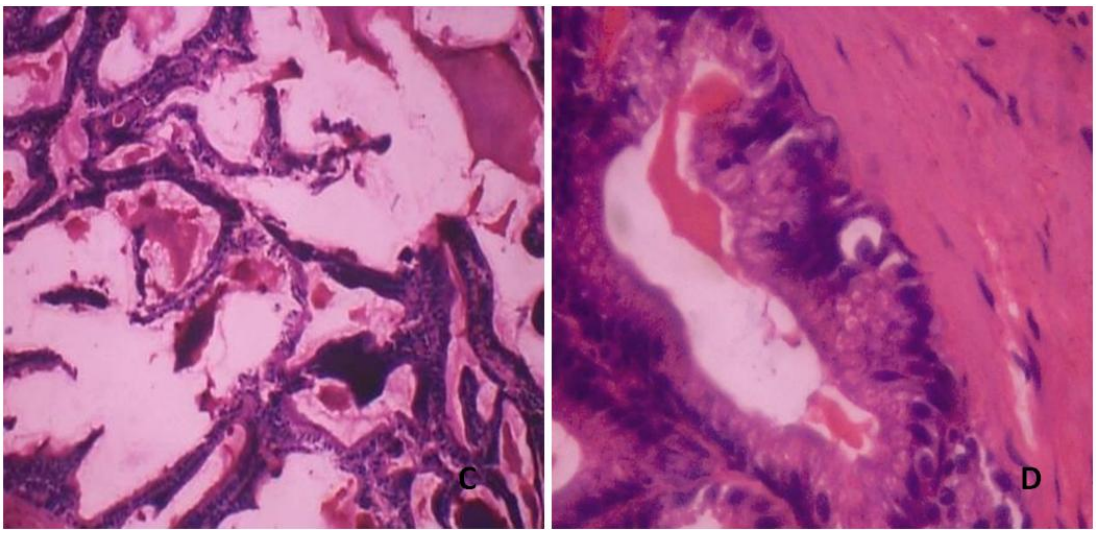
3.8 Effect of *P. betel* Leaf Stalk Extract on Histology of Prostate Glands

Nearly normal histoarchitecture of the prostatic acini were found to be formed of abnormal flattened epithelial layers [21]. The lumen had less secretary material in some follicles [19,20] with conjugated blood cells. Thus the cytoarchitectural perturbation of the accessory sex organs caused by the extract administration is probably the result of the decreased secretary activity supported by histological analysis. This strong inhibitory effect results in reduced availability of androgens.

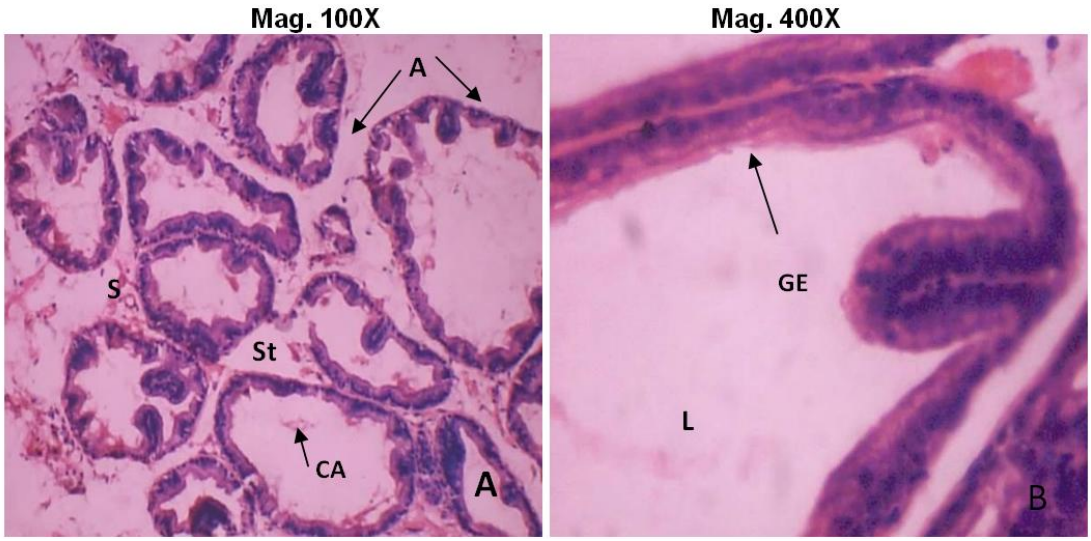


Figs. 4A&B. Transverse section [TS] of Seminal vesicle in control rat showing the normal lumen, filled with secretions and normal glandular epithelium

E- Epithelium *MC- mucosal crypt*
L- Lumen *M- muscular layer*
SP- Secretary pockets *S- secretion*
Stain: H & E

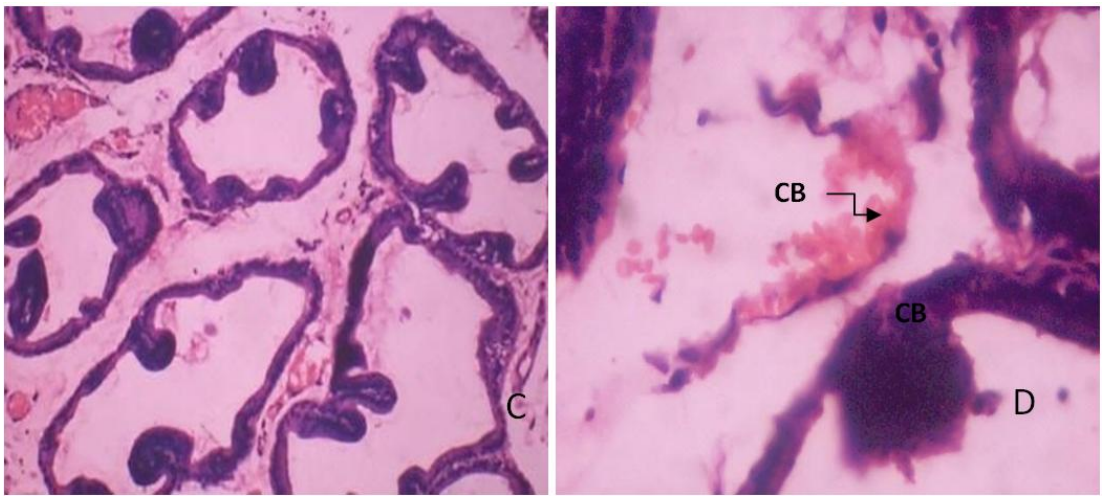


Figs. 4C&D. Transverse section [TS] of Seminal vesicle in *P. betel* leaf stalk extract administered rat showing glandular degeneration and increased fibrosis of intersitium



Figs. 5A&B. Transverse section [TS] of prostate gland in control rats showing a normal alveoli lined by the low columnar glandular epithelium

A- Alveoli, CB- Conjugated blood cells; GE- Glandular Epithelium; L- Lumen with secretions; St- Stroma; CA- Corpora amylacea



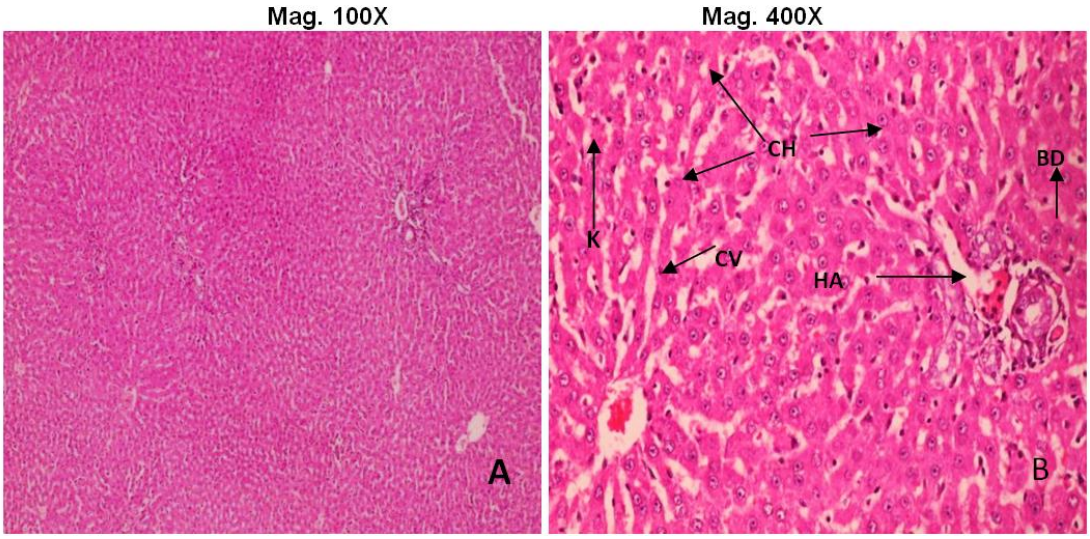
Figs. 5C&D. Transverse section [TS] of prostate gland in *P. betel* leaf stalk extract administered rats showing prostatic acini were found to be formed of abnormal flattened epithelial layers. Most of the inter tubular blood vessels were congested

3.9 Histological Examination of Normal Liver

Control showed normal arrangement of hepatocytes and central vein hepatic cords and portal triad [22]. The histoarchitecture of liver in control animals showed (Figs. 6A & 6B) that the liver lobule contains a number of hepatic acini and each centered on a portal tract. The tissue also contains sparse collagenous tissue acting as cushion for development and functioning of the acini. The hepatic venules were also observed on the terminal of the hepatocytes. The spacing between the hepatic cells was observed to be regular [23]. The Kupffer cells are macrophages that reside in the lining of the liver. The cells play important role in the normal physiology and homeostasis of liver.

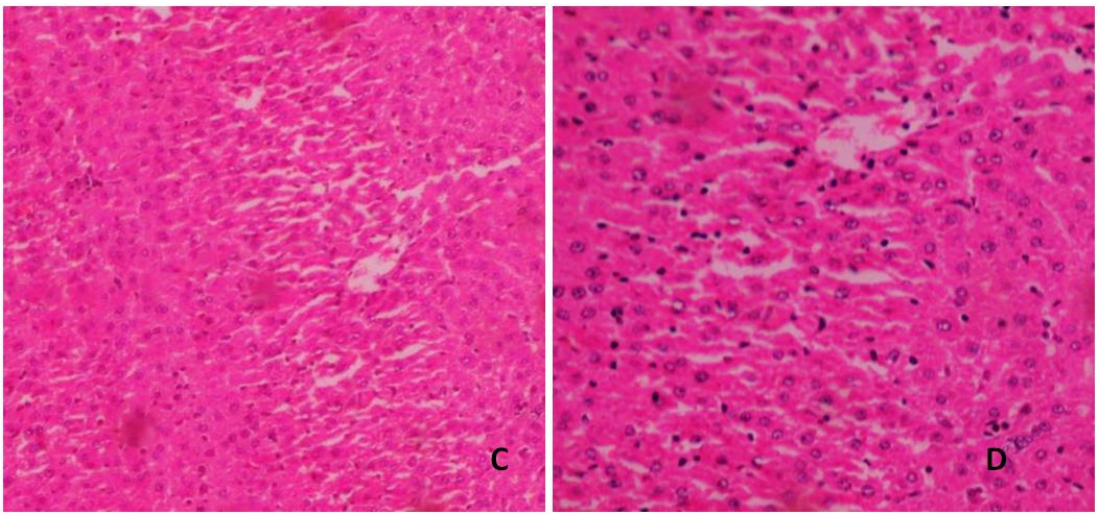
3.10 Effect of *P. betel* Leaf Stalk Extract on Histology of Liver

The administration of betel leaf stalk extract caused hepatocyte proliferation and kupffer cell activation (Figs. 6C & 6D) [24].



Figs. 6A &B. Transverse section [TS] of Liver in control rats showing normal arrangement of hepatocytes and central vein hepatic cords and portal triad

CH – cords of hepatocytes *CV- Central vein*
HA- Hepatic artery BD- Bile duct *K- Kupffer cells*
Si-Sinusoids



Figs. 6C &D. Transverse section [TS] of Liver in *P. betel* leaf stalk extract administered rats showing some hepatocyte proliferation and Kupffer cell activation

4. CONCLUSION

The histometric studies confirmed the anti spermatogenic effect of *betel* leaf stalk extract.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the ethics committee Regd. No. 438/01/a/ CPCSEA/ dt.17/07/2001; Res. No: 30/2012-2013/ (i)/a/ CPCSEA/ IAEC/SVU/CC-VVG Dt: 01-07-2012. Res. No: 29/2012-2013/ (i)/a/CPCSEA/IAEC/SVU/CC-AGN Dt: 01-07-2012.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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