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Research Paper

Effect of Tobacco Leaf Paste on The Histoarchitecture of The Reproductive System of The Male Albino Mice

Zoology

KEYWORDS : spermatogenesis, tobacco paste painting, reducing effect, histology, vitamins

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ABSTRACT

Oral cancer is a problem of paramount importance due to its high incidence in India and its statistically significant relationship with chewing tobacco. The testis controls a myriad of functions in the male.Spermatogenesis is affected in a variety of ways when the testis is subjected to different physiological treatments.The present work aims to study the effect of tobacco paste painting on the spermatogenic activity of the testis, since a part of the painted tobacco paste gets ingested by the animal into the gut. Experiments were designed to include a group which received vitamins in addition to the normal diet, in order to find out whether the addition of vitamins has any reducing effect on the toxic effect of painting tobacco paste.A significant decrease in the spermatogenic stages of the testis was observed in both the experimental groups. The findings suggest that oral tobacco paste painting on mice significantly alters the histology of the testes and epididymis

Introduction

Oral cancer is a problem of paramount importance to our country due to its high incidence in India and its statistically significant relationship with chewing tobacco[1]. Nicotine and related alkaloids of tobacco furnish the habit forming and narcotic effects which account for the general world. Nicotine is rapidly absorbed through the mucous membrane and intact skin but the nicotine salts (sulphates) are absorbed very slowly. It is evident that the action of nicotine is dose dependent and also on the duration of its usage.

The testis controls a myriad of functions in the male. Spermatogenesis is affected in a variety of ways when the testis is subjected to different physiological treatments. A germ cell, in an unfavourable environment will not pause at one step or another of its differentiation, but if it cannot pursue its evolution at the normal rate, it will degenerate and be eliminated from the system2. A reduction in the weight of the testis to 30% was found in the malathion treated rats3. Chlorpromazine has been found to arrest the spermatogenesis at the spermatid or the spermatocyte stage and involute the interstitial cells of the rat tailed bat4. Toxic effect of drugs like acetyl salicylic acid and alcohol are reported to cause testicular dysfunction5,6,7.

The role of defective diet and nutrition in the genesis of oral and pharyngeal carcinomas has been much discussed8,9. Ascorbic acid as needed to collagen synthesis might be required in increased amounts for the protective encapsulation of tumours10.

The present work aims to study the effect of tobacco paste painting on the spermatogenic activity of the testis, since a part of the painted tobacco paste gets ingested by the animal into the gut. Experiments were designed to include a group which received vitamins in addition to the normal diet, in order to find out whether the addition of vitamins has any reducing effect on the toxic effect of painting tobacco paste.

Materials and Methods

Animal model:

For the experimental study male wistar strain albino mice weighing 30-35gms of the age group 30-35 days provided by the animal house were chosen. The animals were acclimatized to the laboratory conditions five days prior treatment. They were maintained at normal room temperature and were provided with laboratory stock diet and water.

Experimental design:

The animals were divided in two major batches. Batch I receiving the normal diet and Batch II were those to the diet of which vitamins were supplemented and both the groups were painted with tobacco. These two experimental batches were further subdivided into three groups of ten animals each belonging to 30, 45 and 60 days of tobacco treatment. The control animals received the normal diet only without tobacco treatment.

Diet:

The normal diet comprised of a mixture of groundnut oil cakes and dry Bengal powder. The vitamin supplemented diet consisted of the addition of powdered multivitamin tablet (Becadex-Glaxo)mixed along with the food. The experimental animals were provided with the above diet ad libitum.

Preparation of tobacco paste:

Commercially available dried tobacco leaves were made to a fine paste. The freshly prepared paste was applied at the rate of 300mg/100gm body weight of each animal with a spatula. The animals were supplied with the food and water approximately an hour after painting to avoid the ingestion of the painted tobacco into the gut along with the food.

Histological study:

The animals were sacrificed after 24 hours duration of the last tobacco treatment. Tissues like the testis and epididymis were fixed immediately in Bouin's fluid for 24 hours and paraffin embedded. The tissues were stained with Haematoxylin and Eosin. A morphometric study was made in the testis and epididymis and a count of the different spermatogenic stages was done.

Morphometric study11:

The surface area and diameter of the seminiferous tubule and diameter of the lumen and tubule height of the columnar epithelial cells of the epididymis was measured using an ocular micrometre calibrated under low and high power.

Mean diameter:

Using the ocularmetre the length and breadth of the tubule was measured. The mean diameter was found using the formula l+b/2.

Surface area:

The diameter of the seminiferous tubules was first measured. Then the surface area was calculated using the formula $\pi r2$ where $\pi = 22/7$ and r the radius of the tubule.

Resultsand Discussion

A significant decrease in the spermatogenic stages of the testis was observed in both the experimental groups (Table 1).The seminiferous tubules were shown to be lined by a few layer of germinal cells after 60 days of tobacco treatment in both the experimental groups (Plates 1, 2a and 2b; 3a and 3b). All the experimental groups showed hypospermatogenesis (Plate 4).

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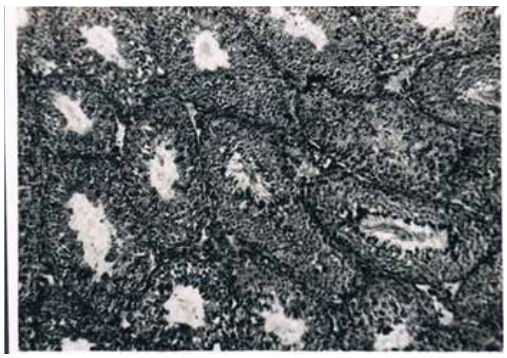

Plate 1: Testis of control mice showing uniform and active spermatogenesis in the seminiferous tubules. (HNE, Mag x 100)

Plate 2a: 60 days normal diet group showing an atrophic lining of many seminiferous tubules in the centre of the field, while a few tubules at the periphery show activespermatogenesis. (HNE, Mag x 100)

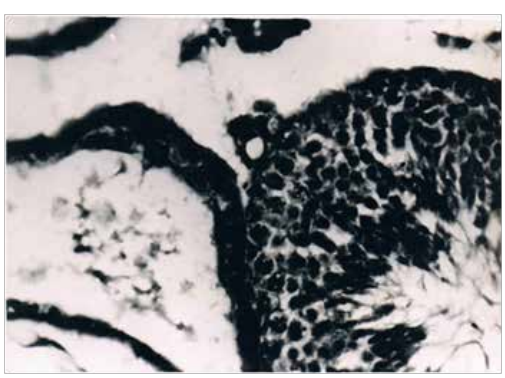

Plate 2b: 60 days normal diet group – two adjoined tubules are shown, one with active spermatogenesis and the other lined by a single layer of spermatogonial cells.

(HNE, Mag x 400)

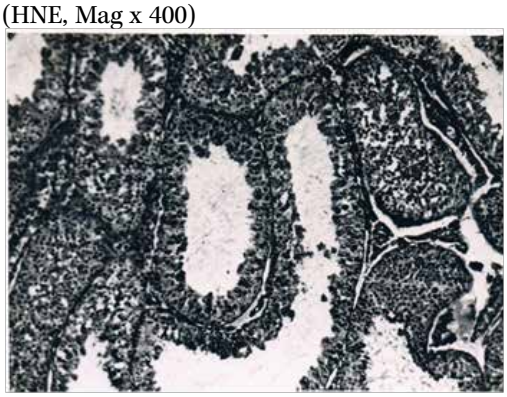

Plate 3a: 60 days vitamin supplemented group showing hypospermatogenesis with no spermatozoa in the lumen. (HNE, Mag x 100)

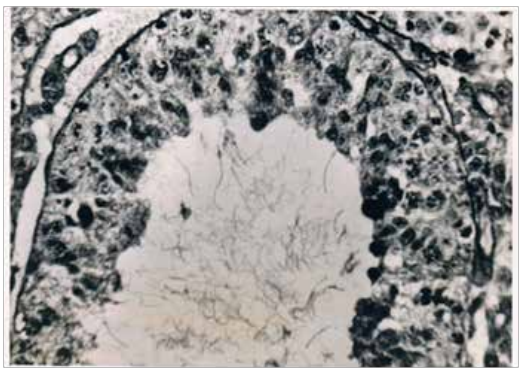

Plate 3b: 60 days vitamin supplemented group showing attenuated epithelium lined by secondaryspermatocytes. Absence of spermatids and spermatozoa. (HNE, Mag x 400)

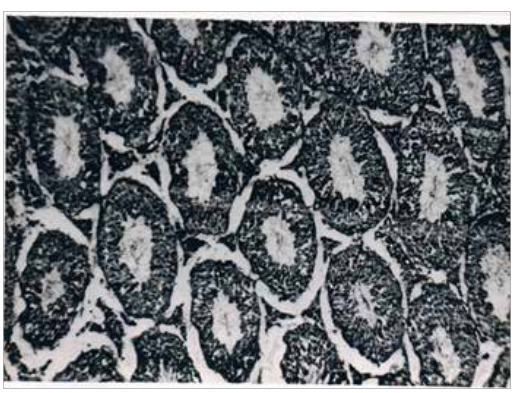

Plate 4: 45 days vitamin supplemented group showing hypospermatogenesis(HNE, Mag x 100)

A decrease of 36% was observed in the vitamin supplemented group and 22% in the normal diet group after 30, 45, and 60 days respectively. The diameter of the seminiferous tubules in both the experimental groups decreased from the control. A significant decrease of seminiferous tubules was observed in the vitamin supplemented group after 30 and 45 days and in the normal diet group after 30 and 60 days.

An increase in the number of leydig cells was found after

30 and 45 days in the vitamin supplemented group and after 30 days in the normal diet group. A significant decrease in the number of spermatogonialA cells was observed in both the experimental groups. An increase in the spermatogonial B cells was observed in both the experimental groups after 30 and 45 days. A decrease was found after 60 days in both the experimental groups.

A significant decrease in the number of primary spermatocytes was observed in both the experimental groups. A significant decrease in the secondary spermatocytes was observed after 45 and 60 days in the vitamin supplemented group and after 30, 45 and 60 days in the normal diet group. A decrease in the number of spermatids was observed in both the experimental groups from the control.

Papillary infoldings of the epithelium of the epididymis were observed in both the experimental groups (Plate 5a and 5b). A significant decrease in the height of the columnar epithelial cells was observed in both the experimental groups (Table 2). A significant decrease in the lumen diameter was found in both the experimental groups.

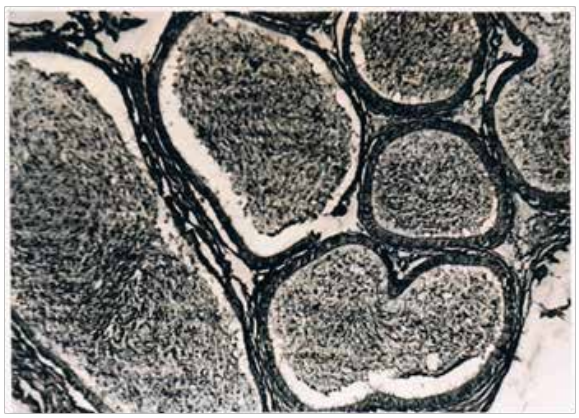

Plate 5a: Epididymis of control mice showing tubules distended with spermatozoa and lined by tall columnar cells. (HNE, Mag x 100)

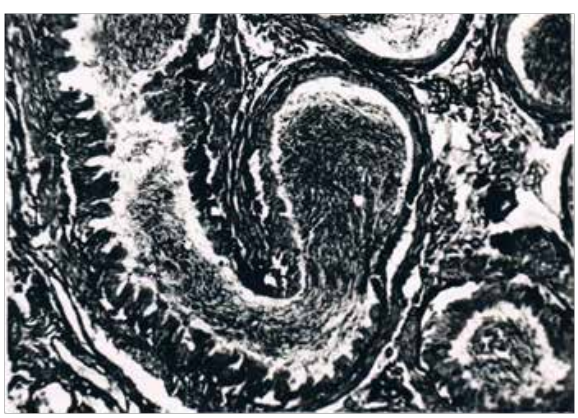

Plate 5b: Epididymis of 60 days normal diet group showing partially collapsed epididymal tubules with reduced number of spermatozoa. Presence of papillary folds. (HNE, Mag x 100)

The testis in both the experimental groups shows a significant decrease in all the spermatogenic stages – spermatogonia, spermatocyte and spermatid.A decrease in the spermatogenic stages is due to an increase in the androgen secretion and hence an increase in the number of leydig cells. The testosterone content per leydig cell increases linearly from 20-90 days of rat testis through pubescence12. Androgens exert a dual effect on the testesby inhibiting the secretion of Gonadotrophins from the Pituitary and serve to injure the seminiferous tubules in the normal animal13. In the hypophysectomised male, androgen administration maintains spermatogenesis.

The significant differences between animals in the diameter of the seminiferous tubules following epididymal obstruction are a reflection of the tubule shrinkage which occurs in testicular degeneration14. In the present work, the toxicity of tobacco pate on the testes could be attributed to testicular degeneration and decrease in the surface area and diameter of the seminiferous tubules.

Histologically the significant decrease in the height of the epithelial columnar cells and diameter of the lumen and tubule of the epididymis reflects on the testicular degeneration. Papillary infoldings observed in the epididymis of tobacco treated mice might be due to testicular shrinkage.

Conclusion

The findings suggest that oral tobacco paste painting on mice significantly alters the histology of the testes and epididymis and definitely infilters on the normal functioning of the testes. It has also been shown that the 60 days effect of oral tobacco paste painting is more striking than the 30 and 45 days duration of tobacco painting.

Table 1: Spermatogenic stages of the testes of the control and experimental mice. Values are expressed as Mean ± S.E.of 25 seminiferous tubules each

Groups	Days of painting tobacco	Surface area of seminiferous tubules(100x) (sq.μ)	Diameter of seminiferous tubules(100x) (μ)	No Of seminiferous tubules(100x)	No of leydig cells(100x)	Components of seminiferous tubules (400x)				
						Spermatogonia A	Spermatogonia B	Primary spermatocytes	Secondary spermatocytes	spermatids
control	-	718 ± 23	225 ± 6.7	21.0 ± 0.5	21.0 ± 0.7	15.0 ± 0.8	19.0 ± 1.2	42 ± 2.3	44 ± 2.3	39 ± 2.3
Vitamin Supplemented group	30	461 ± 9.0[a]	146 ± 2.8[a]	37.0 ± 0.4[a]	25.0 ± 1.3[b]	14.0 ± 0.6[NS]	20.0 ± 1.0	38 ± 0.9[NS]	38 ± 2.0[NS]	33± 1.6[NS]
	45	642 ± 21.0[c]	204 ± 6.8[d]	28.0 ± 0.7[a]	30.0 ± 0.7[a]	13.0 ± 0.7[d]	21.0 ± 1.1[c]	38 ± 2.2[c]	31 ± 2.0[a]	23 ± 1.7[a]
	60	684 ± 22.0[NS]	218±7.0[NS]	18.8 ± 0.5[b]	20.0±0.7[NS]	14.8 ± 0.9[NS]	17.5 ± 0.8[NS]	33 ± 2.0[b]	32 ± 2.3[a]	27 ± 2.8[a]
Normal group	30	563 ± 21.0[a]	179 ± 6.7[a]	28.0 ± 0.9[a]	22.0±1.3[NS]	14.0 ± 0.9[NS]	21.0 ± 1.5[NS]	34 ± 2.0[c]	33 ± 1.7[a]	29 ± 1.4[a]
	45	638 ± 23.0[NS]	220±7.6[NS]	20.8±0.9[NS]	19.0±0.7[d]	13.0 ± 0.6[d]	20.0 ± 1.7[NS]	33 ± 1.6[a]	31 ± 2.2[a]	24 ± 2.2[a]
	60	680 ± 16.0[NS]	216±5.0[NS]	25.0 ± 0.5[a]	19.8±0.5[NS]	13.0 ± 0.5[d]	17.0 ± 0.7[NS]	29 ± 1.9[a]	30 ± 2.5[a]	24 ± 2.4[a]

a P < 0.001 b P < 0.01 c P < 0.02 d P < 0.05 NS – Not significant

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Table 2: Measure of the height of columnar epithelial cells, lumen and tubular diameter of epididymis, expressed in microns (µ). Values are expressed as mean ± S.E. of 25 epididymal tubules each.

Groups	Days of painting tobacco	Height of columnar epithelial cells(100x)	Lumen diameter (100x)	Tubular diameter (100x)
Control	-	35 ± 3.7	568 ± 35.0	814 ± 63.0
Vitamin supplemented group	30	13 ± 0.4 [a]	256 ± 10.0 [a]	297 ± 11.0 [a]
	45	16 ± 1.4 [a]	429 ± 18.0 [a]	471 ± 18.0 [a]
	60	29 ± 2.3 [NS]	412 ± 16.0 [a]	445 ± 17.0 [a]
Normal group	30	12.4 ± 0.2 [a]	269 ± 9.6 [a]	301 ± 9.8 [a]
	45	17 ± 2.1 [a]	293 ± 30.0 [a]	331 ± 33.0 [a]
	60	16 ± 1.2 [a]	267 ± 13.4 [a]	321 ± 13.0 [a]

a $P < 0.001$ NS – Not significant

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