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EFFECT OF EMBELIN ON REPRODUCTIVE ORGANS OF MALE ALBINO RATS

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ABSTRACT

Embelia ribes **Burm (vern.Vidang,Virang,Baivirang,Sanskrit–Kirmighan, chitratundal), a member of family Myrsinaceae, has been reported to possess antioxidant and antifertility activity both in the crude powdered berries as well as in petroleum ether and methanol extracts. Embelin (2,5 Dihydroxy 3-Undicyl, 1-4 benzoquinone) isolated from the berries and administered Sub cutaneously to different groups of rats at doses of 0.4& 0.5 mg/kg body weight for 28 days and 35 days resulted in considerable reduction in the wet weight of testis, epididymis, vas deferens, seminal vesicle and prostate gland. Arrest of spermatogenesis accompanied by large number of empty, collapsed or atrophic seminiferous tubules. Intensely eosinophilic germ cells having 2-9 nuclei and more than one giant cell were observed .Embelin altered the histology of caput and cauda epididymis, vas deferens, seminal vesicle and prostate. The compound is suggested to possess antiandrogenic and antifertility activity in male albino rats.**

KEYWORDS: Embelin, Spermatogenesis, Antiandrogenic and Antifertility

Embelia ribes **Burm**. (vern. *Vidang, Virang, Baivirang*, Sanskrit – *Kirmighan*, chitratundal) a member of family myrsinaceae is an indigenous plant having antioxidant properties (Bhandari etal. 2008) and is commonly used in Ayurvedic formulations for the prevention of pregnancy. One such preparation "Garbhnivaran aushdham" composed of a mixture.of Embelia ribes berries,Piper longam fruits and borax reported to impaired the fertility of female mice and rats (Munshi and rao,1972;Munshi, 1974) ,induced sterility in male mice (Munshi *et al,* 1972, Bhargava and Dixit,1985) and have been reported to possess antifertility activity. Crude powered berries Kholkute et al.1982 Kamboj and Dhawan, (989, Gupta and Sharma 2006). Embelin (2,5dihydroxy 3 Undicyl,1-4 benzoquinone) isolated from the berries altered the testicular histology and suggested to possess anti androgenic properties in albino rats (Agrawal et al. 1986, Gupta et al. 1989, Malhotra and singh 2007).The present study deals with effects of embelin on male reproductive organs.

MATERIALS AND METHODS

Mature male Swiss albino rats (180-250 g) were used in the present investigation.

They were housed in clean and well ventilated animal house with 12hrs light and 12 hrs dark lighting schedule. Tap water and commercial diet (Hindustan Lever Ltd.) were fed to them ad libitum. distributed in 6 groups as reported earlier. Embelin was isolated (Chauhan et al. 1989) Two doses 0.4 and 0.5 mg/ kg body weight were prepared by dissolving 40 and 50 mg of embelin in a weak solution of ammonia. The solution was boiled to evaporate, cooled and administered subcutaneously to different treatment groups. Control rats received vehicle only by the same route for 28 and 35 days. The animals were sacrified 24 hrs after the last treatment using light ether anaesthsia. For histological studies testes, epididymes, vas deferens, seminal vesicle and prostate gland were excised and blotted free of blood, weighed and fixed immediately in alcoholic Bouin's fluid for 5-7 hrs. Paraffin sections were obtained at 6-7μ and stained with haematoxylin- eosin and examined for histological changes.

RESULTS

Testis and Relative Accessory Sex Organ Weights

Reduction in the weight of testis, epididymis, vas deferens, seminal vesicle and prostate gland wasnoted at all the doses and durations (vs control a $p < 0.001$, b $p < 0.01$, c $p < 0.02$ and d $p < 0.05$) (Table 1)

Histopathology

Administration of embelin caused arrest of spermatogenesis at all doses and durations. In addition some of seminiferous tubules at 0.5 mg / kg body wt. show debasement of cells which are huddled together into the lumen. At this dose most of the tubules show a tendency of shrinkage and deformation as a result of which they are separated off from each other. Large number of tubules look

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empty or have collapsed and have assumed atrophic forms and show dysgenesis and dysplasia of seminiferous tubules. Intensely eosinophilic cells having 2-9 nuclei and more than one giant cell have been observed at 0.4 mg / kg body weight dose. At some places more than one Giant cells have been observed surrounded by completely vacuolated cells. (Figure)

Embelin altered the histology of caput and cauda epididymis at both doses and durations. At 0.4 mg / kg b. wt. the epithelium showed hyperplasia with prominent nuclei distributed throughout the epithelial thickness. Such ductules contain scanty sperm. Stromal connective tissue has increased at 0.4 mg/kg dose. In some ductules deep folds are observed which meet across the lumen, giving the impression of partition in lumen. In such ductules nuclear population is highest in the folds. The lumen of the affected ductules mostly contains no sperm but degenerating germs cells and giant cells of various sizes may be observed at 0.5 mg/kg body weight of embelin.(Figure).

At 0.4 mg / kg dose in the luminal epithelium of vas deferens stereocilia are considerably reduced and nuclei show beginning of disorganization while at 0.5 mg/kg dose partial atrophy of the epithelium has been observed. The muscular coat also shows some degree of atrophy not much alteration was seen in the histology of seminal vesicles. doses and Fibromuscular connective tissue hyperplasia noted at 0.4 mg/kg body weight dose but at 0.5.mg/ kg lumen devoid of secretion and cytoplasm shows vacuolation. Prostate showed connective tissue stromal hyperplasia at 0.4 mg /kg dose while 0 .5 mg /kg dose for exhibited acute affect on the epithelial villi and height of epithelium.The luman has been found with very little secretion. The fibromusculuar stroma is normally developed. (Figure)

Table 1: Effect of embelin on reproductive organ weights of male albino rats

(Figures in parentheses represent the number of animals used. All the values are mean±SE)

Groups	Dose administered (mg/kg/bwt/day)	Treatment period (days)	Body weight (g)	Relative organ weights mg/100g b.wt					
				Testis	Epididymis	Vas deferens	Seminal vesicle	Ventral prostate	Dorsal prostate
I	Control (10) (Vehicle only)	28	180±7.07	661.59±24.97	221.85±6.46	47.28±0.75	135.02±7.93	220.79±20.56	86.43±3.06
II	0.4 (7)	28	165±5.63	631.69±24.96	191.71±9.75[c]	44.18±4.59	75.0±7.78[a]	147.88±23.39[d]	81.21±7.46
III	0.5 (7)	28	179±10.05	636.05±13.90	202.95±13.90	42.23±2.02	66.34±9.21[a]	154.56±33.33	81.54±4.64
IV	Control (10) (Vehicle only)	35	187±5.82	694.35±15.40	228.55±6.25	46.22±0.58[c]	133.84±10.04	250.06±15.02	84.33±2.51
V	0.4 (7)	35	192±13.57	628.98±14.81[b]	183.37±6.52[a]	42.94±2.99	67.73±9.61[a]	161.15±11.83[a]	77.76±6.88
VI	0.5 (7)	35	185±22.45	671.22±40.43	171.61±5.94[a]	41.84±3.38	58.06±4.78[a]	150.28±33.85[a]	68.21±7.16[d]

Level of significance: a, p<0.001; b, p<0.01; c, p<0.02; d, p<0.05

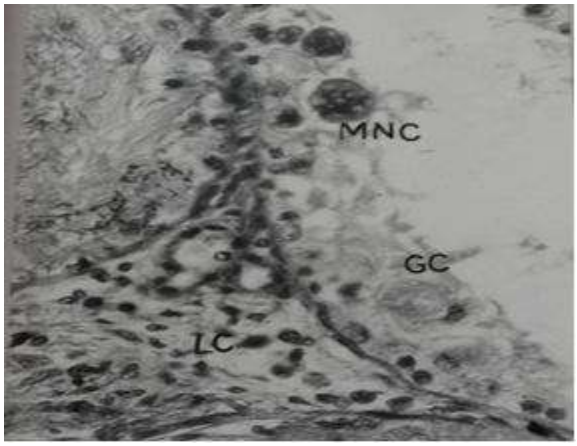

Figure 1: Photomicrograph of the testis of control rat showing normal histological features H.&E.X200

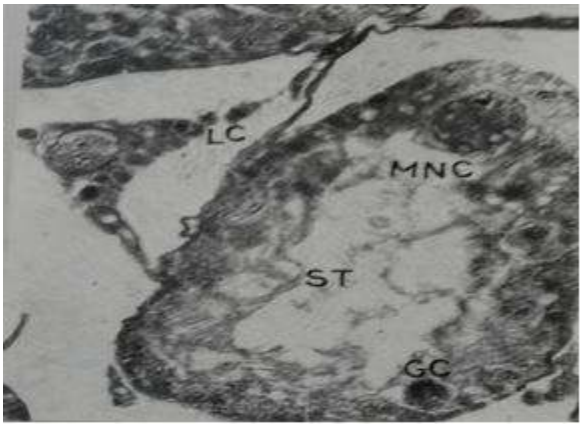

Figure 2: Photomicrograph of testis showing multinucleate giant cells in the lumen.H.&E.X875 Embelin 0.4mg/kg 35 days.

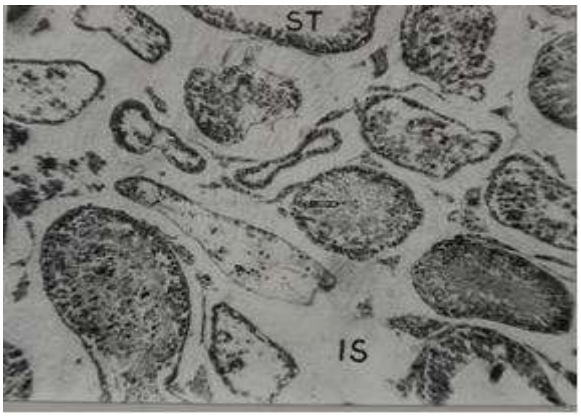

Figure 3: Photomicrograph of th0.4mg/ke test*is showing multinucleate giant cells with peripherally arranged .nuclei H.&E.X875 Embelin 0.4mg/kg 28 days*

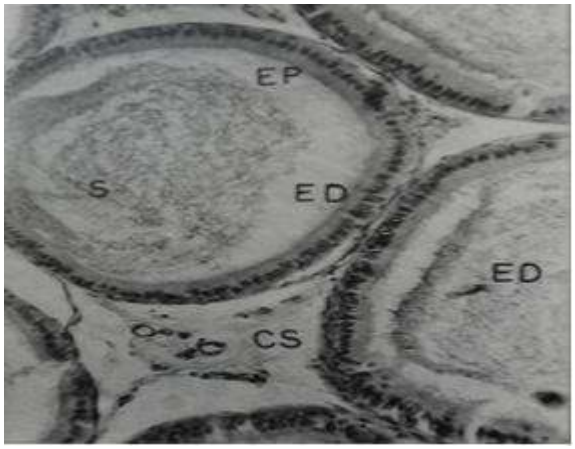

Figure 4: Photomicrograph of the testis ahowing dysgenesis of semi*niferous tubules H.&E.X100 Embelin 0.5mg/kg 35 days*

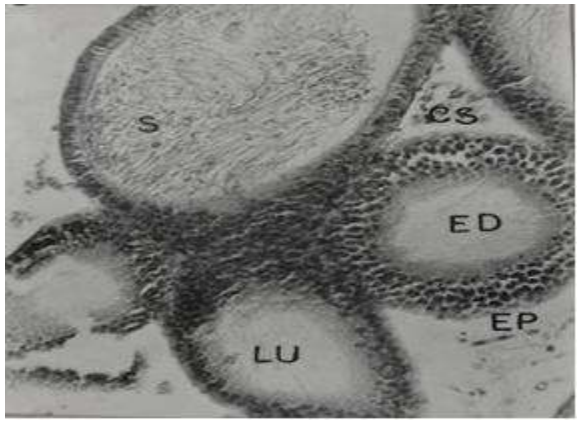

Figure 5: Photomicrograph of the caput epididymis of control rat showing normal histology.H.&E.X200 .

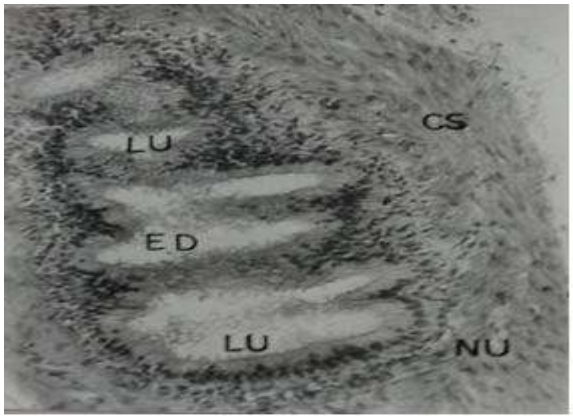

Figure 6: Photomicrograph of the caput epididymis showing epithelial hyperplasia .H.&E.X200 Embelin 0.4mg/kg 35 days.

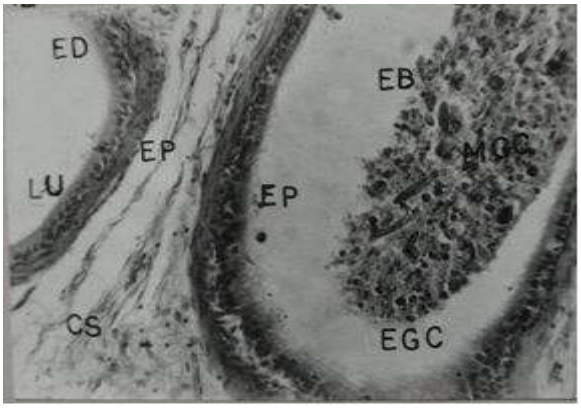

Figure 7: Photomicrograph of the cauda epididymis showing ductular epithelial folds, giving impression of partition in the lumen and oedamatous nature of the fibromuscular connective tissue.H.&E.X200 Embelin0.5mg/kg 35 days

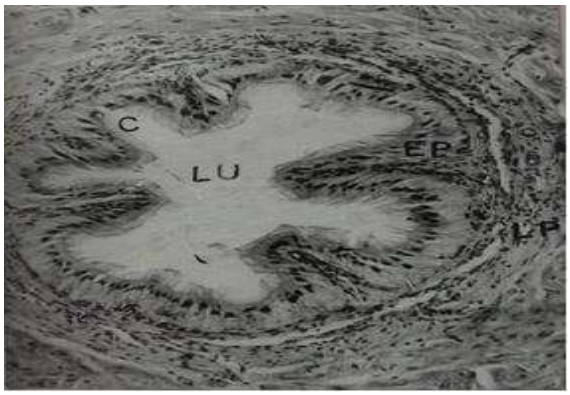

Figure 8: Photomicrograph of the cauda epididymis showing multinucleate giant cells and degenerating germ cells in the ductular lumen.H.&E.X875. Embelin 0.5mg//kg 35 days

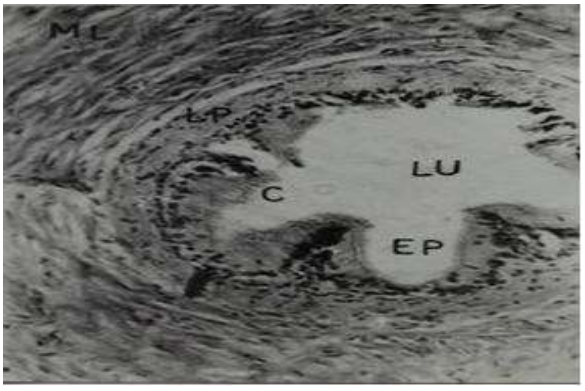

Figure 9: Photomicrograph of thevas deference of control rat showing normal histoarchitecture. H.&E.X100

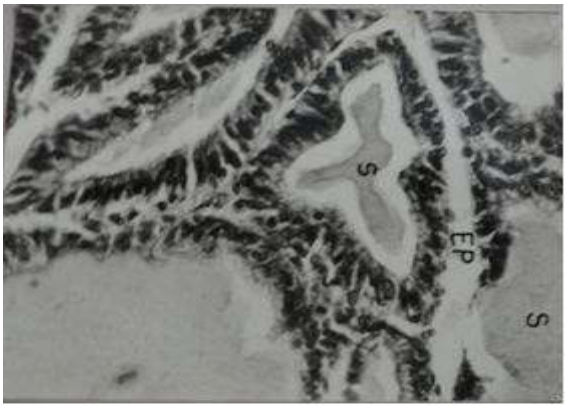

Figure 10: Photomicrograph of the vasdeferens showing disorganization of epithelium with some atrophy in muscle coat. H.&X200. Embelin 0.5mg/kg 35 days

Figure 11: Photomicrograph of the seminal vesicles of the control rat showing normal histology. H.&E.X 443

Figure 12: Photomicrograph of the seminal vesicles showing atrophic changes in a lobe of seminal vesicle and empty lumen. H.&E.X200. Embelin 0.4mg/kg 28 days

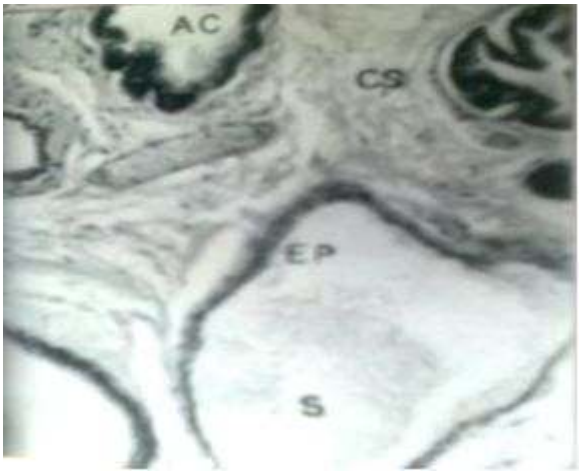

Figure 13: Photomicrograph of prostate gland of control rat showing normal histology.H.&E.X100

DISCUSSION

In the present study, we observed histopathological changes in seminifesrous tubule accompanied with formation of giant cells and reduction in number of germ cells. Seminiferous tubule atrophy and a decreased number of Spermatogenic cells were morphologic indicators of spermatogenesis failure (Park et al.2009). The number of mature Leydig cells has direct bearing on spermatogenesis. Deformation of Leydig cell further indicates the in sufficiency of these cells to synthesize testosterone (Watcho et al. 2001). Reduction in the weight of accessory reproductive organs showed antiandrogenic activity support our previous findings (Agrawal et al. ,1986) ,One of the major observations of present study is formation of bi and multinucleate giants cells in the testis which has been reported earliar upon treatment with or exposure to various drugs or chemicals (Singh and Abe ,1987 , Stanley and Akbarsa,1992, Nakai, M. and Hess, R. A.,1997)

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