_effects of embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) on the male reproductive system, serum chemistry and intestinal absorption of nutrients and digestive enzyme systems of albino rats have been studied during the course of present investigation. For this purpose embelin was extracted from the seeds of *Embelia ribes* Burm (family Myrsinaceae) and chemical characterization was done.

Embelin was administered to rats subcutaneously and detailed histological and biochemical investigations were made on the reproductive organs of albino rats. Effect of embelin on epididymal spermatozoa was also studied both in vivo as well as in vitro. Biochemical studies involved estimation of various metabolites and enzymes of reproductive organs with special emphasis on enzymes of the lipid and carbohydrate metabolism in testis, epididymis and epididymal spermatozoa. Serum lipids, proteins, albumin, globulin, bilirubin and some selected enzymes of clinical importance were also studied. Some aspects of intestinal absorption were also studied following oral as well as subcutaneous administration of embelin.
For this study, adult male rats (Porton strain) in the weight range of 150-200 g were used. The animals were segregated into four groups of 8 animals each. To the animals of Group I and Group II, embelin was administered subcutaneously (20 mg/kg body wt/day suspended in olive oil, 0.2 ml vol) for 15 days (Group I) and 30 days (Group II) respectively. Drug treatment was withdrawn in half of the treated animals in Group II and were kept for recovery for 15 and 30 days which formed Groups III and IV, respectively. For studying the intestinal absorption similar four groups were formed and embelin was administered orally (75 mg/kg body wt/day suspended in 20% alcohol, 2 ml vol) as intestinal absorption was studied both after oral as well as subcutaneous administration. Normal untreated animals served as controls and were administered equal volume of carrier of the drug only. Controls were run with all the treated groups. No significant change in the body weight of the treated animals was observed, but a significant reduction in the weights of testis, epididymis, seminal vesicles and ventral prostates was recorded. The weight in all the organs was regained in 30 days after the cessation of drug treatment. The drug was found more concentrated in the reproductive organs in comparison to kidneys, lungs, heart, spleen, brain and intestine. Embelin retention in the various tissues was more following oral treatment than after subcutaneous administration. The pharmacokinetic pattern of
embelin showed faster turnover and shorter half-life after subcutaneous administration as compared to orally fed rats, as evident by higher rate constant and comparably lower $T_\frac{1}{2}$.

This was one of the reasons for selecting subcutaneous route of administration for antifertility studies.

A distinct testicular damage was caused by embelin treatment in rats. There was observed a reduction in the germinal height and tubular diameter. Embelin also caused spermatogenic arrest by affecting round and elongating spermatids and pachytene spermatocytes which gradually disappeared from the tubules. Tunica propria and tunica albuginea were thickened. Desquamation of germ cells, vacoulation, formation of swollen cell bodies and multinucleate giant cells were also observed after 30 days of treatment. Cytochemically there was observed a marked increase in lipids while the amount of carbohydrates increased moderately.

Cessation of the treatment resulted in the recovery of the testicular histology. The testicular weight improved and the numbers of spermatozoa and round spermatids were greatly increased. The tubules regained their rounded shape and germinal cells were more or less systematically arranged.

Embelin treatment caused the decrease density of luminal contents and thickening of the basement membrane in the epididymis. After 15-days, some residual material/
swollen cell bodies were observed along with some degenerating spermatozoa. After 30-days of treatment, the lumen of caput epididymis was empty but some residual material was still there in the cauda epididymis. Most of the nuclei of the epididymal epithelium became pycnotic. Lipid accumulation was observed in the basal and apical regions. Withdrawal of treatment restored the normal histological architecture of the epididymis.

After embelin treatment (both in vivo and in vitro), a large number of decapitated sperm were observed. The outer membranous sheath of all the regions of the sperm was ruptured and vesiculated.

In in vivo as well as in vitro studies embelin caused a time- and dose-dependent inhibition of sperm motility. At the highest embelin concentration i.e. 1000 \( \mu \text{g/ml} \) to spermatozoa (50 x 10^6 cells) only about 9.5\% motile spermatozoa were observed after 60 min of incubation as compared to 79.8\% motile cells at 0 min without any embelin added and at 60 min control.

The seminal vesicle and ventral prostate histology was not affected much. The seminal vesicle and prostate suffered only a marginal reduction in the epithelial cell height accompanied by a decrease in the number of villi/folds. However, majority of the nuclei became pycnotic.
Biochemical estimations revealed that embelin treatment caused significant increase in total lipid and cholesterol contents in testis, epididymis, seminal vesicles and ventral prostates. An increase in triglycerides and free fatty acids was also observed in the testis and epididymis. Contrary to this there was recorded a significant fall in the phospholipids in testis and accessory organs.

Thin layer chromatography for the analysis of different lipid fractions revealed an increase of lysophosphatidyl choline and phosphatidyl glycerol, while all the other phospholipid fractions (phosphatidyl serine, phosphatidyl inositol, sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine and cardiolipin) showed a decrease in their contents. Among the neutral lipids, the contents of triglycerides, free fatty acids, cholesterol and cholesterol esters were found to increase.

Lipogenic enzymes malate dehydrogenase (MDH) and glucose-6-phosphate dehydrogenase (G6PD) showed a decrease in testis while both were increased in the epididymis. Another lipogenic enzyme, HMG-Co-A-reductase increased both in the testis as well as in the epididymis.

Glycogen content of testis, epididymis and seminal vesicles was elevated while it revealed a fall in the prostates. Sialic acid content was increased in testis but decreased in the epididymis. Citric acid and ascorbic acid levels were decreased in the seminal vesicles. A downward
A trend was noticed in the alkaline phosphatase activity in testis and epididymis and acid phosphatase in the testis. Acid phosphatase activity showed an upward trend in the epididymis. GOT and GPT activities were increased both in testis and epididymis.

Reduction of energy yielding enzymes and impairment of carbohydrate metabolism following embelin treatment could be inferred from the reduced activities of the dehydrogenases (succinate and lactate), adenosine triphosphatase, glucose-6-phosphatase, glycogen phosphorylase, fructose-1, 6-diphosphatase, hexokinase, glucose-6-phosphate isomerase and elevated activity of amylase in testis, epididymis and in the in vivo and in vitro studies of epididymal spermatozoa. The biochemical parameters recovered to varying extents after cessation of the treatment.

Serum revealed no significant changes in total lipids, phospholipids, cholesterol, LDL-C, HDL-C, VLDL-C, triglycerides, free fatty acids, post heparin lipolytic activity, total proteins, albumin, globulin, A/G ratio and glucose. Bilirubin levels showed an insignificant rise but returned to normal values during recovery period. IVFTT after embelin treatment showed a decreased removal rate of lipid from the circulation indicating an impaired tolerance of the animals to circulating fats.

A marginal increase in the intestinal weight and length was caused by embelin (both oral as well as
subcutaneous) administration. A significant increase was observed in the uptake of low molecular weight end-product nutrients, such as glucose, alanine and leucine in intestinal segments after both types of embelin treatments.

BBM-associated enzymes such as sucrase, lactase, maltase, leucine amino peptidase and alkaline phosphatase activities showed a marked increase in the partially purified brush border membrane and intestinal homogenates in oral as well as subcutaneous administration of embelin. The other subcellular enzymes such as glucose-6-phosphatase (microsomal) and lactate dehydrogenase (cytosolic), which were present in much less amount in BBM as compared to homogenates were also increased.

A marked change in the chemical compositions of the isolated BBM fraction was caused by oral as well as subcutaneous administration of embelin. An increase in total lipids, triglycerides, free fatty acids and lipid bound ganglioside-sialic acids level was visible in the isolated BBM fraction. Analysis of phospholipids and cholesterol showed an increase in these membrane constituents but hardly showed any change in phospholipid/cholesterol molar ratio. The changes in the intestinal functional constituents were reversible or at least reached near normal make-up during recovery period in both types of treatment. The changes in
the intestinal functional constituents were more pronounced in an oral administration of embelin as compared to that of subcutaneous administration.

In conclusion, this study illustrated that the administration of embelin, a potential antifertility compound, resulted in certain changes in histological and biochemical parameters in primary and accessory reproductive tissues and serum, which however, return to near normal levels, once the drug regimen was withdrawn. These reversible changes were particularly observed with regard to the lipid and carbohydrate metabolism in the testis and the epididymis and epididymal spermatozoa (including their morphology and motility). Although embelin produced sterility in the sense that it impaired the production of spermatozoa, importantly, the withdrawal of treatment resulted in normalization of spermatogenic processes. The reversible changes in intestinal functional constituents after embelin treatment showed that embelin fulfills the criteria of an ideal contraceptive chemical. Thus the compound has the potential of being considered for human welfare by making use of its capability of producing sterility with reversible mode of action. However, more work needs to be done before it can be used as an effective contraceptive.