ELECTROPHORETIC CHARACTERIZATION OF TESTICULAR AND EPIDIDYMAL FLUID PROTEINS AND GLYCOPROTEINS OF MALE RAT BEFORE AND AFTER ADMINISTRATION OF THE HIBISCUS ROSA SINENSIS FLOWERS EXTRACT

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Testicular and epididymal fluid proteins and glycoproteins of rat before and after administration of the extract prepared from the flowers of a common garden plant Hibiscus rosa sinensis, were characterised with the help of electrophoresis under both non-denaturing and denaturing conditions. Treatment of the extract resulted in changes mainly on testicular specific proteins than those of the proteins and glycoproteins of luminal fluid of epididymal duct showing its effect on spermatogenesis and even in the secretory mechanism of the Sertoli cell.

Hibiscus rosa sinensis Linn., (Family-Malvaceae) generally known as china rose plant is an ornamental plant cultivated widely throughout India and Myanmar (eastwhile Burma). The benzene and alcoholic extracts of the flowers of this plant having effects on spermatogenesis and accessory reproductive organs in rats were reported by Kholkute et al.

In the present study changes on the electrophoretic protein and glycoprotein pattern of the testicular and epididymal fluids were examined to see the effects after administration of the 50% ethanolic extract of the flower parts of Hibiscus rosa sinensis plant.

MATERIALS AND METHODS

Healthy adult male wister strain rats weighing about 300 gm. housed under a 12 hr. light-dark cycle at room temperature and had free access to water and standard food were used in this study. Flowers collected from I.V.R.I. Izatnagar for screening were shade dried and coarsely powdered to an approximately uniform mesh and extract was prepared as follows.
Electrophoretic Characterization of Proteins & Glycoproteins of rat

About 200 gms. of the powdered product was exhaustively extracted with 500 ml. of 50% ethanol in a Soxhlet apparatus. The extract was concentrated under low temperature and reduced pressure in a water-bath. The total dissolved quantity of the plant material in the extract was calculated by subtracting the dry weight of the material after extraction from the original weight put for extraction. According to the quantity of the extract, the concentrated syrupy liquid was then diluted by pouring into hot distilled water so that 0.1 ml of the diluted extract contains 14 mg. of the extractives approximately. The extract was stirred for about half an hour, filtered and preserved in a deep freeze until use.

The animals were divided into three groups consisting of 12 animals in each group. The group I and III animals were administered the crude extract through subcutaneous injection at the daily individual dose of 0.2 ml. for 4 (Group II) and 8 (Group III) weeks. Each of the 4 and 8 weeks treated animal received approximately 2.6 and 5.2 gm/kg body weight of the ethanolic extract respectively. Half of the animals of Group I served as control for Group II and the remaining half for Group III. The next day after completion of the final dose of the treatment period, animals from both treated and control groups were sacrificed under light ether anaesthesia. Serum was collected from the control rats directly by puncturing the heart. The testis and epididymis were dissected out, freed from adhering tissues and seminiferous tubule fluid (STF) was obtained as described by Turner et al.

and fluids from caput (CPF) and cauda (CDF) regions of the epididymis were collected by little modification of the method of Brooks and Higginson by using 10 mM PBS (phosphate buffer saline), pH 7.2. The protein concentration of collected samples of STF, CPF, CDF and serum were determined according to Lowry et al.

Polyacrylamide gel electrophoresis (PAGE) under non-denaturing system and Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions were performed as described by Davis and Weber and Osborn in 8% tube gels. After electrophoresis, for proteins and glycoproteins, staining of the gels were carried out by the method of Fairbanks et al. Molecular weights were determined according to Weber and Osborn. Bovine serum albumin, 66 Kilodalton (KD); Egg albumin, 45 KD; Glycerol-dehyde dehydrogenase, 36 KD; Carbonic anhydrase, 29 KD; Trypsinogen, 24 KD; Trypsin inhibitor, 20.1 KD and Lactalbumin, 14.2 KD were used as molecular weight markers and electrophoresed along with test proteins.

RESULTS

Electrophoretic Protein Pattern of STF, CPF and CDF of Control and 4 to 8 Weeks Flowers extract Administered Rats under Non-denaturing Conditions: Electrophoretic separation of STF, CPF and CDF of control rats under non denaturing conditions revealed 13 protein bands (gel no. 1, 4 and 7, Fig. 1). Albumin, the position of which indicated by the letter A in the figure was particularly noticeable not only in serum (gel no. 10, Fig. 1) but also in STF, CPF, and CDF samples. Though 9 post-albumin bands were found in STF, CPF and CDF samples of control rats, such proteins similar in mobilities were also
Singh, Bisht & Pandey

seen in gel representing serum. However, each of the 3 pre-albumin bands of STF, CPF, and CDF, the position of which are also indicated by the letter B, C and D to the left in the figure had any corresponding bands in serum. Therefore, these pre-albumin proteins (B-D) seem to be secreted solely by testis and epididymis. In the gels representing STF of 4 and 8 weeks treated rats (gel no. 2 and 3, Fig. 1), the three pre-albumin protein bands (B-D) were very much reduced and virtually undetectable. In CPF, the first pre-albumin band B of both 4 and 8 weeks treated ones (gel no. 5 and 6, Fig. 1), was found absent and band C and D were substantially different from those of the corresponding control rats (gel no. 4, Fig. 1), showing decrease in colour intensity. Administration of the extract for 4 weeks also resulted changes in the protein of CDF (gel no. 8, Fig. 1) particularly pre-albumin band B was not detected and band C showed reduction in the colour intensity, however, width of the third pre-albumin band D observed increase when compared with control one (gel no. 7, Fig. 1). After 8 weeks treatment (gel no. 9, Fig. 1), band B was found absent whereas band C resembled with that of the control and band D marked decrease in colour intensity.

Electrophoretic Protein and Glycoprotein of STF, CPF and CDF of Control and 4 to 8 Weeks Flowers extract Administered Rats Under Denaturing Conditions: Electrophoresis of STF, CPF and CDF (gel no. 1, 4, and 7, Fig 2a) of control rats under denaturing conditions separated about 15 to 16 bands, most of which were also found in serum (gel no 10, Fig. 2a). To have a clear picture of this, the diagrammatic presentation of Fig. 2a is shown in Fig. 2b. Those bands in STF which did not comigrate with serum proteins were the bands having molecular weight of 14.4, 17, 20, 21.5, 24, 230, 38.5 and 40.5 KD’s and referred as seminiferous tubule Protein (STP) shown in Fig 2b. No STF protein was found PAS to be positive. In CPF, out of the 15 bands, 7 bands having similar mobilities were not found in serum and they are referred as caput region secreted proteins (CPP) and had the molecular weight of 16, 20, 24, 27.5, 32, 37 and 41 KD’s also shown in Fig 2b of which CPP 27.5, 32, 37 and 41 were found PAS positive (result not produced in the figure). The protein pattern in the case of CDF was also more or less similar with CPF and out of the 16 bands separated, 7 bands having the same molecular weight as in CPF were not found in the serum. These 7 bands accordingly are also named as cauda region secreted proteins (CDP) indicated in Fig. 2b, and 4 of them like in CPF having the molecular weight of 27.5, 32, 37 and 41 KD’s were found to be glycoproteins since the stain with PAS (result for this too not shown in the figure). The CDP 24 was also found slightly PAS positive and this protein marked more prominent than that of the corresponding band of CPF.

The STF protein profiles after administration of the extract were profoundly altered, particularly STP 14.4, 17, 20, 38.5 and 40.5 were decreased in staining intensity in 4 weeks treated rats (gel no. 3, Fig. 2a). In 8 weeks treated rats (gel no. 3, Fig. 2a), STP 38.5 and 40.5 were more sharply decreased in intensity and STP 14.4, 17 and 20 were even barely visible. In CPF, CPP 37 and 41 were sharply reduced after 4 weeks treatment.
Electrophoretic Characterization of Proteins & Glycoproteins of rat

Fig. 1: Electrophoresis under non-denaturing conditions of SF, CPF, and CDF of control and 4 to 8 weeks Hibiscus rosa (sinensis) flowers extract administered rats with serum. All the gels were stained with coomassie blue. Gel 1-STF of control rats; Gel 2 and 3-STF of 4 and 8 weeks treated rats; Gel 4-CPF of control rats; Gel 5 and 6-CPF of 4 and 8 weeks treated rats; Gel 7-CDF of control rats; Gel 8 and 9-CDF of 4 and 8 weeks treated rats; Gel 10-serum. The positions of Albumin (A) and the three pre-albumin protein bands (B-D) are indicated.

Fig. 2a and 2b: Electrophoresis under denaturing conditions of STF, CPF, and CDF of control 4 to 8 weeks flowers extract treated rats with serum and molecular weight standards in Fig. 2a. All the gels were stained with coomassie blue and the sequence of Gel 1 to 10 are same with that of the Fig. 2a. Gel 11-molecular weight standards. In Fig. 2b, the diagrammatic presentation of Fig. 2a, indicating the locations of STF proteins (STP), CPF proteins (CPP) and CDF proteins (CDP) of control rats with molecular weight marker proteins (MW) are shown. The molecular weight values are x 10^3.
DISCUSSION

In the present study albumin and many other serum proteins had mobilities identical with protein present in seminiferous tubule and epididymal luminal fluid. Albumin, immunoglobulin and other serum proteins have been detected in the epididymal fluid of several species \(^{10-16}\). Serum proteins are also present in rete testis fluid of the rat \(^{17}\) and presumably pass into the epididymis by way of the efferent ducts. The presence of serum proteins in STF in present study might have been from the roto testis \(^{18}\) since the rete testis fluid is able to contaminate the tubular protein pattern due to reflux \(^{18}\). The present study observed a more or less similar protein profile as reported by Brooks and Higgins \(^{3}\), therefore the same nomenclature i.e. A for albumin band, B-C for first, second and third pre-albumin bands, have been adopted for all the albumin and pre-albumin protein bands separated in STF, CPF and CDF under non-denaturing conditions but without implying that the proteins are necessarily the same. The pre-albumin bands (B-D) of STF, CPF and CDF (gel no 1.4 and 7, Fig. 1), STP 14.4, 17.20, 21.5, 24, 30, 38.5, 40.5 (gel no 1, Fig. 2a) and CPP and CDP 16, 20, 24, 27.5, 32, 37 and 41 (gel no 4 and 7, Fig. 2a) may be considered as testicular and epididymal specific proteins since they were not detected in the serum (gel no 10, Fig. 1 and 2a) as secretion of specific proteins by testis and specific regions of the epididymis were observed by several workers \(^{17-19,20}\) and many of the epididymal secretions are mostly low molecular weight mass acidic glycoproteins \(^{21-26}\). This study could not really depict which of the pre-albumin bands (B-D) of STF, CPF and CDF under non-denaturing system exactly correspond to which or how many bands of STP, CPP and CDP under denaturing conditions as it lacks the study of estimating the molecular weight of each of the pre-albumin bands (B-C and D) separately, however from the changes found in the electrophoretic protein and glycoprotein patterns after administration of the said Hibiscus rosa sinensis flowers extract (Fig. 1), it becomes clear that the extract affected mainly on testicular specific proteins (STF proteins) though its effect was also seen in CDF proteins. It can affect on spermatogenesis since band C and D in both CPF and CDF under non-denaturing conditions were found having different 16 pictures of relative concentrations, as band 16 and 17 \(^{16}\), which are equivalent to pre-albumin protein C and D of the present study, showed different pictures of relative concentrations at any spermatogenic impairment. Effect of alcoholic extract of the flowers of this plant on spermatogenesis in rat was also reported by Khalkute et al. \(^{12}\). From the effects seen in the present study, it can be suggested that administration of the said flowers extract could have affected even the sertoli cells, the effects more in long term treatment, as the three pre-albumin protein bands of STF under non-denaturing conditions in both 4 and 8 weeks treated rats (gel no.2 and 3, Fig 1) were barely visible and also under denaturing conditions, particularly STP 14.4, 17 and 20 found decreased in colour intensity after 4 weeks treatment (gel no. 2, Fig 2a) as well as their complete disappearance and sharp decrease in intensity of STP 38.5 and 40.5 in 8 weeks treated one (gel no. 3, Fig. 2a). This result can be in agreement with Koskinney et al. \(^{26}\) that specific protein bands were visible even in fluid taken from severely degenerated
tubules. It is the Sertoli cells that elaborate specific proteins\textsuperscript{20,27} and these cells remain intact when the rest of the spermatogenic epithelium degenerates\textsuperscript{26}. Therefore the possible cause for a tremendously affected proteins in the present study could be due to the impaired function of the Sertoli cell after treatment of the extract. The damage of the seminiferous germinal epithelium after administration of the extract, though this study did not produce histological picture, can be assumed from this electrophoretic analysis of STF proteins, because proteins secreted by the Sertoli cells in vivo have direct regulatory roles in germinal cell development\textsuperscript{28}. The effects seen particularly in CDF proteins could be due to testicular fluid and sperm factors since the presence of sperm in the lumen of the epididymal duct affect the production or secretion of the epididymal plasma unique proteins\textsuperscript{16} and the principal cells of the epididymis have also been implicated in the removal of luminal proteins because they accumulate material that stains with PAS reagent when spermatozoa are prevented from entering the epididymis\textsuperscript{29}. These observations implies that amount of glycoprotein secreted by the epididymis is related to the number of luminal spermatozoa and the uptake of proteins by the epididymal epithelium is inhibited by the flow of the testicular fluid\textsuperscript{16}. So, from these changes found in the electrophoretic protein and glycoprotein pattern, it becomes clear that administration of the Hibiscus rosa sinensis flowers extract for both short and long period i.e. 4 and 8 weeks in rats could affect on spermatogenesis and in the secretory mechanisms of Sertoli cells.

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REFERENCES


Singh, Bisht & Pandey