Effect Of Solanum Nigrum L. Fruit Extract On Serum Sex Hormones And Testis Of Male Wistar Rats

Priyanka Meerwal, G. C. Jain

Abstract: Solanum nigrum L. (Family: Solanaceae) commonly called as “Black Nightshade” is found throughout India as weed. S. nigrum has been working as a traditional folk remedy for treating numerous diseases. The current investigation was carried out to examine the outcome of 50% ethanolic extract of S. nigrum on serum sex hormones level and spermatogenesis in the testis. The rats were randomly divided into five groups. Group A conducted by vehicle only and worked as control, although the rats in group B, C, D were treated orally with extract of S. nigrum at 100, 250 and 500 mg/kg doses, for 60 days. Group E rats treated through the dose of 500 mg/kg for 60 days and after that left without treatment for 60 days for recovery analysis. Effect on testes weight, serum testosterone, FSH, LH levels, histomorphometric of the testes was investigated. The results of the study exhibited, the weights of testes, testosterone level, FSH & LH levels significant decrease in extract treatment rat. Histomorphometric study showed a decline in the different germ cell populations in stage VII of the seminiferous cycle and also decline in the seminiferous tubular and Leydig cell nuclear diameters. The histological study of the testis reveals atrophic and degenerative changes. The results of the study indicates that 50% ethanolic extract of S. nigrum fruit get significant negative impact on sex hormones level as well as diminishing of spermatogenesis. However, significant recovery was noticed in all these altered parameters after withdrawal of extract treatment in rats.

Index Terms: Antispermatogenic, Germ cells count, Histopathology, S. nigrum, Sex hormones, Testosterone, Testis

1. INTRODUCTION

Population outburst is a most important reason of poverty and pollution in emerging nations. The regulation of fertility has grown into the big burden of persons of all walks of life. Recently, there has been considerable rise in the use of natural products as an alternative to modern medicines throughout the world (4). During the recent past decades a large number plants, their phytoconstituents have been evaluated for their antifertility effects on males (2). Solanum nigrum Linn. (Family: Solanaceae) Commonly known as black night shade in an annual herb widely distributed throughout the india, srilanka and tropical and warm temperate regions of the world. It is used as a traditional folk drug for handling various ailments for a long history of medicinal usage. The fresh juice of the whole plant is used in treating dropsy, ulcer, gonorrhoea, hemoptysis, piles, dysentery, Enlargement of liver and spleen. Its decoction is considered antispasmodic and narcotic. The warmed leaves are useful in relieving pain and swollen testicles, and the leaf paste is used as a poultice to provide relief for gout, rheumatic joints and skin diseases. The berries are considered to have tonic, diuretic, laxative and cathartic properties. A decoction of the flowers and berries is effective in cough, bronchitis, and diarrhea. The root bark is useful in ophthalmic, ophthalmopathy and hepatitis (3), (4), (5), (6). Experimental studies have exhibited that S. nigrum fruit retains many pharmacological activities, like cytoprotective antinociceptive, and antipyretic anti-inflammatory, antimicrobial, antioxidant, anticancer, hepatoprotective, antidiabetic, cardio protective and antimicrobial (7,8,9). S. nigrum is one of the main constituents of the plant based remedy prescribed for dysfunctional uterine bleeding. It has spasmylic action on the uterus (9). The plant extract have been reported to show uterotrophic and estrogen agonistic effect in in-vivo and in-vitro studies, respectively (10). The berries of S. nigrum have been described to hold a variety of chemical constituents such as glycoalkaloids (5), steroidal glycosides (11), steroidal sapogenins (losoigenin, trigoigenin), steroidal genin (gilogenin), alkaloids, saponins, coumanins, terpenoids, flavonoids, tannin and polyphenolic compounds (12), (13), (6), (7). Since S. nigrum is widely used medicinal plant and the effect of this plant on male reproductive functions are has very limited studies. Hence, the present study was attempted to investigate the effect of S. nigrum fruit extract of on the sex hormone and the testes.

2. MATERIALS AND METHODS

2.1. Plant used: From the regional of “Smriti Van, Jaipur, Rajasthan (India)”, fresh ripe fruits of S. nigrum were collected. The fruits were verified at the “Dept. of Botany herbarium of Rajasthan University” and the certificate number is (RUBL 211341). Alongside 50% ethanol at 55°-60° C for 35 h the fruits were pulverized into coarse powder and soaked. To receive a dark brownish dry viscous mass, that shows extract. Before administering to treated animals the extract was suspended in sterile distilled water.

2.2. Animals: In the present work used weighing of 170-200 g adult healthy, colony bred, male albino rats of Wistar strain. The rats were retained in polypropylene cages and retain below standard handshy situation (12 h light/dark cycle: 23±1°C). Rats were provided Standard laboratory meal “(Aashirwad Food Industries, Chandigarh, India)” and water ad libitum. “The study was allowed by the animal Ethical Committee of the Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur”, For the maintenance and use of the investigational animals, Indian National Science Academy (2000) procedures be there monitored.

2.3. Experimental plan: The rats were branched in five different groups every one having of 7 rats and give orally, allow 60
days treated as follows: Group A: Control group received vehicle (0.5 ml/day of the distilled water), Group B: Fruit extract of S. nigrum at a dose of 100 mg/kg b. wt.一日, Group C: Fruit extract of S. nigrum at a dose of 250 mg/kg b. wt./day, Group D: Fruit extract of S. nigrum at a dose of 500 mg/kg b. wt./day, Group E: Recovery of 60 days later removal of S. nigrum treatment similar to group D.

2.4. Autopsy program: After 24 hours of their last dose, the animals were sacrificed under the mild ether anesthesia, the testes were cut apart freed from adherent tissue part & blood and weighed.

2.5. Hormonal assay: By the cardiac puncture Blood samples were collected and permit to coagulate at room temp. At 2000 rpm serum was isolated through centrifugation for 20 min at 4°C & for hormone assays put in storage at -20°C.Serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured through chemiluminescence immunoassay.

2.6. Histopathological examination: In Bouin’s fluid the testes was fixed, and cut into fragments and processed over ethanol-xylene series and inserted in paraffin wax. The Section were cut at 5µ and stained in hematoxylin- eosin then observed for histopathological changes.

2.7. Histomorphometry
The mean seminiferous tubule diameter were measured through light microscope by way of ocular micrometre calibrated by stage micrometer; 20-40 tubular profiles at X 100. Similarly, Leydig cell nuclear diameter were also measured at X 1000 with ocular micrometer under light microscope. “Described by Leblond and Clermont (1952) (14) Quantitative evaluation of germ cells like, spermatogonia, preleptotene, pachytene spermatocytes and round spermatids were made in stage VII of seminiferous tubule per cross section under X 100 magnification”. By means of an ocular micrometer the nuclei diameters of numerous germ cell forms were restrained.“To obtain the actual numerical density of germ cells a correction factor was used (Abercrombie, 1946)” (15).

2.8. Statistical examination: All the data are expressed as Mean ± SEM. By using one way ANOVA followed by Turkey’s test in the mean was analyzed statistical difference. P <0.05 was accepted by means of statistically significant.

3. RESULTS

3.1. Testes weight: A significant drop of relative wt. of testes (P<0.01; P<0.001; P<0.001) was noticed contrast with control rats. Recovery group showed a significantly (P<0.001) rise in the relative wt. of testes when compared with group IV (500 mg) However, in comparison to control rats it was still low (Fig I).

3.2. Sex hormones: Serum testosterone (P<0.01; P<0.001; P< 0.001), FSH (P<0.05, P<0.001; P<0.001) and LH (P<0.05; P<0.01; P< 0.001) levels were dropped significantly on rats treated with S. nigrum (100, 250 and 500 mg/kg) plant, when compared with control rats. In recovery group, the reduced level of hormones were significantly (P<0.001) recovered as compared to treated rats (Group IV). But, the level was quiet low (P< 0.05) contrast to control rats (Fig I).

3.3 Histomorphometric study
Germ cell count: Changes in germ cell counts i.e. spermatogonia, preleptotene, pachytene as well as round spermatids be there presented in table 1. A significant decrease of spermatogonia (medium and high dose, P<0.05; P< 0.01), preleptotene and pachytene (P<0.05, P<0.01, P<0.001), as well as round spermatids (P<0.05; P<0.001; P< 0.001, respectively) when contrast by control rats. Recovery group (500 mg/kg), a significantly (P<0.001) rise in the germ cell population count when compared with Group-IV. However, in comparison to control rats it was still low. There were as well significantly decrease in the Seminiferous

Figure I: Effect of S. nigrum extract treatment on the testes weight in male rats

Figure II: Effect of S. nigrum extract on FSH, LH and Testosterone levels in male rats

(datas mean ± SEM of seven rats and significantly inequality is: a-P<0.05; b- P<0.01; c- P<0.001 comparison to control, ***- P<0.001 comparison to 500 mg)
tubule (P<0.05; P<0.01; P< 0.001) and Leydig cell nuclear diameter (P<0.01; P<0.001; P< 0.001, respectively) in the rats treated with three separate measure (100, 250 and 500 mg/kg) of S. nigrum extract as compared to control group. However, in recovery group were recover significantly (P<0.001) later 60 days of the extract treatment removal when contrast with Group-IV, but it quiet significantly (P<0.05) low comparison to controls. (Fig III & IV).

<table>
<thead>
<tr>
<th>Table 1: Effect of S. nigrum extract on germ cell population’s count / C.S. seminiferous tubule in male rats</th>
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<td><strong>Treatment</strong></td>
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<td>Control</td>
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<td>Group II</td>
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<td>S. nigrum (100mg)</td>
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<td>Group III</td>
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<td>S. nigrum (Recovery)</td>
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Histopathology of highest dose of S. nigrum (500 mg/kg) showed severe degenerative changes in seminiferous tubules. The seminiferous epithelium showed decline of germ cell populations, vacuolization and sloughing of germ cells. Lumen of the seminiferous tubule was considered by exfoliated germ cells. The Leydig cells exhibited marked degeneracy (Fig 2 d). In recovery group, testicular histopathology express approximately normal structure of seminiferous tubules with all the successive phases of germ cells and Leydig cells near return to normal (Fig 3).

4. Histopathology of testes: The histology of the control rat testes manifest seminiferous tubules normal structure and quality arrangement of all the successive germ cell types with prominent Leydig cells. (Fig 1 a). The histopathology of low dose of S. nigrum extract (100 mg/kg) showed some degenerative changes as presence of less sperms in tubular lumen and slight shrinkage of seminiferous tubules. Nuclear diameter of the Leydig cells also reduced (Fig 1 b). The histopathology of rats treated with S. nigrum (250 mg/kg) showed marked degenerative changes. Reduced in size, decline of germ cells population and showed disruption of normal epithelial organization of seminiferous tubules. Leydig cells were contracted and showed degeneracy (Fig 2 c). The

![Figure III: Effect of S. nigrum extract on Seminiferous tubule diameter in male rats](image)

![Figure IV: Effect of S. nigrum extract on Leydig cell nuclear diameter in male rat](image)

![FIG.1: Histopathology of the control rat testes display normal histoarchitacture (H. E. X 200).](image)
5. DISCUSSION

Testicular weight measurement is one of the important end point test for monitoring antispermatogenic and antiandrogenic activity of any test material (16), (17). In the current work, there was dose dependent drop in the testes relative weight of extract treated rats. Adequate supply of testosterone shows an essential role in the structural and efficient integrity of testis. The decrease in the relative weight of the testis might be due to depletion of testosterone level which was observed in the current study. Parallel to our findings, earlier studies have also shown significant decay in the testis weight in various animal models treated with solasodine (18) and solanine (19) glycoalkaloids, which are also present in S. nigrum berries/fruits. Moreover, "Singh and Teotia (2018) (20) also demonstrated a significant decline in the relative weights of testis in rats treated with Solanum xanthocarpum plant extract". Testicular weight and size are normally regulated by fluid secretion from the Sertoli cells and the mass of differentiated spermatogenic cells. The decline in weight of the testis might be connected with germinal epithelium erosion, loss of spermatids and spermatozoa and impairment of steroid biosynthesis in the Leydig cells (21), (22). The present results are also in consonance with many earlier reports which have shown similar impairment of spermatogenesis and steroidogenesis and drop in the weights of testis in the rats/mice after treatment with various plant (23), (24), (25), (26), and (27). It is well known that reproductive function is regulated by hypothalamic pituitary-testicular (H-P-T) axis function. The hypothalamic secretes gonadotropin releasing hormone (GnRH) which stimulate the pituitary to secrete FSH and LH. FSH acts on the Sertoli cells by binding with FSH dedicated receptors and regulates the function of Sertoli cells, whereas the LH take action on the Leydig cells and encourage the synthesis and secretion of testosterone which also acts on Sertoli cells and exert synergistic effect with FSH in the process of spermatogenesis. The levels of pituitary gonadotrophins (FSH & LH) are managed by negative feedback mechanism medicated by testosterone on H-P-T axis (28). Moreover, recent study have suggested that estrogen also play significant role in testicular physiology and spermatogenesis (29), (30) and the key element of major negative feedback act of androgens is facilitated via aromatization to estrogen (31). Significant drop in the serum pituitary hormones levels (FSH & LH) and testosterone following S. nigrum extract administration in rats indicates the impairment of the H-P-T axis function by virtue of individual or synergistic actions of the phytoconstituents present in the S. nigrum extract. Phytochemical studies have shown the presence of many classes of phytoconstituents in aqueous/alcoholic extract of S. nigrum ripe berries/fruits (32), (6), (12), (13). The extract of S. nigrum berries have been reported to possess estrogenic property (10). These phytoestrogens might interference with estrogen mediated negative feedback mechanism of H-P-T system by estrogen receptors binding in the hypothalamus and pituitary gland thereby reducing the discharge of pituitary FSH, LH and testosterone biosynthesis in the Leydig cells of the testis. A similar decline in circulating FSH, LH and testosterone via impairment of H-P-T axis negative feedback has also been reported in animals treated with estrogen (33), (34) and diethylstilbestrol (DES) (35). Besides affecting H-P-T axis, the
phytochemicals present in the extract may cross the blood-testis barrier and directing act on the Leydig cells resultant in the impairment of the activity of steroidogenic enzymes and consequently testosterone biosynthesis (36). Parallel to present findings, some previous study have also shown antiandrogenic effect of solasodine (glycoalkaloid also present in S. nigrum extract) in bioassay studies (18), (37). “Rao (1988) (38) also reported decline of serum testosterone levels in Solanum xanthocarpum seed extract treated rats”. Moreover, several previous studies using different plant extracts having flavonoids, tannins, terpenes, saponins, phytosterols, coumarins, alkaloids etc. have shown significant decline in circulating FSH, LH and testosterone levels in treated animals (39), (40), (41) (42). In contrast to present finding, “Yubin et al (2011) (19) did not observe any significant effect of solanine treatment on serum testosterone in mice”, although the expression of 3β-HSD (3 β-hydroxysteroid dehydrogenase) was significantly inhibited in Leydig cells. Recently, “Adelakun et al (2018) (43) reported significant increase in blood level of FSH, LH and testosterone as well as sperm function in rats treated with aqueous extract of S. nigrum levels for 28 days. This observed discrepancy in the results might be due to difference in extraction procedure, part used as well as duration of treatment”. Spermatogenesis is a highly ordered sequence of molecular and cellular events taking place in the testes that depends on coordinated interaction between Leydig cells, Sertoli cells, germ cells, peritubular cells and interstitial macrophages as well as proper blood vascularization (44). The normal functions of these components in the testes are regulated by H-P-T axis. Failure in some of these components may compromise the process of spermatogenesis and quality of spermatozoa (45). In the present study, histological examination of the testis in rats examine with distinct doses of extract of S. nigrum ripe fruits exhibited dose related degeneracy effects and lesion in spermatogenesis. The seminiferous epithelium in the highest extract dose treated rats exhibited marked degeneration, vacuolization, disintegration and sloughing of germ cells. The numbers of spermatogonia, preleptotene & pachyten spermatoocytes & spermatids declined significantly. Most of the seminiferous tubule were devoid of spermatozoa and contained sperm debris. The diameters of seminiferous tubules and Leydig cell nuclei were also reduced significantly. The observed histological alteration and arrest of spermatogenesis in the extract treated rats might be related with reduced serum levels of gonadotropins and testosterone. It is thoroughly accepted that Sertoli cells/germ cells needed FSH and testosterone to support spermatogenesis, fall in the nuclear diameter and atrophic alterations in Leydig cells also support decline of testosterone biosynthesis. In extract treatment the diminution in the number of preleptotene, mid-pachytene spermatocytes as well as spermatids, also specify abbreviate intratesticular testosterone, as for post-meiotic division testosterone is necessary and also for spermiation during spermatogenesis (46), (47). The disintegration and sloughing of germ cells could be due to damage of Sertoli cells and interruption of intracellular bridges between the germ cells and Sertoli cells (46), (48). Marked reductions in the diameter of the seminiferous tubules in treated group may be by reason of the loss of developing spermatogenic cells and spermatozoa in the tubules (49) as well as increased germ cell apoptosis due to deprivation of intratesticular testosterone and pituitary gonadotropin hormones (50), (51). The observed antispermatogenic activity of S. nigrum extract in treated rats may be due to individual and/or synergistic effects of the phytoconstituents present in the extract. Antispermatogenic activity of many phytochemicals which are also present in S. nigrum extract such as solasidine (18), (37), solanine (52), (19), saponins (24), flavonoids (53), (23), terpenes (54) and β-sitosterol (27) and many plants having such type of bioactive phytoconstituents have been reported in earlier studies which also corroborates present findings (55), (56), (25), (39), (41). The antispermatogenic effects of crude 50% ethanolic extract of S. nigrum fruits in the present study may possibly due to its estrogenic activity as many synthetic estrogens (33), (34) diethylstilbestrol (DES) (35),phytoestrogens (57), (58) and endocrine disruptor chemicals (59) have been reported to alter sex hormone levels and disruption of spermatogenesis in treated animals. Rats of group of recovery indicated significant rise in the relative weight of testes, sex hormones levels and the histoarchitecture of the testis indicating reversibility of the extract induced adverse effects.

6. CONCLUSION
This work concluded that the 50% ethanolic extract of S. nigrum fruit make significant adverse effect on the testes weight, serum reproductive hormone profile and impairment of spermatogenesis in male rats. Withdrawal of the extract treatment showed reversibility of altered parameters.

7. ACKNOWLEDGEMENT
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