

Synopsis of the Thesis

Possible antigonadal activities of composite extract
of root of *Achyranthes aspera* and leaf of
Stephania hernandifolia in male albino rat

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The combination of a continuing high birth rate and a low death rate is creating a rapid population increase in many countries in Asia, Latin America and Africa. Human population is increasing very rapidly especially in country like India and China. The growth in human population around the world affects all people through its impact on the economy and environment. Hence there is a need to check the rapid growth of the population. Several contraceptive methods are used nowadays of which hormonal method is most popular. It has several side effects. Beside this vaginal contraceptive whose main ingredient is nonoxynol-9 is not free from side effect. So stress is given on contraceptive of herbal origin. In Indian tribal medicine herbal preparation to solve reproductive problem is common. In the present thesis work it is our effort to assess the antitesticular activity of leaf of *S. hernandifolia* and root of *A. aspera* in a composite manner. For assessment of contraceptive efficacy of the composite extract of the two said plants, epididymal sperm count as well as the sperm motility were recorded in male albino rats. To evaluate the effect of the extract on spermatogenesis, different generation of germ cells at stage VII of seminiferous epithelial cell cycle was recorded. To find out the mode of action of the extract, plasma level of testosterone, and the activities of steroidogenic key enzymes i.e. Δ^5 , 3 β -HSD and 17 β -HSD were measured. Activities of ACP of testes and prostate as well as level of seminal fructose are related to plasma testosterone level. Hence to confirm the effect of the extract on testicular androgenesis, these parameters were considered. There is an inverse relationship between plasma testosterone and testicular cholesterol level and hence to confirm about the effect of the extract on pituitary testicular axis by modulating plasma testosterone level, the testicular cholesterol was measured. The extract of the two said plants in composite manner may also affect the male reproduction by directly acting at the testicular level by imposing oxidative stress, hence oxidative stress parameters such as activities of catalase and peroxidase which are the important antioxidant enzymes as well as products of free radicals such as the levels of CD and TBARS were evaluated in testis as well as on mature germ cells. To assess whether the

extract at the effective dose may cause metabolic toxicity, the activity of GOT and GPT as well as ACP and ALP in liver and kidney were measured.

To evaluate the *in vitro* sperm immobilization effect of the extract, epididymal sperm of rat as well as human semen were mixed with the composite extract and sperm motility was evaluated. To find out the mode of action of the extract for the spermicidal action, sperm viability, hypo-osmotic swelling (HOS) test, as well as acrosome status test were performed.

In the present thesis the following experiments were designed:

- i. Comparative study of the anti-testicular effect of the composite extract of leaf of *S. hernandifolia* and root of *A. aspera* in male albino rat using water, methanol, ethanol or hydro-ethanol (1:1) as solvent and to find out the effective solvent extract for this antigonadal activity.
- ii. Ratio dependent experiment was framed to delineate the effective ratio of *S. hernandifolia* and *A. aspera* that exhibit better antitesticular activity. In this experiment the animals were treated with the effective solvent extract of *S. hernandifolia* and *A. aspera* at three different ratios i.e. 1:1, 1:3, 1:7 respectively. The ratio of the two plants which on treatment exhibit maximum inhibition in testicular function was considered as the effective ratio.
- iii. Dose dependent experiment was designed to find out the minimum effective dose of the composite extract of the two plants that inhibit the testicular steroidogenesis as well as spermatogenesis without causing metabolic toxicity. Here rats were treated at three different doses i.e. 40 mg or 80 mg or 160 mg/100 gm body weight.
- iv. Duration dependent experiment was framed to determine the minimum effective or threshold duration required for the anti-testicular activity. In this experiment the animals were treated with the composite extract for four different durations i.e. 7 days or 14 days or 28 days or 56 days.
- v. Human chorionic gonadotrophin (hCG) acts as LH. Ascorbic acid and provitamin-E acts as antioxidant vitamins. To evaluate whether the

composite extract inhibit the testicular function by modulating the pituitary testicular axis alone or the extract may directly acts at the testicular level by imposing oxidative stress, experiments involving hCG or ascorbic acid or provitamin-E co-administration in the extract treated rats were designed.

- vi. Withdrawal experiment was framed to find out the nature of the antitesticular activity of the extract i.e. temporary (reversible) or permanent (irreversible).
- vii. Pituitary testicular axis is very much sensitive at the onset of puberty. So to investigate whether the composite extract is capable of inhibiting the testicular function and modulate the sensitivity of the pituitary testicular axis, the experiment was designed using the immature or pre-pubertal rat.
- viii. *In vitro* experiment was conducted to search out the direct effect of the composite extract on testicular steroidogenesis, by incubating testes in the *in vitro* tubes.
- ix. *In vitro* sperm immobilization experiment was framed to find out the spermicidal activity of the hydro-ethanolic (1:1) composite extract of the two plants as well as its different fraction on rat and human sperm, by treating rat's epididymal sperm and human spermatozoa with the extract.
- x. Fertility efficacy test was performed to evaluate the fertility efficacy of the hydro-ethanolic (1:1) extract of the two plants in composite manner by mating with the extract treated male rat with the fertile female rats at the estrous phase and subsequent noting the implantation site.
- xi. Thin layer chromatography (TLC) study was designed to focus the chemical nature of the hydro-ethanolic (1:1) extract of leaf of *S. hernandifolia* and root of *A. aspera* and their active fraction.

From the results of solvent dependent study, it was indicated that after the treatment with the extract of the two plants in a composite manner using water

or methanol or ethanol or hydro-ethanol (1:1) as solvent, epididymal sperm count, sperm motility, activity of Δ^5 , 3 β -HSD and 17 β -HSD, level of plasma testosterone, activities of ACP in testes and prostate, seminal vesicle fructose level were decreased significantly along with the significant increase in the testicular cholesterol level in the extract treated group in respect to the control. However maximum inhibition in the testicular androgenesis was noted in the hydro-ethanolic (1:1) composite extract treated group in respect to other treated groups using water or methanol or ethanol as solvent for extract preparation. Testicular spermatogenesis was also affected by the extract treatment which was reflected by the decrease in the numbers of germ cells at stage VII of spermatogenic epithelial cell cycle. However, the effect is more prominent after treatment with the composite extract using hydro-ethanol (1:1) as solvent. The extract treatment did not cause any metabolic toxicity which has been indicated by the fact that the activities of ACP and ALP as well as activities of GOT and GPT in liver and kidney were insignificantly differed in respect to the control. The extract treatment imposes oxidative stress which was indicated from the results of testicular catalase and peroxidase activity as well as from the levels of CD and TBARS. The inhibition in the antioxidant enzymes and subsequent imposition of oxidative stress in testis was significantly more in the hydro-ethanolic (1:1) extract treated groups in comparison to the other treated groups. The results of STD and LCNA also supported that the hydro-ethanolic (1:1) composite extract of the two plants exhibits better antitesticular activity than the other extracts prepared using water, methanol or ethanol as solvent. From this experiment it may be hypothesized that the extract may inhibit the testicular activity either by modulating the pituitary testicular axis or by imposing oxidative stress at the testicular level directly.

From the results of ratio dependent study it was revealed that the relative sex organ weight, epididymal sperm count, sperm motility, testicular androgenic key enzyme activity, level of plasma testosterone, ACP activities in testis and prostate, seminal vesicle fructose level were decreased

significantly along with the significant increase in the testicular cholesterol level after treatment with the hydro-ethanolic(1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:1, 1:3 and 1:7 respectively in respect to the control. On comparative analysis among the results of different treated groups, treatment with hydro-ethanolic (1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:3 showed better antitesticular activity in respect to other ratios. The results of different generation of germ cells at stage VII as well as STD and LCNA also indicated that the hydro-ethanolic (1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:3 exhibits better antitesticular activity in comparison to other ratios. The assessment of oxidative stress also indicated that the hydro-ethanolic (1:1) extract of the two said plants at different ratio caused significant inhibition in catalase and peroxidase along with the significant increase in the levels of CD and TBARS in sperm pellet in respect to the control. However, the imposition of oxidative stress by the extract was significantly greater after treatment with hydro-ethanolic (1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:3 in respect to other ratios. So from this experiment it was clear that hydro-ethanolic (1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:3 exhibits better antitesticular effect than the other ratios studied.

From the dose dependent study it was noted that the relative weight of different sex organs, sperm count, sperm motility, activities of Δ^5 , 3β -HSD and 17β -HSD and level of plasma testosterone were decreased significantly along with the increase in testicular cholesterol in the extract treated groups at different doses in respect to the control. However, the said parameters were altered significantly after treatment with the hydro-ethanolic (1:1) composite extract of the two plants at a dose of 80 mg or 160 mg/ 100g body weight in respect to 40 mg/100g body weight dose in rats. The results of seminal vesicle as well as the activities of ACP in testes and prostate also support that hydro-ethanolic (1:1) composite extract of the two plants inhibit testicular androgenesis in a dose dependent manner. The dose dependent testicular inhibitory effect of the extract was also noted from the results of different

generation of germ cells at stage VII of seminiferous epithelial cell cycle. Comparative analysis of GOT and GPT activities as well as ACP and ALP activities in kidney and liver revealed no significant metabolic toxicity after treatment with the composite extract upto 80 mg/ 100g body weight. However, after treatment with the extract at a dose of 160 mg/ 100g body weight there was a significant increase in the activities of these enzymes in respect to the control. Hence the 80 mg/100g body weight in rat is the minimum effective dose for induction of antitesticular activity without causing any metabolic toxicity.

The results of duration dependent study indicated that after treatment with the hydro-ethanolic (1:1) extract of *S. hernandifolia* and *A. aspera* at a ratio of 1:3 and at a dose of 80 mg/ 100g body weight in rat for 28 days is the minimum effective duration for this antigonadal function. Because inhibition in steroidogenesis and spermatogenesis was not further inhibited on extending the duration of treatment upto 56 days over 28days as activities of androgenic key enzymes, plasma level of testosterone, activities ACP in testis and prostate and seminal vesicle fructose level was decreased maximally after 28 days of treatment and no further diminution of this parameter was noted on extending the treatment upto 56 days. The results of this experiment also indicated that beside modulating the pituitary testicular axis, the extract treatment may also directly inhibit testicular function by creating oxidative stress as the activity of catalase and peroxidase in sperm pellet reduced significantly in respect to the control even after extract treatment for a period of 7 days along with the significant elevation in the level of CD and TBARS. Hence from this experiment it is noted that treatment with the hydro-ethanolic (1:1) composite extract for 28 days is the minimum effective duration for the antitesticular activity and it may be postulated that the extract may bring about this testicular inhibition either by modulating the pituitary testicular axis or the extract may directly act at the testicular level by imposing oxidative stress.

To find the validity of our postulation about the dual mode of action of the extract, hCG co-administration experiment as well as co-administration of

ascorbic acid or provitamin-E was performed. Human chorionic gonadotrophin acts as LH while ascorbic acid and provitamin-E are antioxidant vitamins. The results of these experiments indicated a partial recovery of different testicular parameters which support our hypothesis that the extract may act by regulating pituitary testicular axis or the extract may directly act at testicular level by imposing oxidative stress.

From the results of withdrawal experiment, it was noted that the epididymal sperm count, activities of androgenic key enzymes, plasma testosterone level, testicular cholesterol level, different generation of germ cells at stage VII, STD and LCNA as well as antioxidant enzymes in testis as well as sperm cells were recovered towards the control level after cessation of extract treatment for 28 days. So the result of this experiment indicates that the composite extract inhibits the testicular activity but its action is temporary or reversible in nature.

Study conducted by treatment of pre-pubertal rat with hydro-ethanolic (1:1) composite extract of the two plants indicated that the extract are able to inhibit the initiation phase of spermatogenesis and can suppress the pituitary testicular axis which remains in most sensitive condition at the pubertal stage.

The direct effect of the composite extract was confirmed from *in-vitro* experiment. After incubation of the testis with the composite extract it was revealed that the composite extract of the said two plants inhibit the testicular steroidogenic key enzyme activity and increase oxidative stress in the *in-vitro* condition when compared to the control testis.

Regarding the fertility efficacy of the composite extract it was observed that implantation sites of female rats mated with composite extract-treated males did not show any implantation sites. Whereas female rats of control group exhibited implantation sites at an average rate of four/ uterine horn.

Composite extract of the two plants also possess spermicidal effect is evidenced by *in-vitro* experiment. Treatment of rat epididymal spermatozoa and human semen with hydro-ethanolic (1:1) composite extract and their active fraction i.e. hexane fraction resulted sperm immobilization. The results

of this experiment also revealed that sperm immobilization caused by the hydro-ethanolic (1:1) composite extract and their active fraction is irreversible in nature and spermicidal activity of the extract or its fraction may be brought about by disrupting the membrane integrity of the sperm cells.

The TLC study which was conducted to find out the phytochemicals in the plant extracts indicated that the hydro-ethanolic (1:1) extract of leaf of *S. hernandifolia* contains steroids and alkaloids. The hydro-ethanolic (1:1) extract of roots of *A. aspera* contains steroids.

In the hexane fraction of *S. hernandifolia*, three spots were identified in UV 254 nm and in case of *A. aspera*, four spots were identified in UV 254 nm.

So from the different experiments of this thesis work it may be concluded that the hydro-ethanolic (1:1) extracts of leaf of *S. hernandifolia* and root of *A. aspera* in composite manner at a ratio of 1:3 and at dose of 80 mg/ 100g body weight for 28 days in rat can exert better antitesticular activity. Two hypotheses may be proposed for this antitesticular activity. The extract may modulate the pituitary testicular axis by affecting the hormonal milieu or the extract may exert a direct inhibitory effect on the testicular level by imposing oxidative stress. The antitesticular activity is not permanent in nature. The composite extract also seems to possess spermicidal property and thus may be used as vaginal contraceptive. The present research may be a preliminary step in the development of herbal contraceptive or vaginal contraceptive of herbal origin. This thesis work may open new avenues for further research after isolating the compound(s) responsible for antitesticular activity or spermicidal action.