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Evaluation of *Sida rhombifolia* L roots extract on Immobilization stress induced male infertility in rats

Prakash R. Biradar (prakashrb123@gmail.com)

Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi-590010, India https://orcid.org/0000-0001-5573-4595

Ashwini Karade

Department of Pharmacology and Toxicology, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi-590010, India

Research Article

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Abstract

Current study aimed to investigate protective effect of *Sida rhombifolia* L roots hydroalcoholic extract on immobilization stress induced male infertility in experimental rats. Male wistar rats (200-250g) were randomly divided into six groups (n=6). The male fertility enhancing activity was evaluated by sexual behavior, semen parameters, body weight, SGI, hematological parameters, vital and sexual accessory organ weight, histopathology by using standard procedure. GSH, SOD and LPO level were measured in testis tissues to evaluate antioxidant activity. The *S. rhombifolia* L. root hydroalcoholic extract indicated *in vitro* antioxidant activity on scavenging of hydroxy radicle, TAC and NO. Improvement in sperm quality i.e. count, motility, viability, Sexual behaviour, Body weight, sexual and vital organ weight, GSI, seminal fructose, Testicular cholesterol level, haematological parameters observed in treatment groups just like standard group. *S. rhombifolia* showed antioxidant capacity by lowering NO, MDA and elevated SOD in testis tissue. Investigations manifested that improved spermatogenesis in group treated with *S. rhombifolia* L in contrast with immobilization stress induced group resemblance to *in silico* finding study which revealed that SRR extract have adaptogenic and fertility enhancing effect.

Introduction

The desire for bearing children is overwhelming, just as boundless, yet for certain couples its most certainly not effectively to achieve. This is emerging because of
reproductive system illness characterized by a couple's failure to meet clinical pregnancy following a year of continuous unprotected intercourse (Choy et al.2018). The Socio-economic and clinical issues which are influencing in most of the couples might be because female or male factor. As per the literature it was assessed that, the overall 15% of couples managing tribulation of infertility among that half of the couples are experiencing male factor infertility (de Kretser et al.1998).

Various variables recognized like psycho-socioeconomic/ occupational stress are major variables due to globalisation, primarily desk holder like IT profession, bankers etc. suffer from impotency and erectile dysfunction (ED) due to longer duration of immobilization. Many studies successfully represented psyco-physical stress by using immobilization stress model (IMS) and reported for sexual dysfunction (Simo et al. 2019), impaired spermatogenesis (Simo et al. 2019, Lohitha et al. 2009, Mahdivand et al. 2019) and testicular damage (Arun et al. 2018, Lee et al. 2019).

Traditional medicines are well utilized in the management in multiple pathogenic conditions including multiple infectious and non-infectious conditions via multiple approaches i.e. *in vitro* analysis and *in silico* approaches (Khanal et al $2019_{a,b,c,d}$; Khanal et al $2020_{a,b,c,d,e}$; Rodrigues $2020_{a,b}$; Patil et al 2019; Ternikar et al 2020). Similarly, traditional folk medicine *Sida rhombifolia* L. belonging to family Malvaceae is reputed to have numerous applications in traditional system since, this plant root has traditional claim to enhance spermatogenesis activity (Khare 2008); but there is lack of empirical

evidence. Consequently, the present experiment anticipated the potential effect of *Sida rhombifolia* Linn root hydroalcoholic extract (SRR) to treat male infertility with the aid of experimental pharmacology via *in vivo* study on male Wistar rats.

Materials And Methods

Plant collection and authentication

Plant *Sida rhombifolia* L. collected and certified by botanist from ICMR (NITM) Belagavi, Karnataka, India (Voucher number: RMRC-1438). Shade dried roots of plant were reduced to fine powder and subjected for Maceration followed by soxhlet extraction as explained previously (Duyu et al 2020; Khanal $2020_{a, b}$).

In vitro antioxidant assays

Hydroxyl radical scavenging activity was performed as explained by Pavithra & Vadivukkarasi 2015. Nitric oxide scavenging capacity was performed as explained by Boora et al. 2014. Total antioxidant capacity phosphomolybdenum was performed as explained by Kumaran & Karunakaran 2007.

Acute oral toxicity test

Acute toxicity carried out as per the OECD guidelines 423 revised from CPCSEA, Ministry of Social Justice and Empowerment, Govt. of India. Oral acute toxicity did not show any mortality as well as side effect at highest dose (2000mg/kg).

Experimental animals

The complete experimental study assed on healthy male Wistar rats weighing about 160-200 gm, all animals used for the acute toxicity and experiments were approved by the Institutional animal ethical committee of KLE college of pharmacy, Belagavi (Resolution number: KLECOP/CPCSEA-rEG.nO.221/Po/Re/S/2000/cpcsea,Res.28-12/10/2019) and purchased from Bioscience lab Bengaluru, India.

Experimental design

Animals were divided into six group where (n=6) in each group. On the basis of LD_{50} three doses in geometric series were selected i.e. 150 mg/kg, 300mg/kg, and 600mg/kg. Treatment was given by administering extract orally for 30 days. 1. Normal control, 2. Immobilization stress/ Negative control (IMS), 3. Immobilization stress + testosterone (STD) 4. Immobilization stress + 150mg/kg SRR, 5. Immobilization stress + 300mg/kg SRR, 6. Immobilization stress + 600 mg/kg SRR.

Induction model

Animals were exposed regularly for a period of 30 days by immobilization stress for six hours in day time. Food and water were withheld during stress period. IMS was given by using metal framed wire mesh

restrained having size 5 × 5 × 9 cm.

Sexual behaviour study

Sexually experienced male Wistar rats from all groups where used for this test. Female Rats were artificially brought to oestrus phase, by administering suspension of ethinyl oestradiol orally with combine progesterone injection subcutaneously. The dose of ethinyl oestradiol was given 48 hours, prior to the mating and progesterone six hours prior to mating. The experiment was carried out on 0^{th} day, 15^{th} day and 30^{th} day of treatment under deemed light. Animals were introduced in an individual chamber with 1:1 female male ratio. Phases of mating i.e. mount latency (ML), mount frequency (MF), intromission latency (IL), intromission frequency (IF), penile erection frequency (PE), ejaculation frequency (EF) were recorded by cannon video camera for the duration 30 minutes (Zade et al. 2013).

Body weight as well as sexual and vital organ weight

After 30 days of treatment, all groups were observed for fasted body weight. All genitalia like testis, epididymis, vas deference, prostate gland, seminal vesicles and vital organs like liver, kidney, adrenal glands were removed and weighed also, gonadal somatic index (GSI) was calculated for all the animals.

Effect on haematological parameters:

Blood was collected from orbital sinus by using heparinized capillary tube. All blood parameters like RBC count, WBC count, Lymphocyte percentage, Haematocrit was calculated with help of Neubauer's chamber and Haemoglobin estimated by using Sahli's method.

Effect on semen parameters

Sperm count, sperm motility, sperm viability was carried out by using epididymis sperm semen. Fructose estimation was performed using resorcinol were produced pink colour in acidic medium. Intensity of colour was measured at 490nm (Mann 1948).

Biochemical estimations

LPO, GSH, SOD estimation in testicular tissues carried out (Chandrashekhar et al. 2013; Shalavadi et al. 2013) and testis cholesterol level was estimated by *Zaltski* method (El-Desoky et al. 2013).

Histopathology of testis

All animals were sacrificed on 30^{th} day of treatment; testis was isolated, fixed overnight in 10% formalin. Sections of the testis were fixed with, haematoxylin-eosin stain (H&E) technique and observed for pathological changes at 40X magnification**.** Section of testis were observed for diameter of seminiferous tubules, Sertoli cell score per 20 cells, Leyding cells, epithelium thickness of seminiferous tubules and Johnsen's mean testicular biopsy score. (Nna, et al. 2017; Yaribeygi et al. 2017)

Statistical Analysis

Data were expressed as Mean ± SEM were determined via one-way/two-way-ANOVA followed by, Tukey's Multiple Comparison Test & Bonferroni post-tests (Graph Pad -Prism software, version 5.01). *p <0.05* was reflected statistically significant.

Results And Discussion

SRR showed antioxidant activity as hydroxy radicle scavenging activity (IC_{50}) showed by Ascorbic acid and *S. rhombifolia* extract at concentration 52.976 µg/ml, 126.58(µg /ml) respectively. Nitric oxide (NO) scavenging activity (IC_{50}) of *S. rhombifolia* extract was found to be 115 µg/ml and gallic acid at concentration 38.431 µg/ml. Total antioxidant capacity (TAC) activity (IC_{50}) of ascorbic acid and SR extract were found to be 123.5(µg/ml) and 266.4(µg/ml) respectively.

Sexual performance observation revealed that an extended ML and IL along with reduced MF, IF, EF in IMS group whether, 150 mg / Kg, 300 mg / Kg, 600 mg / Kg treated groups results indicated a significant depletion in MI and IL along with rise in the MF, IF, EF, PI on 15^{th} day and 30^{th} day as compared to 0^{th} day data expressed in table S1.

Also, 150mg/kg, 300mg/kg and 600mg/kg treatment were showed significantly increased *(p<0.05, p<0.01, p<0.001*) in percentage increased body weight (Fig. 1), Gonadosomatic index (GSI) and vital organ (Table S2), sexual organ weight (Table S3) contrast with IMS group but Vas deference weight of all treatment group including standard didn't show any significant response in weight respect with IMS group as well as control group and adrenal gland weight declined in treatment group when compared with IMS group. IMS group animals revealed vice versa result contrast to control group.

Treatment and standard groups results indicated, significant increased (*p<0.05, p< 0.005, p<0.001*) in RBC, WBC counts, Lymphocyte, haematocrit percentage as well as Haemoglobin when compared with IMS group. IMS group showed, depletion in all haematological parameters with high significance *(P<0.001*) respect to normal group. Data exhibited in table S4

All treatment groups showed dose dependant increased in sperm count, sperm motility but 300mg/kg treatment group revealed highly significant increase in sperm viability (68.2±1.66%) contrast with IMS. IMS control showed highly significant depletion in sperm count (79.5±0.992million/ml), motility (49.8±0.833), viability (39.2±0.946%) in respect to normal group data expressed in table S5.

Test 300mg/kg and 600mh/kg treated groups result revealed 3.01±0.0742mg/ml, 3.52±0.0808mg/ml fructose content in semen with significant rise (*p<0.01, p<0.001*) in concentration as compared with IMS group i.e. 2.63±0.0348mg/ml as shown in Fig. 2 The IMS group demonstrated a significant decline (*p<0.01, p<0.001 and p<0.001*) with mean value (95.34±3.34, 5.16±0.027) in SOD levels, GSH and cholesterol(0.8±0.4) whereas MDA level (1.14± 0.04) significantly increases (*p<0.001*)in testicular tissue when compared to normal group (160.35±16.8, 13.019± 0.126, 2.93±0.1, 0.37± 0.05) respectively.

300mg/kg treated group significantly increased SOD, GSH (*p<0.05, p<0.001*) with mean value (145.2±6.2, 12.45±0.084) where as300 and 600mg/kg SRR treated groups with mean values (1.6±0.1, 2.59±0.5) indicated highly significant rise (*p<0.001*) in testicular cholesterol and 600mg/kg treated group represent significant decline(*p<0.001*) in MDA level (0.50± 0.026) respect to IMS group indicated Standard group like effect for testicular cholesterol is represented graphically in Fig. 3. The anti-oxidant activity of each group is summarized in table S6.

The IMS group showed reduced in diameters of seminiferous tubules, Number of leyding cells John's testicular biopsy score juxtaposed with normal. Treatment and standard drug manifested same effect as these group indicated rise in seminiferous tubule's diameter, leyding cell number and john's testicular biopsy score respect with IMS group. (Fig. 4)

Male infertility is diagnosed by abnormal semen quality as spermatogenesis plays key role in male impotency so Current experiment was conducted to explore male fertility and sexual performance enhancing capacity of *S. Rhombifolia* plant root hydroalcoholic extract using *in vivo* study. Numerous examinations exhibited that modern lifestyle, prolonged working condition as well as emotional stress are the major components, which antagonistically influences spermatogenesis and ultimately prompts male infertility (Yaribeygi et al. 2017) *IMS* model is used to represent chronic, physical and psychological stress. As previous investigations, *IMS* causes a drastic fall in serum androgen level, which influences sperm quality *and depletion in fructose influenced in motility as* seminal fructose gives wellspring of energy to the sperm for its motility. Depleted body weight, GSI, organ weight revealed similar findings as previous investigations in adult rats (Calvez, J et al. 2011) *due elevated level of* ACTH and corticosterone mediated by receptors on adrenal glands causes anorexia and inhibition of GnRH causes weight loss in sexual accessory organs, vital organ represents common changes of starvation. In stressed circumstance typical defence reaction get activated and the weight of adrenal gland increase after chronic stress is might because of hyperplasia and hypertrophy. Deplete testicular cholesterol, sperm count, sperm motility also sperm viability in IMS group (Hernandez, M.E. et al. 2013) and improved in all the treatment groups reasonable for improved spermatogenesis as cholesterol is a forerunner for important steroid hormone, secreted by the adrenal cortex and testis. Mating *behaviour* study results indicated that there is a significantly decreased in ML and IL alongside increased in MF, IF, EF, PE on 15^{th} day and 30^{th} day when contrasted to 0^{th} day may be due to *the presence* of phytoconstituents like steroids, flavonoids, alkaloids. As steroids *help to increase testosterone* level, flavonoids *help* to increase in dehydroepiandrosterone and alkaloids are responsible to increase NO level leads to dilation of blood vessels and anti-inflammatory action. This could be a potential component for *an improve percentage index* of libido and to overcome ED. Depletion in WBC count and lymphocyte percentage in stressed induced group indicates suppression of immune system due to increased level of corticosteroids in the IMS group similar to previous studies (Srivastava et al. 1993) and increased in the *S. rhombifolia* treatment group showed immunological protective effect. RBC count, Haematocrit and Haemoglobin decreased in the negative control group and increased in treatment group indicates, *S. rhombifolia* extract stimulate erythropoiesis which might be because of increase in erythropoietin level or expanded number of erythropoietin-responsive cells which is

clear sign of increased testosterone level in body as testosterone and its metabolites anabolic activity is answerable for bone marrow maturation and erythropoiesis**.** Rise in testicular SOD, GSH level and depletion in MDA in treatment group suggest good antioxidant capacity as IMS causes oxidative damage to testis also can be observed in histopathology of testis, decreased average seminiferous tubule's diameter, leyding cells in IMS group represented atrophic effect may due to oxidative stress. John's biopsy score increased in treatment group manifested improvement in spermatogenesis.

Conclusion

Our ascertainment's in concurrent experimentation evinced that hydroalcoholic extract of *S. rhombifolia* roots is imperative source in providing potent fertility enhancing capacity due to presence of various bioactive compounds involved in male infertility. *In vivo* Experiment showed increased Sexual performance, sperm parameters, Haematological parameters, vital as well as sexual organ weight, GSI, biochemical investigation as well as histopathological study resemblance to *in silico* finding which revealed that *S. rhombifolia* have adaptogenic effect due to flavonoids, alkaloids, steroids which showed protecting effect on male reproductive system in stressed condition. *S. rhombifolia* roots isolated compounds require a better understanding for possible mechanism involved in male fertility enhancing activity.

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Declarations

Both the Authors of this manuscript do not have any conflict of interest in any financial and non-financial way.

Figures

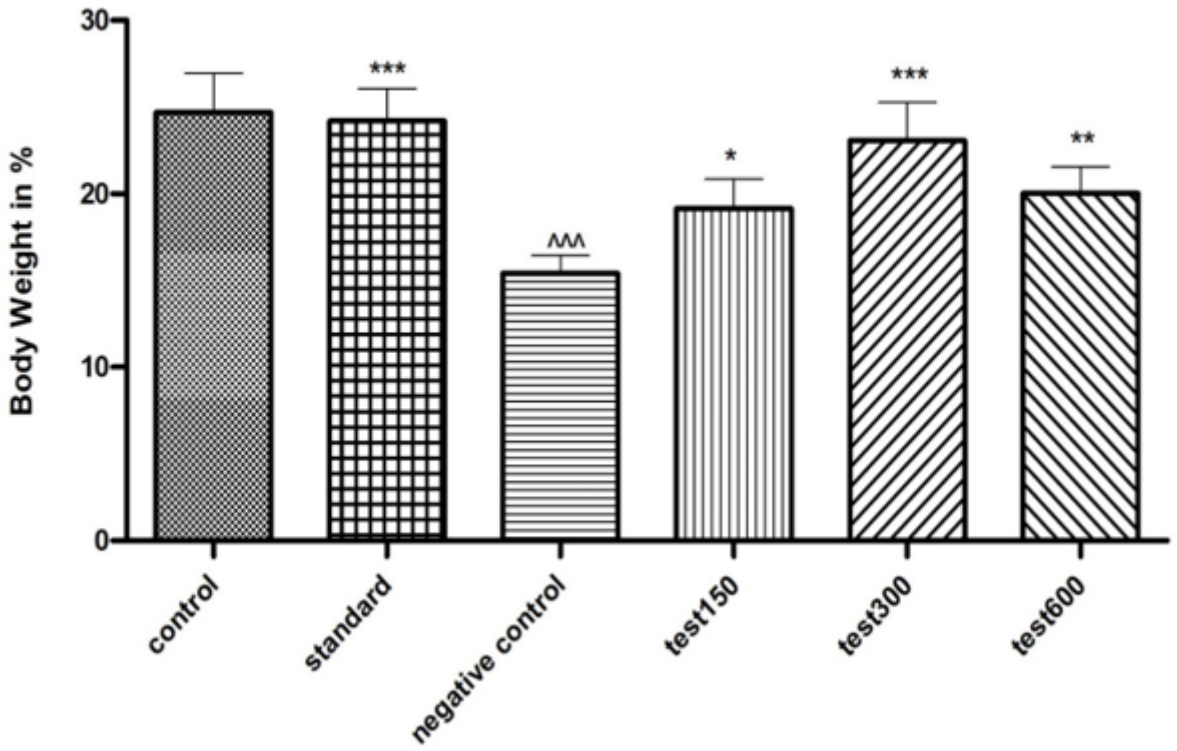

Figure 1

Effect of SRR on percentage increased body weight ***p<0.001 compared to normal, ^^^p<0.001 compared to standard

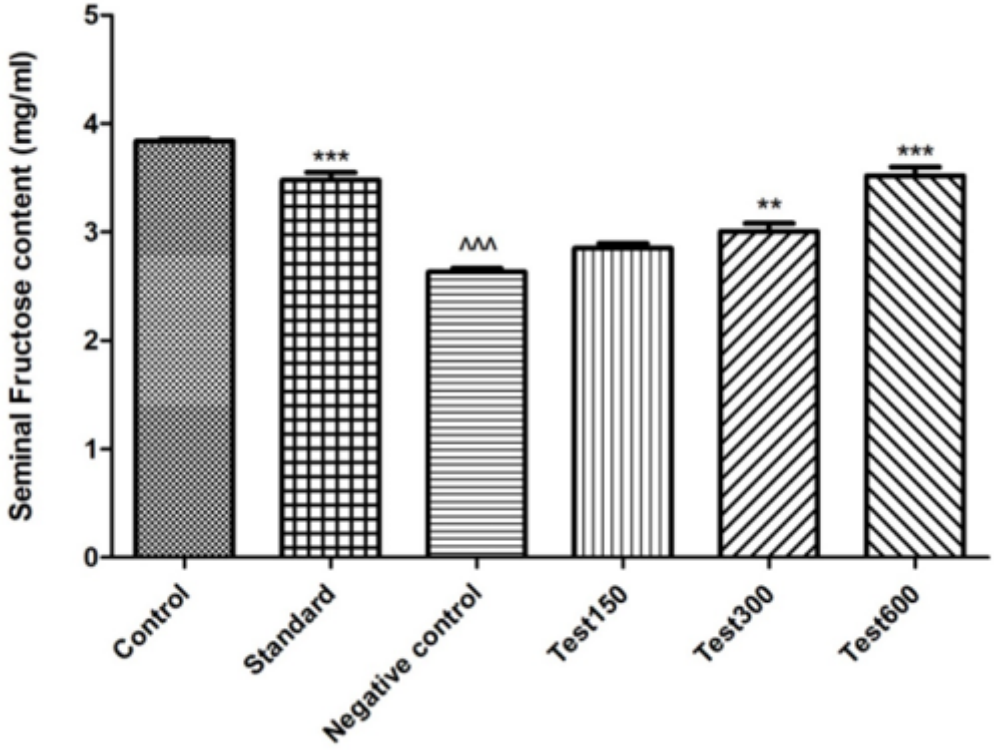

Figure 2

Effect of SRR on seminal fructose content. ***p<0.001 compared to normal, ^^^p<0.001 compared to standard

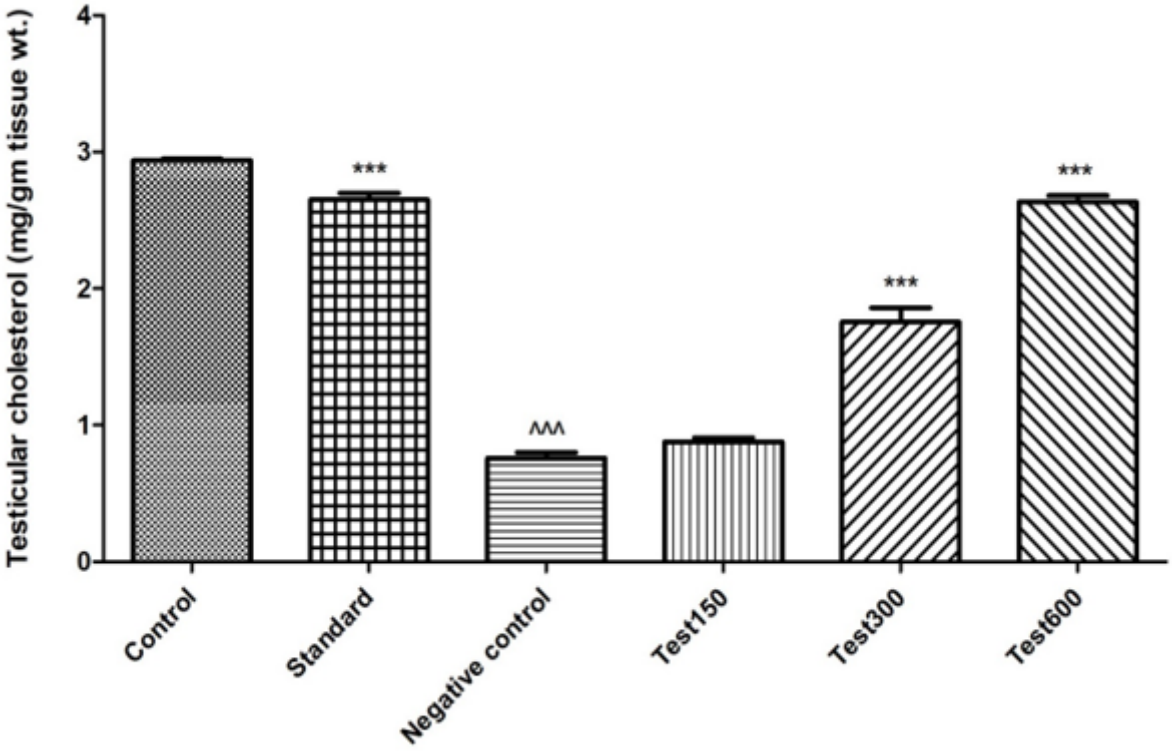

Figure 3

Effect of SRR on testicular cholesterol. ***p<0.001 compared to normal, ^^^p<0.001 compared to standard

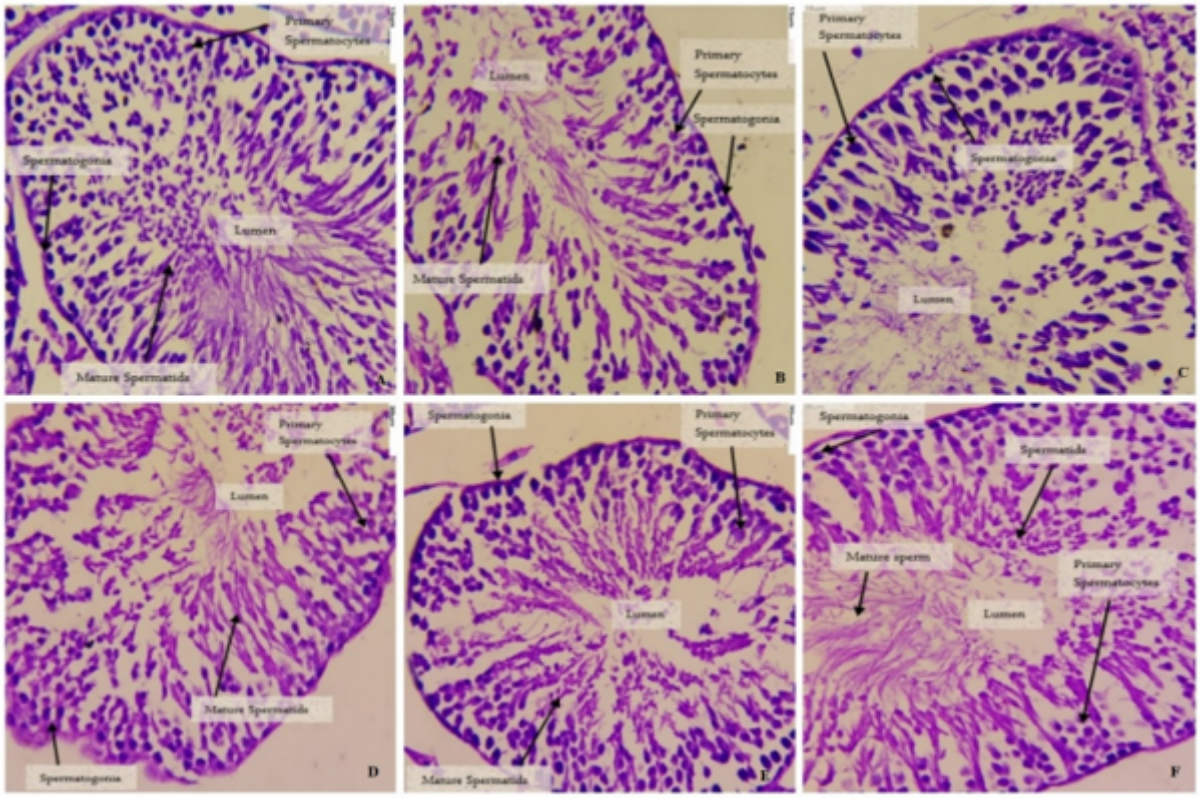

Figure 4

Histopathology of testis. Above images of control group (a) shows normal spermatogenesis also shows presence mature spermatozoa in lumen and negative control shows decreased numer in leyding cells and shows arest of spermatogenesis in 1 st and 2 nd stage . All treatment group i.e. 150mg/kg (d), 300mg/kg (e), 600mg/kg (f), standard (b) shows presence of mature spermatoza in lumen and increased leyding cells represent dose dependent recovey on spermatogenesis. All treatment and control group showed high john's biopsy score than IMS induced group.Also, showed distruption in the seminiferous tubles in IMS group and all treatment group doesnot showed any architectural distruption.

Supplementary Files

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