ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 4(A), pp. 25594-25599, April, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EFFICACY OF NATURAL MOLECULES ON INFERTILITY

Suresh Sirvi[1]., Swati Patil*[2]., Hanumanthrao Palep[3] and Raisa Shaikh[4]

[1,2,4]Department of Pharmacognosy, K. M. Kundnani College of Pharmacy
[3]Dr. Palep's Medical Research Foundation Pvt. Ltd, Lower Parel, Mumbai

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0904.1889

ARTICLE INFO

Article History:
Received 12th January, 2018
Received in revised form 21st February, 2018
Accepted 05th March, 2018
Published online 28th April, 2018

Key Words:
Infertility, Spermatogenesis, *Mucuna pruriens, Tribulus terrestris,* Pro-fertility, sodium arsenite rat model.

ABSTRACT

Infertility can be defined as a lack of pregnancy after one year of unprotected intercourse, and it is the manifestation of one or more pathologic conditions of male or female origin. Reduced spermatogenesis and defective sperm function are the most prevalent causes of idiopathic male infertility. Many environmental, physiologic, endocrine, and genetic factors have been reported as underlying poor sperm function and male factor infertility. At present, there is a dearth of research evaluating the use of herbal medicines as pro-fertility agents. There are many well-tested Ayurveda and other traditional herbs, which have a long standing reputation as a cure for sexual dysfunction and which have been used in numerous preparations for improving sexual performance and fertility. *M. pruriens* seeds & *Tribulus terristris* fruits have been found to be powerful aphrodisiacs and are known to promote sexual vigour and semen. Formulations containing the combination of these have shown promising results in the management and treatment of sexual disorders.
The present study is aimed at demonstrating spermatogenic restorative efficacy of above herbs and its major constituent, and finding the possible mechanism of action thereof in a rat model
The results thus throws light on use of ethnobotanical herbs for treatment of infertility.

INTRODUCTION

Fertility is the natural capability to produce offspring. As a measure, fertility rate is the number of offspring born per mating pair, individual or population. A lack of fertility is infertility. Treatments of infertility will depend on many factors, including the age of the patient(s), how long they have been infertile, personal preferences, and their general state of health.

There are many well-tested Ayurveda and other traditional herbs, which have a long standing reputation as a cure for sexual dysfunction and which have been used in numerous preparations for improving sexual performance and fertility.

There are a multitude of herbs available that greatly help rebuild a strong nutritional base and can potentially eliminate male infertility. System tonics that increase and nourish vitality and help reverse low adrenal energy are the best place to begin your healing journey.

Literature survey revealed Tribulus terrestris & Mucana pruriens amongst many herbal drugs used to treat male infertility. *M.pruriens* seeds &*Tribulus terrestris* fruits have been found to be powerful aphrodisiacs and are known to promote sexual vigor and semen.

Furostanolic type of saponin present in Tribulus terrestris increases luteinzing hormone, motivates spermatogenesis and results in stimulation of testosterone. These activities results in increases in quality and quantity of sperm significantly. *Mucuna pruriens* contains L-Dopa and *Tribulus terrestris* contains MAO inhibitor. Combination of L-Dopa with MAO helps making L-Dopa available to brain for conversion into Dopamine. Optimum Dopamine level increases testesterone, growth hormone, elevates mood, increases libido and strength promotion. Hence it was hypothesized that a combination of these two drugs will work wonders for treatment of infertility. Formulations containing the combination of these have shown promising results in the management and treatment of sexual disorders. *M. pruriens* seed powder contains high amount of L-DOPA (L-3, 4 dihydroxy phenylalanine). *Tribulus terrestris* fruits contain diosgenin as the major constituent. A combined extract was used in the current study for evaluation of profertility activity.

Corresponding author:* **Swati Patil
Department of Pharmacognosy, K. M. Kundnani College of Pharmacy

MATERIALS AND METHODS

Plant materials

Fresh fruits of *Tribulus terrestris* and fresh seeds of *Mucuna pruriens* were collected from authorized dealer and authenticated by Dr.Harshad M Pandit, formerly Head and Associate Professor, Department of Botany, at Guru Nanak Khalsa College, Mumbai. The sample matched with the herbarium specimen no #: srs p 1050806 and is identified as the fruits of Tribulus terrestris L. of family: *Zygophyllaceae* and the *Mucuna pruriens* sample matched with the herbarium specimen no #: arp p 1050804 is identified as that of *Mucuna pruriens L* of Family: *Fabaceae*.

Standardization

Standardization of *Mucuna pruriens* extract with respect to Levodopa as biomarker:

Using UV spectrophotometry: Levodopa was used as marker compound. It was diluted with 0.1N HCl to get 10µg, 20 µg, 30 µg, 40 µg, 50µg, 60µg, 70 µg/ml solutions. Absorbance was recorded by UV at 280 nm using UV-VIS spectrophotometer so as to obtain linear curve.

Using HPLC: High performance liquid chromatography method development and validation was carried out using phosphate buffer and methanol (70:30) by using Ortho Phosphoric Acid to adjust pH 2.8.

Standardization of *Tribulus terrestris* extract with respect to Diosgenin as biomarker:

Using UV spectrophotometry: Diosgenin was used as marker compound. It was diluted with 0.1N HCl to get 10µg, 20 µg, 30 µg, 40 µg, 50µg, 60µg, 70 µg/ml solutions. Absorbance was recorded by UV at 203 nm using UV-VIS spectrophotometer so as to obtain linear curve.

Using HPLC: High performance liquid chromatography method development and validation was carried out using Acetonitrile: water (90:10) as mobile phase.

In vitro evaluation

Free radical scavenging potential of methanolic extract of *Tribulus terrestris* was evaluated against a methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) and the degree of discoloration was read at 517nm, as a measure of the free radical scavenging activity.

In vivo evaluation

The Animals were divided into following groups:

- ***Group 1:*** Control group received standard diet and 0.9% saline water orally.
- ***Group II (12 animals):*** Negative control (0.1mg/L sodium arsenite) (where sperm count is reduced)
- ***Group III:*** Treated with Polyherbal formulation (0.1mg/L sodium arsenite+300mg/kg herbal formulation)
- ***Group IV:*** Treated with Polyherbal formulation (0.1mg/L sodium arsenite+600mg/kg herbal formulation)
- ***Group V:*** Treated with combined extract of *Mucuna pruriens* and *Tribulus terrestris*(0.1mg/L sodium arsenite+300mg/kg herbal extract)
- ***Group VI:*** Treated with combined extract of *Mucuna pruriens* and *Tribulus terrestris*(0.1mg/L sodium arsenite+600mg/kg herbal extract).

The control group animals received normal saline (0.9% NaCl). The treatment group II, III, IV, V, & VI received 0.10mg/L of Arsenic in the form of sodium arsenite (from sigma Aldrich). This was given for 30 days.

Polyherbal formulation was given to group III and IV in dose of 300mg/kg and 600mg/kg respectively.

Extract of Mucuna pruriens and Tribulus terrestris(300mg/kg) was administered to group V animals and extract of Mucuna pruriens and Tribulus terrestris(600mg/Kg) was administered to group VI animals.

After 30 days, 6 animals from group II of Arsenic exposure were randomly selected, blood withdrawn from retro-orbital plexus and thereafter humanely sacrificed in carbon dioxide chamber. The testis was dissected and extracted for sperm count. The remaining animals from groups II were humanely sacrificed in carbon dioxide chamber after 60 days. After one month of toxicant treatment, blood was collected from retro-orbital plexus and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 300 C for 15 min and used for estimation of testosterone level. At the end of the treatment period, blood was again collected from retro-orbital plexus and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 300 C for 15min and used for estimation of testosterone level.

After collection of blood samples, all animals were humanely sacrificed in carbon dioxide chamber. The semen sample was collected from the epididymis which was separated from the testes by blunt dissection. The epididymis was cut open longitudinally and with gentle pressure on the serosa.A drop of semen was collected on slide for semen analysis parameters namely sperm count, sperm motility & sperm viability.

RESULTS AND DISCUSSION

Percentage yield of methanolic extract of seed of *Mucuna pruriens* and *Tribulus terrestris* was found to be 5.35%w/w and 9.06%w/w respectively.

Standardisation of tribulus Terrestris Fruit Extract Indicated In Figure 1

HPLC analysis

Validation Parameters of Developed Method

Validation of developed method was carried out as per ICH guidelines. Parameters such as Linearity, Accuracy, Precision, specificity, LOD and LOQ were taken up as tests for analytical method Validation.

Linearity

The linearity was evaluated by analysing different concentration of the standard solutions of Diosgenin. The linearity range was found to be 5-25 µg/ml as shown in figure 2 and 3

Accuracy: Average percent recovery was found to be 91.55% as shown in table 1

Precision: Percent relative standard deviation: 0.28% as shown in table 2. Intra-day and inter day precision was found as given in table 3 and 4.

LOD & LOQ

Limit of detection = 1.13 (µg/ml)
Limit of Quantitation = 3.44(µg/ml)

Specificity and Selectivity

The method was quite selective for Diosgenin. There was no other interfering peak around the retention time of Diosgenin. The baseline did not show any significant peak as indicated in figure 4.

As given in table 5 and figure 5, quantitative estimation of Diosgenin from *Tribulus terrestris* extract by HPLC method showed that 3.66%w/w diosgenin is present in fruits of *Tribulus terrestris.*

Diosgenin was found to be present in highest quantity in fruits of *Tribulus terrestris* apart from other constituents, so diosgenin was selected as a standard for standardization of extract by HPLC.

DPPH free radical scavenging activity

Antioxidant activity of methanolic extract of Tribulus terrestriswas found out as given in table 6, figure6 and figure 7

The degree of reduction in absorbance measurement indicates the radical scavenging (antioxidant) power of extract that means lower the absorbance at 517nm indicate higher radical scavenging activity. In the present study, the evaluation and comparison of antioxidant activity of Ascorbic acid and *Tribulus terrestris* fruit extract by DPPH scavenging activity showed increase free radical scavenging activity. The good scavenging activity of *Tribulus terrestris* scan be attributed due to presence of flavonoids and phenolic in seed extract.

Tribulus terrestris extract profertility activity may be enhanced due to its good potential as free radical scavenger.

In vivo study

1. Biochemical estimation of serum testosterone level
2. Estimation of serum testosterone level as given in table 7 and figure 8
3. Estimation of Sperm Count as given in table 8 and figure 9
4. Estimation of Sperm motility as given in table 9 and figure 10
5. Estimation of sperm viability as given in table 10 and figure 11

- ALL values are mean ±SEM ; N=6 in each group one –way ANOVA followed by Bonferroni's multiple comparison test applied for statistical analysis
- a <0.001 when toxicant control compared with normal control
- a <0.05 when experimental group compared with toxicant group
- a <0.001when experimental group compared with toxicant group

It is known that the number of spermatids present in the cauda epididymis and the total daily sperm production are important indicators of male fertility potential. In this study it was evaluated the toxicity sodium arsenite (10mg/ml) exposure on the reproductive system in male Wistar rats. It was found that a significant increase in sperm anomalies with decreased sperm count, motility, sperm viability. The epididymis plays an important role in sperm development and sperm maturation, where it depends on the luminal environment of the epididymis, including its specific proteins. Taken together, these findings indicate that exposure to arsenite induced testicular changes. These changes were immediate, as there was no time for reduced number of sperm to be generated in the testis, and subsequently directed to the caput/corpus epididymis, to arrive in the cauda. Further, it is important to consider that the cauda has the characteristic of storing sperm from several cycles of the seminiferous epithelium which may influence the result of sperm count. Oxidative stress has direct effect on sperm count and sperm motility as excess of ROS produced germ cell apoptosis. Administration of combined extract of *Mucuna pruriens* and *Tribulus terrestris,*we observed significant increase in sperm count and sperm motility. Both *Mucuna pruriens and Tribulus terrestris* attributed to its nutritional, adaptogenic, aphrodisiac and restoring properties. *M.pruriens* thus has the potential to treat compromised fertility characterized by by loss of sperm count and sperm motility also provides immunity against such complication and Furostanolic type of saponin present in *Tribulus terrestris* increases luteinzing hormone, motivates spermatogenesis and results in stimulation of testosterone. These activities results in increases in quality and quantity of sperm significantly.

The major effect of combined extract of*Mucuna pruriens* and *Tribulus terrestris* on spermatogenesis was mediated by recovery of the endocrine axis suppressed as a result of sodium arsenite administration. MP helped an early recovery of the endocrine axis and spermatogenesis. At the end of the treatment period the sperm count and motility regain up to normal mean value and sperm viability regain only in HF high dose which could be due to elevated hormone levels promoting the process of spermatogenesis due to reduce number of sperm elimination during quality control of epididymis as a result qualitatively better sperm production .

Histopathological study of testis in rat as indicated by figure 12, 13, 14, 15 and 16

Animals receiving sodium arsenite administration exhibited difference in the testicular histology, particularly the number and arrangement of the Sertoli and Spermatogenic cells in the seminiferous tubules. The lumina of the seminiferous tubule were particularly enlarged with Sertoli cells shrinking towards the basement membrane coupled with significant number of reduction of spermatids in the lumen. In auto-recovery group, the Sertoli cells regained their normal architecture and lumina ere occupied by the spermatids. Treatment with HF (high) and MP (high) resulted in much better recovery of the luminal architecture, with the Sertoli cells regaining their normal position and the spermatids completely filing the lumina.

Control group showed normal testicular histology with the lumen of the seminiferous tubules filled with sperm. Sodium arsenite group showed significant compromise in

spermatogenesis with almost empty lumens and degeneration of Sertoli cells. Toxicant 2 (auto-recovery) groups showed poor recovery in comparison to complete recovery in HF and MP+TT groups evidenced by densely filled seminiferous tubules and sodium arsenite administration increased the ROS level significantly.

- Mucuna pruriens, a combination of Mucuna &*Tribulus terristris*, & Rejuspermin capsule were evaluated for their profertility activity using sodium arsenite induced antifertility model.
- Of all the above evaluated, Rejuspermin –a polyherbal capsule showed maximum reversal of fertility activity.
- The experiments conducted indicate that the various herbs present in the formulation may be acting by
- Supplying nutrients to sertoli cells which inturn are responsible for spermatogenesis.
- Stimulate FSH & LH & hence increase levels of testosterone.
- Reduce the oxidative stress on enzymes responsible for spermatogenesis.
- The results thus throws light on use of ethnobotanical herbs for treatmentof infertility.

Tables

Table 1

Drug	Initial amount(µg/ml)	Added amount(µg/ml)	Amount recovered±SD (µg/ml) n=3	% Recovery
Diosgenin	10	8	17.24	92.47
	10	10	19.17	91.74
	10	12	21.04	90.46

Table 2

SR.NO.	CONC	AREA	%RSD
1	10	138648	
2	10	138426	
3	10	137564	0.28
4	10	137992	
5	10	138524	

Table 2

Sr no	Conc (µg/ml)	0 hr		3 hr		6 hr		Mean	SD	%RSD
		Rt min	Area	Rt min	Area	Rt min	Area			
1	2	14.1	25448.1	14.1	25859.21	14.1	25354.9	25553.67	219.3	0.85
2	6	14.1	84331.1	14.1	84256.65	14.1	84562.4	84383.54	130.2	0.15
3	10	14.1	138386	14.1	138514.4	14.1	138715	138538.2	135.7	0.09

Table 4

Sr. no.	Conc µg/ml	Day 1		Day 2		Day 3		Mean	SD	%RSD
		Rt min	Area	Rt min	Area	Rt min	Area			
1	2	14.06	25448	14.06	24590	14.06	25856	25298	528.1	2
2	6	14.06	84331	14.06	84982	14.06	84561	84624	269.1	0.31
3	10	14.06	138386	14.06	138427	14.06	138624	138478	104.4	0.07

Table 5

Peak name	Rt (min.)	Mean Area(n=3)	NTP	Symmetry
Extract	14.06	144652.7	3497.34	1.47

Table 6

Concentration(µg/ml)	% inhibition of DPPH radical		IC50	
	Extract	Ascorbic acid	Extract(µg/ml)	Ascorbic acid (µg/ml)
5	16.66	29.84		
10	27.77	39.49		
30	47.88	69.28	51.99	
50	81.13	89.20		65.53
80	86.55	107.83		

Table 7

GROUPS	1st day	30th day	60th day
Control	172.26±0.79	172.38±0.47	172.58±0.671
Toxicant 1	173.61±1.066	82±0.7	83.38±0.48
Toxicant 2(AR)	174.45±1.19	83.6±0.51	94.25±1.83
HF (high dose)	174.91±0.4	82.6±0.51	146.4±3.32***
HF (low dose)	175.39±0.3	84.38±0.3	170.64±0.35***
MP+TT (high dose)	174.21±0.79	81.54±1.3	172.60.±0.52***
MP+TT(low dose)	172.98±1.32	83.32±0.67	139.11±0.47***

Table 8

GROUPS	Sperm count at 60th day(million/ml)
Control	153.2±3.71
Toxicant 1	89.71±7.38
Toxicant 2(AR)	104.75±2.90
HF (high dose)	146.3±3.91
HF (low dose)	139.35±2.20
MP+TT(low dose)	129.38±2.64

Table9

GROUPS	Sperm motility at 60th day(%)
Control	83.33±1.74
Toxicant 1	50.66±2.82
Toxicant 2(AR)	65.66±2.23
HF (high dose)	77±2.42
HF (low dose)	69.66±1.72
MP+TT (high dose)	73.66±1.19
MP+TT(low dose)	67.33±2.23

Table 10

GROUPS	Sperm viability at 60th day(%)
Control	86±1.77
Toxicant 1	63.16±2.24
Toxicant 2(AR)	69.66±3.06
HF (high dose)	82±3.35
HF (low dose)	71.5±1.44
MP+TT (high dose)	73.66±2.59
MP+TT(low dose)	66.5±2.65

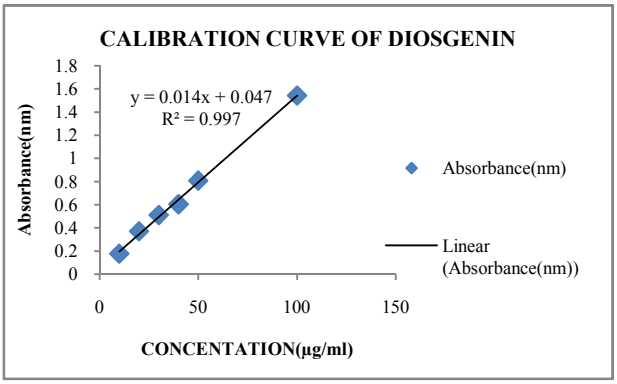

Figure 1 Calibration curve of Diosgenin

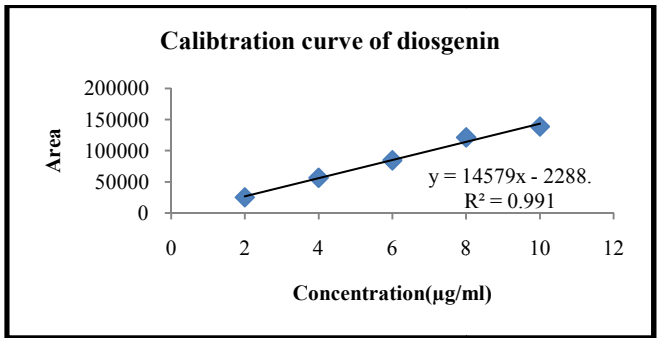

Figure 2 Linearity of Diosgenin

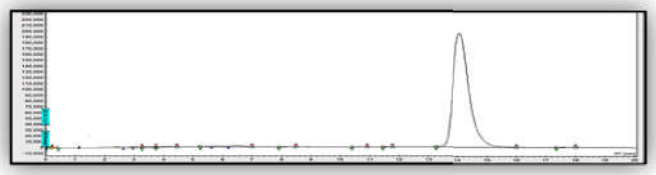

Figure 3 HPLC chromatogram for working standard diosgenin 10µg/ml

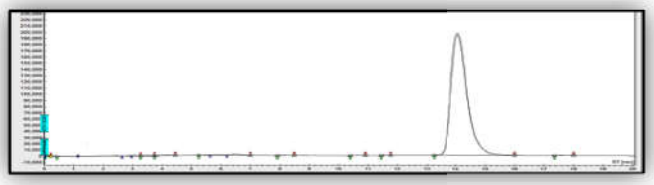

Figure 4 HPLC chromatogram of standard Diosgenin

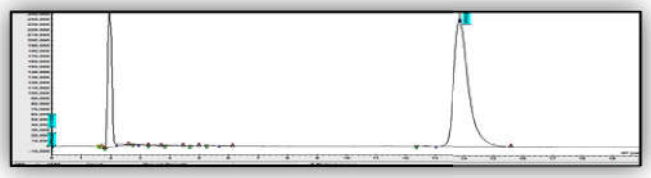

Figure 5 HPLC chromatogram of *Tribulus terrestris* extract

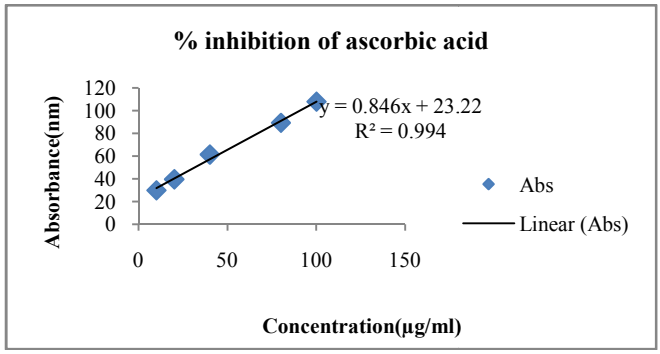

Figure 6 Calibration curve of ascorbic acid

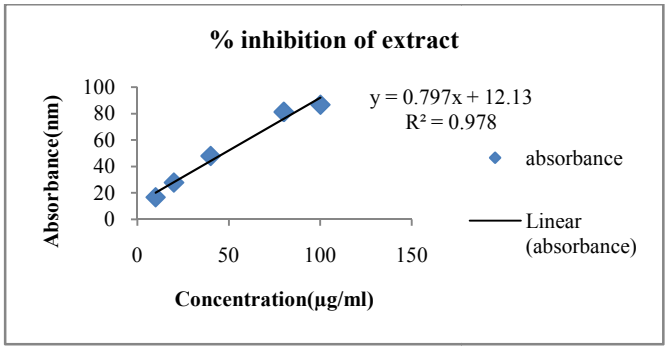

Figure 7 Calibration curve of extract

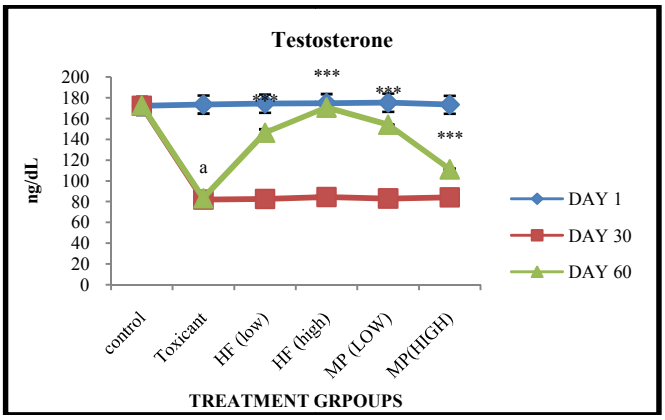

Figure 8 Line graph of testosterone level in serum sample

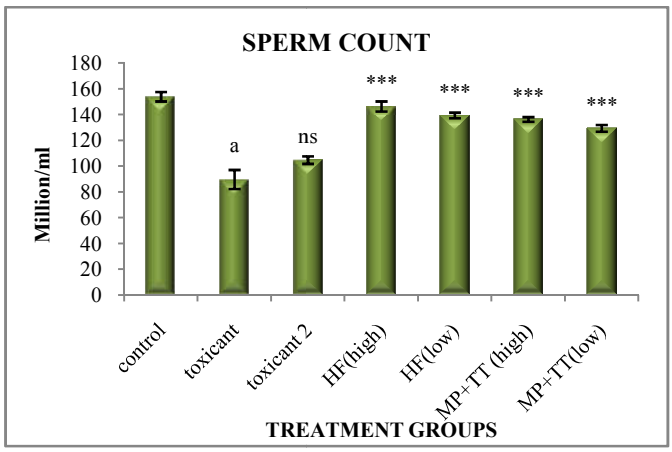

Figure 9 Bar graph of sperm count in treatment groups

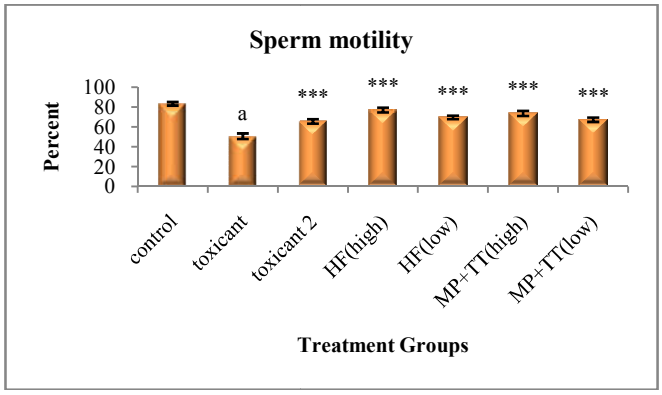

Figure 10 Bar graph of sperm motility in treatment groups

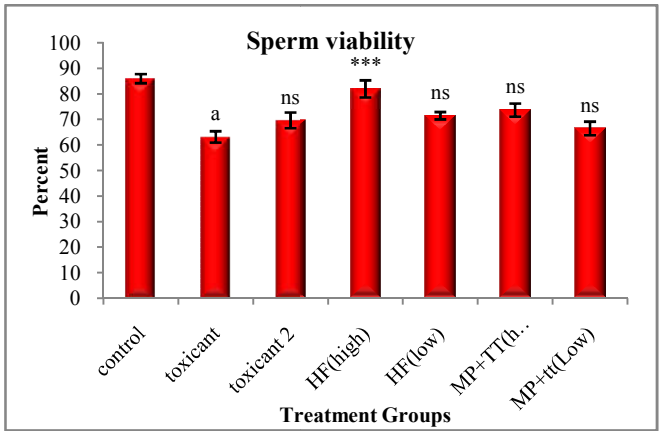

Figure 11 Bar graph of sperm viability in treatment groups

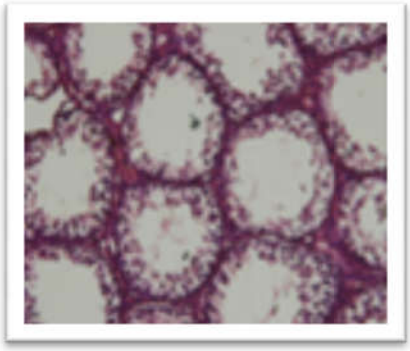

Figure 12 Normal Control group

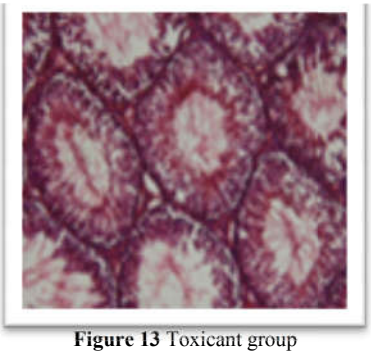

Figure 13 Toxicant group

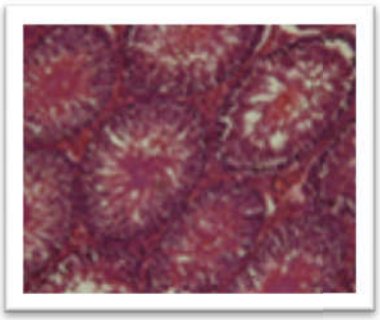

Figure 14 Toxicant group (Auto recovery)

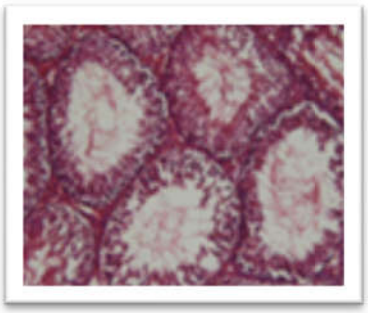

Figure 15 MP+TT (high dose) group

Figure 16 HF (high dose) group

References

1. Jungwirth, A.; Diemer, T.; Dohle, G. R.; Giwercman, A.; Kopa, Z.; Krausz, C.; Turnaye, H. Male Infertility. *Eur. Urol*. 2012, 62 (2), 324-332.
2. Ivanova, A.; Lazarova, I.; Mechkarova, P.; Tchorbanov, B. HPLC Method for Screening of Steroidal Saponins and Rutin as Biologically Active Compounds in *Tribulus terrestris* L. *Biotechnol. Biotechnol. Equip*. 2014, 24 (sup1), 129-133.
3. Chhatre, S.; Nesari, T.; Somani, G.; Kanchan, D.; Sathaye, S. Phytopharmacological Overview of *Tribulus terrestris*. *Pharmacogn Rev*. Jan-Jun; 2014, 8 (15), 45–51.
4. G, G.; Verma, P.; Bj, P.; Pk, P. *Tribulus terrestris* Linn.: A Phyto-Pharmacological Review. *J. Ayurveda Holist. Med*. 2013, 1 (3), 37-43.
5. Fossato da Silva, D. A.; Teixeira, C. T.; Scarano, W. R.; Favareto, A. P. A.; Fernandez, C. D. B.; Grotto, D.; Barbosa, F.; Kempinas, W. D. G. Effects of Methylmercury on Male Reproductive Functions in Wistar Rats. *Reprod. Toxicol*. 2011, 31 (4), 431-439.
6. Yanala, S. R.; Sathyanarayana, D.; Kannan, K. A Recent Phytochemical Review - Fruits of *Tribulus terrestris Linn. J. Pharm. Sci. Res*. 2016, 8 (3), 132-140.
7. Deokar, G.; Kakulte, H.; Kshirsagar, S. 50 © Pharmaceutical and Biological Evaluations Phytochemistry and Pharmacological Activity of *Mucuna pruriens* : A Review Phytochemistry and Pharmacological Activity of *Mucuna pruriens* : A Review. 2016, No. April.
8. Chemistry, D.; Solution, S. S.; Extraction, D. Chapter III Diosgenin Extraction and Characterization in *Dioscorea prazeri*. 85-119.
9. Vilas B Warke., Tushar A Deshmukh.*, V. R. P. Development and Validation of RP-HPLC Method for Estimation of Diosgenin in Pharmaceutical Dosage Form. *Asian J. Pharm. Clin. Res*. 2011, 4 (9), 126-128.
10. Kavitha, C.; Thangamani, C. Amazing bean "Mucuna Pruriens": A Comprehensive Review. *J. Med. Plants Res.* 2014, 8 (2), 138-143.
11. Renuka; Shukla, V. J.; Prajapati, P. K. Validation Aspect in Multicomponent Fraction from Gokshur (*Tribulus terrestris* Linn.) by RP-HPLC Method. *Int. J. PharmTech Res*. 2013, 5 (3), 1286-1293.

****