



ISSN: 2456-2912
VET 2019; 4(2): 36-42
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www.veterinarypaper.com
Received: 26-01-2019
Accepted: 27-02-2019

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***Tribulus terrestris*: Herbal medicated feed as uterine tonic in wistar rats**

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Abstract

Efficacy study of herbal medicated feed containing *Tribulus terrestris* on 8 female wistar rats of age 5 weeks were grouped in 2 groups each containing 8 female rats and observed for period of 63 days.

No adverse changes were observed. Though statistically significant alterations were observed in Hb, TEC, TLC, DLC, PCV, Blood Clotting Time, ALT, AST, TP, BUN, Creatinine and glucose on day 0th, 14th and 63rd of the experiment and all the changes were within normal physiological limits. An elevation in mean serum progesterone level was observed in treatment group and even in control group animals on day 14th and 63rd day. This is an indicative of positive effect of medicated feed on female reproductive system.

Higher number of pups in all the treatment group animals against the control group animals is indicative of increased fecundity percentage because of feeding of herbal medicated feed.

Keywords: Medicated feed, *Tribulus terrestris*, herbs for pregnancy maintenance, herbal nutraceuticals

1. Introduction

One calf per year is dream of every dairy farmer. However, the main hurdle in achieving this is infertility in animals. Anoestrus, repeat breeding, retention of placenta, prolapsed of uterus are the major constraints and repeat breeding is second major problem in dairy industry.

Fertilization failure accounts for about 40 per cent of the repeat breeder cows and it may be due to failure of ovulation, delayed ovulation, defects in the ovum, inflammatory conditions and anatomical defects of the genital tract of animal, unhealthy uterus and lack of hormones etc. Above both causes contribute a lot in the economic loss to farmer and to overcome such losses it is necessary to avoid such problems in the herd, and further it is necessary to have a healthy pregnancy and foetus.

Gokhru (*Tribulus terrestris*) is referred as uterine tonics. Though the exact mechanism of action of this herbs is still unknown in female reproductive system, This plant has several medicinal values including antimicrobial, antibacterial, antioxidant and antitoxic activities used in the treatment of cardiovascular diseases, diabetes, tumors, articular pains and respiratory diseases. *Tribulus terrestris* increases libido in addition to prevent reluctance, infertility and menopausal disorders. Researchers have shown that dioscin present in *Tribulus terrestris* increases male sexual ability by increasing free testosterone levels and balancing levels of estrogen, progesterone and pregnenolone. It is considered that this herb provide nutritional support to uterus. A novel concept of "Healing through Nutrition" by using Nutraceuticals is an emerging concept. Medicated feed is the feed which contains drug ingredients intended or presented for cure, mitigation, treatment, or prevention of disease of animals or which contains drug ingredients intended to affect the structure or any function of the body of animals (AAFCO, 2000) [1].

Considering current trend of using herbal medication in the present investigation, it was proposed to formulate laboratory animal feed medicated with uterine tonic herb and aphrodisiac i.e. *Tribulus terrestris* and evaluate its efficacy in female reproductive performance.

2. Materials and Methods

This topic describes the materials and methods used to formulate and evaluate the reproductive performance of herbal medicated feed by using *Tribulus terrestris* in female Wistar rats under

experimental conditions. The study included blood biochemical, hematological parameters and hormonal level and gross as well as histopathological changes in liver, kidney, spleen, lungs, brain, genital organs, and Intestine and heart tissues.

The present study was conducted on 16 Female Wistar rats of age 5 weeks and having 80-120 g body weight. The live body weight range was within ± 10 percent of the mean body weight at the time of randomization. The Wistar rats were housed in standard laboratory conditions like environmentally controlled room with $22 \pm 3^{\circ}$ C temperature and 30-70 percent relative humidity in polypropylene cages and grouped into two groups i.e. control group and treatment group with 2% *Tribulus terrestris* and animals were provided with food and water *ad-libitum* in the experimental room of Laboratory animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. The experimental animals were kept under constant observation for a period of 7 days as acclimatization period before commencement of the experiment. All standard

management procedures were adopted to keep the animals free from stress.

After the acclimatization period, animals were fed with herbal medicated feed formulated and prepared as per the study design. Animals at the age of 6th weeks were kept for mating for a period of two weeks using 1:1 as male female ratio. (OECD-421, 2015) [36]. after two weeks of mating male rats were separated and the female pregnancy was confirmed with the physical examinations of individual female rats.

Conception rate was confirmed followed by number of pups parturated and survival of pups was confirmed with the experimental female animals. All the pups were weaned after 3 weeks of age and female rats were sacrificed for the gross and histopathological investigation on 63rd day of the experiment.

2.1. Drinking water and diet

Feed formulation as per daily nutrient requirement of Wistar rats is given in Table No: - 1. Reddy (2016) [38]

Table 1: Feed formulation for (100kg of feed) as per nutritional requirement of Wistar Rats (BIS 2007).

Sr No.	Ingredient	Level (%)	CP (%)	ME (Kcal)	Ca (%)	P (%)	Lysine (%)	Methionine (%)
1.	Maize	57	5.13	1903.8	0.011	0.15	0.12	0.10
2.	DOC (CP%-52)	35	18.2	805	0.10	0.22	0.94	0.22
3.	DORB	5	0.67	110	0.0035	0.075	0.03	0.012
4.	Oil	0.49		4348.2				
5.	Limestone	0.48	-		0.48	-	-	-
6.	Salt	0.5	-	-		-	-	-
7.	Mineral mixture	1.30						
8.	Vitamin Premix	0.2						
	Total	100	24	7167	0.6	0.4621	1.10	0.3426

Purified water and specially formulated herbal medicated laboratory animal feed i.e. feed added with 2% *Tribulus terrestris* seed powder was prepared as per the daily nutrient requirement of animals from M/S L.R. Pharmaceuticals, A-51, MIDC, Parbhani and provided *ad-libitum* throughout the experimental period.

2.2. *Tribulus terrestris*

Botanical name : *Tribulus terrestris*
 Family : *Zygophyllaceae*
 Common name : Bindii, Bullhead, Gokharu caltrop, devil's-thorn,
 English : *Tribulus*
 Hindi : Burragokhur, Chhotagokhru
 Sanskrit : Bahukantaka, Bhakshataka
 Marathi : Gokhru, Lahanagokharu
 Malayalam : Neringil
 Telgu : Chirupalleru

2.3. Botanical description

Tribulus terrestris is known as Puncture vine is a native yearling lying plant which has been widely spread in Mediterranean area and warm areas of Europe, Asia, America, Africa and Australia. It has been widely used in the traditional medicines of China, India, Iraq, Bulgaria, South Africa and also Iran. (Sharma *et al.*, 2003)

Studies show that *Tribulus terrestris* contains steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, nitrate potassium, aspartic acid and glutamic acid Martino *et al.* (2010) [32]. This plant has several medicinal values including antimicrobial, antibacterial,

antioxidant and antitoxic activities used in the treatment of cardiovascular diseases, diabetes, tumors, articular pains and respiratory diseases. (Turan *et al.*, 2007) [44]

Tribulus terrestris is a flowering plant of the family Zygophyllaceae. It is about 30 to 70cm high. It grows as a summer annual, has pinnately compound leaves, yellow flowers and stellate shaped carpel fruits. (Bashir *et al.*, 2009) [9].

2.4. Collection of Sample

Blood samples were collected on the 0th day, 14th day & on the termination day of experiment (63rd day), from retro-orbital plexus with the help of capillary tube for estimations of haematological, biochemical and hormonal levels.

Rats were sacrificed on termination day of experiment by using excess dose of inhalation anesthesia (di-ethyl ether) at the end of experiment. Gross necropsy examination was performed. Liver, kidney, spleen, lungs, brain, uterus, skin, genital organs such as ovaries and heart tissue were collected in 10% formalin for histopathological examination.

2.5. Parameters studied

In this experiment parameters viz. general observations (behavioral changes, and organ weight), serum biochemical estimations, Progesterone hormone estimation, haematological and pathological observations (gross pathology and histopathology) were performed. The data obtained from various parameters from all groups were analyzed as per the method suggested by Panse and Sukhatme (1967) [37] the present experiment was carried to assess the efficacy of using completely randomized block design (CRD).

3. Results and Discussion

The present experiment was carried to assess the efficacy of herbal medicated feed on female reproductive system in wistar rats under experimental conditions. The results are interpreted as follows.

3.1 Behavioral Changes

The animals from control group remained active and alive throughout the experimental period. No visible signs of toxicity or no mortality was observed in the treatment group throughout the experimental period.

All the experimental rats belonging to treatment group when observed daily post treatment did not reveal any observable behavioral changes or any abnormalities.

3.2 Haematological and Biochemical parameters

The various haematological parameters viz. haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC), Packed cell

volume (PCV), Blood Clotting Time values were analyzed from blood collected and biochemical parameters viz. alanine transaminase (ALT), aspartate aminotransferase (AST), total protein (TP), blood urea nitrogen (BUN), creatinine (CREA) and glucose (GLU) values were analyzed from blood collected at 0th, 14th and 63rd day of the experimental period.

All the haematological values in control and treatment group were within normal physiological limits. Beneficial effects of herbal medicated feed were seen in treatment group.

Though small alterations were observed in mean AST, ALT, BUN, Creatinine, Total protein and Glucose values in control and treatment group on day 0th, 14th and 63rd no statistical significance was observed in any and all the values were observed to be within normal physiological range.

However, the findings in the present investigation revealed no significant alterations in blood biochemical parameters and treatment with herbal medicated feed containing *Tribulus terrestris* indicative of maintaining normal health and physiology of animals.

Table 1: Showing mean values of Haematological parameters of experimental rats studied at 0th, 14th and 63rd day of intervals of study.

Parameter	Day	Control Group	<i>T. terrestris</i> Group	CD	Statistics
Haemoglobin (g/dl)	0 th	13.61+1.21	13.93 +1.02		NS
	14 th	13.71+1.00	^{ab} 14.31+1.01	(0.05)=0.648	S
	63 rd	13.75+1.22	^a 15.04+1.00	(0.05)=0.599 (0.01)=0.790	S
Total Erythrocyte Counts(10 ⁶ /cmm)	0 th	6.45+0.15	6.64+1.23		NS
	14 th	6.76+1.05	6.89+1.02		NS
	63 rd	^b 6.56+1.25	^a 7.03+1.03	(0.01)=0.347 (0.05)=0.258	S
Total Leukocytes Counts(10 ³ /cmm)	0 th	7.65+0.61	8.07+1.25		NS
	14 th	9.40+1.37	8.76+1.00		NS
	63 rd	9.93+1.25	9.11+1.01		NS
Packed Cell Volume (%)	0 th	33.81+0.71	34.13+1.02		NS
	14 th	33.78+1.00	33.71+1.01		NS
	63 rd	^b 32.86+1.40	^a 35.37+1.04	(0.01) = 1.575 (0.05) = 1.187	S
Clotting Time (Sec)	0 th	1.06+0.11	1.31+1.00		NS
	14 th	1.58+1.35	1.69+1.00		NS
	63 rd	1.56+1.03	1.77+1.33		NS
Lymphocyte Counts (%)	0 th	76.87+2.74	77.50+1.00		NS
	14 th	74.37+1.22	77.37+1.21		NS
	63 rd	78.75+1.34	80.75+1.01		NS
Neutrophils Counts (%)	0 th	^d 14.12+1.41	^c 17.62+1.03	(0.01)=3.995 (0.05)=2.997	S
	14 th	^{bc} 19.25+1.06	^{ab} 20.75+1.38	(0.05)=3.571	S
	63 rd	20.62+1.00	17.37+1.08		NS
Eosinophils Counts (%)	0 th	3.5+1.32	2.62+1.01		NS
	14 th	2.25+1.03	1.75+1.42		NS
	63 rd	2.87+1.25	1.37+1.03		NS
Monocyte Counts (%)	0 th	^b 0.5+0.18	^a 1.87+1.28	(0.05)=1.092	S
	14 th	1.00+1.00	0.62+1.42		NS
	63 rd	1.12+1.07	0.75+1.01		NS
Basophill Counts (%)	0 th	0.12+0.12	0.12+1.01		NS
	14 th	0.25+1.024	0+0		NS
	63 rd	0+0	0.12+1.01		NS

Superscripts a, b, c shows significant difference within the column (between different groups on specific day) (p = 0.05)

Table 2: Showing mean values of Biochemical parameters of experimental rats studied at 0th, 14th and 63rd day of intervals of study.

Parameter	Day	Control Group	<i>T. terrestris</i> Group	CD	Statistics
Serum AST (IU/L)	0 th	61.31+1.00	60.94+1.00		NS
	14 th	61.60+1.10	61.20+1.00		NS
	63 rd	61.35+1.00	60.78+1.01		NS
Serum ALT (IU/L)	0 th	31.89+1.01	30.96+1.01		NS
	14 th	31.74+1.11	31.49+1.35		NS
	63 rd	31.44+1.21	31.74+1.01		NS
Blood urea nitrogen (mg/dl)	0 th	21.23+1.00	21.16+1.36		NS
	14 th	21.42+1.00	21.17+1.17		NS
	63 rd	21.28+1.19	21.27+1.00		NS
Creatinine (mg/dl)	0 th	0.81+1.20	0.83+1.03		NS
	14 th	0.81+1.00	0.84+1.15		NS
	63 rd	0.82+1.01	0.83+1.03		NS
Serum Total Protein (gm/dl)	0 th	6.54+1.19	6.53+1.01		NS
	14 th	6.57+1.09	6.43+1.10		NS
	63 rd	6.57+1.14	6.50+1.03		NS
Blood glucose level (mg/dl)	0 th	113.98+1.06	114.09+1.00		NS
	14 th	113.84+1.01	114.67+1.08		NS
	63 rd	114.31+1.01	113.60+1.11		NS
Serum Progesterone level (ng/ml)	0 th	30.00+4.49	37.75+9.29		NS
	14 th	78.38+3.77	110.75+6.36		NS
	63 rd	^c 93.24+1.75	^a 356.13+31.11	(0.01)=75.905(0.05)=56.774	S

Superscripts a, b, c shows significant difference within the column (between different groups on specific day) (p = 0.05)

3.3 Hormonal assay

The hormonal assay like serum progesterone values were analyzed from blood collected at 0th, 14th and 63rd day of the experimental period.

3.4. Serum progesterone level

An increase in mean serum progesterone level was observed in treatment group and even in control group animals on day 14th. Where, the 14th day values were observed to be, 110.75+6.36ng/ml, as against day 0 values that is 37.75+9.29 ng/ml, further elevation in these values to 356.13+31.11ng/ml on day 63rd was observed.

No statistically significant difference was observed in all the treatment group rats on day 0 and 14th when compared against control group values. However, the elevation on day 63rd in the treatment group rats was observed to be statistically significant when compared against control group values.

Agoreyo fo; onwegbu (2015) [4] reported elevation in the progesterone level during pregnancy in albino rats. However, Elevation in mean progesterone level in non-pregnant rats in the present experiment are supported by the observations recorded by Lee J. Grota and Kristen B Eik-Nes (1967) [25] where they have reported elevation in progesterone level during pregnancy and lactation.

Agoreyo fo; onwegbu (2015) [4] reported that the elevation in progesterone level is a result of converting the endometrium to its secretory stage. It also makes the vaginal epithelium and cervical mucus thick and impenetrable to sperm and it also decreases contractility of the uterine smooth muscle.

Thus an increase in the progesterone level in the treatment group animals in the present investigation is an indicative of positive effect of individual and combination of these herbs on female reproductive system.

3.5 Reproductive Performance

3.5.1 Conception Rate

In treatment and control group animals in the experiment contained 8 female rats and were met using 1:1 male to female ratio. Animals were kept for mating during 8th to 10th week of age and male Wistar rats then were separated assuming the pregnancy in female Wistar rats. The pregnancy

was confirmed by physical examination and conception rate was calculated based on parturition.

All the female rats in control and treatment group were pregnant indicating 100% conception rate, so the treatment with herbal medicated feed when compared with control the results were observed to be at par.

3.5.2 Survival Rate

The total number of pups born in control and treatment group was observed to be 53, 82 respectively. However, all the pups in both the groups survived indicating 100% survival rate in control as well as treatment group.

3.5.3 Male: Female ratio in pups

Number of male pups born in treatment groups were significantly higher as compared to control group and Number of female pups born in treatment groups were also significantly higher as compared to control group.

Though no literature on conception rate, survival rate and male: female pups born ratio is available., the higher number of pups in the treatment group animals against the control group animals was indicative of increased fecundity percentage by feeding of herbal medicated feed.

Tribulus terrestris fed group showed highest number of pups born when compared with control group.

3.6 Gross Pathological investigations

The gross pathological examination of liver, Kidney, Brain, heart, lung, Intestine, Spleen, uterus and ovaries did not show any appreciable change in either of the organ except occasional minimal congestion in liver and kidneys of experimental rats of control as well as treatment group.

3.6.1 Organ weight

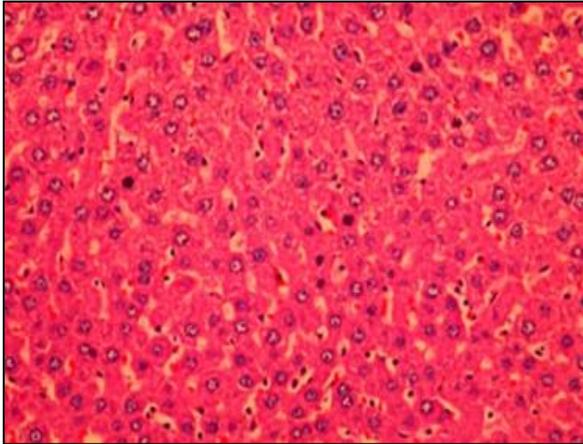
Mean organ weight in grams of liver, kidney, spleen, lungs, heart, brain did not show any significant changes but

The weight of uterus in control group animals was observed to be 1.62+0.02 gm. statistically significant higher values of uterine weight in treatment group 2.81+0.05gm. Increase in the weight of uterus in treatment group animals in comparison with control group was an indicative of positive effect of treatment on uterine weight. Similar observations were

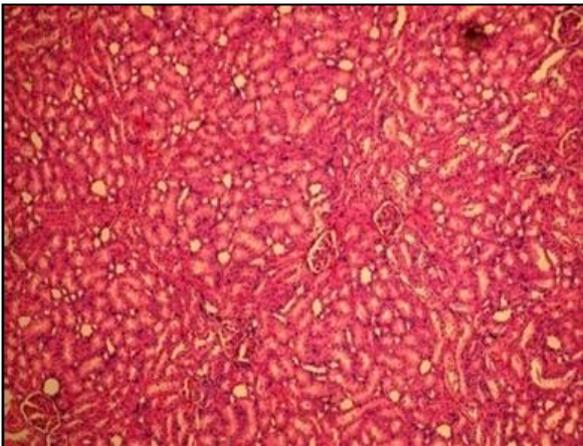
reported by Bashir (2009) ^[9], Khulbe (2015), Chitra *et al.* (2017), Prabhu (2014) indicating the positive effect of herbs on weight gain of organs.

3.7 Histopathological Investigation

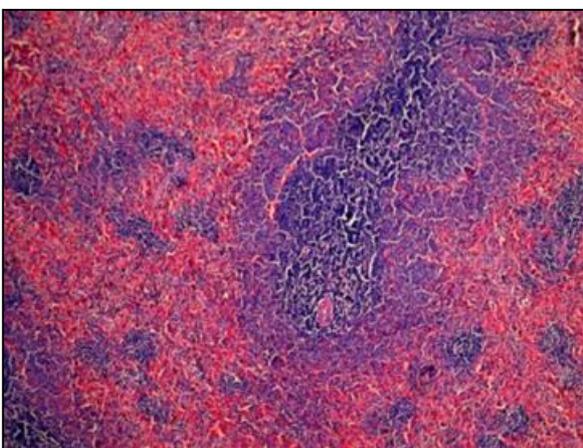
The histopathological investigations of liver, Kidney, spleen, intestine, heart, brain, ovary and uterus of experimental rats of almost groups was attempted at the end of study period and revealed that control as well treatment group showed minimal congestion and focal MNC infiltration. None organ could show neither gross nor histomorphological treatment related changes.



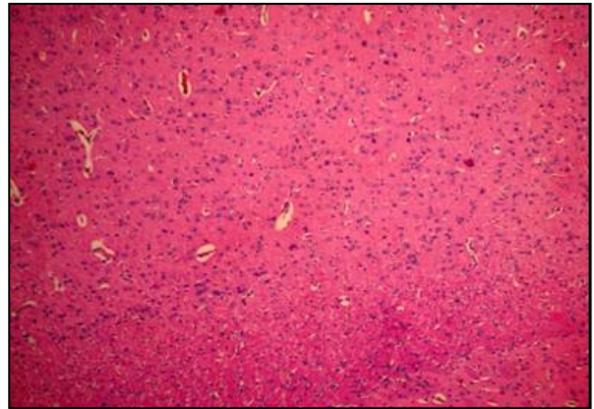
Focal MNC infiltration in Liver of *Tribulus terrestris* treated group (400X) H&E Stain



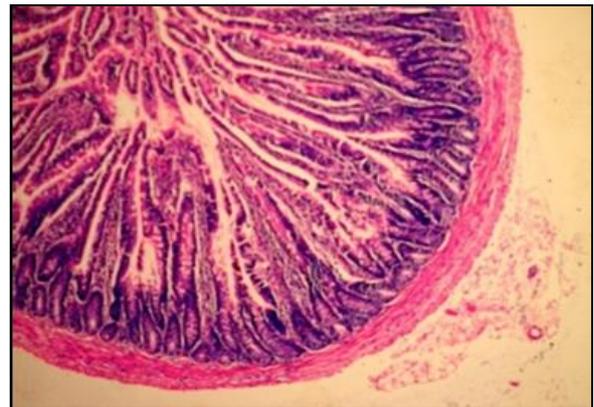
Focal MNC infiltration in Kidney *Tribulus terrestris* treated group (100X) H&E Stain



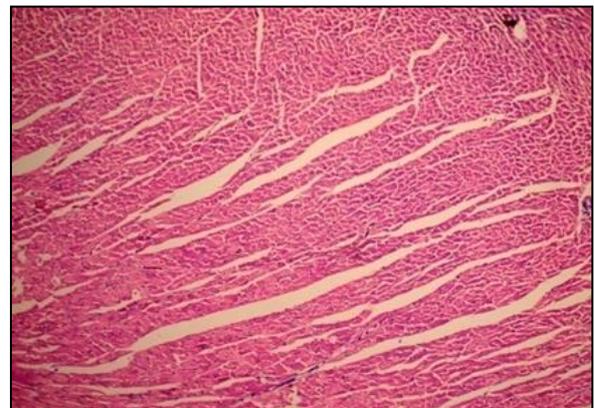
Histopathological Examination of Spleen treated with *Tribulus terrestris* group (100X) H & E Stain



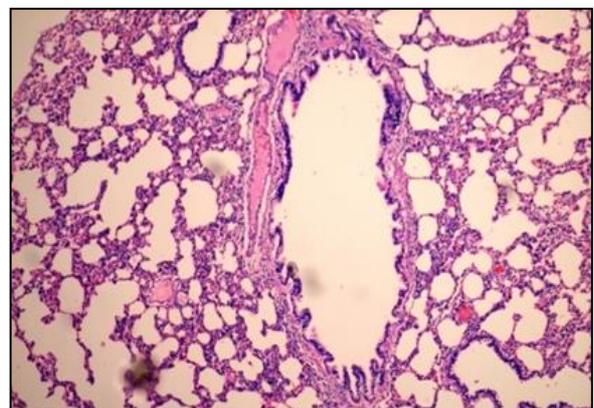
Focal MNC infiltration in brain of *Tribulus terrestris* treated group (100X) H & E Stain



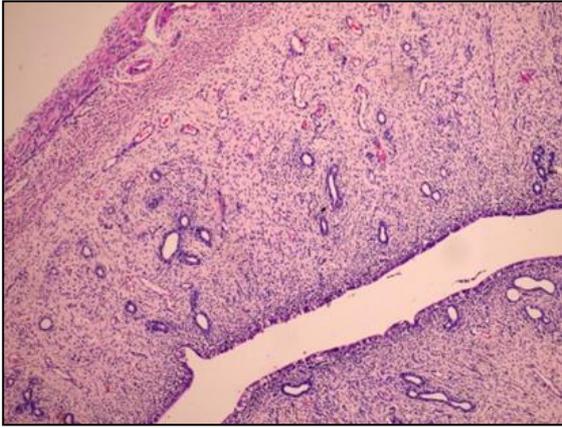
Histopathological Examination of Intestine of *Tribulus terrestris* treated group (100X) H & E stain



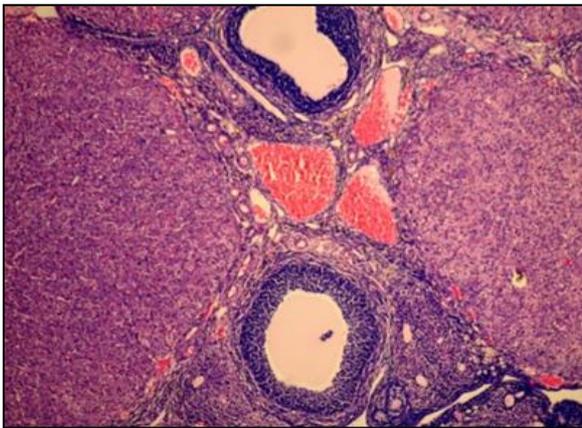
MNC infiltration in Heart of *Tribulus terrestris* treated group (100X) H & E stain



Histopathological Examination of Lung of *Tribulus terrestris* treated group found at par with control group (100X) H & E stain.



Histopathological Examination of Uterus of *Tribulus terrestris* treated group (100X) H & E Stain



Histopathological Examination of Ovary of *Tribulus terrestris* treated group (100X)

4. Summery

In the present investigation feed medicated with uterine tonic herb was evaluated for its efficacy in female reproductive performance.

From the present investigation it can be concluded that

1. Herbal medicated feed formulated and prepared by using *Tribulus terrestris* herb with the nutritional requirement of Wistar rats was observed to be effective uterine tonic.
2. The herbal medicated feed improves the progesterone level in treatment group when compared with the control group animals. The blood progesterone level was observed to be highest in *Tribulus terrestris* treated group.
3. The conception rate and survival rate in all the experimental animals were 100 per cent. However the number of pups born in the treatment group fed with herbal medicated feed was increased as compared to control group animals. Highest number of pups were observed in a group fed with *Tribulus terrestris* herb.
4. No statistically significant alterations were observed in Hematological and blood biochemical parameters in treatment group as well as control group.

Looking to the positive effects of this herb with or without combination, their usefulness is to be evaluated in other domesticated animals, considering the future prospect in Nutraceuticals.

5. Acknowledgements

Authors are thankful to Associate Dean, College of Veterinary and Animal Sciences, Parbhani for providing

facilities during conduct of research and M/s. L.R. Pharmaceuticals Company, Parbhani, Maharashtra, India for providing standard feed pellet for experimental rats to conduct present research.

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