WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 628-642. Research Article ISSN 2277– 7105

STUDIES ON ACUTE TOXICITY PROFILE AND UTERINE MORPHOMETRIC ANALYSIS OF A SINGLE INTRA-UTERINE INJECTION OF RISUG, A NANOTECHNOLOGY BASED BIOMEDICINE IN RATS

Falgun Wanganuji Bansode[1], Anil Kumar Meena[2] and Rama Kant Singh[2]*

Division of Endocrinology[1] and Division of Toxicology & Experimental Medicine[2]
CSIR- Central Drug Research Institute, Sector- 10, Jankipuram Extension, Sitapur Road, Lucknow-226031, India.

Article Received on
23 May 2018,
Revised on 13 June 2018,
Accepted on 03 July 2018
DOI: 10.20959/wjpr201814-12564

***Corresponding Author**
Dr. Rama Kant Singh
Division of Toxicology & Experimental Medicine
CSIR- Central Drug Research Institute, Sector-10, Jankipuram Extension, Sitapur Road, Lucknow-226031, India.

ABSTRACT

The non-hormonal contraceptive, named RISUG (an acronym for Reversible Inhibition of Sperm under Guidance) has expected to provide a valuable addition to the current options of male contraception. A recent study on intra-uterine infusion of RISUG has shown antifertility effects. Present study was conducted to assess the toxic effects of the RISUG in female Charles Foster rats. Healthy adult female rats of Charles Foster strain were randomly divided into two groups (Gr.) consisting 10 animals each. Gr. I. rats were injected with vehicle only (DMSO) in both uterine horns served as Control. Rats in G.II were injected with RISUG in the uterus bilaterally and observed for a period of 14 days. The initial (on day 0) and final (on day 15) body weights, food and water consumption in control and treated animals were recorded. Blood samples were collected in EDTA-coated vials for haematological and biochemical analysis. On day 15, autopsy of animals was done and body organs (viz. brain, heart, liver, lungs, kidney, adrenal, spleen, uterus and ovaries) were dissected out, weighed and fixed in formalin for histopathological study. Results showed that the body weights, absolute/relative organ weights, food and water consumption, hematological parameters, viz. Hgb(g%), RBC($x10^6/mm^3$), Hct(%), MCV($micron^3$), MCHC(g%), TLC($x10^3/mm^3$), DLC(%) of Polymorphs, Lymphocytes, Macrophages, Eosinophils and Platelets ($x10^3/mm^3$), and biochemical analysis of marker enzymes, did not show any significant changes in RISUG-treated as compared to control rats. There were no

any toxic effects or mortality observed during the entire treatment period. The microscopic examination of histological slides of the vital organs and uterine morphometric analysis did not reveal any significant difference but showed normalcy as compared to controls. Results of the study show that intra-uterine infusion of RISUG did not show any adverse effects during 14 days toxicity study in rat, thus, indicate that it is safe to use for further studies.

KEYWORDS: RISUG, Intra-uterine infusion, Toxicity profile, Uterine morphometry, Rats.

INTRODUCTION

The non-hormonal contraceptive, named RISUG (an acronym for Reversible Inhibition of Sperm Under Guidance) has expected to provide a valuable addition to the currently limited options of male contraception.[1] RISUG (Mark sans Pharma, Mumbai, India) consists of a co-polymer styrene maleic anhydride (SMA) dissolved in 99.9% pure dimethylsulphoxide (DMSO) has been developed by Prof. S.K. Guha and his team at I.I.T. Kharagpur.[2] Earlier studies have been shown the reproductive functional success, safety of vas occlusion by RISUG, and its reversal by dimethylsulphoxide (DMSO), followed by multigenerational (F1-F3) teratogenicity studies in rats when RISUG - a co-polymer of styrene maleic anhydride (SMA) dissolved in 0.01 ml DMSO was injected into the lumen of the vas deferens bilaterally at the dose levels of 0.25, 0.50 and 1.00 mg/vas/rat.[3] Previous studies with RISUG have been demonstrated the spermicidal activity and its non-toxicity[4,5] and teratogenic safety[6] in rats. Injection of RISUG in vasa causes degenerative changes to sperm acrosome when its contents come in contact with the polymer. The positive and the negative charges on the polymer surface leads to the sperm surface burst, makes it immotile and incapable to fertilize an egg.[7,9]

RISUG is long time effective, non-invasively reversible and controllable. It also shows antimicrobial, anti HIV and anti-prostate cancer activity in males.[10,11] In monkeys, long term vas occlusion with RISUG resulted in necroasthenoterato-zoospermic, suggesting instant sterility in ejaculated sperms. An additional advantage of this technique is that it causes a partial blockage of the vas deferens with concomitant flow of functionally inactive cells.[8,12] RISUG is retained in the folds of the inner wall of the vas deferens for a long period of time despite not being tissue adherent. Phase I[13] and Phase II[14] clinical trials have been successfully completed and currently a Phase III multicentre trial is underway.[15] The short term studies on semen and accessory gland function in phase III clinical trials subjects

confirmed azoospermia between 1-4 months post-injection period and absence of pregnancy during 6 months study period.[16]

Rcently, Intra-uterine infusion methods have been used to study implantational and decidual events during early pregnancy.[17,18] Levonorgestrel intrauterine system (LNG-IUS) applied to control atypical and non-atypical endometrial hyperplasia.[19] The medicated intrauterine systems are superior to inert devices and today a number of active principles, such as copper and progestogens, have been incorporated and tested when released from an intrauterine device (IUD). Copper-releasing devices last more than 10 years, with cumulative pregnancy rates of between approximately 5 and 3, and cumulative expulsion rates between approximately 12 and 8. With all IUDs, bleeding and pain are the most common reasons for a request to withdraw a device. There is agreement that fertility after removal of a copper-IUD is not impaired. Finally, the overall risk of ectopic pregnancy is reduced in IUD users, compared with using no contraception. The system is one of the most effective methods of contraception available today: large clinical studies indicate a Pearl index of 0.1 per 100 woman-years. Although a post-fertilization effect cannot be excluded, in a majority of cases, intrauterine systems act as true contraceptives, preventing fertilization.[20]

However, information on effect of RISUG in females is lacking, although its intra-uterine administration has been shown to cause female anti-fertility effects. The present study was undertaken to evaluate its toxic effects on body/organ weights, food and water intake, haematological parameters, histopathology and uterine morphometric analysis after a single intra-uterine injection of RISUG in rats.

MATERIALS AND METHODS

Chemicals

The test compound RISUG, a male anti-fertility agent was provided by Dr. Sujoy K. Guha, Professor of Biomedical Engineering at the School of Medical Science and Technology, Indian Institute of Technology (IIT), Kharagpur, India. All other chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Company (India).

Animals

Total of 20 female adult rats (170-180 g body weights) of Charles Faster strain used in this study were obtained from Institute's animal house. Animals were acclimatized for 1 week, maintained in standard laboratory conditions (24 ± 2^0C) with 12**:**12 h light and dark cycles in

individual polypropylene cages and fed with pelleted standard rat diet (Lipton India Ltd., Bangalore) and water *ad libitum*. Experimental protocol was approved by the 'Institutional Animal Ethical Committee' (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India (CPCSEA Approval No. AIEC/2016/65, dated 12.5.16).

Experimental Design

In the present study, healthy and disease-free rats were selected on the basis of initial health check-up. Adult female rats were divided into two groups (Gr.) consisting of 10 animals each. Gr. I rats were injected with vehicle (0.01 ml DMSO) only in the lumen of uterine horns, served as Control. Rats in Gr. II were subjected to bilateral intra-uterine infusion of RISUG (SMA-DMSO Complex) under ether anesthesia. The abdomen was exposed by a single median incision surgically and RISUG (dissolved in 0.01ml DMSO) was injected into the both uterine horns. The uterine horns of control and treated rats were placed properly in their original position; incision was closed by stitching with catgut internally and upper skin incision with nylon thread. Post-operative care was taken by dressing with Neosporin antibiotic powder and merbromin solution (2% w/v) and anti-inflammatory drugs. The antibiotic, Terramycin (Pfizer Ltd, Bombay) was injected intramuscularly to each rat for 5 consecutive days as per method of Sethi *et al.*[4]

The body weights, food and water consumption were recorded initially on day 0 before the beginning of the experiment and after 14 days post-injection period (on day 15). On day 15, blood samples were collected for biochemical analysis and hematology and all animals were sacrificed. Hematological parameters were studied initially and terminally. Urine Analysis (Colour, Sp. Gravity, pH, Protein, Bilirubin, Glucose, Ketone, Occ. Blood, Urobilinogen Microscopy Examination- E, P, M, R, O, C, A) was carried on day 0 and 15 before autopsy of animals. The body organs (viz. brain, heart, liver, lungs, kidney, adrenal, spleen and gonads) were dissected out freed from connective tissues /blood clots in chilled saline and weights recorded. The tissues from different organs were fixed in 10% formalin for histopathology purpose.

Histomorphometry

Formalin fixed uterine tissues from control and treated rats were dehydrated in graded series of ethanol, cleared in xyline and infiltrated and embedded in moulton paraffin wax (at 58^0C). Tissue sections/Uterine sections (5µM) were cut and stained with routine haematoxylin-

eosin. Measurements of morphometric parameters (viz. uterine luminal and glandular epithelial cell height, average diameter of endometrial glands and number of glands/section) were observed under Olympus Trinocular Microscope (BX51, Olympus Singapore Pvt. Ltd., Singapore) as per methods reported earlier[21,22] and uterine sections were micro photographed. In this process at least two uterine tissue blocks from each animal, 5 transverse sections from each block and 10 microscopic fields per section were measured at 400x magnifications under Olympus Trinocular Microscope (BX51, Olympus Singapore Pvt. Ltd., Singapore).

Statistical Analysis

Data were expressed as mean ± S.D. Student's '*t*' test and one-way ANOVA (one factor analysis of variance) was applied for statistical significance and comparisons between control and treated groups of rats. P values < 0.05 considered as significant.

RESULTS

General health check-up and mortality

Animals belonging to control and treated group were generally active and healthy throughout the period of the study. No mortality was seen in either control or treated group of rats. The DMSO injected control and RESUG- injected animals had adopted the normal behavior within a week of surgery. None of them showed hypo- and hyper- excitability of nervousness during implants, post-operative reversal and handling thereafter.

Food and water consumption

Measurement of the initial water and leftover water and pellets given to the animals, did not show any significant change in the average 24-h water and food intake of animals in RISUG-treated group as compared to control (Table 1).

Table 1. Average food Intake (gm/day/rat) and average water intake (ml/day/rat) of female rats after 14 days toxicity study of intra-uterine RISUG injection (Mean ± S.D., n = 10 number of animals) .

Group No.	Initial Food intake	Final Food intake
Group I (Control)	17.2	17.4
Group II (RISUG- inj.)	18.6	18.0

Group No.	Initial Water intake	Final Water intake
Group I (Control)	32.3	31.6
Group II (RISUG- inj.)	33.6	33.5

Biochemistry

The biochemical parameters 'marker' for general metabolic functions included glucose, cholesterol, triglycerides, total protein, albumin and globulin, did not show any significant change in RESUG-injected as compared to control group of rats. Similarly, the animals from both the groups did not show any significant variation in the biochemical parameters of kidney function (blood urea nitrogen, criatinin, calcium and phosphate) and liver function (globulin, ALT, AST, ALP, T-Bill) (Table 2).

Table 2. Terminal Serum Biochemistry (Mean ± S.D.) after 14 days toxicity study of intra-uterine RISUG injection in Female Rats.

Group No.		General Metabolic Functions					Liver Functions				Kidney Functions				
		GLU (mg/dl)	CHOL (mg/dl)	TG (mg/dl)	TP (mg/dl)	ALB (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	TBIL (mg/dl)	Urea (mg/dl)	BUN (mg/dl)	CRTN (mg/dl)	Ca (mg/dl)	P (mg/dl)
Group I (Control)	Mean	137.83	63.77	97.78	6.99	3.38	69.20	175.16	352.27	0.15	40.91	19.06	0.58	10.60	7.45
	±S.D.	18.87	8.79	23.71	0.34	0.15	8.83	25.46	45.61	0.05	2.89	1.34	0.04	0.57	1.34
Group II (RISUG- inj.)	Mean	148.78	64.05	49.47	6.66	3.28	55.32	150.37	368.46	0.13	36.01	16.79	0.52	10.36	6.82
	±S.D.	22.83	7.13	12.76	0.54	0.21	8.45	30.64	98.47	0.03	4.99	2.33	0.06	0.76	0.69

Haematology

There were no significant changes observed in any of the haematological parameters viz. Hgb(g%), RBC (x106/mm^3), Hct(%), MCV(micron^3), MCHC(g%), TLC(x10^3/mm^3), DLC(%) of polymorphs, lymphocytes, macrophages and eosinophils and platelets (x10^3/mm^3) of RISUG-treated as compared to control animals before (Initial-day 0) or 14 days after (Final-day 15) a single intra-uterine injection of RISUG (Tables 3 and 4).

Table 3. Initial Haematology (on day 0) before 14 days of intra-uterine RISUG- injection in female rats (Mean ± S.D.).

Group No.		Red Blood Cells						White Blood Cells						Platelets (x10^6/mm^3)
								TLC (x10^6/mm^3)	Differential Leucocyte Count (%)					
		Hgb (g%)	T-RBC (x10^6/mm^3)	Hct (%)	MCV (micron^3)	MCH	MCHC (g%)		P	L	M	E	B	
Group I (Control)	Mean	14.56	7.57	43.84	58.12	19.26	33.15	21.06	13.70	78.10	5.80	2.40	0.00	885.10
	± S.D.	1.75	0.92	4.55	3.01	0.70	1.11	3.93	1.77	2.56	1.03	0.70	0.00	24.06
Group II (RISUG- inj.)	Mean	15.04	7.72	44.55	57.94	19.61	33.79	17.92	14.50	76.50	6.20	2.80	0.00	853.40
	± S.D.	0.58	0.37	1.51	2.23	0.86	0.99	2.54	2.17	2.55	1.23	0.63	0.00	140.37

Table 4. Final Haematology(on day 15) after 14 days of intra-uterine RISUG- injection in female rats (Mean ± S.D.)

Group No.		Red Blood Cells						White Blood Cells						Platelets (x10⁶/mm³)
								TLC (x10⁶/mm³)	Differential Leucocyte Count (%)					
		Hgb (g%)	T-RBC (x10⁶/mm³)	Hct (%)	MCV (micron³)	MCH	MCHC (g%)		P	L	M	E	B	
Group I (Control)	Mean	13.99	7.34	42.79	57.46	19.18	32.86	15.72	14.10	77.50	6.00	2.40	0.00	750.30
	± S.D.	0.62	0.42	2.53	4.77	1.09	1.05	4.08	3.67	4.48	0.82	0.84	0.00	214.49
Group II (RISUG-inj.)	Mean	13.73	7.32	43.30	59.86	18.80	29.75	13.05	13.80	77.60	5.60	3.00	0.00	840.80
	± S.D.	1.14	0.72	2.51	8.86	0.80	9.33	5.65	4.08	4.88	1.17	1.05	0.00	159.76

Urine Analysis

Compared to the respective control values, there were no variations in the values of the Urine parameters (Colour, Sp. Gravity, pH, Protein, Bilirubin, Glucose, Ketone, Occ. Blood, Urobilinogen Microscopy Examination- E, P, M, R, O, C, A) of the RISUG-treated rats and were well within the limit of normalcy (Tables 5 and 6).

Table 5. Initial Urinalysis (day 0) before 14 days of intra-uterine RISUG- injection in female rats (Mean ± S.D.).

Group		Colour	Sp.Gravity	pH	Protein	Bilirubin	Glucose	Ketone	Occ.Blood	Urobilinogen	Microscopy						
											E	P	M	R	O	C	A
Group-I (Control)	Mean	Straw	1.0	7.4	14.5	0	0	0	0	0.2	1	0	0	0	0	0	0
	±SD	Straw	0.0	0.7	13.4	0	0	0	0	0.0	0	0	0	0	0	0	0
Group-II (RISUG-inj.)	Mean	Straw	1.0	7.5	15.0	0	0	0	0	0.2	1	0	0	0	0	0	0
	±SD	Straw	0.0	0.7	12.9	0	0	0	0	0.0	0	0	0	0	0	0	0

Table 6. Final urinalysis (on day 15) after 14 days of intra-uterine RISUG- injection in female rats (Mean ± S.D.).

Groups		Colour	Sp.Gravity	pH	Protein	Bilirubin	Glucose	Ketone	Occ. Blood	Urobilinogen	Microscopy						
											E	P	M	R	O	C	A
Group-I (Control)	Mean	Straw	1.0	6.9	15.0	0	0	0	0	0	1	0	0	0	0	0	0
	±SD	Straw	0.0	0.6	12.9	0	0	0	0	0	0	0	0	0	0	0	0
Group-II (RISUG-inj.)	Mean	Straw	1.0	6.9	20.0	0	0	0	0	0.2	1	0	0	0	0	0	0
	±SD	Straw	0.0	0.7	12.9	0	0	0	0	0.0	0	0	0	0	0	0	0

Body and organ Weights

The data obtained on body and organ weights are represented in Tables 7-9. Results showed that there was no any significant change ($P < 0.1$-0.2) in body weights before (day 0) or after 14 days intra-uterine single injection of RISUG and comparable gain in body weights among

the animals of both control and treated groups was seen (Table 7). Similarly, the vital organs weights viz. adrenal, brain, heart, liver, lungs, kidney, spleen and gonads(uterus-cervix-ovaries), did not show any significant change in its absolute or relative organ weights in RESUG-injected as compared to control rats (Tables 8 and 9).

Table 7. Body Weights (g) from control (Gr. I) and RISUG-treated (GR.II) female rats after 14 Days Toxicity Study (Mean ± S.D., n = 10 number of animals)

Group No.		Initial Body Weight	Final Body Weight
Group I (Control)	Mean	214.6	254.7
	± S.D.	7.71	14.11
Group II (RISUG- inj.)	Mean	215.4	245.7
	± S.D.	8.24	14.78

Table 8. Absolute Organ Weights of Rats after 14 days toxicity study of RISUG in female Rats (Mean ± S.D., n = 10 number of animals) .

Groups		Adrenal		Brain	Ut+Cx +Ovaries	Heart	Kidney		Liver	Lungs	Spleen
		Rt	Lt				Rt	Lt			
Group-I (Control)	Mean	0.029	0.030	1.807	0.506	0.823	0.785	0.811	9.725	1.287	0.794
	±SD	0.001	0.001	0.154	0.152	0.070	0.062	0.062	0.896	0.188	0.105
Group-II (RISUG-inj.)	Mean	0.029	0.028	1.966	0.644	0.748	0.862	0.852	9.458	1.341	0.786
	±SD	0.001	0.003	0.073	0.102	0.045	0.080	0.069	1.409	0.119	0.229

Table 9. Relative organ weights of rats after 14 days of RISUG-injection in female rats (Mean ± S.D., n = 10 number of animals) .

Groups		Adrenal		Brain	Ut+Cx +Ovaries	Heart	Kidney		Liver	Lungs	Spleen
		Rt	Lt				Rt	Lt			
Group-I (Control)	Mean	0.012	0.012	0.709	0.200	0.323	0.308	0.319	3.813	0.505	0.312
	±SD	0.001	0.001	0.042	0.063	0.020	0.020	0.025	0.182	0.069	0.742
Group-II (RISUG-inj.)	Mean	0.012	0.011	0.829	0.262	0.305	0.351	0.347	3.845	0.546	0.320
	±SD	0.001	0.002	0.058	0.036	0.016	0.031	0.023	0.587	0.041	1.552

Histopathological Examination

The microscopic examination of the histological slides of the vital organs (viz. brain, heart, liver, lungs, kidney, adrenal and spleen) did not reveal any pathological changes in RESUG-injected rats as compared to control rats (Figures not shown).

Effect of RISUG on uterine histo-morpholmetric analysis

In control group of rats, uterine endometrium showed large number of endometrial glands with wide lumen and blood vessels embedded in endometrial stromal matrix. The uterine luminal epithelium was columnar and showed infiltration of leukocytes. The uterine stroma represented few decidual-like cells, abandoned spindle shaped cells, leucocytes and blood vessels/venules (Figure 1A-C). In RISUG-injected rats after a period of 14 days, the uterine luminal and glandular epithelium was observed to be columnar and uterine luminal space was wide similar to controls. Similarly, the stromal compartment showed leucocytes infiltration and blood vessels (Figure 1D-F).

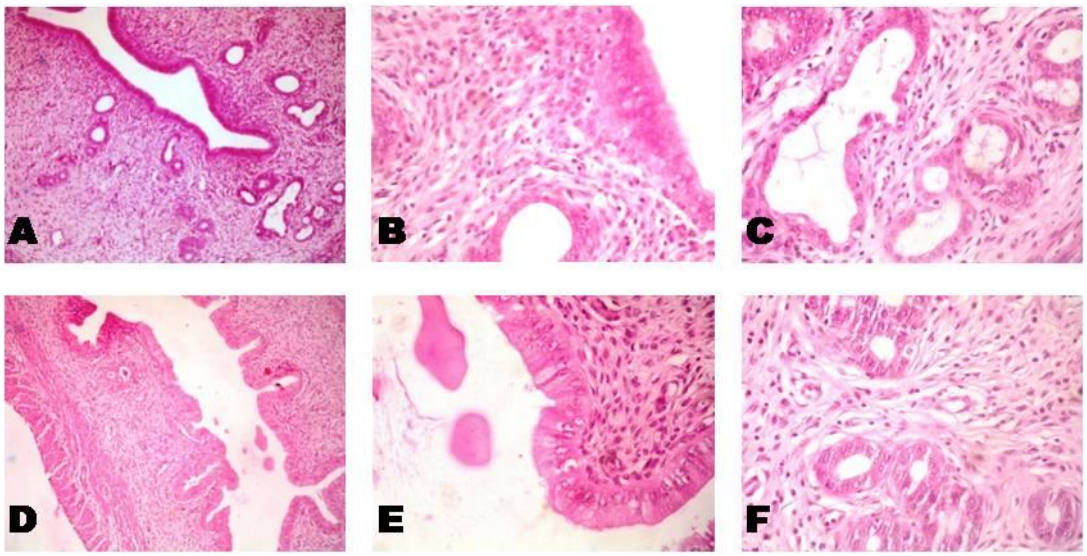

Figure 1: T. S. of uterus in control rat showing uterine luminal epithelium and stroma with abandoned endometrial glands and blood vessels (A). (B) Showing columnar luminal epithelium and (C) glandular epithelium with wide lumen and leukocytes infiltration, abandoned spindle shaped cellsin stroma. In RISUG-injected rats after a period of 14 days (D-F), the uterine luminal and glandular epithelium was observed to be columnar and uterine luminal space was wide similar to controls (D). The stromal compartment showed stromal cells, leucocytes infiltration and blood vessels (E,F) similar to controls (Magnification x100(A,D) and x400(B,C,E,F); H-E stained).

The morphometric measurements viz. number of endometrial glands, average diameter of glands and uterine luminal and glandular epithelial cell height was not significantly changed in RISUG-treated as comparable to control rats (Figure 2).

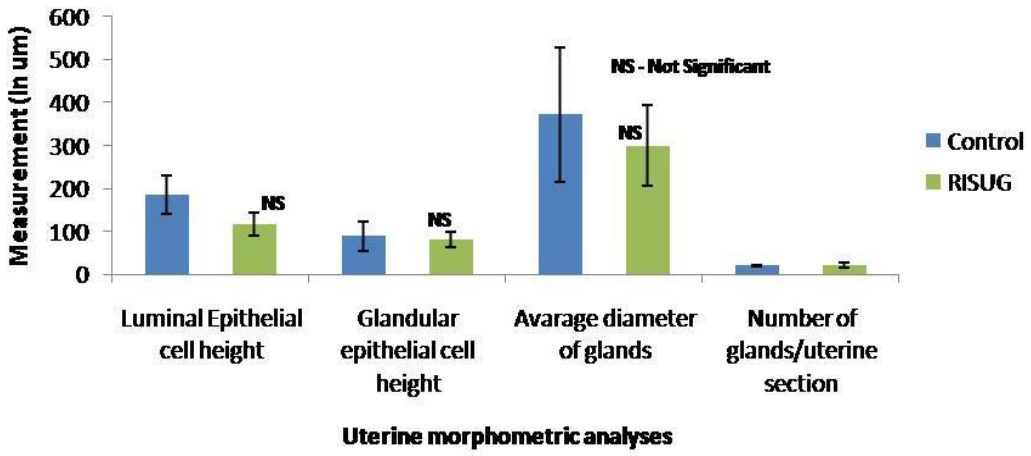

Figure 2: The morphometric measurements viz. number of endometrial glands, average diameter of glands and uterine luminal and glandular epithelial cell height in RISUG-injected and control rats.

Fallopian tube /ovarian histology

Fallopian tube was normal in structure showing folded (Convoluted) epithelium and outer circular muscle layer (Figure 3A, B). Follipian tube was similar to control in structure showing convoluted epithelium, lumen and outer muscle layer (Figure 3C, D).

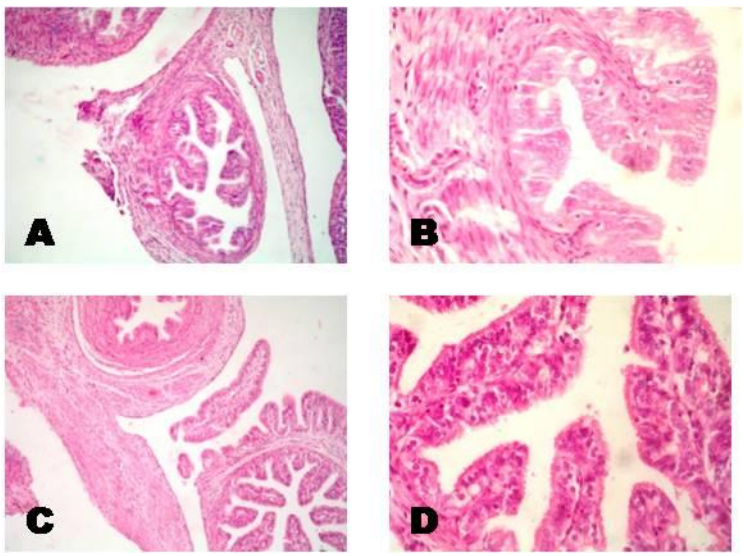

Figure 3: Cross section of fallopian tube showing normal histology in control (A,B) and treated (C,D) group of rats exhibiting convoluted epithelium, lumen and outer muscle layer(Magnification x100(A,C) and x400(B,D); H-E stained).

In control rats, ovarian histology showed large number of C.L.s with luteal cells and leucocytes as well as attretic follicles (Figure 4 A-C). In RESUG-injected rats, ovary showed large number of attretic follicles and C.L.s similar to in controls (Figure 4D-F).

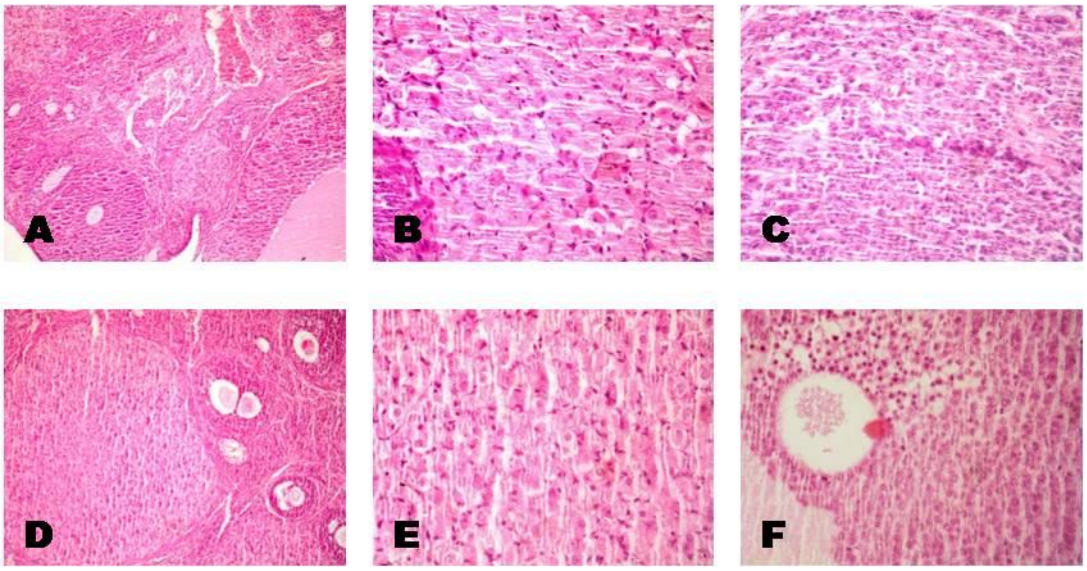

Figure 4: In control rats, ovarian histology showing large number of C.L.s with luteal cells and leucocytes as well as attretic follicles (A-C). In RESUG-injected rats, ovary showed large number of attretic follicles and C.L.s (D-F) similar to in controls (Magnification x100(A,D) and x400(B,C,E,F); H-E stained).

DISCUSSION

Recently, chemical occlusion of the vas deferens by RISUG has been considered to be an ideal male contraceptive method that is in Phase III clinical trial after successful completion of phase I and II clinical trials. The polymer SMA can be used to block the lumen of the vas deferens over an extended period of time and its systemic toxicity evaluation had been studied in detail in rat, rabbit and rhesus monkeys previously. Findings indicated that SMA-injection did not cause any systemic toxicity, male mediated teratogenicity and multigenerational teratogenicity in experimental animals.[3,4,23,27] Results of the present study on acute toxicity profile in rats with intra-uterine RESUG injection did not show any significant change in gross behavior, food and water intake, haematological parameters and biochemical analysis of marker enzymes for kidney, liver and metabolic function. Further, the histoarchitecture of vital body organs did not show any pathological changes in RESUG-injected as compared to DMSO-control rats after 14 days of post-injection period. The uterine morphometric measurements viz. number of endometrial glands, average diameter of glands and uterine luminal and glandular epithelial cell height was not significantly changed in

RISUG-treated as comparable to control rats. Also, the histoarchitecture of uterine endometrium showed normal histoachitecture exhibiting large number of endometrial glands, blood vessels, infiltration of leukocytes and stromal cells in endometrial stromal matrix as well as columnar uterine epithelium was evident in both treated control group of rats. In addition, fallopian tube and ovary also showed normal histoarchitecture in both the groups.

Previous studies have been shown that the male gamete, spermatozoa and its morphology play a significant role in fertilization process, especially the anterior part, acrosome which secrets three important key enzymes – 5'-nucleotidase (5'-NT), hyaluronidase and proacrosin-acrosin system which facilitate sperm-oocyte interaction. Any change in it by means of antifertility agents, acrosin/hylronidase inhibitors and spermicides leads to impairment of gamete interaction and fertilization of ova.[9,28,30] The treatment of RISUG have been shown to cause significant inhibition in plasma membrane-associated enzymes, 5'-Nucleotise, hyaluronidase and acrosin from the acrosomal membrane[9] leading to spermicidal action.[31] Our earlier study have shown that RISUG injection in uterus causes antifertility effect and did not implant the ovum when mated with males which may be due to its spermicidal activity leading to acrosome degeneration. Further, Follopian tube occlusion by RISUG also caused inhibition of implantation in rats leading to degeneration of Zona pellucida of Eggs in rat (Unpublished data). Present study on uterine morphometry did not show any significant change in uterine structure including endometrial stroma and epithelium. The uterine epithelial cell height, number of endometrial glands and average diameter of glands in RISUG-injected rats were similar to in controls after a period of 14 days of post-injection period. Further, there were no histo-pathological changes were evident in the ovaries and fallopian tubes as well.

In conclusion, results on 14 days toxicity study of RISUG-injection on gross behavior, food and water consumption, body and organ weights, haematology, biochemistry, histopathology and uterine morphometry, indicate that the intra-uterine infusion of RISUG (SMA- DMSO complex) is safe in rats.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Sujoy K. Guha for kindly providing the compound, RISUG for the above study and the research grant in the form of collaborative research project to our Institute.

REFERENCES

1. Ananthaswamy A. Now it's men's turn. A contraceptive injection makes its debut in India. New Scientist, 2002; 174: 5-5.
2. Guha SK. Contraceptive for use by a male. United States Patent, 1996; 5488075; Jan. 30.
3. Singh RK, Bansode FW, Meena AK. Studies on Testis, Sperm Characteristics and Teratogenic aspects after Vas occlusion by a co-polymer of styrene maleic anhydride - RISUG and its reversal in rats. International Journal of Innovative Research in Science, Engineering and Technology, 2014; 3(5): 12474-85.
4. Sethi N, Srivastava RK, Singh RK. Safety evaluation of male injectable antifertility agent, styrene maleic anhydride in rats. Contraception, 1989; 39: 217-26.
5. Sethi N, Srivastava RK, Singh RK. Chronic toxicity of styrene maleic anhydride, a male contraceptive in rhesus monkeys (*Macaca mulatta*). Contraception, 1990; 42: 337-48.
6. Sethi N, Srivastava RK, Nath D, Singh RK. Teratological evaluation of an injectable male antifertility agent styrene maleic anhydride in rats. Int J Fertil, 1992; 37: 183-7.
7. Guha SK, Singh G, Ansari S, Kumar S, Srivastava S, Koul V, et al. Phase II clinical trial of a vas deferens injectable contraceptive for the male. Contraception, 1997; 56: 245-50.
8. Sharma U, Chaudhury, Jagannathan NR, Guha SK. A proton NMR study of the effect of a new intravasal contraceptive RISUG on seminal plasma metabolites. Reproduction, 2001; 122: 431-6.
9. Chaudhury K, Bhattacharya AK, Guha SK. Studies on the membrane integrity of human sperm treated with a new male injectable contraceptive. Hum Reprod, 2004; 19: 1826-30.
10. Sharma S, Sen P, Mukhopadhyay SN, Guha SK. Microbicidal male contraceptive – RISUG induced morphostructural damage in *E.coli*. Colloids Suface, 2003; 32: 43-50.
11. Guha SK. RISUG^{TM} (reversible inhibition of sperm under guidance) – An antimicrobial as male vas deferens implant for HIV free semen. Med Hypotheses, 2005; 65: 61-4.
12. Chaudhury K, Sharma U, Jagannathan NR, Guha SK. Effect of a new injectable male contraceptive on the seminal plasma amino acids studied by proton NMR spectroscopy. Contraception, 2002; 66: 199-204.
13. Guha SK, Singh G, Anand S, Ansari S, Kumar S, Koul V. Phase I clinical trial of an injectable contraceptive for the male. Contraception, 1993; 48: 367-75.
14. Guha SK. An improved reversible contraceptive for male and female. Canada Patent, 2007; 2367414.
15. Sharma RS, Rajanna A, Singh BK, Mathur AK, Mukherjee AK. Current status of development of RISUG: an intravasal injectable male contraceptive. In: Sharma RS,

Rajanna A, Rajalakshmi M, editors. *Proceedings of the Conference on Recent Advances and Challenges in Reproductive Health Research*. New Delhi, India: Indian Society for the Study of Reproduction and Fertility (ISSRF); Feb. 19–21, 2007; pp. 127–140.

16. Chaki SP, Das HC, Misro MM. A short-term evaluation of semen and accessory sex gland function in phase III trial subjects receiving intravasal contraceptive RISUG. Contraception, 2003; 67: 73-8.
17. Friberg J, Nilsson O. Changes in fertility and morphology of sensitized rat uterine endometrium upon an intra-uterine injection of antigen. Ups J Med Sci, 1972; 77(3): 133-5.
18. K. Bridget Brosnihan, Victor M. Pulgar, Manish S. Bharadwaj, Liomar A. A. Neves and Liliya M. Yamaleyeva. Local intra-uterine Ang-(1–7) infusion attenuates PGE2 and 6-keto PGF1α in decidualized uterus of pseudopregnant rats. Reproductive Biology and Endocrinology, 2016; 14: 68, DOI 10.1186/s12958-016-0202-9.
19. Haoula ZJ, Walker KF, Powell MC. Levonorgestrel intra-uterine system as a treatment option for complex endometrial hyperplasia. Eur J Obstet Gynecol Reprod Biol, 2011; 159(1): 176-9.
20. Benagiano G, Gabelnick H, Farris M. Contraceptive devices: intravaginal and intrauterine delivery systems. Expert Review of Medical Devices, 2008; 5(5): http://dx.doi.org/10.1586/17434440.5.5.639
21. Gaytan F, Aceitero J, Bellido C, Pinilla L, Aguilar R, Aguilar E. Are the eosinophil leucocytes involved in the estrogenic response of the post-natal rat epididymis. Int J Androl, 1990; 13: 500-07.
22. Ghosh D, De P, Sengupta J. Effect of RU 486 on the endometrial response to deciduogenic stimulus in ovariectomized rhesus monkeys treated with estrogen and progesterone. Hum Reprod, 1992; 7: 1048-060.
23. Sethi N, Srivastava RK, Singh RK. Male mediated teratogenic potential evaluation of new antifertility compound SMA in rabbit (Oryctolagus cuniculus). Contraception, 1990a; 42: 215-23.
24. Sethi N, Srivastava RK, Singh RK. Histological changes in the vas deferens of rats after injection of a new male antifertility agent "SMA" and its reversibility. Contraception, 1990b; 41: 333-39.
25. Sethi N, Srivastava RK, Singh RK, Nath D. Long-term toxicity studies of styrene maleic anhydride in rats. Biomed Environ Sci, 1990c; 3: 452-57.

26. Sethi N, Srivastava RK, Singh RK, Bhatia GS, Sinha N. Chronic toxicity of styrene maleic anhydride, a male contraceptive, in rhesus monkeys (Macaca mulatta). Contraception, 1990d; 42: 337-47.
27. Sethi N, Srivastava RK, Nath D, Singh RK. Preclinical toxicity study of a male injectable antifertility agent (styrene maleic anhydride) in rhesus monkeys, Macaca mulatta. J Med Primatol, 1991; 20: 89-93.
28. Cheng CY, Mruk DD. Cell junction dynamics in the testis: Sertoli – germ cell interactions and male contraception development. Physiol Rev, 2002; 82: 825-74.
29. Srivastav A, Chandra A, Singh M, Jamal F, Rastogi P, Rajendran SM, Bansode FW, Lakshmi V. Inhibition of hyaluronidase activity of human and rat spermatozoa in vitro and antispermatogenic activity in rats *in vivo* by *Terminalia chebula*, a flavonoid rich plant. Reprod Toxicol, 2010; 29: 214-24.
30. Pandey RR, Srivastava A, Pachauri SD, Khandelwal K, Naqvi A, Malasoni R, Kushwaha B, Kumar L, Maikhuri JP, Pandey G, Paliwal S, Gupta G, Dwivedi AK. Design and synthesis of γ-butyrolactone derivatives as potential spermicidal agents. Bioorganic & Medicinal Chemistry Letters, 2014; 24: 3903-06.
31. Jha RK, Jha PK, Rana SVS, Guha SK. Spermicidal action of styrene maleic anhydride polyelectrolyte in combination with magnetic and electrically conductive particles. Inter J Pharm, 2009; 5(1): 1-12.