STUDIES ON ACUTE TOXICITY PROFILE AND UTERINE HISTOPATHOLOGICAL CHANGES AFTER UTERINE IMPLANTATION OF SMA-COATED AND NON-COATED COPPER T (CU-T) IN RATS

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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. Present study was conducted to assess the toxic effects after uterine implantation of SMA-coated Cu-T and non-coated Cu-T in female Charles Foster rats. Healthy adult female rats of were randomly divided into two groups (Grs.) consisting 10 animals each. Gr. I. rats were implanted with non-coated Cu-T in both uterine horns served as control. Rats in G.II were implanted with SMA-coated Cu-T 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, hematological parameters, viz. Hgb(g%), RBC(x10⁶/mm³), Hct(%), MCV(micron³), MCHC(g%), TLC(x10³/mm³), DLC(%) of Polymorphs, Lymphocytes, Macrophages, Eosinophils and Platelets (x10³/mm³), and biochemical analysis of marker enzymes, did not show any significant changes in SMA-coated Cu-T as compared non-coated Cu-T group of 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 uterus, ovary and fallopian tubes did not reveal any significant pathological changes as compared to controls. Results of the study show that uterine implantation of SMA-coated Cu-T and non-coated Cu-T did not reveal any adverse effects during 14 days toxicity study in rat. Thus, indicate that it is safe to use for further studies.

**KEYWORDS:** SMA-coated Copper T, Non-coated Copper T, Intra-uterine implantation, Toxicity profile, Rats.

**INTRODUCTION**

RISUG, an acronym for Reversible Inhibition of Sperm Under Guidance (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.[1] It has been expected to provide a valuable addition to the currently limited options of male contraception.[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.[12] An additional advantage of this technique is that it causes a partial blockage of the vas deferens with concomitant flow of functionally inactive cells.[8,9] 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.[15,16] 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.[17]

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 postfertilization effect cannot be excluded, in a majority of cases, intrauterine systems act as true contraceptives, preventing fertilization.[18] Levonorgestrel intrauterine system (LNG-IUS) applied to control atypical and non-atypical endometrial hyperplasia.[19] Intra-uterine infusion methods have been used to study implantational and decidual events during early pregnancy.[20] The IUDs although are contraceptive in a number of laboratory and domestic animals, the apparent mode of action varies widely. In rodents and primates, the presence of an IUD does not alter the sexual cycle to a significant degree and a major antifertility effect seems to be exerted between the time the embryo enters the uterus and the time of implantation.[21,22] In contrast, insertion of large diameter (uterus-distending) IUDs in sheep, cattle and guinea pigs results in an alteration of the estrous cycle by shortening the functional lifespan of the corpus luteum[23,24] and inhibit sperm transport and fertilization in ewes.[25,26] In our previous experiments intra-uterine administration of SMA-coated Cu T has been shown to cause female antifertility effects. The introduction of an intrauterine device into the uterine cavity induces a foreign body reaction in the surrounding endometrium which is characterized by the infiltration of polymorphonuclear leucocytes and macrophages into the endometrial stroma and subsequently through the surface epithelium. Leucocyte migration is greater with copper IUDs than with inert IUDs. Ulceration of the surface epithelium, haemorrhage of erythrocytes and microthrombosis of stomach capillaries occur in the functional endometrium in contact with inert and copper IUDs. In endometrium adjacent to, but not in contact with, the IUD gaps appear in the endothelial lining of small blood vessels without a haemostatic response. The most striking response in endometrium exposed to progesterone-releasing IUDs is the
occurrence of dilated, thin-walled vesicles, associated with a thinning of the surface epithelium and a decidual reaction in the stroma.\cite{27}

The present study was conducted to determine toxic effects of uterine implantation of SMA-coated Cu-T and non-coated Cu-T in female rats after a period of 14 days. The parameters studied will be the body and organ weights, food and water intake, haematological and biochemical parameters, histopathology of uterus, ovary and fallopian tube after intra-uterine implantation of SMA-coated Cu-T and non-coated Cu-T in rats.

**METHODS**

**Chemicals**

The test compound 10 SMA coated and 10 Plain Copper T were received from Prof. S.K. Guha, SMST, IIT, Kharagpur which surgically inserted in the uterus of rats. 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°C) 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 implanted with non-coated copper T in the lumen of uterine horns surgically, served as Control. Rats in Gr. II were subjected to bilateral implantation of SMA-coated Copper T under ether anesthesia. The abdomen was exposed by a single median incision surgically and SMA-coated copper T was inserted surgically into the lumen of 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. liver, lungs, uterus, ovary and fallopian tubes) 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. Formalin fixed uterine tissues from control and treated rats were dehydrated in graded series of ethanol, cleared in xylene and infiltrated and embedded in moulton paraffin wax (at 58°C). Tissue sections (5µM) were cut and stained with routine haematoxylin-eosin and micro photographed 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 (Non-coated Cu-T) and treated (SMA-coated Cu-T) groups were generally active and healthy throughout the period of the study. No mortality was seen in either control or treated group of rats. The animals from both the groups 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. Fur coat, nasal mucosa, eyes and conjunctivae remained normal. There was no redness or discharge from the mucosal membranes and body orifices.
Food and water consumption

Initial and final monitoring by measurement of the initial and leftover water and solid diet (pellets) in the containers revealed irregular variations in the average 24-hour water and food intake of animals in the control and treated groups. There was no evidence of any effect of treatment with the test material (Table 1).

### Table 1. Average food intake (gm/day/rat) and average water intake (ml/day/rat) after 14 days toxicity study of uterine implantation of non-coated Cu-T (Group I) and SMA-coated Cu-T (Group II) in rats (Mean ± S.D., n = 10 number of animals).

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Initial Food Intake</th>
<th>Final Food Intake</th>
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<tbody>
<tr>
<td>Group I</td>
<td>18.5±1.5</td>
<td>17.7±2.7</td>
</tr>
<tr>
<td>Group II</td>
<td>18.4±1.4</td>
<td>18.3±1.3</td>
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<tr>
<th>Group No.</th>
<th>Initial Water Intake</th>
<th>Final Water Intake</th>
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<tr>
<td>Group I</td>
<td>31.2±1.2</td>
<td>32.8±2.2</td>
</tr>
<tr>
<td>Group II</td>
<td>32.7±1.7</td>
<td>33.9±2.4</td>
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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 SMA-Coated Cu-T group as compared to non-coated Cu-T 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, creatinin, calcium and phosphate) and liver function (globulin, ALT, AST, ALP, T-Bill) in both the groups (Table 2).

### Table 2. Terminal serum biochemistry (Mean ± S.D.) after 14 days toxicity study of uterine implantation of non-coated Cu-T (Group I) and SMA-coated Cu-T (Group II) in female Rats.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>General Metabolic Functions</th>
<th>Liver Functions</th>
<th>Kidney Functions</th>
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<tbody>
<tr>
<td>Group I</td>
<td>GLU (mg/dl)</td>
<td>CTKH (mg/dl)</td>
<td>TG (mg/dl)</td>
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<tr>
<td>Mean</td>
<td>137.85</td>
<td>62.77</td>
<td>97.8</td>
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<tr>
<td>±S.D.</td>
<td>18.7</td>
<td>9.77</td>
<td>21.71</td>
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| Group II  | Mean     | 145.78       | 64.52      | 50.47    | 6.76      | 3.25    | 58.32     | 160.37    | 358.46   | 0.13     | 38.01     | 16.91      | 0.62      | 10.38      | 7.52      |
| ±S.D.     | 20.83    | 7.15         | 12.47      | 0.56     | 0.25      | 8.52    | 31.64     | 82.47     | 0.03     | 4.91      | 2.31       | 0.02      | 0.68       | 0.52      |
Haematology
There were no significant changes observed in any of the haematological parameters viz. Hgb(g%), RBC (x106/mm³), Hct(%), MCV(micron³), MCHC(g%), TLC(x10³/mm³), DLC(%) of polymorphs, lymphocytes, macrophages and eosinophils and platelets (x10³/mm³) of SMA-coated Cu-T group of rats as compared to non-coated Cu-T group of animals (Tables 3 and 4).

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 SMA-coated Cu-T rats and were comparable with non-coated Cu-T group (Tables 5 and 6).
Table 5. Initial urinalysis (day 0) before 14 days of uterine implantation of non-coated Cu-T (Group I) and SMA-coated Cu-T (Group II) in female Rats (Mean ± S.D.).

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<tr>
<td></td>
<td>Mean</td>
<td>Straw</td>
<td>1.0</td>
<td>7.4</td>
<td>14.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>1</td>
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<tr>
<td>±SD</td>
<td>Straw</td>
<td>0.0</td>
<td>0.7</td>
<td>13.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Group</td>
<td>Mean</td>
<td>Straw</td>
<td>1.0</td>
<td>7.5</td>
<td>16.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>1</td>
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<tr>
<td>±SD</td>
<td>Straw</td>
<td>0.0</td>
<td>0.7</td>
<td>12.8</td>
<td>0</td>
<td>0</td>
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Table 6. Final urinalysis (on day 15) after 14 days of uterine implantation of non-coated Cu-T (Group I) and SMA-coated Cu-T (Group II) in female Rats (Mean ± S.D.).

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<tr>
<td></td>
<td>Mean</td>
<td>Straw</td>
<td>1.0</td>
<td>6.9</td>
<td>15.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>1</td>
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<tr>
<td>±SD</td>
<td>Straw</td>
<td>0.0</td>
<td>0.7</td>
<td>12.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Group</td>
<td>Mean</td>
<td>Straw</td>
<td>1.0</td>
<td>6.9</td>
<td>18.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td>±SD</td>
<td>Straw</td>
<td>0.0</td>
<td>0.6</td>
<td>12.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Body and organ Weights

Mean values of body weights, absolute (g) and relative (g/100g b. wt.) weights of vital organs as well as reproductive organ weights (viz. liver, lungs, uterus, ovary and fallopian tubes) showed no significant variations and remained comparable in SMA-coated as compared to in non-coated Cu-T group (Tables 7-9).

Table 7. Body weights (g) after uterine implantation of non-coated Cu-T (Group I) and SMA-coated Cu-T (Group II) after 14 days in female rats (Mean ± S.D., n = 10 number of animals)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
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<tbody>
<tr>
<td>Group I</td>
<td>Mean 214.6</td>
<td>264.7</td>
</tr>
<tr>
<td></td>
<td>± S.D. 7.71</td>
<td>14.71</td>
</tr>
<tr>
<td>Group II</td>
<td>Mean 215.4</td>
<td>255.7</td>
</tr>
<tr>
<td></td>
<td>± S.D. 8.24</td>
<td>14.74</td>
</tr>
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The microscopic examination of the histological slides of the vital organs (viz. liver, lungs) did not reveal any pathological changes in SMA-coated as compared to non-coated Cu-T groups of rats.

### Histopathological Examination

The microscopic examination of the histological slides of the vital organs (viz. liver, lungs) did not reveal any pathological changes in SMA-coated as compared to non-coated Cu-T groups of rats.

### Effect of uterine implantation of non-coated and SMA-coated Cu-T on uterine, ovarian and Fallopian tube histology

In normal control rats, uterine endometrium showed large number of endometrial glands and stromal matrix with infiltration of leukocytes and blood vessels embedded. The uterine stroma represented few decidual-like cells, abandoned spindle shaped cells, leucocytes and blood vessels/venules. The uterine luminal epithelium was columnar, and showed epithelial peaks and luminal closer. (Figure 1 A, B). In rats implanted with non-coated Cu-T in uterus, showed increased infiltration of leucocytes in endometrial stroma and luminal epithelium as
well as in wide luminal space after a period of 14 days of post-implantation. The uterine luminal and glandular epithelium showed proliferation with increased epithelial cell height and luminal leucocytes infiltration (Figure1 C, D). In rats implanted with SMA-coated Cu-T in both uterine horns, showed normal uterine histoarchitecture, exhibiting number of endometrial glands embedded in endometrial stroma. The uterine luminal epithelium was low columnar, folded and lumen was wide with increased infiltration of leucocytes as compared to normal rats but was similar to non-coated Cu-T group of rats. Uterine stroma represented few decidual-like cells, abandoned spindle shaped stromal cells, leucocytes infiltration and blood vessels/venules similar to non-coated and normal control rats (Figure 1 E, F).

![Figure 1](image)

Figure 1: Cross sections of uterus in normal control rats (A, B) and in rats implanted with Non-coated Cu-T (C, D) and SMA-Coated Cu-T (E, F). Magnification- x100 (A, C, E), X 400(B), X200(D, F); H-E Staining.

**Ovarian histology**

In normal control rats, ovarian histology showed large number of C.L.s with luteal cells and leucocytes as well as attretic follicles (Figure 2A). In non-coated Cu-T group of rats, ovarian histology showed large number of attretic follicles and few C.L.s and oocytes (Figure 2B,C). In rats implanted with SMA-coated Cu-T, the ovarian histology showed large number of corpus luteum (C.L.s) with luteal cells and leucocytes as well as few attretic follicles (Figure 2 D-F) similar to controls.
Figure 2: Cross sections of ovary in normal rat (A), rats implanted with Non-coated Cu-T (B, C) and SMA-Coated Cu-T (D, E, F). Magnification- x100 (A, B, D, E), X200(C, F); H-E Staining.

Fallopian tube histology
In normal control rats, fallopian tube was normal in structure showing convoluted epithelium, luminal space and outer circular muscle layer (Figure 3A,B). In non-coated Cu-T group of rats, fallopian tube was normal in structure showing folded (convoluted) epithelium with wide lumen and outer circular muscle layer (Figure 3C,D). In SMA-coated Cu-T group of rats, frollipian tube was similar to normal control and non-coated Cu-T group of rats in structure showing convoluted epithelium, lumen and outer muscle layer (Figure 3E,F).

Figure 3: Cross sections of fallopian tube in normal rat (A,B) and after uterine implantation of Non-coated Cu-T (C,D) and SMA-Coated Cu-T (E,F). Magnification-x 100 (A, C, E), x 400(B, D, F); H-E Staining.
Microscopic examination revealed did not reveal any pathology. Also no irritation of SMA coated Copper T was recorded at the site of insertion and adjacent areas of the uterus except leucocytic infiltration in lumen in both the groups.

**DISCUSSION**

The intrauterine device (IUD) is a long-acting reversible contraceptive method with a favorable impact on reducing unwanted pregnancy. It is usually well tolerated, especially if it conforms to the uterine cavity.\textsuperscript{[28]} Expulsion may be related to a number of factors, including insertion technique and the relationship between the size of the IUD and that of the uterine cavity.\textsuperscript{[29]} An uncommon problem is that of uterine perforation, which is potentially serious and is variously reported as occurring in every 1,000–2,500 insertions or with even greater frequency.\textsuperscript{[30,31]} Results of the present study on acute toxicity profile in rats with uterine implantation of SMA-coated Cu-T 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 as compared to non-coated Cu-T group of rats. Further, the histoarchitecture of vital body organs did not show any pathological changes as compared to non-coated Cu T rats after 14 days of post-insertion period. Also, Present study did not show any histopathological changes in uterine endometrial stroma and epithelium in SMA-coated as compared to non-coated Cu-T group except increased leucocytic infiltration in both groups in lumen when compared with normal rats. Further, there were no pathological changes in ovarian and fallopian tube histology as well. All intrauterine devices (IUDs) that have been tested experimentally or clinically induce a local inflammatory reaction of the endometrium whose cellular and humoral components are expressed in the tissue and the fluid filling the uterine cavity. It has been reported that Levonorgestrel released from an IUD causes some systemic effects, but local effects such as glandular atrophy and stromal decidualization, in addition to the foreign body reaction, are dominant. Copper ions released from an IUD enhance the inflammatory response and reach concentrations in the luminal fluids of the genital tract that are toxic for spermatozoa. In the human, the entire genital tract appears affected due to luminal transmission of the noxa that accumulates in the uterine lumen. This affects the function and viability of gametes, decreasing the rate of fertilization and lowering the chances of survival of any embryo that may be formed, before it reaches the uterus.\textsuperscript{[32]} Our previous study have shown that RISUG (SMA-DMSO Complex) 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 as well as of degeneration of zona pelucida of ovum. Further, Follopian tube occlusion by RISUG also causes inhibition of implantation and degeneration of Zona pellucida layer of Eggs.

Previous studies have shown that chemical occlusion of the vas deferens by RISUG has been considered to be an ideal male contraceptive method where the polymer SMA can be used to block the lumen of the vas deferens and spermatozoa activity over an extended period of time. The male gamete, spermatozoa and its morphology play a significant role in fertilization process, especially the anterior part, acrosome which secretes 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/hyaluronidase inhibitors and spermicides leads to impairment of gamete interaction and fertilization of ova.\[9,33-35\] 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.\[36\] 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.\[3,5,37,41\]

In conclusion, results of the present study on 14 days toxicity study on gross behavior, food and water consumption, body and organ weights, haematology, biochemistry and histopathology indicate that the uterine implantation of SMA-Coated Cu-T is well tolerated similar to non-coated Cu-T and no irritation was seen, thus indicate that it is safe in rats.

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REFERENCES
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 -
Rama et al. World Journal of Pharmaceutical Research


