Research Article

Tartrazine Suppresses the Functions of Female Reproductive System by Inducing Structural Alterations and Functional Impairment of the Ovary and Uterus in Albino Rat

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ABSTRACT

Indiscriminate usage of the synthetic azo food dye tartrazine (TAZ) as a color additive has become an integral part of the present century. Humans get often exposed to this anthropogenic chemical through the consumption of TAZ-tainted foodstuffs, beverages and medicines, which draws attention to examine the effects of TAZ exposure on the female reproductive function. Therefore, the aim of the present study has been set to examine the probable effect of TAZ on the female reproductive system in albino rats. From the results, we observed that TAZ exerts positive cytotoxic effects on the cell line. We have also found significant alterations in the duration of each phase of the estrous cycle compared to the control group of rats along with prominent cytoarchitectural changes in vaginal smear component phases in a dose dependent manner. Further, the serum levels of FSH, LH and estradiol have also been significantly increased in TAZ-exposed groups of rats in a dose dependent manner. TAZ reconstructs the contractile movement of uterine visceral smooth muscle in each phase of the estrous cycle. Hence, it might be concluded that TAZ-induced impairment of the female reproductive functions probably involves the inhibition of the set point functions of the anterior pituitary ovarian axis.

Keywords: Tartrazine, cell viability, estrous cycle, serum hormones, smooth muscle, uterus, ovary.

1. INTRODUCTION

Numerous synthetic dyes have been in use since time immemorial in different industries especially paper, leather, foods, medicines, dyes, plastic, and textile materials to impart color either for necessity in its applicability or to increase consumer demands through visual appearance or both of these purposes[1]. In the modern era also, various anthropogenic azo dyes constitute the major proportion of synthetic colorants. Tartrazine (TAZ) is one of those anthropogenic azo dyes consisting of its characteristic -N:N-linkage (-N=N-), being vastly used for inherent bright orange or lemon-yellow colors. Its use is even reported in combination with the other colored chemical compounds to generate different color shades of yellow, pink, green, or blue[2-3]. The following two major factors contribute to the prevalence of the use of TAZ. Being the trisodium salt of tartrazine acid, TAZ gains its higher water solubility. Hence, making the applicability of the salt broad spectrum by using water as the solvent. Alongside, TAZ can maintain the coloration stability of the salt solution even at extreme physicochemical conditions including low pH environments and high-pressure situations, further increasing its preference as a dye[4]. Widespread use of TAZ as a food colorant in foodstuffs viz., bread, breakfast cereals, pasta, processed cheese, sauces, jam, jellies, cookies, cake mixes, cotton candy, ice cream; beverages including soft and sports drinks, fruit juices, and mustard oil has already been established. It is also used in cosmetic products and various pharmaceutical products such as medicine capsules, antacids, cough syrup, vitamins, throat lozenges, and certain prescription drugs[5]. The presence of TAZ has been detected in various food samples collected from many countries[6-7]. Joint FAO/WHO Expert committee on food additives and European Union (EU) scientific committee for food have standardized the acceptable daily intake (ADI) value for tartrazine to be 7.5 mg/kg body weight to restrict its unregulated usage because the high concentration of TAZ in foods causes adverse health effects on humans[8]. The use of TAZ has been banned in several countries like Austria, Germany and Norway[9]. However human beings are often exposed to TAZ probably in a daily basis through the consumption of TAZ-tainted foods and medicines[10]. Reports are present for TAZ exposure to cause asthma, urticaria, angioedema, atopic dermatitis, blurred vision, and migraine in adults; and hyperactivity in children[11].

Earlier studies have reported several hazardous effects of TAZ exposure on various physiological systems. Acute oral exposure to TAZ in rat has been reported to induce DNA damages in the glandular stomach, colon, and gastrointestinal organs at a low dose (100 mg/kg) [12-14]. Oral administration of TAZ on male Swiss albino rats has been reported to cause damage to spermatogenic cells associated with the absence of spermatozoa in the lumen; affect the structure of the cerebellum; alter the biochemical markers of brain tissue; cause the dilation of glomerular capillaries and collecting tubules in renal medulla that exhibited presence of flattened epithelial cells[15-19]. In intestinal smooth muscles of male albino rats, TAZ exposure at the dose of 10 mg/kg induced histopathological and structural damages[20]. In female

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Wistar rats, TAZ administration by oral gavage has been reported to decrease the fetal body weight and length, triggering fetal resorption and swelling, leading to the deaths of the fetus[21]. Though TAZ has been reported to induce adverse effects on several mammalian organ systems, till to date, there is no report available about the probable toxic effects of TAZ on mammalian female reproductive system.

Reproduction ensures the continuation of a species. In females, reproduction is guided and assisted by the rhythmic changes occurring in ovaries and uterus known as the ovarian cycle and endometrial cycle, respectively. These cycles control hormonal and morphological changes in the ovaries and uterus[22]. Any disturbances in the hormonal profile and/or structural degeneration of reproductive organs lead to impairment of reproductive function and may even lead to infertility[23]. The female reproductive system is already under the threat of various industrial chemicals. So, the present study has been designed to examine the probable adverse effects of TAZ on the functions of the female reproductive system including ovary and uterus, the two primary female reproductive organs, along with the functions of hypothalamo-hypophyseal-gonadal axis in female albino rats.

2. METHODS AND MATERIALS

2.1. Reagents and chemicals

All of the chemicals used for this study were of analytical grade. Tartrazine (Purity 85%, CAS No. 1934-21-0) was purchased from Sigma-Aldrich USA. All other reagents and chemicals were procured from Merck, India Pvt. Ltd. and Sisco Research Laboratory Pvt. Ltd. respectively. MTT was procured from Sigma-Aldrich (St. Louis, MO, USA). Streptomycin and penicillin were purchased from Thermo Fisher Scientific Pvt. Ltd.

2.2. Animals

Studies were carried out on adult virgin female Charles Foster strain of albino rats, weighing between 110-130 grams. In animal house, animals were nourished with standard laboratory chow and water *ad libitum* as per the guidelines of the Animal Ethics Committee of Kalyani University and kept in an equal light-dark cycle (12L:12D) at room temperature of 25°±2°C.

2.3. Experimental Design

After one week of acclimatization to the laboratory environment, the animals were divided into eight groups for *in vivo* study. The doses used in this study were based on the established LD_{50} value of TAZ in the rat model[24]. They were administered with different doses of TAZ according to the specifications mentioned in the Table 1. Oral administration was applied to the animals for exposure to TAZ. Two exposure durations of 15 day and 30 day were selected as the experimental duration. After the completion of the exposure duration of the study, the animals were sacrificed by cervical dislocation.

Table 1: Group division of animals for exposure to TAZ by oral gavage for the two exposure durations of 15 day and 30 day.

Name of the groups	Specifications of the groups	
	Dose of exposure to TAZ for 15 day	**Dose of exposure to TAZ for 30 day**
Control	Received distilled water (not exposed to TAZ).	Received distilled water (not exposed to TAZ).
TAZ-2	Received 0.8 gm TAZ/kg BW/day i.e., (2% of LD_{50}).	Received 0.8 gm TAZ/kg BW/day i.e., (2% of LD_{50}).
TAZ-4	Received 1.6 gm TAZ/kg BW/day i.e., (4% of LD_{50}).	Received 1.6 gm TAZ/kg BW/day i.e., (4% of LD_{50}).
TAZ-8	Received 3.2 gm TAZ/kg BW/day i.e., (8% of LD_{50}).	Received 3.2 gm TAZ/kg BW/day i.e., (8% of LD_{50}).

2.4. Cytotoxicity study of tartrazine (TAZ)

(3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) MTT based colorimetric assay was used to determine the effect of tartrazine on the cell viability using adherent murine macrophage cell line RAW 264.7. RAW 264.7 cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 µg/ml streptomycin and 100 U/ml penicillin. RAW 264.7 cells (1×10^5/well) in 96 well plates were exposed to various concentrations of tartrazine (0–16 µM) for 24 hours. Then 20 µl of MTT (5 mg/ml) was added to each well and incubated for an additional 4 hours. The supernatant from each well was removed and formazan crystals were dissolved with DMSO (100 µl/well). The optical density was determined with a microplate reader (Bio-Rad, USA) at 570 nm. The percentage of viable cells was calculated after considering the untreated control as having 100% viability.

2.5. Gonadosomatic Index

The TAZ-exposed groups of rats were weighed before the initiation of the exposure to TAZ and after the completion of the final exposure to TAZ prior to the sacrifice. Moreover, after the sacrifice, the ovaries of each group of rats were excised and weighed. Gonadosomatic index (GSI) was calculated by the following formula: GSI= weight of ovaries/body weight×100.

2.6. Collection of vaginal smears and microscopic observation

The vaginal fluid was collected for the study of estrous cycle physiology throughout the exposure durations of TAZ by using the pipette smear technique. Vaginal lavage was performed by using a pipette filled with isotonic saline (0.9 gm% NaCl). The study of the estrous cycle was carried out according to the method of Marcondes et al., (2002) with

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slight modification[25-26]. The smears were analysed under the bright-field microscope (100× magnification) and images were captured by a digital camera (SLR Olympus, E-620) fitted with the microscope (Olympus CH20i). The diestrus index was calculated by using the following formula: Diestrus index= (number of days with clear diestrus smear/total duration of treatment) ×100.

2.7. Serum hormonal study

After completion of the experimental period, the TAZ-exposed groups of rats and control group of rats were sacrificed and blood samples of sacrificed rats were collected by cardiac puncture technique. Then the acquired blood samples were centrifuged at 1500 rpm for 30 min at 4℃ using a cooling centrifuge (Remi Elektrotechnik Limited, Vasai, India). The serum obtained from the centrifugation of the blood samples was stored at -20℃. The serum hormonal level of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol was estimated by the method of chemiluminescence immunoassay (CLIA) using the kit of Lilac Medicare Pvt. Ltd, India, according to the manufacturer's instruction[27].

2.8. Tracing of uterine smooth muscle contraction

The uterine smooth muscle contraction of each TAZ-exposed groups and control group of rats was recorded according to the laboratory standard method[28-29]. After the completion of the exposure durations uterine samples were collected immediately from each rat by cervical dislocation. Isolation of uterus was performed by transverse incision after opening the abdominal cavity following the sacrifice of rat. In the 50 ml organ bath of Dale's apparatus containing Tyrode's solution, a segment of the uterus was placed longitudinally with the help of two metal hooks pierced through it. The Tyrode's solution consists of 8.0 g/L NaCl, 0.2 g/L KCl, 0.2 g/L $CaCl_2$, 0.1 g/L $MgCl_2$, 1.0 g/L $NaHCO_3$, 0.05 g/L NaH_2PO_4 and 1.0 g/L glucose (pH-7.4). The Dale's bath was maintained at a temperature of 37°±0.5℃ and was supplied with continuous bubbling of 95% O_2 and 0.5% CO_2. Recording of the uterine movement was continuously achieved with an isotonic transducer (IT-2245) apparatus coupled with RMS Polyrite D analysing software (RMS India) to study the effect of TAZ in uterine contraction at different phases of the estrous cycle.

2.9. Statistical Analysis

All the data obtained from this study were expressed as mean±standard error of the mean (SEM). The statistical analysis of the data obtained from the all the groups were done by Tukey's test of one-way analysis of variances (ANOVA), using the software Microcal origin version 7.0 for windows.

3. RESULTS AND DISCUSSION

3.1. Effect of TAZ on the cell viability in RAW 264.7 cells

We have examined the effects of TAZ on the viability of macrophage cell line (RAW 264.7) *in vitro* to understand the probable cytotoxic effect of TAZ. We have observed a significant decrease in the number of macrophages in RAW 264.7 macrophage cell line in a dose dependent manner as obtained from MTT based colorimetric assay after a period of 24 hours incubation with different concentrations of TAZ. Furthermore, at higher concentration levels, TAZ showed prominent cytotoxic effects on the quantity of the macrophage cells (Figure 1).

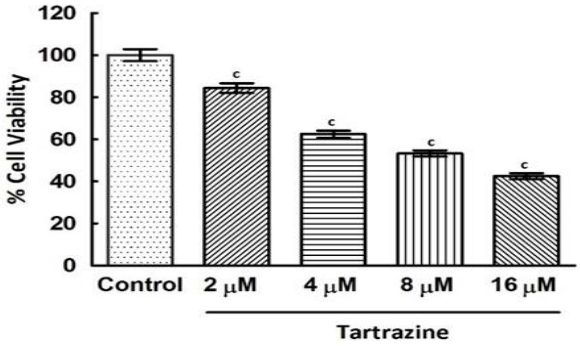

Figure 1: Graphical representation showing the percent changes in viability of RAW 264.7 macrophage cells in response to graded doses of tartrazine ranging from 2-16 μM for 24 hours. Data are represented as mean±SEM, $^{c}p < 0.001$ vs Control.

The MTT assay is used to measure cellular metabolic activity and serves as an indicator for cell viability, cytotoxicity, and cell proliferation (cytokines and nutrients). So, this result indicates that TAZ is probably a cytotoxic chemical. Therefore, TAZ might exert cytotoxic effects by altering the gene functions. Our result is consistent with a previous study reporting about the cytotoxic and genotoxic effects of TAZ on the human lymphocyte[30]. From the study it can be hypothesized that TAZ could exert toxic effects on tissues of primary reproductive organs.

3.2. Effect of TAZ on the gonadosomatic index

Gonadosomatic index (GSI) of the ovary of TAZ-exposed female rats has been studied to examine the probable role of TAZ on the growth and development of the ovary. We have observed a significant decrease in the GSI in TAZ-exposed groups of rats compared to the control group of rats in a dose dependent manner in all the exposure durations (Figure 2).

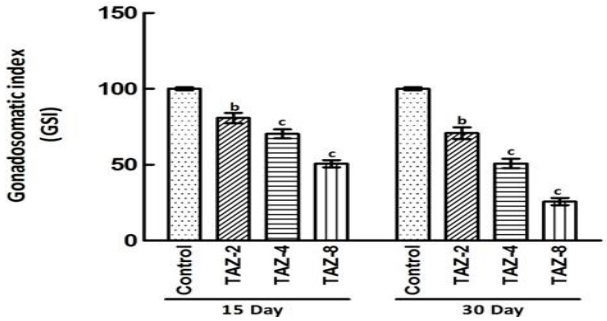

Figure 2: Graphical representation showing the gonadosomatic index (GSI) of TAZ exposed groups of rats for the duration of 15 day and 30 day. Data are

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represented as mean±SEM (n=6), $^{b}p < 0.01$ and $^{c}p < 0.001$ vs Control.

GSI is a tool for measuring the reproductive maturity of the ovary. In our study, the GSI of the ovary in TAZ-exposed groups of rats decreased significantly in comparison with the ovary of control group of rats. This result suggests that TAZ could impair female reproductive functions of the ovary probably by reducing the size and weight of the ovary.

3.3. Effect of TAZ on estrous cycle physiology

3.3.1. Effect of TAZ on the durations of estrous cycle

Estrous cycle physiology has been evaluated to know the probable effects of TAZ exposure on the functions of the ovary in different TAZ-exposed groups of rats by monitoring the durations of estrous cycle. We have observed a significant decrease in the duration of proestrus (Phase I), estrus (Phase II) and metestrus (Phase III) phases in TAZ-exposed groups of rats compared to the control group of rats in a dose dependent manner in both the TAZ exposure durations (i.e., 15 day and 30 day). We have also observed a significant increase in the duration of the diestrus phase (Phase IV) in each estrous cycle following the TAZ exposure. The results indicate a significant increase in the Diestrus index (Di) in TAZ-exposed groups of rats in a dose dependent manner compared to control group of rats in both the exposure durations (Table 2).

3.3.2. Effect of TAZ on the cellular changes of estrous cycle

The estrous cycle physiology has also been examined to analyse the effects of TAZ exposure on characteristics of cells present in vaginal smears of different groups of rats by visualizing cellular alterations of estrous cycle. We have observed a dose dependent decrease in the number of nucleated epithelial cells, cornified epithelial cells, leukocytes, and non-nucleated epithelial cells in proestrus, estrus and metestrus phases of the estrous cycle in TAZ-exposed groups of rats in comparison with control group of rats in both TAZ-exposed durations. Further, a dose dependent increase in the accumulation of leukocytes in diestrus phases of the estrous cycle has also been observed in TAZ-exposed groups of rats compared to the control group of rats in both exposure durations (Figure 3).

Table 2: Durations of the phases of estrous cycle and Diestrus index in control and TAZ-exposed groups of rats. Data represented as mean±SEM (n=6), $^{a}p < 0.05$ and $^{b}p < 0.01$, $^{c}p < 0.001$ vs Control.

Group	No. of cycle	Different phases of estrous cycle				
		Proestrus	Estrus	Metestrus	Diestrus	Diestrus index
15 day exposure duration						
Control	1.246±0.441	5.993±1.997	4.891±1.630	8.180±2.726	3.158±1.052	27.05
TAZ-2	1.309±0.463	3.752±1.25^{b}	3.952±1.31^{b}	4.924±1.641	4.155±1.385^{c}	39.92
TAZ-4	1.512±0.535	3.012±1.004	3.752±1.250^{c}	4.155±1.385^{a}	5.993±1.997^{c}	45.25
TAZ-8	1.604±0.567	3.158±1.052^{b}	3.058±1.019^{a}	2.859±0.953^{a}	6.497±2.165^{c}	48.23
30 day exposure duration						
Control	1.246±0.441	5.790±2.047	6.901±2.440	8.349±2.952	7.332±2.592^{b}	32.5
TAZ-2	1.852±0.655	5.212±1.843^{a}	6.261±2.213^{a}	7.498±2.651^{b}	9.405±3.325^{c}	42.2
TAZ-4	2.053±0.726	4.959±1.753^{b}	5.899±2.085^{b}	7.267±2.569^{a}	9.561±3.380^{b}	52.1
TAZ-8	4.276±1.512	4.250±1.109^{b}	5.304±1.875^{c}	6.034±2.133^{b}	13.614±4.813^{c}	64.12

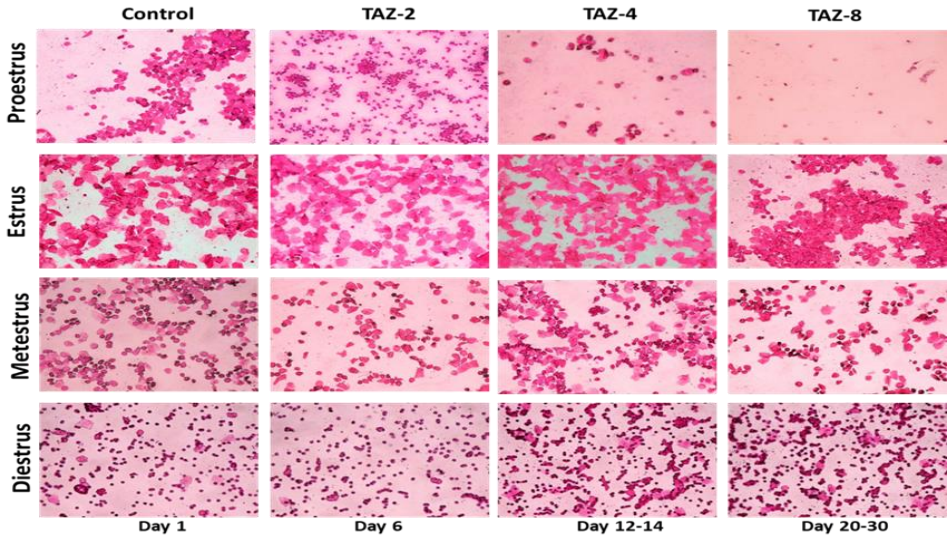

Figure 3: Photomicrographs (magnification 40×10) of hematoxylin and eosin-stained vaginal smears of TAZ-exposed groups and control group of rats showing the cellular characteristics of different phases of representative estrous cycle.

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In rodents, the reproductive cycle is known as estrous cycle that represents the cyclical pattern of ovarian activity. The estrous cycle consists of four phases i.e., proestrus, estrus, metestrus and diestrus phase. It takes about four to five days to complete an estrous cycle. In every month generally six complete cycles occur[31]. In our study, we have found a significant decrease in the durations of estrous cycle as well as decrease in the number of cells in proestrus, estrus and metestrus phases of estrous cycle in TAZ-exposed groups of rats for all doses tested. We have also found a significant increase in the duration of diestrus phase associated with an increase in the number of accumulated leucocytes in diestrus index of TAZ-exposed groups of rats for all doses tested. An augmentation of gonadotropin-releasing hormone has previously been reported to alter estrous cycle physiology[32]. From our results it is speculated that TAZ alters the estrous cycle physiology probably by modulating the endocrine functions of the anterior pituitary ovarian axis. To find out how TAZ exerts an impact on the endocrine functions of the anterior pituitary ovarian axis, the serum hormonal levels of FSH, LH and estradiol have been studied in both control and TAZ-exposed groups of rats.

3.4. Effect of TAZ on the level of serum FSH, LH and estradiol

The serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol have been determined in TAZ-exposed and control group of rats to examine the involvement of TAZ in FSH, LH and estradiol mediated regulation of estrous cycle. We have found significant increase in the levels of serum FSH, LH and estradiol in TAZ-exposed groups of rats in comparison with control group of rats in a dose dependent manner in both the durations. (Figure 4).

The FSH is one of the gonadotropin hormones released from the gonadotrophic cells of anterior pituitary. FSH plays an important role to stimulate the recruitment and growth of developing ovarian follicle. Another gonadotropin hormone, LH, is released from arcuate and preoptic area of nucleus in anterior pituitary that triggers the ovulation, ovarian function and promotes the secretion of estradiol from ovaries and progesterone from theca cells. The hormone estradiol released by the ovaries, plays an important role in the development and growth of Graafian follicle. Estradiol also exerts a double feedback control on gonadotropin releasing hormone (GnRH). The gonadotropins FSH and LH, modulate the ovarian and uterine changes by acting directly and/or indirectly via ovarian hormones[33]. In our study, we have found a significant increase in the levels of LH, FSH and estradiol in TAZ-exposed groups of rats for all doses tested. These results indicate that TAZ probably impairs the function of pituitary gonadal ovarian axis by augmenting the secretion of FSH, LH from anterior pituitary and estradiol from ovary. The increased level of gonadal hormones indicates that TAZ acts on glutamatergic neurons, which is responsible for release and synthesis of follicle stimulating hormone releasing hormone (FSHRH) and luteinizing hormone releasing hormone (LHRH) (also known as GnRH) that increases the secretion of FSH and LH respectively from anterior pituitary thereby augmenting the secretion of estradiol from ovary through positive feedback mechanism.

3.5. Effect of TAZ on the contraction of uterine smooth muscle during different phases of the estrous cycle of rats

To examine the probable role of TAZ on the contraction of uterine smooth muscle, the contractions of uterus of TAZ-exposed groups of rats and control group of rats have been studied. In our study, we did not observe any significant changes in amplitude and frequency of contraction of uterus in proestrus phase of estrous cycle in TAZ-exposed groups of rats compared to control group of rats. Further, we have observed a significant increase in amplitude of contraction along with a significant decrease in frequency of contraction of uterus of TAZ-exposed groups of rats in estrus, metestrus and diestrus phases of estrous cycle compared to control group of rats (Figure 5A-D).

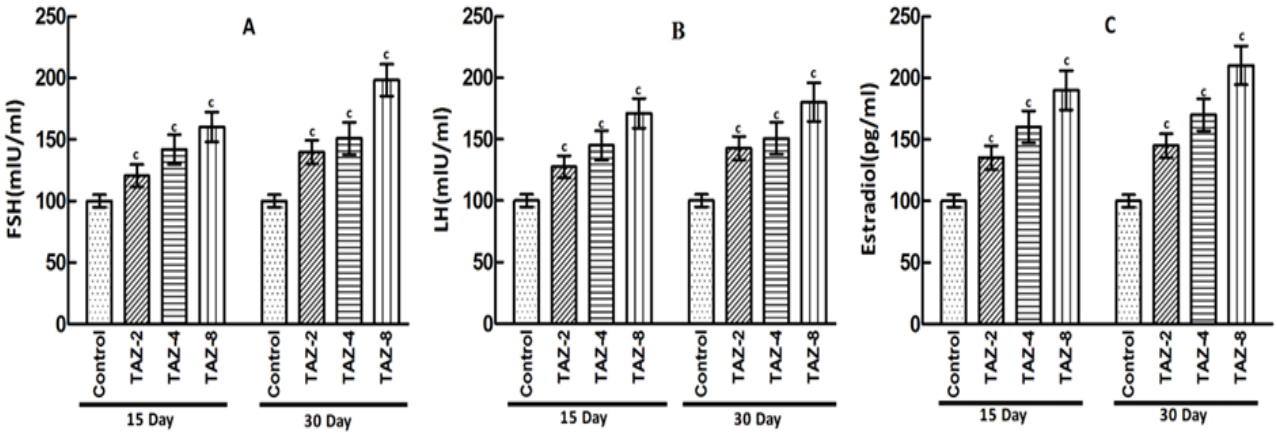

Figure 4: Graphical representations showing the increase in the levels of follicle stimulating hormone (A), luteinizing hormone (B) and estradiol (C) in TAZ- exposed groups of rats compared with serum levels of FSH, LH and estradiol of control groups of rats. Values are represented as mean±SEM (n=6), $^{c}p < 0.001$ vs Control.

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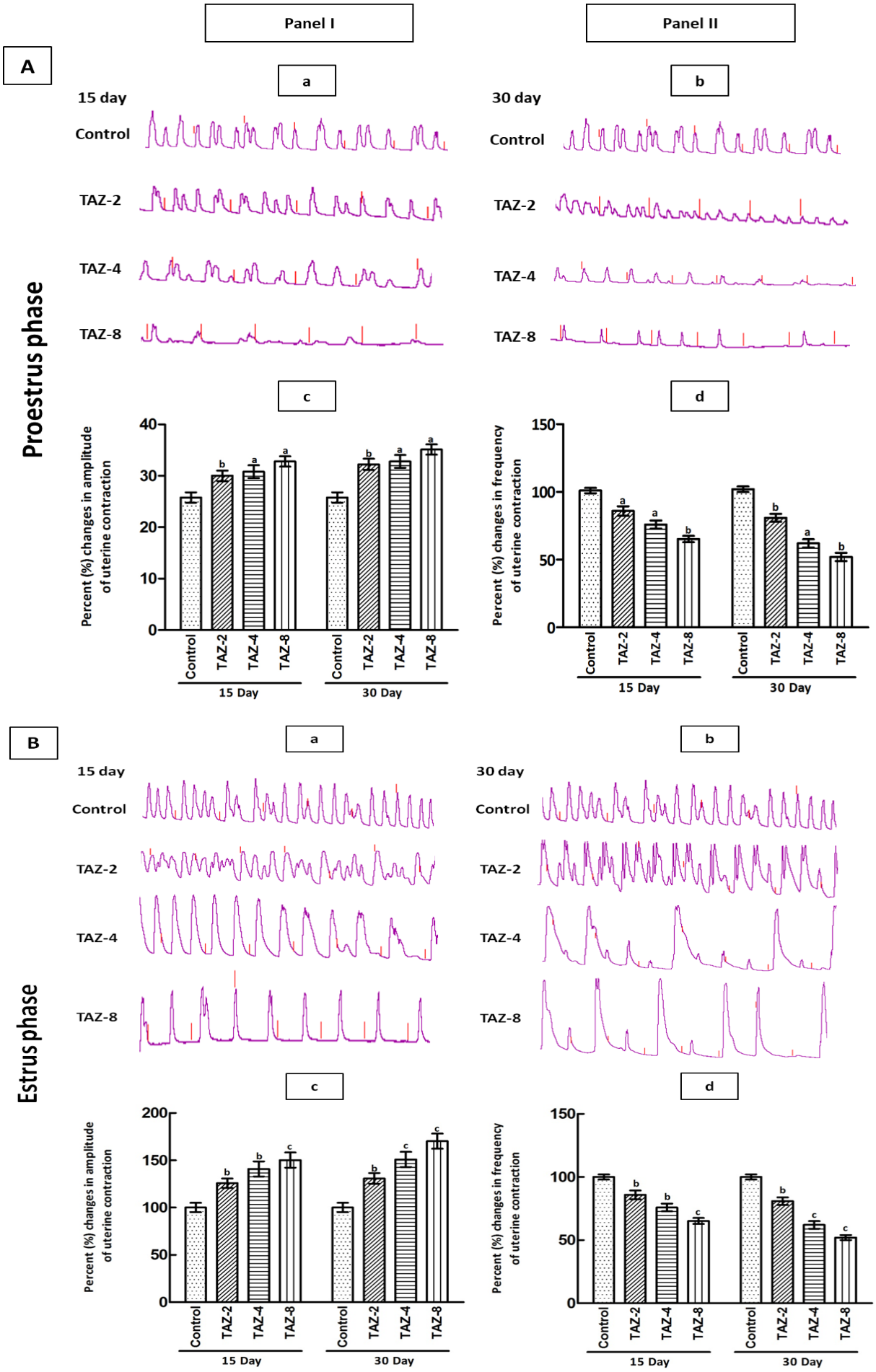

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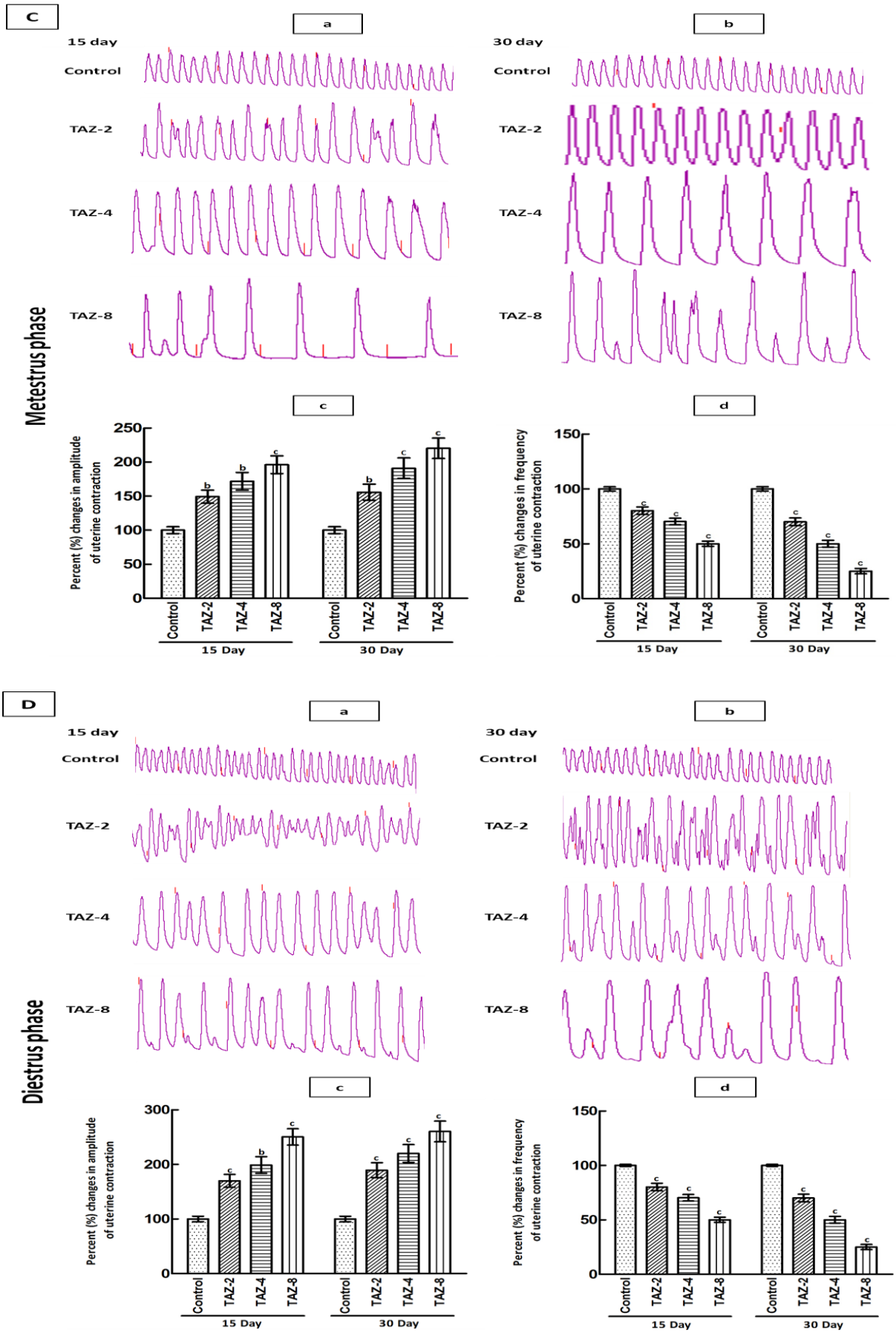

Figure 5: Representative records showing the contractions of the uterus *ex vivo* in TAZ-exposed groups of rats in the proestrus (5A), estrus (5B), metestrus (5C), and diestrus (5D) phases of the estrous cycles. In panel I-subset (a) represents the contractile responses of uterus in TAZ-exposed groups of rats in each phase of estrous cycle for 15 day exposure

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duration in response to three different doses. In panel II-subset (b) represents the contractile responses of uterus in TAZ-exposed groups of rats in each phase of estrous cycle for 30 day exposure duration in response to three different doses. In panel I-subset (c) represents the amplitude of the uterine smooth muscle contraction in TAZ-exposed groups of rats for 15 day and 30 day exposure duration in response to three different doses. In panel II-subset (d) represents the frequency of the uterine smooth muscle contraction in TAZ-exposed groups of rats for 15 day and 30 day exposure duration in response to three different doses. Values are represented as mean±SEM (n=6), $^{a}p < 0.05$ and $^{b}p < 0.01$, $^{c}p < 0.001$ vs Control.

The uterus is one of the primary female reproductive organs in rat. It facilitates fertilization and provides the space for implantation and growth of the embryo. Besides, uterine contraction plays a major role in delivering the developed fetus and uterus helps in the process of parturition. The contraction of the uterus is provided by the contraction of single unit smooth muscles found in the wall-structure of uterus. The contraction of uterus is controlled by intrinsic nerve plexus and hormone and/or neurotransmitters secreted from the endocrine glands and/or intrinsic nerve endings. In our study, we have found significant alterations in amplitude and frequency in TAZ-exposed groups of rats in both the exposure durations for all doses tested in all phases of the estrous cycle except the proestrus phase. In proestrus phase, we did not find any significant change in force and frequency of contraction in uterine smooth muscle. As TAZ potentiates the force of contraction by promoting the secretion of oxytocin from hypothalamic neurons in posterior pituitary, we could hypothesize that TAZ probably does not influence the secretion of oxytocin from hypothalamic neuron of posterior pituitary in proestrus phase. However, in estrus, metestrus and diestrus phases, we have found a significant increase in the amplitude and a significant decrease in the frequency of contraction of the uterus in both the TAZ-exposed groups of rats for all doses tested. These results indicate that TAZ probably impairs the function of uterus by potentiating the force of contraction of uterine smooth muscle and decreasing the frequency of contraction of uterine smooth muscle. The hormone oxytocin released from the magnocellular hypothalamic neurons in posterior pituitary promotes or potentiates the force and frequency of contraction of uterine smooth muscle[34-35]. Hence, from the results, it could be hypothesized that TAZ probably stimulates the secretion of oxytocin from hypothalamic neurons of posterior pituitary. The decrease in frequency of contraction in TAZ-exposed smooth muscle might be due to depression of TAZ-mediated inhibition of slow wave rhythmicity of intrinsic pacemaker cells found in unitary uterine smooth muscles. Alternatively, TAZ probably promotes the force of contraction of uterine smooth muscle by inducing the estradiol mediated release of oxytocin from hypothalamic neurons in posterior pituitary.

From all these results it can be concluded that TAZ suppresses the female reproductive functions through impairing the functions of the ovary by promoting cytotoxicity; following the augmentation of release of FSH and LH from anterior pituitary, and estradiol from the ovary. Moreover, TAZ also alters uterine contraction by impairing the contractile functions of uterine visceral smooth muscles. So, this study might help to give a clear account of the effect of TAZ on the female reproductive system. The probable mechanisms of alteration in the reproductive functions by TAZ have been illustrated in Figure 6.

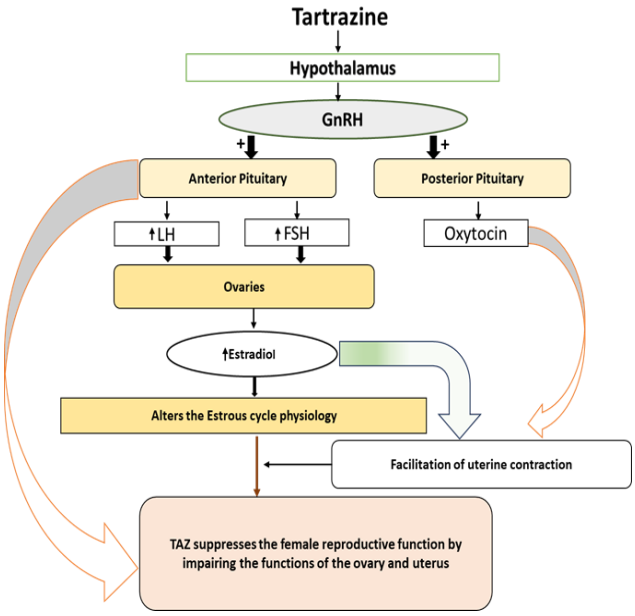

Figure 6: Schematic representation showing the probable mechanism of action of TAZ induced impairment of the female reproductive function in albino rat. GnRH= Gonadotropin hormone-releasing hormone; FSH= Follicle stimulating hormone; LH=Luteinizing hormone; (+) = indicates stimulated; (↑) = indicates increased.

4. CONCLUSION

Tartrazine produces cytotoxicity that leads to the impairment of several functions of the female reproductive organs. It induces the alteration of the estrous cycle physiology by promoting the release of FSH, LH and estradiol hormones along with the reduction of the size and weight of the ovaries. TAZ also impairs the contractile function of the uterus through increasing the force of contraction and decreasing the frequency of contraction of uterine visceral smooth muscle probably by modulating the endocrine set point function of the gonadal pituitary ovarian axis.

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