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Short Communication

Teratogenesis Induced by Trimethoprim Sulfamethoxazole in Mice

Madeeha Arshad[1], Asmatullah[1], Chaman Ara[1], Shagufta Andleeb[2] and Naveed Ahmad[3,*]

[1]*Developmental Biology Lab, Department of Zoology, University of the Punjab, Lahore*
[2]*Department of Zoology, University of Education, Lahore*
[3]*Department of Environmental Sciences, COMSATS Institute of Information and Technology, Vehari Campus, Vehari*

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MA performed experimental work. Asmatullah planned the research. CA and SA did statistical analysis. NA edited the manuscript.

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ABSTRACT

Antibiotic therapy is the most common for treating the UTIs during gestation, Trimethoprim Sulfamethoxazole (TMP-SMX) is one of antifolate used in such therapies. Current study was done to test the ability of TMP-SMX to induce developmental defects in mice fetuses. Different concentrations of the drug, 0.00, 4.10, 8.33 and 16.66 µg/g B.W. were administered orally to the dams on days 6-12 of gestation, and fetuses were recovered on day 18. Morphological observations of fetuses from 4.10, 8.33 and 16.66 µg/g B.W. dose groups showed abnormalities such as skin hemorrhages, microphthalmia, limb deformities, hygromas, kyphosis, curved and short tail and fluid filled abdominal cysts. Significant increase in intrauterine growth retardation and resorptions of fetuses, with increasing dose concentration, was also observed. Morphometric observations of fetal body parts like head circumference, eye circumference, forelimb and hindlimb size and tail length demonstrated a significant ($P<0.005$) decrease with increasing dose concentrations in comparison with control group. The fetal body weight and CR length reduced significantly ($P<0.005$) in all dose group. Trimethoprim Sulfamethoxazole has significant potential to cause congenital defects in developing embryo.

Since the invention of antibiotics, these are most commonly prescribed drugs to treat infections all over the world. These drugs are given during gestation period as frequently as in routine manner, can easily cross the placenta and as a result fetus exposure becomes undoubted. Antibiotics administered during gestation should be under highly necessitated conditions (Korzeniowski, 1995).

Urinary tract infections (UTIs) are the most common infections during pregnancy (Mittal and Wing, 2005). It is observed that one out of three pregnant women undergo UTIs during gestation period. Antibiotic therapy is the most common therapy for treating the UTIs. But this can lead to some damages to the mother as well as the fetus (Gilstrap and Ramin, 2001). Thus the safety of the drug should be assessed before giving it (Duarte *et al*., 2008). Among the most commonly used antibiotics to treat UTIs is Trimethoprim Sulfamethoxazole. It is abbreviated as (TMP-SMX) or co-trimoxazole, having common name as Septra DS. It is categorized as C category drug by the food and drug administration department (FDA) (Murase *et al*., 2014). Experimental data especially from the stand point of view of teratology is not well established. So, it is unavoidable to check its embryo toxic potential (Mahadevan, 2007). TMP/SMX is a choice of antibiotic therapy from the last 30 years as it can be effective for both gram negative and gram positive bacteria. It is a multifunctional drug that can perform fungicidal action or also has the ability to kill bacteria (Master *et al*., 2003). TMP-SMX is used during pregnancy when the benefits of this drug take over the risks factor induced by this drug (Forna *et al*., 2006). Sulfamethoxazole, is a dye derivative that has the ability to kill the bacterial agents. But this is a folic acid inhibitor that can lead to many defects. By combining Trimethoprim; a diaminopyrimidine, enhances the killing ability of pathogens by TMP-SMX. It is cheap and easily available commercially but excessive misuse of this drug provide the bacteria to develop resistance against this drug (Libecco and Powel, 2004). Route of administration, drug absorption, its distribution among different tissues, its metabolism and how it is excreted out of the body plays very important role. Trimethoprim sulfamethoxazole can cross the placenta so it can harm the neonates as it is a folic acid antagonist (Sipos *et al*., 2011). It can also disturb the metabolism of mother that leads to neural tube anomalies (Wen *et al*., 2008).

The evolution of TMP-SMX as a broad spectrum

* Corresponding author: naveedahmad@ciitvehari.edu.pk
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antibiotic has been exciting and conventional to current standards. It is a drug that has sold itself to a large extent, but certainly needs a careful evaluation by clinical investigators and researchers for definition of its proper role.

Materials and methods

Swiss albino mice (*Mus musculus*) were used to conduct the present studies. The animals were placed in steel cages under highly controlled conditions of temperature (27±2°C), humidity (40-45%) and light dark (12h) cycles. A total number of 20 females were used during this experiment which were about 7-9 weeks old having body weight of about 30±2g, 05 females for each dose group and 05 for control. All the protocols of animal husbandry were followed approved by the medical ethics committee of Punjab University, Lahore. For experiment, first objective was to mate male and female under controlled conditions so that exact conception time and day 0 of gestation can be determined. Males and females were kept together for at least 5-7 days before mating would occur, so that estrous cycle of females is regulated. Usually two estrous females were kept with one male in cages. Next morning, these females were examined for presence of vaginal plug, which was considered as confirmation of fertile mating. These females were isolated in separate cages and marked as day "0" of gestation. Trimethoprim sulfamethoxazole (TMP-SMX) is available in the form of tablet with the trade name as "Septra D.S" (GlaxoSmithKline Pakistan). Different dose groups were managed as control, S-I, S-II, and S-III by dissolving TMP-SMX in distilled water in such a way that 0.1ml of dose administered to the mice contained 0.00 µg/g B.W. for control, 4.10 µg/g B.W. for S-I, 8.33 µg/g B.W. for S-II, and 16.66µg/g B.W. for S-III dose groups.

Dams were exposed to TMP-SMX with single dose, daily from 6th-12th day of gestation. The treated mice were kept singly in different cages till day 18 of gestation. On 18th day of gestation, dams were weighed and then anaesthetized with ether. Uteri of dams were dissected out and were placed in 0.9% saline solution after weighing. After counting the implantation sites, the fetuses were recovered from the uteri, fixed in Bouin's fixative for 48 h and then preserved in 70% alcohol for morphological and morphometric studies (Arshad *et al*., 2017). Morphological and morphometric studies involved wet weights as well as crown-rump (CR) length measurements of each fetus. Similarly, head circumferences, eye circumferences, forelimb lengths, hindlimb lengths and tail lengths were also subjected to morphometric analyses. The average per litter values were obtained for every parameter in each dose group separately. These values were statistically analyzed by ANOVA using IBM-SPSS 16.

Table I.- Developmental defects produced in 18-days old mice fetuses by TMP/SMX, administered orally to pregnant mice on days 6-12 of gestation.

Dose group TMP-SMX (µg/g BW)	No of fetuses recovered (n)	Malformed fetuses (%)	Resorbed fetuses (%)
Control	20	0.00	0.00
4.1	22	54.54	0.00
8.3	27	70.37	7.40
16.66	24	54.16	44.83

Results and discussion

Morphological observations showed control group as normal in size and form (ears, eyes, limbs and skeleton). There was no resorption observed in control group (Table I). In S-I (4.1µg/g B.W.), as compared to control, the morphological defects *i.e.* distorted axis, hyperextensions of hind and fore limbs, hemorrhages, and hygromas were observed. The percentage of malformed fetuses in this group was 54.54 with no resorbed fetus (Table I). S-II (8.33µg/g B.W.) showed defects like abdominal cysts, extensions of limbs, low set arms, limb displacements, hemorrhages and hygromas. The malformed and resorbed fetuses percentages were 70.37 and 7.40, respectively (Table I). Defects observed in S-III (16.66µg/g B.W.) were *i.e.* kyphosis, distorted axis, micromelia, extenions of fore and hind limbs, microcephaly, micro-ophthalmia and intrauterine growth retardation. The percentage of malformations in S-III was 54.16 and of resorptions was 44.99 (Table I).

These results are supported by Crider *et al*. (2009) study, who studied limb defects occurred in 87 out of 537 pregnancies implants by using this drug. Similarly micro-ophthalmia and neural tube defects were also observed. Among all defects, limb deficiencies were the highest ones in our studies. This is further supported by the findings of Czeizel *et al*. (2001), that out of 66 pregnancies, there were 10 with neural tube defects and 2 were having limb defects along with other defects.

Morphometric studies showed that there is significant ($p < 0.05$) decrease in the head circumference, eye circumference, fore limb and hind limb sizes, tail sizes, and fetal weights in all treatment groups when compared with the control groups (Table II).

The current morphometric analysis showed that Septra D.S has effects on the fetal growth as our results show the fetuses obtained from the pregnant mice treated with Septra D.S were significantly different from controls in their body weight, crown rump length and other morphometric parameters. These findings are

Table II.- Effects of TMP-SMX on development of 18-days old fetuses recovered from pregnant mice, administered orally with different concentrations on 6-12 days of gestation.

Dose groups	Body weight (mg) (n=5)	C.R length (mm) (n=5)	Head size (mm^2) (n=5)	Eye size (mm^2) (n=5)	Forelimb size (mm) (n=5)	Hindlimb size (mm) (n=5)	Tail length (mm) (n=5)
Control	1381.42±24.36	21.78±0.85	38.35±0.69	3.54±0.16	6.50±0.28	7.29±0.19	8.88±0.46
S-I	607.10±111.19***	13.48±0.61*	17.84±1.07***	2.68±0.15*	4.25±0.20*	5.32±0.19*	6.96±0.27*
S-II	578.78±62.49***	12.61±0.47**	17.25±1.2***	2.62±0.23*	4.21±0.24*	5.27±0.32*	6.26±0.37**
S-III	495.49±98.60***	10.42±3.20**	13.06±3.6***	1.86±0.53***	3.87±1.04**	4.45±1.23**	4.44±1.42**

Mean±SEM; Significance: ***, P < 0.001; **, P < 0.01; *, P < 0.05. S-I, 4.1µg/g BW; S-II, 8.3µg/g BW; S-III, 16.66µg/g BW.

in accordance with the findings of Wen *et al*. (2008) and Hernandez-Diaz *et al*. (2001). They studied the teratogenic effects in relation to folic acid antagonist's administration. There are many resorbtions observed during the present study which are also observed by Matok *et al*. (2009), who observed abortions in 44 pregnancies and termination of pregnancies due to the use of these folic acid antagonists during the first trimester of pregnancy. Their findings also showed that there are also greater risks of urinary tract and cardiovascular abnormalities induced by Septra D.S.

Hemorrhages on different body parts such as brain, abdomen, foot and sacral regions were also induced by Septra D.S in the fetuses of treated mothers. These observations are similar to Dickerman (1984) that Septran D.S has been implicated as one of the agents responsible for hemorrhagic disease of newborn due to glucose 6 phosphate deficiency. It binds to billirubin in the plasma resulting in coagulation of blood, consequently hemorrhagic spots occurred.

Conclusion

The present study has clearly shown that oral administration of TMP-SMX 4.10, 8.33 and 16.66 µg/g B.W. of mice has potential to induce developmental defects in mice embryos particularly if such exposure occurs at the beginning of organogenesis. Therefore, it is strongly recommended that Septran D.S should be used under highly necessitated conditions in human beings; especially its use should be checked during pregnancy. If its use is unavoidable then folic acid should be used as therapeutic agent.

Statement of conflict of interest

Authors have declared no conflict of interest.

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