Evaluation of developmental toxicity of guaifenesin using pregnant female rats

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ABSTRACT

Objectives: Guaifenesin possesses expectorant, muscle relaxant, and anticonvulsive properties. To the best of our knowledge, the promising data regarding the developmental toxicity of guaifenesin are scarce. The current study investigates the developmental toxic effects of guaifenesin in detail using female rats.

Materials and Methods: Twenty-five dams were divided into five groups. Group 1 served as a control, while Group-2, -3, -4, and -5 were administered with 250, 350, 500, and 600 (mg/kg b.w.) doses of guaifenesin, respectively, starting from gestation day 6 to day 17. Half of the total recovered fetuses was subjected to morphologic and morphometric analysis, while other half was subjected to skeletal examination.

Results: A significant reduction in maternal weight, and food/water intake, was observed, however, no mortality and morbidity were observed. About 14 dead fetuses were found in Group-3 and -4 each, while 26 in Group 5. Morphological analysis revealed 21.2%, 45.4%, 67.2%, and 86.9% of total fetuses having hemorrhagic spots in Group-2, -3, -4, and -5, respectively. Dropping wrist/ankle and kinky tail were found in Group-4 and -5 only. Morphometric analysis showed a significant decline in fetal weight, full body length, skull length, forelimb length, hindlimb length, and tail length in all guaifenesin treated groups. Skeletal examination displayed that only Group 5 fetuses had increased intercostal space between 7th and 8th rib. We also observed improper development of carpals, metacarpals, tarsals, and metatarsals of the Group 5 fetuses.

Conclusion: Guaifenesin showed a significant developmental toxicity at selected test doses; therefore, a careful use is suggested during pregnancy.

KEY WORDS: Developmental toxicity, embryotoxicity, fetotoxicity, guaifenesin, teratogen, toxicology

Introduction

Teratology is associated with inborn developmental and congenital abnormalities caused by xenobiotics or physical agents and is now becoming the area of interest for medical research, which seeks to minimize preventable birth defects. A large number of people in the world are affected by birth defects. Around 7–10% children in the world need medical care for the diagnosis and treatment of birth defects, affecting the quality of lives of millions of people globally. During pregnancy, nearly all the drugs are capable of crossing the placental barrier and entering in the fetus circulation, resulting in deleterious effects to the newborn until proved otherwise.[10] In addition to drugs, a variety of other agents such as X-rays, radioactive isotopes, metals, and inorganic elements can alter the embryo maturation in both human and animals resulting in numerous inborn defects.

Several classes of drugs including expectorants, decongestants, and antihistamines have been utilized by the
Dextromethorphan is a nasal decongestant drug known to cause gastoschisis alone and in combination with acetaminophen. Pseudoephedrine is recognized as the safest antitussive during pregnancy. However, a previous study suggested to avoid the use of dextromethorphan during pregnancy due to the risk of fetal abnormalities associated with it. Guaifenesin is classified as an expectorant and is being used in several cough suppressant medications. It also possesses anticonvulsive and muscle relaxant activities and is used in the treatment of fibromyalgia. Briggs et al. reported an association between increase in the expected cases of inguinal hernia and increase in the use of guaifenesin by pregnant females during the 1st trimester. Another study reported that the evidence in support of an association between congenital abnormalities and guaifenesin was weak; however, the authors suggested to use the drug only if the favorable effects to the mother surpass the risk to the fetus. To the best of our knowledge, promising data regarding the developmental toxicity of guaifenesin are scarce and therefore, in the current study, we intended to evaluate the developmental toxic effects of guaifenesin in detail using pregnant female rats.

**Materials and Methods**

**Animals**

Twenty-five primiparous female pregnant rats and eight sexually mature male Sprague-Dawley rats, all aging 6–8 weeks, were used for evaluation of developmental toxicity of guaifenesin. All the animals were kept in the animal house of Faculty of Pharmacy, The University of Lahore, and standard environmental conditions (Temperature: 22 ± 3°C and humidity: 50% ± 10%) were ensured. Rats were subjected to 12 h artificial light and dark cycles. They were given free access to clean filtered water and fed with commercially prepared standard diet pellets. All the experiments were approved by the Institutional Animal Ethics Committee, The University of Lahore (Ref. # IAEC.2015.04).

**Determination of Estrous Phase**

Vaginal smear analysis test was used in order to confirm the estrous phase. A pipette filled with 10 μl of normal saline was gently and not deeply inserted into the rat vagina. Vaginal fluid was poured onto the glass slide and examined under the microscope. The estrous phase was confirmed by the dominance of anucleated cornified cells under microscopic examination.

**Confirmation of Conception and Gestational Day 0**

After the estrous phase confirmation, estrous females were kept with male rats in a separate cage for mating. 12 h light/dark cycle was maintained and in the next morning, pregnancy was confirmed via the presence of vaginal plug. The presence of vaginal plug was taken as an indication of successful mating and considered as day 0 of the gestation.

**Experimental Design**

All the rats were individually weighed and randomly divided into five groups. Each group contained five pregnant female rats. We selected four doses of guaifenesin, i.e., 250 mg/kg (low dose), 350 mg/kg (medium dose), 500 mg/kg (high dose), and 600 mg/kg (toxic dose). Doses used for human oral conventional cough preparation were converted to rat equivalent doses according to a method used in previously published literature. Group 1 was denoted as control group containing healthy pregnant rats and was administered with distilled water only. Group-2, -3, -4, and -5 were assigned to low, medium, high, and toxic doses, respectively. Guaifenesin was administered per oral and once daily to all the groups. The administration of guaifenesin was started at gestational day 6 and ended at gestational day 17.

**Observation of in Life Parameters**

Each of the pregnant rats was examined for different parameters such as maternal weight gain/loss, food and water intake, clinical conditions, and morbidity or mortality.

**Recovery and Processing of Fetus**

On day 20, all the dams were individually weighed and anesthetized using ether. The euthanization was performed by cervical dislocation. Cesarean section was executed, uterus horns were removed, and placed in normal saline. All the fetuses were removed and placentas were cut down from fetuses. Number of fetuses was counted and various parameters were noticed, such as fetal weight, number of implantation, number of live/dead fetuses, early and late resorptions. Half of the fetuses were preserved in 70% alcohol for morphological studies, while others were subjected for skeletal examination.

**Morphological Examination of Fetuses**

The preserved fetuses were analyzed for morphological changes. Various fetal parameters were examined, such as distorted axis, eye defects, forelimb and hindlimb defects, abdominal distention, tail defects, and hemorrhagic spots were examined. Any other signs of anomalies or malformation were also examined.

**Morphometric Examination of Fetuses**

Morphometric changes were also determined in all groups. Various fetal parameters, such as fetal weight, fetal length, tail length, forelimb length, hindlimb length, and skull lengths were examined.

**Staining and Skeletal Examination of Fetuses**

The other half of the total fetuses was subjected to staining in order to investigate any sign of skeletal deformities. For this purpose, skin of each fetus was gently peeled off and viscera were removed. The skeleton was then subjected to staining. For staining, all the fetuses were given a bath for 24 h with 1% KOH solution. The KOH solution was drained off after 24 h and replaced with 0.005% Alizarin Red Solution. The solution was also drained off when all the bones were adequately stained. The stained fetuses were preserved in pure glycerin and were examined for any possible signs of skeletal deformation.

**Statistical Analysis**

Data were statistically analyzed using one-way analysis of variance followed by Tukey’s test, GraphPad Prism version 6 software was used for statistical analysis and $P < 0.05$ was considered as statistically significant.

**Results**

**Treatment with Guaifenesin Significantly Inhibited the Maternal Weight, Growth, and Food and Water Intake**

Increase in the maternal weights was found in all groups; however, treatment with guaifenesin significantly inhibited the maternal weight growth as compared with control group [Figure 1d]. In general, reduced food and water intake were
also observed in treatment groups as compared with control group. No signs of morbidity and mortality were observed in all female rats. The mean body weights of female rats at GD 0 and GD 20 are given in Table 1.

**Treatment with Guaifenesin Caused Fetal Mortality, Hemorrhagic Spots, and Limb Defects**  
About 50, 47, 44, 48, and 46 fetuses were recovered after cesarean section in Group-1, -2, -3, -4, and -5, respectively. Fourteen dead fetuses were found in Group-3 and -4 each, while 26 dead fetuses were found in Group 5. No early and late resorptions were found. Distorted axis, eye defects, and abdominal distortion were not observed in any group. Forelimb defects, hindlimb defects, and tail defects were found in Group-4 and -5 only. These defects were characterized as dropping wrist/ankle (low set arm) and kinky tails. Hemorrhagic spots at different places were found in all the treated groups. In Group 2, hemorrhagic spots were observed in subcutaneous and abdominal regions. In Group 3, hemorrhagic spots were found in subcutaneous and hindlimb regions. In addition to these three regions, hemorrhagic spots were also found in brain region in Group-4 and -5 [Figure 1a-c and Table 2].

**Treatment with Guaifenesin Significantly Reduced Fetal Weights**  
A significant reduction (P < 0.001) in fetal weights of Group 2 (2.436 ± 0.081 g), Group 3 (1.488 ± 0.037 g), Group 4 (1.520 ± 0.040 g), and Group 5 (1.580 ± 0.027 g) was determined as compared with control group (3.66 ± 0.072 g) [Figure 5].

**Treatment with Guaifenesin Significantly Decreased Fetal Length, Fetal Skull Length, and Tail Length**  
A significant attenuation (P < 0.001) in fetal lengths of Group 2 (2.660 ± 0.097 cm), Group 3 (2.420 ± 0.308 cm), Group 4 (2.140 ± 0.050 cm), and Group 5 (2.120 ± 0.080 cm) was found as compared with control group (3.020 ± 0.136 cm) [Figure 2b].

A significant alleviation was observed in fetal skull length of rats of Group 2 (1.140 ± 0.050 cm; P < 0.05), Group 3 (0.980 ± 0.060 cm; P < 0.01), Group 4 (0.880 ± 0.178 cm; P < 0.01), and Group 5 (0.790 ± 0.033 cm; P < 0.01) as compared with control group (1.320 ± 0.020 cm) [Figure 2c].

**Treatment with Guaifenesin Significantly Reduced Fetal Forelimb and Hindlimb Lengths**  
A significant reduction (P < 0.001) in fetal forelimb length of Group 2 (0.660 ± 0.040 cm), Group 3 (0.580 ± 0.020 cm), Group 4 (0.620 ± 0.037 cm), and Group 5 (0.600 ± 0.0316 cm) rats was determined as compared with control group (1.00 ± 0.000 cm) [Figure 2e].

We also found a significant reduction (P < 0.001) in fetal hindlimb length of Group 2 (0.700 ± 0.0316 cm), Group 3 (0.560 ± 0.067 cm), Group 4 (0.660 ± 0.024 cm), and Group 5 (0.640 ± 0.040 cm) rats as compared with control group (1.100 ± 0.00 cm) [Figure 2f].

**Treatment with Guaifenesin Increased Intercostal Space and Caused Improper Development of Limbs**  
Five fetuses from each group were subjected to skeletal examination, and we found no skeletal deformation in Group-1, -2, -3, and -4. There was well-developed bone infrastructure in all the regions, such as skull, forelimb, hindlimb, vertebrae, and the ribs. The whole skeleton was intact and uniform in appearance. In Group 5, we found increased intercostal space between 7th and 8th ribs on the right side in all the fetuses. It was also observed that carpals, metacarpals, tarsals, and metatarsals of the fetuses were not properly developed [Figure 1e and f].

**Table 1:**  
Treatment with Guaifenesin significantly inhibited maternal weight growth along with food and water intake. We did not find any mortality and morbidity in any group

| Groups | No. of female rats | Mean body weight at GD 0 (Gram±S.E.M) | Mean body weight at GD 20 (Gram±S.E.M) | Food and water intake | Morbidity/mortality status Yes/No |
|--------|--------------------|---------------------------------------|---------------------------------------|-----------------------|-----------------------------------|
| 1 (Control) | 5 | 164.0±7.314 | 246±4.626 | †† | No |
| 2 | 5 | 163.6±6.983 | 228±5.992 | † | No |
| 3 | 5 | 160.4±6.439 | 216.2±5.093 | † | No |
| 4 | 5 | 152.0±6.907 | 216.2±5.093 | † | No |
| 5 | 5 | 159.2±4.188 | 218±6.921 | † | No |

**Table 2:**  
Treatment with Guaifenesin caused fetal mortality, limb defects, tail defects, and hemorrhagic spots

| Groups | No. of fetuses recovered | No. of alive fetuses | No. of dead fetuses | Distorted axis (%) | Eye defects (%) | Forelimb defects (%) | Hindlimb defects (%) | Abdominal distortion (%) | Tail defects (%) | Hemorrhagic spots (%) |
|--------|--------------------------|---------------------|---------------------|-------------------|----------------|---------------------|----------------------|-----------------------|------------------|---------------------|
| 1 | 50 | All | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| 2 | 47 | All | Nil | Nil | Nil | Nil | Nil | Nil | Nil | 21.2 |
| 3 | 44 | 30 | 14 | Nil | Nil | Nil | Nil | Nil | Nil | 45.4 |
| 4 | 48 | 34 | 14 | Nil | Nil | 31.2 | 20.8 | Nil | 10.4 | 67.2 |
| 5 | 46 | 20 | 26 | Nil | 32.6 | 21.2 | Nil | 15.4 | 86.9 |
Discussion

Most of the developmental toxicity occurred when the pregnant mothers were exposed to various toxic agents during their trimester periods. The gestational period varies among different species, such as in humans and animals. In animals, especially rats, there is an increased risk of teratogenic effects when the embryo is exposed to toxic agents during their gestation period, i.e. from day 6 to 15, which is the period of organogenesis starting from the development of neurons till the hardening of cleft palate.¹⁴
We neither find any mortality nor any signs of intoxication in pregnant rats during the study period. We determined significantly less maternal weight growth in guaifenesin treated groups as compared with control group. Previously, it was reported that decrease in food and water intake might cause a decrease in body weight in pregnant rats.\textsuperscript{10,11} Reduction in carbohydrate, protein, and fat consumption, and increased catabolism can also lead to decrease in body weight.\textsuperscript{12} A significant reduction in food and water intake was observed in this study in guaifenesin-treated group. The decrease in maternal weight growth may be attributed to less food and water consumption. Our results are in line with the findings of Mokhtar \textit{et al}.\textsuperscript{13,14}

The morphological observations were also carried out in this study. We found several hemorrhagic spots at different regions, such as brain, abdominal, hindlimb, and subcutaneous regions as compared with control group. Previous studies have described that guaifenesin possesses antiplatelet properties and is responsible for platelet dysfunction.\textsuperscript{15} In the current study, we found several hemorrhagic spots on fetuses at different locations and this could be due to the anti-coagulant activity of guaifenesin. The proposed mechanism was unknown but this could be due to the interference of guaifenesin with the endogenous adenosine di-phosphate (ADP) release. ADP acts on platelet receptors and initiates the cascade of events required for normal platelet aggregation process. ADP initiates three signal transduction pathways as a result of its interaction with platelet receptors. The ADP causes activation of phospholipase C by binding on P2 × 1 receptors present on platelet cell membrane. This leads to the release of calcium by inositol triphosphate, which is formed as a result of phosphoinositol bisphosphate degradation. The second pathway involves the interaction of ADP with P2T receptors, which results in the activation of GpIb/IIIa complex and leads toward platelet aggregation. The third mechanism includes inhibition of adenyl cyclase, which results in the inhibition of cAMP production and subsequently leads to prevention of the GpIb/IIIa complex activation.\textsuperscript{16,17} Thus, ADP antagonists cause the inhibition of all these three signal transduction pathways, and as a consequence, inhibition of platelet aggregation pathways occurs. Previous studies have shown that different drugs that exhibit anti-coagulant activity also cause developmental toxicity. Aspirin also causes fetal hemorrhage by inhibiting thromboxane A2 production in platelets through inhibition of cyclooxygenase enzymes.\textsuperscript{18}

Limb displacements, like low set arm, hindlimb displacement, and kinky tail were also found in Group-4 and -5. We also found a significant reduction in fetal forelimb, hindlimb, and tail length in all treated groups as compared with control during morphometric analysis. It could be due to the effect of drugs during the ossification process that leads to interference in the developmental process.\textsuperscript{19} Guaifenesin is known to enhance the excretion of phosphate, calcium, and oxalate.\textsuperscript{20} It can be assumed that increased excretion of minerals, such as calcium and phosphate that are the major constituents in bone development, might have led to the reduced ossification in rat fetuses.

Our results also showed a significant decrease in fetal weight in all guaifenesin-treated groups as compared with control group. Previous findings suggested that decrease in weight and length of different body parts could be due to the deficiency of nutrients in fetus, which is usually supplied through placenta. This reduced supply could be due to the exposure of mother to test drug that may interfere with placental supply because of its capability of crossing placental barrier.\textsuperscript{10} Fetal size is also influenced by the rate of placental growth and placental volume. Inhibitory effect of different drugs on cell growth is another factor that can lead to a reduction in body weight. The major part of fetal mass and volume are made up of extracellular liquid. Other studies reported that the decrease in fetal weight could be due to the reabsorption of extracellular liquid.\textsuperscript{21}

We also performed skeletal examination and no signs of prominent skeletal deformities were found in fetuses of the females who were administered with guaifenesin up to the test dose of 500 mg/kg body weight. However, increased intercostal space and improperly formed carpal, metacarpals, tarsals, and metatarsals were found in Group-5, which was administered with 600 mg/kg body weight guaifenesin. It was suggested that guaifenesin has anticonvulsant and muscle relaxant properties because of its tendency to block the excitatory amino acid, such as glutamate and amino acid derivative N-methyl-D-aspartate (NMDA).\textsuperscript{22} NMDA receptors are inotropic glutamate receptors and are excitatory in nature. NMDA receptors are usually in sedentary state at resting potential because of the obstruction created by Magnesium ion. As the action potential begins, magnesium ions are deported from the channels, resultant entries of sodium and calcium into the channels are allowed. NMDA causes osteoblast cell differentiation and thus plays a major role in bone development. Previously, it was found that drugs having NMDA blocking activities also inhibit osteoblast cell differentiation.\textsuperscript{23} It can be assumed that NMDA and glutamate blocking properties of guaifenesin might have caused skeletal deformities observed in high-dose-treated group. There are several agents that are NMDA antagonists and cause skeletal malformation in the fetus during pregnancy. Alcohol also has NMDA blocking activity and is responsible for causing fetal alcohol syndrome. Bone deformation, for example, reduction in bone size and weight, and metacarpal defects are prominent features of fetal alcohol syndrome.\textsuperscript{24} In this study, an increased intercostal space was found between 7th and 8th ribs in Group-5, which correlates with the previous finding of Mishra \textit{et al}.\textsuperscript{25} who also testified the increase in intercostal space with the use of alcohol during pregnancy.

**Conclusion**

Guaifenesin causes fetal mortality at the test dose of 350 mg/kg b.w. and above. We also found a significant prenatal developmental toxicity, which is characterized by a reduction in fetal weights, full body length, skull length, forelimb length, highlimb length, and tail length, found in our study. Guaifenesin also caused hemorrhagic spots, dropping wrists and ankles, kinky tail, increased intercostal space, and inappropriate development of carpal, metacarpals, tarsals, and metatarsals. All the test doses of guaifenesin used in this study showed fetal and maternal toxicity. Therefore, no-observed-effect-level (NOEL) of guaifenesin was not obtained in this study and future studies are suggested using lesser doses to evaluate NOEL.
Based on the results of this study, highly careful and cautious use of guaifenesin is also suggested during pregnancy.

**Financial Support and Sponsorship**

Nil.

**Conflicts of Interest**

There are no conflicts of interest.

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