

Prenatal and developmental toxicity study of meclizine and caffeine combination in female albino wistar rats

Sandeep M & Alvin Jose M*

Department of Pharmacology, Swamy Vivekanandha College of Pharmacy, Tiruchengode 637 205, Tamil Nadu, India

Received 30 July 2013; revised 27 October 2014

Meclizine and caffeine combination is used for the treatment of morning sickness. Both compounds are teratogenic and caffeine is known to possess anti-fertility activity also. The present study was undertaken to evaluate the reproductive toxic effect of meclizine and caffeine combination. Three doses were taken for the study; low dose (LD; meclizine 3.7 mg/kg and caffeine 3 mg/kg) was selected from commercially available formulation, middle dose (MD; meclizine 37 mg/kg and caffeine 30 mg/kg) and high dose (HD; meclizine 370 mg/kg and caffeine 300 mg/kg). The mixture was administered 1-7 days and 8-14 days for fertility and embryotoxic studies respectively. Laparotomy was done on 10th day of gestation period. Number of implants and corpora lutea were counted, pre and post-implantation losses were determined. In embryo toxicity study fetuses were evaluated for external, skeletal and visceral examination. High dose was removed from both fertility and embryotoxicity studies due to its severe toxicity to the dam. Significant anti-fertility activity was observed at middle dose. Embryotoxicity study showed significant reduction in fetal body weight, body length and body mass index, dam body weight gain on gestation day 14. Absolute kidney weight in MD and absolute and relative spleen weight in both LD and MD were significantly reduced. There was no increase in external or internal congenital anomalies at both LD and MD. The results suggest that prescription of meclizine and caffeine for morning sickness in early pregnancy should be reviewed carefully.

Keywords: Antifertility, Caffeine, Embryo toxicity, Meclizine, Morning sickness, Reproductive toxicity

Morning sickness is commonly regarded as an unpleasant and often incapacitating sideeffect of pregnancy and common in the first trimester¹. Nausea and vomiting in early pregnancy (NVP) is a common phenomenon affecting 80% pregnant women². Symptoms generally begin around 4-9th week of gestation, increases around 7-12th week and typically cease by week 16. However, up to 15% pregnant women experience persistent symptoms until delivery. Treatment of this disorder ranges from dietary and lifestyle changes to vitamins, anti-emetics, and hospitalization for intravenous therapy³.

Even though drugs are given to the mother during pregnancy, child is always a recipient. A drug given to the mother reaches the fetus through complex but integrated set of variables like uterus, placenta, amniotic fluid and fetus⁴. These complex set of variables makes it difficult to evaluate drug effects on fetus, because they go through a lot of changes during pregnancy⁵. Thalidomide and Benedictin[®], which is a combination of doxylamine succinate, dicyclomine and pyridoxine were removed from the market due to

fetal effects^{6,7}. Presently, no drug has been approved by the FDA for treating NVP, and no standard treatment protocol exists. Unfortunately, the safety concern of the drugs used for NVP is uncertain. Because as per FDA, most of the drugs used for NVP (FDA class-C) are having little or no evidence available regarding the safety in pregnant women⁸.

To treat morning sickness, meclizine (Piperazine, 1-[(4-chlorophenyl) phenylmethyl]-4-[(3 methylphenyl)]), a well known H₁ receptor antagonist is prescribed in combination with other drugs like caffeine in India. Meclizine has been reported as a teratogen at relatively large doses^{9,10}. Caffeine (1,3,7-trimethylxantine) is the most commonly used substance in the stimulant group, and there is a large toxicology data base that shows good prenatal tolerability when taken in doses lower than 300 mg/day¹¹⁻¹⁵. Caffeine prevents implantation in uterus¹⁶. The effect of meclizine and caffeine combination in implantation and embryo toxicity is unknown and needs evaluation.

The anti-fertility activity and embryo toxic effects of meclizine and caffeine combination which represents the Ist and IInd segments of ICH tripartite guidelines have been examined. The fertility study is

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*Correspondence:

E-mail: deepu.pharma08@gmail.com; pharmjose@gmail.com

concerned with the effect of combination in early embryonic development before implantation on the uterus and implantation period. The embryo toxicity study is concerned to evaluate the effect of combination in organogenesis and fetal growth¹⁷.

Materials and Methods

Chemicals—Meclizine (purity >99%) was purchased from UCB Pharma Private Ltd, Mumbai, India, and Caffeine (purity >99%) from Sigma-Aldrich, St Louis, USA.

Animals and treatment—This experiment was designed in accordance with international guidelines¹⁸. The protocols received prior approval from the institutional animal ethical committee (approval number SVCP/IAEC/M.Pharm/03/2011) and experiments were conducted in accordance with guidelines set by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India. Adult albino rats (40 and 80) of Wistar strain of 13-14 weeks of age were obtained from the animal house of Swamy Vivekanandha College of Pharmacy (Namakkal, India). The rats were acclimatized to laboratory conditions for two weeks prior to the experiment. The animals were maintained under standard conditions of 20-25 °C and 40-70% RH and a 12-12 h L:D cycle 06.00-18.00 h with free access to food and water. Vaginal smears of the female were taken daily for assessment of their estrus cycle. Adult female albino Wistar rats of appropriate weight with established fertility in pro-estrus and estrus were housed with mature male rats of appropriate weight with established fertility (two females for one male) for approximately 14 h. Each female was examined for the presence of spermatozoa in early morning vaginal smears. The female rats which had mated were separated and caged singly. The first presence of sperms in the vaginal smear was taken as day 1 of pregnancy.

The meclizine and caffeine doses were selected directly from the commercially available formulation, which is also minimal human dose and converted into animal dose. Meclizine 3.7 mg/kg and caffeine 3 mg/kg body wt/day were selected as low dose (LD). The middle dose and high dose were 10 and 100 times the low dose. Accordingly, 37 and 30 mg/kg body wt/day of meclizine and caffeine, respectively were selected as middle dose (MD); and 375 mg/kg (meclizine) and 300 mg/kg (caffeine) body weight/day were selected as higher dose (HD). All the

drug suspensions were prepared by using 1% (w/v) of carboxy methyl cellulose (Loba Chemie) in water. The suspension was administered in a volume of 10 mL/kg body weight through oral route using oral needle. The animals in the control group received only CMC-water suspension in volumes similar to that of mixture treatment groups i.e. 10 mL /kg body weight per day^{17,19}.

Fertility study—The mated females were randomly divided into four groups i.e., control and three treatment groups (LD, MD and HD) consisting of 10 animals each. The mixture of drugs were administered orally once daily from day 1 until day 7 of pregnancy. The control group received 1% w/v of carboxy methyl cellulose suspension in a volume of 10 ml/kg body weight per day from day 1 until day 7 of pregnancy. The animals underwent laparotomy on gestation day 10 and the implants present in both uterine horns as well as the corpora lutea (CL) on each ovary were counted. The animals were allowed upto the gestation day 21. On gestational day 21 dam was sacrificed by decapitation and laparotomy was done to deliver the uterus. The number of fetuses present in the uterus was recorded. The percentages of pre- and post-implantation loss and the anti-fertility activity were calculated using the following formula^{20,21}.

$$\text{Pre-implantation loss (\%)} = \frac{\text{Number of CL} - \text{Number of implant}}{\text{Number of CL}} \times 100$$

$$\text{Post-implantation loss (\%)} = \frac{\text{Number of implants} - \text{Number of litters}}{\text{Number of implants}} \times 100$$

$$\text{Anti-fertility activity (\%)} = \frac{\text{Number of CL} - \text{Number of litters}}{\text{Number of CL}} \times 100$$

Embryotoxicity study—The mated female rats were randomly divided into three groups consisting of 10 animals each. The mixture of drugs were administered orally once daily from day 8 until day 14 of pregnancy. The control group received only CMC-water suspension. The suspension was administered intra-gastrically in a volume of 10 mL/kg body weight. The daily food intake of the experimental animals was noted. Dam body weight gain was monitored on gestation days 1, 8, 14, and 21. On gestation day 21, dams were sacrificed by decapitation and the fetuses were delivered by laparotomy. Liver, kidney and spleen weights of dam were recorded^{22,23}. The uterus was visually inspected for the presence and position of resorption sites and dead and live fetuses. Each uterus was stained with

5% ammonium sulphide (Sigma) to reveal possible early resorption, when visible evidence of pregnancy was not obtained. Fetuses were removed from the uterus by detaching them from placenta and examined under magnification (Illuminated magnifier) for external malformations. The weight of fetuses (HPB 220/310/410, Wensar Weighing Scales Limited), body length, tail length (Mitutoyo Digimatic Caliper-Japan-CD-6öCS) and sex of the fetus were checked and body mass index (BMI) was calculated²³.

Half of the fetuses were taken for the skeletal examination and they were stained by double staining technique using alizarin red S and alcian blue. Remaining fetuses were taken for visceral examination and fixed in Bouin's solution. Skeletal examination and visceral examination was done as per the procedure given in the Wallace Hayes principles of toxicology. Processed fetuses were examined for visceral (Nikon binocular stereozoom microscope-C-Dss230-japan) and skeletal examination (Leika stereozoom microscope Germany-541006). Findings were classified as malformations and variations. Lack of any staining in double-stained skeletons was defined as missing bone²⁴.

Statistical analysis—The unit for statistical analysis was pregnant female or the litter. Quantitative continuous data were compared among experimental groups using one-way ANOVA followed by Dunnett's multiple comparison test. Inhomogeneous data were analysed by using one-way ANOVA followed by Kruskal Wallis post test. $P < 0.05$ was considered as statistically significant. Data were analysed by using Graph pad 4 software.

Results

Fertility and embryotoxicity studies were designed with three dose levels, the minimal human dose was selected as LD and the remaining two doses were geometrically spaced from LD (LD:MD:HD = 1:10:100). Since, three mated female animals dosed

with HD died within 15 minutes after drugs treatment, it was removed from both fertility study and embryotoxicity study.

Fertility study—There was no statistical significance between control group and both treatment groups in case of percent pre-implantation and post-implantation loss. MD showed significant anti-fertility activity ($P < 0.01$) in comparison with control group (Table 1).

Embryotoxicity study—Maternal effects: HD mortality indicates that the drugs combination was highly toxic to the dam. There was no significant change in the feed intake of mixture treated animals in comparison with control group. Relative weight of kidney, absolute weight of spleen and absolute and relative weight of liver were not affected in both doses. Absolute kidney weight in MD and relative spleen weight in both doses were significantly decreased ($P < 0.01$) in comparison with control group. In MD, dam body weight gain was significantly decreased ($P < 0.001$) in comparison with control group (Table 2).

Fetal effects: There was no fetal mortality in both groups. Fetal body weight ($P < 0.001$), body length ($P < 0.01$) and BMI ($P < 0.05$) were significantly decreased in MD significantly, but insignificantly decreased in LD group in comparison with control group. There were no external malformations observed in any of the treatment groups. Resorption sites observed in MD were more than that of LD treatment group but not significant in comparison with control group. Skeletal and visceral examination did not reveal any significant anomalies in the mixture treated groups as compared to that of control group. Resorption sites observed in MD were more than those of LD group and both were not significant in comparison with control group (Table 3; Fig. 1).

There were no significant skeletal and visceral anomalies in the mixture treated groups when compared to control group. There was a malpositioned kidney in control and MD and also a

Table 16 Effect of meclizine and caffeine combination on fertility

[Values mean \pm SE from 10 observations each]

Parameters	Control	Low dose	Middle dose
Pre-implantation loss (%)	31.50 \pm 3.61	12.82 \pm 6.34	53.13 \pm 8.60
Post-implantation loss (%)	21.03 \pm 7.03	38.55 \pm 16.78	62.50 \pm 16.07
Anti-fertility activity (%)	44.85 \pm 7.46	38.12 \pm 11.03	83.28 \pm 4.95**

Group I: Normal control (0.25% carboxy methyl cellulose), Group II: Low dose (3.7 mg/kg meclizine + 3mg/kg caffeine). Group: Middle dose III: (10 \times low dose). One way ANOVA followed by Dunnett's multiple comparison test. ** $P < 0.01$ when compared to control.

Table 26 Effect of meclizine and caffeine combination on maternal parameters
[Values mean± SE from 10 observations each]

Parameters	Control	Low dose	Middle dose
Relative food intake (g)	73.3±4.78	63.86±5.14	56.42±6.80
Maternal weight gain on Gestation day 14 (g)	35±5.62	20±5.16	3.33±6.14***
Maternal weight gain on Gestation day 21 (g)	51.66±10.13	35±13.35	21.66±12.49
Absolute kidney weight (g)	1.45±0.09	1.25±0.03	1.15±0.03**
Relative kidney weight (% bw)	0.59±0.02	0.57±0.03	0.58±0.04
Absolute liver weight (g)	8.07±0.96	6.53±0.67	6.90±0.66
Relative liver weight (% bw)	3.17±0.16	3.14±0.14	3.14±0.06
Absolute spleen weight (g)	0.66±0.08	0.65±0.04	0.67±0.06
Relative spleen weight (% bw)	0.65±0.10	0.32±0.03**	0.31±0.03**

Group I: Normal Control (0.25% Carboxy methyl cellulose), Group II: Low dose (3.7 mg/kg meclizine + 3mg/kg caffeine). Group: Middle dose III: (10 × low dose). One way ANOVA followed by Dunnett's multiple comparison test. *P* values: **<0.01, ****P*<0.001 when compared to control

Table 36 Effect of meclizine and caffeine combination on Fetal parameters
[Values mean± SE from 10 observations each]

	Control	Low dose	Middle dose
Litters	10	9	8
Resorptions	0	1.16±0.74	1.66±0.71
Resorptions /Litter	0	0.91±0.12	0.27±0.10
Fetal length (cm)	3.70±0.05	3.59±0.08	3.48±0.04**
Fetal Body weight (g)	3.74±0.15	3.43±0.14	2.82±0.09***
Tail Length (cm)	1.35±0.03	1.27±0.02	1.25±0.02
Body Mass Index (kg/m ²)	2.71±0.07	2.66±0.05	2.34±0.06**
Percentage of sex distribution			
Male	55	53	45
Female	45	47	55

Group I: Normal Control (0.25% Carboxy methyl cellulose), Group II: Low dose (3.7 mg/kg meclizine + 3mg/kg caffeine). Group: Middle dose III: (10× low dose). One way ANOVA followed by Kruskal wallays multiple comparison test. *P* values: *<0.05, **<0.01, ***<0.001 when compared to control

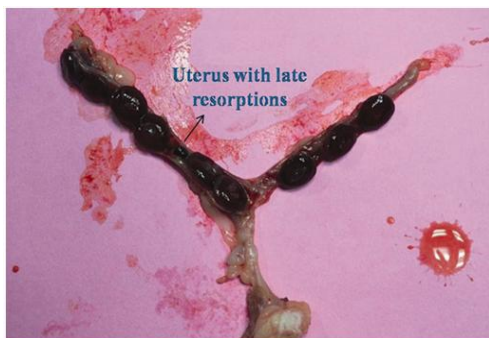


Fig. 16 Uterus with resorptions

hematoma in control group. Reduced ossification in frontal, parietal, hyoid bone, metacarpal and metatarsal bodies was observed in all treatment groups. Reduced ossification of interparietal bone and supraoccipetal bone were found in control and MD

groups (Table 4; Figs. 2-4). The findings in both visceral and skeletal examination were spontaneous and unrelated to the examined test mixture.

Discussion

The minimal human dose i.e., LD and 10 times the LD i.e., MD has not shown any dam mortality. However HD, which is 100 times LD shown severe toxicity, hence it is discontinued. This lethal effect can be due to possible drug interaction between the two components; emphasising the importance of further safety pharmacological investigation.

Meclizine and caffeine combination at MD shown significant anti-fertility activity (*P*<0.05) and consistent with Pollard *et al.*¹⁶, who reported the reduced fertility rate of preconceptual caffeine exposed rats due to failure of litter implantaion.

Table 46 Effect of meclizine and caffeine combination on fetal skeletal and visceral examination

	Control	Low dose (LD)	Middle dose (MD)
Total litters examined	10	9	8
Total number of fetus examined	84	40	34
Total fetus for skeletal examination	42	20	17
Total fetus for visceral examination	42	20	17
Visceral variations:			
Hematoma	1	1	2
Malpositioned kidney	1	0	1
Skeletal variations:			
Frontal bone reduced ossifications	0	1	1
Parietal bone reduced ossification	1	1	2
Interparietal bone unossified	1	0	1
Supraoccipetal bone reduced ossification	1	0	1
Hyoid bone reduced ossification	0	1	2
Metacarpal bodies un-ossified	1	0	2
Metatarsal bodies un-ossified	1	1	1

Group I: Normal Control (0.25% Carboxy methyl cellulose), Group II: Low dose (3.7 mg/kg meclizine + 3mg/kg caffeine). Group III: Middle dose (10× low dose). n=10. All fetuses were examined for external malformations, including cleft palate. A single fetus may be represented more than one time in listing individuals.



Fig. 26 Normal control group (0.25% Carboxy methyl cellulose) double stained fetus. Photographs showing fetus skeleton stained with alizarin red and alcian blue. Ossified frontal (F), parietal (P), Interparietal (Ip), supraoccipetal (So), metatarsal (Mt) and metacarpal (Mc) bones.



Fig. 36 Low dose group (3.7 mg/kg meclizine + 3mg/kg caffeine) double stained fetus. Photographs showing fetus skeleton stained with alizarin red and alcian blue. Ossified frontal (F), parietal (P), Interparietal (Ip), supraoccipetal (So), metatarsal (Mt) and metacarpal (Mc) bones.



Fig. 46 Middle dose group (10× low dose) double stained fetus. Photographs showing fetus skeleton stained with alizarin red and alcian blue. Reduced ossification of frontal (F), parietal (P), Interparietal (Ip) supraoccipetal (So) and metacarpal bones; ossified metatarsal (Mt) bones.

As per Chahoud *et al.*²⁵ the toxicity shown on spleen and kidney were purely theoretical and there won't be any casual relationship exists between maternal and fetal embryo toxicity. Since there was reduced ossification in control group also, the reduced ossification in treated groups can be interpreted as spontaneous developmental variation unrelated to the test mixture and they were unlikely to effect the survival or health.

Till now, no studies were conducted on the effect of meclizine and caffeine combination, however done with single drug. For the first time, King *et al.*²⁶ found meclizine teratogenicity. They found cleft palate and other oral abnormalities in Sprague-Dawley rats at 25-50 times the human therapeutic dose²⁶. In addition to that it is confirmed through metabolic studies, norchlorcyclizine is the active metabolic form of meclizine responsible for teratogenicity in rat^{27,28}. However, meclizine has not increased the incidence of congenital abnormalities in mice, rabbits, pigs and monkeys in contrast to its developmental effects in rats²⁹⁻³¹. The large toxicology database of caffeine suggesting 60 µg/mL caffeine concentration in dam blood causes teratogenicity to the fetus and it takes 80 mg/kg/day through oral, 330 mg/kg/day in drinking water and 250 mg/kg/day through intraperitoneal route to cause teratogenicity³²⁻³⁷. In a skeletal study conducted by Burdan³⁸ caffeine showed significant fetal length reduction at 7 mg/kg free of skeletal, external or internal malformations¹⁷. Even, caffeine exposure throughout the gestation period at 16-17 and 25-33 mg/kg/day showed free of embryo or fetotoxicity³⁸. On the other hand, caffeine reported to increase the pre-natal toxicity of 5-bromodeoxyuridine, mitomycin C, chlorambucil, cyclophosphamide, di(2-ethylhexyl)phthalate, 2-ethylhexanol, 2-thylhezanoicacid, valproic acid and acetazolamide³⁹⁻⁴³. Caffeine's half life in a 12-15 day gestation rat was found to be 1.7 h at 80 mg/kg of single oral dosage. The ratio of embryo to maternal blood caffeine concentration was found to be 1, confirming the free movement of caffeine to the fetus. As per Kimmel *et al.*⁴⁴ one of the possible mechanism behind the reduction in fetal body weight, body length and BMI could be due to the capacity of caffeine to reduce uterine blood flow in pregnant animals. However, in our study along with fetus body length, there was significant reduction in body weight and BMI at MD. It confirms that meclizine and caffeine prenatal and developmental toxicity increased in combination when compared to the drugs alone.

No effect level (NOEL), reproductive NOEL and teratogenic NOEL of caffeine in rodents was found to be 30 mg/kg, 80-120 mg/kg and 80-100 mg/kg/day, respectively. The reproductive NOEL for meclizine and caffeine combination was found to be 3.7 and 3 mg/kg and NOEL for malformations and variations were found to be 37 and 30 mg/kg. Since, maternal toxicity was found in all the treatment groups NOEL for dam was not obtained⁴⁵⁻⁴⁸.

FDA classifies meclizine as a class B drug for pregnancy safety classification, which means it is a drug which had not resulted fetal risk in animal studies without controlled studies in pregnant women or animal studies showed adverse effects that have not been observed or confirmed in controlled studies involving pregnant women. Moreover, the clinical efficacy studies of meclizine and other anti-histamines efficacy were conducted during 1951-1975; the rates of malformations in those studies were not homogenous. So new additional, larger, randomized, control trials of the more common antihistamines like meclizine, would be helpful in evaluating the efficacy of these agents for treating NVP⁴⁹.

In conclusion, pre-natal administration of meclizine and caffeine at human dose level (LD) did not produce any toxicity, whereas MD showed significant fetal toxic effects of decreased body weight, body length and BMI, so MD level caused intrauterine growth retardation but did not cause any external malformations or internal congenialities. Based on these observations, unscrupulous use of meclizine and caffeine combination for morning sickness in early pregnancy should be reviewed carefully. It is therefore important to conduct proper Pharmacovigilance studies, may be carried out with the use of combination of meclizine and caffeine in early pregnancy for morning sickness. Necessary warning may be indicated on the label of such products based on these studies.

Acknowledgement

Thanks are due to Dr. D Vijayaraj, College of Veterinary Science, Guwahati for help in visceral and skeletal examination and support.

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