# Evaluation of the Developmental Toxicity of Amlodipine Besylate in Mice

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**Amlodipine besylate, a dihydropyridine calcium antagonist, was evaluated for its potential to cause embryonal fetal toxicity and teratogenicity in pregnant mice. Amlodipine was administered in drinking fluid at dose levels of 0.2, 0.8 and 1.6 mg/kg body weight on days 1 through 21 of gestation. Litters were examined on gestational day 21. There were significant ($p<0.05$) decreases in the absolute and relative weights of the maternal heart, liver, pancreas and vagina in the 0.8 and 1.6 mg/kg body weight amlodipine, and non-significant decreases in these measurements in the 0.2 mg/kg body weight dose level. There was no fetal growth retardation as shown by the crown rump length in the 0.2 and 0.8 mg/kg body weight amlodipine. At dose size of 1.6 mg/kg body weight, amlodipine caused embryo lethality. The no-observed-adverse-effect level (NOAEL) was found to be 0.2 mg/kg amlodipine.**

**Key words** —— amlodipine besylate, teratogenicity, maternal toxicity

## INTRODUCTION

The most important toxic effects of calcium channel blockers are a direct extension of their therapeutic actions. Excessive inhibition of calcium influx can cause cardiac depression including cardiac arrest, bradycardia, atrioventricular block and congestive heart failure.[1] Amlodipine has been reported to reduce glomerular filtration rate (GFR), albumin excretion rate (AER) and to induce incipient nephropathy during antihypertensive therapy.[2] In a study with valsartan — a new angiotensin II antagonist and the calcium channel blockers amlodipine and felodipine, it was reported that the incidence of drug-related dependent edema was somewhat higher in the amlodipine group, particularly at a dose of 10 mg per day than other comparative drugs in use.[3] Packer and his colleagues[4] have reported a high incidence of morbidity and mortality associated with amlodipine in severe chronic heart failure. Amlodipine also precipitates cardiovascular stress responses.[5]

There is a dearth of information on the teratogenic effect of amlodipine.[6] However, studies on gestational periods of rats using amlodipine showed a delayed parturition and prolonged labour.[7] Khera analysis of teratology studies indicated that some agents that prolonged labour and delayed parturition are associated with maternal toxicity, weight loss, abortion and/or death.[8] The aim of the present study was to investigate the teratogenic effect and developmental toxicity of amlodipine besylate in mice with a view to determining the no-observed - adverse-effect level (NOAEL).

## MATERIALS AND METHODS

**Animals** —— Female albino-mice (12—15 weeks old) obtained from the Toxicology Unit Breeding Laboratory (Nnamdi Azikiwe University, Nnewi Campus, Nigeria) were used in this study. The animals were housed under standardized conditions of temperature (22—24℃), relative humidity (70—76%) and 12 h dark/light cycle. They weighed between 20—25 g and were divided into four weight-matched groups of ten mice each. Pfizer rat cubes (Pfizer PLC, Lagos, Nigeria) and water were provided *ad libitum*.

**Drug** —— Five mg of amlodipine besylate [Pfizer (Neimeth) Pharmaceuticals PLC, Lagos, Nigeria] was dissolved in 90 ml of distilled water and 10 ml absolute ethanol. The stock-drug solution was stored in a brown bottle at 0℃, in the dark. The following drug concentrations were used in the study: 0.2, 0.8 and 1.6 mg/kg.

**Experimental Design** —— The female mice were primed prior to breeding[9] by placing a single male mouse in a cage in the same animal room as ten females to acclimatize the females. Forty-eight hours later, each female was placed overnight in the home

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cage of a single housed male for mating, and examined the next morning for the presence of a vaginal plug.[10] Plug-positive mice assigned to the same experimental group were group-housed (maximum of ten per group).

The day of the vaginal plug was recorded as gestational day 0 "GDO". From GDO, the test groups received 0.2, 0.8, 1.6 mg/kg/body weight of amlodipine for 21 days. The control group received ethanol in distilled water (1 part ethanol: 9 parts water). The drug and control solutions were given orally in drinking water. The amount of fluid and feed intake by the animals was recorded daily while the maternal weight was recorded weekly. At the end of the 21-day treatment period, the pregnant mice were sacrificed under chloroform anesthesia, laparatomised and the live litters delivered from the gravid uterus.

The following maternal toxicity parameters were monitored: emaciation, dehydration, piloerection, lethargy, rough coat, red vaginal exudate, change in pupil size, and urine and fecal staining. The developmental endpoints monitored were: number of live and dead litters, implantation sites, crown rump length (CRL), number of growth-retarded embryos, prenatal mortality, and cleft palate.

The litters collected after laparatomy were examined for external abnormalities, then fixed, cleared, stained with Alizarin Red S, and examined for skeletal abnormalities. CRL of the embryo was measured from the intersection of parietal and occipital skull sutures to the base of the tail on postnatal day 1 (PND 1). An embryo with a CRL of less than 20% of the mean CRL of control was defined as growth retarded.

**Statistical Analyses** —— Data are expressed as mean±S.E.M. and analysed using Student's $t$-test and analysis of variance (ANOVA). Significant values were determined at $p<0.05$.[11]

## RESULTS AND DISCUSSION

Table 1 shows the effect of amlodipine treatment on maternal body weight and on fluid and feed intake. There were no dose-dependent significant effects on any of these parameters. There were significant differences ($p<0.05$) in the relative and absolute weights of the following maternal organs-heart, liver, pancreas, uterus, lungs and vagina between the control and the treated groups (Table 2). There was no evidence of maternal toxicity as shown by the absence of emaciation, dehydration, rough coat, red vaginal

**Table 1.** Effect of Amlodipine Besylate on Maternal Body Weight and Fluid and Feed Intake

| Treatment | Total fluid intake (ml) | Total feed intake (g) | Maternal body weight (g) |
|---|---|---|---|
| 10% v/v EtOH+water | 151.7±0.57 | 225±0.86 | 23.67±0.25 |
| 10% v/v EtOH+water+0.2 mg/kg AML | 162.5±0.62 | 215±0.44 | 21.95±0.38 |
| 10% v/v EtOH+water+0.8 mg/kg AML | 187.6±0.92 | 266±0.56 | 22.07±0.45 |
| 10% v/v EtOH+water+1.6 mg/kg AML | 165 ±0.75 | 218±0.68 | 21.4 ±0.22 |

For each treatment $n=10$, values are expressed as mean±S.E.M., EtOH, ethanol; AML, amlodipine.

**Table 2.** Effect of Amlodipine Besylate on Some Maternal Organ Weights

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10% v/v EtOH+water | | 10% v/v EtOH+water+0.2 mg/kg AML | | 10% v/v EtOH+water+0.8 mg/kg AML | | 10% v/v EtOH+water+1.6 mg/kg AML | |
| Maternal organ | Absolute weight (g) | Relative weight (%) | Absolute weight (g) | Relative weight (%) | Absolute weight (g) | Relative weight (%) | Absolute weight (g) | Relative weight (%) |
| Heart | 0.21±0.01 | 0.88±0.05 | 0.18±0.02 | 0.81±0.04 | 0.10±0.01* | 0.48±0.02* | 0.11±0.01* | 0.48±0.08* |
| Liver | 1.49±0.05 | 6.37±0.40 | 1.44±0.08 | 6.43±0.42 | 1.14±0.02* | 5.20±0.25* | 1.27±0.08* | 5.75±0.22* |
| Pancreas | 0.13±0.01 | 0.56±0.03 | 0.10±0.01 | 0.45±0.02 | 0.08±0.01* | 0.37±0.01* | 0.09±0.03* | 0.41±0.33* |
| Vagina | 0.20±0.04 | 0.85±0.04 | 0.20±0.01 | 0.89±0.06 | 0.15±0.02* | 0.68±0.02* | 0.09±0.01* | 0.41±0.03* |
| Uterus | 0.53±0.02 | 2.24±0.08 | 0.38±0.05 | 1.44±0.06 | 0.31±0.05* | 1.41±0.06* | 0.74±0.04[a] | 3.35±0.14[a] |
| Lungs | 0.11±0.01 | 0.47±0.02 | 0.10±0.01 | 0.46±0.03 | 0.08±0.01 | 0.46±0.13 | 0.23±0.01[a] | 1.03±0.04[a] |

For each treatment $n=10$, values are expressed as mean±S.E.M., *Significantly decreased compared to control at $p<0.05$. *a)* Significanlty increased compared to control at $p<0.05$, EtOH, ethanol; AML, amlodipine.

**Table 3.** Fetal Effects of Amlopidine Besylate

| Developmental parameter | Treatment | | | |
|---|---|---|---|---|
| | 10% v/v EtOH+ water | 10% v/v EtOH +water+0.2 mg/kg AML | 10% v/v EtOH +water+0.8 mg/kg AML | 10% v/v EtOH +water+1.6 mg/kg AML |
| Implantation site | 48 | 48 | 42 | 0 |
| No. of litters | 48 | 48 | 42 | 0 |
| No. of live litters | 48 | 48 | 42 | 0 |
| No. of dead litters | 0 | 0 | 0 | 0 |
| CRL (cm) | 1.20±0.04 | 1.25±0.03 | 1.30±0.04 | 0 |
| No. of growth-retarded embryos | 0 | 0 | 0 | 0 |
| Prenatal mortality | 0 | 0 | 0 | 0 |
| Missing ribs | 0 | 0 | 0 | 0 |
| Fused ribs | 0 | 0 | 0 | 0 |
| Cleft palate | 0 | 0 | 0 | 0 |

For CRL, value is expressed as mean±S.E.M., and $n=48$ for control and 0.2 mg/kg group, and 42 for 0.8 mg/kg group. EtOH, ethanol; AML, amlodipine; CRL, crown rump length.

exudate, and urine and fecal staining.

The fetal effects of amlodipine besylate are shown in Table 3. The 0.2 mg/kg body weight amlodipine treatment did not show any statistical difference in the number of implantation sites from the control. There was loss of pregnancy in two mice in the 0.8 mg/kg body weight amlodipine, and no embryo development in the 1.6 mg/kg body weight dose size. There was a non-significant increase in the CRL, no missing or fused ribs, and no cleft palate.

Khera's analysis of teratology studies showed that agents that cause missing or fused ribs, cleft palate, retarded CRL or non-development of the fetus are all associated with fetal toxicity, while maternal toxicity is marked by emaciation, dehydration, piloerection, lethargy, weight loss, rough coat, red vaginal exudate, decrease in pupil size, and urine and fecal staining.[8] Pharmacological or toxicological behavioural signs, and abortion or death are associated with either fetal or maternal toxicity.[8] In the present study, there was no evidence of maternal toxicity. There was, however, evidence of fetal toxicity in the 1.6 mg/kg body weight group which was manifested by non-development of any embryo. There was abortion in the 0 8 mg/kg body weight group which could be attributed to either fetal or maternal toxicity. However, the abortion occurred early in the embryological development as evidenced by resorption of the implantation site, which tends to favour fetal rather than maternal toxicity, since the mother continued with the dosing for the remaining period without signs of toxicity.

Manifestation of developmental toxicity cannot be presumed to be constant or specific across species. Any manifestation of exposure-related developmental toxicity on animal studies is indicative of a variety of responses in human.[12] When more common outcomes (*e.g.*, embryonic death, abortion, intrauterine growth retardation) are taken into consideration, a more sensitive appraisal of toxicity can be obtained.

Amlodipine besylate at the 0.2 mg/kg body weight level produces no maternal or developmental toxicity, but causes severe fetal effects at the 1.6 mg/kg body weight dose. These observations indicate that the NOAEL of the drug in mice is 0.2 mg/kg body weight.

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