



## Embryotoxic Effects of Atorvastatin on Mouse Fetus

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### Abstract

Although the biokinetics, metabolism, and chemical toxicity of atorvastatin calcium are well known, until recently little attention was paid to the potential toxic effects of atorvastatin calcium on re-production and development in mammals. In recent years, it has been shown that atorvastatin calcium is a developmental toxicant given orally or subcutaneously (SC) to mice. Decreased fertility, embryo/fetal toxicity including teratogenicity, and reduced growth of the offspring have been observed following atorvastatin calcium exposure at different gestation periods. On the other hand, an *in vitro* study using fetal isolated mitochondria nowadays has been recognized as a confident tool to evaluate the developmental toxicity of a number of chemicals. Although the developmental toxicity induced by atorvastatin has been investigated, the precise cellular mechanism of atorvastatin -induced embryo-toxicity has not been thoroughly recognized yet. For investigating the effects of atorvastatin calcium on pregnant animals, three groups (control, sham and test) of NMRI mice were chosen. In test group, 50mg/kg, 100mg/kg and 150mg/kg of atorvastatin calcium were intraperitoneally administered at 11 day of gestation, in sham group only normal saline injected to interior peritoneum as indicated in the test group and in Control group which was considered as the comparison base line of our research, no injection was made. Caesarean sections were performed at 15 day of the gestation; and their placentas were examined externally. Based on our results atorvastatin calcium caused significant external anomalies, and caused a significant decrease ( $p < 0.001$ ) in the weight and diameter of placentas, the number of the embryos, their body weight and crown-rump length of fetuses.

**Keywords** : Atorvastatin calcium, Embryotoxicity, Liver enlargement, Morphological anomalies, Umbar vertebrae.

### 1. Introduction

Atorvastatin is a member of the drug class

known as statins, which catalyzes the conversion of HMG-CoA to mevalonate and is used for decreasing blood cholesterol [1]. Animal studies have produced conflicting evidence on the potential teratogenicity during the first trimester of pregnancy of statins. As intensive statin therapy is likely to be a mainstay of cardiovascular disease prevention strategy, gestational exposure to statins is an issue of significant clinical importance [2, 3].

In developmental toxicity studies, a few HMG-CoA reductase inhibitors have been shown to be teratogenic in rats at maternally

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toxic doses, For example, atorvastatin decreased fetal body weight, survival rate of the offspring lower than normal and unusual neonatal development [4]. the result of the studies showed that fluvastatin also induced weight loss, decreased neonatal weight gain, and an increased incidence of stillbirths and neonatal deaths [5, 6]. Besides, lovastatin caused maternal weight loss, fetal skeletal malformations (for example, vertebrae and ribs), and gastroschisis and an increased resorption rate in rabbits and decreased mean fetal body weight and maternal weight gain in rats [7]. The result of post-marketing surveillance showed that exposure to simvastatin induced spontaneous abortions, fetal death, and congenital anomalies (polydactyly, unilateral cleft lip, balanic hypospadias, trisomy, and clubfoot) [8, 9, 10, 6]. Animal models (rats, mice, rabbits) have provided evidence for the teratogenic effects of statins on pregnancy outcome, including fetal death, intrauterine growth retardation, malformations, and abortions. Finally, it was shown that atorvastatin seemed to be the safest statin, is the cause of developmental toxicity in rats and rabbits [11]. Therefore, it is important to know about the potential hazards and their cellular mechanisms in pregnancy, because in common point of view, atorvastatin has the safest position among all statins for human lipid lowering drug therapy. Therefore, toxicological and pathological research to determine the effects of atorvastatin in fetus and its possible mitochondrial damage in embryonic cells, will be more than necessary. Outcomes of this research will provide protective strategies which can counter the toxic effects of atorvastatin in high risk pregnant women.

## 2. Materials and Methods

### 2.1. Devices and Tools

Binocular optical microscope, research microscope model M3, manual rotary microtome, One scale balance with an accuracy of 0.1 g, analytical balance with an accuracy 0.0001 g, water distillation apparatus, loop binoculars, timer, surgical sets (forceps, scalpel, scissors, dissection tray etc.), laboratory glassware (flask, graduated cylinders, desiccators etc.), molding wax for molding glass, tissue warming plate, the oven, metal cages for keeping the animals, cComputer and printer, slide and cover slip, filter paper, digital camera, latex gloves, hydrophilic cotton .

cators etc.), molding wax for molding glass, tissue warming plate, the oven, metal cages for keeping the animals, cComputer and printer, slide and cover slip, filter paper, digital camera, latex gloves, hydrophilic cotton .

### 2.2. Compounds

Atorvastatin calcium, medical alcohol, toluene, paraffin with a melting point of 60-56°C, antler glue, xylene, Picric acid, bouin's fixative solution, haupt's gelatin, Glacial acetic acid, formalin, hematoxylin, eosin powder, gelatin, ether, were purchased from Sigma-Aldrich Co. (Taufkirchen, Germany). All other chemicals were of the highest commercial grade available. Normal saline and distilled water were offered as a generous gift by Daroo Pakhsh Co.Ltd. Tehran, Iran.

### 2.3. Animals

The animals used in this research, were mice of NMRI race, purchased from Institute Pasteur (Tehran, Iran). Firstly, the limited numbers of healthy mice were separated; their feeding and housing were done in special cages whose floor were covered by woody chips. The food consisted of ready pallets. Also, their necessary water was supplied by special bottles. In order to prevent any kind of pollution, the woody chips in floor of cage changed at least once a week and their consuming water bottle was replaced 3 or 4 times in this period of time. Every few days, the cages disinfected by Formalin 6%, ethanol or liquid bleach. The temperature and moisture of the room were regularly determined. All mice were housed in a room at a constant temperature of 25°C on a 12/12 hr light/dark cycle with food and water available. All of the experiments were conducted according to ethical standards and protocols approved by the Committee of Animal Experimentation of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The ethical standards were based on "European Convention for the Protection of Vertebrate Animals Used for Test and other Scientific Purposes" Acts of 1986, and the "Guiding Principles in the Use of Animals in Toxicology," adopted by the Society of Toxicology in 1989, for the acceptable use of test animals.

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 86 – 23, revised 1985). The mice were monitored during the study period; the weak and sick mice were distinguished and eliminated. In this study, in order to investigate the effect of Atorvastatin calcium on growth of mice fetus, 3 groups each contained 6 female adult mice selected. Before that, the pregnant mice were chosen by observing the vaginal smear and plug. In this project, the following groups were considered and studied:

1) Test group: in this group, half of 6 adult female mice which did not have the experience of mating were. The Atorvastatin calcium with the concentration of 50, 100, 150 mg/kg/day was intraperitoneally injected. This solution was injected at 11 day of gestation.

2) Sham group: in this group, only DMSO was injected to interior peritoneum as indicated in the test group.

3) Control group: this group which was considered as the comparison base line, grew up in a quite normal condition.

The animals were anesthetized at 15 day of gestation. Following laparotomy, the uterus was exteriorized and the number and location of fetuses and resorption were noted, then their weight and length (crown-rump length) were measured. Individual fetuses were carefully examined for external anomalies then fetuses were stained by hematoxylin-eosin method and investigated by stereomicroscope for skeletal malformations. The incidence of skeletal malformations and other histological lesions were determined and compared in the groups.

#### *2.4. Surgery and Tissue Preparation*

Mice were supine in description tray after being anesthetized by ether or chloroform. The uterus including fetuses and placenta are visible after opening abdominal skin in front of the vagina. The tissue sections were prepared in series in order to investigate the microscopic variations due to toxic effect of Atorvastatin calcium on ovary. In the following, the different stages of preparation were

summarized.

##### *2.4.1. Tissue Fixation*

The tissue samples should be fixed for preparing to maintain their natural shape and avoid marked alterations. Leaving tissues in the air may cause evaporation and this water loss may cause shrinkage. On the other hand, putting the samples in water causes swelling or osmotic pressure [12].

In order to fix tissues obtained from fetuses, we rinsed them by physiologic serum and put them in bouin's fixative solution for 18 hours then we continue with dehydrating stages.

##### *2.4.2. Dehydration, Clearing, Infiltration*

The process of removing cellular and tissues water is called dehydrating which proceed by medicinal ethyl alcohol. After dehydrating, the alcohol replaced with cellular and tissues water should be extracted. Toluene is used for extracting alcohol. After clearing stage, we introduce paraffin in to tissues in the way the tissues are inserted in the paraffin bath and oven [12].

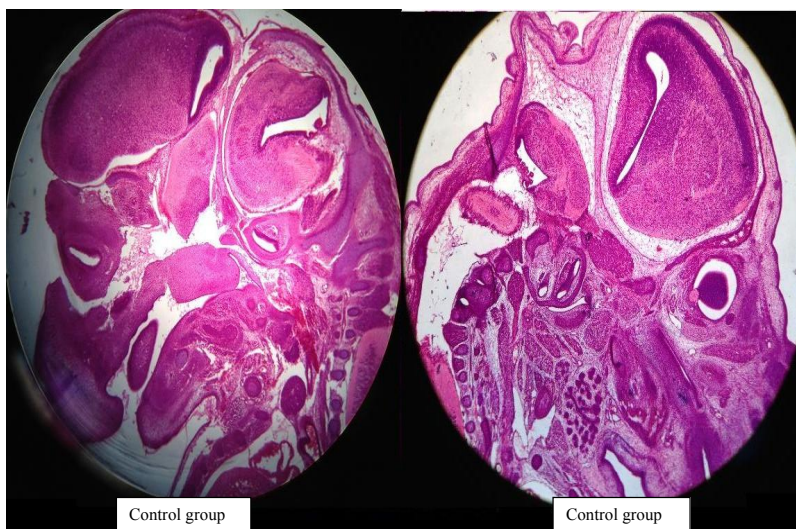
##### *2.4.3. Embedding*

Once tissues saturated completely by paraffin we started embedding process. For this purpose, the samples were taken out from paraffin bath 2 and put it into glassy molds which are specified for embedding and contain melt paraffin. This stage should be immediately and carefully done in order to prevent bubble formation. Besides, the samples should be located in the middle of the mold (minimum distance of 1mm from bottom) [12].

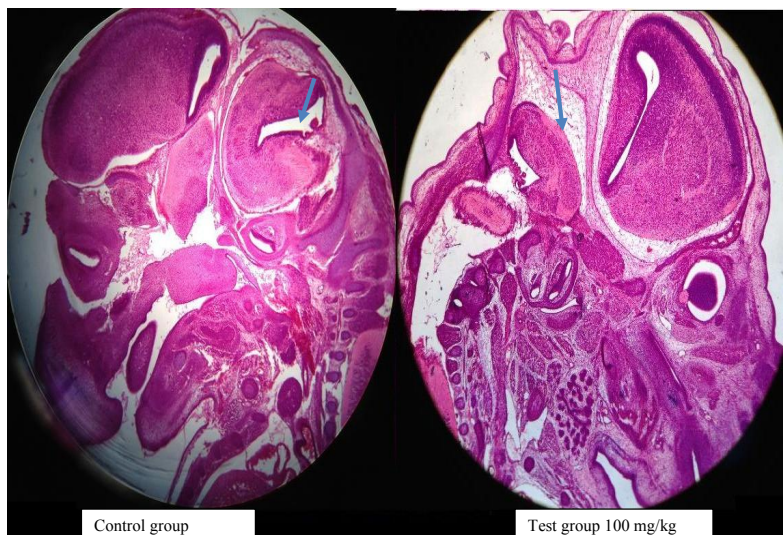
##### *2.4.4. Sectioning*

Before cutting, the molds contain paraffin were shaped into small trapezoid. In other word, the molds were shaped into neat and regularized forms, we call

this procedure trimming. Then the molds were fixed in special tray for installing on microtome. After fixing molds on microtome and sectioning, the paraffinic bands containing tissue sections with thickness of 10 micrometer for fetus, were achieved in series [12].



**Figure 1.** Comparison of the cerebral cortex curve (top left) in fetuses of test and control groups (100 x magnification).



**Figure 2.** Comparison of size of fourth ventricle of the fetus brain in test and control groups (100 x magnification).

#### *2.4.5. Sticking the paraffinic Bands on Slides*

The obtained paraffinic bands were transferred on slides which covered by Haupt's gelatin glue. In order to eliminate the shrinks on paraffinic bands, the surface of slides should already be covered with formalin 2%. We put paraffinic bands to float on these slides and finally, the slides were located on warming plate whose heat is adjusted to 40 °C (10-15°C lower than the melting point of paraffin). After complete spreading and drying the sample, we took the slides from

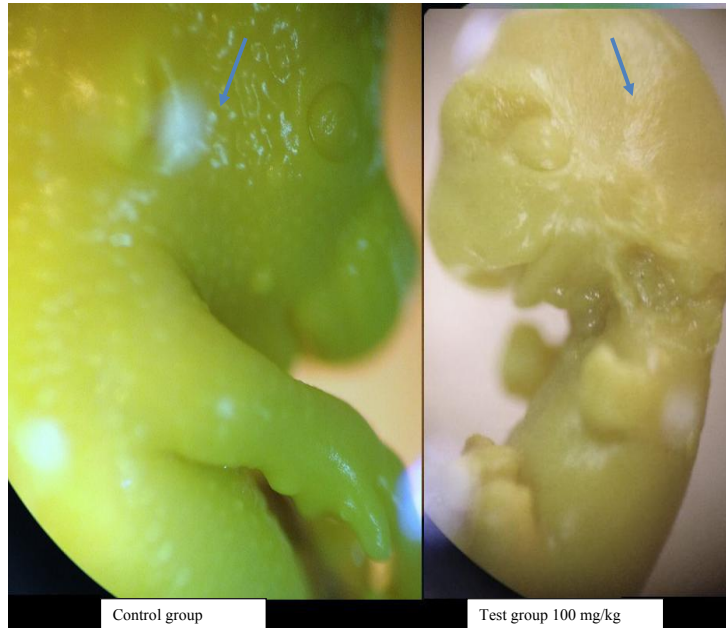
warming plate [12].

#### *2.4.6. Staining*

Not only the nuclei and cytoplasm of cells but also the connective tissues surrounding the cells were stained using hematoxylin-eosin.

#### *2.4.7. Mounting the Cover Slips*

As soon as finishing the staining process and before drying the samples, we stuck the samples with antler glue on slides. This procedure should be done carefully in order



**Figure 3.** Comparison of fetal head size in the test group (100mg/Kg) and control groups (40 x magnification).



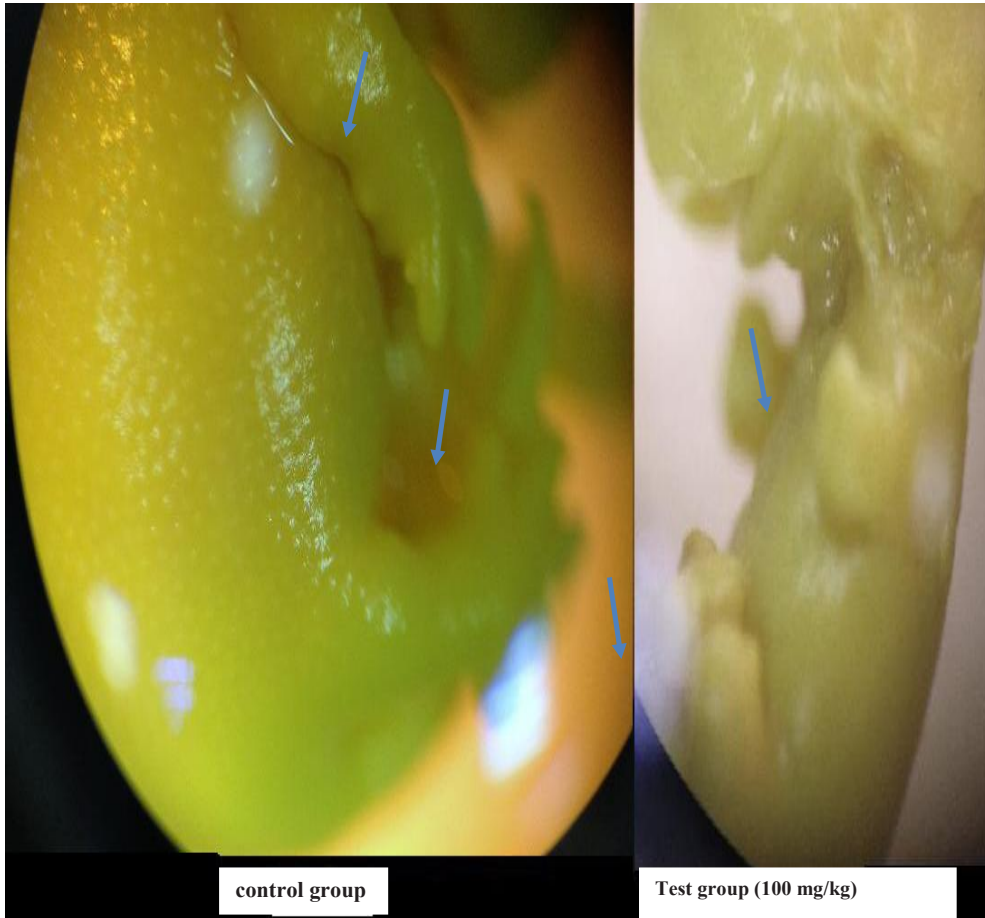
**Figure 4.** Comparison of the finger development in the embryos of the test (100 mg/kg) and the control groups (100 x magnification).

to preventing the bubble formation on the slides. Then the cover slips were mounted on slides.

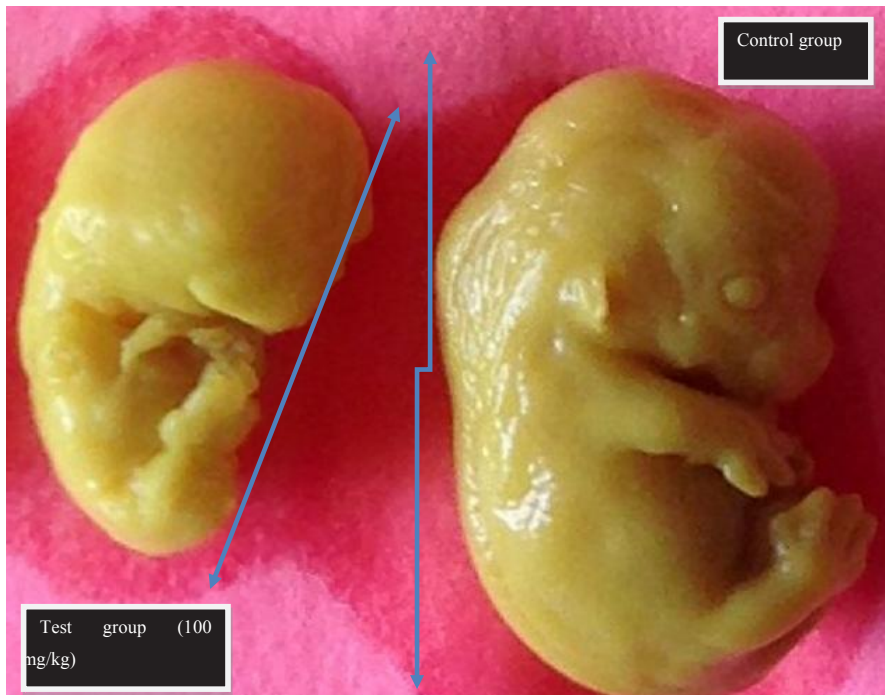
#### 2.4.8. Microscopic investigation

The stained sections were investigated microscopically and the fetal microscopic

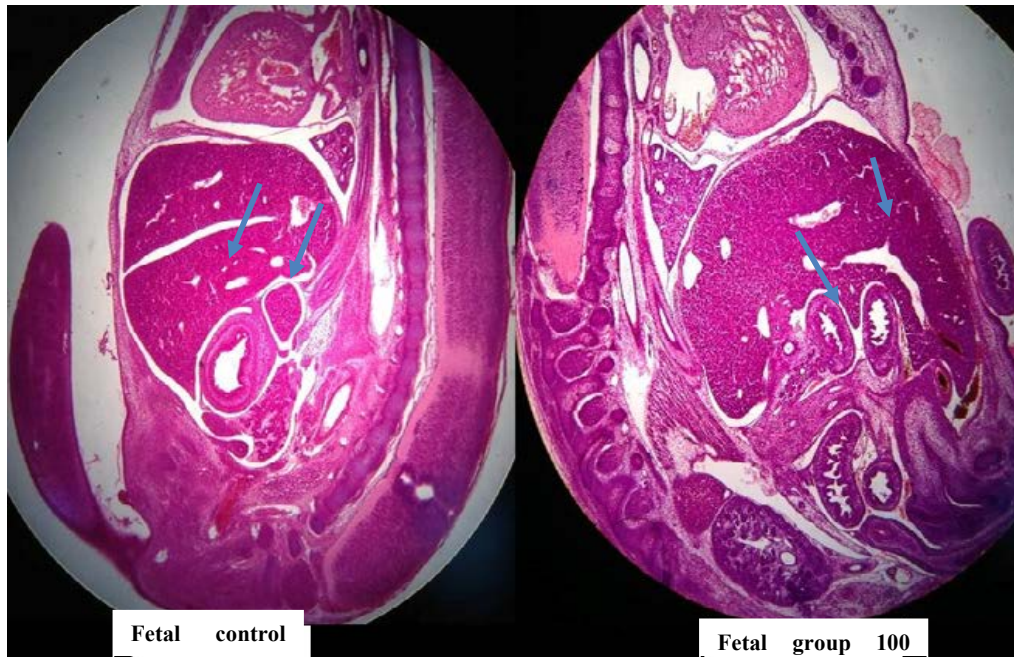
parameters that belong to sham, test and control group were compared. Parameters compared included weight of fetus, weight of placenta (measured by digital balance), placenta diameter and size of fetus C-R (Crown - Rump Length) measured microscopically by caliper.



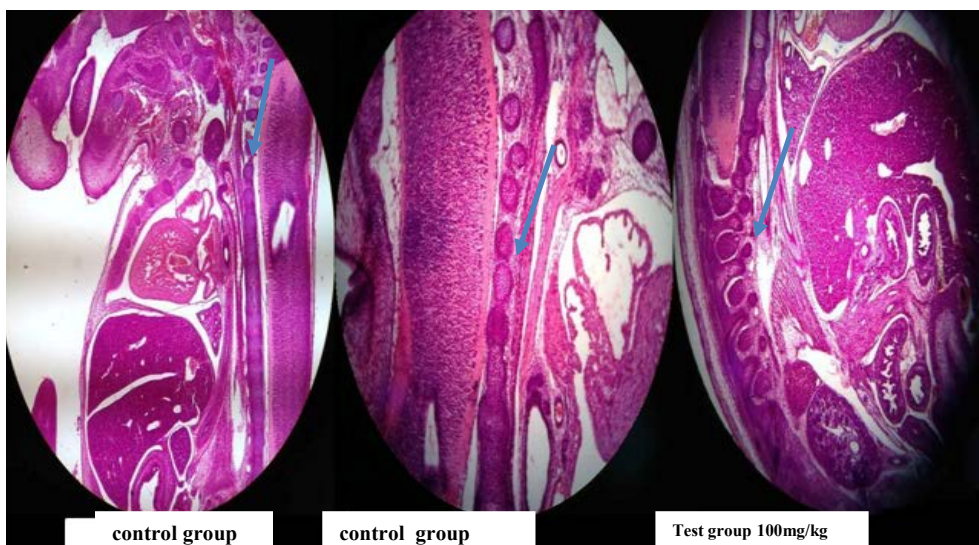
**Figure 5.** Comparison of fetal limbs in the test (mg/kg100) and control groups (40 x magnification).



**Figure 6.** Comparison of crown-rump length (CR) in both test (100 mg/kg) and control groups (40 x magnification).



**Figure 7.** Comparison of liver size and intestinal lobes location in both test (100 mg/kg) and control groups (100 x magnification).



**Figure 8.** Comparison of spinal cord vertebrae in both test (100 mg/kg) and control groups (100 x magnification).

### 2.5. Statistical analysis

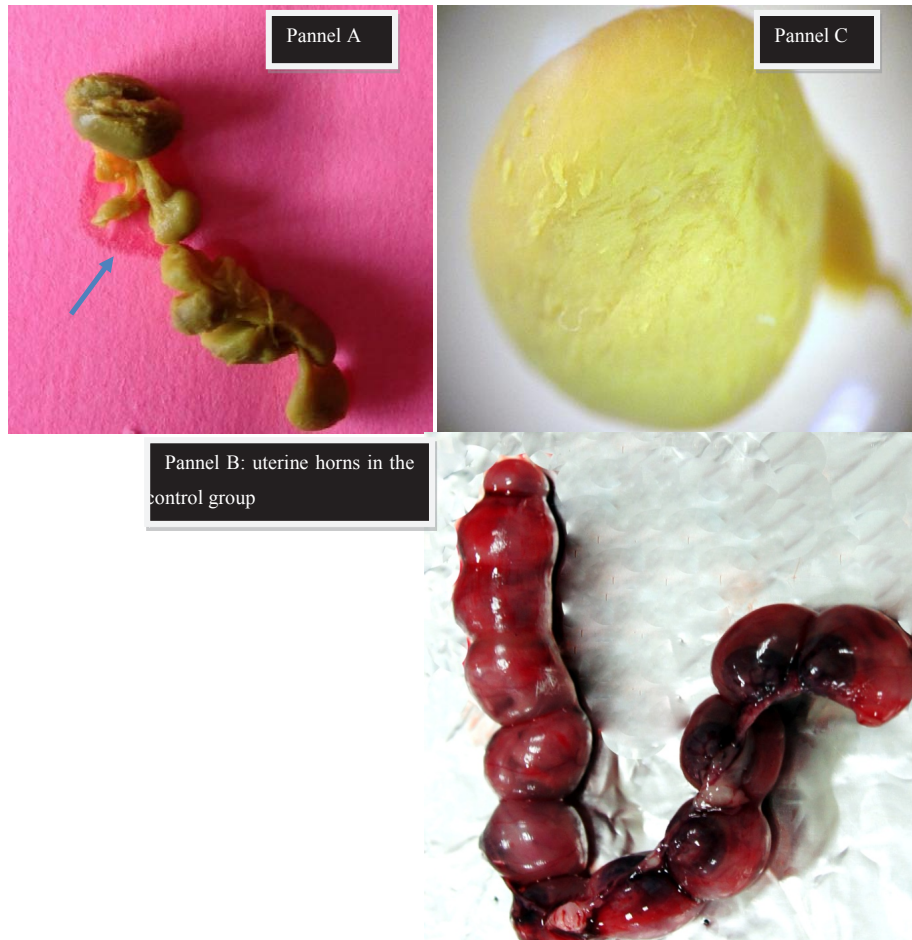
Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD as the post hoc test. Results were presented as mean $\pm$ S.D. of triplicate samples. The minimal level of significance chosen was  $p < 0.001$ .

### 3. Results and Discussion

In this research, the teratogenicity of Atorvastatin calcium on mouse fetus of

NMRI race were studied. Live fetuses were examined for external, visceral, and skeletal malformations and variations.

The photomicrographs were prepared from samples of test, Sham, and control groups. In the following, the important findings in only (100 mg/kg) atorvastatin treated group compared to sham & control groups are discussed. Due to atrophied nature of fetuses and lack of clear morphology in the higher (150 mg/kg) atorvastatin treated test



**Figure 9.** A comparison of normal mouse uterine in the control group with the mouse uterine in the atorvastatin treated (150 mg/kg) test group (40 x magnification). Panel A demonstrates one uterine horn in the atorvastatin treated test group (150 mg/kg) containing the atrophied fetuses. Panel B shows the normal uterine horns in the control group containing normal fetuses. Panel C shows one node of the uterine horn in control group.

group, we were not able to demonstrate morphological, histopathological and metrical data of this test group in the article figures. Besides, in the lower (50 mg/kg) atorvastatin treated test group, there was no significant change ( $p < 0.001$ ) in the morphological, histopathological, and metrical findings compared to control.

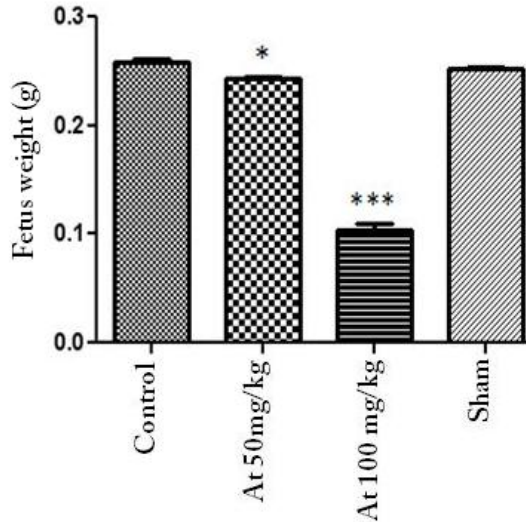
As shown in figure 1 (top left), cerebral cortex in the atorvastatin treated test (100 mg/kg) group has a curvy internalized shape compared to cerebral cortex in the control group.

As shown in figure 2 (see the arrows), the size of the fourth ventricle of the brain in the atorvastatin treated test group (100 mg/kg) is smaller than its normal size in the control group.

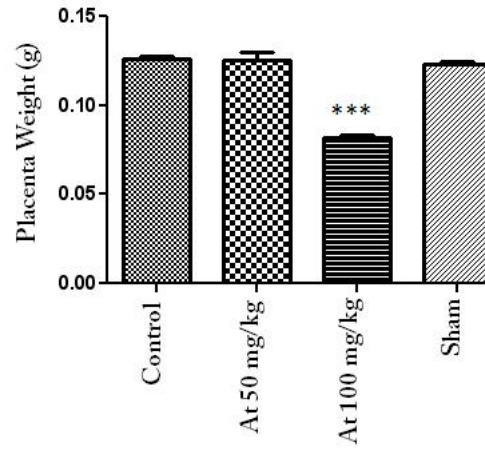
As shown in figure 3 (see the arrows), in contrast to control group the fetus head in the atorvastatin treated (100 mg/kg) test group is larger than trunk and the head/trunk ratio in the test group (100mg/Kg) is quite different compared to the normal condition in the control group.

As shown in figure 4 (see the arrows), appropriate grooves between fingers in the atorvastatin treated test group (100mg/Kg) were not appeared compared to the control group indicating that the normal fetus growth and development was delayed.

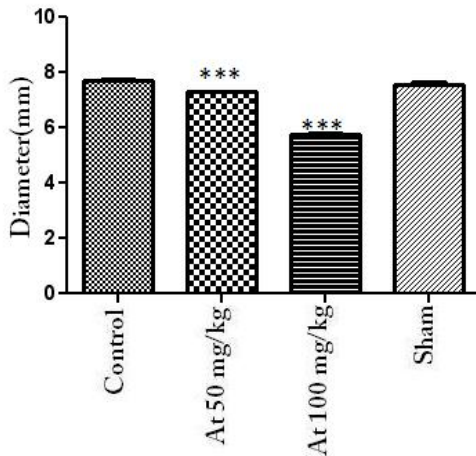
As shown by the arrows in figure 5, limbs (hands and feet) in the atorvastatin treated test group (100 mg/kg) look quite undeveloped compared to control group. This indicates an obvious delay in fetus growth in-



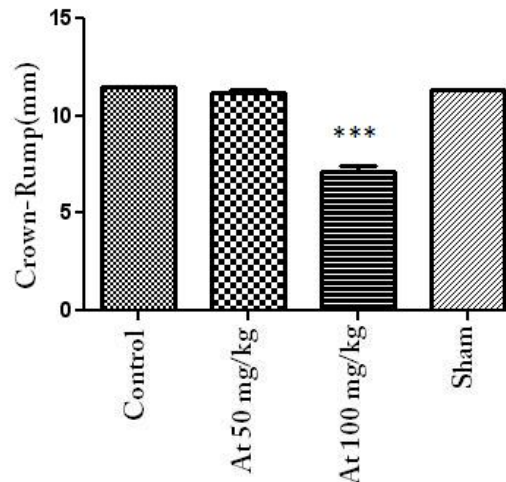
**Figure 10.** Comparison of weight of fetuse between the atorvastatin treated test group (150 mg/kg), sham and the control groups. Values represented as mean±SD (n=3). \*  $p < 0.001$  compared with sham and the control groups.



**Figure 11.** Comparison of weight of placentas between the atorvastatin treated test group (100 mg/kg), sham and the control groups. Values represented as mean±SD (n=3).



**Figure 12.** Comparison of diameter of placenta between the atorvastatin treated test group (150 mg/kg), sham and the control groups. Values represented as mean±SD (n=3).



**Figure 13.** Comparison of length of Fetuses (CR) between the atorvastatin treated test group (100 mg/kg), control and sham groups. Values represented as mean±SD (n=3). \*  $p < 0.001$  compared with sham and the control groups.

duced by atorvastatin in the test group.

As shown in figure 6, fetus crown-rump length (CR) and also weight in the atorvastatin treated test (100 mg/kg) group is less than the control group. Quite different to normal condition in the control group, the head was larger than trunk in the test (100 mg/kg) group.

As shown by the arrows in figure 7, Liver volume in the atorvastatin treated test group

(100 mg/kg) is bulkier than its normal expected size in the control group. Quite different to the control group, the normal boundary between the intestinal lobes and liver is absent and also intestinal lobes entered in the liver tissue in the test group (100 mg/kg).

As shown by the arrows in figure 8, the number of lumbar vertebrae in the fetus of atorvastatin treated test group (100 mg/kg) is apparently less than those of control group,

an obvious sign of atorvastatin induced spina befigda.

Figure 9 compares the normal uterine horns in the control group containing normal fetuses with the uterine horn in the atorvastatin treated test group (150 mg/kg) containing the atrophied fetuses. Due to atrophied nature of fetuses and lack of clear morphology in the (150 mg/kg) test group, we were not able to demonstrate morphological, histopathological and metrical data of this test group in the article figures.

In addition to morphological and histological findings, alterations in the length and weight of fetuses, weight and diameter of placentas were also determined. As shown in figure. 10, the total weight of fetus in the atorvastatin treated test group (100 mg/kg) were significantly ( $p<0.001$ ) decreased compared to control and Sham groups. As shown in figure11, the weight of placenta was apparently decreased in the atorvastatin treated test group (100 mg/kg) but this decrease was not significant ( $p<0.001$ ) compared to control and Sham groups.

As shown in the figure 12, placenta diameter in test group was increased but again this increase was not significant ( $p<0.001$ ) compared to control and Sham group.

As shown in figure 13, the length of fetus (size of crown-rump) was significantly ( $p<0.001$ ) decreased in the atorvastatin treated test group (100 mg/kg) compared to Sham and Control groups.

Complex lower-limb anomalies including both long- bone shortening and aplasia or hypoplasia of the foot structures were already reported in two simvastatin- exposed cases [11]. The infant in one of these cases and a lovastatin-exposed infant also had rare forms of the VACTERL association (i.e., three or more of the following findings: vertebral, anal, cardiac, tracheal, esophageal, renal, and limb defects) [11]. Our findings in the current research also confirms that limbs (hands and feet) in the atorvastatin treated test group (100 mg/kg) were quite undeveloped compared to the untreated control group. This indicates an obvious delay in fetal normal growth induced by atorvastatin. Our results also showed that the number of lumbar vertebrae in the fetus of atorvastatin treated test

group (100 mg/kg) were apparently less than those of the untreated control group, an obvious sign of atorvastatin induced vertebral defect, known as spina befigda.

Holoprosencephaly (HPE, once known as arhinencephaly) which already reported as an obvious sign in the (HMG-CoA) reductase inhibitors embryo-toxicity is a cephalic disorder in which the prosencephalon (the forebrain of the embryo) fails to develop into two hemispheres [13]. Normally, the forebrain is formed and the face begins to develop in the fifth and sixth weeks of human pregnancy. The condition also occurs in other species like rodents. The condition can be mild or severe. According to the National Institute of Neurological Disorders and Stroke (NINDS), in most cases of holoprosencephaly, the malformations are so severe that babies die before birth. When the embryo's forebrain does not divide to form bilateral cerebral hemispheres (the left and right halves of the brain), it causes defects in the development of the face and in brain structure and function [14]. Holoprosencephaly and the VACTERL association have been linked to inhibition of cholesterol biosynthesis, down-regulation of the cholesterol dependent sonic hedgehog morphogenetic pathway, or both of these mechanisms. These malformations as well as neural-tube and cardiac defects are also associated with maternal diabetes. Thus, diabetes might confound the association between statin use and these malformations. However, maternal diabetes was identified in only 7 of 178 case reports and 1 of 20 cases of malformation (spina bifida) [15]. Our findings are also somehow in agreement with previous reports, since we figured out that size of the fourth ventricle of the brain in the atorvastatin treated test group (100 mg/kg) was smaller than its normal size in the control group. We also found that cerebral cortex in the atorvastatin treated test (100 mg/kg) group had a curvy internalized shape compared to cerebral cortex in the control group. These 2 unique outcomes indicate a serious defect in the development of the brain structure. We also showed out that the number of lumbar vertebrae in the fetus of atorvastatin treated test group (100 mg/kg) is apparently less than those of control group, an obvious

sign of atorvastatin induced spina bifida.

#### 4. Conclusion

In the morphological and macroscopic point of view, we got some findings in this research which is quite novel including;

1) Cerebral cortex in the atorvastatin treated test (100 mg/kg) group had a curvy internalized shape compared to cerebral cortex in the control group.

2) Size of the fourth ventricle of the brain in the atorvastatin treated test group (100 mg/kg) was smaller than its normal size in the control group.

3) In contrast with control group, the fetus head in the atorvastatin treated (100 mg/kg) test group was larger than trunk and the head/trunk ratio in the test group (100mg/Kg) is quite different compared to the normal condition in the control group.

4) The appropriate grooves between fingers in the atorvastatin treated test group (100mg/Kg) were not appeared compared to the control group indicating that the normal fetus growth and development was delayed.

5) Liver volume in the atorvastatin treated test group (100 mg/kg) was bulkier than its normal expected size in the control group. Quite different to the control group, the normal boundary between the intestinal lobes and liver as well as intestinal lobes entered in the liver tissue in the test group (100 mg/kg) were absent.

#### Acknowledgments

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#### References

[1] Henck J W, Craft WR, Black A, Colgin J, Anderson JA. Pre- and Postnatal Toxicity of the HMG-CoA Reductase Inhibitor Atorvastatin in Rats. *Toxicological sciences* 1998; 41:88-99.

[2] Kazmin A, Garcia-Bournissen F, Koren. Risks of Statin Use during Pregnancy: A Systematic Review. *J Obstet Gynaecol Can* 2007; 29(11):906-908.

[3] Topol EJ. Intensive statin therapy—a sea changes in cardiovascular prevention. *N Engl J Med* 2004; 350:1562-4.

[4] Henck JW, Craft WR, Black A, Colgin A, Anderson JA. Pre- and postnatal toxicity of the HMG-CoA reductase inhibitor atorvastatin in rats. *Toxicol Sci* 1998; 41:88-99.

[5] Hrab RV, Hartman HA, Cox RH. Prevention of fluvastatin-induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology* 1994; 50:19-29.

[6] Sandoz Canada Inc, Astra Pharma Inc. Pharmacokinetics. In: Product monograph. Lescol: fluvastatin sodium. Dorval, Que, Mississauga, Ont: Sandoz Canada Inc. Astra Pharma Inc 1993:1-11.

[7] Minsker DH, MacDonald JS, Robertson RT, Bokelman DL. Mevalonate supplementation in pregnant rats suppresses the teratogenicity of mevulinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Teratology* 1983; 28:449-56.

[8] Wise LD, Prahalada S, Robertson RT, Bokelman DL, Akutsu S, Fujii T. Simvastatin (MK-0733) oral teratogenicity study in rabbits. *Yakuri to Chiryō* 1990; 39(2):159-67.

[9] Minsker DH, Robertson RT, Bokelman DL. Simvastatin (MK-0733): oral late gestation and lactation study in rats. *Yakuri to Chiryō* 1990; 39(2):169-79.

[10] Manson JM, Freyssinges C, Ducrocq MB, Stephenson WP. Postmarketing surveillance of lovastatin and simvastatin exposure during pregnancy. *Reprod Toxicol* 1996; 10(6):439-46.

[11] Dostal LA, Schardein JL, Anderson JA. Developmental toxicity of the HMG-CoA reductase inhibitor, atorvastatin, in rats and rabbits. *Teratology* 1994; 50(6):387-94.

[12] Di Renzo F, Broccia ML, Giavini E, Menegola E. Antifungal triazole derivative triadimefon induces ectopic maxillary cartilage by altering the morphogenesis of the first branchial arch, *Birth Defects Res B Dev. Reprod Toxicol* 2007; 80: 2-11.

[13] Taguchi N, Rubin E T, Hosokawa A, Choi J, Ying A Y, Moretti M E., Shinya Ito G K. Prenatal exposure to HMG-CoA reductase inhibitors: Effects on fetal and neonatal outcomes. *Reprod Toxicol* 2008; 26: 175-177.

[14] Totori-Donati P, Rossi A, Biancheri, R. In C. Pediatric Neuroradiology: Brain, Head, Neck and Spine, Brain Malformations; Totori-Donati, Paolo; Rossi, Andrea; Raybaud, Springer, 2005; pp, 92-95.

[15] Edison R J, Muenke M. Central nervous system and limb anomalies in case reports of first trimester statin exposure. *New England Journal of Medicine* 2004 ; 350: 1579-1582.

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