Evaluation of the Teratogenic Potentials of Muscle Relaxant Myolginin Albino Rats

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ABSTRACT---- ***Teratology is the study of abnormal development in embryos, and the causes of congenital malformation or birth defects. Musculoskeletal conditions are very common to cause tenderness and muscle spasms as fibromyalgia, tension headaches, myofascial pain syndrome, and mechanical low back or neck pain. Skeletal muscle relaxants are one of several classes of medications such as anti-inflammatory drugs and pain relievers that are used to treat these conditions.However insufficient information is available regarding its safety during pregnancy. Therefore, this work was initiated to study the effect of prenatal exposure of mylogine on fetuses of female rats. The study was conducted on pregnant rats to observe the safety profile ofmylogine in comparison to control. Pregnant albino rats (Rattusnorvegicus) were administrated during organogenesis period with therapeutic dose. Fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformation as well as histological study of liver, kidney and brain. Results showed significant reduction in placental weight of pregnant rats treated with mylogine. Fetal growth retardation during gestational period was recorded also some skeletal anomalies were observed. These abnormalities included weak ossification of the skull bones roof and bones forming girdles and limbs. Histopathological studies of fetuses during gestation revealed changes in liver histology such as presences ofclumping of the hepatocytes with hyperchromaticnuclei and increases in the number of megakaryocytes were seen, kidney tissue revealed numbers of mitotic activity in the nuclei of the tubular lining epithelium, coagulative necrosis in the lining tubular epithelium of the proximal convoluted tubules at the cortexand the cerebrum showed severe congestion in the meningeal blood vessels.Our findings suggest the need for great caution to handle mylogine especially during pregnancy.***

Keywords--- Muscles relaxants, Teratogenicity, Gestation and Albino Rats

1. INTRODUCTION

The study of these malformations isknown as teratology. Teratogens are the factors that influence and cause such abnormalities **(Sadler 2000).**Teratogenic agents cause approximately 7% of congenital malformations. A teratogenic agent is a chemical, infectious agent, physical condition, or deficiency that, on fetal exposure, can alter fetal morphology or subsequent function. Teratogenicity depends upon the ability of the agent to cross the placenta. The most vulnerable period for malformation to take place is the period of organogenesis. Agents given duringthis period are more likely to cause birth defects. This critical time of fetal development in rats and mice is from 6-12 days of their gestation **(Somer, 1962; Farris, 1967**).
Almost all therapeutic agents cross placental barrier **(Schlegel *et al*. 1991)** and enter fetal circulation. Every agent given during pregnancy, therefore, is likely to produce some sort of structural abnormality until proved otherwise.There is little information available on the safety of myloginusage during pregnancy.

Myolginis indicated for the relief of severe skeletal muscle spasm and pain associated with such medical and orthopaedic problems as: sprains and strains, myalgias, torticollis, tension headaches, traumatic muscle injuries, low back pain, fibrositis, cervical root and disc syndromes. The objective of this study is to assess the teratogenic risk of Myolgin on albino rat fetuses during gestation.

2. MATERIALS AND METHODS

Experimental animals

The present experimental study is carried out on the albino rat (*Rattusnorvegicus*). The standard guidelines of the Institutional Animal Care and Use Committee (IACUC) were used in handling animals.

Females of 11-13 weeks old were selected for the present study and vaginal smears were prepared every morning and examined under light microscope (according to the method of **Snell (1956)**) for 5 days to select the female with regular estrus. Two females with regular estrus cycle were selected in the pro-estrus stage and caged together with one male overnight under controlled environmental conditions of temperature, humidity and light. The first day of gestation was determined by the presence of sperms in the vaginal smear **(McClain & Becker, 1975)**.

Experimental design

Myolgin was purchased from UCB pharmaceutical sector (Chemin du Foriest, Belgium). The route of administration was oral. The time of administration was scheduled from the 5th day of gestation, daily until the end of gestation.

Experimental groups

Group A: Control group received distilled water from 5th day of gestation to 19th day of gestation. (Eight female rats)

Group B: Treated group received 500mg/kg of Myolginfrom the 5th day of gestation, daily until the end of gestation. (Twelve female rats)

Developmental observations

On the 20th day of gestation, all pregnant rats of groups A and B were sacrificed and total implantation sites, fetal mortality rate (resorped or still birth) and living fetuses were recorded. Fetal body weight, body length, tail length and external malformation were also recorded.

Skeletal examination

Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (blue) and bone (red) according to the method described by **Deinz*et al.*, (1995)**.

Histological examination

Autopsy samples were taken from liver, kidney and brain of fetuses in different groups on the 20th day of gestation. They were fixed in 10% formal saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 °Cin hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin& eosin stain for routine examination then examination was done through the light electric microscope **(Bancroft*et al*., 2002).**

3. RESULTS

A. Morphological studies

The average weight of placenta of all treated pregnant rats groups decreased as compared to control (Table 1& Fig. 1). There was significant ($P \leq 0.05$) reduction in the placental weight of animals that received Myolgin.

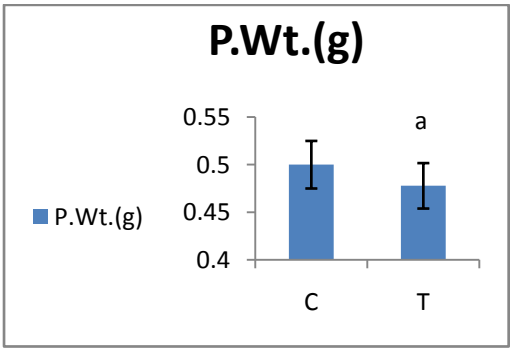


Fig. 1: Histogram showing effect of Mylogin on placenta weight (P.W) at 20th day of gestation.

Values are expressed as mean. The statistical differences were analyzed by Duncan's test. a= P ≤ 0.05 compared with control.

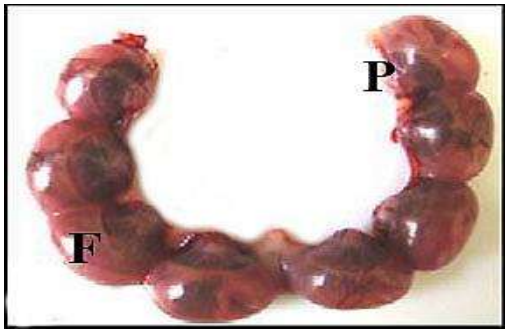
The uterus of control pregnant rats on day 20 of gestation showed normal distribution of the implanted fetuses between the two horns (Fig. 2). The uterus of pregnant rats treated with 500mg/Kg showed asymmetrical distribution of fetuses in the two uteri, completely resorbed fetuses were also observed (Fig. 3).

Table 1: Showing effect of Mylogin on fetus weight, fetus length, tail length and placenta weight on the 20th day of gestation.

Group	Fetus weight (F.WT)	Fetus length (F.L)	Placenta weight (P.WT)
Control (A)	5.00± 0.130	6.00± 0.081	0.500± 0.000
500 mg/Kg (B)	4.433± 0.074	2.90± 0.000	0.477± 0.049

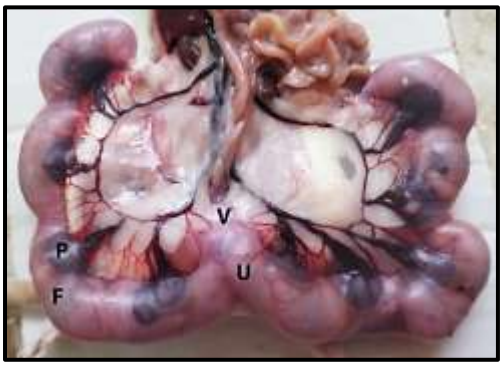
Values are expressed as Mean. The statistical differences were analyzed by Duncan's test. a= P ≤ 0.05 compared with control.

Fig. 2: A photograph of uterus of control pregnant rat on the 20th day of gestation.



Showing normal symmetrical distribution of fetuses in the two uteri horns.
P=Placenta, F=Fetuses

Fig. 3: A photograph of uterus of pregnant rat treated with 500mg/Kg of Myolginon the 20th day of gestation.

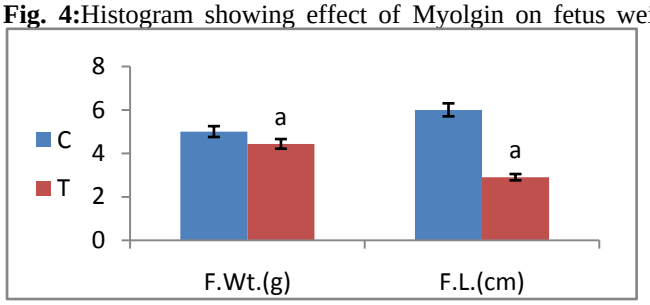


Showing normal shape uterus (U) with symmetrical distribution of fetuses (F).

P= Placenta and V= Vagina.

The morphological examination of the fetuses showed that the Myolgincaused growth retardation represented by adecrease in fetal body weight and body length (Table 1 &Fig. 4). There was a significant (P≤ 0.05) reduction in fetus weight and fetus length in treated groupwhen compared with the control group (A).

Fig. 4: Histogram showing effect of Myolgin on fetus weight (F.W) and fetus length (F.L) at 20th day of gestation.



Values are expressed as mean. The statistical differences were analyzed by Duncan's test. a= P ≤ 0.05 compared with control.

The fetus from control animals appeared with normal shape, correct weight and length (Fig. 5), also appeared straight dorsally. The malformations are not recorded in fetuses from treated group (Fig. 6).

Fig. 5: A Photograph of fetus of control mother on 20th day of gestation.Fetus exhibited normal morphology and normal length.

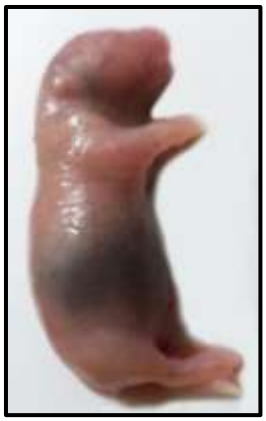


Fig. 6: Photographs of fetuses of maternally treated with 500mg/Kg of Myolginon 20th day of gestation.

Fetal mortality:
Total mortality rate included resorbed fetuses and stillbirth (dead fetuses at birth) and was recorded for both control and treated groups in table 2.

Table 2: Effect of Myolginon fetal mortality on the 20th day of gestation.

Groups	Total no. of pregnant rats	No. of implantation sites	No. of live fetuses		No. of resorbed fetuses		No. of dead fetuses	
Control (A)	6	54		100%	0	0 %	0	0 %
Treated Groups (500 mg/Kg) (B)	4	24	6	25 %	18	75 %	0	0 %

Values are expressed as Mean ± SEM. The statistical differences were analyzed by independent samples T test. a= $P \leq 0.05$ compared with control.

Skeletal anomalies
On the 20th day of gestation, the cleared cartilage and bone preparations of control rat fetuses have designated that in all parts of the axial skeleton skull, vertebrae and ribs as well as appendicular skeleton comprising the fore and hind limbs, pectoral and pelvic girdles, both chondrification and ossification processes have been obviously completed. The cartilaginous parts of the skull included the nasal region (Fig. 7). On the other hand, fetuses maternally treated with 500mg/Kg of Myolginshowed lack of ossificationof the skull roof (frontal and parietal), delay ossification of the fore and hind limbs, pectoral and pelvic girdles, vertebrae and ribs(Fig. 8).

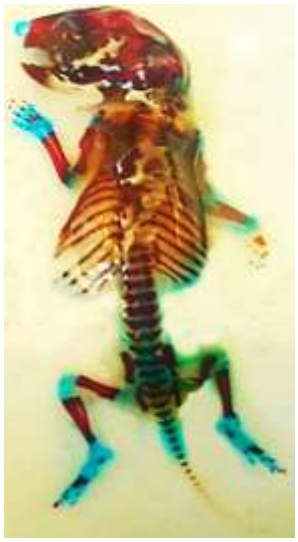


Fig. 7: A Photomicrograph of a skeleton of a fetus from control mother. Double stain.

Showing completeossification in all parts of the axial and appendicular skeleton.

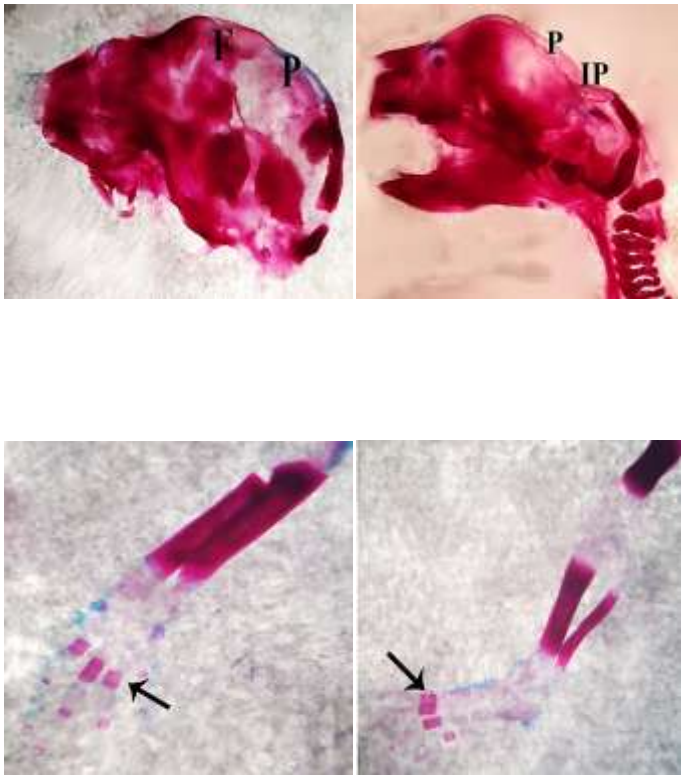


Fig. 8: A Photomicrograph of a skeleton of a fetus from treated mother. Double stain.

Showinglack of ossificationof the skull roof (frontal and parietal), delay ossification of the fore and hind limbs, pectoral and pelvic girdles, vertebrae and ribs.

Histological studies of fetuses

Liver

Light microscopic examination of sections of the fetal livers of controlgroup using H&E stain showed ill-defined demarcation of hepatic lobules andthe interlobular connective tissue was poorly developed. In each hepatic lobule, hepatocytes were arranged as irregular, branching and interconnected cords originating from a central vein and goes peripherally. Blood sinusoids were seen between the hepatic cords. The cytoplasm was abundant, granular and stained acidophilic. The nucleus was euchromatic, located centrally, rounded, and contained one or more nucleoli (Fig. 9).

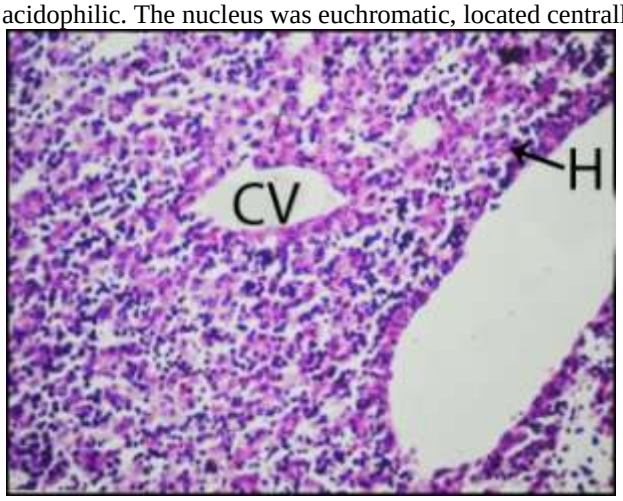


Fig. 9: A Photomicrograph of a section of liver of a fetus from control mother. H&E stain.Showing normal architecture of the liver tissue, the hepatic lobules that can be only distinguished by their central veins (CV) and hepatocytes (H) and numerous erythroblasts (arrow).100X

In liver tissuemultiple numbers of multinucleated megakaroblastswere detected in between the degenerated hepatocytesin the groups receiving Myolgin during the pregnancy.Yellow pigments were detected in between the hepatocytes(Fig. 10).

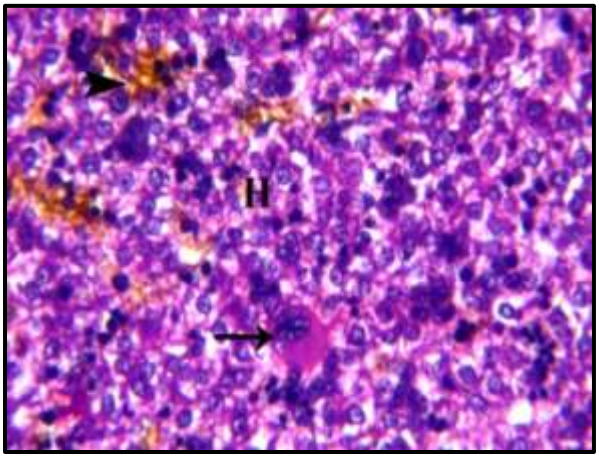


Fig. 10:A Photomicrograph of a section of liver of a fetus from treated mother. H&E stain. Megakaryoblasts (arrow) were detected in between the degenerated hepatocytes (H) in diffuse manner all over the parenchym and yellow pigments (head arrow) were detected in between the hepatocytes (H).400X

Kidney

Examination of the kidney of control fetus revealed that it is differentiated in to outer cortex and aninner medulla, which is formed of conicalpyramids. Each medullary pyramidwith the corresponding part of the cortexrepresents a renal lobe which consists of theuriniferous tubules and stromal tissue.The uriniferous tubule is composed of thenephron which is formed of the Malpighiancorpuscle, the proximal convoluted tubule, thedescending and the ascending limbs of Henle'sloop and the distal convoluted tubule. Eachcorpuscle consists mainly of a tuft of bloodcapillaries, or glomerulus and a Bowman'scapsule which is a double walled cup formedof two layers, an outer parietal layer and aninner visceral layer separated by a clear, distinctcapsular space (urinary space) (Fig. 11).

Examination of the kidney of fetus maternally treated with 500mg/Kg showed numbers of mitotic activity in the nuclei of the tubular lining epithelium as well as the nuclei of the endothelial cells lining the glomerular tuftsin Fig. 12.

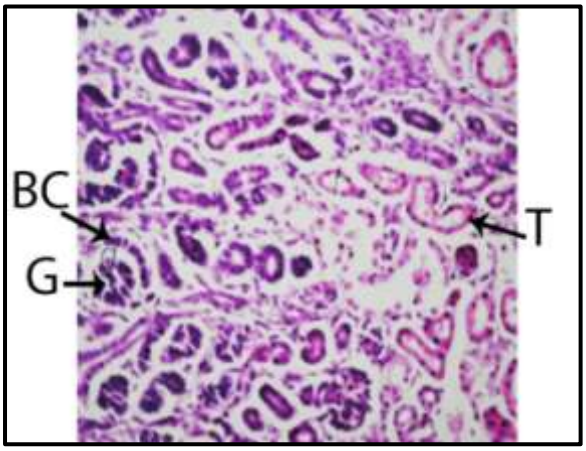


Fig. 11:A Photomicrograph of a section of kidney of a fetus from control mother. H&E stain.Showing a part of the cortical region containing, a glomeruli (G) within Bowman's capsule (BC) tubules (T).100X

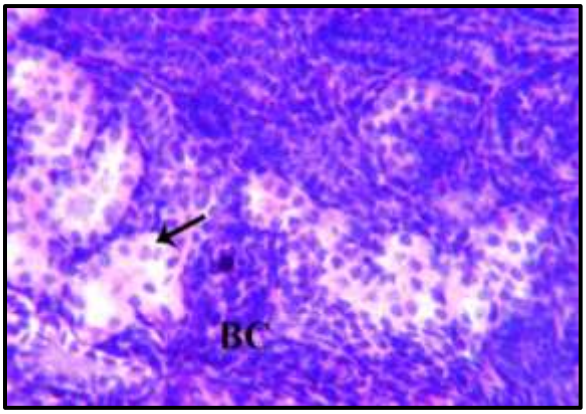


Fig. 12:A Photomicrograph of a section of kidney of a fetus from treated mother. H&E stain. There was degeneration in the tubular lining epithelium (arrow).

BC= Bowman's capsule. 400X

Brain

The brain tissues of fetuses from control pregnant rats showed normal features under microscopic observation (Fig. 13). Examination of the brain of fetus maternally treated with 500mg/Kg, the cerebrum showed ill developed wall of the blood vesselsas recorded in Fig. 14, leading to the picture of severe congestion of the meningeal vessels.

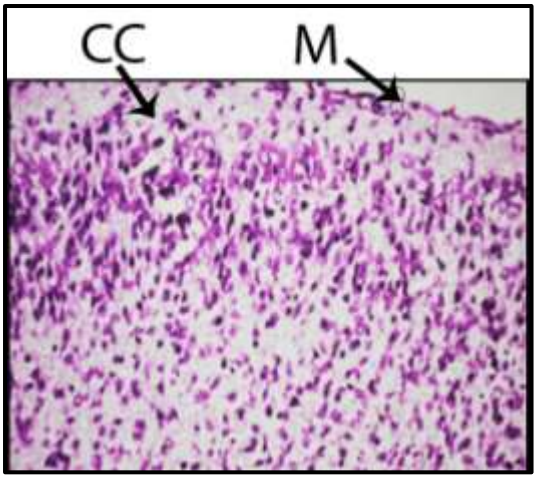


Fig. 13: A Photomicrograph of a section of brain of a fetus from control mother. H&E stain.Showing normal structure of brain tissue, CC= cerebral cortex.400X

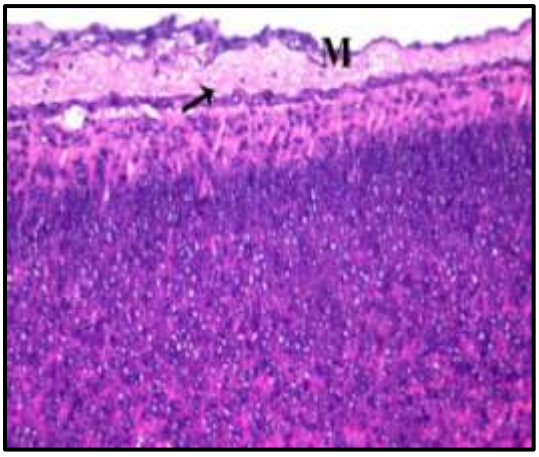


Fig. 14: A Photomicrograph of a section of brain of a fetus from treated mother. H&E stain.Sever congestion was detected in the meningeal blood vessels(arrow). M= mening. 100X

4. DISCUSSION

The present study is carried out to evaluate the teratogenic potential of Myolgin on the fetuses maternally treated with 500mg/Kg of the used drug during the gestation.

Teratology, the study of abnormal prenatal development and congenital malformations induced by exogenous chemical or physical agents, is a growing area of medical research in the quest for the eradication of preventable birth defects. Birth defects are known to occur in huge numbers; roughly 7∼10% of all children requiring extensive medical care to diagnose or treat a birth defect; this compromises the quality of life of millions of people worldwide **(O'Rahilly, 2001).** Almost all therapeutic agents cross placental barrier and enter fetal circulation. Every agent given during pregnancy therefore has a tendency to produce some sort of structural abnormality in the neonate at birth until proved otherwise **(Schlegel *et al.*, 1991)**. A birth defect or a congenital malformation is a structural abnormality of any type present at birth. It may be macroscopic or microscopic, on the surface or within the body **(Moore, 1988).** During the past few decades, it has become increasingly evident that human and animal embryos are subjected to the toxic effects of many drugs, such as the use of some antibiotics in the treatment of serious diseases occurring during pregnancy.

In the present study, oral treatment of pregnant rats with Myolginduring the gestation led to a significant reduction in the weight, length and tail length of fetuses. The observed fetal growth retardation may be arising from the direct action of the used drug on embryos and fetal tissues.

The present work showed no cases of external malformations and revealed skeletal abnormalities as un-ossified skull bones and weakossification of phalanges.

The results obtained from the present study showed that administration of the therapeutic doses in organogenesis period induced various changes in liver and kidney of fetuses maternally treated with 500mg/kg of Myolginfrom 5th day to 20th day of gestation. Liver showed degeneration of the cytoplasm of hepatocytes and increase in the number of megakaryocytes. The fetal liver assumes the primary role of blood cell development at mid- and late-gestation. The histopathological changes in liver that were observed with drug treatment may affect haematopoietic cell trafficking from the liver to other sites. Kidney revealed degeneration in the tubular lining epithelium with swelling in the endothelial cells lining the tufts of the glomeruli within the Bowman's capsule.

5. CONCLUSION

It was evident that the use ofMyolgin in rat females during the "critical period" of gestation caused fetal growth retardation and histopathological alternations in main fetal tissues. Therefore, more scientific and clinical knowledge in the first trimester of gestation are needed to reduce macro- and microscopic alteration in fetuses.

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