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HEPATOTOXIC CHANGES INDUCED BY PRENATAL ADMINISTRATION OF ZIDOVUDINE IN SWISS ALBINO MICE

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ABSTRACT

Introduction – Zidovudine is the first anti retroviral agent approved by FDA for treatment of HIV. Now a day it is used along with other anti retro viral agents to prevent maternal to child transmission. But its safety margin is yet to be ascertained.

Materials and methods – Pregnant albino mice was given zidovudine in the dose of 50mg/kg, 100mg/kg, 50mg/kg by oral gavage from 6th - 16th day of gestation and control mice was fed distilled water during the same period. On day 17th maternal blood was collected for liver function test. On day 19th the mice was sacrificed and foetuses were taken out .The liver of foetuses were dissected, formalin fixed, processed and stained with H&E and PAS for histological study.

Results- The treated foetal liver shows fatty degeneration, hepatocytic degradation and loss of cytoarchitecture in a dose dependent manner. The serum bilirubin and transaminases were increased and serum albumin was decrease in dams denoting toxic effect of this drug on the mother.

Conclusion- Zidovudine causes hepatoitoxicity in both mother and fetus if given to mother during pregnancy and should be used with caution in pregnancy.

Zidovudine is a nucleoside reverse transcriptase inhibitor (NRTIs) which is widely used in the treatment of patients infected with Human Immuno deficiency Virus (HIV). It is a prodrug which must be phosphorylated to Zidovudine triphosphate by thymidilate kinase which is its active constituent. Zidovudine is also used as a constituent in combination chemotherapy to prevent maternal to child transmission (MTCT) of HIV. [1, 2]

In mammalian embryo the entire gestational period is divided into two parts, embryonic period and fetal period. The embryonic period is further subdivided into pre-organogenesis period and organogenesis period. The pre-organogenesis period coincides with first two weeks of embryonic life and any toxic insult at this stage might lead to

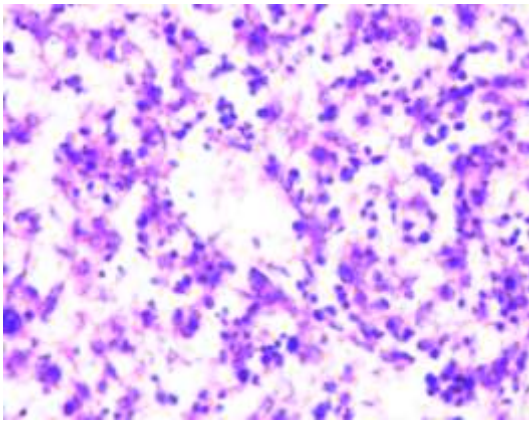

Figure 1. Control liver showing central vein, scattered hepatocytes with developing sinusoids, progenitor cells and developing portal triad. (H&E x400)

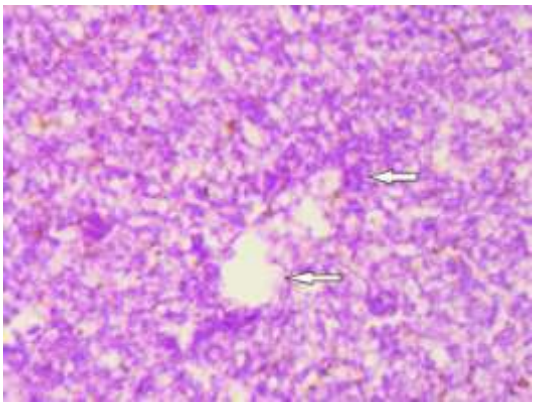

Figure 2. Control liver showing central vein and hepatocytes containing purple stained glycogen granules (→). (PAS X400)

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the death of the conceptus. The organogenesis period corresponds to third to eighth week of intra-uterine life. This period is susceptible to any injury leading to congenital malformation or even death depending upon the intensity of insult. [3,4]

Animal studies have shown rapid placental transfer of zidovudine and it even crosses human placenta. As it inhibits DNA replication and retards cell division so it may damage early embryonic development. Zidovudine has been found to be genotoxic in humans as it exerts a dose related cytotoxic effect, causes metabolic disruption and mitochondrial DNA depletion on human cells in-vitro. It has been classified as class- C drug by FDA which means that it has been found to be safe in animals but studies in human beings are inconclusive.[5]

Use of zidovudine during pregnancy is a dilemma faced by the physician as the safety profile of this drug has not yet been established. So, in the present study we intend to observe the toxic effect of zidovudine on liver of the embryo when given to the mother during pregnancy.

MATERIALS AND METHODS

Prior approval of institutional ethical committee was taken before the start of the present study. For this study swiss albino female mice were taken and were kept with male mice for mating overnight in the ratio of 3:1. Presence of vaginal plug was considered to be the first day of gestation (GD 0). The pregnant female mice were divided into four groups for the present study.

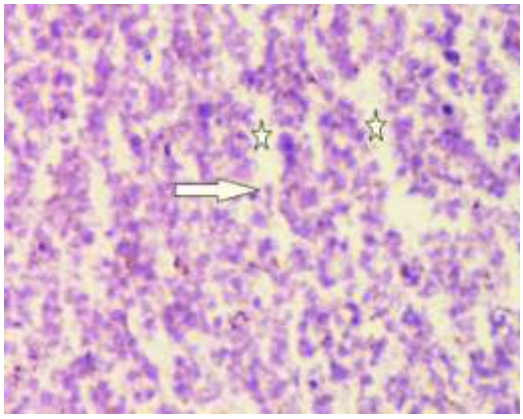

Figure 3. 50mg/kg Zidovudine treated liver showing loss of hepatocytes and progenitor cells (→) and irregular sinusoids and central vein development (*). (H&E x400)

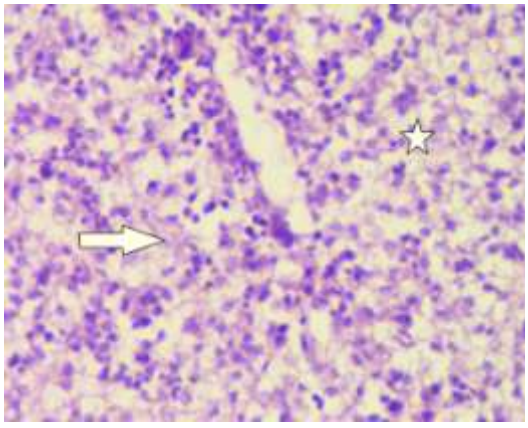

Figure 5. 100mg/kg Zidovudine treated liver showing diminished cellularity and fatty degeneration (*) and presence of cellular debris (→). (H&E x400)

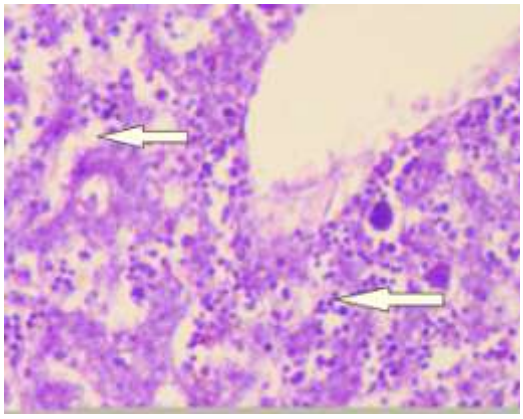

Figure 4. 50mg/kg Zidovudine treated liver showing loss of hepatocytes with deficient glycogen content and ill demarcated central vein and sinusoidal pattern (→).(PAS x400)

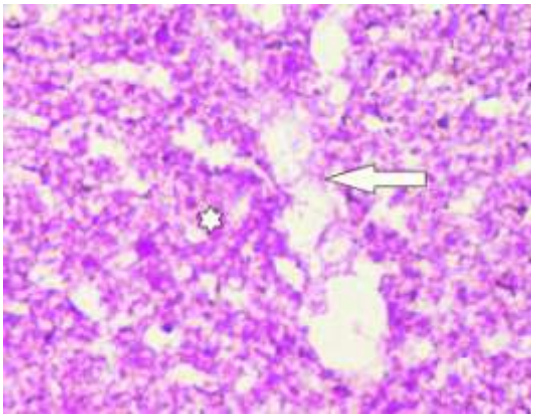

Figure 6. 100mg/kg Zidovudine treated liver showing degenerated hepatocytes (*) and empty lacunar spaces (→). (PAS x400)

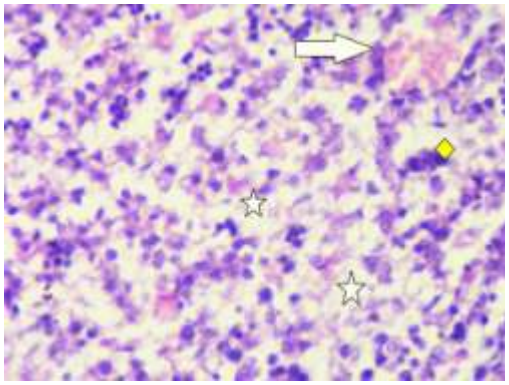

Figure 7. 150mg/kg Zidovudine treated liver showing dilated engorged central veins (→), marked hepatocytic necrosis (*) and lacunar spaces (◊). (H&E x400)

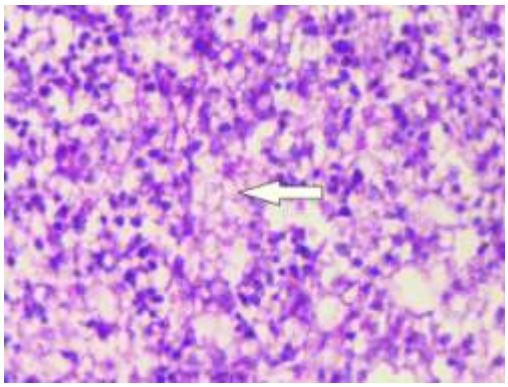

Figure 8. 150mg/kg Zidovudine treated liver showing highly deficient hepatocyte with lacunar spaces (→). (PAS x400)

The first group was designated as control and was given tap water by gavage from day6 to 16 of gestation. The other three groups were designated as treated and were given zidovudine in the dose of 50mg/kg, 100mg/kg and 150mg/kg respectively by gavage for the same period. Maternal blood (1ml) was obtained from retro-orbital vene puncture to evaluate the hepatic transaminases in it on day 17th of gestation. On day 18th of gestation the female mice was sacrificed by cervical dislocation and uterotomy was done to extract the embryos. The liver of the embryos were dissected out and kept in formalin for fixation. For histological study the liver was processed, sections were cut at 8µm and stained with hematoxylin and eosin (H&E) as well as periodic acid Schiff (PAS).

RESULTS

a. Low dose (50mg/kgbw)

The treated liver of this group shows loss of hepatocytes and haemopoetic stem cells. The granulocyte cell concentration in subcapsular and perivascular areas is also diminished. The sinusoid pattern between the hepatocyte cords is less demarcated. On PAS staining glycogen content is reduced. (Fig1,2)

b. Medium dose (100mg/kgbw)

Medium dose treated liver shows marked pathological changes. Hepatocytic cellularity is further decreased and there is an appreciable fatty degeneration in them.

There are empty lacunar spaces seen due to degeneration of hepatocytes which are present as cell debris. The haemopoetic stem cells are further decreased as compared to the low dose group and the megakaryocytic population is also diminished. The granulocytes are less appreciated as compared to low dose and they are scattered randomly in the liver parenchyma. PAS staining depicts further reduction in glycogen content in hepatocytes. (Fig 5,6)

c. High dose (150mg/kgbw)

In the high dose treated liver, the entire cyto-architecture of the liver is lost. There is marked necrosis of hepatocytes with pyknotic nuclei and heavy cell debris. Degeneration of hepatocytes has given an empty lacunar space inside the liver parenchyma which is giving a spongiform

TABLE-1 : Values of different variables of Liver Function Test of the dams.

Variable	Control (gr-1) Mean (*SD*)	Group-2 (ZDV,50mg/kg) Mean (*SD*)	Group-3 (ZDV,100mg/kg) Mean (*SD*)	Group-4 (ZDV,150mg/kg) Mean (*SD*)	F value
Bilirubin (Total)	0.60 (*1.00*)^{A}	9.94 (*0.12*)^{B}	10.72 (*0.01*)^{C}	14.18 (*0.01*)^{D}	27692.12***
Total Protein	43.20 (*0.84*)^{A}	12.40 (*0.07*)^{B}	11.04 (*0.11*)^{C}	10.16 (*0.05*)^{D}	7124.80***
Albumin	21.40 (*0.55*)^{A}	4.32 (*0.08*)^{BBB}	3.88 (*0.13*)^{BBB}	3.82 (*0.45*)^{BBB}	4643.04***
SGOT (AST)	185.60 (*0.55*)^{A}	75.08 (*1.07*)^{B}	335.00 (*1.00*)^{C}	251.40 (*0.55*)^{D}	87992.19***
SGPT (ALT)	45.40 (*0.55*)^{A}	2.04 (*0.11*)^{B}	18.60 (0.55)^{C}	5.94 (*0.09*)^{D}	12359.475***

***** p < 0.000 , $^{A, B, C, D}$ - Common superscripts show no significant difference (Post hoc using Tukey HSD)**

appearance. The haemopoetic stem cells are fewer in number and granulocytic population is extremely low. There is presence of negligible glycogen on PAS staining. (Fig 7,8)

Liver Function Test:

Serum bilirubin and hepatic transaminases were significantly raised in a dose related manner in the treated group. There was a significant drop in the serum levels of total protein and albumin. (**Table-1**)

DISCUSSION

The main toxicity of Zidovudine results from its ability to cause mitochondrial DNA depletion resulting in mitochondrial myopathy causing lactic acidosis in association with hepatic abnormality including raised liver enzymes and hepatic failure.[6]

Zidovudine causes raised creatine kinase and hepatic kinase level in the serum of a HIV infected male who had been on zidovudine therapy. On liver biopsy macrovacuolar and microvacuolar degeneration of hepatocytes were seen. It was suggested that this could be due to mitochondrial DNA depletion in the hepatic tissue giving rise to β-oxidation defects and respiratory chain anomalies resulting in lactic acidosis, hepatic steatosis and raised liver enzymes. It was further opined that mitochondrial disease can lead to multiorgan degeneration like skeletal muscle, liver and kidney.[6-8] Further studies elucidated that this mitochondrial damage may be due to formation of intracellular zidovudine-monophosphate, which is a toxic metabolite of zidovudine. This may be due to the fact that zidovudine monophosphate is an alternative substrate inhibitor of thymidine monophosphate. This may result in decreased dTTP in the mitochondria and thus decrease mitochondrial DNA synthesis.[9-12]

On microscopic examination of the fetal liver we observed that there was a dose dependent degeneration of hepatocytes and hemopoetic stem cells, fatty degeneration, cell death and loss of cyto-architecture. On Periodic acid Schiff staining there seems to be deficient glycogen staining of hepatocytes in the treated group. This explanation is further supported by the fact that considerable increase in serum transaminases and bilirubin level in the treated dams was observed. This could be explained both by depletion of mitochondrial DNA leading to β-oxidation defects and respiratory chain anomalies and increased zidovudine monophosphate levels in the hepatocytes leading to afore said toxic changes. .

Thus it will be prudent to say that zidovudine causes toxic changes in the fetal liver and should be cautiously prescribed by the physician during pregnancy especially to ladies suffering from any liver disease or predisposed to oxidative stress.

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