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# Azidothymidine induces severe hematological toxicity and hepatic injury in Charles Foster rats

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**ABSTRACT**

**Background:** The present study aimed at evaluating the effects of azidothymidine (AZT) on hematologic and biochemical parameters in Charles Foster rats.

**Methods:** Twelve adult healthy Charles Foster rats comprising of six male and six females were selected for study. Test rodents were divided into four groups containing three rodents each. Three males and three females served as control and remaining received AZT drug. Rodents were acclimatized for 10 days and drug was administered for 28 days. After the completion of drug administration, blood samples were collected and analyzed for hematologic parameters, i.e., Hemoglobin (Hb), Packed cell volume (PCV), red blood cell (RBC), Mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), Mean corpuscular volume (MCV), Platelet count (Plt) using a Fully Automatic Fully Digital Hematology Cell Counter. In addition, biochemical parameters, were measured to assess the effects of AZT on rodent physiology. In-vivo histopathological studies were also performed on vital organs of rodents to understand the effects of drug at tissue level.

**Results:** When compared with the control group, the data indicated a outstanding decrease in Hb, PCV, RBC, TLC and platelets in all test groups, whereas MCHC did not show any major reduction but MCV data suggested a slight increase. Among biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). were found to be remarkably elevated along with elevated bilirubin and reduced albumin, pointing towards a possible liver damage which was later corroborated by liver histopathological study.

**Conclusions:** Above results indicate azidothymidine to be a myelosuppresive and hepatotoxic drug and its usage as an anti-retro viral during highly active anti-retro viral therapy (HAART) regime should be strictly monitored.

**Keywords:** AZT, Hematotoxicity, Hepatotoxicity, Subacute toxicity

## INTRODUCTION

Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by the human immunodeficiency virus and it remains one of the world's most significant public health challenges, particularly in low and middle-income countries.[1] As per a WHO report, in 2015 about 36.7 million people were living with HIV and it resulted in 1.2 million deaths Most of those infected live in sub-Saharan Africa.[2] According to a statistical report of CDC

(centre for disease control) between its discovery and 2014 AIDS has caused an estimated 39 million deaths worldwide.[3]

AIDS is a lethal multi-system disease that has become a major public health problem since its recognition in 1981.[4,5] The etiological agent of AIDS is a retrovirus referred to as HIV.[6] There is no certain cure or vaccine for the treatment of HIV; treatment consists of highly active antiretroviral therapy (HAART) which slows progression of the disease.[7] Current HAART options are combinations

(or "cocktails") consisting of at least three medications belonging to at least two types, or "classes," of antiretroviral agents. HIV medicines are grouped into six drug classes according to how they fight HIV.

The six drug classes are:

1. Non-nucleoside reverse transcriptase inhibitors (NNRTIs),
2. Nucleoside reverse transcriptase inhibitors (NRTIs),
3. Protease inhibitors (PIs),
4. Fusion inhibitors,
5. CCR5 antagonists (CCR5s),
6. Integrase strand transfer inhibitors (INSTIs)

These six drug classes include more than 25 HIV medicines that are approved to treat HIV infection. Azidothymidine commonly referred to as AZT was the first drug to be approved by FDA for AIDS treatment.[8-10] AZT is a synthetic analogue of thymidine (3'-azido-3'-deoxythymidine) and a reverse transcriptase inhibitor used in combination with other agents in the therapy and prophylaxis of the human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS).[11] AZT is phosphorylated intracellularly and then acts in competing with the natural substrate, thymidine triphosphate, for incorporation into growing HIV DNA chain causing inhibition of the viral reverse transcriptase and chain termination. Recently, AZT has been replaced by better tolerated nucleoside analogues and it is no longer commonly used in developed countries but that's not the case with developing or under developed countries because of the drug's affordability.[12]

The most adverse effect of AZT are anaemia and granulocytopenia, which are believed to reflect bone marrow toxicity.[8,13] Two types of anaemia may occur with AZT therapy marcrocytic megaloblastic anaemia and normochromic anaemia.[14,15] Several subacute and subchronic rodent toxicity studies have demonstrated that the primary toxicity of AZT is myelosuppression. A recent study evaluated the effects of AZT on hemostatic and hematologic parameters in Wistar rats and found out a significant decrease in RBCs, PCV and platelet count.[16] In another study it was demonstrated that AZT treatment can cause hepatotoxicity in Wistar rats.[17] They reported highly significant increase in alanine transaminase, alkaline phosphatase, argininosuccinic acid lyase and bilirubin in serum of Wistar albino rats. In a similar kind of study it was reported that AZT can cause significant increase in serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).[18] Apart from rodent models, AZT has also shown to cause severe clinical problems in humans as well. A group of researchers reported AZT associated toxicity in HIV infected patients, they reported severe anaemia in significantly higher number of patients, moreover female patients were reported to be more prone to anaemia as compared to male patients.[19] In the present study, we have investigated the effects of an anti-retro viral drug

Azidothymidine on hematological parameters of Charles foster rats. We have also studied the hepatotoxic effects of Azidothymidine on the liver of Charles Foster rats by histopathological studies and quantifying important serum marker enzymes along with bilirubin and albumin.

## METHODS

The drug which was tested for hematotoxicity in the present study was Azidothymidine (AZT) Brand name: Zidovir (Cipla pharmaceuticals) is available in 300 mg Tablet form and was purchased from local pharmacy shop in Lucknow, India.

### In vivo cytotoxicity studies

#### Experimental rodents

6 Male and 6 Female Charles foster strains of Rats, weight ranging from 200 to 300g were used. These rodents were supplied for the experiment, by the Division of Laboratory animals, CDRI, Lucknow. Proper approvals were obtained from ethical committee of CDRI, Lucknow before starting of this work. Rats were quarantined for 10 days and it was confirmed that they were free of pathogen (Ecto and Endo Parasites- Mycoplasma, Pasteurella, Rat pneumenitis virus etc.) After acclimatization, 12 healthy young and active albino rats of charles foster strain comprising of 6 male and 6 female rats were selected based on their body weight and by assessing initial hematological parameters by collecting 1ml fresh blood sample in 0.1% EDTA collection vials. The rodents which looked sick or those that had initial hematological parametric values out of the range were discarded. The female rats were nulliparous and non-pregnant. Rats were divided in to two groups of 6 rats having 3 males and 3 females, and were kept under conventional condition (open system) and housed in plastic cages (floor area 800 cm$^2$, height 14cm.) on sterilized rice bran bedding. They were provided with standard rodent pellets diet procured from Ashirvad Ltd. and filter water from Aqua guard in 300 ml bottles (24 hrs). Rats were housed in a room where room temperature was kept at 22 degree Celsius, relative humidity was controlled between 50-70% and 12-hour light and 12-hour dark photo period was provided. The first group served as control group (Group I) and second as treated (Group II).

#### Dose

The dose selected was 180mg AZT per kilogram body weight. The drug was mixed with appropriate amount of water and grinded in mortar and pestle (glass). The fine drug solution was then given to the rats of treated group (oral route) with the help of cannula.

### In-vivo hematological studies

Important hematological parameters were assessed at the end of the experiment i.e. 28 days of dosing using MS9 Fully Automatic Fully Digital Hematology Cell Counter.

Version 3.5 × E from Melet Schloesing Laboratories, France. Hemoglobin, Hematocrit (PCV), T-RBC, MCHC, TLC, MCV and Platelet were the hematological parameters studied.

### Biochemical analysis

The cardiovascular blood was removed after sacrificing the rodents. The Blood was allowed to clot and was subjected to centrifugation at 3000 rpm for 10 minutes. The serum obtained was further analyzed in BECKMANN Synchron clinical system CX4/CX5, made in USA for the following parameters: Glucose, Cholesterol, Triglycerides, Total Protein, Albumin, Alkaline aminotransferase, Aspartate aminotransferase, Alkaline Phosphatase, Total Bilirubin, Blood Urea Nitrogen, Creatinine, Calcium and Phosphate.

### Dissection

The rats were anesthetized by anesthetic ether and then were dissected. The cardiovascular blood was collected and allowed to clot for serum isolation and further biochemical analysis. The different organs removed and further processed were: Brain, Trachea, Lung, Heart, liver, spleen, adrenal gland, kidney, reproductive system. Absolute and relative organ weights were recorded.

### In vivo histopathological studies

Systemic studies of the Drug were analyzed by standard Histopathological techniques including Dissection, Tissue processing and Slide preparation.

## RESULTS

### Hematotoxicological manifestations of AZT

Table 1 and Table 2 shows the data of hematological parameters of AZT treated as well as control rats. It was observed that following AZT administration for 28 days, the mean values of Hemoglobin, packed cell volume, RBC, WBC and platelets remarkably reduced in male as well as female treated rats, when compared to control.

**Table 1: Recording of hematological parameters of AZT treated rats.**

| | Hb (g/dl) | | PCV (%) | | RBC ($10^6$cells/ml) | | MCHC (g/dl) | | TLC ($10^3$ cells/ml) | | MCV (μ) | | Plt ($10^3$ cells/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day |
| 1F | 12.8± 1.8 | 4.5± 0.5 | 30.8± 5.6 | 12.9± 1.8 | 4.5± 0.9 | 1.8± 0.3 | 41.6± 6.4 | 39.8± 4.1 | 11.5± 1.6 | 4.6± 0.9 | 69.2± 6.9 | 72.2± 5.2 | 384± 23 | 143± 12 |
| 2F | 13.7± 1.7 | 5.6± 0.9 | 36.1± 5.2 | 12.9± 3.2 | 5.8± 1.0 | 2.3± 0.2 | 38.0± 6.2 | 42.5± 3.7 | 9.9± 1.0 | 3.2± 0.6 | 62.2± 7.1 | 67.3± 4.1 | 315± 34 | 108± 11 |
| 3F | 12.5± 2.1 | 5.5± 1.1 | 35.4± 6.3 | 15.6± 3.8 | 5.9± 1.2 | 1.9± 0.4 | 35.4± 3.0 | 38.1± 3.0 | 12.8± 1.8 | 3.5± 0.5 | 60.0± 4.5 | 65.3± 4.9 | 272± 28 | 155± 16 |
| 7M | 15.8± 2.2 | 6.1± 0.8 | 43.1± 7.8 | 14.9± 2.9 | 6.1± 1.2 | 1.9± 0.4 | 36.8± 4.1 | 37.1± 3.9 | 10.3± 2.1 | 4.9± 0.6 | 63.2± 5.9 | 68.9± 5.3 | 328± 31 | 135± 15 |
| 8M | 15.7± 1.6 | 6.2± 0.6 | 40.5± 6.9 | 19.7± 4.4 | 6.2± 1.3 | 1.8± 0.3 | 38.8± 3.4 | 37.8± 4.2 | 11.6± 1.5 | 5.8± 0.8 | 64.9± 5.1 | 67.5± 4.5 | 419± 39 | 238± 32 |
| 9M | 14.9± 2.3 | 7.4± 0.9 | 39.1± 4.3 | 17.2± 3.2 | 6.1± 1.3 | 2.3± 0.2 | 38.2± 5.4 | 36.5± 5.1 | 9.4± 1.3 | 4.3± 0.7 | 63.8± 6.1 | 70.2± 4.9 | 343± 22 | 125± 18 |

**Table 2: Recording of hematological parameters of control rats.**

| | Hb (g/dl) | | PCV (%) | | RBC ($10^6$cells/ml) | | MCHC (g/dl) | | TLC ($10^3$ cells/ml) | | MCV (μ) | | Plt ($10^3$ cells/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day | 1st day | 28th day |
| 4F | 14.4± 1.4 | 14.3± 1.3 | 33.1± 3.2 | 39.7± 4.2 | 4.7± 0.8 | 4.9± 0.6 | 43.5± 2.9 | 42.3± 3.7 | 7.8±0. 6 | 8.3± 0.7 | 70.0± 6.3 | 68.5± 5.1 | 176± 22 | 173± 24 |
| 5F | 13.9± 1.1 | 14.5± 1.5 | 37.1± 3.3 | 38.2± 4.0 | 5.6± 0.9 | 5.8± 0.9 | 37.3± 3.5 | 38.0± 3.0 | 11.2± 0.9 | 10.4 ±0.8 | 66.0± 4.3 | 66.3± 5.4 | 348± 30 | 494± 44 |
| 6F | 13.3± 1.5 | 12.9± 1.1 | 37.5± 2.9 | 34.3± 3.7 | 5.9± 0.7 | 5.3± 0.7 | 40.8± 3.1 | 37.5± 3.9 | 10.4± 1.1 | 9.7± 0.7 | 63.3± 4.8 | 61.0± 4.4 | 311± 45 | 289± 23 |
| 10M | 15.7± 0.9 | 15.7± 1.8 | 43.0± 5.6 | 41.9± 3.0 | 6.7± 1.2 | 6.2± 0.5 | 36.5± 3.2 | 37.6± 3.1 | 10.4± 1.0 | 15.1 ±1.1 | 64.1± 5.3 | 62.3± 3.3 | 304± 27 | 641± 57 |
| 11M | 13.3± 1.3 | 13.9± 1.4 | 36.6± 3.9 | 36.6± 4.2 | 6.1± 1.0 | 5.8± 0.7 | 36.4± 3.3 | 38.0± 3.0 | 9.9±0. 8 | 11.3 ±0.8 | 59.8± 4.9 | 58.6± 4.9 | 221± 19 | 278± 26 |
| 12M | 11.6± 1.2 | 16.1± 1.6 | 31.3± 4.1 | 44.6± 4.4 | 4.5± 0.8 | 6.2± 0.9 | 37.2± 3.3 | 36.1± 4.2 | 7.8±0. 9 | 12.1 ±1.0 | 69.2± 5.4 | 70.5± 5.0 | 280± 33 | 586± 41 |

### Serum marker enzymes and bilirubin

The standard serum enzymes predicting hepatotoxicity and their respective activities are presented in Table 3 and Table 4. Results suggest a notable elevation in the levels of all three serum marker enzymes i.e. ALT, ASP, ALP along with total bilirubin content in AZT treated rats. Male as well as female rats showed the similar pattern of elevation as compared to control rats.

**Table 3: Recording of biochemical parameters of AZT treated rats.**

|  | ALB (g/dl) | ALT (IU/l) | AST (IU/l) | ALP (IU/l) | TBIL (mg/dl) |
|---|---|---|---|---|---|
| 1F | 1.6±0.2 | 75±4.1 | 301±23 | 224±14 | 0.6±0.09 |
| 2F | 2.0±0.1 | 70±3.3 | 283±21 | 216±8 | 0.7±0.1 |
| 3F | 1.3±0.1 | 66±2.9 | 288±26 | 264±12 | 0.5±0.1 |
| 7M | 1.4±0.2 | 72±3.6 | 367±31 | 236±16 | 0.6±0.08 |
| 8M | 1.7±0.1 | 69±3.9 | 292±29 | 250±7 | 0.6±0.1 |
| 9M | 1.7±0.2 | 73±3.2 | 350±21 | 230±11 | 0.8±0.2 |

ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;
ALP: Alkaline phosphatase; TBIL: Total bilirubin

**Table 4: Recording of biochemical parameters of control rats.**

|  | ALB (g/dl) | ALT (IU/l) | AST (IU/l) | ALP (IU/l) | TBIL (mg/dl) |
|---|---|---|---|---|---|
| 4F | 3.6±0.2 | 38±1.8 | 160±14 | 95±6 | 0.1±0.0 |
| 5F | 3.8±0.4 | 42±2.1 | 166±11 | 114±10 | 0.2±0.0 |
| 6F | 4.0±0.3 | 40±3.4 | 159±13 | 120±12 | 0.15±0.0 |
| 10M | 4.1±0.2 | 41±3.1 | 171±12 | 114±9 | 0.12±0.01 |
| 11M | 4.3±0.3 | 42±2.7 | 161±9 | 134±11 | 0.22±0.05 |
| 12M | 4.8±0.1 | 44±4.2 | 160±15 | 131±13 | 0.25±0.03 |

ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase;
ALP: Alkaline phosphatase; TBIL: Total bilirubin

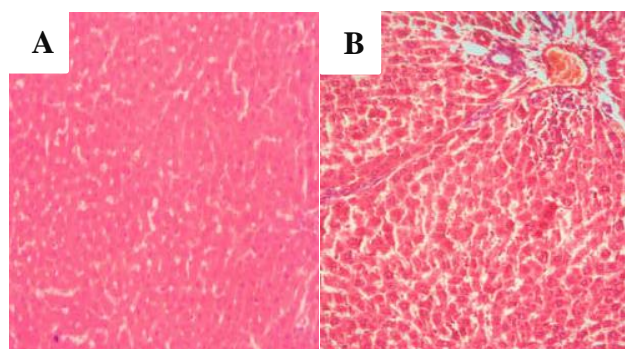### Effect of AZT on histopathology of liver



**Figure 1: A) Control rat, B) AZT treated rat; Histopathology of Charles Foster rat liver.**

The histopathological effects of AZT on the liver of Charles foster rat is presented in Figure 1. The liver of treated rat is showing inflammatory cells around the central vein owing to AZT toxicity, the image also shows an enlargement of the central vein of the liver. The liver is highly susceptible to xenobiotic-induced injury because of its central role in foreign chemical substance metabolism.

## DISCUSSION

The findings suggest AZT to have an inhibitory effect on hemopoiesis as reported previously.[16] AZT therapy is probably the most common cause of anaemia in HIV-infected patients. In the clinical trial studies of AZT in patients with advanced stage of HIV, statistically significant reductions in hemoglobin levels occurred in 34% of subjects receiving AZT (1,200mg per day) following 6 weeks of therapy.[13] Our data suggests a drastic reduction in haemoglobin level of AZT treated rats as compared to control. A similar kind of study reported an increase in erythrocyte mean corpuscular volume (MCV) which finds agreement with our data where AZT treated male and female rats have slightly increased MCV as compared to control rodents.[13] Apart from anaemia, the most common effect observed in-vivo during AZT treatment is neutropenia, which seems to have direct suppressive effect on heme synthesis.[20] The high dose of heme analogue compounds which are used for treatment of heavy metal induced hematotoxicity, may cause anaemia because of their modulatory effects on the enzymes involved in heme synthesis.[21] Some studies report increased WBC counts, following AZT administration while some authors report otherwise.[16,22-24] Increased WBC count maybe attributed to inflammation due to chronic infection, but in the present study WBC count was reduced post drug administration. Our data suggests that AZT may have a leucopenic inhibitory effect in Charles foster rat. Our study also indicated a reduction in the platelet count as reported previously.[16,23] A previous study also reported platelet degradation upon high dose of AZT administration.[25]

Preceding literature suggests AZT induced elevation in the levels of transaminases and ALP in human and albino rats has already been reported.[18,26] The data presented in this report indicates towards a hepatic injury in AZT treated Charles Foster rats. Elevated bilirubin levels were reported in humans and rats upon AZT administration, and our results are in agreement with these reports.[18,27] Low titers of serum albumin in AZT treated rats also point towards a possible liver injury. Albumin is produced by hepatocytes and hence determining serum albumin levels is often considered "tests of liver function." This is mainly because hepatic synthesis of albumin tends to decrease in end-stage liver disease.

Liver occupies a portal location within the circulatory system.[28] Hepatotoxicity is the most adverse reaction of AZT treatment but still there is no clear explanation of its mechanism mainly because manifestations of AZT induced hepatotoxicity are like other conditions of hepatic injuries but it is very much evident from the images that

AZT caused an abnormal morphology of the liver.[17,29] Transaminases and ALP are considered important markers of hepatic injury because their increased level in blood serum is observed immediately post hepatic necrosis and hepatocellular membrane damage.[27,30] In the present study, AZT treated rodents showed a highly remarkable increase in ALT and ALP and these results are in agreement with the above report. In the present study, AZT treated rodents showed increased bilirubin levels and this observation demonstrate that this toxin-induced insult to the liver could also result in hyperbilirubinemia in addition to hepatocellular necrosis.

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