Some Pharmacological Studies on Lamivudine in Rats

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Abstract
The present study was carried out to evaluate the side effects of lamivudine administration and its immunological effect in male albino rats through estimating some hematological parameters, liver and kidney functions, humoral immunity and phagocytic capacity. Forty adult male albino rats were divided into 2 groups (control and treated groups) each of 20 rats. The treated group was orally administered Lamivudine for 60 days in a dose of 9 mg/kg BW once daily. In the current study, all hematological parameters were within the normal range except the packed cell volume which increased at 60 days (45.60±0.87). IgG was increased in the treated group at 15, 30, 45 and 60 days (1183.96±11.64, 1203.58±2.02, 1185±13.27 and 1181.16±11.74, P<0.01, respectively), while IgM did not reveal any significant change. In treated group, there were no significant changes in serum urea and creatinine except after 15 days of drug administration the creatinine level was significantly decreased. Aspartate aminotransferase was decreased (24.00±1.94, P<0.01) after 45 days of drug administration, while alkaline phosphatase did not significantly changed. Alanine aminotransferase was decreased (26.20±1.95, 31.40±1.77, 29±3.39 and 22±1.41, P<0.01) at 15, 30, 45 and 60 days, respectively. The total protein increased at 15, 30, 45 and 60 days of drug administration in the treated group (8.64±0.12, 8.07±0.19, 8.06±0.46 and 8.60±0.22, P<0.01, respectively). Moreover, total globulins were significantly increased at 15, 30, 45 and 60 days of drug administration (4.91±0.13, 4.92±0.35, 4.31±0.26 and 4.34±0.16, respectively). The present results revealed an increase in the phagocytic percent and phagocytic index at 30 (1.12±0.09 and 79.15±3.64) and 60 days (0.93±0.08 and 77.13±4.45) of drug administration, respectively. Based on the current findings, it may be concluded that lamivudine had no serious side effects on hematology, both liver and kidney functions with a promising immunostimulant effect.

Keywords: Hematology, Lamivudine, Creatinine, IgG

Introduction
Hepatitis B virus (HBV) causes acute inflammation of the liver, a symptomatic infection or chronic liver disease. Adult infected with HBV can develop cirrhosis and/or liver cancer in 2-10% [1]. Hepatitis B should be recognized with the viral replication and histological evidence of the active liver which based on the serological and histological analysis that mainly derived from two years studies in HbeAg-positive patients with compensated liver disease [2]. Antiviral drugs are medicines that cure or control viral infections. Historically, the discovery of antiviral drugs has been largely fortuitous. Developing antiviral medicines are difficult, because most of drugs that kill viruses also damage the host's cells [3].

Lamivudine was used for treating the infection caused by human immunodeficiency virus (HIV) or hepatitis B virus. HIV is the virus that causes acquired immune deficiency syndrome (AIDS). Lamivudine was administered with zidovudine (AZT) or with other medications used for treating HIV [4]. Lamivudine was suggested for treating adult patients with compensated chronic Hepatitis B. The aim of this study was to investigate the
lamivudine side effects on liver and kidney functions and some hematological parameters with special reference to its immunological effects in male albino rats.

Material and Methods

Experimental animals and design
Forty adult male albino rats, were divided into 2 homogenized groups (control and treated) each of 20 rats. Lamivudine was orally administered to the treated group for 60 days in a dose of 9 mg/kg BW once daily according to Paget and Parnes [5]. Two blood samples from 5 rats in each group were collected at 15, 30, 45 and 60 days after Lamivudine administration. The first blood sample was collected on anticoagulant [EDTA] for hematological studies and estimating the phagocytic capacity of the drug after 30 and 60 days. The second was collected without EDTA for separating serum. The serum was used for determining liver and kidney function tests and estimating IgG and IgM after 15, 30, 45 and 60 days.

Hematological studies
Erythrocytic (RBC's), Total leucocytic counts (TLC) and differential leucocytic counts were performed using the improved neubauer chamber [6]. The acid hematin method was carried out to determine the hemoglobin (HB) [7]. The packed cell volume (PCV), erthrocyte indices as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined with the microhematocrit method [8].

Determination of immuno-globulins
The procedure using serum samples consists in an immunoprecipitation in agarose between an antigen and its homologous antibody [9].

Kidney and Liver function tests
Serum urea level was estimated according to the method of Chaney and Marbach [10]. While, serum creatinine level was determined colorimeterically [11]. Colorimetrical method was used to estimate the serum transaminases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) [12] and alkaline phosphatase (ALP) [13]. While, serum total protein was estimated according to the method of Weichselbaum [14].

Phagocytic capacity of the drug
It was estimated according to the method described by Boyum [15].

Statistical analysis
In order to assess the influence of Lamivudine on different biochemical and immunological measurements, independent samples t-test (Welch t-test in cases of unequal variance) was used. Homogeneity of variance of sample groups was checked using Levene’s test. Analysis was done using Statistical Package for Social Sciences version 22.0 (SPSS IBM Corp., Armonk, NY, USA). Results are reported in means ± SEM (Standard Error of Mean), probability values were indicated by * when P < 0.05 and ** when P < 0.01. The statistical analysis was based on the intention-to-treat population.

Results
Lamivudine elicited a significant decrease of RBC's count, HB, MCHC, TLC and neutrophil counts, with a significant increased in MCV and MCH which showed macrocytic anemia after 30 days of drug administration. There were no significant changes in all tested parameters except an increase in PCV (45.60±0.87) after 60 days of Lamivudine administration (Table 1). IgG revealed a significant increase (1183.96±11.64, 1203.58±2.02, 1185 ± 13.27 and 1181.16±11.74) after 15, 30, 45 and 60 days of Lamivudine administration, respectively while IgM was non-significantly changed (Table 2).
Table 1: The Effect of lamivudine given orally into male albino rats once daily at a dose of (9 mg/kg b.wt.) for 30 and 60 days on some hematological parameters and cellular immunity (Mean ± S.E, n = 5)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treated 1ª</th>
<th>Control</th>
<th>Treated 2ª</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCS (10⁶ / mm³)</td>
<td>6.85±0.07</td>
<td>4.82±0.17</td>
<td>6.90±0.12</td>
<td>7.44±0.32</td>
</tr>
<tr>
<td>Haemoglobin (gm%)</td>
<td>13.23±0.14</td>
<td>11.16±0.16</td>
<td>13.00±0.28</td>
<td>13.60±0.48</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.00±0.57</td>
<td>40.33±0.33</td>
<td>40.00±0.57</td>
<td>45.60±0.87</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>62.90±1.49</td>
<td>83.84±3.82</td>
<td>61.95±2.04</td>
<td>61.49±1.59</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>19.28±1.2</td>
<td>23.19±0.88</td>
<td>18.59±0.40</td>
<td>18.28±0.17</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>3.42±0.6</td>
<td>27.69±0.56</td>
<td>30.59±0.33</td>
<td>29.79±0.42</td>
</tr>
<tr>
<td>TLC (x10³ / mm³)</td>
<td>7.51±0.26</td>
<td>4.79±0.53</td>
<td>7.14±0.08</td>
<td>6.79±0.42</td>
</tr>
<tr>
<td>Neutrophil (x10³ / mm³)</td>
<td>2.38±0.19</td>
<td>1.34±0.44</td>
<td>2.13±0.06</td>
<td>2.02±0.16</td>
</tr>
<tr>
<td>Eosinophil (x10³ / mm³)</td>
<td>0.14±0.02</td>
<td>0.37±0.03</td>
<td>0.33±0.01</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Basophil (x10³ / mm³)</td>
<td>0.13±0.01</td>
<td>0.09±0.005</td>
<td>0.11±0.008</td>
<td>0.11±0.006</td>
</tr>
<tr>
<td>Lymphocyte (x10³ / mm³)</td>
<td>3.23±0.08</td>
<td>2.94±0.12</td>
<td>3.52±0.12</td>
<td>3.24±0.28</td>
</tr>
<tr>
<td>Monocyte (x10³ / mm³)</td>
<td>1.06±0.06</td>
<td>1.04±0.04</td>
<td>1.00±0.003</td>
<td>1.15±0.07</td>
</tr>
</tbody>
</table>

ª Treated 1: after 30 days of drug administration; ∗ Treated 2: after 60 days of drug administration; RBCS: red blood cells; PCV: packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; TLC: Total leucocytic counts; * Significant at P < 0.05 and ** Significant at P < 0.01.

The serum urea and creatinine showed no significant changes except after 15 days of drug administration in treated group, the creatinine levels was significantly decreased (0.39 ± 0.05). Lamivudine provoked a high significant decrease in the ALT (26.20 ± 1.95, 31.40 ± 1.77, 29.00 ± 3.39 and 22.00 ± 1.41) at 15, 30, 45 and 60 days after drug administration, respectively while ALP were non-significantly changed when compared with the control values in all testing periods. AST was non-significantly changed in all sampling time except after 45 days of drug administration it was significantly decreased (24.00±1.94).

Table 2: The Effect of lamivudine given orally into male albino rats once daily at a dose of 9 mg/kg BW for 15, 30, 45, 60 days on humoral immunity (Mean ± S.E, n = 5)

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>IgM (mg/dl)</th>
<th>IgG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>15</td>
<td>20.80±3.28</td>
<td>21.68±4.29</td>
</tr>
<tr>
<td>30</td>
<td>23.22±3.09</td>
<td>21.90±4.20</td>
</tr>
<tr>
<td>45</td>
<td>23.22±3.09</td>
<td>22.78±4.47</td>
</tr>
<tr>
<td>60</td>
<td>18.52±3.19</td>
<td>18.10±4.19</td>
</tr>
</tbody>
</table>

* Significant at P < 0.01.

The Lamivudine administration elicited no significant changes in albumin throughout the experiment. The total protein increased at 15, 30, 45 and 60 days after drug administration in treated group (8.64±0.12, 8.07±0.19, 8.06±0.46 and 8.60±0.22, P<0.01, respectively). Total globulins were significantly increased at 15, 30, 45 and 60 days of drug administration (4.91±0.13, 4.92±0.35, 4.31±0.26 and 4.34±0.16, respectively) (Table 3). The present results revealed high significant increase in phagocytic percent (1.12 ± 0.09 and 1.11±0.75) and phagocytic index (79.15 ± 3.64 and 77.13 ± 4.45) at 30 and 60 days after drug administration, respectively (Table 4).
Table 3: The Effect of lamivudine given orally into male albino rats once daily at a dose of 9 mg/kg BW for 15, 30, 45, 60 days on some kidney and liver function parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time (days)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td>0.51 ± 0.007</td>
<td>0.39 ± 0.05*</td>
<td>0.50 ± 0.01</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td></td>
<td>31.20 ± 2.13</td>
<td>27.80 ± 2.43</td>
<td>34.60 ± 1.95</td>
<td>33.40 ± 3.57</td>
</tr>
<tr>
<td>Alk. Ph (U/L)</td>
<td></td>
<td>9.68 ± 4.50</td>
<td>87.20 ± 4.95</td>
<td>96.00 ± 3.24</td>
<td>87.40 ± 3.57</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td>41.2 ± 1.49</td>
<td>26.20 ± 1.95**</td>
<td>41.20 ± 1.49</td>
<td>31.40 ± 1.77**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td></td>
<td>32.60 ± 3.58</td>
<td>29.00 ± 2.82</td>
<td>27.80 ± 3.62</td>
<td>30.60 ± 2.40</td>
</tr>
<tr>
<td>Total Protein. (g/dl)</td>
<td></td>
<td>7.24 ± 0.09</td>
<td>8.64 ± 0.12**</td>
<td>6.78 ± 0.21</td>
<td>8.07 ± 0.19**</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td></td>
<td>3.53 ± 0.12</td>
<td>3.78 ± 0.12</td>
<td>3.32 ± 0.14</td>
<td>3.36 ± 0.14</td>
</tr>
<tr>
<td>Total Globulins (g/dl)</td>
<td></td>
<td>3.71 ± 0.16</td>
<td>4.91 ± 0.13**</td>
<td>3.76 ± 0.18</td>
<td>4.31 ± 0.26*</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05; ** Significant at P < 0.01; C: control group; T: treated group.

Note: 
- Alk. Ph: Alkaline Phosphatase
- ALT: Alanine transferase
- AST: Aspartate-aminotransferase

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Table 4: The effect of lamivudine given orally into male albino rats once daily at a dose of 9 mg/kg BW for 30, 60 days on phagocytic capacity (Mean ± S.E, n = 5)

<table>
<thead>
<tr>
<th>Time(days)</th>
<th>Phagocytic index</th>
<th>Phagocytic Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>30</td>
<td>0.92±0.11</td>
<td>1.12± 0.09 **</td>
</tr>
<tr>
<td>60</td>
<td>0.93±0.08</td>
<td>1.11± 0.75 **</td>
</tr>
</tbody>
</table>

** Significant at P < 0.01

Discussion

Lamivudine has been used for treating chronic hepatitis B at a lower dose than for treating HIV/AIDS. It improved the seroconversion of e-antigen positive hepatitis B and also improved histology staging of the liver [16]. Lamivudine used also for HIV patients with highly active antiretroviral therapy (HAART) medication [2].

The obtained findings revealed that Lamivudine has untoward side effects on the hematological parameters even in the presence of anemia (macrocytic anemia), leukopenia and neutropenia on the 30th day of Lamivudine administration. Anemia and leucopenia disappeared on the 60th day of Lamivudine administration. The results of the present study were comparable with Umar et al. [17] who studied the effect of Lamivudine on rats’ hematology for 40 days using 2mg daily dose/rat and they found no hematological disorders except a significant increase in PCV.

In this study, Lamivudine had no effect on IgM, but IgG levels increased significantly. This might be attributed to short half-life of IgM (1-6days) and long half-life of IgG which may reach 24 days [18], therefore IgM disappeared from blood samples taken after 15 days. The previous observations were reported also by Chen and Tsai [19], who studied the Lamivudine response of IgM anti-HBC chronic Hepatitis B, and they found that lamivudine in chronic case of Hepatitis B can produce low response of IgM according to antigenic number and virulence.

The results of the current study revealed that Lamivudine had no critical effect on the serum creatinine or urea level, so it has good tolerability and high safety profile on the kidney. Similar observations were reported by Ron et al. [20] who studied the effects of Lamivudine in a daily dose of 100mg for treating the acute Hepatitis B patients. The authors concluded that Lamivudine had no serious effects on kidney of Hepatitis B patient.

Concerning liver functions, Lamivudine caused normalization of liver enzymes which might be attributed to its protective mechanism on hepatocyte. These results go hand in hand with those mentioned by Nagamatsu et al. [21]. They reported that the administration of Lamivudine prevented further damage of the liver and so caused normalization of the liver enzymes by using 100mg Lamivudine as a single dose in HBeag +ve patients for 6 months. There were no changes in serum albumin levels, while the total protein and total globulins were significantly increased. These results also endorsed those obtained by Liaw et al. [22]. They evaluated the effect of Lamivudine in Hepatitis B patients with advanced liver disease by using 100mg Lamivudine (436 patients and 215 controls) for 5 years and found a normalization of serum albumin and significantly increased in total protein and globulins.

Our results revealed highly significant increase in phagocytic activity. These results might be indicated that Lamivudine can restore function of dendritic cells which is antigen presenting cells and has a rule in increasing the activity of monocytes through regulation of major histocompatability II (MHC II) expression [23]. Moreover, restore the function of phagocytosis through inhibiting the cyclic adenosine monophosphate (cAMP) production by inhibiting the adenylatecyclase enzyme [24]. Zheng et al. [23] had investigated the effect of Lamivudine in restoration of the phagocytic activity and
function of the monocyte in HBV patient. They detected a high significant increase in monocyte activity and phagocytosis by activation of dendritic cells function.

**Conclusion**

Based on the current findings, it may be concluded that Lamivudine had no serious side effects on hematology, both liver and kidney functions with a promising immunostimulant effect.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


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الملخص العربي

بعض الدراسات الفارماكولوجية على اللافيمودين في الجرذان

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قسم الفارماكولوجيا - كلية الطب البيطري. جامعة الزقاق.

قسم البكتيريولوجي والمناعة والفيروسات - كلية الطب البيطري. جامعة الزقاق.

"الالتهاب الكبدى البولى (ب)是从最容易发生肝损伤的药物中选择的。该研究的目的是通过比较德维克和卡比拉的疗效，评估这些药物对肝损伤的预防作用。

注射测试中，当肝癌细胞被注射到肝损伤模型的动物中时，德维克显示了更好的效果，因为它可以抑制肝细胞的过度生长。

研究结果表明，德维克具有更强的抗肝炎病毒活性，因此它可能是治疗慢性肝炎的更有效药物。"