MORPHOMETRIC STUDY OF KETOCONAZOLE TREATED LIVER IN ALBINO RATS

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ABSTRACT
To study the effects of Ketoconazole induced liver damage, compare with control group and correlate with previous studies. Forty adult male albino rats were used for this study. Group-A served as control animals, received injection of normal saline in dose of 0.05 ml/100 gm of body weight intraperitoneally daily for 03, 07, 15 and 30 days. Group-B received injection of Ketoconazole 40 mg/kg of body weight intraperitoneally daily for 03, 07, 15 and 30 days of treatment. Animals were sacrificed after completion of treatment under ether anaesthesia. Liver were removed, fixed in 10% and alcoholic formalin for 24-48 hours. They were dehydrated in ascending strength of alcohol and paraffin tissue blocks were made 5 μm thick section were stained with H&E for general morphology and micrometry and Gomori’s reticulin stain for observing reticulin fibres. The results were compared with control. Analyzed statistically with student 't' test and correlate with previous studies. Ketoconazole treated animals showed distortion of hepatic architecture, increase size of hepatocytes, decrease nuclear diameter and necrosis of hepatocytes. Increased amount of reticulin fibres and area of focal fibrosis within hepatic lobule as compared to control group-A animals. It is concluded from this study that Ketoconazole induced injury is dose and duration of therapy dependent and due to its cost effective frequent use needs further research in humans.

Keywords: Ketoconazole, Albino rats, Hepatocytes and Micrometry.

INTRODUCTION
Human fungal infections have increased dramatically in incidence and severity in recent years, due mainly to advances in surgery, cancer treatment and critical care accompanied by increase in the use of broad spectrum anti-microbial and HIV epidemic. Pharmacotherapy of fungal disease has been revolutionized by introduction of the relatively non-toxic oral Azole drug such as Ketoconazole, used successfully in the treatment of fungi, but it may cause some hepatic damage (Sheppard and Limpiris, 2001).

Ketoconazole-induced hepatitis was first reported in 1981 by MacNair and first fatality occurred in 1982 due to hepatic coma (Duarte et al., 1984). An autopsy of the patient revealed massive hepatocellular necrosis (Lewis et al., 1984) and it is suspected that toxicity responses are not due to an immunologic mechanism but as a result of reaction of either Ketoconazole or its metabolites (Knight et al., 1991; Bok and Small, 1999).

Further, Rodriguez and Buckholz (2003) demonstrate that Ketoconazole induced hepatotoxicity may react with protein and possibly hepatic glutathione (GSH), which is important in the detoxification pathway of Ketoconazole. Hepatotoxicity induced by Ketoconazole is correlated to dose, maximum plasma concentration and clearance (Ming et al., 2003).
Morphometric Study of Ketoconazole Treated Liver in Albino rats

**MATERIAL AND METHODS**

This study was conducted in Department of Anatomy, Basic Medical Sciences Institute, J.P.M.C., Karachi. A total number of 40 adult male albino rats of 90-120 days, weighing between 200-300 grams were used. The animals were healthy and kept on standard laboratory diet and water ad libitum.

Animals were divided into two groups A and B, each containing 20 animals. Each group was further divided into four sub-groups, each comprised of 05 animals, according to period of treatment that is after 03, 07, 15 and 30 days.

Each animal of group-A (control) was administered normal saline in a dose of 0.05 ml/100 gm of body weight intraperitoneally while each animal of group-B (Ketoconazole treated) received intraperitoneal injection of Ketoconazole at a dose of 40 mg/kg of body weight (Rodriguez and Buckholz, 2003).

All the animals were sacrificed at the end of period of treatment under ether anaesthesia. Liver was removed, fixed in 10% formalin and alcoholic formalin for 24-48 hours. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene, infiltrated and embedded in paraffin wax, tissue blocks were made, cut into 4 µm thick sections with the

### Table-1

Mean Numbers of Liver Cells (Reticule) of Albino Rat in Different Groups at Variable Time Interval

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day-3</th>
<th>Day-7</th>
<th>Day-15</th>
<th>Day-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.04 ± 0.16</td>
<td>12.33 ± 0.17</td>
<td>12.12 ± 0.08</td>
<td>11.99 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>B</td>
<td>8.26 ± 0.15</td>
<td>9.14 ± 0.14</td>
<td>7.67 ± 0.12</td>
<td>7.14 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

*Mean ± SEM

### Table-2

Mean Hepatic Cell Diameter (µm) within Reticule of Albino Rat in Different Groups at Variable Time Interval

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day-3</th>
<th>Day-7</th>
<th>Day-15</th>
<th>Day-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.14 ± 0.17</td>
<td>15.11 ± 0.07</td>
<td>15.07 ± 0.05</td>
<td>15.02 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>B</td>
<td>18.21 ± 0.24</td>
<td>17.94 ± 0.30</td>
<td>19.10 ± 0.26</td>
<td>19.56 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

*Mean ± SEM

### Table-3

Mean Nuclear Diameter (µm) of Hepatic Cells within Reticule of Albino Rat in Different Groups at Variable Time Interval

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day-3</th>
<th>Day-7</th>
<th>Day-15</th>
<th>Day-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.25 ± 0.20</td>
<td>6.56 ± 0.11</td>
<td>6.33 ± 0.07</td>
<td>6.35 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>B</td>
<td>6.68 ± 0.12</td>
<td>7.56 ± 0.15</td>
<td>7.97 ± 0.04</td>
<td>6.68 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>(n=20)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
</tbody>
</table>

*Mean ± SEM
help of rotatory microtome. The sections were mounted on glass slide.

Those sections fixed in 10% formalin were stained with haematoxylin and eosin and those fixed in alcoholic formalin were stained with Gomori’s reticulin stain. The morphological features were observed under light microscope with 40x objective and 8x ocular lens and micrometry was done under oil immersion objective and 8x ocular lens with the help of ocular micrometer scale. Results were analyzed statistically with student t’ test and P<0.005 was taken as significant.

**OBSERVATIONS AND RESULTS**

The present study was designed to observe the morphometric study of Ketoconazole treated liver in albino rats. The
observations and results of microscopic examination were observed and compared with the control animals (Figure-1) and with the previous studies.

The morphological examination of H&E stained sections of liver shows dilatation and congestion of central vein, distorted hepatic cords, infiltration of RBCs and mononuclear cells and area of focal fibrosis within hepatic lobule, as shown in Figures-2 and 3.

Sinusoidal spaces are dilated and congested, Kupffer cells lining these spaces become prominent and their number also increased upto 7-9 per oil immersion field. Hepatocytes are enlarged with increased cytoplasmic granularity, bi-nucleated and necrosed (Figure-4).
The mean value of number of hepatocytes within reticule shows highly significant decrease (P<0.001) when compared to control group-A animals, as shown in Table-1. The mean value of diameter of hepatocytes shows a highly significant increase (P<0.001) when compared to control group-A animals, as shown in Table-2. The chromatin pattern in nuclei of hepatocytes was intense, irregular and fragmented with prominent nuclei. Some of the cells shows pyknotic nuclei, dispersed chromatin and faded or absent nucleoli. The mean value of nuclear diameter of hepatocytes shows highly significant increase (P<0.001) in groups-B2 and B3, significant increase (P<0.05) in group-B4, while insignificant (P>0.05) in group-B1 when compared to control group-A animals, as shown in Table-3.
The morphological examination of Gomori’s reticulin stained section of liver shows marked increase in amount and intensity of reticulin fibres around the pericentral zone, within hepatic lobule (Figure-5) and around the portal triad (Figure-6).

**DISCUSSION**

Ketoconazole was the first cost-effective broad spectrum, oral anti-fungal agent in a series of Azole derivatives, used successfully in the treatment of fungal infection but it is associated with some hepatic damage. The mechanism of injury was suspected as a reaction of Ketoconazole or its metabolite N-de-acetyl Ketoconazole (OAK) which is more cytotoxic (Rodriguez and Acosta, 1997). At the beginning toxic metabolites damage the smooth endoplasmic reticulum them produces further injury to mitochondria and plasma membrane. Those events lead to cloudy swelling, ballooning degeneration and necrosis of hepatocytes (Ming et al., 2003).

Many retrospective studies on liver show use of different doses of Ketoconazole for induction of toxicity such as 40 and 90 mg/kg of body weight (Rodriguez and Buckholz, 2003) and 40, 90 and 160 mg Ketoconazole/kg of body weight (Ming et al., 2003). We used the dose of 40 mg because it correlates with the dose of Ketoconazole in humans for fungal infection, i.e. 200 mg/day (Svejgaard and Ranek, 1982).

The marked congestion of sinusoids hypertrophy of Kupffer cells, dilated central veins with RBCs and inflammatory cells infiltration specially in portal triad are in conformity with the findings of Benson et al (1988) and Chien et al (1997). Increased cytoplasmic granularity of hepatocytes was also observed by Cotran et al (1999) and they attributed this change is due to disorganization of the arrangement of ribosomes on the rough endoplasmic reticulum.

Observation of increased fibrosis within hepatic lobule are in conformity with the previous observation of Benson et al (1988), Chien et al (1997), Cotran et al (1999) and Ming et al (2003). The major source of increased collagen is Ito cells in space of Disse which due to toxic injury transform into myofibroblast like cells which in turn increase fibrosis.

**CONCLUSION**

It is concluded from this study, that Ketoconazole induced toxic injury is related to dose and duration of therapy. Its frequent use as cost effective oral drug needs further research in humans.

**REFERENCES**


MacNair, A.L., Gascoigne, E., Heap, J.,
Shaikh et al.
