MORPHOMETRIC STUDY OF KETOCONAZOLE TREATED LIVER IN ALBINO RATS

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ABSTRACT

Objective:

To study the effect of ketoconazole induced liver damage, compare with control group and correlate with previous studies.

Materialand Methods:

Experimental study conducted during year 2005 in the Department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre,Karachi. Forty adult male albino rates 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 inraperitoneally daily for 03, 07, 05 and 30 days. Group-B received injection of ketoconazole 40 mg/kg of body weigh 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 the result were compared with control. Analyzed satistically with student 't' test and correlate with previous studies.

Results:

Ketoconazole treated animals showed distortion of hepatic architecture increase size of hepatocytes, decrease nuclear diameter and necrosis of hepatocytes within hepatic lobule as compared to control group-A animals.

Conclusion:

It was sconcluded 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.

Key Words:

Ketoconazole, Albino rats, Hepatocytes and Micrometry.

INTRODUCTION

Human fungal infections have increased dramatically in incidence and severity in recent years, mainly due to advances in surgery, cancer treatment and critical care accompanied by increase in the use of board spectrum anti-microbial and HIV epidemic. Pharmacotherapy of fungal disease has been revolutionized by introduction of the relatively non-toxic oral anti-fungal drug such as Ketoconazole, used successfully in the treatment of fungi, but it may cause some hepatic damage[1] and alteration in hepatic enzyme level[2]

Ketocanazole is an Azole derivative beside the anti fungal treatment also successfully used in the treatment of advanced prostate cancer by decreasing the testosterone level.[3]

Ketoconazole-induced hepatitis was first reported in 1981 by MacNair[4] and first fatality occurred in 1982 due to hepatic coma[5]. An autopsy of

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the patient revealed massive hepatocellular necrosis[6] and it is suspected that toxicity responses are not due to an immunology mechanism but as a result of reaction of either Ketoconazole or its metabolites[7and8].

Ketoconazole induced hepatotoxicity may react with protein and possibly hepatic glutathione (GSH), which is important in the detoxification pathway of Ketoconazole[9]. Hepatotoxicity induced by Ketoconazole is correlated to dose, maximum plasma concentration and clearance[10].

Hepatoxicity due to anti fungal drugs is overlooked by many physician in Pakistan. This study focused the hepatotoxic effects of ketoconazole (antifungal) . The objective of the study to observe the morphological and morphometric changes produced by ketoconazole in rat liver

MATERIAL AND METHODS

This study was conducted in Department of Anatomy, Basic Medical Sciences Institute, J.P.M.C., Karachi in the year 2005. A total number of 40 adult male albino rats 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 animals of group-A (control) was administered normal saline in a dose of 0.05 ml/100 gm of body weight intraperitoneally while each animals of group-B (ketoconazole treated) received intraperitoneal injection of Ketoconazole at a dose of 40 mg/kg of body weight [9].

All the animals were sacrificed at the end of period of treatment under ether anaesthesia. Liver was removed, fixed in 10% formalin for 24-48 hours. The tissue 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 help of rotatory microtome. The sections were mounted on glass slide.

The sections were stained with haematoxylin and eosin. The morphological features were observed under light microscope with 40x objective lens and micrometry was done under oil immersion objective and 8x ocular lens with the help of ocular micrometer scale. Result were analyzed statistically with student 't' test and $P<0.05$ was taken as significant.

RESULTS

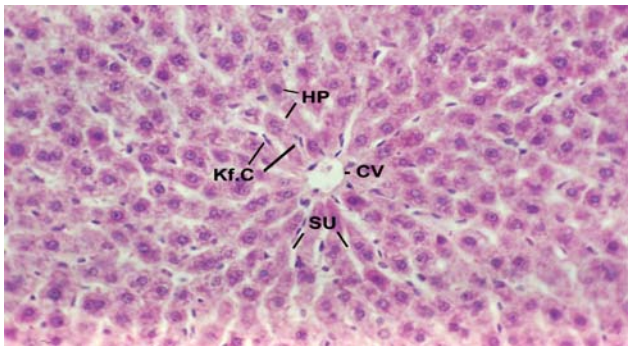
The present study was designed to observe the morphometric study of ketoconazole treated liver in albino rats. The observations and result of microscopic examination were observed and compared with the control animals (Figure 1).

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 within hepatic lobule, as shown in Figure-2 and 3.

Sinusoidal spaces are dilated and congested, Kupffer cells lining these spaces become prominent and their number also increased up to 7-9 per oil immersion field. Hepatocytes are enlarged with increased cytoplasmic granularity, polyploidy and necrosis (Figure-4).

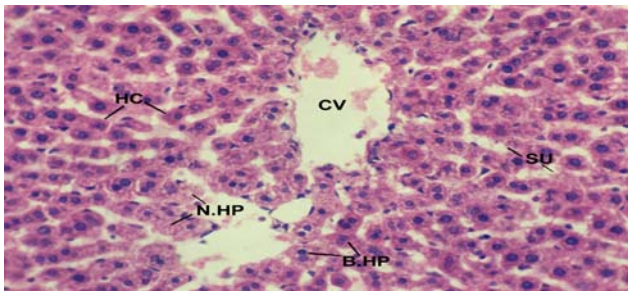
The mean value of number of hepatocytes within reticule shows highly significant decrease ($p<0.001$) when compared to control group-A animals, as shown in Table-1. The mean value of diameter of hepatocytes showed a highly significant increase ($P<0.001$) when compared to control group-A animals, as shown in Table-2. The chromatin pattern in nuclei of hepatocytes was intense, irregular and fragmented with prominent nuclei. Some of the cells showed pyknotic nuclei, dispersed chromatin and faded or absent nucleoli. The mean value of nuclear diameter of hepatocytes showed 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.

FIGURE- 1



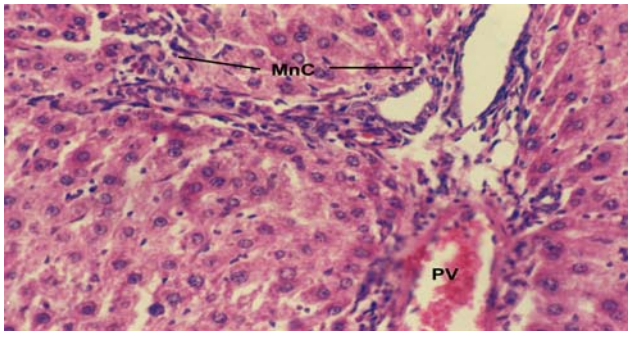
H&E stained 4 µm thick section of liver showing normal appearance of central vein (CV), hepatocytes (HP) arranged in radiating cords, sinusoids (SU), Kupffer cells (Kf.C) of control albino rat. (Photomicrograph x400)

FIGURE- 2



H&E stained 4 µm thick section of liver showing dilated and distorted central vein (CV) disturbed and irregular pattern of hepatic cords (HC) irregular and congested sinusoids (Su), necrosed hepatocytes (N.HP) and bi-nucleated hepatocytes (B.HP) in ketoconazole treated albino rat. (Photomicrograph x400)

FIGURE- 3



H& E stained 4 µm thick section of liver showing portal triad with highly dilated portal vein (PV) containing RBS's, high infiltration of mononuclear cells (MnC), necrosis of hepato cytes (N.HP) in Ketoconazole treated albino rat. (Photomicrograph x400)

FIGURE-4

H& E stained 4 µm thick section of liver showing irregular and dilated sinusoids (Su), enlarged hepaocytes (E.HP), enlarged and prominent nuclei (E.nc) or pyknotic nuclei (Pk.Nc) dispersed chromatin with faded nucleoli (Nol), bi-nucleated hepatocyte (B.HP) and hypertrophy of Kupffer cells (Kf.C) in Ketoconzole treated albino rat. (Photomicrograph x400)

TABLE- 1
MEAN HEPATIC CELL DIAMETER (µM) WITHIN RETICULE OF ALBINO RAT IN DIFFERENT GROUP AT VARIABLE TIME INTERNAL

Groups	Day-3	Day-7	Day-15	Day-30
A	15.14+0.17	15.11+0.07	15.07+0.05	15.02+0.13
(n=20)	(n=5)	(n=5)	(n=5)	(n=5)
B	18.21+0.24	17.94+0.30	19.10+0.26	19.56+0.20
(n=20)	(n=5)	(n=5)	(n=5)	(n=5)

TABLE- 2
***MEAN HEPATIC CELL DIAMETER (µM) WITHIN RETICULE OF ALBINO RAT IN DIFFERENT GROUP AT VARIABLE TIME INTERNAL**

Groups	Day-3	Day-7	Day-15	Day-30
A	6.25+0.20	6.56+0.11	6.33+0.07	6.35+0.10
(n=20)	(n=5)	(n-5)	(n=5)	(n=5)
B	6.68+0.12	7.56+0.15	7.97+0.04	6.68+0.11
(n=20)	(n=5)	(n=5)	(n=5)	(n=5)

TABLE-3
***MEAN NUCLEAR DIAMETER (µM) OF HEPATIC CELLS WITHIN RETICULE OF ALBINO RAT IN DIFFERENT GROUPS AT VARIABLE TIME INTERVAL**

Groups	Day-3	Day-7	Day-15	Day-30
A	6.25±0.20	6.56±0.11	6.33±0.07	6.35±0.10
(n=20)	(n=5)	(n-5)	(n=5)	(n=5)
B	6.68±0.12	7.56±0.15	7.97±0.04	6.68±0.11
(n=20)	(n=5)	(n=5)	(n=5)	(n=5)

*Mean±SEM

DISCUSSION

Ketoconazole was the first cost-effective board 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 (DAK) which is more cytotoxic[11]. At the beginning toxic metabolites damage the smooth endoplasmic reticulum then produces further injury to mitochondria and plasma membrane. These events lead to cloudy swelling, ballooning degeneration and necrosis of hepatocytes[10]. In present study post katacanazol treated rat liver showed marked irregularity in hepatic lobular pattern with distorted hepatic cords, dilated sinusoids and cellular necrosis. This is an agreement with Kumar et al[12] who described similar type of changes with hepatocytic destruction leads to cellular degeneration, apoptosis and necrosis.This is also in confirmation with Kasper etal who also describes the similar type of drug indused hepatocellular changes[13].

The marked congestion of sinusoids hypertrophy of Kupffer cells, in portal tried is in conformity with the findings of Benson[14] and Chien[15]. This is also in agreement with the Gillman etal[16] who also discussed the sinusoidal and hepatic lobular changes.Increased cytoplasmic granularity of hepatocytes was also observed by Cotran[17] and they attributed this change is due to disorganization of the arrangement of ribosomes on the rough endoplasmic reticulum. The present study also shows some fibrotic changes in hepatocytes which also in confirmation with Kim et al[18] who also showed similar type of necrotic changes in hepatic lobule.

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.

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