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Histological and electron microscopic examination of the effect of Dexketoprofen Trometamol on liver in rats

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Author's contribution to the manuscript

- Arzu Esen Tekeli: She made a significant contribution to experiment design. She has made contribution to acquisiton of datas, analysis and drafting of the manuscript. She has made a substantial contribution to interpretation of datas and revising the manuscript for intellectual content. She prepared the manuscript.
- Hatice Yağmurdur: She has made contribution to acquisiton of datas, analysis and drafting of the manuscript.
- Erçin Öngen: He has made contribution to acquisiton of datas, analysis and drafting of the manuscript.
- Ahmet Tekeli: He has made part of the experimantal animals experiment. He prepared the manuscirpt.
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ABSTRACT

Background: The current study was performed for histological and electron microscopic examination of the effects of different doses of dexketoprofen trometamol on liver in rats.

Material and Methods: Shame group consisted of rats administered 1 ml of 0.9% NaCl twice a day via intraperitoneal route, 8 mg/kg/day was used in Dexketoprofen Trometamol low-dose group, and 16 mg/kg/day was used in Dexketoprofen Trometamol high-dose group. 30 healthy Wistar albino type male rats were used in the study as animal materials.

Results: The presence of TUNEL positive cells was increased with the increasing dose level of Dexketoprofen Trometamol and TUNEL positive hepatocytes distributed all over the tissue. Diffuse degeneration was determined in the liver sections of the group administered high-dose. Necrotic areas became more apparent particularly in regions close to the central vein. PCNA involvement was detected to be considerably increased compared to the shame and low-dose groups. Electron microscopic image of liver in the group administered high-dose drug showed that all hepatocytes present with highly active cell structure. Hepatocyte mitochondria were observed to be highly developed and to grow large and fuse from place to place. Granulated and smooth endoplasmic reticulum tubulus and cisternae displayed a highly-dilated appearance. Bile canaliculi were distinguished as dilated and its lumen was covered with microvilli. There were many vacuolar formation in addition to lipid droplets in the cytoplasms of ito cells.

Conclusion: Dexketroprofen Trometamol drug administration was determined to increase activation particularly in parenchymal cells depending on dose and cause degeneration in liver tissue with heavy activity.

Key words: Dexketroprofen Trometamol, liver, histology, electron microscope, rat

1. INTRODUCTION

Dexketoprofen trometamol is a highly water-soluble salt of rac-ketoprofen and non-steroidal anti-inflammatory drug (NSAID) used for pain.[1] 6% of adults in the USA were reported to use NSAID in a month, and 24% of this group was reported to use over the counter ibuprufen.[2] Epidemiological risk of clinically apparent liver injury was reported to be low in patients using NSAID. However,

the results may be serious and cause diagnostic confusion.[3] Bessone[4] reported that NSAI drugs trigger liver diseases, as demonstrated by clinical trials. When the results were compared, the risk of hospitalization due to liver seems to be in the range of 3-23%. NSAID display a wide range of liver injury from asymptomatic, to transient hyper-transaminasemia, and to fulminant liver failure. Female patients over 50, patients with autoimmune disease and people who use concomitant medications are more prone[3]. Several NSAID (bromfenac, ibufenac and benexaprofen) were removed from the market as they cause hepatotoxicity.[4] Neutropenia is an infrequent side effect of NSAID.[5] NSAIDs were demonstrated to decrease the risk chronic inflammation and many cancer types. However, the risk of death due to chronic liver disease and hepatocellular carcinoma is yet to be determined.[6]

NSAIDs are commonly used drugs which are associated with hepatotoxicity. The data from the histological and particularly electron microsopic retrospective-prospective trials on the liver injury due to NSAIDs are insufficient. Ekici et al.[7], on cartilage and synovium of intra-articular injected dexketoprofen trometamol (6.25 mg) in rat, Hacibeyoğlu et al.[8], the rabbit knee joint injected of dexketoprofen trometamol (6.25 and 12.5 mg), it was determined that there is no significant histopathological damage on articular cartilage. Literature studies indicate that further animals and electron microscopic studies are needed in order to determine the effective and adverse dose to be used in humans. The effects on liver damage of recommended clinical dose level and increasing cumulative high dose level have not been previously investigated. The current study aimed to provide a histological and electron microscopic examination of the effects of different repeated doses of Dexketoprofen trometamol which is newly started to be used in clinical practice and has analgesic properties on liver.

2. MATERİAL AND METHODS

2.1. Animal material

30 healthy Wistar albino type male rats weighing 220 gr on average were used in the study as animal materials. The study was performed in Ankara University experimental animal laboratory after the approval of Ankara University, Animal Studies Local Ethics Committee. Before the study, the diet, environment, day-night conditions of the rats were standardized under optimal conditions. During the trial, animals with pellet form mixed food consisting of 2700 Metabolic Energy kcal/kg and 24% Crude Protein, and rats were given food and water ad libitum. Rats were separated into 3 groups with randomization as to each group has 10 rats. The trial lasted 7 days. The rats were sacrificed with 80 mg/kg Ketamine Hydrochloride (Ketalar, Pfizer) administration and their livers were removed.

2.2. Histological Examination

The tissue samples were preserved into 10% formalin solution for the electron microscopic and immunohistochemical examination.

2.3. Immunohistochemistry for apoptosis

The extent of apoptosis was evaluated using a previously described immunohistochemical method that identifies cell death as reflected by DNA fragmentation. [9]

2.4. Immunohistochemistry for proliferation

Proliferation was determined by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) [10].

2.5. Electron microscopic method

Tissue samples were evaluated by reflecting the findings.[11]

3. RESULTS

3.1. Apoptag Findings

In the TUNEL staining performed in the liver tissue of the shame group; generalized TUNEL positive cell presence was detected all over the tissue starting from the surroundings of central vein In the low-dose group; TUNEL positive cells were found to be reduced compared to the previous groupIn the high-dose group, TUNEL positive cells were determined to be even more reduced (Figure 1a, b).

3.2. Immunohistochemistry Findings

PCNA involvement was observed in the hepatocytes in the liver section of the Shame group. Sinusoids were observed to be normal. PCNA involvement was also observed in a few endothelial cellsi In the low-dose group, PCNA involvement in the liver sections was

found to be at a similar level and distribution to control group PCNA involvement was detected to be considerably increased compared to the other two groups (Figure 2).

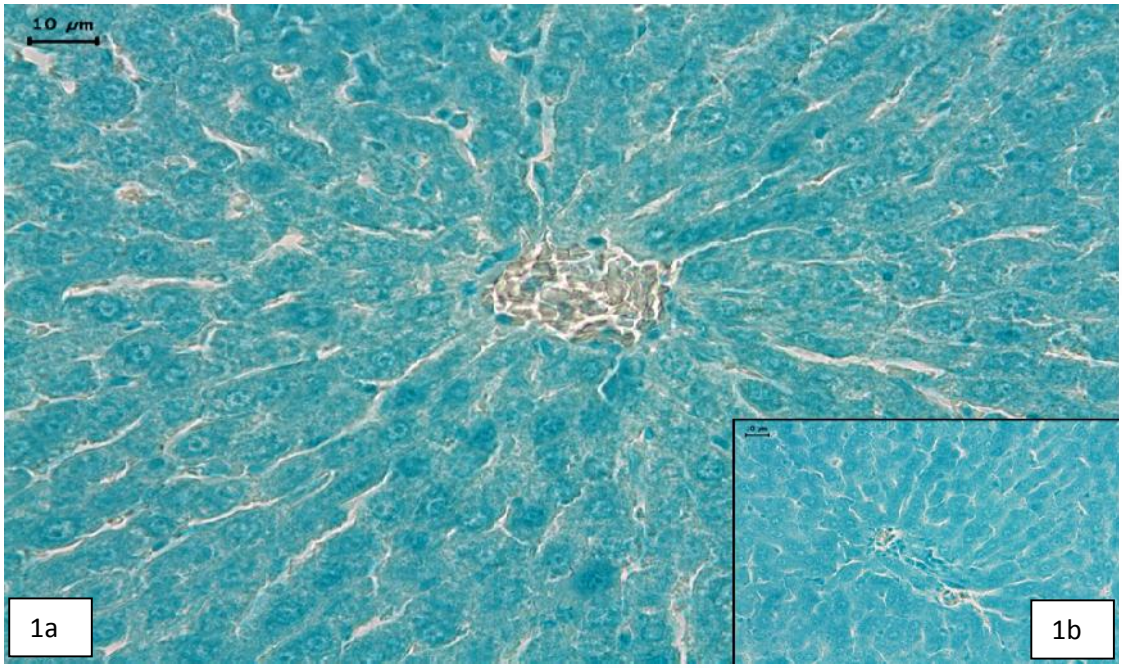

Figure 1a,b
High-dose group (1a,b; TunelX400).

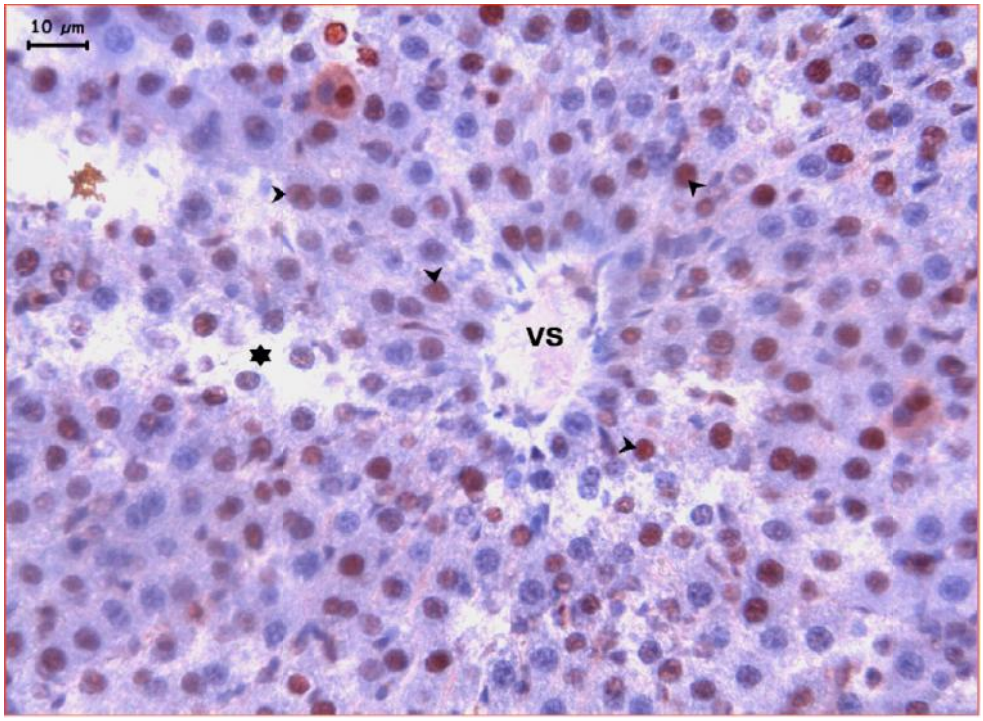

Figure 2
Liver section of the high-dose group ✸: Necrotic area, ➤: PCNA positive cells, CV: central vein (Immunoperoxidase – Hematoxylin X 400)

3.3. Electron Microscopic Findings

Hepatocytes in the liver sections of the shame group were observed to have mitochondria with normal crista characteristics, an abundant amount of granulated endoplasmic reticuli, smooth endoplasmic reticuli and normal nucleus structures

While hepatocyte nuclei were observed to have normal structure in this group, absence of apparent nucleolus structure drew attention. While smooth endoplasmic reticuli were observed to have active tubular appearance, granulated endoplasmic reticuli had apparent distribution particularly around the nucleus. Ito cells were characterized with large and small lipid droplets and vacuolar formations

Hepatocyte mitochondria were observed to be highly developed and to grow large and fuse from place to place. Granulated and smooth endosplasmic reticulum tubulus and cisternas displayed a highly-dilated appearance. There were many primary and secondary lysosomes observed in cytoplasm. Bile canaliculi were distinguished as dilated and its lumen was covered with microvilli. There were many vacuolar formations in addition to lipid droplets in the cytoplasms of ito cells (Figure 3).

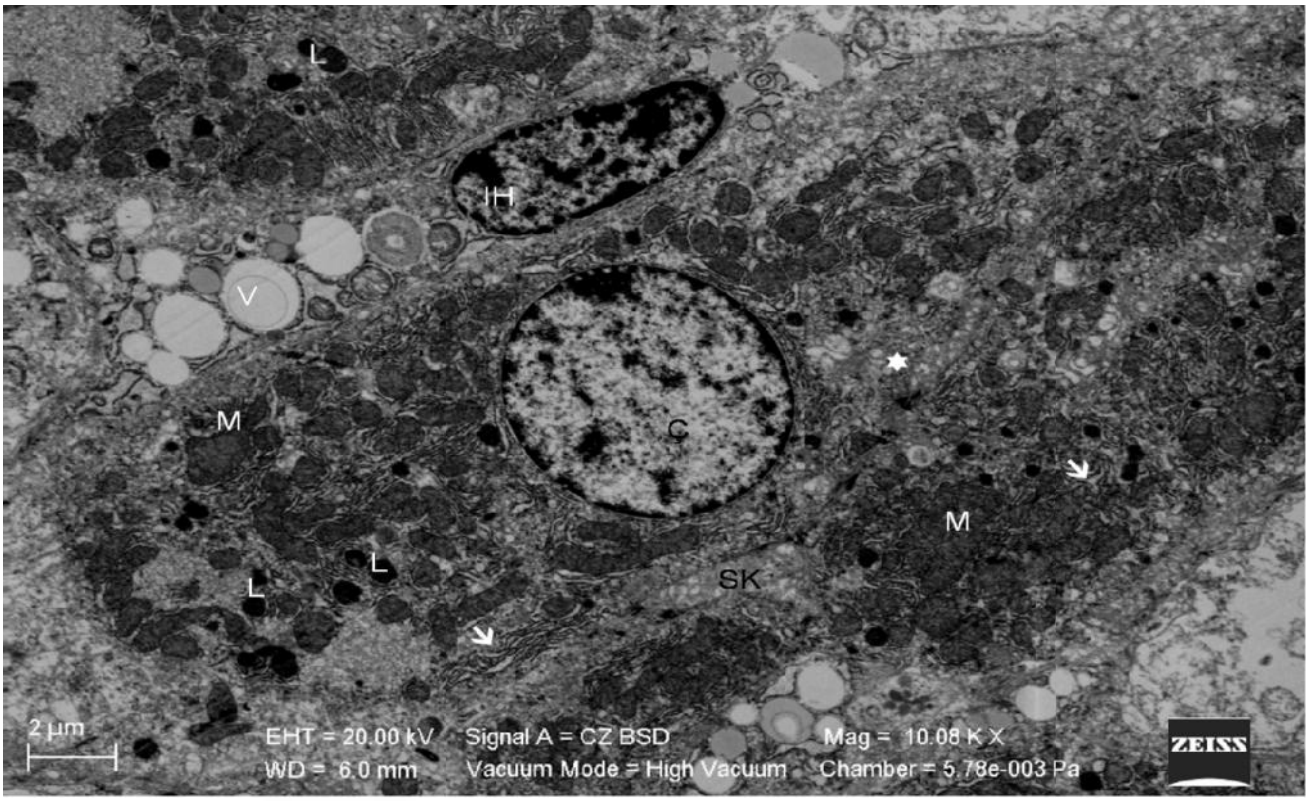

Figure 3

Electron microscope image of liver in the high-dose group. N: hepatocyte nucleus, M: mitochondria which grew larger and fuse from place to place, L large amount of primary and secondary lysosomes, ↑: dilated granulated endoplasmic reticulum cisternae, ✸: dilated granulated endoplasmic reticulum cisternae, BC: dilated bile canaliculus, IC: ito cell with a large amount of vacuols (Uranyl acetate – lead citrate)

4. DISCUSSION

Drug induced liver injury (DILI) is a different and complex entity, and occurs 5 to 90 days of drug intake. In the present study, the trial was terminated on day 7 due to sudden deaths on day 5 in the high dose group. Acetaminophen, anti HIV, troglitazone, anti-convulsants, pain-killers, antibiotics and anti-cancer medications are the most common causes of fatal DILI.[12] Schmeltzer et al.[13], in their study performed to determine the effects of NSAIDs on liver injury, performed histological examination on the livers of 10 cases, and they reported acute hepatitis due to diclofenac use in 3 cases, oxaprozin use in 2 cases, and cholestatic hepatitis due to celecoxib, ibuprofen, meloxicam, diclofenac and valdecoxib use in five cases. In their study, Sahasrabuddhe et al.,[6] reported that NSAIDs other than aspirin only decreases the risk of death due to chronic liver disease, and aspirin use decreases the risk of death due to chronic liver disease and development of hepatocellular carcinoma. In a retrospective trial, the risk of liver injury due to

NSAIDs used in the treatment of rheumatoid arthritis found to be 10 times higher than the risk liver injury due to NSAIDs used in the treatment of osteoarthritis.[3] Kishida et al. [14], similarly reported that cell injury may occurs due to the effect of acidic fragment of NSAIDs or NSAIDs metabolites on essential cell proteins. Recently, several in vitro animal studies were performed to determine the possible mechanisms of NSAID related hepatotoxicity. Rat liver mitochondrion and pure rat liver cell were isolated and it was demonstrated that diphenylamine which is commonly found in NSAID structure, separates oxidative phosphorylation from each other, decreases hepatic ATP synthesis, and induces liver cell injury.[15,16] These data are correlated with the findings of maintaining hepatocyte tissue integrity despite crista loss from place to place in hepatocyte mitochondria in the electron microscopic examination in the low-dose group, presence of advanced active cell structure in the hepatocytes in the high-dose group, growing large and fusion from place to place of hepatocyte mitochondria, presence of large amount of primary and secondary lysosomes observed in cytoplasm. Therefore, our study became a guide as its histological and electron microscopic findings demonstrate the toxicity of high doses of dexketoprofen trometamol on liver.

Valles et al.[1], reported that single and repeated doses (25 mg) of Dexketoprofen trometamol decreases urine excretion in patients with liver conditions. Dose adjustment for dexketoprofen trometamol is recommended in patients with liver function disoerder. Inal et al[17], in rats, investigated the toxicities of dexketoprofen trometamol (DEXT), meloxicam (MEL) and diclofenac sodium (DIC) use at clinical doses on liver and kidney by using histopathological and biochemical parameters to see their different effects on bone healing. Consistent with our findings when histopathological examination of liver was compared with DIC group, high level of parenchymal necrosis was shown in DEXT and MEL groups.

5. CONCLUSION

Drug administration was determined to accelerate the metabolic process, increase the activation particularly in parenchymal cells in a dose-dependent manner, and high dose administration was found to be toxic enough to increase necrotic process in liver. New studies on the detection of cross reaction with other drug groups in patients who developed liver injury induced by NSAIDs, and also on the effective dose detection of drugs which may be hepatotoxic even at therapeutic doses are necessary.

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