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# CONCOMITANT USAGE OF HIGH DOSE OF ACETAMINOPHEN WITH THERAPEUTIC DOSE OF KETOROLAC, AND THEIR TOXIC EFFECTS IN RATS

# YÜKSEK DOZ ASETAMİNOFEN İLE TERAPÖTİK DOZ KETOROLAK'IN BİRLİKTE KULLANIMI VE SIÇANLARDAKİ TOKSİK ETKİLERİ

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*The effects of therapeutic dose of ketorolac on acute toxic effects of acetaminophen have been evaluated by using rats as an animal model setting. Acetaminophen oral doses (1 g/kg and 2g/kg) were administered to rats with or without i.p. ketorolac (40 mg/kg). Blood acetaminophen levels were measured at the end of 24 h period by the method of fluorescence polarization immunoassay with TDx Analyzer. Histopathologic examinations on kidneys, liver stomach, intestine, lungs, spleen and myocardium were also performed, 3 days after the treatment. It has been concluded that therapeutic dose of ketorolac worsens the renal and hepatic injuries in case of acute acetaminophen intoxications.*

*Sıçanlar deney hayvanı modeli olarak alınmış ve terapötik doz ketorolak'ın akut asetaminofen intoksikasyonundaki etkileri değerlendirilmiştir. Ratlara oral 1 g/kg ve 2 g/kg asetaminofen tek başına veya 40 mg i.p. ketorolak dozu ile birlikte uygulanması 24 saatlik peryodun sonunda kan asetaminofen düzeyleri floresans polarizasyon immunoessey yöntemi ile TDx analizörü kullanılarak ölçülmüştür. Ayrıca böbrekler, karaciğer, mide, barsak, akciğer, dalak ve miyokard üzerinde ilaç verilmesinin ardından 3 ncü günün sonunda histopatolojik incelemeler yapılmıştır. Terapötik doz ketorolak'ın akut asetaminofen zehirlenmelerine bağlı ortaya çıkan renal ve hepatik harabiyetleri daha da artırdığı sonucuna varılmıştır.*

**Keywords** : *Acetaminophen; Ketorolac; Acute acetaminophen intoxication; Organ injury; Rats*

**Anahtar kelimeler:** *Asetaminofen; Ketorolak; Akut asetaminofen zehirlenmesi; Organ harabiyeti; Sıçanlar*

## Introduction

Mixed drug interactions are the major problems in emergency units in hospitals. Obtaining a complete and accurate history is one of the most crucial steps in the initial diagnosis and subsequent management of the poisoned patient. Acute poisoning, unfortunately, is often mismanaged. Overtreatment of the poisoned patient with large doses of antidote, sedatives or stimulants often does far more damage than the poison itself. Thus, identifying the poisons as soon as possible, enhance the quality of the overdosed patients management in the emergency units(7).

Mixed drug intoxications make up almost 50% of all adult overdoses. Sedative-hypnotics and analgesic drugs are the most frequently used drugs, as single or mixed pharmaceutical forms, which may lead to toxic manifestations among people by ingesting intentionally or accidentally. The potential for concurrent administration of those drugs are also considerable in overdosed patients. Interaction between nonsteroidal antiinflammatory drugs (NSAIDs), and other drugs relatively frequent because of the wide use of NSAIDs. Such interactions with drugs of narrow therapeutic index used in serious disease states may lead to toxicity (19).

Acetaminophen is one of the popular NSAIDs (6). Since its relative safety and freedom from side effects when taken in therapeutic doses, the wider utilisation of acetaminophen also increased the incidence of toxicity to overdose. In acute overdose, the main site of organ injury is the liver. Mild hepatic toxicity may also lead to acute renal failure. In the use of therapeutic dose, neither the drug itself nor its metabolites which are eliminated are toxic. A small amount, 4% of any given dose is metabolized by the hepatic cytochrome P 450 mixed function oxidase system to an active intermediate metabolite, N-acetyl-benzoquinone, which produces hepatotoxicity. In te therapeutic dose, this metabolite is detoxified by glutathione.

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In the overdose situation, glutathione stores drop to less than 30% of normal, the unconjugated toxic metabolite can bind covalently to various hepatocellular constituents and produce hepatic degenerations. In this case, acetaminophen is also metabolized in the renal medulla to toxic reactive metabolites which may produce renal toxic manifestations (7).

Ketorolac is a member of NSAIDs which is widely used in emergency units and clinics during the postoperative period because of its analgesic effects and lack of central nervous system activities (11). Ketorolac inhibits synthesis of prostaglandins and may exert its analgesic effects peripherally (17). The drug is extensively bound (>99%) to plasma proteins. Ketorolac is mainly metabolized through glucuronidation and oxidation. The primary route of ketorolac and its metabolite (p-hydroxy metabolite) is in the urine as conjugates (91%) and the remainder is excreted in the feces. Decrease in serum albumin, such as in liver cirrhosis, would be expected to change ketorolac clearance. Ketorolac is indicated for the short term management of pain. It is not recommended for longer then 5 days usage because of the possibility of severe adverse reactions (3,16).

In this study, we wanted to evaluate the effects of therapeutic dose of ketoroloac on acute toxic effects of acetaminophen by using rats as an animal model setting.

## Materials and Methods

Experiments were performed on female Wistar rats with an average body weight of 175-200 g. Rats were housed in an environment maintained at 20±2°C, isolated from noise, and with a 12-h light/dark cycle. Diet and tap water were given ad libitum. Acetaminophen (Sigma, USA) was suspended in a 1% tylose solution and instilled by stomach tube (20). First group of animals (n=8) were received 1 g/kg acetaminophen, and 5 of them were sacrificed for the determination of blood acetaminophen levels at the end of 24 h period. All blood samples were treated and analyzed by the method of fluorescence polarization immunoassay with TDx Analyzer (Abbott, USA) (5). Remaining 3 animals were sacrificed 3 day after the treatment for the histopathologic examinations. Kidneys, liver, stomach, intestine, lungs, heart and spleen were removed and examined macroscopically. Representative sections of each organ were fixed in10% buffered formalin, and embedded in paraffin, sectioned at 5-6 μm, and stained with hematoxyline-eosin for the histopathologic examination (18). Second group of animals were received 40 mg/kg ketorolac (Deva, İstanbul) intraperitoneally and 1 g/kg acetaminophen as described above. In a same manner, 2 g/kg acetaminophen alone or plus ketorolac were given to the third and the fourth groups of animals. Blood acetaminophen level measurements and the histopathologic examinations were performed as depicted previously. The last group of animals were used as controls, and received only 1% tylose solution orally and normal saline intraperitoneally. Data were expressed as mean±SD. Statistical comparisons were made by student's test.

## Results and Discussion

Histopathologic findings of some organs taken from 1 g/kg acetaminophen treated rats were as follows: In the liver; portal veins were hyperemic and diffusional paranchimal degenerations in hepatocytes were observed. In the kidneys; vascular hyperemia in cortex, adhesions to Bowman's capsule in some glomeruli, hyperemic glomerular capillaries and vacuolisations were seen. Additionally, sparse mononuclear cells in some glomeruli (glomerulitis acuta) were remarkable. Necrobiotic alterations were also prominent in tubular epithelia in medullar and cortical areas (Fig 1). Gastritis cattarrhalis acuta in the stomach, enteritis catarrhalis acuta in the intestines, interstitial pneumonie in the lungs, petechial haemorrhagie in myocardium were also found. Any histopathological changes was observed in the spleen.

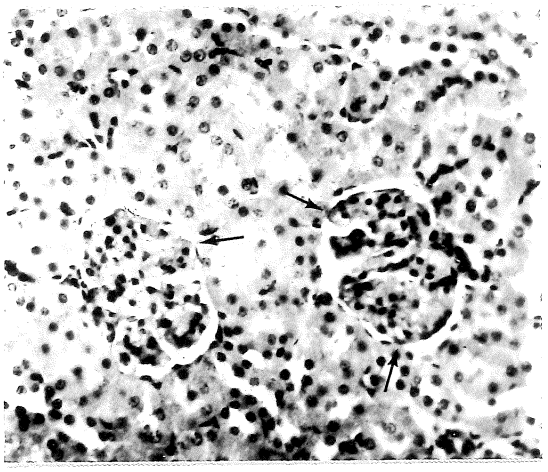

Fig 1. Necrobiotic alterations in tubuli, vacuolisation and adhesions to Browman's capsule in glomerulus (arrows). Hematoxylin-eosin stain (HxE)X200

In 2 g/kg acetamionophen treated animals, histopathologic examination results were; in the liver, hyperemic portal veins and the activation in Kupffer's cells were prominent. Paranchimal degenerations in hepatocytes, especially in v.centralis region, and advanced degenerative changes or necrosis were also observed (Fig 2a). In addition, nuclei of

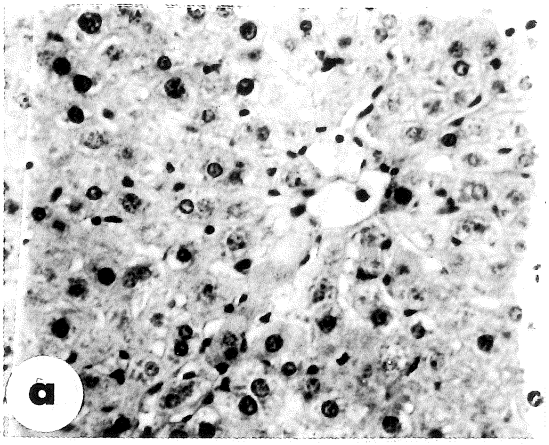


Fig 2.a) Necrobiotic alterations of hepatocytes around v.centralis in liver

hepatocytes were picnotic and dissociation on remark cordons were present. Mononuclear cell infiltrations through some portal areas were seen (Fig 2b). In the kidneys, stomach,

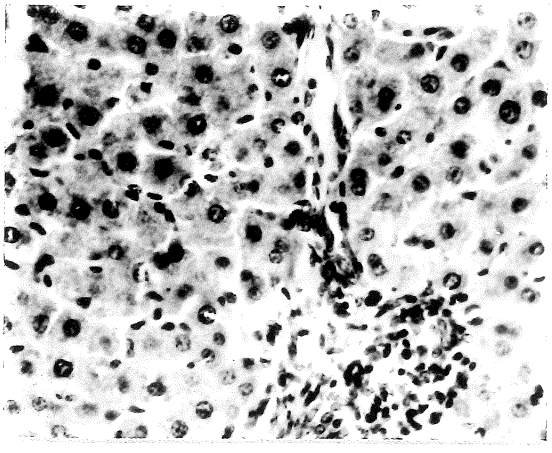

Fig 2. b) Mononuclear cell infiltrations in portal area

intestine, lungs, spleen and myocardium, observed histopathologic changes were similar in those 1 g/kg acetaminophen treated animals. Only difference was dilatations in cortical tubuli in the kidney (Fig 2c).

Acetaminophen (1 g/kg and 2 g/kg) and ketoroloc treated animals were also examined histopathologically. All kind of histopathologic

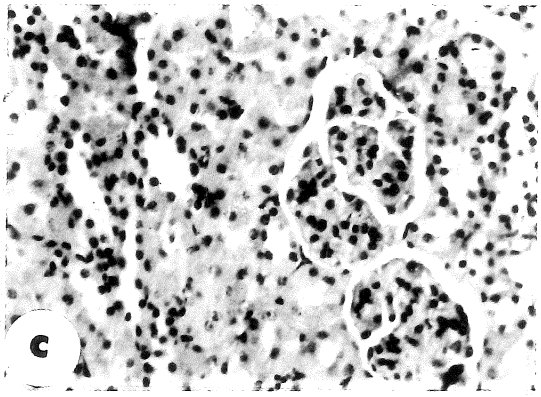


Fig 2. c) Tubuli dilatations in kidney. HxE X 200

alterations were as similar as the results obtained from only acetaminophen treated animals, but the severity of this kind of degenerations were advanced (Fig 3, 4a and 4b).

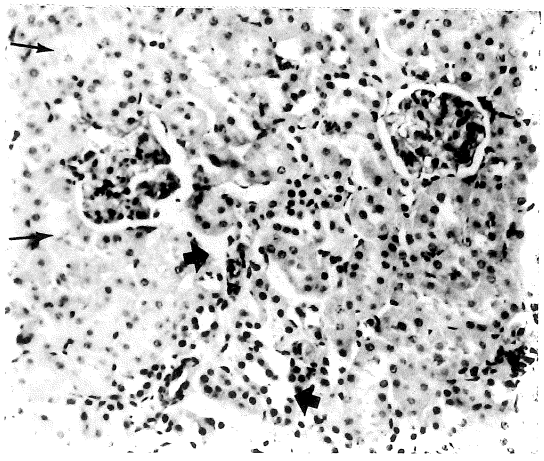

Fig 3. Tubuli dilatations (thick arrow) and necrobiotic alterations of tubuli epithelia (thin arrow) in kidney. HxE X 150

Blood acetaminophen levels were seen in Table 1. Two different high acute acetaminophen doses were well tolerated by all animals, and characterized histopathologic alterations were observed in such high doses

of acetaminophen alone or combined with ketorolac.

The success of ketorolac as a nonnarcotic anagesic is likely to propagate its widespread use to control moderate to severe postoperative pain. Indeed, of the patient treated with

Table 1. Blood acetaminophen levels of rats.

| Group (n=5) | Blood acetaminophen levels (µg/ml) ($\bar{x}$±SD) | Significance |
|---|---|---|
| Acetaminophen (1 g/kg) | 24.84±15.27 | N.S. |
| Acetaminophen (1g/kg) +Ketorolac | 24.04±4.79 | |
| Acetaminophen (2 g/kg) | 78.03±19.41 | N.S. |
| Acetaminophen (2 g/kg) + Ketorolac | 68.83±8.70 | |

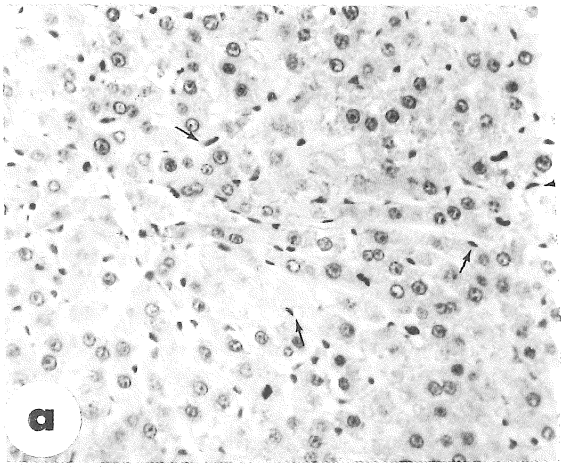


Fig 4 a) Activation in Kupffer's cells (thin arrow) and necrobiotic alterations in liver. HxE X 150

ketorolac and described in the medical literature, nearly 90% had had a major surgical procedure (15).

Ketorolac exhibits some organ selectivity with a particularly high activity in the kidneys (13). Acute and chronic impairment of renal function have been reported as complications of thearpy with NSAIDs. Renal prostaglandins play an increasingly important role in maintaining renal function when there is decreased

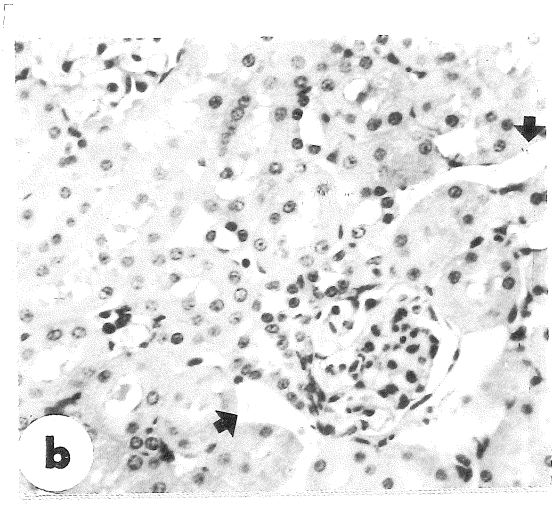


Fig 4. b) Tubuli dilatations in kidney (thick arrow), vacuolisation and adhesions to Bowman's capsule in glomerulus. HxE X 250

renal function (14, 17). The elderly exhibite reduced clearance of keterolac (1). Renal insufficiency may cause an accumulation of ketorolac in plasma(2). Pottle et all (12) presented a case report on renal failure following short term usage of keterolac in a patient with no known risk factors who underwent an uncomplicated laparotomy. Acute and chronic renal failure related to ketorolac is transient and improved after discontinuation of this durg (8).

Postoperative ketorolac therapy should be avoided in patients who have conditions that predispose to NSAIDs nephrotoxicity. Likewise in nonsurgical patients the some degree of caution should be used with ketorolac as with any oral NSAID (4,15).

It was reported that hepatic disease might not affect the pharmacokinetics of ketorolac (2). At present, there is no study in the literature about acetaminophen induced hepatic insufficiency and the concomitant usage of keterolac. Acetaminophen is available without prescription, and it has earned a prominent place as a common household analgesic. However acute overdosage causes fatal hepatic damage and the number of self-poisoning and suicides with acetaminophen has grown alarmingly in recent years (10). Therefore

therapeutic benefit of using paracetamol should be carefully evaluated, taking into consideration its potential for inducing acute, chronic and genotoxic effects (9). About 1 to 10 percent of patient treated only with supportive care suffer acute renal failure, usually, in conjunction with severe hepatic failure. It may be that acetaminophen is metabolized in the renal medulla to toxic reactive metabolites. The most serious adverse effect of acute overdosage of acetaminophen is a dose dependent (7).

Our study shows that overdose acetaminophen and therapeutic dose of ketorolac enhance the predicted toxic effects on kidney and liver in case of their concomitant usage comparing with control group of animals (Fig 1-4). On the other hand, ketorolac has no apparent effect on acetaminophen plasma levels (Table 1).

In conclusion, during the treatment of acute acetaminophen intoxication in emergency units, concomitant usage of ketorolac has a special importance. It is worthy of note that therapeutic dose of ketorolac worsens the renal and hepatic injury in case of acute acetaminophen intoxication.

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