

# Acute Toxicity Study of Indomethacin and Oxytetracycline in Rabbits

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## Abstract

**Background:** The toxic effect of nonsteroidal anti-inflammatory drugs (NASIDs) and broad-spectrum antibiotics in human and animal science has been studied widely. The advantages and the adverse impact of these medications could be determined by measuring some biochemical parameters such as the level of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN). In addition, the changes in some hematoimmunological parameters could also be measured; for instance, red blood cell (RBC) count, hemoglobin level, white blood cell (WBC) count, and differential WBC count. **Objectives:** The aim of this study is to evaluate the effect of both indomethacin, as a NSAID example, and oxytetracycline, as a broad-spectrum antibiotic separately and together, on some biochemical and hematological parameters. **Materials and Methods:** Sixteen male albino rabbits were used, with age about 1 year and body weight about 1.5 kg. They were divided into four groups and each group contains four rabbits. The first group served as a control group, which receives only food and water. One of the groups were administered with oxytetracycline at a dose of 50 mg/kg. The second was administered with indomethacin at a dose of 5 mg/kg, and the final group was administered with both oxytetracycline and indomethacin with the same previous dose. All the drugs were dissolved in the distilled water before administration of dose to each animal in the group orally using a stomach cannula for 14 days. The rabbits were kept for 14 days under the room temperature with free access to food and water. Serum was separated from the clotted blood obtained by ear vein. Total RBC and WBC counts were made by the hemocytometer method. The differential WBC counts were made by finding the percentage average of the different types of cells counted in ten fields from Giemsa-stained slides. Serum enzymes such as ALP, AST, and ALT were determined by procedures of Sigma Diagnostics, BUN by method of Crocker. **Results:** The level of ALP has shown great increase in all groups, while the level of AST and ALT was increased only in the group that administered with indomethacin. However, both parameters showed a significant decrease in the other groups. Regarding hematological changes, WBC increased in all groups, while RBC and Hb level showed sharp decrease. Lymphocytopenia occurred in only the group that administered with oxytetracycline. **Conclusion:** The results showed toxic effect of these two most popular using drugs in animals specifically when it is used chronically. In addition, there is no great difference when it is used separate or together. Further study is required to investigate the level of damage that happens in the organs.

**Keywords:** Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, white blood cells, and red blood cells

## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, naproxen, and indomethacin, are widely used for their analgesic and anti-inflammatory effects. This occurs through their inhibitory effect in the synthesis of prostaglandin.<sup>[1]</sup> At the same time, broad-spectrum antibiotics are considered as one of the most important and commonly used antimicrobial agents. Apart from the advantages of these medications, their chronic use could have an adverse effect on liver activity, and this could be

determined by measuring some biochemical parameters such as the level of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN).<sup>[2]</sup> In addition, the changes in some hematoimmunological parameters could also be measured; for instance, red blood cell (RBC) count, white blood cell (WBC)

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count, differential WBC count, and C-reactive protein (CRP) level.<sup>[3]</sup>

It has been experimentally proven that aspirin and other NSAIDs block the formation of colon cancer in animals, and there is evidence that prolonged usage of NSAID decreases the incidence of colorectal cancer in humans.<sup>[4]</sup> Biliary excretion can be used as an indicator for direct determination of the toxicity of some compounds. For instance, indomethacin can cause intestinal lesions. The toxic response of different species is directly related to the indomethacin level that is excreted into the bile.<sup>[5]</sup> The route of elimination of these compounds could be different according to the different species. For example, in dogs, indomethacin is excreted through feces. Moreover, the greater portion of a dose is excreted in bile as conjugates.<sup>[6]</sup>

At least, three different types of nephrotoxicity have been associated with NSAID administration. These include acute renal failure, which occurs within hours of a large dose of an NSAID; analgesic nephropathy which occurs from chronic consumption of NSAID<sup>[7]</sup> and interstitial nephritis, which is characterized by diffuse interstitial edema with infiltration of inflammatory cells.<sup>[7,8]</sup>

Oxytetracycline, on the other hand, is the most common broad-spectrum antibiotic that is used in veterinary clinics to treat intestinal and respiratory infections. Oxytetracycline is considered as one of the members of tetracycline group; it has an effect on Gram-positive and Gram-negative microorganisms, *Rickettsia* species, *Mycoplasma* species, and *Protozoa* species. Due to the fact that the intestine microflora resists the chronically use of this medication, the prolonged use could have a negative impact on the treatment process. Nowadays, it is widely used for treating respiratory infection.<sup>[9]</sup>

Oxytetracycline is cheap and can be administered in three different routes: oral, subcutaneous, and intramuscular. It is also used topically on the skin. However, oxytetracycline is known to have a toxic effect on the pancreas, as it causes pancreatitis. This may lead to the fact that it might have an adverse impact on the liver and its functions.<sup>[10]</sup> It has been proved that the production of CRP is also inhibited by oxytetracycline.<sup>[11,12]</sup> Oxytetracycline could have some effects on the level of CRP and also on the level of WBCs. Although NSAIDs are referred to as being anti-inflammatory, their effect on CRP levels is still poorly documented in the literature.<sup>[13]</sup>

The study is, therefore, carried out to evaluate the effect of both indomethacin, as an NSAID example, and oxytetracycline, as a broad-spectrum antibiotic separately and together, on some biochemical and hematological parameters.

## MATERIALS AND METHODS

### Experimental animals

Sixteen male albino rabbits were used, with age about 1 year and body weight about 1.5 kg. They were divided into four groups, and each group contains four rabbits. The first group

served as a control group, which receives only food and water. One of the groups was administered with oxytetracycline at a dose of 50 mg/kg. The second group was administered with indomethacin at a dose of 5 mg/kg, and the final group was administered with both oxytetracycline and indomethacin with the same previous dose. All the drugs were dissolved in distilled water before administration of dose to each animal in the group orally using a stomach cannula for 14 days. The rabbits were kept for 14 days under the room temperature with free access to food and water.

### Test drugs and chemicals

Drugs used were indomethacin (Global Pharmaceuticals, GMBH, Yantai, China), at a dose of 5 mg/kg and oxytetracycline at a dose of 50 mg/kg which is provided by Ranbaxy, New Delhi, India. All the drugs were dissolved in the distilled water before oral administration of dose to each animal in the groups using a stomach cannula for 14 days. The animals were observed in their cages daily. Three samples were taken from the ear vein; the first one on day 1 and the second one on day 7 and the third one on day 14 for hematological and biochemical analyses.

### Determination of biochemical and hematological parameters

Serum was separated from the clotted blood obtained by ear vein. Total RBC and WBC counts were made by the hemocytometer method.<sup>[14]</sup> The differential WBC counts were made by finding the percentage average of the different types of cells counted in ten fields from Giemsa-stained slides.<sup>[15]</sup> Serum enzymes, such as ALP, AST, and ALT, were determined by the procedures of Sigma Diagnostics and BUN by the method of Crocker.<sup>[16]</sup>

## RESULTS

Animals group, which administered with indomethacin showed some clinical signs such as reduced feed intake, sluggishness, unthrifty appearance, and diarrhea with some mortality.

Effect of the NSAIDS (indomethacin) on the serum biochemical parameters showed a slight decrease in the BUN levels (45–26). All the NSAIDS used produced significant increases in the level of ALP (144–190), AST (43–58), and ALT (86–94) [Figure 1].

The group, which was administered with oxytetracycline, showed a significant decrease in the level of AST (110–88) and ALT (83–65) while greater increase in the level of ALP (229–284). There were not significant differences in the level of BUN [Figure 2].

There is not much difference in using both medications with using tetracycline, as the result almost the same [Figure 3].

Regarding hematological parameters, the group that treated with oxytetracycline produced a significant increase in WBC (7.5–17). On the other hand, the level of RBC (6–5), Hb (12.7–8), and lymphocyte (4.6–2.7) decreased [Figure 4].

Figure 5 shows the group which is administered with indomethacin. All of the parameters increased significantly, which were WBC (6–9.2), RBC (3.1–5.5), and lymphocyte (3.2–5.3). However, there was a greater decrease in the level of Hb (11.5–7).

The result for using both medications is also the same as using oxytetracycline alone [Figure 6].

## DISCUSSION

The usage of medication, such as indomethacin which is NSAIDs, belongs to the group of the most abused drugs by virtue of combining the pharmacological actions of anti-inflammatory and analgesia because they can easily be bought over-the-counter.<sup>[17]</sup> In addition, beta-lactam antibiotic, such as oxytetracycline, is also considered safe. This study has shown that indomethacin and oxytetracycline when improperly used for long period could be a source of harm to animals. This is because indomethacin has shown a significant increase in the level of liver enzymes (ALP, AST, and ALT).

The increase that occurs in ALP level is occurred in using both medications, and this is connected to the damage of the bile duct damage.<sup>[7,18,19]</sup> This suggests that both medications caused severe damage to the liver. However, indomethacin caused greater damage because it also produced a significant increase in the level of serum enzyme such as ALT and AST. Increases in the serum levels of AST and ALT (especially ALT) are reported to be associated with liver damage.<sup>[18,20]</sup> It has been also confirmed that indomethacin causes periportal hepatic necrosis and Kupffer cell proliferation, which are the main signs of hepatotoxicity,<sup>[7,21,22]</sup> and may suggest that indomethacin is a hepatotoxicant in rats and the effect on the liver may be one of the causes of death. There has been a small change of group treated with indomethacin, which may cause due to liver damage. The animals, which are treated with oxytetracycline, showed noticeable decrease in AST and ALT level, which might be associated with some cirrhotic changes and fatty infiltration in the hepatic cells.

The toxic effect of indomethacin was administered in the blood parameters such as RBC and Hb, which showed greater reduction. This might cause due to damage to

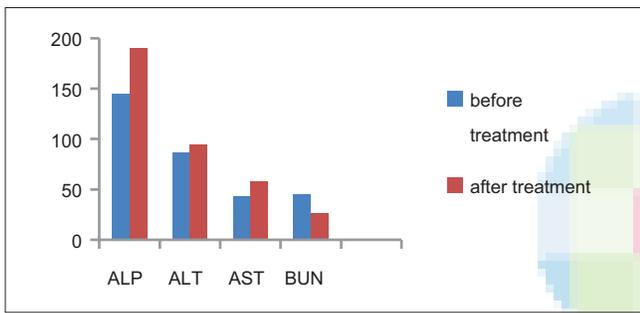


Figure 1: The effect of indomethacin on biochemical parameters

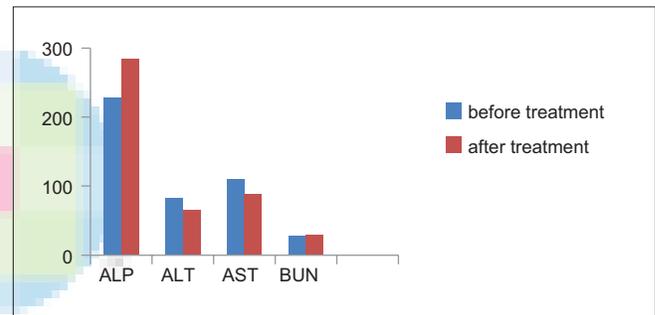


Figure 2: The effect of oxytetracycline on biochemical parameters

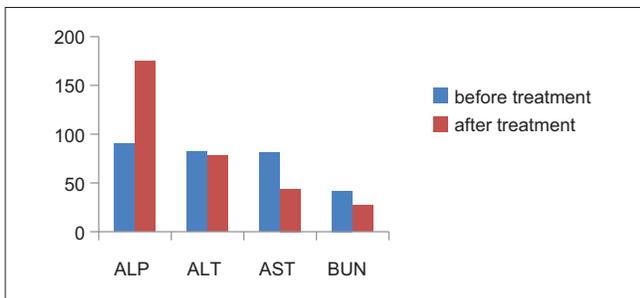


Figure 3: The effect of both indomethacin and oxytetracycline on biochemical parameters

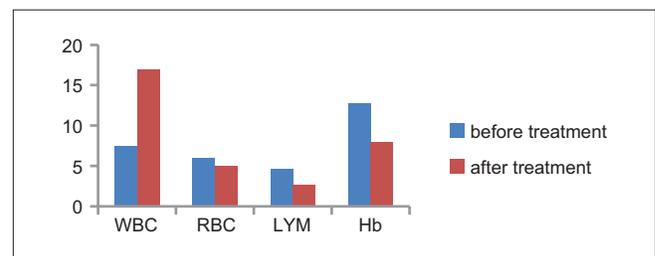


Figure 4: The effect of oxytetracycline on hematological parameters

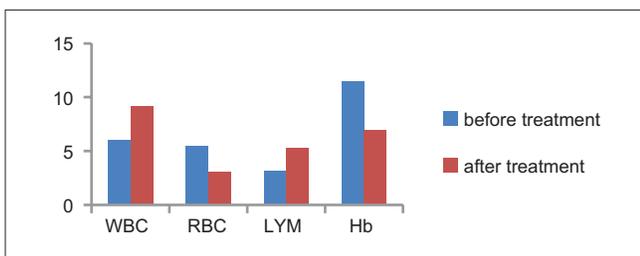


Figure 5: The effect of indomethacin on hematological parameters

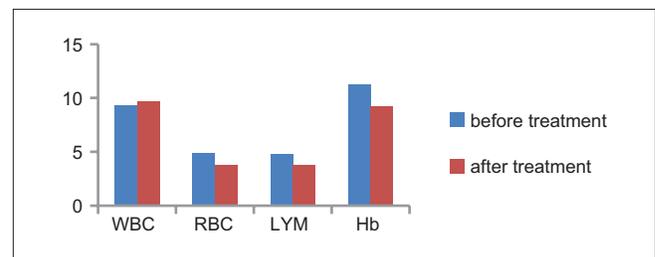


Figure 6: The effect of both indomethacin and oxytetracycline on hematological parameter

the gastrointestinal tract, which leads to blood loss and hemorrhagic anemia. This may consequently cause low blood oxygen, anoxia, and respiratory acidosis, which may lead to death.<sup>[23]</sup>

The adverse effects of these may involve hemorrhage and acute perforation of colonic diverticula, nausea, and dizziness.<sup>[24]</sup> That there was an increase in the WBC following hemorrhage. It also needs to be stressed that following the administration of indomethacin there was an increase in the level of lymphocytes (lymphocytosis), while the group administered with oxytetracycline showed lymphopenia, which means decrease in the level of lymphocytes. This occurred due to the severe stress that the animals were subjected to.<sup>[17]</sup> In addition, indomethacin inhibits the motility of polymorphonuclear leukocytes. This may account for this observation, i.e., lymphopenia and monocytopenia.

## CONCLUSION

Analysis of data from the current study revealed that the most of the types of generally used antibiotics have toxic effect on most of the organs of the animals such as liver, kidney, and gastrointestinal tract. However, there were not significant changes when they were used as a mixture or separate. Further studies are needed to investigate the organ activity of the animals that received these medications for a long period.

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## Conflicts of interest

There are no conflicts of interest.

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