

## **Biochemical and Histopathological Effects of Olive Oil on The Small Intestine Injury Induced by Tartrazine in Iraqi Local Male Rabbits.**

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### **Abstract**

Tartrazine is an artificial colorant used in the food, drug, and cosmetic industries in addition to its many benefits through color, but as an artificial dye, it may cause many health problems for humans and animals. The current study was planned to demonstrate the suspected effect of tartrazine on small intestines, in addition to the obvious the properties of olive oil in reducing biochemical effects and histopathological changes on small intestines. Fifteen male rabbits are classified into three groups; each group consists of five rabbits. The biochemical results showed a significant increase ( $P < 0.001$ ) in the second tartrazine group when compared with the control group. While the third group treated with olive oil showed slight changes in liver enzymes (ALT, AST, and ALP) compared to the control group and the second group treated with tartrazine ( $P < 0.001$ ). The histopathological results indicated that the intestinal structures in the control group had a normal histological appearance. The intestine of the second group, which received tartrazine treatment for 21 days, displayed desquamation of mucosal and submucosal layers, distortion of goblet cells, and hemorrhage. The third group, receiving treatment with olive oil and tartrazine, displayed a comparatively normal histological appearance of the intestine. This study demonstrated the harmful effects of tartrazine, as well as the many benefits of olive oil.

**Keywords:** Tartrazine, Olive oil, Intestine, Liver enzyme, Rabbits.

## Introduction

Tartrazine is a food dye colorant. It is commonly used in the nutrition industry, as it is found in ice cream, cakes, some types of industrial milk, and gelatin. It is usually yellow in color (1). Moreover, many medications with added colorants, such as pills, capsules, and some multivitamins, contain tartrazine (2). Tartrazine is used in the manufacture of cosmetics such as soap and shampoo, in addition to mouthwash and toothpastes (3). Tartrazine, despite its widespread use as a food dye in the pharmaceutical, food, and cosmetic industries, poses risks to the public's health and animal welfare. Researchers have documented allergic reactions to tartrazine (4) and reported cases of asthma (5). Some studies reported the occurrence of gastric damage in rats and mention when the use of tartrazine for a long period can cause cancer in the stomach (6). In humans, some pathological symptoms have been recorded as a result of the use of tartrazine, such as pain in the extremities, diarrhea, and itching (7). Some studies have indicated the presence of psychological and neurological changes. Researchers have attributed this to the fact that tartrazine affects the normal level of zinc in the blood, which causes these symptoms (8). Olive oil is monounsaturated fatty acids found in a liquid fat; it consists of oleic acid, with a small percentage of palmitic acid, linoleic acid, and other (9). Olive oil is beneficial because it contains antioxidants that can reduce inflammation and have anti-inflammatory characteristics. Oleocanthal, a compound found in olive oil, functions as an

antioxidant and shares an analgesic effect with ibuprofen. The antioxidant properties in olive oil can also inhibit the action of some proteins and genes that cause body inflammation (10). Also, reduce the oxidative properties of free radicals, which lead to cancer initiation. Olive oil can also contest cancer cells. But this research is required to regulate whether olive oil compounds reduce the risk of cancer (11). This study reveals the biochemical and histopathological effects of olive oil on the small intestine injury induced by tartrazine in Iraqi local male rabbits.

## Material and Methods

In this experimental study, we used olive oil syrup (Asorubal.S.A. Safax, Tunisia) and tartrazine dye from Indian food industries. The dose of tartrazine is 1 gram/kg (12,13). The biochemical test kits are from Pelta, Poland, for ALT, AST and ALP tests. Fifteen male rabbits were classified into three groups: the control group (5 rabbits that received normal saline orally). The second group (5 rabbits) received tartrazine dissolved in distilled water in a dose of 1 gram/kg. The third group, consisting of five rabbits, received 2 ml/kg (14) of olive oil orally, followed by tartrazine after two hours. The experimental study lasted 21 days and was conducted in rabbit-friendly conditions.

## Biochemical analysis

After the end of the experimental study with 24 hours, the blood for biochemical tests was collected, coagulated, centrifuged to

obtain the serum for ALT, AST, and ALP tests (15).

### Histopathological procedures

After 24 hours from the last dose of olive oil and tartrazine, the animal under general anesthesia with ketamine, scarified, exposed to internal organs and taking 1 cm from the intestine of all rabbits, put in 10% neutral buffer formalin for fixation. All histopathological procedures according to (16).

### Statistical experimental analysis

The study's results were displayed in the mean slandered division. Statical significance used for differences between groups using SPSS version 18.

### Results

Liver enzymes (ALT, AST, and ALP) showed a significant increase ( $P < 0.001$ ) in

the tartrazine second group when compared with the control group. The third group, the liver enzyme, which is mentioned above, showed better results than the second tartrazine group, but also these parameters were less than the first group ( $P < 0.001$ ) (Table 1) (Diagram 1).

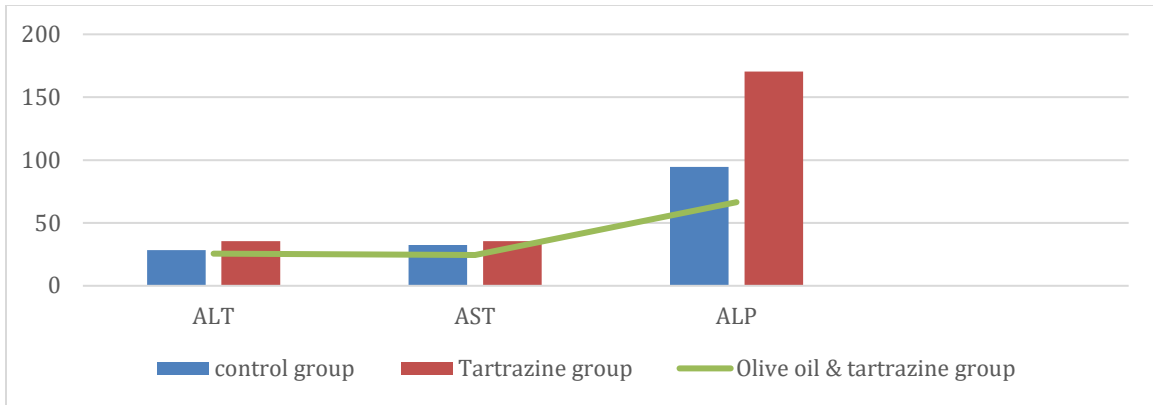
### Histopathological results

The control group showed normal histological appearance of small intestinal structures (ffgure 1). In the second group, which was treated with tartrazine for 21 days, the intestine showed desquamation of mucosa and submucosa layers, distribution of goblet cells, and hemorrhage (Figure 2). The third group, which was treated with olive oil and tartrazine, showed a relatively normal histological appearance of the intestine (Figure 3).

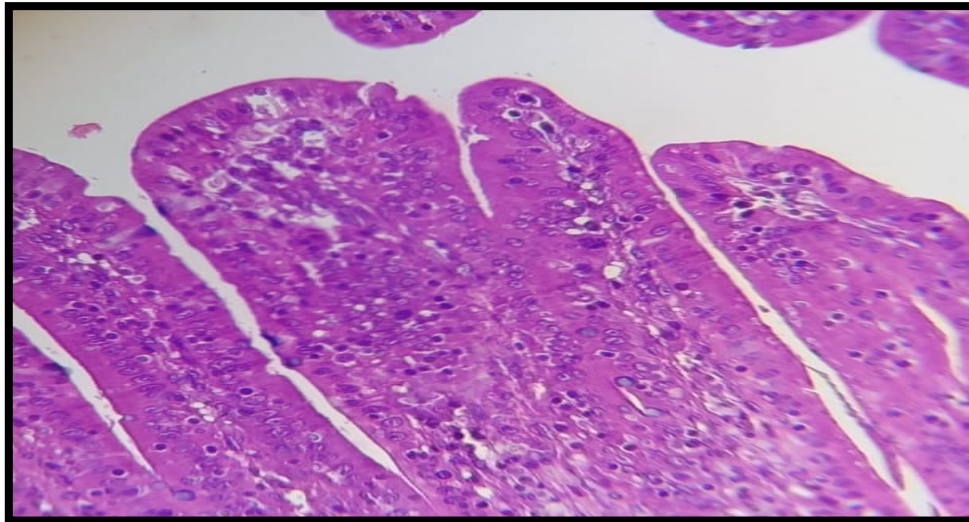
**Table 1: ALT, AST and ALP for three experimental groups used (Mean  $\pm$  SE).**

	ALT (U/L)	AST (U/L)	ALP (U/L)
Control group	28.55 $\pm$ 2.22	32.50 $\pm$ 12	94.50 $\pm$ 0.50
Second group	35.67 $\pm$ 0.50	35.50 $\pm$ 1.2	170.50 $\pm$ 1.50
Third group	25.55 $\pm$ 0.50	24.50 $\pm$ 0.50	66.50 $\pm$ 0.50

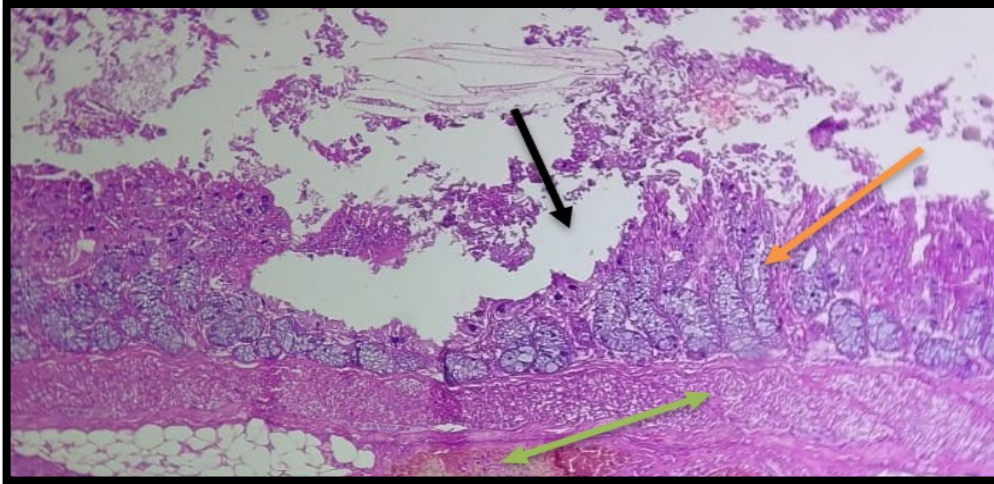
**The significant difference between group at  $P < 0.05$ .**



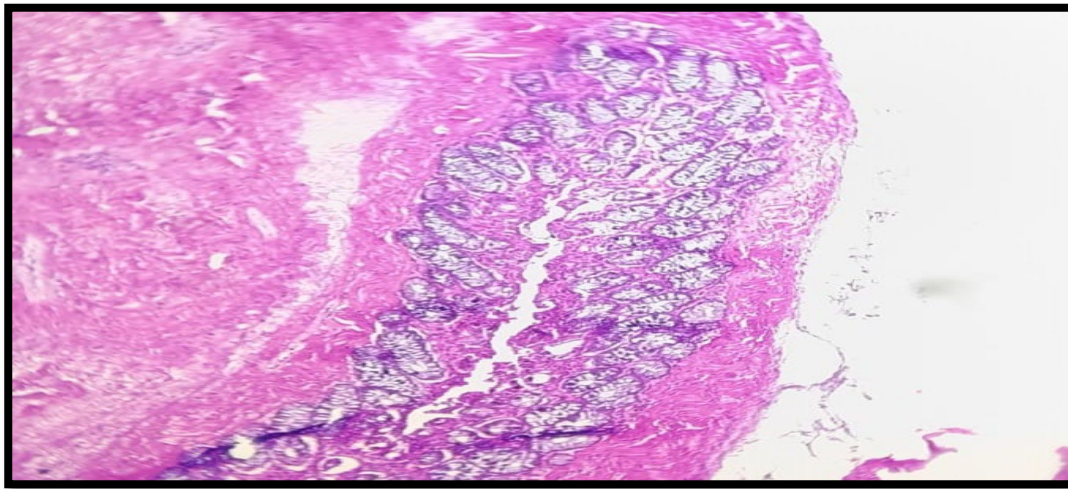
**Diagram 1. ALT, AST and ALP liver enzyme in this study.**



**Figure 1: Section of rabbit intestine of control group appears normal histological structure of the small intestine in control group (H&E stain 40X).**



**Figure 2: Section of rabbit small intestine (Tartrazine second group) appears desquamation of mucosa and submucosa layers (black arrow), distribution of goblet cells (orange arrow) and few hemorrhage (green arrow), (H&E stain 40X).**



**Figure 3: rabbit small intestine (olive oil & tartrazine group) appears relatively normal histological appearance of intestinal structures (H&E stain 40X).**

## Discussion

Tartrazine is currently used widely in the food colorant dye, drug, and cosmetic industries (17). Tartrazine affects the biochemical parameters and histology of the intestine by causing multiple

histopathological disorders, a finding that is similar to a 2020 study conducted in humans(18). Tartrazine also causes histological damage to fish guts (19). The tartrazine caused the mucosal and submucosal layers to shed, goblet cells to spread, and only a few hemorrhages in this

study. This is similar to what other studies have found, where goblet cells became distracted and bleeding happened (20). Tartrazine effect on the liver enzymes ALT, ALP, and AST that revealed a significant increase ( $P < 0.001$ ) in the tartrazine second group when compared with the control group (21). In the third group, the liver enzyme mentioned above showed results better than the second tartrazine group, but also these parameters were less than the control group ( $P < 0.001$ ), (22). \ Some biochemical parameters in this study stayed the same when olive oil was used compared to the control group, with only a very small drop in significant value. On the other hand, AST, ALT, and APT levels significantly rose when compared to the control group when tartrazine was used (23). Tartrazine has a harmful effect on the small intestine by producing free radicles which lead to pathological damage to it (24). The use of olive oil improved the biochemical parameters in this study, which is consistent with other studies (25). Olive oil led to enhanced biochemical value is which showed a significant decrease compared with the tartrazine group and returned to normal, which may be due to antioxidant properties; this may be due to the composition of olive oil (phenolic and oleic compounds), which prevent free radicles and product antioxidant activity (26).

## Conclusions

The olive oil showed antioxidative properties against damage caused by tartrazine by improving the biochemical

parameters used in this study and returning intestinal tissue to near normal.

## Conflicts of interest

The authors declare that there is no conflict of interest.

## Ethical Clearance

This work is approved by The Research Ethical Committee.

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## التأثيرات الكيموحيوية والنسجية المرضية لزيت الزيتون على إصابة الأمعاء الدقيقة المحدثه بالتارترازين في ذكور الأرانب المحلية العراقية.

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### الخلاصة

التارترازين هو ملون غذائي صناعي يستخدم في الصناعات الغذائية والأدوية ومستحضرات التجميل بالإضافة إلى فوائده العديدة من خلال اللون، إلا أنه كصبغة صناعية قد يسبب العديد من المشاكل الصحية للإنسان والحيوان. صممت هذه الدراسة لبيان تأثير التارترازين على الأمعاء الدقيقة، بالإضافة إلى دور زيت الزيتون في تقليل التأثيرات البيوكيميائية والتغيرات النسيجية المرضية على الأمعاء. أظهرت النتائج الكيموحيوية لأنزيمات الكبد (ALT، AST، ALP) زيادة معنوية ( $P < 0.001$ ) في مجموعة التارترازين الثانية مقارنة مع مجموعة السيطرة. المجموعة الثالثة أظهرت إنزيمات الكبد المذكورة أعلاه نتائج أفضل وأقرب إلى الطبيعي من مجموعة التارترازين الثانية ولكن هذه المعايير كانت أقل من مجموعة السيطرة ( $P < 0.001$ ). أظهرت النتائج التشريحية المرضية أن مجموعة السيطرة بينت مظهر نسجي طبيعي للأمعاء. المجموعة الثانية التي عولجت بالتارترازين لمدة 21 يوماً، أظهرت الأمعاء انسلاخاً في الطبقات المخاطية وتحت المخاطية، تشتت الخلايا الكأسية، ونزيفاً. المجموعة الثالثة التي تعاملت بزيت الزيتون والتارترازين أظهرت مظهر نسجي طبيعي نسبياً للأمعاء. واتفقت هذه الدراسة مع العديد من الدراسات حول وجود تأثير ضار للتارترازين على الأمعاء الدقيقة، بالإضافة إلى وجود العديد من فوائد زيت الزيتون، بحسب دراسات أخرى.

**الكلمات المفتاحية:** التارترازين، زيت الزيتون، الأمعاء، إنزيم الكبد، الأرانب.