

## The effect of olive oil (*Olea europaea*) on ibuprofen induced hepatotoxicity in female rats

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Key words: Ibuprofen, Hepatotoxicity, Olive oil, Rats.

### Abstract

Ibuprofen, a propionic acid derivative, is one of the most commonly Non-steroidal anti-inflammatory drugs, which are among the most frequently prescribed medications worldwide. The aim of this study is to investigate the protective effect of olive oil against ibuprofen-induced hepatotoxicity in female albino rats. In this study we used 24 female white rats and divided them into 4 equal groups. Each experimental group consisted of 6 animals. Group1,control they were fed on diet and water without any treatment ,group2, ibuprofen given at dose 40 mg/kg/day orally by gastric tube for 30 days, group3,olive oil 2 ml/kg/day (oral administration), group 4, ibuprofen at dose of 40 mg/kg/day and olive oil 2 ml/kg/day (oral administration).Treatments were administered once daily for 30 days. After 30 days, biochemical and histopathological analysis were conducted to evaluate hepatotoxicity. Serum levels of albumin, total proteins, and activity of AST, ALP, ALT and total bilirubin were measured. Animals treated with ibuprofen alone showed a significant increase in serum levels of ALT, AST and ALP and significant decrease in albumin and total proteins. Treatment of rats with olive oil showed significant improvement in hepatic function , presumably as a result of decreased biochemical parameters associated with ibuprofen-induced hepatotoxicity. Histopathological examination of the rats liver confirmed these observations. Therefore olive oil may protect against ibuprofen-induced hepatotoxicity.

أثر زيت الزيتون على التسمم الكبدي المستحث باعطاء الايبوبروفين لأنثى الجرذان

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الكلمات المفتاحية: الايبوبروفين، التسمم الكبدي، زيت الزيتون، الجرذان.

### الخلاصة:

الايوبروفين وهو مشتق حمض بروبيونيك، هو احد من الادوية غير الستيرويدية المضادة للالتهابات الاكثر شيوعا، وهي من بين الأدوية الموصوفة في كثير من الأحيان في جميع أنحاء العالم. الهدف من هذه الدراسة هو التحقق من الآثار الوقائية لزيت الزيتون ضد التسمم الكبدي الذي يسببه الايبوبروفين في الجرذان. في هذه الدراسة استخدمنا 24 أنثى من الفئران البيضاء وقسمناها إلى 4 مجموعات متساوية على النحو التالي: المجموعة الاولى: الضابطة على الغذاء

والماء دون أي علاج ، المجموعة الثانية: الايبوبروفين 40 ملغم/كغم/يوم يعطى شفويا بواسطة انبوب المعدة، المجموعة الثالثة: زيت الزيتون 2 مل/كغم/يوم يعطى بواسطة انبوب المعدة، المجموعة الرابعة: ايبوبروفين 40 ملغم/كغم/يوم، زيت الزيتون 2 مل/كغم/يوم يعطى شفويا بواسطة انبوب المعدة، تم إعطاء العلاجات مرة واحدة يوميا لمدة 30 يوما. بعد 30 يوما، تم إجراء تحليل كيميائي ونسجي لتقييم التسمم الكبدي. تم قياس مستويات مصّل الدم من الألبومين، والبروتينات الكلية، ونشاط أسبارتيت أمينوترانسفيرس، الانين ترانسفيرس، الكلاين فوسفيت و البيليروبين الكلي. أظهرت الحيوانات المعاملة بالإيبوبروفين وحدها زيادة كبيرة في مستويات مصّل أسبارتيت أمينوترانسفيرس، الانين ترانسفيرس، الكلاين فوسفيت وانخفاض كبير في الألبومين والبروتينات الكلية. وأظهرت معاملة الجرذان بزيت الزيتون تحسنا ملحوظا في وظيفة الكبد، ويفترض ذلك نتيجة لانخفاض المعاملات البيوكيميائية المرتبطة بالتهاب الكبد الناجم عن الايبوبروفين. وأكد الفحص النسيجي لكبد الجرذان هذه الملاحظات. ولذلك قد يحمي زيت الزيتون من التسمم الكبدي الناجم عن الإيبوبروفين.

## Introduction:

Ibuprofen, a propionic acid derivative, is one of the most commonly non-steroidal anti-inflammatory drugs, which are among the most frequently prescribed medications worldwide<sup>1-2</sup>. It is used for the relief of fever, pains, arthritis, and inflammatory conditions<sup>3</sup>. The mechanism actions of NSAIDs have long been established to be via inhibition of cyclooxygenase (COX) enzyme activity<sup>4-5</sup>. However, its frequent use is limited by a risk of serious side effects such as hepatotoxicity and nephrotoxicity. Previous studies have shown the adverse effects of different NSAIDs to the kidney<sup>6-7</sup>. In addition, Lateef , et al<sup>8</sup>. have reported that NSAIDs may also alter liver function, causing elevations of serum aspartate and alanine aminotransferases and necrosis of hepatic cells.

Olive oil tree (*Olea europaea*) is native to the Mediterranean region and has its medicinal properties. Historically, the products of *Olea europaea* have been used as aphrodisiacs, emollients, laxatives, nutritives, sedatives, and tonics. Olive oil has antioxidant properties, hypotensive, hypoglycemic and cardiovascular, nephron and hepato protective effects. In addition to that it was also known for its antimicrobial activity and anti-inflammatory properties<sup>9-10</sup>.

The aim of this study is to investigate the protective effects of olive oil against Ibuprofen induced hepatotoxicity in female rats by biochemical assaying and histopathology of liver tissues.

## Materials and Methods:

### Chemicals:

Ibuprofen was obtained from the essential drug company (Baghdad, Iraq), (Each 5ml contains 100mg) and given as orally at dose of 40 mg/kg body weight as previously described by Sydney<sup>11</sup>. Olive oil was purchased from local market (Kerbala, Iraq), provided by ZER Company/ Turkey .Olive oil was given by gavages at a dose of 2 ml/kg as described by necib<sup>12</sup>.

### Experimental animals:

In this study, we used 24 Wister albino 230-240g female Rats brought from the University of Babylon College of Science. Rats were left in our laboratory for seven days before beginning the experiment. The rats were housed in wire bottom cages, free diet, tap water and with a 12 h light / dark cycle for 4 weeks . The experimental protocol and

procedures used in this study were approved by the Ethics Committee of the Karbala University , Kerbala , Iraq for the care and use of laboratory animals .The animals were randomly divided into four groups. Each experimental group consisted of six animals:

**Group1.**Control group (n=6): They were given only normal saline for 30 days.

**Group2.** Ibuprofen (n=6):Animals of this group were given Ibuprofen as given orally by gavage at a dose level of 40 mg / kg body weight, every day for 30 days.

**Group3.** Olive oil (n=6): Animals of this group were given olive oil via gavage at a dose level of 2 ml/kg body weight, every day for 30 days.

**Group4.** Ibuprofen + Olive oil-treated group: Rats were treated with Ibuprofen (40 mg / kg) and Olive oil (oral administration) (2 ml / kg) daily for 30 days.

At the end of the experiment rats were given ketamine 10% for anesthesia and were sacrificed 24 h after the last olive oil and ibuprofen received, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at ( 60 RPM) for (7 min). The serum was collected in plastic tubes and stored in a frozen at  $-8^{\circ}$  for biochemical analysis.

#### **Histopathological examination:**

The animal was killed under anesthesia, the liver was excised and the specimens were fixed in formalin 10% solution. After fixation, the tissues were washed under running tap water and dehydrated with concentrated ethanol. After the application of xylol, the specimens were made into paraffin blocks.5-6 micron thick sections were rehydrated and dyed with eosin and hematoxylin and examined under light (Olympus BX51) microscope.

#### **Biochemical analysis:**

When measuring biochemical analysis, an automatic device (veterinary chemoanalyser) was used for this task. Several parameters were measured and included liver function tests (AST, ALT, ALP, total proteins, total bilirubin, globulin and Albumin).

#### **Statistical analysis:**

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means  $\pm$  MSE and  $P < 0.05$  was considered to be statistically significant.

#### **Results:**

Table (4.1) shows the effect of olive oil on the hepatic functions among the different groups. Ibuprofen administration influenced the hepatic function as assessed by significant increase ( $P < 0.05$ ) of serum ALT, AST, ALP and TB, with decrease of serum TP, ALB and GLOB. All the previous changes were significantly different from the corresponding values

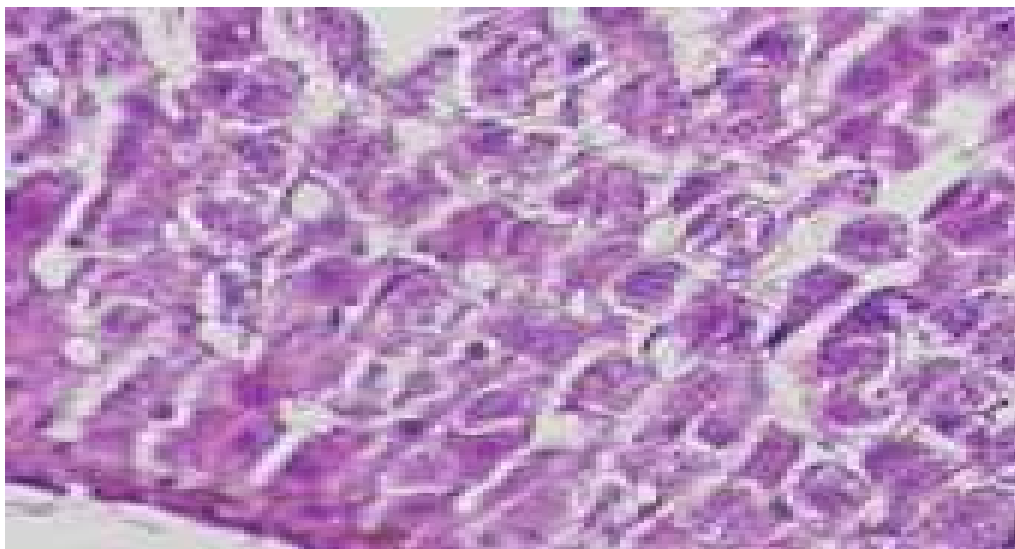
in the control group. Olive oil administration significantly attenuated ( $P<0.05$ ) hepatic dysfunction in ibuprofen- treated rats as assessed by decreased serum ALT, AST, ALP and TB, with increase of serum TP, ALB, and GLOB.

**Table (4.1) Effect of olive oil on the serum concentration of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), albumin (ALB), globulin (GLOB) in ibuprofen-treated rats and control rats.**

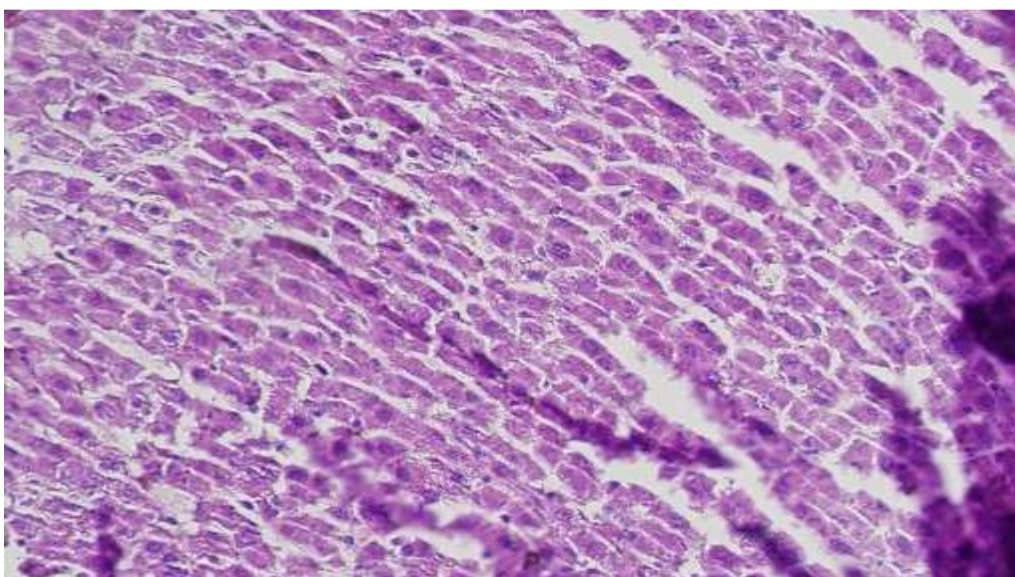
	Control group	Ibuprofen group	Olive oil group	(Ibuprofen+ olive oil) group
<b>Parameter</b>	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
<b>ALT(U/L)</b>	82 $\pm$ 7.31	258.66 $\pm$ 20.11*	80.16 $\pm$ 7.08	83.66 $\pm$ 3.72
<b>AST(U/L)</b>	129 $\pm$ 6.29	358.5 $\pm$ 20.98*	127.33 $\pm$ 7.06	132.83 $\pm$ 6.36
<b>ALP(U/L)</b>	92.33 $\pm$ 5.04	198.83 $\pm$ 12.78*	91.33 $\pm$ 6.62	93.5 $\pm$ 6.62
<b>TB (<math>\mu</math>mol /L)</b>	4.38 $\pm$ 0.38	15.41 $\pm$ 1.92*	4.03 $\pm$ 0.48	4.53 $\pm$ 0.5
<b>TP (g/L)</b>	68.16 $\pm$ 5.34	42.33 $\pm$ 4.71*	69.5 $\pm$ 3.61	66.66 $\pm$ 4.96
<b>ALB (g/L)</b>	37 $\pm$ 4.14	23 $\pm$ 2.68*	37 $\pm$ 1.89	36.33 $\pm$ 2.25
<b>GLOB (g/L)</b>	31.16 $\pm$ 2.78	19.33 $\pm$ 3.61*	32.5 $\pm$ 4.18	30.33 $\pm$ 3.93

#### **Histopathological examination of liver tissue:**

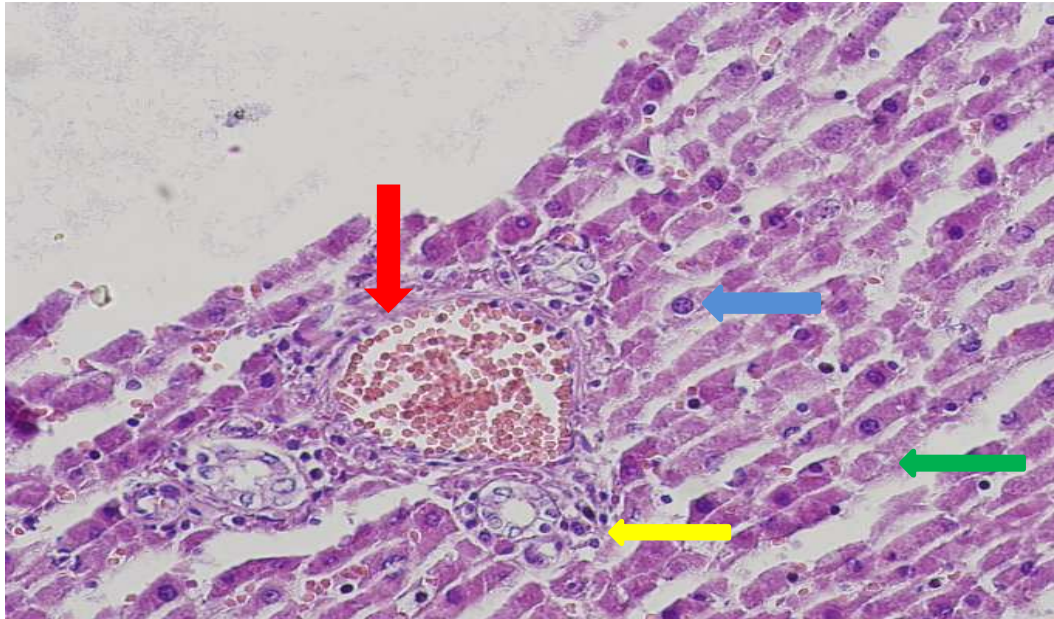
Histopathological examination showed normal histological appearance of hepatocytes structure in section of the liver tissue (stained with eosin and hematoxylin) of olive oil when compared with the liver tissue of the control group (Figures 4.1 and 4.2). On the other hand, we founded that treatment with ibuprofen led to significant congestion, focal hydropic degeneration with single hepatocyte necrosis. Biliary stasis with only mild chronic inflammatory cell infiltration of portal tracts (Figure 4.3) . But these changes were treated by giving olive oil with ibuprofen (Figure 4.4).



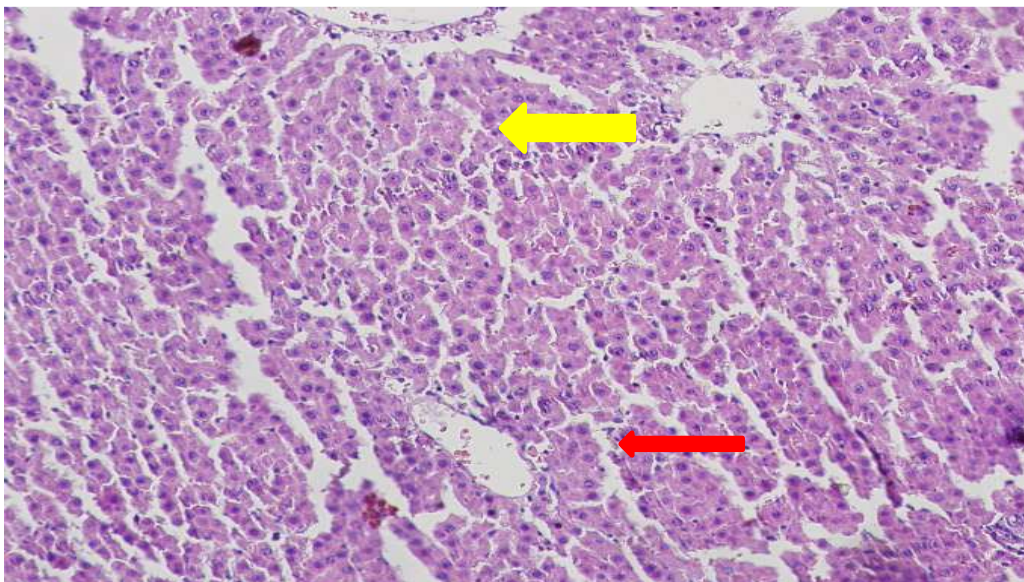
**Figure (4.1):** Photomicrograph of liver from the control rat showing normal histological, no significant changes in tissue. Magnification is (200x).



**Figure (4.2):** Photomicrograph of liver from olive oil - treated rat showing normal histological as compared to control rat. Magnification is (200x).



**Figure (4.3):** Photomicrograph of liver from ibuprofen- treated rat showing significant congestion and portal tracts (red indicator), focal hydropic degeneration (blue indicator), single hepatocyte necrosis (green indicator), mild chronic inflammatory with biliary stasis (yellow indicator). Magnification is (400x).



**Figure (4.4):** Photomicrograph of liver from (olive oil + ibuprofen)- treated rat showing decrease in congestion (red indicator), with mild decrease in degeneration and necrosis effect (yellow indicator). Magnification is (200x).

## Discussion:

The aim of this study is to investigate the protective effects of olive oil against Ibuprofen -induced hepatotoxicity in female rats. In this study, we investigated liver function tests by determination of serum ALT, AST, ALP activities, total bilirubin, albumin and total protein levels.

The liver is the largest organs in the body and the main site for metabolism and excretion<sup>13</sup>. It plays a main role in detoxification and excretion of many endogenous and exogenous compounds, the major roles of the liver are carbohydrate, fat and protein metabolism, detoxification, secretion of bile and storage of vitamin<sup>14</sup>. Chemicals that cause liver injury are called hepatotoxins. More than 800 drugs have been implicated in causing liver damage and it is the most common reason for a drug to be withdrawn from the market<sup>15</sup>. Hepatic reactions have been of concern because of serious liver damage being reported with some NSAIDs and coxibs, e.g., diclofenac, sulindac , celecoxib, and lumiracoxib<sup>16</sup>.

Our study reported that ibuprofen administration at a dose of 40 mg/kg/day for 30 days leads to a significant increase in serum levels of liver function tests consist of ALT, AST, ALP and total bilirubin, and significant decrease in serum levels of albumin and total protein compared with control group that evidence of hepatotoxicity. Liver dysfunction and toxicity induced by ibuprofen administration it may be due to a generation of free radicals. The aminotransferases (transaminases) are sensitive indicators of liver cell damage and are most helpful in recognizing acute hepatocellular diseases such as hepatitis. The pattern of the aminotransferase elevation can be helpful diagnostically<sup>17</sup>. Although serum levels of both ALT and AST become elevated whenever disease processes affect liver cells, ALT is the more liver- specific enzyme. Elevation ALT activity persist longer than do those of AST activity<sup>18</sup>. Measurement of both ALT and AST has some value in distinguishing hepatitis from other parenchymal lesions. Accordingly, higher dose levels of ibuprofen exposure would increase hepatic toxicity, and the elevation of serum AST levels may become significant, as a result of increased release of the enzyme from damaged hepatocytes<sup>19-20</sup>. And significant decrease in serum levels of albumin and total protein, A decrease in serum levels of albumin, which is synthesized in the rough endoplasmic reticulum of hepatocytes, maybe decreased hepatic production due to decreased liver function following hepatocellular dysfunction<sup>21</sup>.

The mechanism blamed for adverse liver effects by NSAIDs is thought to be due to NSAIDs induced idiosyncratic liver damage and change in metabolism of liver which might be due to enterohepatic recirculation of NSAIDs (ibuprofen)<sup>22-23</sup>. The hepatotoxicity associated with ibuprofen has been considered to be the lowest among commonly used NSAIDs. The most common type of liver injury caused by ibuprofen is hepatocellular and cholestatic. It has been associated with prolonged cholestatic and vanishing bile duct syndrome.

In the present study, the histopathological changes which occurred in the liver included of significant congestion, focal hydropic degeneration with hepatocyte necrosis. This results agree with<sup>24</sup>.

(Poudyal et al,<sup>25</sup> reported the olive oil has the ability to protect damage cells of the liver from some toxic agents. In this study, it was founded the giving of olive oil along with ibuprofen caused a significant decrease in ALT, AST and ALP activities and total bilirubin levels and this suggested the protective effect of olive oil that is reported by<sup>26</sup>. In addition to that the giving of olive oil with ibuprofen resulted in a significant increase in GLOB, ALB and TP levels, that agree with study reported by<sup>27</sup>.

### Conclusion:

In the present study, AST, ALT, ALP activity, total proteins, total bilirubin and albumin levels were analyzed, it was concluded that olive oil has significant hepatoprotective activity against ibuprofen- induced hepato toxicity in femal rats.

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