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Effect of hazelnut oil on hematological parameters and liver histopathological changes induced by naproxen in local male mice

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ABSTRACT

This study designed to identify the outcome use of naproxen on hematological parameters and liver histopathological changes and the effect of hazelnut oil on these parameters in local male mice. Eighteen mice were divided equally into three groups: and each group involved of 6 mice. The first group is control group which received distal normal saline while the second group was treated with naproxen at 20 mg/kg of mice body weight. The third was group treated with hazelnut oil 3g/kg of mice body weight, after two hours followed by naproxen 20 mg/kg of mice body weight. The experimental study continues for 21 days. The hematological results exhibited a significant decrease in WBC, RBC, hemoglobin and hematocrit at $P < 0.05$ in the second group treated with naproxen compared to the control group. The third group treated with hazelnut oil followed by naproxen showed a significant increase in hemoglobin and hematocrits at $P < 0.05$ compared to the second group but the hematological values in the third group did not show a significant difference compared to the control group. The histopathological results showed normal liver histology in first group the end of the experimental study. The second group which was received the naproxen showed congestion of blood vessels, hemorrhage inflammatory cells infiltration, necrosis and the presence of Kupffer cells. The third group showed a histological appearance of the liver relatively similar to the control group. The hazelnut oil improved the hematological parameters in this study and preserved the histological structures of the liver from being affected by naproxen.

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KEYWORDS: Naproxen, hazelnut oil, hematological parameters, histopathological changes, liver.

INTRODUCTION

Naproxen is non-steroidal anti-inflammatory drug, anti-inflammatory action by preventing and or inhibiting proinflammatory factors. Naproxen inhibit cyclooxygenase 1 and 2 which is related to prostaglandin E which responsible for pain and inflammation in the body [1]. Naproxen is used to relief the pain of various condition such as head ache, muscles pain, tendonitis and gingivitis [2]. Naproxen may causes gastric ulcers, vomiting, diarrhea and other abnormal body signs [3]. In veterinary medicine, naproxen is very toxic to dogs which causes vomiting and melena when injected with 11 mg/kg of mice body weight [4]. The use of naproxen in mice experimentally led to the inhibition of inflammation, decreased proliferation of tumor cells and enhanced apoptosis [5]. In the another

study, naproxen exhibits binding to the nucleoproteins and RNA which is characterized by antiviral properties [6]. Also, the same analgesic prevents fibrosis in mice by preventing formation of bleomycin [7]. In humans, naproxen causes hepatitis and cholestasis [8]. Naproxen causes inhibition of heart rate, hepatotoxic injury and other abnormalities in zebrafish [9]. In another study, naproxen causes the liberation of free radicles, inhibition of liver enzymes and liver toxicity in rats [10]. On the other hand, hazelnut oil has beneficial properties including decreasing fatty liver syndrome and hyperlipidemia in hamsters [11], enhancing muscles cells action in mice [12], and preventing the formation of free radicals that affect the brain by improving memory and diminishing nervous signs [13]. Hazelnut oil inhibits the formation of cancer cells by interfering with apoptotic mechanisms in colorectal cancer [14]. In rabbits, hazelnut oil enhanced lipid profile value by preventing the formation of aortic cholesterol and disadvantageous lipids [15]. In this study, we identify the effect of hazelnut acid on the hematological parameters and liver histopathological changes induced by naproxen in local male mice.

MATERIAL AND METHODS

Naproxen drug was manufactured by Kontam Pharmaceuticals (Zhongshan), LTD and supply by AL-dyar pharmacy. Hazelnut oil was supplied by Albaraka company for natural oil.

Animals

Five weeks old Iraqi local male mice, weighing 24g, housed in an animal house under standard conditions, were used in this study. An acclimation period of two weeks with slandered food and water were given before the experimental study was performed on the mice.

Animal study design

Eighteen mice were classified into three groups (each group 6 mice). The control group received oral distilled water for 21 days. The second group received naproxen at a dose of 20 mg/kg [16] mixed with the diet for 21 days. The third group received hazelnut oil 3g/kg of BW [17] mixed with the diet followed by naproxen at a dose of 20 mg/kg 2 hours post-meal for 21 days.

Hematological analysis:

One ml syringe was used for collecting blood for each animal which contained an anticoagulating agent, white blood cells and red blood cells measurement according to [18], hemoglobin and hematocrit according to [19].

Histopathological procedures:

After 24 hours, all animals were sedated with xylazine and ketamine for general anesthesia. Then the animal was sacrificed and 0.5cm pieces of the liver were obtained from each anima, preserved in buffer formalin (10%) followed by histopathological procedures according to [20].

Statistical analysis:

Mean slandered error (SD) revealed the results while the deference between groups using one-way ANOVA. The significant differences (SD) by p values ≤ 0.05 were reported as significant.

RESULTS

Hematological parameters

The result showed that naproxen causes a change in the hematological values by affecting the liver. The control group showed normal values of WBC, RBC, HB and hematocrit. The second group which was received with naproxen at a dose of 20 mg/kg showed significant decrease in WBC, and a significant decrease in RBC, HB and hematocrits at $P < 0.05$ compared with the first group. In the third group, the use of hazelnut oil 3g/kg of B.W showed the WBC, RBC, HB, and hematocrits to the normal value resembling the first group (Table 1), (figure 1).

Table 1.

Hematological value of the first, second and third group after 21 days of experimental study.

Value	WBC (mm3)	RBC (×106/ul)	HB (g/dL)	Hematocrit (%)
Control G.	7.550 ± 173	6.200±0.47	15±0.5	47.2±1.5
Second G.	7.400±120	4.430±0.27	11±1.0	37±3.00
Third oil G.	7.450±183	6.100±0.7	16±0.6	47.5±0.50

***Significant difference (SD) showed of 1st group (p<0.05) revealed white blood cells. red blood cells. Hemoglobin and Hematocrit concentration in the first, second and third group.**

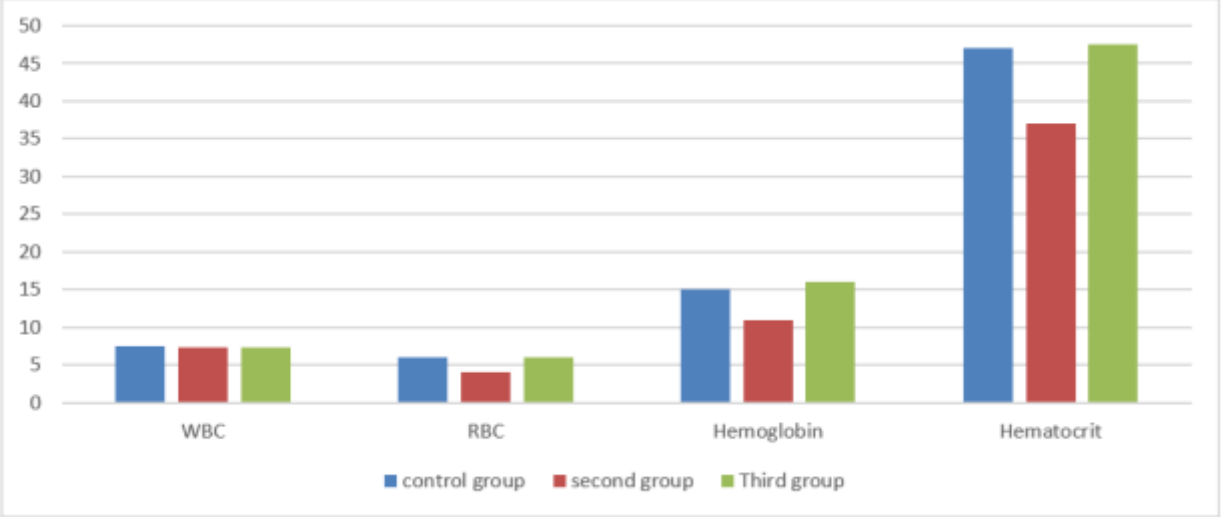

Figure 1. showed hematological value of first, second and third group after 21 days of experimental study.

Histopathological changes:

The histopathological changes showed normal histological architectures of the liver in control group after 21 days after the end of the experimental study (Figure 2). The second group treated with naproxen showed congestion of blood vessels, hemorrhage and inflammatory cells infiltration (Figure 3), necrosis and the presence of Kupffer cells (Figure 4). The third group showed a histological appearance of the liver relatively similar to the control group (Figure 5).

DISCUSSION

Naproxen is an anti-inflammatory and analgesic drug that is used to reduce pain in many diseases' such as arthritis, tendonitis and gingivitis. This study is designed to identify the ability of hazelnut oil to reduce the effect of naproxen on hematological parameters and hepatotoxic injury in mice. The hematological results showed a significant decrease of WBC and highly a significant decrease of RBC, hemoglobin and hematocrit in the second group treated with naproxen compared with the control group, this results were comparable to other studies which had shown the effect of naproxen in humans [1] and in rats [21]. On the other hand the result of [22] showed a slight decrease in WBC by naproxen in rats, while the [23] reported an elevation of WBC by naproxen, this may belong to the deference of animal or dose, and duration of action in animals.

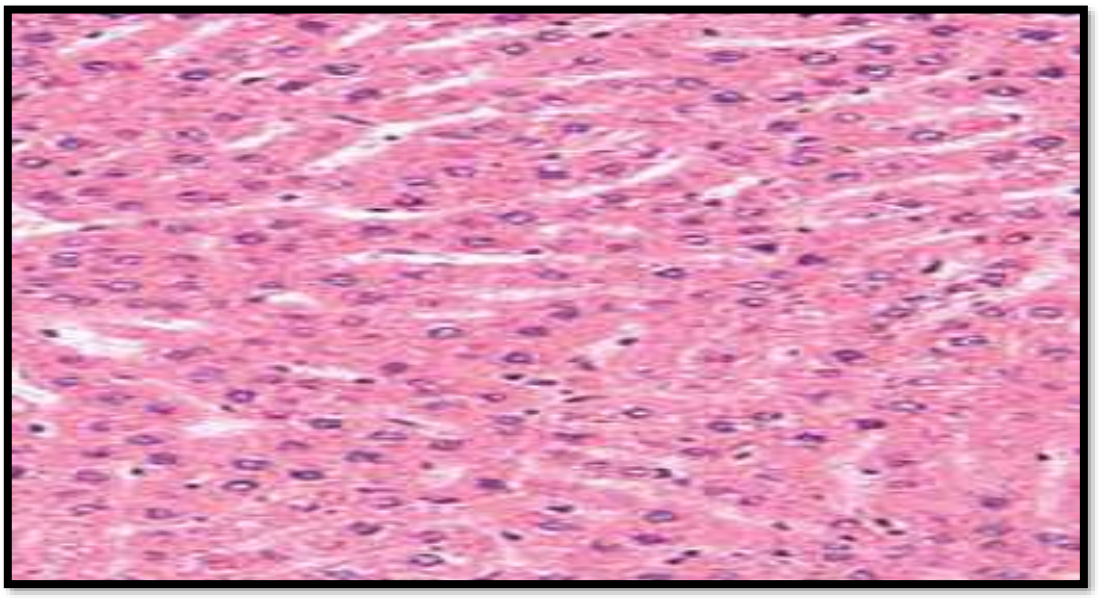

Figure 2. Normal histological architectures of mice liver of the1st control group (H&E stain 20X).

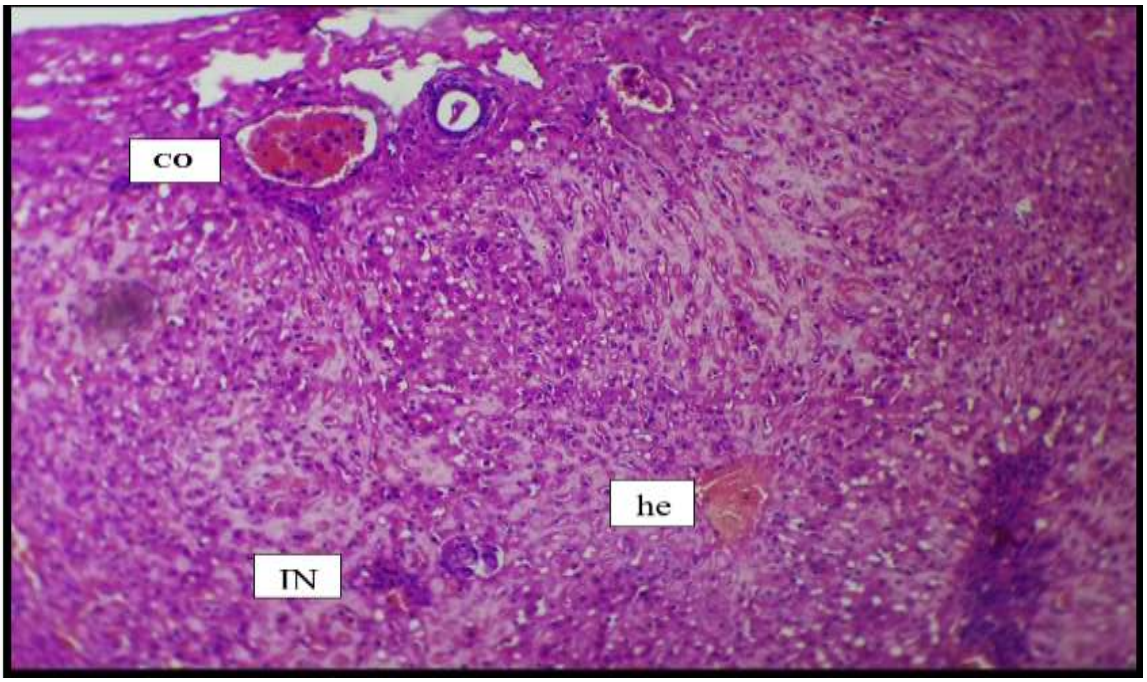

Figure 3. Photomicrograph of mice liver of second group showed congestion (CO), hemorrhage (he) and inflammatory cells infiltration (IN), (H&E stain 20X).

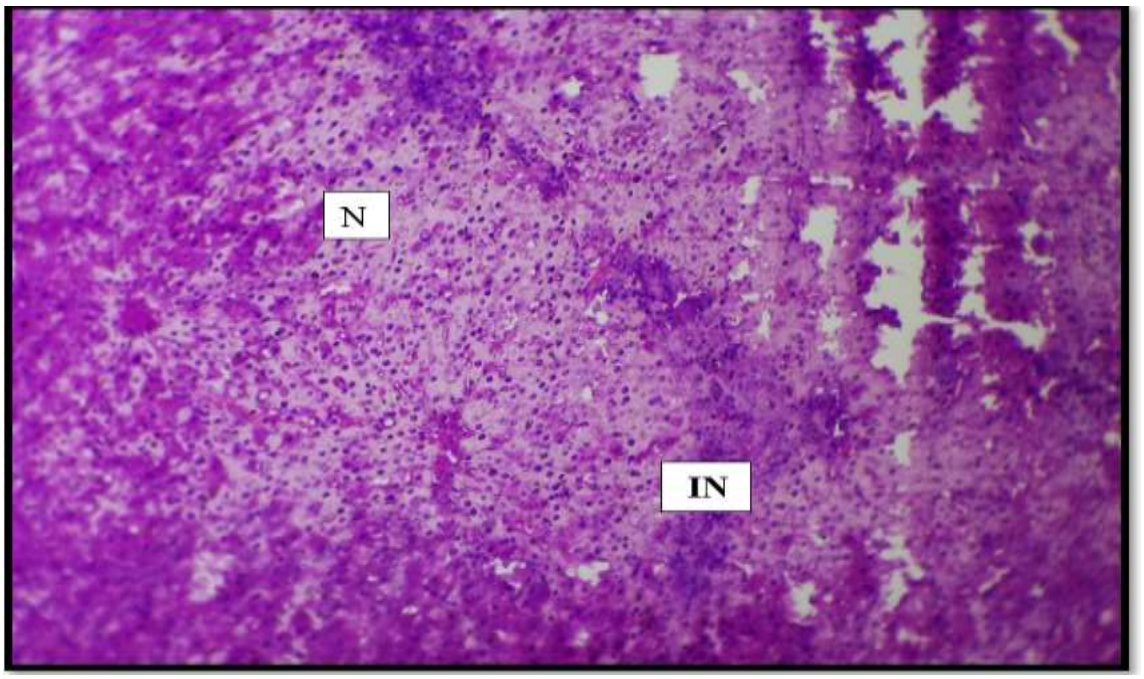

Figure 4. Photomicrograph of mice liver of second group showed necrosis (N), and inflammatory cells (IN), (H&E stain 20X).

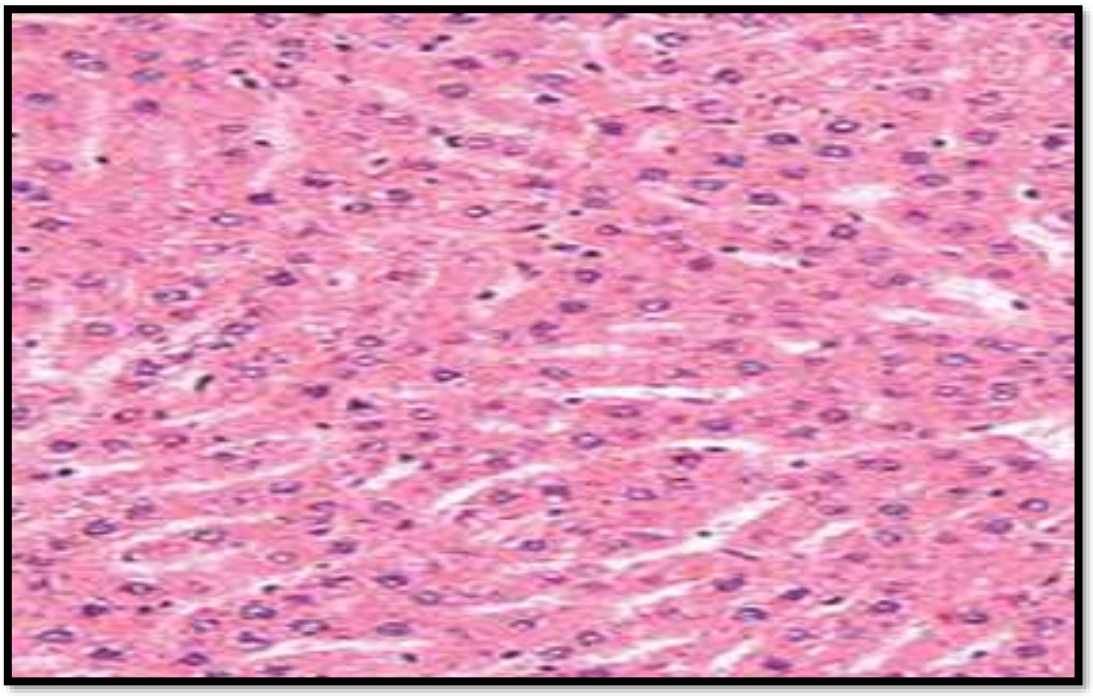

Figure 5. Photomicrograph of mice liver of third group showed relatively normal histological architectures of liver, (H&E stain 20X).

In this study, the RBC decreased in the second group treated with naproxen, A study reported the production of free radicals by interacts with cells membrane of RBC leading to hemolysis and distraction of RBC [24]. Naproxen causes a decrease in hemoglobin due to destruction of red blood cells and this accepted with [25]. In this study naproxen causes a decrease in hematocrit, which results from failure of platelet aggregation due to the effect of

naproxen as reported in human studies [26].The histopathological changes showed normal histological architectures of the liver in control group. The second group which was treated with naproxen showed congestion of blood vessels, similar to another study in the rats [27], bleeding and inflammatory cells [28], in addition to necrosis and the presence of Kupffer cells [8]. In the current study, the treatment of mice with hazelnut oil led to a significant decrease in WBC, RBC, hemoglobin and hematocrits compared to the second group treated with naproxen. The study in rabbits showed generally the beneficial effect of hazelnut oil on blood composition [29] In this study, hazelnut oil improved the value of red blood cells in the third group compared to decreasing value in the second group, this is because of the ability of hazelnut oil to prevent the destruction of RBC, hemoglobin and hematocrits and improve its function [30]. In this study, the histology of the liver in the control group is normal but exposure to naproxen in the second group led to the presence of hemorrhage and congestion, this agreement with [31], that naproxen leads to hepatotoxic injury and destruction of the hepatocytes. The use of naproxen for 21 days led to presence the of necrosis, reported in a 57 years old women who showed hepatic cells necrosis after signal dose of naproxen [32]. The treatment of experimental animals with hazelnut oil resulted in improvement and returned of the hematological value relatively to normal. Hazelnut oil is composed of phenolic acid (catechin, ferulic acid naringenin, protocatechuic acid, luteolin and caffeic acid) and fatty acid with vitamin E provide good antioxidant activity [33]. The treatment enhanced the values in the third group by preventing the free radicles from affecting the hematological parameters and histopathological changes such as those induced by naproxen in the second group.

CONCLUSIONS

Naproxen is an anti-inflammatory and antipyretic drug which has many beneficial properties but when used for a long period causes changes in hematological parameters and histopathological changes. Hazelnuts oil leads to the preservation of hematological parameters and histopathological properties by acting as an antioxidant agent to prevent the production of free radicals.

RECOMMENDATIONS

Future studies to explore other benefits of hazelnut oil in other organs are recommended.

CONFLICT OF INTEREST: None.

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