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Studying the protective and therapeutic role of Rutin on the histological structure of the liver and some physiological parameters in Rats treated with ciprofloxacin

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ABSTRACT— Antibiotics have great benefits, however, not free from side effects. Its side effects, especially those related to liver damage and increased levels of liver enzymes, have prompted the world to search for a traditional medicinal plant such as Rutin to find preventive and curative agents against multi-organ dysfunction (unwanted effects) of Ciprofloxacin. The aim of this study is to evaluate the preventive and therapeutic effect of Rutin in inducing liver injury in animals by Ciprofloxacin. The study included Six groups, each group contains 5 male albino rats and the dose was taken orally daily, The control group were given normal drinking water for 14 days, While The first treatment group (T1) were given Rutin concentration of 50mg/kg of body weight for 14 days, and The second treatment group (T2) were given Ciprofloxacin antibiotic, and it's concentration equal 14mg/kg of body weight for 14 days, The third treatment group (T3) were given Rutin concentration of 50mg/kg of body weight for 14days and then Ciprofloxacin oral given with a dosage of 14mg/kg of body weight for 14 days, The fourth treatment group (T4) were given Ciprofloxacin antibiotic concentration of 14mg/kg of body weight and then Rutin oral given with a dosage of 50mg/kg of body weight in combination for 14 days and The fifth treatment group (T5) was given Ciprofloxacin antibiotic concentration of 14mg/kg of body weight for 14 days and then Rutin with concentration equal 50mg/kg of body weight for 14 days. histopathological changes occurred in the liver of the animals of the group (T2) represented with Loss of the normal hexagonal arrangement of hepatocytes, cellular dissociation, congestion in a central vein, fatty necrosis, and lymphoid infiltration compared with other groups. Rutin treatment at various doses has hepatoprotective effect by maintaining the functional integrity by improving the cellular antioxidant status, reducing oxidative stress, and preserving the integrity of the histomorphological structure of the liver. It was concluded that Rutin at 50mg/kg has an important hepatoprotective effect against injury induced by Ciprofloxacin in the liver.

KEYWORDS: Rutin, Ciprofloxacin, induced dysfunctions, liver.

1. INTRODUCTION

Ciprofloxacin is an extensively ordered Fluoroquinolone with wide antimicrobial coverage and an oral broad bioavailability effect [1], [2]. Much adversative consequences were linked to its use in the syndrome of Stevens-Johnson, tendon rupture, interstitial kidney injury, and hepatitis [3], [4]. Mainly, the liver damage which is linked to Ciprofloxacin is limited to an asymptomatic elevation of liver enzymes. In rare cases, it can also manifest as an acute hepatitis. Although man is now in the industrial era and social progress, man still remains close to a large number of substances that are toxic, for example, Drugs, which not only damage one organ but also pose a threat to other organs simultaneously.

The adverse effect of Ciprofloxacin is a well-established model for the study of liver damage and dysfunction. Ciprofloxacin causes liver injury like clinical viral hepatitis. Hence, this model is used to explore and develop hepatoprotective factors [1]. Sometimes Ciprofloxacin can cause acute hepatitis within two days to two weeks after starting antibiotic treatment. Even though the exact mechanism of Ciprofloxacin-induced hepatitis is currently still unknown, hepatocellular necrosis leading to raised liver enzymes has been empirical. The prototype of injury can be cholestasis, hepatocellular, or mixed. The most common pattern in the acute setting is hepatocellular, which is associated with significantly elevated levels of alanine transferase. The cholestatic type of liver injury typically happens after extended administration of antibiotics [5]. [6] suggested that the hepatitis caused by drugs and toxins is due to their direct toxic effect or their own interaction. The direct toxic effect is a specific reaction that can be metabolic or immunological, dose-dependent, and can occur at any dose. In most cases, the hypersensitivity response is especially likely due to the short latency period, recurrent immune-susceptibility characteristics, and severe illness upon re-exposure. Traditional medicine plants are recognized to endorse the natural curative process of internal organs [7] because of the production of Polyphenols or a group of secondary metabolites that have produced significant changes in drug expansion research's [8]. More than 4000 diverse secondary metabolites have been recognized from plants [9] and classified into Flavonols, Flavonoids, Flavones, etc., to date [10]. Flavonoids affect human health [11] by providing a high level of protection against ROS [12]. Rutin or vitamin P is one of the most common Flavonol glycosides that was discovered in many plants and vegetables in the usual human diet [10]. In recent times, the researchers were interested in exploring its medicinal potential as antioxidant [10], vascular prophylactic, antiproliferative [13], and anticoagulant [14], cell protector [15], anti-inflammatory [9], antibacterial [16], antiviral [17], antiulcerogenic [18] Neuroprotective [19], cardioprotective [20], hepatoprotective, and renal protective potential [21]. a wide variety of the biological and pharmacological activities of Rutin has been studied, which reveals that Rutin may be used as a key molecule for an advanced clinical use. It is an enormous need to investigate pharmacological effects of Rutin against a variety of diseases, so that it can be exploited against multi-organ injury with safer clinical use. This enthusiastic feature increased the pharmacological ability of Rutin with more empirical verification. Therefore, in the current study, we investigate the protective effect of Rutin against exposure to Ciprofloxacin-induced defect in the liver.

2. Methodology

2.1 Study design

The study was applied in the department of animal house of Biology/Education college/University of Al-Qadisiyah, using 30 female albino rat -healthy and sexually mature- (Six weeks aged) and average weight (180-200) g. Animals collected at room (12m2) in plastic cages and exposed for the similar conditions of temperature (20-25)ºC, which was controlled by the air conditioner, and lighting rate (12 hour light: 12 hour dark).

Intensive diet and water by a free method were given to animals, and then random spreaded and left for one week to accommodate and then weighed to determine the suitable dosage, it divided into

1. The Control group (C) included: five animals with normal drinking water for 14 days.
2. The first treatment group (T1) include: five animals with Rutin concentration (50mg/ kg) body weight for 14 days.
3. The second treatment group (T2) included: five animals with Ciprofloxacin antibiotic concentration (14mg/ kg) body weight for 14 days.
4. The third treatment group (T3) included: five animals with Rutin concentration (50mg/kg) of body weight for 14 days, and then Ciprofloxacin oral given with a dosage (1mg/kg) body weight for 14 days.

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5. The fourth treatment group (T4) included: five animals with Ciprofloxacin antibiotic concentration of 14mg/kg body weight and then Rutin oral given with a dosage of 50mg/kg body weight in combination for 14 days.
6. The fifth treatment group (T5) included: five animals with Ciprofloxacin antibiotic concentration (14mg/kg) body weight for 14 days, then Rutin concentration (50mg/kg) of body weight for 14 days.

2.2 Chemicals

Rutin was prepared by Sigma- Aldrich chemical company (St Louis, MO, USA).

2.3 Drug

Medication doses of the antibiotic Ciprofloxacin (1.4mg/kg/day) was determined according to the equal to the human therapeutic dose appropriate with the Food and Drug Administration (FDA) (Guidance for Industry and Reviewers, 2002). The dose of Ciprofloxacin and Rutin was determined According to body weight, and then dissolved in water and the doses were given to animals by using gastric tube with a rate of 1 ml/ animal.

2.3 Determination of transaminases (ALT, AST and ALP)

The activity of transaminases (ALT, AST and ALP) in plasma was measured using commercially kits (COBAS, COBAS E, ELECSYS and PRECICONTROL Roche).

2.4 Histological and morphometric analysis

According to the standard protocol, histological analysis was routinely performed [24] with using a light microscope. For the histological and morphometric analysis of each tissue, 15 sections from each block were considered using the systematic random sampling method.

DATA ANALYSIS

The data of current study was statistically analyzed using F-test at probability level 0.05 to find out the significance of the differences between the C-group and treatment groups (Al-Rawi and Khalaf Allah, 2000). Differences were tested using the least significant difference (LSD).

3. RESULTS AND DISCUSSION

3.1 PHYSIOLOGICAL STUDY

Table (1) shows the effect of Ciprofloxacin and Rutin on some liver enzymes.

Group	alkaline phosphatase enzyme (IU\L)	alanine transaminase (IU\L)	aspartate transaminase (IU\L)
control	182.45±2.47 D	34.75±1.88 BC	188.00±4.26 BC
T1	121.17±5.45 B	33.75±0.47 C	181.50±5.43 C
T2	227.62±6.40 A	51.50±4.73 A	232.50±4.40 A
T3	201.25±4.35 C	40.25±1.08 B	193.50±2.39 B

T4	198.07±1.32 C	38.00±0.81 BC	188.00±1.08 BC
T5	205.15±3.11 C	39.75±1.10 B	192.00±0.81 B
LSD	10.48	5.48	8.75

* (A, B, C) indicate the significant difference present of means between the groups

Table 2: Values, 95% confidence level for mean ± SD (margin of errors) of transaminases for rats tested for the effect of Rutin as a prophylaxis and treatment agent in rats given and not given ciprofloxacin as an inducting agent of acute liver injury.

Transaminases Mice Groups	AST(Aspartate transaminase) Mean ± SD IU/L Margin of error	ALT(alanine transaminase) Mean±SD IU/L Margin of error	ALP(alkaline phosphatase). Mean ± SD IU/l Margin of error
Control group Reference	213.5±19.428 (±9.10%)	40±4.234 (±10.59%)	231.95±101.165 (±43.62%)
T1: Rutin 50 mg/ kg 14 days.	181.5±10.661 (±5.87%)	38±8.581 (±22.58%)	165.925±60.458 (±36.44%)
significance	0.027*	0.68	0.30
T2:Ciprofloxacin 14 mg/kg/14 days	146.875±62.73 (±42.71%)	81.75±74.279 (±90.86%)	146.875±62.73 (±42.71%)
significance	0.088	0.03	0.20
T3: Rutin 14days \after ciprofloxacin 14 days.	123.75±22.021 (±17.79%)	30.75±6.807 (±22.14%)	194.75±77.136 (±39.61%)
significance	0.0008*	0.05*	0.57
T4:Ciprofloxacin 14days/compination Rutin 14 days	107±28.572 (±26.70%)	21.75±8.815 (±40.53%)	120.575±29.135 (±24.16%)
significance	0.0008*	0.009*	0.07
T5:ciprofloxacin 14 days and after 14 days/ Rutin for 14 days	137.25±27.069 (±19.72%)	19±3.099 (±16.31%)	201.65±83.878 (±41.60%)
significance	0.0037*	0.0002*	0.65

Based on the SEM, the following are the margins of error (or confidence intervals) at different confidence levels. Depending on the field of study, a confidence level of 95% (or statistical significance of 5%) is typically used for data representation.

3.2 HISTOLOGICAL STUDY

By comparing the histological examination of the liver tissues of two groups, the first is given

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Ciprofloxacin for 14 days, and the second is given Rutin for 14 days followed by Ciprofloxacin for an additional 14 days. The first group showed more significant histopathological changes in the liver tissues, including hemorrhage and constriction of blood vessels in the central vein, in addition to the development of sebaceous cysts and necrosis.

In Figure 2 the histological examination showed that in the T4 group (dosage ciprofloxacin 14 days and afterward Rutin combination 14 days) and T5 group (dosage Ciprofloxacin 14 days/ Rutin combination) groups, there was a repair of liver tissue that made it almost similar to normal liver tissue with Minor blood congestion and minor dilation in the blood capillaries in T5 group.

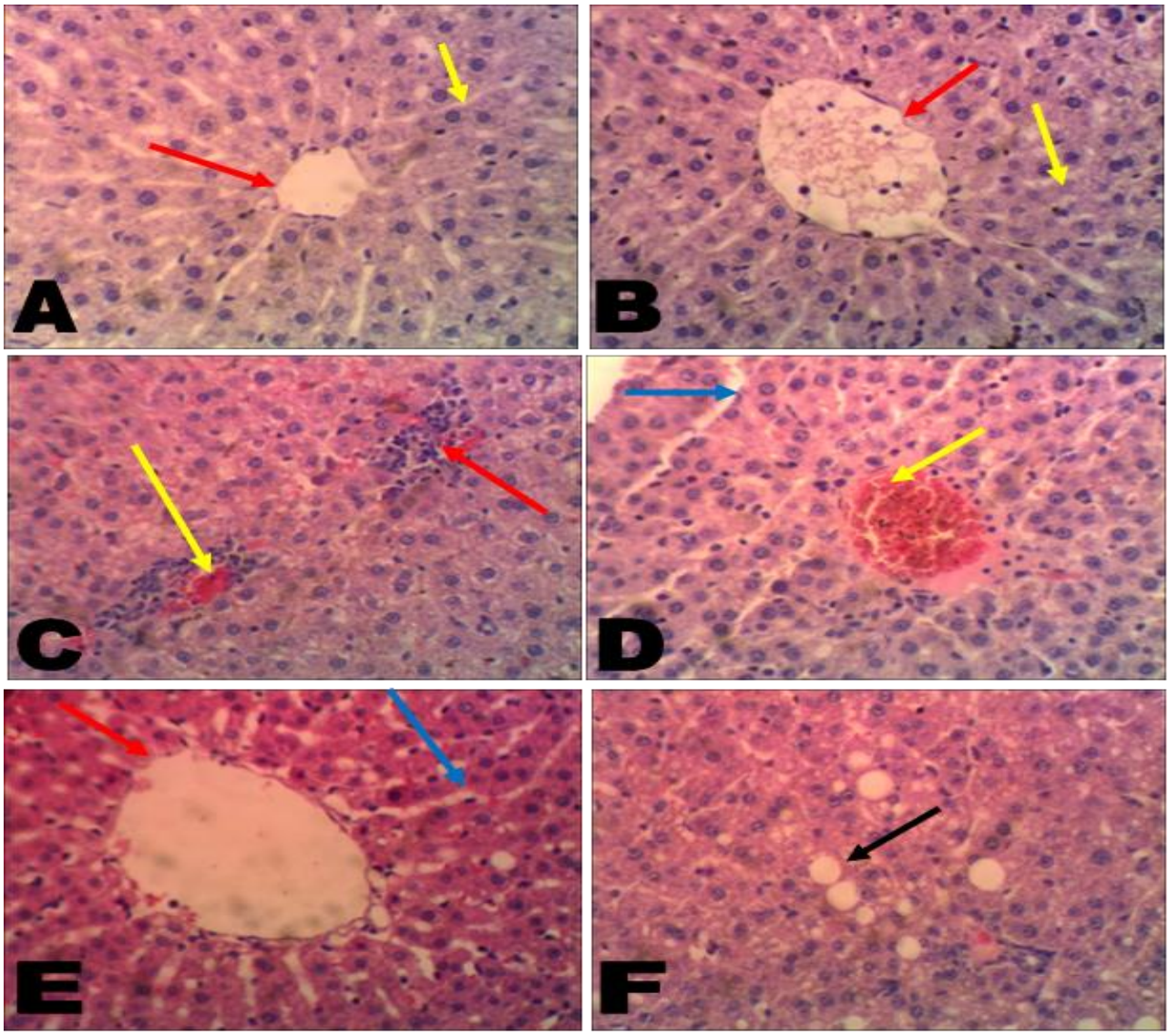

Figure (1) shows liver tissue in rats: picture (A) is the control group, demonstrated normal arrangement of the hepatic cells (Yellow arrow) around the central vein (red arrow). picture (B) T1 group shows Normal cellular arrangement pattern, (Yellow arrow) regularly arranged around the central vein and the blood capillaries seen clearly (red arrow). picture (C,D and F) T2 group shows Loss of the normal hexagonal arrangement of hepatocytes, cellular dissociation(blue arrow), congestion in central vein (Yellow arrow), fatty necrosis (black arrow) and lymphoid infiltration (red arrow). Picture E T3 group shows A minor dilation in the blood capillaries (blue arrow) and in the central vein (red arrow).

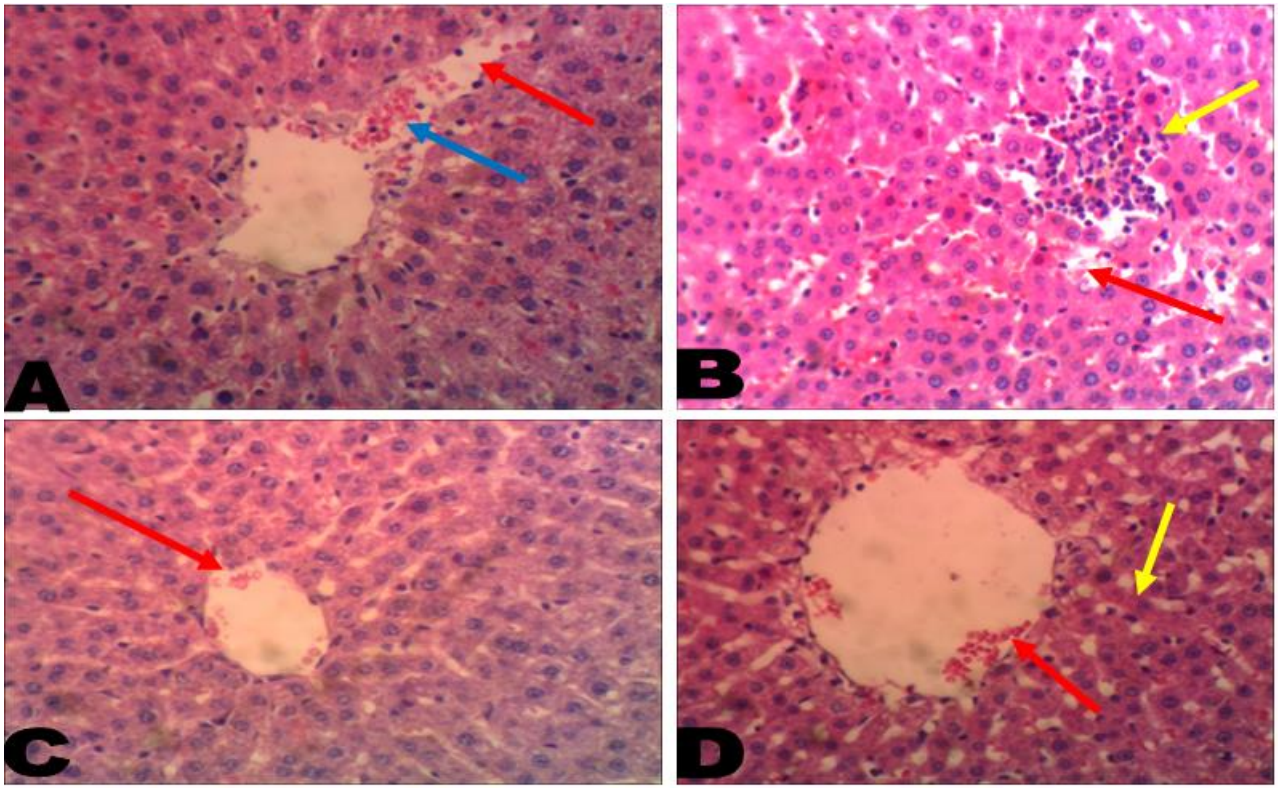

Figure (2): picture (A and B) is the T2 group shows lymphoid infiltration (yellow arrow), necrosis (red arrow) and bleeding (blue arrow). Picture C T4 group shows Nearly normal cellular arrangement and normal distribution around the central vein, Picture D T5 group shows Minor blood congestion (red arrow) and minor dilation in the blood capillaries (yellow arrow).

In the current study, considering the second group (T2), which consisted of five animals, the dose of Ciprofloxacin antibiotic was given 14mg/kg body weight for 14 days. There were histopathological changes in the liver tissues, including hemorrhage and constriction of blood vessels in the central vein, the hepatocytes lost their normal pattern in addition to the development of sebaceous cysts, necrosis and accumulation of inflammatory cells, and hepatocyte dissociation. In addition, the mean AST ± SD in this group was 146.875 ± 62.73 IU/L with a very wide margin of error of ± 42.71% and the mean ALT ± SD in this group was 81.75 ±74.279 IU/L with a very wide margin of error of 90.86% indicating an elevated level of these two transaminases with histological result as evidence of hepatocyte fractionation due to Ciprofloxacin (this demonstrates hepatocellular injury) (Table 1, Figure 2). [23], [25], [26] confirmed that the type of damage was done by Ciprofloxacin and other substances in humans can be confirmed by elevated liver function tests. If ALP is increased more than ALT/ALP, this indicates cholestatic hepatitis. The prognosis is worse if bilirubin levels raise more than twice their standard level in combination with increased serum transaminase level [25], [26]. The results of the current study are consistent with the facts that fluorinated quinolones which included Ciprofloxacin are hepatic metabolized and kidneys excreted. Ciprofloxacin has been cleared to cause a temporary 1% to 3% increasing of liver enzymes. Patients with acute liver damage caused by Ciprofloxacin have symptoms equal to those with acute non-drug-induced liver damage. These include pain in right upper quadrant abdominal, fatigue, loss of appetite, fever (low-grade), nausea, vomiting, and concentrated urine. Many cases may also present with thrombosis and/or hepatic coma. Enlarged liver and jaundice may be marked on physical examination [4]. In the current study, we kept our animals and gave them Ciprofloxacin for 14 days. This should be a sufficient period to develop or cause liver injury as confirmed by the findings of the National Institutes of Health and Schmid et al. they found that Ciprofloxacin sometimes lead to acute liver damage -two days to two weeks- after starting

antibiotic therapy. In spite of the accurate pathway of Ciprofloxacin-induced liver damage is still unknown, hepatocellular necrosis leading to increased liver enzymes has been noticed, The injury can be cholestasis, hepatocellular, or mixed. The most common shape in the acute condition is hepatocyte breakdown and increased alanine transferase levels. The biliary type of liver damage usually follows a long time of an antibiotic therapy. Therefore, the drugs and toxins may cause hepatic injury either due to their toxic effect or specific interaction. The direct toxic effect is dose dependent, while the specific reactions: metabolic or immunological and can be done at any dose of the drug. In most cases, the hypersensitivity response is due to the short latency period, recurrent immune-susceptibility characteristics, and severe illness when re-exposure is occurred [5], [6]. In human studies, researchers have found that hepatitis caused by Fluoroquinolone is usually non-fatal and self-resolving after stopping the drug and treating symptoms. Only four previous results of Ciprofloxacin-induced liver damage were reported in the literature [27]. At least one death from Ciprofloxacin-induced hepatitis has been reported in 74-year-old woman with treatment for a urinary tract infection. In her case, symptoms started after using Ciprofloxacin. Nevertheless, she was given a 2nd course of the medication due to a urinary tract infection that did not resolved [27]. Hepatic enzymes should be monitored until they return to normal. Corticosteroids can be included with different success. In human, Corticosteroids may be more helpful in relieving complains in cases with hypersensitivity. Because acute hepatitis caused by Ciprofloxacin is a classic effect, patients who presented with drug-induced hepatitis liver damage after using Ciprofloxacin should avoid more use of -not only- Ciprofloxacin but also Fluoroquinolones [22], [27].

Considering treatment group (T3) consisting of five animals, Rutin was administered at a concentration of 50mg/kg bw for 14 days and then the animals were given oral Ciprofloxacin at a dose of 14mg/kg b/w for 14 days. A significant decrease of AST was found (123.75 ± 22.021) with a margin of error equal to ± 17.79% ($p = 0.0008$ *), and histological examination also showed slight histopathological changes in liver tissue, with some development in liver tissue when Rutin was followed by Ciprofloxacin, indicating the protective effect of Rutin, which prevented the breakdown of hepatocytes when using the antibiotic. This result confirmed Rutin as a hepatoprotective, and renal protective potential [21], medicinal potential as antioxidant [10], vascular prophylactic, antiproliferative [13], and anticoagulant [14], cell protector [15], anti-inflammatory [9], antibacterial [16], antiviral [17], antiulcerogenic [18], Neuroprotective [19], cardioprotective [20]. The researchers concluded that Rutin has a wide variety of biological and pharmacological activities and has the potential to be a key molecule for advanced practical use [22], [23]. In this study, the treatment group (T4) and (T5) was considered which showed the protective and neutralizing role of Rutin in neutralizing the toxicity of Ciprofloxacin on the liver (Table 1). These results are similar to those reported by [23] The preventive efficacy of Rutin on impaired hepatic, renal and mental function was evaluated, where Rutin (5,10, 20mg/kg) given continuously to test animals for 6 days then a single dose of D-galactosamine (300mg/kg) was evaluated. kg i.p. and lipopolysaccharide (50µg/kg i.p.) on day 6 [23]. The protective role of Rutin can be explained when it prevents spread of AST, ALT, ALP and bilirubin from hepatocytes into the systemic circulation, reflecting its protective effect on the cell membrane of vital organs [8], [9], [23]. Routine pretreatment also modulated the regular metabolic pathways and controlled glycogen level. Rutin selectively regulates elevated cholesterol by improving metabolism and preserving structural integrity due to its free radical scavenging activity [28], [29]. It may be a prophylactic treatment of Rutin-protected vital organs from injuries caused by Ciprofloxacin and control diagnostic variables due to its strong antioxidant properties [30- 32].

4. CONCLUSIONS

Hepatitis caused by Ciprofloxacin in laboratory animals, often a special reaction that causes necrosis of hepatocytes. Rutin pretreatment at various doses protected liver by maintaining functional integrity by

improving antioxidant status of the cell, reducing oxidative stress, and preserving the integrity of the liver histomorphology structure. However, the 50mg/kg dose of Rutin is the most acceptable dose as hepatoprotective and against dysfunctions by drug-induced injury.

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