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Original Article

An Examination of the Effect of Rutin against Neurotoxicity Induced by Ciprofloxacin Antibiotic in Wistar Rats

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Abstract

Rutin is a citrus flavonoid that exists in different types of food, such as fruits, tea, as well as vegetables, and is considered a natural antioxidant. This study aimed to investigate the therapeutic role of Rutin in oxidative stress-induced in Wistar rats exposed to Ciprofloxacin (CPX). The study included 36 healthy adult Wistar rats, which were randomly divided into six groups (n=6). The control group (C) received normal drinking water for 20 days. The first treatment group (T1) received Rutin at a dose of 50 mg/kg of b.w for 20 days. The second treatment group (T2) received CPX antibiotic at a dose of 14 mg/kg of b.w for 20 days. The third treatment group (T3) received Rutin at a dose of 50 mg/kg of b.w for 20 days, and afterward, they received CPX antibiotic at a dose of 14 mg/kg of b.w for 20 days. The fourth treatment group (T4) received CPX antibiotic at a dose of 14 mg/kg of b.w for 20 days, and then, they received Rutin at a dose of 50 mg/kg of b.w for 20 days. The fifth treatment group (T5) received CPX antibiotic at a dose of 14 mg/ kg of b.w and Rutin at a dose of 50 mg/ kg of b.w together for 20 days. All the treatments were administrated by oral gavage. Analysis of the recorded data showed a significant increase (P<0.05) in the concentration of Methylenedioxyamphetamine (MDA) in the T2 group, compared to the other groups. The MDA level significantly (P<0.05) increased in the T3 group (2.29±0.04), compared to the C (1.71±0.01), T1 (1.54±0.04), T4 (1.18±0.02), and T5 (1.29±0.03) groups. However, there were no significant differences (P<0.05) between the C (1.71±0.01) and T1 (1.54±0.04) groups, as well as the T4 (1.18±0.02) and T5 (1.29±0.03) groups with regards to the MDA. The results clarified a significant increase (P<0.05) in the antioxidant activity, Glutathione (GSH), Superoxide dismutase (SOD), and Catalase (CAT) contents in the T1 group, determined at 5.91±0.26, 5.78±0.02, and 1.98±0.05, respectively, compared to the other groups. The lowest antioxidant activity, GSH, SOD, and CAT contents were recorded in the T2 group, in comparison with the other groups (P<0.05). The findings revealed that the level of SOD, GSH, and CAT in the T4 and T5 groups significantly (P<0.05) increased, compared to the T2 and T3 groups. Histological examination of the slides obtained from the brain demonstrated that in the T2 group, some histopathological changes were observed, compared to the C, T1, T4, and T5 groups. These changes were as follows: 1) damaged and clear blood vessel congestion with the deposition of fibrous networks, 2) brain edema, 3) multiple necrotic foci, 4) accumulation of neutrophils, and 5) simple histopathological changes in the brain of animals in the T2 group, compared to the other groups. It is, therefore, concluded that Rutin supplementation at a dose of 50 mg/kg b.w can be the most appropriate dose in protecting brain tissue against tissue damages caused by CPX.

Keywords: Central nervous system, Ciprofloxacin, Flavonoid Rutin, Neurotoxicity

1. Introduction

Fluoroquinolones are a type of antibacterial antibiotics that are used to treat different infections throughout several mechanisms (1). They have their broad-spectrum activity against different bacterial infections, such as joint and bone infections, urinary tract infections, intra-abdominal infections, infections of the skin, typhoid fever, as well as respiratory tract

infections (2). Ciprofloxacin (CPX) is a fluoroquinolone antibiotic that can be utilized for the treatment of a wide range of Gram-negative and Gram-positive bacteria. It inhibits cell division by its adverse effect on DNA function through inhibiting bacterial DNA gyrate and topoisomerase IV, which are essential to the replication of bacterial DNA, transcription, recombination, and repair (3).

CPX has equivalent or greater bioavailability, compared to other fluoroquinolone antibiotics; it has an enhanced pharmacokinetic, higher plasma concentrations, and tissue penetration. Furthermore, CPX is found in different concentration levels in body organs, including the liver, kidney, pancreas gland, skeletal muscle, adipose tissue, testes, as well as cartilage (4). CPX is the only fluoroquinolone antibiotic that exists in the list of "Essential Medicines for Children" by the World Health Organization. It was agreed that CPX can be used for children aged 1 to 17 in 2004, but not for newborns (3). CPX side effects include gastrointestinal discomfort, juvenile joint toxicity cutaneous reactions, and negative effects on the central nervous system, probably due to the oxidative stress produced from different medications, such as CPX (5).

Traditional medicinal plants have always been an important part of folk medicine ever since the ancient era (6). Flavonoids are a unique category of therapeutic compounds due to their varied pharmacological properties. Nearly 4,000 kinds of flavonoids have been detected in plants (7). Rutin, also called vitamin P or rutoside, has been reported to have a lot of therapeutic effects (8). It exists in some foods, such as fruits, tea, and vegetables (9), and it is believed to have vital therapeutic roles in brain ischemia. The use of Rutin causes the reduction of "ischemic neural apoptosis" due to the embarrassment of lipid peroxidation and p53 gene expression, along with an increase in the endogenous enzymatic antioxidant defense systems, such as Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (10). Rutin is also utilized in the therapy of Alzheimer's disease by diminishing the Interleukin 1 beta and Tumor necrosis factor (TNF-α) formation in microglia. Rutin protects the cell membranes by scavenging free radicals and prohibiting their oxidative damage (9). This study, therefore, aimed to focus on the therapeutic role of Rutin in oxidative stress-induced in adult male Wistar rats exposed to CPX.

2. Materials and Methods

2.1. Experiment Design

The present study was conducted in animal husbandry laboratories in the Department of Biology, College of Education, University of Al-Qadisiyah, Al Diwaniyah, Iraq. A total of 36 healthy adult Wistar rats (12 to 14 weeks old, 190 to 200 g) were randomly divided into six groups (n=6). The following standard laboratory conditions were considered for animal housing during the study: a temperature of 21.00±2.00˚C, relative humidity of 40.00% to 55.00%, as well as photoperiod of 12 h light and 12 h dark. All animals were fed with a standard diet and water *ad libitum*. The grouping of animals was conducted as follows:

1. Animals in the control group (C) received normal saline by oral gavage for 20 days.
2. Animals in the first treatment group (T1) received Rutin at a dose of 50 mg/kg of b.w for 20 days.
3. Animals in the second treatment group (T2) received CPX at a dose of 14 mg/kg of b.w by oral gavage for 20 days.
4. Animals in the third treatment group (T3) received Rutin at a dose of 50 mg/kg of b.w by oral gavage for 20 days, and afterward, they received CPX by oral gavage at a dose of 14 mg/kg of b.w for 20 days.
5. Animals in the fourth treatment group (T4) received CPX by oral gavage at a dose of 14 mg/kg of b.w, and then, they received Rutin by oral gavage at a dose of 50 mg/kg of b.w together for 20 days.
6. Animals in the fifth treatment group (T5) received CPX by oral gavage at a dose of 14 mg/kg of b.w for 20 days, and afterward, Rutin was administered to them by oral gavage at a dose of 50 mg/kg of b.w for 20 days.

2.2. Chemicals

Rutin (CAS Number: 207671-50-9) was purchased from Sigma-Aldrich chemical company (St Louis, MO,

USA). A medication dosage of the antibiotic CPX (i.e., 1.4 mg/kg a day) was determined according to the equivalent therapeutic dose of humans based on the Food and Drug Administration guidelines (11). The CPX and Rutin were weighed according to the bodyweight of the rats. Afterward, they were dissolved in distilled water and were directly administered to animals at a volume of 1 ml for each animal by oral gavage.

2.3. Determination of some Biochemical Parameters

2.3.1. Catalase

The reactive oxygen species (ROS) and hydrogen peroxide are degraded through CAT. CAT is considered an important antioxidant enzyme. It is well documented that this antioxidant enzyme is widely expressed in living organisms that contain the cytochrome system. The antioxidant role of CAT is expressed in the catalysis decomposition of hydrogen peroxide into water and oxygen. The CAT concentration was measured, according to the methodology previously described by Aebi (12).

2.3.2. Glutathione

The level of Glutathione (GSH) in serum was determined based on the methodology previously described by Hakuna, Doughan (13). Briefly put, the GSH extraction involved the following steps: 1) extraction, 2) reduction with immobilized tris (2-carboxyethyl) phosphine, 3) deproteinization, and 4) concomitant fractionation of GSH using size exclusion chromatography.

2.3.3. Superoxide Dismutase

The SOD concentration was measured using commercially available Enzyme-Linked Immunosorbent Assay RANSOD kits (Randox Company Ltd., UK), as previously described by Momen Beitollahi, Mansourian (14).

2.3.4. The Level of Lipid Peroxidation (Malondialdehyde)

The malondialdehyde concentration as an index of lipid peroxidation was measured by the thiobarbituric acid reaction, according to the methodology previously described by Guidet and Shah (15).

2.4. Histological Study

Histological slides of the brain tissue were prepared based on the methodology of Banchroft, Stevens (16).

2.5. Microscopic Evaluation and Imaging

The slides were examined by a light microscope to determine the pathological changes in the brain tissue, and they were imaged by a microscope equipped with a digital camera at a magnification force of 100× and 400×.

2.6. Statistical Analysis

The data of the current study were statistically analyzed by the F-test at a probability level of 0.05 to find out the significance of the differences between the C and treatment groups (17). Differences were tested using the Least Significant Difference (LSD).

3. Results and Discussion

The statical analysis of the recorded data revealed a significant increase (P<0.05) in the concentration of MDA in the T2 group, compared to the other groups (Table 1). The MDA level significantly increased (P<0.05) in the T3 group (2.29±0.04), compared to the C (1.71±0.01), T1 (1.54±0.04), T4 (1.18±0.02), and T5 (1.29±0.03) groups. However, there were no significant differences (P<0.05) between the C (1.71±0.01) and T1 (1.54±0.04) groups, as well as the T4 (1.18±0.02) and T5 (1.29±0.03) groups, with regards to MDA.

The results clarified a significant increase (P<0.05) in the antioxidant activity, GSH, SOD, and CAT contents in the T1 group, determined at 5.91±0.26, 5.78±0.02, and 1.98±0.05, respectively, compared to the other groups (Table 1). The lowest antioxidant activity, GSH, SOD, and CAT contents were recorded in the T2 group, compared to the other groups (P<0.05) (Table 1). The recorded data showed that the level of SOD, GSH, and CAT in the T4 and T5 groups significantly increased (P<0.05), compared to the T2 and T3 groups.

Histological examination of the slides obtained from the brain demonstrated that in the T2 group, some histopathological changes were observed, compared to the C, T1, T4, and T5 groups. These changes were as follows: 1) damaged and clear blood vessel congestion with deposition of fibrous networks, 2) brain edema, 3) multiple necrotic foci, 4) accumulation of neutrophils (Figures 1 and 2), and 5) simple histopathological changes in the brain of animals in T3 group, compared to the other groups (Figure 3). These histopathological changes occurred due to the oxidative stress caused by ROS produced from the CPX antibiotic (19). Previously published studies showed the oxidative stress induced by medication may lead to abnormal structural changes in brain tissue, cellular proteins, membrane lipids, as well as DNA and RNA, as important mechanisms underlying oxidative stress-induced neurotoxicity (30-32). On the other hand, it is well documented that CPX causes histopathological changes in brain tissue, including blood vessel congestion and perivascular edema (1).

Table 1. Effect of Ciprofloxacin and Rutin on some indicators of oxidative stress

No.	SODU / mL	GSH nmol/mg	CAT ng/dl	MDA nmol/mg
C	2.96 ± 0.09^{c}	2.57 ± 0.06^{d}	0.70 ± 0.01^{c}	1.71 ± 0.01^{c}
T1	5.78 ± 0.02^{a}	5.91 ± 0.26^{a}	1.98 ± 0.05^{a}	1.54 ± 0.04^{c}
T2	1.38 ± 0.08^{d}	1.26 ± 0.02^{f}	0.39 ± 0.02^{c}	3.83 ± 0.16^{a}
T3	1.96 ± 0.05^{d}	1.69 ± 0.06^{e}	0.59 ± 0.006^{c}	2.29 ± 0.04^{b}
T4	3.99 ± 0.12^{b}	3.97 ± 0.05^{b}	1.61 ± 0.06^{b}	1.18 ± 0.02^{d}
T5	3.45 ± 0.23^{bc}	3.16 ± 0.13^{c}	1.38 ± 0.06^{b}	1.29 ± 0.03^{d}
LSD	0.67	0.31	0.36	0.17

Different letters a, b, and c indicate the significant difference between the means of the groups.

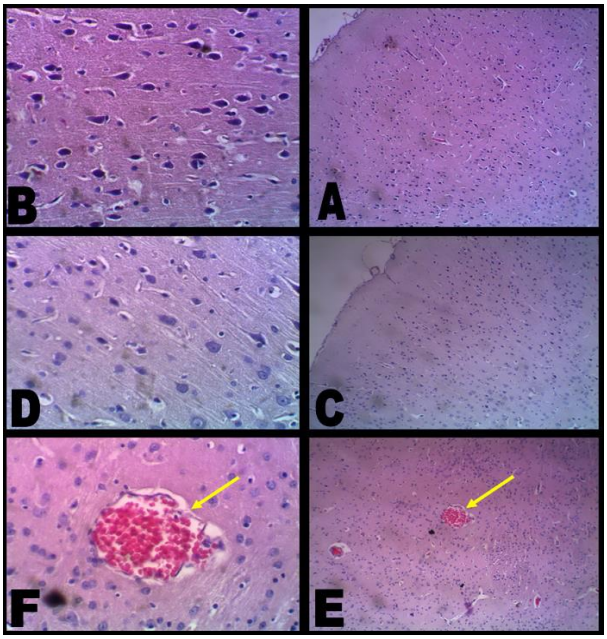

Figure 1. Brain tissue in rats: (**A** and **B**) The C group demonstrates normal brain cells without any significant occupied lesion (SOL); (**C** and **D**) T1 group shows normal texture in the section without any SOL; (**F** and **E**) T2 group shows clear blood vessel congestion (Yellow arrow) (H and E, 10× and 40×)

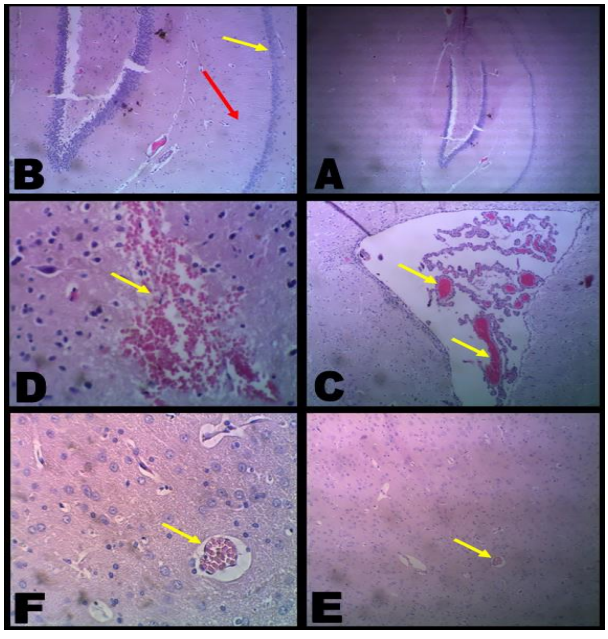

Figure 2. Brain tissue in rats: (**A** and **B**) T2 group shows the accumulation of structureless, homogenous fluid (brain edema, red arrow) surrounded by a fibrous capsule (Yellow arrow); (**C** and **D**) T2 group shows clear blood vessel congestion (Yellow arrow) with dilated brain venous diameter to appear elongated in shape and hemorrhage (Red arrow); (**F** and **E**) T3 group shows simple blood vessel congestion (Yellow arrow) (H and E, 10× and 40×)

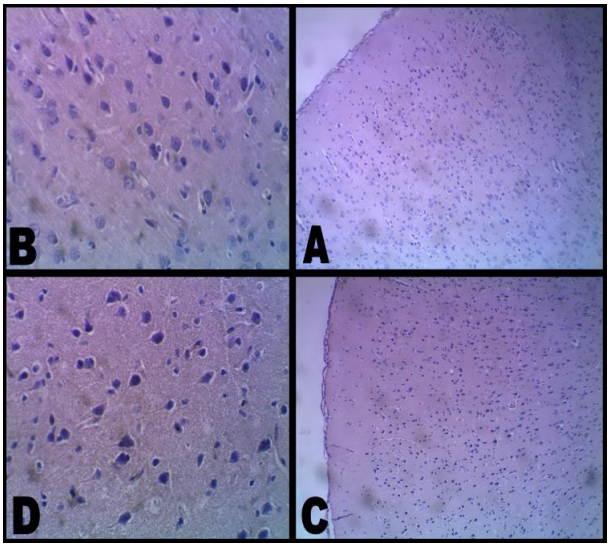

Figure 3. Brain tissue in rats: (**A** and **B**) T4 group shows normal texture in the section without any significant SOL; (**C** and **D**) T5 group shows normal brain tissue texture in the section without any SOL. (H and E, 10× and 40×)

As for the T4 and T5 groups, examination of the tissue slides taken from their brains did not show any pathological and histological changes, and brain tissues were normal. This may be due to the therapeutic role of Rutin in protecting brain tissues against medication (33). Almutairi, Alanazi (34) demonstrated that Rutin has beneficial effects and can reduce cisplatin medicine neurotoxicity with possible mechanisms by its antioxidant nature. On the other hand, Rutin reduces degeneration of pyramidal neurons and accumulation of polymorphonuclear leucocytes due to brain tissue injuries while removing edema and other histopathological changes. Furthermore, Rutin plays an essential role in decreasing oxidative stress, as well as proinflammatory brain tissue damages, and prevents negative effects on the brain (35). Previous studies have documented that Rutin can inhibit proinflammatory NF-κBand TNF-α generation (36).

It is concluded that Rutin supplementation at a dose of 50 mg/kg can be the most appropriate dose in protecting brain tissue against tissue damages caused by CPX.

Authors' Contribution

Study concept and design: A. J. A.

Acquisition of data: H. A. A.

Analysis and interpretation of data: H. A. A.

Drafting of the manuscript: H. A. A.

Critical revision of the manuscript for important intellectual content: J. K. A.

Statistical analysis: A. J. A.

Administrative, technical, and material support: A. J. A.

Ethics

All ethical procedures were approved by the ethics committee of the University of Al-Qadisiyah, Al Diwaniyah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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