REVIEW ARTICLE

Defensive Activity of Hesperidin against Ciprofloxacin Induced Hepatic Injury in Rabbits

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ABSTRACT

This study was conducted to find out hepatoprotective activity of hesperidin (HES) 100mg/kg body weight (b.w.) against ciprofloxacin (CPX) 100 mg/kg induced hepatotoxicity in local breed rabbits. CPX is a broad-spectrum antibiotic used for the treatment of many bacterial infections. Twenty four male rabbits were divided into four groups ,group1: control, (1 ml/kg saline orally) group 2: CPX (100 mg/kg orally) for (14) consecutive days , group 3: HES (100 mg//kg) orally for (14) consecutive days group 4: CPX (100 mg/kg orally) plus HES (100 mg/kg orally) for (14) consecutive days. All the rabbits were killed on the (15) day of the experiment, and then the blood and livers samples were taken. CPX induced hepatotoxicity was proved by a significant ($p < 0.01$) reduction in the body weight, and a significant ($p < 0.01$) increased serum aspartate transaminase (AST), alanine transaminase (ALT), Malonaldehyde enzyme (MAD) and histopathological changes. Protective hepatic toxicity effect and oxidative damage caused by CPX significantly ($p < 0.01$) increasing in body weight and significantly ($p < 0.01$), decreasing AST, ALT, MAD, and improving tissue morphology in HES (100 mg//kg). These results assure that HES (100 mg/kg) antioxidant effects can protect CPX-induced hepatotoxicity in rabbits.

Keywords: Anti-oxidant, Ciprofloxacin, Hesperidin, Rabbits.

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INTRODUCTION

CPX is a synthetic broad-spectrum antibacterial belonging to the class of quinolones.[1] It well absorbed orally, and it induced its antibacterial action mainly by inhibiting DNA gyrase, a type II topoisomerase. Topoisomerase IV enzymes fundamental to separate bacterial DNA, thereby inhibiting cell division.[2] CPX is one of the most generally used antibiotics for different types of infections in the lower respiratory tract, skin, bone, joint, urinary tract, and infectious diarrhea.[3, 4] CPX is generally well tolerated. The most common adverse reactions occur in the gastrointestinal tract, central nervous system, and hematological system. Recently, rising cases of CPX associated organ toxicities have been reported.[5] Studies in experimental animals exhibit various side effects after CPX such as chondrotoxicity[6] and damage in testicular structure and function.[7] Renal side effects as crystal nephropathy was reported in some clinical cases after high CPX dose.[8]

Flavonoids are a naturally occurring group of compounds that are highly present in foods of plant origin. Flavonoids have a variety of biological effects in numerous mammalian cell systems, in vitro as well as in vivo. They have been shown to exert anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumor activities.[9] The pharmacological effects inhibiting ability on certain enzymes and also, their antioxidant activity.[10] Hesperidin is a flavanone glycoside derived from the word "hesperidium, " the kind of fruit produced by citrus trees as it is found abundantly in citrus fruits.[11] Hesperidin is mainly used as an antioxidant, as it uncommonly prevented indicators of oxidative stress, such as the reactive oxygen species (ROS) and lipid peroxidation levels in a dose-dependent manner.[12] Hesperidin may be related to potential benefits in the prevention of many diseases, such as decreasing capillary permeability, anti-inflammatory, antimicrobial, and anticarcinogenic effects. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase.[13-16] Moreover, it is reported as anticancer antihyperlipidemic,[17] antihypertensive[18] and cardioprotective activity in ischemic heart disease in diabetic rats.[19]

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MATERIALS AND METHODS

This study was conducted at the period of November 2018 in the physiology department of veterinary medicine of AL-Qassim green university.

Drugs

Hesperidin (HES), 100% natural, was purchased from BULK Supplements.com USA. Ciprofloxacin (CPX), 20% as DUFACIPROFLOX, was purchased from Dutch Farm-Holland. Ketamine 10% inj. from KEPRO-HOLLAND. Xylazine, XYL-M2, VMD- Belgium.

Experimental rabbits

The number of laboratory animals used in the experiment are twenty four healthy male (local breed) rabbits at weighing 1375-1385 g and aged nine months were used in this study, obtained from the animal house of the College of Veterinary Medicine, Baghdad University, were kept for 10 days as acclimatization period before the beginning of the experiment. The animals were maintained at standard housing conditions and fed a standard pellet diet and water.

Experimental design

After a quarantine period of 10 days, 24 rabbits were randomly divided into four equal groups, each group consists of 6 rabbits, and they received the treatment as follows:

Group I: Control (1 mL/kg b.w Saline orally).

Group II: CPX (100 mg/kg b.w orally) for 14 days.[20, 21]

Group III: HES (100 mg/kg b.w orally) + for 14 days.[22, 23]

Group IV: CPX (100 mg/kg b.w orally) + HES (100 mg/kg b.w orally) for 14 days .

Body weight

All animals were weighed before and after treatment using the digital electronic balance.

Serum Preparation

At the end of the experimental period, rabbits were fasted for (10) hours, anesthetized with ketamine (75 mg/kg) combined with xylazine (2.5 mg/kg).[24] Blood samples were collected by heart puncture in non-heparinized tubes, centrifuged at (4000) rpm for 10 minutes.[25] After separation the serum from the clot, using a sampler, the samples were used to measurement of AST, ALT, and MDA level concentration. The rats were sacrificed by cervical dislocation, and the abdominal cavity was immediately opened, livers were removed and processed for histopathological studies.

Histopathological techniques

Sections were taken from livers tissues from different animals in each group immediately after sacrificed. These tissues were washed with the normal saline solution to remove blood, then fixed in 10% neutral formalin for 24 hrs, dehydrated in different concentrations of alcohol, and processed for paraffin embedding. Sections of 5 µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series stained with Haematoxylin and Eosin, cleared in xylene, and examined microscopically according to D. Bancroft, *et al.*[26]

Statistical analysis

The statistical analysis was carried by using Complete Randomized Design (CRD) method, according to AL-Rawi and Kalaf-Allah, 2000.[27] The mean differences between the averages of the studied traits were determined at the probability level of (0.01) using the Duncan test.[28] Statistical data were analyzed using the (SAS 2010).[29]

RESULTS

Bodyweight

No deaths were observed in the groups of rabbits that were given CPX, HES either alone or in summation. Administration

Table 1: Effect of HES on CPX induced change of the weight /gram of rabbits

Traits	*Control* *Mean ± SE*	*CPX (stress drug)* *Mean ± SE*	*HES (antioxidant)* *Mean ± SE*	*CPX + HES (ciprofloxacin + Hespeidin)* *Mean ± SE*
No. of rabbits	6	6	6	6
Initial weigh at 1 day/(gm).	1377.366 ± 3.26 A	1377.870 ± 1.76 A	1377.350 ± 1.85 A	1377.438 ± 0.72 A
final weight at 15 days/gm.	1394.350 ± 1.58 A	1360.332 ± 1.79 C	1395.178 ± 1.60 A	1388.352 ± 2.02 B

The averages of traits which have carried different levels horizontally indicates high significant at 0.01

Table 2: Effect of HES on AST, ALT and MAD in the serum of control and CPX treated rabbits

Traits	*Control* *Mean ± SE*	*CPX (stress drug)* *Mean ± SE*	*HES (antioxidant)* *Mean ± SE*	*CPX + HES (ciprofloxacin + Hespeidin)* *Mean ± SE*
AST (U/L) .	35.834 ± 0.30 D	49.892 ± 0.58 A	38.620 ± 2.19 B	41.620 ± 0.37 C
ALT (U/L) .	25.954 ± 0.22 D	37.224 ± 0.34 A	27.030 ± 0.37 C	28.544 ± 0.35 B
MAD (µmol/L).	2.372 ± 0.01 C	3.652 ± 0.11 A	2.192 ± 0.02 C	2.804 ± 0.06 B

The averages of traits which have carried different levels horizontally indicate high significant at 0.01

of CPX in group II after 14 days, produced significant ($p < 0.01$) decrease in the body weight compared to control and treated groups, group HES they were closest to the control group (Table 1).

Effect of HES on CPX induced alterations in hepatic function parameters

Effect of CPX induced reduction in liver function in rabbits. A significant ($p < 0.01$) increased in serum AST, ALT, levels compared to the control group and significant ($p < 0.01$) increase in MDA levels compared to control was observed after 14 days of treatment with CPX Whereas. Treatment with HES (100 mg/kg) prevented CPX induced increase in serum AST, ALT levels ($p < 0.01$) and produced significant ($p < 0.01$) reduction on the MDA compared to CPX control rabbits. However, HES (100 mg/kg) has a good effect on body weight, serum AST, ALT, levels and MDA level significantly ($p < 0.01$) on CPX caused hepatic alteration when compared to CPX control rabbits (Table 2).

Histopathological Examination

Light microscopic of liver examination using H&E (400X) stain in control rabbits, showed the normal histological structure of the liver tissue. (Fig: a and b). Histopathological effects of CPX on the liver of treated rabbits are presented in rabbits treated with CPX for 14 days, showed fatty changes, congestion, inflammatory cell, and dilation of sinusoids. (Fig :c and d). Histopathological effects of HES on the liver of treated rabbits are presented in rabbits treated with HES for 14 days, showed no clear lesion, only mild vacuolation and nuclear division also seen. (Fig: E and F). Histopathological effects of combination CPX plus HES on liver of treated rabbits are presented in rabbits treated with CPX plus HES for 14 days, showed mild congestion, dilation of sinusoids and regeneration of hepatocytes (Figures G and H).

DISCUSSION

Quinolones considerably are used to treat infections due to their broad-spectrum of bactericidal activity.[30] Hepatotoxicity

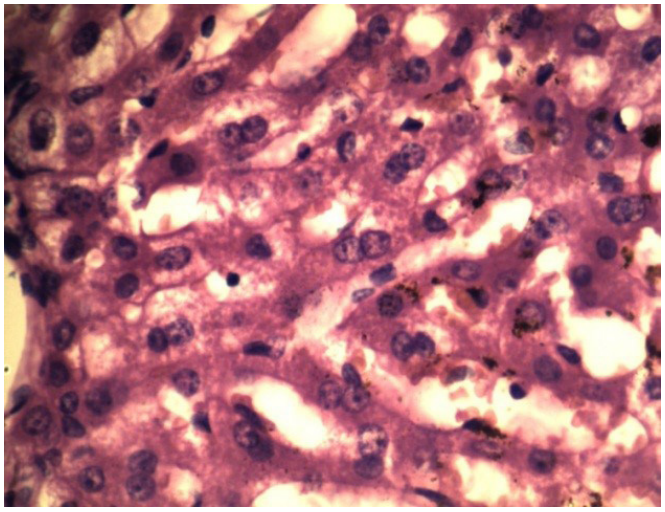

Fig A: H&Ex400

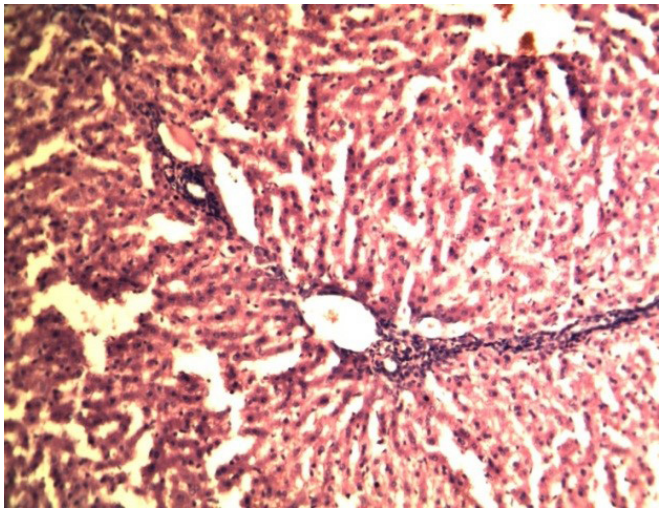

Fig B: H&E x100

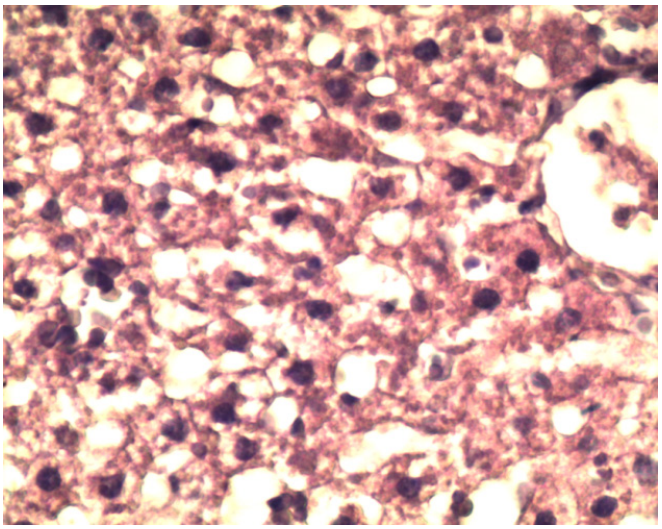

Fig C: H&E x400

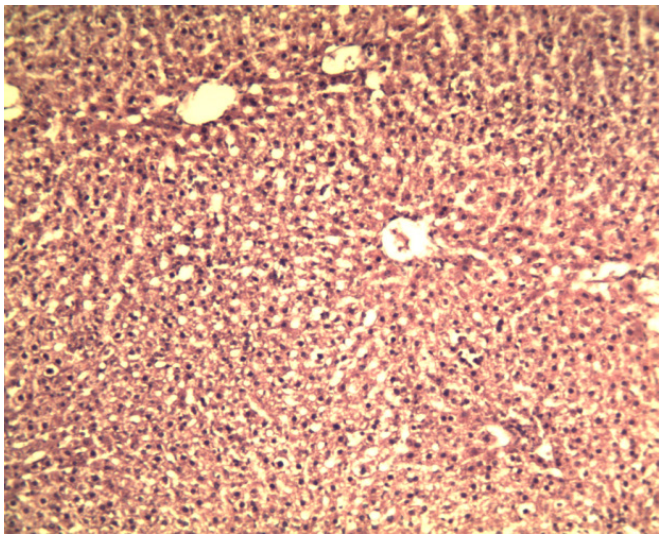

Fig D: H&E x100

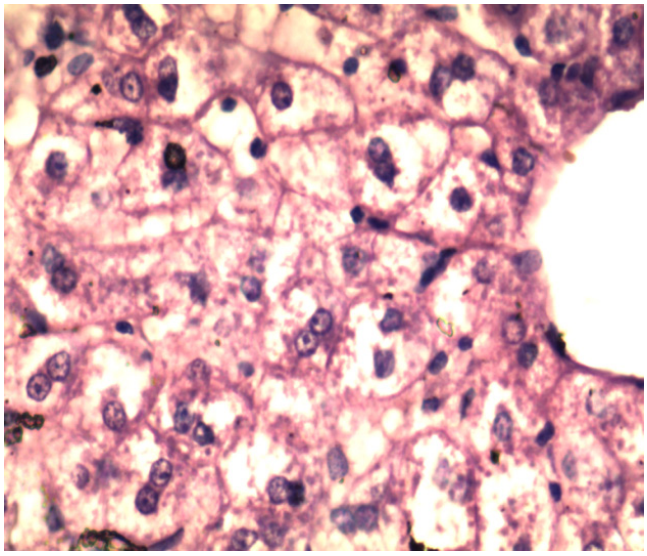

Fig E: H&E x400

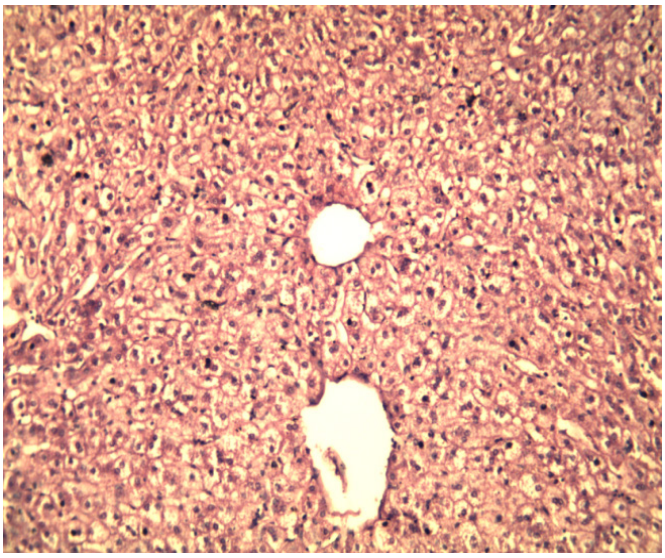

Fig F: H&E x100

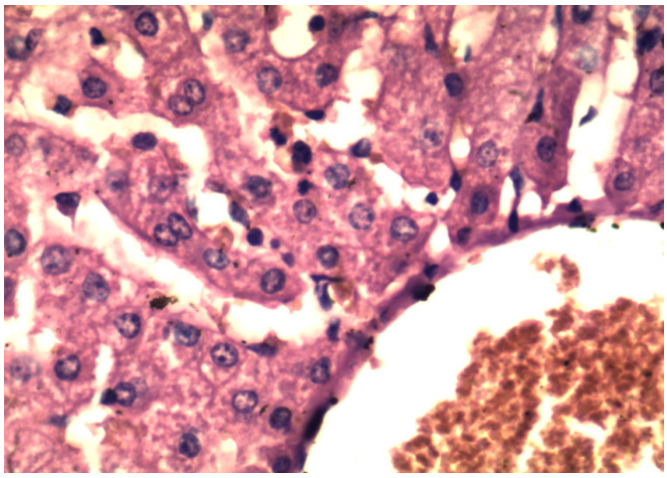

Fig G: H&E x400

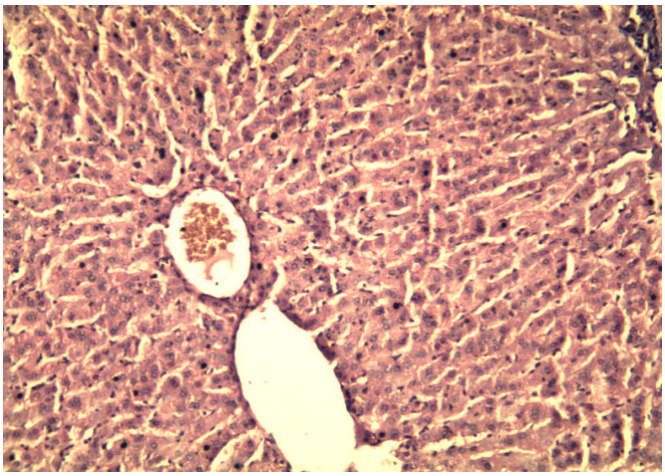

Fig H: H&E x10

caused by fluoroquinolones generally causes minimum symptoms, significant morbidity, acute liver failure, or death have been reported occasionally.[31,32] Liver injury correlated with CPX has been reported in experimental animals.[33] Hesperidin is mainly used as an antioxidant, as it uncommonly prevented indicators of oxidative stress, such as the reactive oxygen species (ROS) and lipid peroxidation levels in a dose-dependent way.[34] In our study, orally CPX (100 mg/kg) in rabbits produced a significant decrease in the body weight when compared to control ($P < 0.01$). Our results are in acceptance with previous findings of K.N. Agbafor, *et al*., Thomas Bourgeois Anne, *et al*., Hamida Hamdi, *et al*.,[35,36,37] and controversy with A. Nashwa, *et al.*[38] noted that the administration of CPX at a dose of 12.5 mg/kg.b.wt. for 65 days (5 days/week), it did not affect the bodyweight of the animals but caused a significant reduction in the weights of testes, epididymis, and seminal vesicles relative to the control group. According to,[39] increased catabolism and anorexia are responsible for decreased food intake and causes body weight loss Further, following a loss of the tubular cells, involved in renal water reabsorption leads to dehydration and decreases body weight.[40] In the existent study, the administration of CPX for 14 days produced a significant elevation of serum AST and ALT levels. These results are in acceptance with those obtained by other investigators.[41,42] AST and ALT are a better parameter for detecting liver injury and largely used as the most common biochemical markers to evaluate the liver injury.[43] It has been reported that the development of CPX induced liver injury may result from the production of free radicals, which initiate the process of lipid peroxidation and oxidative stress. This may lead to destroying the membranes of hepatocytes leading to the release of their cytosolic enzymes into the blood.[44] We found that CPFX treatment caused increased MDA levels, these results are in acceptance with those obtained by other investigators,[45,41] MDA is a stable metabolite of the free radical-mediated lipid oxidation cascade. It is used widely as a marker of oxidative stress and destruction of lipids.[46] Lipid oxidation is an important cause of the destruction of cell membranes and is thought to participate in the development of tissue injury.[47] Histopathological changes induced by CPX,

which add with clearly elevated levels of biochemical liver markers AST, ALT and MDA activities. Similar findings were noticed by E Taslidere, *et al.*, A Basaran, *et al.*[48,49] who noticed the administration of CPX for (14) days resulted in rabbits liver injuries, with histological features of fatty changes, congestion, inflammatory cell, and dilation of sinusoids. Oxygen-free radicals appear to be involved in the development of CPX induced liver injuries.[50] We investigated the role of free oxygen radicals in CPX induced hepatotoxicity. Although the mechanism of CPX induced hepatic damage is not fully understood, it is believed that ROS play a significant role. Damage due to oxidative stress may increase owing to increased free radical or decreased antioxidant levels (45). Treatment with HES for 14 days reduced the CPX injured liver at a dose of (100 mg/kg) produces a significant ($p < 0.01$) decreased in serum AST, ALT, and MDA levels. Histopathological changes induced by CPX have reduced the mild livers congestion, dilation of sinusoids, and regeneration of hepatocytes in HES (100 mg/kg) treated rabbits. Our results show the ameliorative effect of HES (100 mg/kg) on CPX for (14) days induced liver toxicity in the rabbits. HES is a natural and pharmacologically active bioflavonoid found in citrus fruits, with antioxidant and free radical scavenging property.[51] The ability of HES to scavenge free radicals which led to oxidative stress that adversely affects cell structure and function. Oxidative stress triggers inflammation, which further potentiates oxidative stress in a vicious cycle, triggering various life-threatening diseases ranging from cardiovascular and neurodegenerative and cancer. HES not only scavenges ROS but can also stimulate the endogenous antioxidant defense mechanisms. Hence, HES provides invaluable support in conditions associated with oxidative stress and protects against stress-inducing treatments such as chemotherapy and radiation therapy.[52,53] HES are able to reduce various pathologically elevated inflammatory markers effect. That has been predominantly associated with their antioxidant activity and ability to inactivate the pro-inflammatory cascade initiated by free radicals, also effective in decreasing the synthesis of pro-inflammatory cytokines as tumor necrosis factor alpha as well as pro-inflammatory enzymes such as inducible nitric oxide synthase ((iNOS), that yields nitric oxide-NO) and cyclooxygenase-2 (COX-2).[54]

It was recently determined that the hesperidin is the active component responsible for, which increases ghrelin secretion. Ghrelin, often referred to as the hunger hormone, stimulates appetite, gastric motility, and gastric acid secretion.[55] Also, they act as antifungal viral and bacterial infections.[56] This can be explained on the antioxidant, anti-inflammatory and antimicrobials activity effect of HES in protects cells from damages such as free radicals from CPX induced oxidative stress, it can HES (100 mg/kg, bw) administration showed a marked hepatoprotective activity. The protective effects of HES (100 mg/kg, bw) may be due to its anti-inflammatory effects or antioxidant effects with antimicrobials activity individually or synergistically.

CONCLUSION

In conclusion, the administration of CPX and HES at doses 100 mg/kg, bw protect hepatic dysfunction through inhibiting free-radical formation and restoration of the antioxidant defense systems.

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