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Physiological and Hematological Changes induced by the Administration of Ciprofloxacin in Mice

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Abstract

The aim of the present work was to study the effect of Ciprofloxacin administration on hematological and biochemical alterations in blood mice. The results showed that red blood cell (RBC) count and hemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacin. Similar changes were observed with the mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH). Meanwhile, White blood cell (WBC) count and the temporary hematocrit (PCV%) were significantly elevated in the two doses of Ciprofloxacin and remained elevated until the end of the experiment. The result showed also, that The administration of cadmium at 0.5 and 1 mg/kg/day in mice, caused a significant elevation in serum aminotransferases (AST and ALT) enzymatic activity in treated mice. There was a significant decrease in serum total protein in treated mice when compared with the control group. The liver glycogen recorded a significant decrease in treating mice and significant rises in serum uric acid and creatinine in treated mice compared with control. Ciprofloxacin treatment either at 0.5 and 1 mg/kg/day in mice causes biochemical disturbances in the major glycolytic–gluconeogenic pathways, hepatic marker enzymes.

Keywords: Mice**,** Ciprofloxacin, Hematological, White blood cell (WBC), Hematocrit (PCV%), aminotransferases, Uric acid and creatinine biochemical analysis.

Contents

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1. Introduction

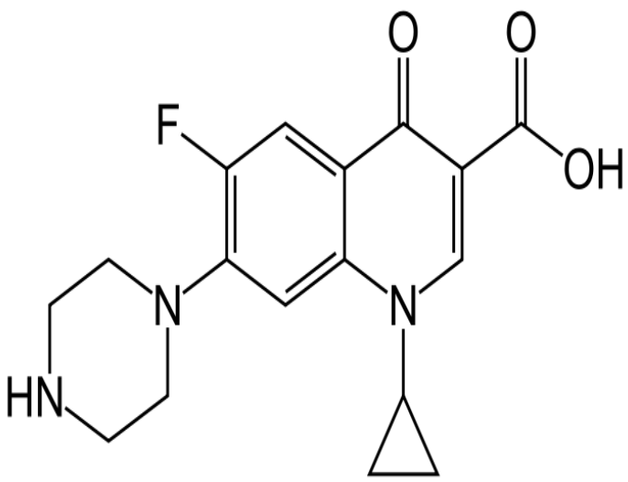
In modern medicine uses antibiotics are among the drugs that which is used in the treatment of disease by killing bacteria. It uses more than 100 different antibiotics are available to doctors to cure life threatening infections and the first Antibiotics used is penicillin. Ciprofloxacin is a synthetic antibacterial agent belonging to the second generation of the family of fluoroquinolones with a very broad spectrum against of microbial pathogens, especially Gram negative infectious diseases, that has been approved in more than 100 countries world-wide [1]. Ciprofloxacin is well absorbed orally and induced its antibacterial action mainly by inhibition of DNA gyras, which is equivalent to topoisomerase II in mammalian cell [2]. Ciprofloxacin used I the treatment of urinary, biliary, intestinal, and pulmonary infections. Drug-induced liver injury accounts for about 10% of adverse drug reactions. Injury may result from direct toxicity to hepatocytes or biliary epithelial cells, causing necrosis, apoptosis, or disruption of cellular function through hepatic conversion of a xenobiotic to an active toxin or through immune mechanisms, usually by a drug or a metabolite acting as a hapten to convert a cellular protein into an immunogen [3].There is a general agreement that energy metabolism is the fundamental global measure of the vital activity in all organisms [4] which in many ways is regulated by nucleotide level and the degree of the phosphorylation of nucleotide pools [5]. Pyruvate Kinase (PK) and Phosphoenolpyruvate carboxy kinase (PEPCK) play key roles in energy metabolism because they direct the flow of carbon of phosphoenolpyruvate (PEP) into the end products of anaerobic metabolism, the non enzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) is major contributors to hyperglycemia induced cell damage) to form advanced glycosylated end product (AGE) Which interferes with cell integrity by modifying protein function [6].

Therefore, the aim of the present work was to study the effect of Ciprofloxacin administration on haematological and biochemical alterations in blood and tissues of mice.

2. Materials and Methods

2.1. Drug

Ciprofloxacin (Cipro) is antimicrobial agents for oral administration manufactured by Bayer healthcare. Ciprofloxacin hydrochloride, USP, a fluoroquinolone, is the monohydrochloride monohydrate salt of 1-cyclopropy1-6-Fluoro-1,4- dihydro-4-Oxo-7-(1- piperaziny1) -3 quinolinecarboxylic acid. It is light yellow crystalline substance, the molecular weight is 385.8 and the empirical formula is C17 H18FN3O3.HCL.H2O



The structure of Ciprofloxacin

2.2. Toxicant Solution Preparation

A stock solution was prepared by dissolving Ciprofloxacin in deionizer water and from each stock solution a measured amount of metal solution was taken and thoroughly mixed with distilled water to make it up to 100 microgram/ml solution from which the amount of the daily dosage was prepared

2.3. Animals

Thirty matures mice matched by age with mean body weight of 27.5 g, were obtained from Schistosome Biological Supply Center, Theodor Bilharz Research Institute.

2.4. Experimental Design

The thirty mice were divided randomly into three groups, Mice of the experimental group were given Ciprofloxacin ingestion; one group (10 mice) 0.5 mg/Kg/day of Ciprofloxacin and Two group 1 mg/kg/day of Ciprofloxacin for four weeks period. Animals of Third group (control group) were given an equal dosage of distilled water for the same period. They were housed in a quiet room, maintained at a temperature of about 24 $\pm$ 1^{o}C, with lighting on schedule of 12 hr light and 12 hr dark.

2.5. Blood Samples

At the end of the experiment two blood samples were obtained from each mouse, one collected in heparinized tubes for determination of hemoglobin, hematocrit, red blood cell (RBC) and white blood cell (WBC) counts. The other sample was collected in sterilized centrifuge tubes without anticoagulant for separation of sera for biochemical analysis (To obtain clear serum, the blood samples were left to clot at room temperature, then centrifuged for about 15 minutes at 3000 r.p.m).

2.6. Preparation of Liver Tissue Homogenates

Each the following enzyme parameter assay is 0.34g from each liver aluminium package was taken and homogenizes in 2.5 ml of the specific recorded solution to give 10% concentration and then used for assay. The homogenate was centrifuged for 5 minutes at 3000 x g at 4^{o}C and the supernatant was used for different enzyme assays

2.7. Analytical Procedures

Blood cell counts were estimated using an improved neubauer haemocytometer [7]. Haemoglobin was measured (packed cell volume) using the cyanmethaemoglobin method [8]. Hematocrit values (PCV) were read with a microhaematocrit reader to avoid the swelling of cells, which can occur in anaerobically stored mice blood; hematocrit was determined according to Soivivo, et al. [9].

2.8. Enzyme Assays Methods

The serum glucose concentration was determined according to Trinder [10] The total serum protein and albumin contents were determined according to Gasbarro, et al. [11]. The serum creatinine was measured according Henry [12].The serum uric acid was measured according to Barham and Trinder [13].The serum uric acid was measured using enzymatic determination according to Barham and Trinder [13]. The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to Retiman and Frankel [14]. Glycogen content of the liver was determined according to the method adopted by Seifter, et al. [15]. The serum alkaline phosphatase was determined according to alkaline phosphatase and acid phosphatase (AP) according to Wattiaux and De Duve [16].Lactic dehydrogenase (LDH) activity (nmol of pyruvate reduced/ min/mg protein) was determined according to Anon [17]. Pyruvatekinase (PK) activity was determined according to McManus and James [18]. phosphoenolpyruvate carboxy kinase (PEPCK) activity was measured according to the method of Suarez, et al. [19]. phosphofructokinase (PFK) activity was measured according to Zammit, et al. [20]. Fructose -1, 6-diphosphatase (F-1, 6-ase) [21]. Glucose phosphate isomerase (GPI) [22] and Glucose phosphate isomerase (GPI) [22]

2.9. Statistical Analysis

The data were analyzed using one-way ANOVA [23]. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ was considered statistically significant.

3. Results

Data in Table (1) concerning the effect of Ciprofloxacin when administration daily for 4 weeks on the blood haemogram parameters. The table shows that red blood cell (RBC) count and haemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacin. Similar changes were observed with the mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). White blood cell (WBC) count and the temporary hematocrit (PCV %) were significantly elevated in the two doses of Ciprofloxacin and remained elevated until the end of the experiment.

Table-1. Hematological characteristics of mice treated with Ciprofloxacin

Dose mg/kg/day	RBC x 10^6/*ul*	WBC x 10^3/*ul*	PCV%	Haemoglobin g/dl	MCH pg/cell	MCV	MCHC g/dl
0 (control)	5.2 ± 0.15	7.2 ± 0.11	32.1 ± 0.32	12.4 ± 0.23	27.4 ± 1.44	60.12 ± 3.13	53 ± 1.43
0.5	4.12 ± 0.12	8.2 ± 0.32	36.1 ± 0.43	10.15 ± 0.43	27.1 ± 1.5	72.3 ±3.4**	41.2 ± 1.12*
1	4.12 ± 0.13*	8.9 ± 0.13*	43.2 ± 0.41*	8.65± 0.13*	25.2 ± 1.1*	98.44 ±1.22***	30.2 ± 1.5***

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

**MCV = mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration.

Results presented in Table (2) indicate that the administration of Ciprofloxacin at 0.5 and 1 mg/kg/day into mice, caused significant elevation in serum aminotransferases (AST and ALT) enzymatic activity in treated mice. Ciprofloxacin administration resulted in significant change in other biochemical aspects in treated mice when compared with the untreated ones. Thus, there was significant decrease in serum total protein of treated mice when compared with control group. The results revealed also hyperglycemia in mice exposed to 0.5 and 1 mg/Kg/day of Ciprofloxacin. The liver glycogen recorded significant decrease in treated mice. Moreover, the present results showed significant rises in serum uric acid and creatinine in treated mice compared with control.

Table-2. Effect of Ciprofloxacin treatment on liver and kidney functions parameters in serum and liver of treated mice.

	Control	0.5 mg/Kg/day	%change	1 mg/Kg/day	% change
ALT (u/l)	17.12 ± 0.52	20.5 ± 0.3	-19.7	28.4 ± 0.22**	-56..9
AST (u/l)	13.21 ± 0.22	18.22 ± 0.42*	- 37.9	25.2 ± 0.21**	- 90.76
Glucose (mg/dl)	135.2 ± 0.78	184.2 ± 0.65*	- 34.8	206.1 ± 0.31*	- 52.4
Total protein (g/dl)	5.11 ± 0.91	3.43 ± 0.95*	- 32.9	2.5 ± 0.16**	+ 51.1
Creatinine (mg/dl)	2.05 ± 0.85	3.21 ± 0.73*	- 56. 6	4.1 ± 0.1**	- 100
Uric acid (mg/dl)	33.2 ± 0.43	37.2 ± 0.85	- 12	44.2 ± 0.65*	- 33.13.
Glycogen (in liver) (mg/mg tissue)	6.13 ± 0.21	4.13 ± 0.27*	- 32.6	2.22 ± 0.13**	+63.8

(+) reduction, (-) Increase , * $p<0.05$, ** $p<0.01$

The present results indicate that significant elevated level of glycolytic (PK,PFK and GPI) and gluconeogenic enzyme activities (F-1-6,D-Pase and PEPCK) and significant reduction in PK/PEPCK ratio with in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacin. A significant difference between doses of Ciprofloxacin and a significant effect of

the interaction between them were observed as well.PK/PEPCK ratio exhibited insignificant change was observed between 0.5 and 1mg/kg doses. The observed results (table 3) recorded significant decrease in LDH and G-6-Pase upon treatment with Ciprofloxacin for both doses. Higher doses showed the most potent effect on enzymes reduction.

Table-3. Effect of Ciprofloxacin on some glycolytic enzymes, gluconeogenic enzymes and hepatic marker enzymes in male mice liver.

Dose (mg)	Glycolytic enzmes (umole/mg protein/min.)			Gluconeogenic enzymes (umole/mg protein/min.)			Hepatic marker enzymes (umole /mg protein /min.)	
	PK	PFK	GPI	Faructose -1,6-diphos-phatase	PEPCK	PK/ PEPCK	Lactate dehydrogenase	Glucose -6-phosphatase
0 (control)	4.23±0.12	7.11 ^{A}±0.13	123.23 ±1.33	9.31 ±0.45	2.11 ±0.12	2.1 ±0.02	111.23±1.22	30.20 ±0.25
0.5	9.34±0.32	16.23 ^{E}±0.12	311.23 ±2.44	12.11 ±0.31	3.22 ±0.45	6.12±0.03	64.3 ±0.41	15.22 ±0.48
1	15.32 ±0.1	25.13 ^{F}±0.43	422.43±12.55	18.16 ±0.43	6.12 ±0.44	9.2±0.45	32.12 ±0.34	10.11±0.43

4. Discussion

The effect of Ciprofloxacin when administration daily for 4 weeks on the blood haemogram parameters showed that red blood cell (RBC) count and haemoglobin concentration were significantly decreased in mice exposed to 0.5 and 1 mg/kg of Ciprofloxacin. The reduction in RBC count may be due to macrocytic or normocytic anemia [24] and also the decrease is a common effect for toxicity with various types of pollutants on different animal species [25-28]. Similar changes were observed with the mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). These results agree with those obtained by Berberian and Enan [29] who studied the effects of some antimoulting compounds on the haematological picture in male rats and found significant reductions in Hb, RBC, MCH and MCHC, while increases of PCV and MCV were reported. The increase in the haematocrit values may be attributed to the significant decrease in RBC's and swelling of RBCs as revealed by the significant increase in MCV. The reduction in MCH indicated the mobilization of small poor haemoglobin erythrocytes from the erythropoietic organs into the circulation. White blood cell (WBC) count was significantly elevated in the two doses of cadmium and remained elevated until the end of the experiment and the temporary hematocrit (PCV%) increased. In this respect, these findings agree with those obtained by Guilhermino, et al. [30] who reported that when rats were exposed to cadmium the number of WBC's was increased significantly.

As regard to the enzymatic activities, the increase of AST and ALT activities could be explained by general tissue damage, particularly liver and kidney [31]. This increase could be regarded as a factor to increase the permeability and subsequent leakage of cellular enzyme [32]. The significant decrease in serum total protein in treated mice coincide with the results of Guilhermino, et al. [30].

Ciprofloxacin administration resulted in significant change in other biochemical aspects in treated mice when compared with the untreated ones. Thus, there was hyperglycemia in mice exposed to 0.5 and 1 mg/Kg/day of Ciprofloxacin. The liver glycogen recorded significant decrease in treated mice. The source of such hyperglycemia seems to be the liver glycogenolysis, resulting from the increase plasma catecholamines and corticosteroid hormones [33]. The present results showed significant rises in serum uric acid and creatinine in treated mice compared with control. Serum creatinine and uric acid can be used as a rough index of the glomerulus filtration rate [34]. High values of creatinine and uric acid indicate several disturbances in the kidney Ahmed and Gad [35]. The obtained results agree with that of Guilhermino, et al. [30] who reported that acute cadmium exposure caused increase in the levels of creatinine and urea in rodents.

The current investigation revealed significant enhancement of PK,PFK and GPI the rate limiting glycolytic enzymes, in liver of Ciprofloxacin treated mice compared to control group accompanied by a marked increase in the gluconeogensis ; PEPCK and F,1-6-diphosphtase. The increase in gluconeogenic enzymes may be also responsible for the production of glucose during drugtreatment [36]. Stimulation of PK in drug toxicity ascertained the enhancement of glycolytic flux previously reported by Ahmed and Gad [35]. Drug acts as a respiratory enzyme poison and causes respiratory distress in rainbow trout and Nile tilapia [37].

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