Plant Archives Volume 20 No. 2, 2020 pp. 8072-8076 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IMMUNE AND ANTIOXIDANT EFFECTS OF BROMELAIN WITH CIPROFLOXACIN IN BROILER CHICKS.

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Abstract

This study aimed to examine the possible protective effect of bromelain (BROM) on renal and hepatic toxicity induced by ciprofloxacin (CPX) 100 mg/kg in broiler chicks. CPX is a broad-spectrum antibiotic used for the treatment of many bacterial infections. One hundred and twenty (120) female broiler chicks Ross 308 at one day old were divided into four groups: first group (control group) (1 ml/kg Saline in drinking water) second group CYPX (100 mg/kg in drinking water), third and fourth groups were treated with basal diet; basal diet was supplemented with (CYPX. plus 20 mg/kg BROM) and (CYPX. plus 40mg/kg BROM) respectively. Body weights were significantly increased by BROM (20 mg/kg, 40mg/kg) respectively, the blood was collected killed after (21) days of the experiment. CYPX induced hepatic and renal toxicity was proved by a significant ($p<0.01$) reduction in the body weight and a significant ($p < 0.01$) increased serum glutamic oxaloacetic transaminase (GOT), serum glutamic-pyruvic transaminase (GPT), Total protein (TP), Serum creatinine (CR), Blood urea nitrogen (BUN), Urea (UR), Malonaldehyde enzyme (MAD). Also CYPX cause decrease in hematological parameters Protective hepatic and renal toxicity effect and oxidative damage caused by CYPX by improving significantly ($p < 0.01$) by increasing in body weight and decreasing GOT, GPT, TP, CR, BUN, UR, MAD also increasing hematological parameters in BROM (20 mg//kg) while, BROM (40 mg//kg) with less effect and presented significant ($P < 0.05$) rises in antibody titers against ND virus, while group G1 had less significant effects compared to the other groups These results assure that BROM antioxidant effects can protect CYPX-induced hepatic and renal toxicity with (20 mg//kg).

Key words: Bromelain, Ciprofloxacin, Anti-oxidant, Broiler chicks.

Introduction

CPX is a synthetic broad-spectrum antibacterial belonging to the class of quinolones. (Naeem *et al*., 2010). It well orally absorbed and the mainly antibacterial action by DNA gyrase inhibiting, a topoisomerase type II and IV enzymes fundamental to inhibiting cell division to separate bacterial DNA (Liu and wang, 1999). Generally, CPX is common antibiotics used for several lower respiratory tract types infections, joint, infectious diarrhea, skin, urinary tract and bone. Halawa, (2010) who recorded the CPX is generally well tolerated. The most known side effect occur in the central nervous system, hematology and gastrointestinal tract. CPX-related toxicities of the organ was increased recently (Adikwu and Brambaifa, 2012). Vary of adverse effect were showed in experimental studies after CPX such as chondrotoxicity (Li *et al*., 2004) and testicular structure and function damage (Demire *et al*., 2006). Renal side effects as crystal nephropathy were reported in some clinical cases after a high CPX dose (Markowitz and Perazella, 2005). Pineapple plant (Ananas comosus) is the origin of BROM that contains a mixture of non-enzymatic and proteolytic enzymes substances. Many native cultures were used of BROM as a folk remedy such as Hawaii and the Philippines. BROM has a beneficial effects in several conditions (Mahbubur *et al*., 2018). The importance of BROMs is evidence by a beneficial effect for treating of inflammation, pain resulted from surgery and trauma, swelling and bruising. BROM has fewer adverse effects than (NSAIDs) drugs (Ali *et al*., 2015; Erdaw *et al*., 2018). BROM contains, among other compounds, various closely related thiol endopeptidases, offer various fibrinolytic, antithrombotic and anti-inflammatory activities *in vitro* and *in vivo* (Taussig and Batkin, 1988).

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Materials and Methods

Drugs

BROM 100% Natural was purchased from Superior

Labs. Inc. San Diego USA. GNT, Gentaject 10% was purchased from KEPRO- HOLLAND. Ketaminej 10% inj. from KEPRO- HOLLAND. Xylazine, XYL-M2, VMD- Belgium.

Experimental animals

A total of 120 one-day-old female broilers Ross 308 was taken from a commercial hatchery for this study. The experiment was conducted during the period from 18 March to 13 April 2019 at the farm of Al-Qasim Green University, Babylon, Iraq. For housing, a cage system with an environmentally controlled room was used. We are monitoring the ventilation and lighting regiment continuously. Birds had access to water and feed ad libitum. Relative humidity was 50% during the period of the experiment. For the first three days, we maintained the room temperature at 34°C reduced gradually thereafter to 32°C, then maintained there for 21 days.

Experimental design

After a quarantine period of (21) days, the broiler chicks were divided into the four groups, each group consisting of (30) broiler chicks and CYPX. was given by drinking water at the dose of 100 mg/kg/day for (21) consecutive days. The dose of CYPX. was selected according to previous studies that demonstrated significant hepatic toxicity. (Freitas *et al.,* 2011), and they received the treatment as follows:

Group I: Control (1 ml/kg Saline in drinking water) for (21) days..

Group II: CYPX. (100 mg/kg in drinking water) for (21) days.

Group III: CYPX.+ BROM (20 mg/kg orally in food) for (21)days.

Group IV: CYPX.+ BROM (40 mg/kg orally in food) for (21) days.

Body weight

Body weight (BW) was measured weekly. We calculated the feed intake by measuring the weighed of feed supplied and the feed remaining in each pen. Birds were weighted weekly. Weight gain was measured by the difference between the initial weight and the final weight.

Table 1: Effect of Bromelain on the weight chicks /gram.

Traits	Control Mean ± SE	CYPX. Mean ± SE	CYPX.+BROM. 20 Mean ± SE	CYPX.+BROM. 40 Mean ± SE
Weight at 1 day (g/animal)	49.40 ± 1.28 A	50.41 ± 1.11 A	50 ± 1.57 A	49 ± 1.58 A
Weight at 35 day (g/animal)	1720.60 ± 0.81 A	1412.90 ± 0.33 C	1700.40 ± 1.50 A	1692.40 ± 1.07 B

Biochemical and hematology parameters

Birds were prohibited from food for 6 h at the end period of the experiment (week 3), then randomly selected per treatment group and collected 4 ml blood from the jugular vein. Sharpened knives were used for manual slaughter and birds were extracted for determining the yield of the carcass. Manually, we sharpened the beginning of knives and washed before slaughter and extracted for each bird. Hematology analyser (MH Medical Co., Ltd., Jilin, China) was used to measure the hematological parameters, Also the Blood samples were collected by in non- heparinized tubes, centrifuged at (4000) rpm for (10) minutes (Laessig *et al*., 1976). After separating the serum from the clot, using a sample, the samples were used to measurement of GOT), (GPT), (TP), (CR), (BUN), (SU) and MAD level concentration.

Statistical analysis

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

Results

Body weight

No deaths were observed in the groups of rats that were given CYPX. Administration of CPX in group II after (21) days produced significant decrease in the body weight compared to control ($P<0.01$). The animals were weighed before and after the experiments, whereas, treatment with BROM (20 mg/kg) produced significantly improved on the body weight ($P<0.01$) compared to CYPX control broiler chicks (Table 1).

Effect of BROM on CYPX. induced alterations in hepatic and renal function parameters

Effect of CYPX. Induced reduction in liver and kidney function in chicks. A significantly ($p<0.01$) increased in serum (GOT), (GPT), (TP), (CR), (BUN), (UR) levels compared to the control group and significant ($p < 0.01$) increase in (MDA) levels compared to control was observed after (21) days of treatment with CYPX. Whereas, treatment with BROM (20 mg/kg) prevented CYPX. induced increase in serum (GOT), (GPT), (STP), (CR), (BUN), (UR) levels ($p < 0.01$) and produced significant ($p < 0.01$) reduction on the (MDA) compared to CYPX. control broiler chicks. However, BROM

Table 2: Effect of Bromelain on CYPX. induced change in liver and kidney function of chicks.

Traits	Control Mean ± SE	CYPX. Mean ± SE	CYPX. + BROM. 20 Mean ± SE	CYPX. + BROM. 40 Mean ± SE
GPT (U/L)	48.276 ± 0.43 C	112.125 ± 0.68 A	46.038 ± 0.52 C	70.109 ± 0.50 B
GOT (U/L)	73.610 ± 0.59 C	191.103 ± 1.14 A	74.099 ± 0.64 C	118.744 ± 0.83 B
TP (mg/dl)	0.850 ± 0.09 C	1.534 ± 0.03 A	0.906 ± 0.08 C	1.111 ± 0.06 B
CR (mg/dl)	0.570 ± 0.09 C	3.004 ± 0.13 A	0.851 ± 0.02 C	1.220 ± 0.07 B
BUN (mg/dl)	16.132 ± 0.21 C	60.860 ± 0.43A	18.582 ± 0.31 C	38.646 ± 0.23 B
UR (mg/dl)	56.918 ± 0.85 C	67.966 ± 0.70 A	57.086 ± 0.30 C	59.150 ± 0.51 B
Malonaldehyde (nMole/L)	0.362 ± 0.07 C	3.920 ± 0.07 A	0.590 ± 0.01 C	2.304 ± 0.16 B

Table 3: Effect of bromelain CYPX. Induce change in hematology parameters.

Parameters	Control	CYPX.	CYPX.+ BROM. 20	CYPX.+ BROM. 40
R.B.C. (×106µL)	7.52 ± 0.02 A	4.54 ± 0.02 D	6.82 ± 0.02 C	6.35 ± 0.02 B
Hb. (g/dL)	13.62 ± 0.02 A	8.53 ± 0.009 C	11.19 ± 0.01 B	11.18 ± 0.01 B
Hte %	43.12 ± 0.11 A	21.80 ± 0.11 D	38.57 ± 0.13 B	36.73 ± 0.14 C

Table 4: Effect of bromelain CYPX. In antibody titre (log2) against NDv in all groups.

Age of Birds (days)	Control	CYPX.	CYPX.+ BROM. 20 mg	CYPX.+ BROM. 40 mg
20	4.36±0.22 A	4.01±0.50 A	3.95±0.38 A	3.99 ±0.39A
27	3.25±0.16 A	3.45±0.45 A	3.63±0.37 A	3.92±0.33 A
4	3.62±0.30 A	3.77±0.37 A	4.88±0.20 A	4.25±0.32 A
42	3.26±0.27 A	4.15±0.52 A	4.55±0.22 A	4.15±0.44 A

(40 mg/kg) it had less effect than, BROM (20 mg/kg) on body weight, serum (GOT), (GPT), (TP), (CR), (BUN), (UR) levels and (MDA) level significantly ($P<0.01$) compared to CYPX. control chicks (Table 2).

Immunological and Hematological parametrs

There were a significant ($p < 0.01$) differences in the mean values of hematological parameters between all groups in this experiment. The results revealed that both groups which treated with BROM. (20, 40mg/kg) respectively had significantly ($p<0.01$) higher hematological parameters values than the control and CYPX. groups while the second group which treated with CYPX. had significantly ($p<0.01$) lower hematological parameters values than all the other groups (Table 3), also there is no significant between groups in immune status (Table 4).

Discussions

Quinolones have a broad-spectrum bactericidal activity used to treat infections (Bedford and Schulze, 1998). Hepatic and renal toxicity caused by fluoroquinolones generally causes minimum symptoms, significant morbidity, acute liver failure or occasionally death has been reported (Nori *et al*., 2004). Liver and kidney injury correlated with CPX has been reported in experimental animals (Nordmann *et al*., 1981). Whereas, BROM (20 mg/kg) produced a significant effect on the body weight compared to CYPX. control chicks. However, BROM (40 mg/kg) not produce significant changes in body weight compared to CYPX. control chicks. In our study orally CPX (100 mg/kg) in chicks, produced a significant decrease in the body weight, when compared to control ($P<0.01$). The results are in acceptance with previous findings of (Agbafor *et al*., 2015) and controversy with (Erdem *et al*., 2000) noted that the, administration of CPX at a dose of 12.5 mgD kg.b.wt for 65 days (5 days/week), did not affect the body weight of the animals but caused an epididymis, weights of testes reduced significantly and seminal vesicles correlated to the control. In the existent study, the administration of CPX for (21) days produced a significant elevation of serum GOT, GPT, TP, CR, BUN and UR levels. These results are in acceptance with those obtained by other investigators (Faried *et al*., 2019), GOT, GPT, TP, CR, BUN and UR are a better parameter for detecting liver and kidney injury and largely used as most common biochemical markers to evaluate both organ injuries (Girish *et al*., 2009). The liver and kidney injury was induced by CPX due to free radical production, which initiates the process of oxidative stress and lipid peroxidation. This may lead to destroying the hepatocytes membranes causing release the cytosolic enzymes into the blood (Adikewu and Brambeifa, 2012). We found that increased levels of MDA by CPFX treatment, these results are in acceptance with those obtained by other investigators (Weyers *et al.,* 2002 and Faried *et al*., 2019), the stable metabolite of the free radical-mediated cascade of lipid oxidation is called MDA and widely used as lipids destruction and oxidative stress marker (Sahna *et al*., 2006). The oxidation of lipid causes destroys cell membranes and is thought to participate in tissue injury development (Parkes and Granger, 1988). Treatment with BROM for (21) days reduced the CYPX injured liver

and kidney at a dose of (20 mg/kg) produces a significant ($p < 0.01$) decreased in serum GOT, GPT, TP, CR, BUN, UR levels and significant ($p < 0.01$) decreased in (MDA) levels. Our results demonstrate the ameliorative effect BROM (20 mg/kg) on CYPX for (21) days induced liver and kid both organ toxicity in the chicks. This can be explained on the anti-inflammatory and analgesic activity effect of BROM activity is due to decreasing levels of bradykinin and prostaglandin E2 at sites of inflammation and cysteine that has antioxidant properties (Oh-Ishi *et al*., 1979). Also, considers an important precursor in the glutathione production, which protects cells from free radicals damages such as from CYPX induced oxidative stress (Piste, 2013). As for BROM (40 mg/kg), it had little effect. Based on the advance findings, it can deduce that, CYPX had adverse effects on the liver and kidney. BROM (20 mg/kg) administration showed a marked hepatic and renal protective activity. The protective effects of BROM (20 mg/kg) maybe due to its anti-inflammatory effects individually or antioxidant effects or synergistically.

The number of red blood cells is very low in CYPX treated chicks compared to the results of Boukerche *et al*., (2004), those authors have founded the number of red blood cells to be 8.45.106 / mm3 in healthy Wistar rats. The antibiotic effected the RBCs number, leading to anemia in the intoxicated animals. Indeed, liver dysfunction induces by CYPX and abnormal blood cells formation including red blood cells, platelets and leukocytes in intoxicated animals (Kurtovec and Rordan, 2003). In the group intoxicated with CYPX and treated with BROM (20mg/kg), there is remarkable of progressive regeneration cell in chicks. A similar result was observed by (Yi and Liu, 2011) in chicks intoxicated CYPX results were obtained by Viala, (1998), regarding hematocrit and hemoglobin. The platelets and white blood cells were decreased, especially in CYPX treated chicks. The toxic action of CYPX causes thrombocytopenia and leukopenia in the severe liver dysfunction cases, these results recorded by Moiling *et al*., (2006). The significant decrease of hemoglobin in chicks of CYPX, indicate a trend towards hypochromia, this lowered of blood cells was corrected significantly in chicks of BROM (20, 40 mg/kg) following the action in BROM. As a result, the BROM has a potential anti-oxidant and lowered (over 30%) oxidant stress (Szito *et al*., 2002). This property of BROM promotes its favorable action on the liver in regulating hematopoiesis, also there are no significant differences in antibody titre between all groups, there was no effect on antibody titres in birds after administration of therapeutic or double therapeutic dosages of ciprofloxacin (Niyogi *et al*., 2000).

Conclusion

The results of statistical analysis of hepatic and renal hormones of the four groups indicated that both bromelain (40 mg\kg) and (20 mg\kg) had antioxidant effects but the BROM (20 mg\kg) more effective as an antioxidant effect can protect CYPX-induced hepatic and renal toxicity in broiler chicks.

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