***Original Research Article***

# NEUROTOXIC EFFECT OF CIPROFLOXACIN ON ALBINO RAT

**Sayed M Rawi[1]*, Nasser M. Alshibly[1] and Fatema Seif El-Nasr[2]**

1. Department of Biology, Faculty of Sciences and Arts, Khulais King Abdulaziz University, Saudi Arabia
2. Zoology Department, Faculty of Science, Cairo University

## ABSTRACT

Flouroquinolones are one of the most commonly used antibacterial antibiotics for the treatment of various infections throughout several mechanisms. The CNS side effects, however, which is the molecular target organ is still not exactly known under therapeutic and higher dose exposure. Therefore, the objective of this study was to explore the neuronal biochemical effects of ciprofloxacin at different doses. Five groups of Sprague Dawley rats (150-170 g) were employed; one received 20 mg/kg/24 hours for 14 days and the others were received a single oral dose of either 50,100,200 or 300 mg/kg and decapitated post 24 hours. At the therapeutic dose level, ciprofloxacin was significantly lowered whole brain total protein, glutamate, GABA, glycine and alanine and increased aspartate, taurine, histidine and serine. At the same dose level, brain serotonin, AChE, $Na^+$, $K^+$-ATP-ase, plasma total protein and glucose were decreased. Reduced-GSH was decreased and oxidized-GSH was increased in the studied brain areas. Noradrenaline and dopamine were decreased only in cerebral cortex and increased in the hippocampus. At the higher tested doses, there was a vigorous similar pattern effect on the concentration of the whole-brain amino acids, and the higher potent effect was attained at 200 and 300 mg/kg dosing groups.

***Keywords*:** Ciprofloxacin, Neurotransmitters, Brain, Rat.

***Corresponding Author*: Sayed M Rawi**, Faculty of Sciences and Arts , Khulais, King Abdulaziz University, Saudi Arabia, P.O.Box 355 Khulais 21921; Tel:+966552072192;E-mail address: rawisayed@yahoo.com

## INTRODUCTION

In the current practices of anti-infective therapy, ciprofloxacin (Ciprofloxacin) is a very popular fluoroquinolone having a broad-spectrum of activity and diverse therapeutic prospects. In recent years, over 20 million outpatient prescriptions were written for ciprofloxacin. In USA, ciprofloxacin is the 35th most commonly used drug, and the 5$^{th}$ most commonly prescribed antibiotics [1].The available clinical evidence suggests the potential enhanced efficacy of this drug for the treatment of various communities acquired and nosocomial infections, e.g. urinary, biliary, respiratory, skin and neuronal pathogens [2].The pharmacological effects, however, results from inhibition of the enzymes topoisomerase II(DNA-gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, strand supercooling repair, and recombination [3].As compared to other quinolones, the pharmacokinetic profile of ciprofloxacin demonstrates equivalent or greater bioavailability, higher plasma concentrations,

and increased tissue penetration. According to Khan et al.[4], the absolute bioavailability of ciprofloxacin is approximately 70% with no substantial loss by first-pass metabolism. Furthermore, several investigators have postulated the abundant distribution of ciprofloxacin at different levels in the body organs, including, kidney, liver, skeletal muscle, pancreas, testes, cartilage, adipose tissue [5]. Moreover, at steady-state, there was a differential distribution for ciprofloxacin in brain tissue and cerebro-spinal fluid as reported by Nau et al.[6].

Consequent to the broad-spectrum, quinolones are associated with a variety of adverse events. There are several reports of biochemical, clinical and epidemiologic studies, which indicated that treatments with quinolones, including ciprofloxacin may cause serious liver failure [7] increased intracranial pressure [8], peripheral [9] and central neuropathy[10] .In addition, there has been a particular concern about convulsions that produced after exposure to different types of quinolones including, ciprofloxacin [11] .The mechanisms of toxicity, however, have been attributed to different causes, including, interactions with GABA-receptor [12], glycine-receptor and N- methyl-D-aspartate receptors [13].Another explanation has been attributed to the toxic effects of on the antioxidant's activity [14] and quinolone structural similarities to kynurenic acid and other compounds which are endogenous ligands of the glutamate receptor [15].

As previously outlined, there is a good evidence that quinolones exhibits unusual modes of toxic action in the CNS, thus, it appears questionable that what extent did the pharmacological effects of quinolones at distinctive dosages on the concentrations of different brain neurotransmitters like monoamines, excitatory and inhibitory amino acids in regard to the possibility of oxidative stress in the whole brain and selected brain areas of male albino rat. The hippocampus and cerebral cortex areas were therefore selected in order to obtain a more-reliable ranking with respect to the different chemical structures and to get more insight into the possible excitatory potency of a broad range of fluoroquinolones.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats (150-170 g) were obtained from the animal house of King Fahd Research center at King Abdulaziz University. Animals were maintained at atmospheric temperature (25±3°C) as well as normal daylight and dark. They were fed on a standard diet and water ad libitum. All animal procedures were approved by our Institute of Ethical Committee.

### Drug

Ciprofloxacin: manufactured by Bayer's health care pharmaceuticals as ciprofloxacin hydrochloride. It is a faintly yellowish to light yellow crystalline substance of molecular

Weight 385.8 and C17H18FN3O3•HCl•H2O empirical formula.

### Animal Grouping and tested doses

After two weeks of acclimatization to the laboratory environment, the animals of nearly similar weights were selected and divided into control and treated groups. The first was daily orally administered the therapeutic dose value (20 mg/kg) of ciprofloxacin as calculated according to FAD (Guidance for Industry and Reviewers) [16], divided into two subgroups and used to throw light the prolonged exposure effect to the therapeutic dose on the whole brain amino acids, and cerebral cortex and hippocampus brain catecholamine, AChE, reduced and oxidized glutathione

and $Na^+$-$K^+$-ATPase activities. For both treated and control subgroups, plasma was collected and used to elucidate total protein and glucose concentration changes. On the other hand, the animals of the 2nd, 3rd, 4th and 5th groups were administered either with 50, 100, 200 or 300 mg/kg of ciprofloxacin as a single dose, decapitated after 24 hours and used to elucidate brain free amino acids changes after exposure to acute single doses of ciprofloxacin. At all experimental groups, the control animals were administered 2 ml distilled water and processed in the same manner and circumstances conducted in treated groups. For all animals, the drug or distilled water was executed by the gastric intubation technique via the mouth, and the animals were fasted for 12 h before sampling. All animal procedures were approved by our Institute of Ethical Committee.

**Tissue Sample preparation**

At the end of the experimental periods (3 and 14 days), the animals of the 1st group were divided into two equal subgroups, the 1st was used only for the determination of whole-brain amino acids, and the brains of the second subgroup was dissected, cerebral cortex and hippocampus were removed carefully, each was divided equally, one was used for monoamine determination and the second for oxidized and reduced , glutathione, $Na^+$-$K^+$-ATPase and AChE activities in the cerebral cortex and hippocampus. For each experimental group six animals were sacrificed by decapitation, brains were quickly excised over ice-cold glass slides and quickly homogenized on 1/10 weight/volume 75% aqueous HPLC grade methanol. The homogenate was spun at 4000 rpm and used for amino acids determination by HPLC using the precolumn PTC derivatization technique as described by Heinrikson and Meredith [17]. In the same experimental group, whole blood was collected into a dry clean tube and centrifuged at 4000 rpm for 10 min. The collected plasma were used for determination of total protein content according to the method of Gornal et al. [18] and the plasma glucose level as described by Trinder [19].For the 3rd, 4th, 5th and 6th animal treated groups after scarification, brain was dissected in the same way and was, homogenized, centrifuged and was immediately used for the determination of whole brain free amino acids.

For dopamine, norepinephrine, serotonin, glutathione, AChE and Na+,K+-ATP-ase determination in cerebral cortex and hippocampus after prolonged exposure to the therapeutic dose level at the appropriate time periods, six animals were selected at the appropriate experimental time periods, brains were excised, cerebral cortex and hippocampus tissue samples were separated, weighed and homogenized in 20 mmol-phosphate buffer (PH 7.6), the homogenate was divided equally, one was used for monoamines determination according to the method of Pagel et al.[20] and reduced-GSH and oxidized-GSH as described by Jayatilleke and Shaw [21] by HPLC.The homogenate of the other half was used for determination of both AChE [22] and $Na^+$,$K^+$ATP-ase [23] .activities

**Statistical Analysis**

Data were presented as Mean±SE and analyzed statistically by a student's t-test using SPSS program version 16.

## RESULTS

**General Toxic observations**

At the higher tested doses, the animals displayed a noticeable neurotoxicological change as excitation, convulsions, and disturbances of the locomotors activity.

**Effect on the Brain-Free amino acids**

Data presented in Figs. (1-3) showed durational time effect of ciprofloxacin at the therapeutic dose level on the whole brain amino acid concentration. The data showed a significant decrease of glutamate, GABA, glycine and alanine and increased aspartate, taurine, histidine and serine concentrations. Moreover, the acute treatment with ciprofloxacin at different dose levels, successively, induced the previously mentioned pattern effect (Table 1). At the higher tested dose level, respectively; glutamate, GABA, glycine and alanine were decreased by -25.09%, -19.04%, -53.13%, and-31.51% and aspartate, taurine, histidine and serine were increased by 36.62%, 42.70%, 109.80% and 31.70%, respectively.

**Table1: Amino acids concentration (µmole/g fresh tissue) in the whole brain of male albino rat after treatments with different doses of ciprofloxacin.**

| Amino acid | Control | dose (mg/kg) | | | |
|---|---|---|---|---|---|
| | | 50 | 100 | 200 | 300 |
| **Glutamate** | 9.14±0.56 | 8.96±0.46 | 8.72±0.30 | 7.10±0.38*** | 7.02±0.58*** |
| **Aspartate** | 3.50±0.15 | 3.98±0.12*** | 4.17±0.19*** | 4.51±0.19*** | 4.54±0.14*** |
| **Taurine** | 5.68±0.24 | 5.89±0.37 | 6.32±0.38** | 7.12±0.33*** | 7.81±0.27*** |
| **GABA** | 2.41±0.16 | 2.28±0.21 | 2.19±0.23 | 1.98±0.23** | 1.97±0.18** |
| **Glycine** | 1.17±0.06 | 1.22±0.14 | 1.13±0.03 | 0.99±0.09** | 0.56±0.02*** |
| **Alanine** | 0.57±0.02 | 0.51±0.02** | 0.50±0.03*** | 0.46±0.03*** | 0.42±0.03*** |
| **Serine** | 1.26±0.06 | 1.33±0.09 | 1.51±0.13** | 1.52±0.10*** | 1.56±0.16** |
| **Histidine** | 0.13±0.02 | 0.18±0.02*** | 0.21±0.01*** | 0.21±0.02*** | 0.22±0.01*** |

Each value is mean ±S.E.M. of six animals

* P≤ 0.05 significant;**P≤ 0.01 highly significant;***≤ 0.01 more highly significant

**Effect on Total Protein, Glucose, Glutathione and Enzymes Bioassay**

At the lower tested dose level, sera total protein (Fig.4) and glucose (Fig. 5) contents were decreased significantly by -26.9% ($p<0.001$) and -16.11($P<0.05$), respectively. Also, the administered dose level investigated a significant decreased effect on the activities of AChE (Fig.6) and $Na^+$,$K^+$-ATPase (Fig.7) as well as reduced-GSH (Fig.8) contents in both cerebral cortex and hippocampus. On the other hand, there was a significant increased effect in the concentration of oxidized-GSH in both areas, at the end of the tested period; it was increased by 20.655 and 16.306 in cerebral cortex and hippocampus.

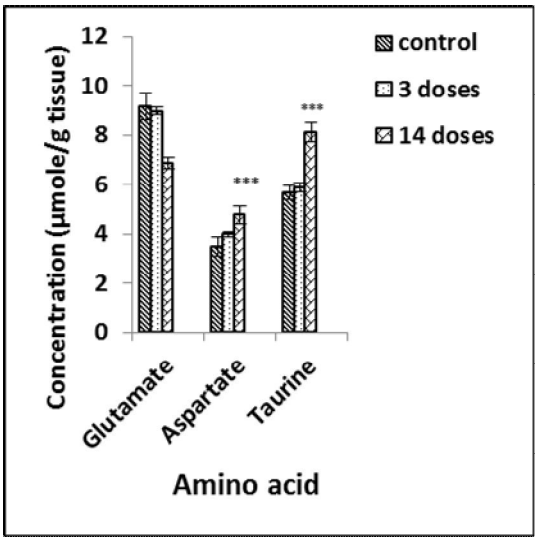


**Figure 1. Glutamate, aspartate and taurine concentrations in the whole brain of male albino rat after daily administration of ciprofloxacin at the dose level 20 mg/kg. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

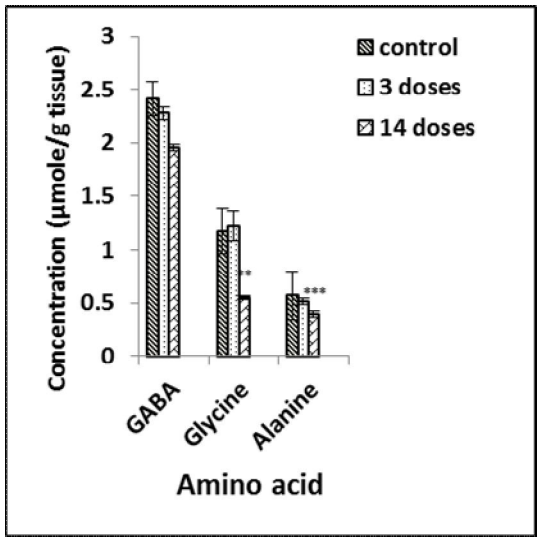


**Figure 2. GABA, glycine and alanine concentrations in the whole brain of male albino rat after daily administration of ciprofloxacin at the dose level 20 mg/kg. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

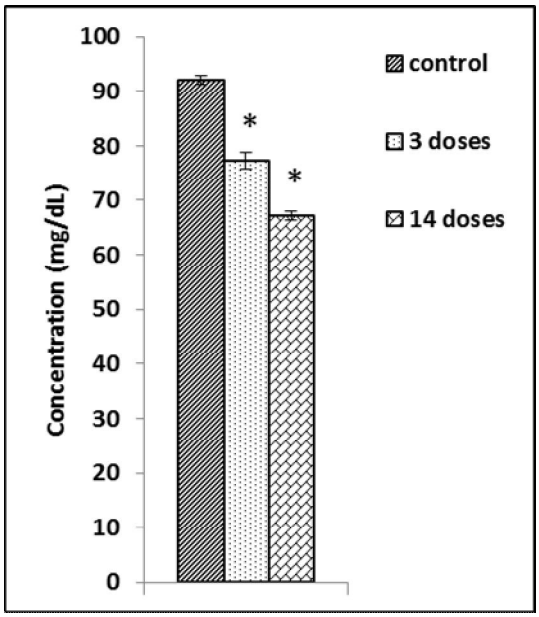


**Figure 5. Serum glucose concentration of male albino rat after daily administration of ciprofloxacin at the dose level 20 mg/kg. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

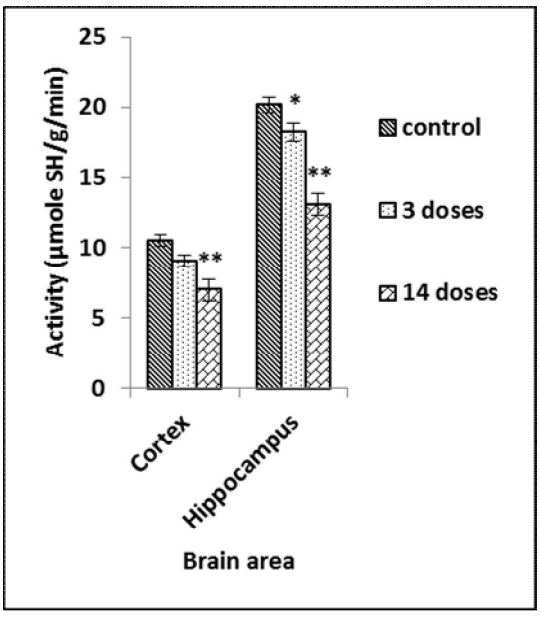


**Figure 6. Effect of ciprofloxacin at the dose level 20 mg/kg in AChE activity in brain cortex and hippocampus of albino rat. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

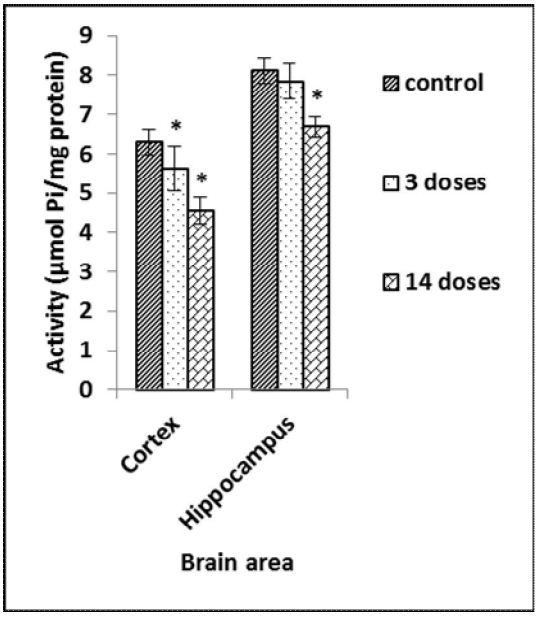


**Figure 7 .Effect of ciprofloxacin at different doses in $Na^+$, $K^+$-ATPase activity in brain cortex and hippocampus of albino rat. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

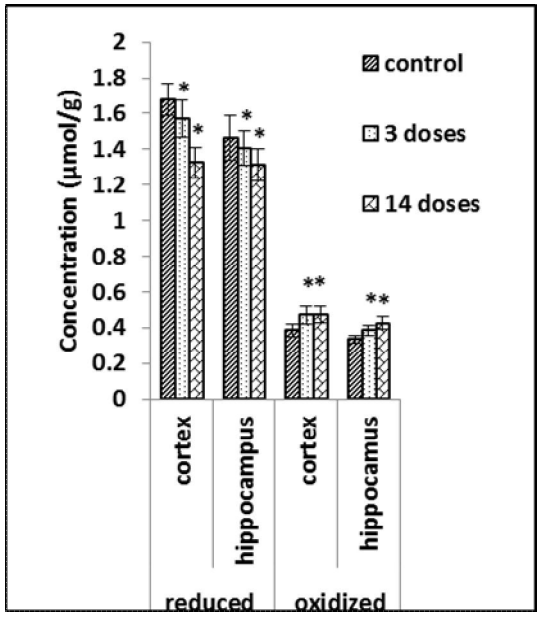


**Figure 8. Effect of ciprofloxacin at different doses in oxidized and reduced glutathione in brain cortex and hippocampus of albino rat. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

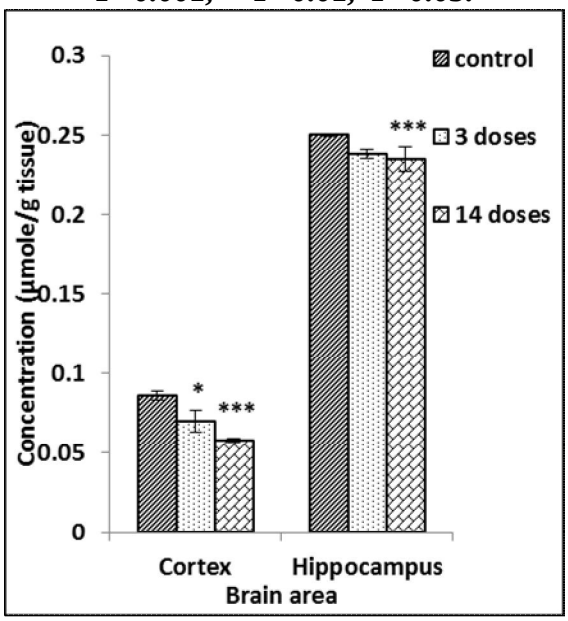


**Figure 9. Effect of ciprofloxacin at different doses in the concentration of serotonin in brain cortex and hippocampus of albino rat. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

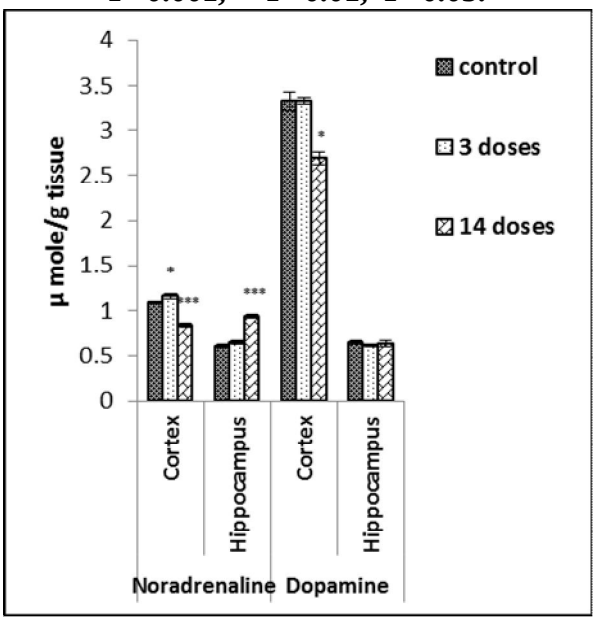


**Figure 10. Effect of ciprofloxacin at different doses in the concentration of adrenaline and dopamine of the whole brain cortex and hippocampus of albino rat. Each bar represents the mean and veridical lines above denote the S.E. of six animals*** P<0.001, **P<0.01,*P<0.05.**

### Effect on Monoamines

In rats that treated with ciprofloxacin, there was a general decreased effect in the concentration of serotonin in the cerebral cortex and hippocampus (Fig.9). On the other hand, as regarded to dopamine and noradrenaline contents in the studied brain areas (Fig.10), the data recorded showed highly versatile output according to the type of brain area and duration of exposure. The finding that, after seven doses most of the data recorded being insignificantly affected but after fourteen days, while dopamine and noradrenaline were significantly decreased in the cortex, they were increased in the hippocampus

## DISCUSSION

The wide-spread use of ciprofloxacin in medicinal treatments increases the risk of its exposure. There are many concerns that exposure to ciprofloxacin causing serious failures including irreversible peripheral neuropathy [24] and epileptogenic activity [25]. In recent years extensive in vivo and in vitro experiments have been performed in an attempt to explain the neurotoxic and neurotransmitters molecular target effect of quinolones observed under therapeutic and acute exposure. In the instant study 4% of cases, of the treated rats being hyper excitable and suffering from diarrhea, and the single dose level 300 mg/kg induced 16 % mortality rate of rats. These findings were quite similar to the observations previously presented by some other investigators [11].and may be explained by the suggestion that ciprofloxacin at the higher tested doses may have intracellular vigorous disturbances and mortality toxic effect As reported by Nagai et al.[26], the lethal toxic single doses of T-3762, "a quinolone" was between 260 and 391 mg/kg for rat when administered intravenously. According to the same author in survived animals, there was a decrease in locomotors activity, irregular respiration, staggering gait, tonic convulsion and the animals were dying within about 90 minutes after dosing. The present study also showed a hyper excitability which was done along with the significant decrease in AChE activity, which plays an important role in neurotransmission and Na+,K+- ATPase which regulates sodium and potassium ions across the plasma membrane. The decreased effect of both enzymes had been hypothesized to be involved in various neurological disorders that lead to behavioral abnormalities. In addition, several lines of evidence indicated that, excitation of CNS tissue by pharmacological agents like quinolones can be induced by many physiological factors like neuronal voltage-dependent activation and inactivation of Na+ channels [27] and Ca++ channel [28]. Furthermore, according to Ahmed and Simmons [29], quinolones affect neuromuscular transmission both pre-synaptically and post-synaptically and at the presynaptic level, there is a reduction of acetylcholine release due to blockade of voltage dependent sodium channels. The recorded data, however, agree well with the present findings. At the lower tested dose level, the consecutive doses of 20 mg/ kg for 14 days decreased AChE activity by -33.99 % to -35.96 in cerebral cortex and hippocampus, respectively. By this inactivation, the animals are shifted to hyperpolarized potential by quinolones.

The present study has also revealed reduction in norepinephrine, dopamine and serotonin in the frontal cortex. In the hippocampus, there were an elevation in norepinepherine and dopamine and reduction of serotonin levels after exposure to ciprofloxacin at the therapeutic dose level. The data recorded may be a supplement to the studies of Agbaht et al. [25] and validated the seizure induction through the assumption about the pharmacological treatments that affect monoamine levels in the brain. Several lines of evidence indicate that quinolones are strong inhibitors for

MAO [30] and lowered brain monoamines concentration [31] in a dose-dependent manner. These effects may be attributed to the effect of the tested compound on chromaffin cells that may be decreased after ciprofloxacin treatment. Furthermore, quinolones can elicit vigorous autonomic and neuro-endocrine response due to an indirect action on the hypothalamic-pitutary axis as reported recently by Jeong et al.[32].

A second group of neurotransmitters that can be also interfered with ciprofloxacin mental disorders is the free amino acid neurotransmitters. The overall pattern effect of GABA, glycine and alanine concentration which is the major inhibitory amino acids was a general reduced effect. These findings are in accordance with Arafa et al.[33] that ciprofloxacin administration induced decreased brain GABA and glycine concentration. Blockage of GABA-Eric neurotransmission and antagonistic inhibition of GABA receptors are thought to be responsible [34]. In addition, GABA reduction can be mediated through GABA synthesis mediated inhibition and increased activity of glutamate decarboxylase as reported by De Sarro and De Sarro [12].

One of the concerns regarding glycine and alanine has been their dual role in the central nervous system (CNS). In the present study, both are significantly decreased. According to Chen et al. [35] glycine can exert its effect on both synaptic (NMDARs) and extrasynaptic receptors (GlyRs) via distinct binding sites. The results of Dimpfel et al. [36] have pointed to an involvement of the glycine site of the central NMDA receptor in the development of side effects by different quinolones. On the other hand, according to Wolfson and Hooper [37], elevations in alanine aminotransferase, and aspartate aminotransferase are most commonly reported after quinolones exposure. The degradation of glycine and alanine may be explained on the reverse of the synthetic pathways and or involves oxidative deamination of glycine by glycine dehydrogenase and alanine aminotransferase (ALT). According to Pidathala et al. [38] both enzymes are highly affected with quinolones.

Taurine concentration in brain tissue was recorded to increase in all treatments under investigation. Although there has been long standing controversy as whether taurine function as a neurotransmitter, or a neuromodulator, its effects on nervous tissue are well established. Taurine increase may have antioxidant effect. In this regard, it may react with the toxic agents like ciprofloxacin via the amino group to detoxify them; secondly, it may also exert a direct protective effect in preventing the ionic and water shifts that result in cellular damage.

Following ciprofloxacin administration, there was a vigorous increase in histidine concentration. Histidine and its precursor histamine have been reported to induce a number of typical pharmacological responses at several peripheral sites. It is also colonized with a number of substances like glutamate decarboxylase (GAD), GABA, and GABA-transaminases and immune-stimulators. Dalhoff and Shalit [39], have reported fluoroquinolones affect in-vivo cellular and humoral immunity by attenuating cytokine responses. However, increased brain histidine content encountered during our experiments might be attributed to the previously mentioned reasons.

The release of the excitatory amino acid aspartate and decrease of glutamate as recorded in the present study may be explained through the glucose homeostasis abnormalities associated with ciprofloxacin intoxication [40]. Electrophysiological experiments using hippocampal slices have demonstrated that when glucose concentration was reduced, stimulation of the Schaffer collaterals gave an aspartate-mediated NMDA response [41] and indicated a functional role of

aspartate released from excitatory nerve endings. During hypoglycemia, the excitatory and inhibitory nerve terminals released aspartate which activates NMDA receptors on synaptic and extrasynaptic sites. Moreover, as reported by Takahashi et al.[42],glutamate transporters in neurons and glia cells play may play a crucial role in controlling the extracellular glutamate concentration in the brain. During hypoxia or ischaemia, glutamate transporters can run backwards, releasing glutamate into the extracellular space, triggering the death of neurons and thus causing mental and physical handicap. In this concern the histopathological results which are recorded in our previous studied [31] showed highly swollen and hypercellularity of the examined neuronal cells comes in agreement with that recorded for aspartate and glutamate. On the other hand, as reported by many investigators, glutamate is adequate supply for other compounds, including amino acids, ornithine and proline, which have been affected by quinolones[43] Moreover, reduced serum total protein content encountered during the present study may give another interpretation of the change which seen in the studied amino acids. Theoretically, if protein synthesis is blocked, protein breakdown should occur and free amino acids throughout the body should arise. Furthermore, if the natural synthetic mechanisms are unimpaired, great increase or alteration in the concentration of the free amino acids were attained. Indeed, we could conclude the decrease in total protein content with ciprofloxacin administration as a result of blockage of protein synthesis due to interference with DNA and RNA replication and this will definitely be mirrored on the level of brain free amino acids.

## CONCLUSION

Quinolones medications have been implicated to produce serious neurotoxic adverse effects. The present study has demonstrated that ciprofloxacin at different doses alters whole brain and selected brain regions, excitatory and inhibitory amino acids, brain monoamines, serum total protein, serum glucose level, reduced and oxidized glutathione, AChE, and Na+, K+-ATPase, activities.

## GUIDELINES FOR ETHICAL PUBLICATIONS

Authors declare that this manuscript was consistent with the guidelines and principles of ethical committee in King Abdulaziz University Saudi Arabia.

## ACKNOWLEDGMENT

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, The authors, therefore, acknowledge with thanks DSR technical and financial support.

## REFERENCES

1. Gyawali, S; R. Shankar, A. Saha and L. Moha, 2009. Study of prescription of injectable drugs and intravenous fluids to inpatients in a teaching hospital in Western Nepal. Mcgill. J. Med, 12(1): 13-20.
2. John, A and M.D. Weigelt, 2007. Empiric treatment options in the management of complicated intra-abdominal infections. Cleveland Clinic. J. of Medicine, 74: S29.

3. Fisher, L.M and X.S. Pan, 2008. Methods to assay inhibitors of DNA gyrase and topoisomerase IV activities. Methods Mol. Med, 142: 11-23.
4. Khan, H and G. Mustafa, 2009. Bioavailability of ciprofloxacin tablets in humans and its correlation with the dissolution rates. Pak. J. pharm. Sci, 22(1): 329-334.
5. Ooie, T; T. Terasaki, H. Suzuki and Y. Sugiyama, 1997. Quantitative brain microdialysis study on the mechanism of quinolones distribution in the central nervous system. Drug Metab. Dispos, 25(7): 784-789.
6. Nau, R; F. Sörgel and H. Eiffert, 2010. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin. Microbiol. Rev, 23(4): 858–883.
7. Olsson, R; A. Danielsson, G. Järnerot, E. Lindström, L. Lööf, P. Rolny, B.O. Rydén,.C. Tysk and S. Wallerstedt, 1991. Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis. Gastroenterology, 100 (5): 1319-1323.
8. Naora, K; N. Ichikawa, H. Hirano and K. Iwamoto, 1999. Distribution of ciprofloxacin into the central nervous system in rats with acute renal or hepatic failure. J. Pharm. Pharmacol, 51(5): 609-616.
9. Cohen, J.S, 2001. Peripheral neuropathy associated with flouroquinolones. Ann. Pharmcother, 35(12):1540-1547.
10. Owens, Rc and .P.G. Jr, Ambrose. 2005. Antimicrobial safety: focus on fluoroquinolones. Clinical Infectious Diseases, 41(2): 144–157.
11. Oliphant, C.O and G.M. Green, 2002. Quinolones: a comprehensive review. Am. Fam. Physician, 65 (3): 455-465.
12. De Sarro, A and G. De Sarro, 2001. Adverse reactions to fluoroquinolones: An overview on mechanistic aspects. Curr. Med. Chem, 8 (4): 371–384.
13. Danysz, W and C.G. Parsons, 1998. Glycine and N-methyl-d-aspartate receptors: physiological significance and possible therapeutic applications. Pharmacol. Rev, 50 (4): 597-664.
14. Chae, S.C; J. Lee, U.N. Sang and S.U. Park, 2013. Recent studies on flavonoids and their antioxidant activities. Excli. Journal, 12: 225-230.
15. Williams, P.D and D.R. Helton, 1991. The proconvulsive activity of quinolone antibiotics in an animal model. Toxicol Lett, 58: 23-28.
16. Guidance for Industry and Reviewers, 2002. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. FDA, 1-26.
17. Heinrikson, R.L and S.C. Meredith, 1984. Amino acid analysis by reverse-phase high-performance liquid precolumn derivatization with phenylisothiocyanate. Anal. Biochem, 136(1): 65-74.
18. Gornal, A.G; G.S. Bardwil and M.M. David, 1947. Determination of serum proteins by mean of Biuret reactions. Biochem, 177: 751-766.
19. Trinder, P, 1969. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol. 22(2): 246.

20. Pagel, P; J. Blome and H.U. Wolf, 2000. High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. J. Chromatogr, 746 (2): 297–304.
21. Jayatilleke, E and S.A. Shaw, 1993. High-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. Anal. Biochem. 214(2): 452-457.
22. Gorun, V; I. Proinov, V. Baltescu, G. Balaban and O. Barzu, 1987. Modified Ellman procedure for assay of cholinesterase in crude enzymatic preparation. Anal. Biochem, 86: 324-326.
23. Bowler, K and R. Tirr, 1974. The temperature characteristics of synaptic membrane ATPases from immature and adult rat brain J. Neurochem, 23(3): 611-613.
24. Eric, V; M.D. Granowitz, B. Richard and M.D. Brown , 2008. Antibiotic adverse reactions and drug interactions. Crit. Care Clin, 24: 421-442.
25. Agbaht, K; B. Bitik, S. Piskinpasa, M. Bayraktar and A. Topeli, 2009. Ciprofloxacin-associated seizures in a patient with underlying thyrotoxicosis: Case report and literature review. Int. J. Clin. Pharmacol. Ther, 47 (5): 303-310.
26. Nagai, A; T. Shibata, M. Nagasawa , N. Shiotani, M. Miyazaki,Y. Kawamura ,T. Kodama and T. Onimaru, 1998. Single dose intravenous toxicity studies of T-3762, a novel parenteral quinolone antimicrobial agent, in rats, dogs and monkeys. Jpn. J. Antibiot, 51(10): 583-599.
27. Thomas, D; B. Gut, G. Wendt-Nordahl and J. Kiehn, 2002. The antidepressant drug fluoxetine is an inhibitor of human Ether-A-Go-Go-Related Gene (HERG) Potassium Channels. JPET, 300(2): 543–548.
28. Eichenbaum, G; M.K. Pugsley , D.J. Gallacher , R. Towart ,G. McIntyre ,U. Shukla, J.M. Davenport, H.R. Lu, J. Rohrbacher and V. Hillsamer 2012. Role of mixed ion channel effects in the cardiovascular safety assessment of the novel anti-MRSA fluoroquinolone JNJ-Q2. Br. J. Pharmacol, 166(5): 1694-1707.
29. Ahmed, A and Z. Simmons. 2009. Drugs which may exacerbate or induce myasthenia gravis: a clinician's guide. The Internet Journal of Neurology, 10 (2).11.
30. Han, X.H; S.S. Hong, J.S.Hwang, M.K.Lee, B.Y.Hwang and J.S. Ro, 2007. Monoamine oxidase inhibitory components fromCayratia japonica. Arch. Pharmacal. Res, 30 (1): 13-17.
31. Rawi, S.M; I.M. Mourad, N.M.S. Arafa and N.I. Alazabi , 2011. Effect of ciprofloxacin and levofloxacin on some oxidative stress parameters in brain regions of male albino rats. AJPP, 5(16): 1888-1897.
32. Jeong, H.G; M.S. Lee, H.Y. Lee, Y.H. Ko, C. Han and S.H. Joe, 2012. Changes in sexual function and gonadal axis hormones after switching to aripiprazole in male schizophrenia patients: a prospective pilot study. Int. Clin. Psychopharmacol, 27(4): 177-183.
33. Arafa, N.M; M. Abdel-Rahman , M.F. El-Khadragy and R.B. Kassab, 2013. Evaluation of the possible epileptogenic activity of ciprofloxacin: the role of nigella sativa on amino acids neurotransmitters. Neurochem. Res, 38(1): 174-185.

34. Carbon, C, 2001. Comparison of side effects of levofloxacin versus other fluoroquinolones. Chemotherapy. 47:9-14.

35. Chen, R; S. Wang, W.Yao, J. Wang, J.I. Fang, J. Yan, S. Ren, Z. Chen, S. Liu and W. Lu, .2011. Role of glycine receptors in glycine-induced ltd in hippocampal ca1 pyramidal neurons. Neuropsychopharmacology, 36(9): 1948–1958.

36. Dimpfel, W.A; A. Dalhoff and E. von Keutz, 1996. In vitro modulation of hippocampal pyramidal cell response by quinolones: effects of HA 966 and gamma-hydroxybutyric acid. Antimicrob. Agents Chemother, 40 (11): 2573-2576.

37. Wolfson, J.S and D.C. Hooper, 1991. Overview of fluoroquinolone safety. Am. J. Med, 9(6A): 153S-161S.

38. Pidathala, C; R. Amewu, B. Pacorel, G.L. Nixon, P.W. Gibbons, W.D.Hong , S.C. Leung, N.G. Berry, R. Sharma, P.A.Stocks, A. Srivastava, A.E.Shone, C. Charoensutthivarakul, L. Taylor, O. Berger, A. Mbekeani, A. Hill, N.E. Fisher, A.J. Warman, G.A. Biagini, S.A. Ward and P.M. O'Neill, 2012. Identification, Design and biological evaluation of bisaryl quinolones targeting plasmodium falciparum Type II NADH: Quinone Oxidoreductase (PfNDH2). J. Med. Chem, 55(5): 1831-1843.

39. Dalhoff, A and I. Shalit, 2003. Immunomodulatory effects of quinolones. Lancet Infect. Dis, 3(6): 359-371.

40. Jose, J; B. Jimmy and K. Saravu, 2007. Dysglycemia associated with the use of fluoroquinolones-focus on gatifloxacin. J. Clin. Diagnostic Res, 3: 185-187.

41. Zappettini, S; M. Grilli, A. Salamone, E. Fedele and M. Marchi M. 2011 Pre-synaptic nicotinic receptors evoke endogenous glutamate and aspartate release from hippocampal synaptosomes by way of distinct coupling mechanisms. Br. J. Pharmacol, 161(5): 1161–1171.

42. Takahashi, M; B. Billups , D. Rossi, M Sarantis , M. Hamann and D. Attwell, 1997. The role of glutamate transporters in glutamate homeostasis in the brain. J. Exp. Biol, 200(2): 401-409.

43. Walters, J.D; F. Zhang and R.J. Nakkula, 1999. Mechanisms of fluoroquinolone transport by human neutrophils. Antimicrob. Agents Chemother, 43(11): 2710-2715