Relationship between Ciprofloxacin and blood glucose level, glucose transporters in adult male rats

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Abstract: The present study was designed to investigate the effect of oral administration of ciprofloxacin on blood glucose level and glucose transporters in adult male albino rats. The animals were divided into four equal groups (n= 10 per group). The first group, kept as control. The second group; hypoglycemic ciprofloxacin treated rats. The third group; diabetic ciprofloxacin treated rats. The fourth group; ciprofloxacin treated rats. The rats were sacrificed at the end of the experiment (7 days treatment with ciprofloxacin), serum was obtained for determination of blood glucose level, and tissues (brain, heart and kidney) were taken for determination of glucose transporters (GLUT 1, 2, 3, 4). The results revealed a significant increase in blood glucose in the second, third and fourth group compared with the control, while, the level of glucose transporters (GLUT 1, 2, 3, 4) was significantly lower compared with control. in conclusion: this study revealed that disturbed glucose transporters function may be the cause of the dysglycemic effects of ciprofloxacin. Moreover, patients with diabetes should be aware in the use of ciprofloxacin.
[Nora E Abdel-Hamaid and Mostafa, I. M. **Relationship between Ciprofloxacin and blood glucose level, glucose transporters in adult male rats.** *J Am Sci* 2015;11(3):132-137]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 18

Key words; ciprofloxacin, hypoglycemia, diabetes, glucose transporters

Introduction:

Ciprofloxacin is one of the few broad spectrum antimicrobials available in both intravenous and oral formulations (Davis et al., 1996). It belonged to the second generation of the family fluoroquinolones, which had a broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profils (Wolfson and Hooper, 1989). Four generation classification of quinolone drugs takes into account of the expanded antimicrobial spectrum of the newer fluoroquinolones and their clinical indication. First-generation drugs (e.g; nalidixic), achieve minimal serum levels. Second generation (e.g; ciprofloxacin), have increased gram-negative and systemic activity. Third generation drugs (e,g; lvofloxacin) have expanded activity against gram-positive bacteria and atypical pathogen. Fourth generation quinolone drugs (currently only trovafloxacin) which add a significant activity against anaerobes (Just, 1993).

The results of clinical trials with ciprofloxacin have confirmed its clinical efficacy, where it is effective in the treatment of a wide variety of infections, particularly those caused by Gram-negative pathogens, including; complicated urinary tract infections, sexually transmitted diseases (gonorrhea and chancroid), skin and bone infections, gastrointestinal infections caused by multiresistant organisms, lower respiratory tract infections (including those patients with cystic fibrosis), febrile neutropenia, intra-abdominal infections(which combined with an anti anaerobic agents) and malignant external otitis (Davis et al.,1996). Moreover, ciprofloxacin is considered the most potent fluoroquinolones against pseudomonas aeruinosa because of its penetration into bone (Stein and Ensberg, 1998).

On the other hand, the most common adverse effects of fluoroquinolones are nausea, vomiting and diarrhea which occur in 3-6% of recipients, central nervous system effects including; headache, confusion and dizziness (Stahlmann and Lode, 1999), photo toxicity (more common with sparfloxacin, cardiotoxicity and hepatotoxicity (Fitton,1992). In addition, hyperglycemia may occur with any fluoroquinolones (Mehlhorn and Brown, 2007).

Glucose is a vital source of energy for all life, the first step in glucose utilization is its uptake into the cell, which can be mediated by either the sodium-coupled glucose transporters (SGLTs, now known as the SLC5 family) or the facilitative glucose transporters (GLUTs, now known as the SLC2 family. The latter are used by most mammalian cell types to transfer various hexose molecules (Antonina and Kelle, 2011). the facilitative glucose transporters (GLUT family) has been classified into three sub classes; class I; GLUT1-GLUT4, class II; GLUT5, 7,9,11, class III; GLUT6, 8,10,12 (Bell et al., 1990).

GLUT1; is responsible for low level of basal glucose uptake required to sustain respiration in all cells, it is widely distributed in fetal tissues. In the adult, it is expressed at high levels in erythrocytes and also in the endothelial cells of barrier tissues as the

blood-brain barrier (Bell et al., 1990; Klepper et al., 2001).

GLUT2; is a facilitative bidirectional transporter, it is passively transport intracellular glucose and galactose across the basolateral membrane of the cells including; hepatocytes, pancreatic beta cells, renal tubular cells and intestinal epithelial cells by moving the carbohydrate molecules down its concentration gradient (Klepper and Leiondecker, 2007; Kellett et al., 2008).

GLUT3; (neuronal GLUT), which expressed mostly in neurons, furthermore it has been studied in other glucose requirements including; sperm, preimplantation embryos, circulating white blood cells and carcinoma cell lines (Simpson et al., 2008).

GLUT4; is the only member that is regulated by insulin and it is found in adipose tissues, striated muscle (skeletal and cardiac muscles) (James et al., 1988; Herman and Kahn, 2006).

The present study was designed to study the effect of ciprofloxacin (under therapeutic level) on blood glucose level and glucose transporters which may explain a cause for the different side effects of ciprofloxacin.

2. Materials & Methods

Animals;

This study was carried out on adult male albino rats, weighing (200-250gm), obtained from the laboratory animal house, Faculty of Veterinary Medicine, Zagazig University**.** The animals were housed in stainless steel cages and were allowed to acclimate under the laboratory conditions two weeks before the beginning of the experiment. The animals were maintained on 12-hour light-dark cycle, room temperature ($25^{o}c \pm 1^{o}c$) and kept under hygienic conditions. Commercial pelleted diet and water was offered add-libitum throughout the experiment

Drugs;

1-Ciprofloxacin (cipro);

Ciprofloxacin hydrochloride manufactured by the Egyptian International Pharmaceutical Industries Company (EIPICO), 10^{th} of Ramadan city, Egypt. Tablets are synthetic broad spectrum antimicrobial agents for oral administration. It is a faint yellowish to light yellow crystalline substance, the molecular weight is 385.8 and the empirical formula is $C_{17}H_{18}FN_3O_3.HCL.H_2O$.

2- Streptozotocin; obtained from Sigma chemical company, Egypt.

Experimental design;

a- Induction of diabetes;

Diabetes was induced by intraperitoneal injection of Streptozotocin (70 mg/kg body weight) one week before the experiment (Camps et al., 1992).

b- Induction of hypoglycemia;

Hypoglycemia induced by fastening the animals 48 hours with access to water (Camps et al., 1992).

Before ciprofloxacin administration, blood samples were taken from the orbital plexus of veins by capillary microtubules and left to clot for separating serum for estimation of blood glucose level.

Rats were divided into four equal groups, each of ten rats. The first group was orally administered 2 ml distilled water daily and kept as control. The second group was hypoglycemic rats orally administered 80 mg / kg body weight ciprofloxacin dissolved in 2 ml distilled water daily for one week. The third group was diabetic rats orally administered 80 mg / kg body weight ciprofloxacin dissolved in 2 ml distilled water daily for one week. The fourth group was rats orally administered 80 mg / kg body weight ciprofloxacin dissolved in 2 ml distilled water daily for one week.

Blood sampling and serum collection;

After one week of ciprofloxacin treatment, the animals were sacrificed by cervical dislocation, blood was collected and left to clot for separating the serum after centrifugation at 3000 rpm for 15 minutes, and the serum samples were directly kept frozen at $-20^{o}C$ until used to estimate blood glucose level.

Preparation of tissue homogenates;

The tissues taken from the animals depends upon different types of GLUTs, where brain was used as a source for GLUT1 and GLUT3, heart used as a source for GLUT4 and kidney as a source for GLUT2 (Uldryl et al.,2001). 1oo mg tissue was rinsed with PBS, homogenized in 1 ml of PBS and stored overnight at $-20^{o}C$ two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 rpm at $2\text{-}8^{o}C$, the supernatant was removed and assayed immediately or stored at $-20^{o}C$ or $-80^{o}C$. Centrifuge the sample again after thawing before the assay and avoid repeated freeze-thaw cycles.

Determination of glucose level in serum;

The blood glucose level was estimated by using enzymatic glucose kit according to method of Trinder (1969). **Determination of glucose transporters (GLUT 1, 2, 3, 4);**

Was carried out by using ELISA kits for glucose transporters (GLUT 1, 2, 3, 4) Bioassay.

Principle of the assay;

This ELIZA kit uses Sandwich-ELIZA as the method. The micro ELIZA plate provided in this kit has been pre-coated with an antibody specific to (GLUT 1, 2, 3, 4). Standards or samples are then added to the appropriate micro-ELIZA plate wells and bound by the specific antibody, and then a biotinylated detection antibody specific for GLUTs and Avidin- Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and

incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain GLUT, biotinylated detection antibody and Avidin-HRP conjugate will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm ± 2 nm. The OD value is proportional to the concentration of GLUT. The concentration of the GLUT can be calculated by comparing the OD of the samples to the standard curve.

Statistical analysis;

Data was collected, arranged, summarized and then analyzed using the computer program; Statistical Package for Social Science (SPSS / PC+ 2001, version 16). The statistical method was ANOVA test (one way analysis of variance). The data expressed means ±SE.

All differences were considered as statistically different at ($p \leq 0.05$).

3. Results and Discussion

To confirm hypoglycemia and diabetes induced either by fasting or injection of Streptozotocin**, table (1) and figure (1)** showed the blood glucose level as compared with control.

Table (1): Blood glucose level in hypoglycemic, diabetic and control rats.

Group	Glucose level mg /dl
Control	109.15 ± 3.49^{b}
Hypoglycemic rats	75.15 ± 2.59^{c}
Diabetic rats	206.20 ± 6.61^{a}

Means within the same column carrying different superscripts are significant at ($P \leq 0.05$).

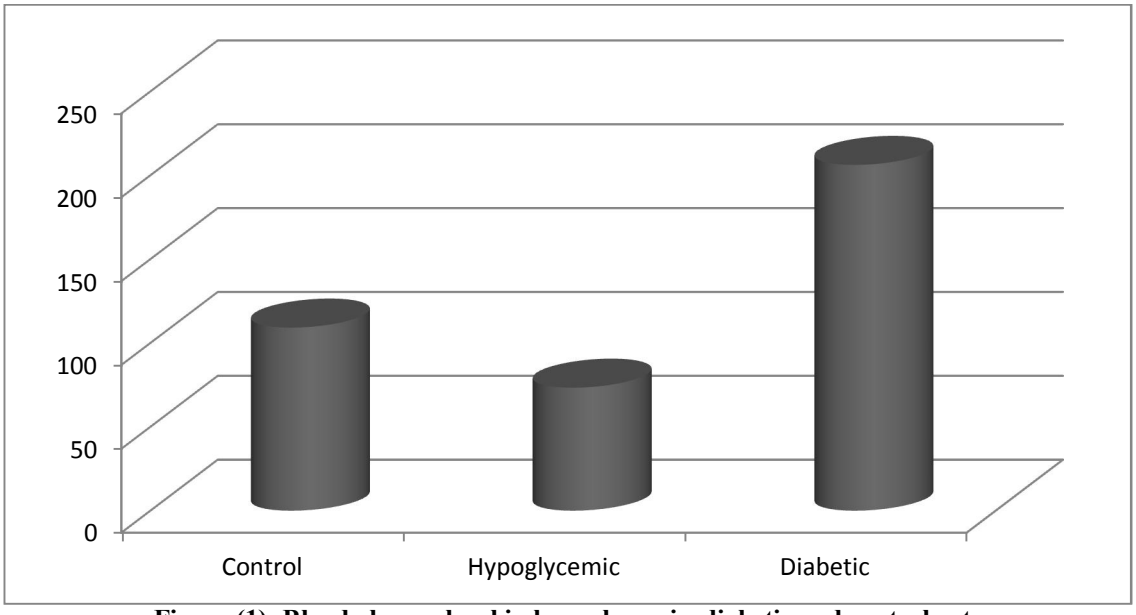

Figure (1): Blood glucose level in hypoglycemic, diabetic and control rats

It is obvious from table (1) and figure (1); that the blood glucose level showed a significant decrease in hypoglycemic rats (75.15 ± 2.59) and a significant increase in diabetic rats (206.20 ± 6.61) as compared with control (109.15 ± 3.49).

Table (2); Blood glucose level in hypoglycemic, diabetic and rats treated with ciprofloxacin (80 mg / kg body weight) daily for one week as compared with control.

Group	Blood glucose level mg / dl
control	109.15 ± 3.49^{d}
Hypoglycemic ciprofloxacin treated rats	140.56 ± 8.66^{c}
Diabetic ciprofloxacin treated rats	248.14 ± 6.62^{a}
Ciprofloxacin treated rats	188.55 ± 4.52^{b}

Means within the same column carrying different superscripts are significant at ($P \leq 0.05$).

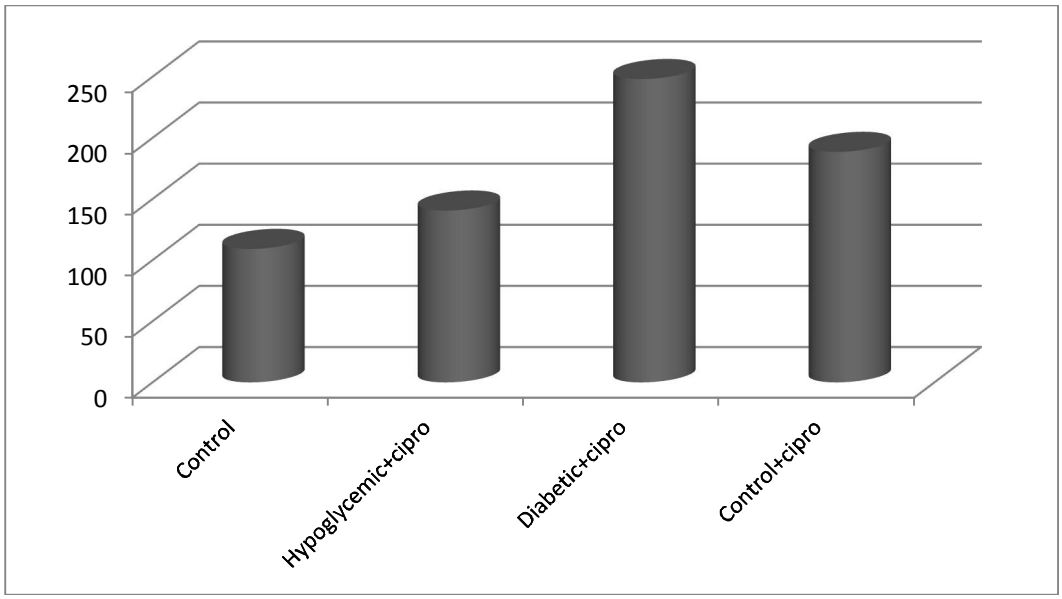

Figure (2); Blood glucose level in hypoglycemic, diabetic and rats treated with ciprofloxacin (80 mg / kg body weight) daily for one week as compared with control.

The prescribing information for quinolones (ciprofloxacin, levofloxacin and moxifloxacin) include information about altering serum glucose levels and suggest caution regarding their use in patients with diabetes (Mays and Stephen, 2011).

Regarding, the blood glucose level after the treatment with ciprofloxacin as shown in table (2) and figure (2), the current study revealed a significant increase ($p \leq 0.05$) in hypoglycemic, diabetic rats treated with ciprofloxacin (140.56 ± 8.66; 248.14 ± 6.62 respectively) and ciprofloxacin treated rats (188.55 ± 4.52) as compared with control (109.15± 3.49).

These results were in agreement with the previous finding of Mehlhorn and Brown, (2007) who reported that hyperglycemia might occur with any fluoroquinolone. Moreover, Allana and Dana, (2007) reported that ciprofloxacin might cause a serious changes in blood glucose levels especially in patients with diabetes, found that symptoms of hyperglycemia such as excessive thirst, frequent urination, increased hunger, blurred vision and dry skin and mouth occur with ciprofloxacin treatment which indicate high blood glucose level.

On the other hand, Tayama et al., (2007) demonstrated that, ciprofloxacin did not show a significant effect on serum glucose and suggest that the cytotoxic effects of ciprofloxacin and tacrolimus cause a decrease in insulin secretion, leading to glucose intolerance. Furthermore,, Allana and Dana, (2007) reported that ciprofloxacin might cause a serious changes in blood glucose level and found that symptoms of hypoglycemia such as headache, cold sweat, fast heart beat, irritability and confusion can also a companied ciprofloxacin treatment. It had been theorized that quinolones indirectly cause hypoglycemia through blockage of adenosine 5¯ triphosphate (ATP)-sensitive K channels in the pancreatic B-cells that regulate calcium influx that enhanced insulin release in a dose dependent manner (Maeda et al., 1996). Moreover, as quinolones are excreted primarily by the renal rout, patients with renal insufficiency may be at high risk for developing hypoglycemia if accumulation occurs particularly in the elderly (Greenberg et al., 2005).

Table (3): Glucose transporters concentration (ng / ml tissue extract) in hypoglycemic, diabetic and rats treated with ciprofloxacin (80 mg / kg body weight) daily for one week as compared with control.

Group	GLUT1	GLUT2	GLUT3	GLUT4
Control	7.83 ±0.35^{a}	25.45±1.08^{a}	1.51±0.09^{a}	29.81±0.56^{a}
Hypoglycemic ciprofloxacin treated rats	2.12± 0.14^{c}	3.41±0.13^{c}	1.08±0.07^{b}	8.06±0.18^{c}
Diabetic ciprofloxacin treated rats	^{c} 1.86 ± 0.03	2.47±0.14^{c}	1.22± 0.12^{b}	5.91±0.23^{d}
Ciprofloxacin treated rats	4.75± 0.05^{b}	16.22±0.51^{b}	1..03± 0.07^{b}	18.99±0.40^{b}

Means within the same column carrying different superscripts are significant at ($P \leq 0.05$).

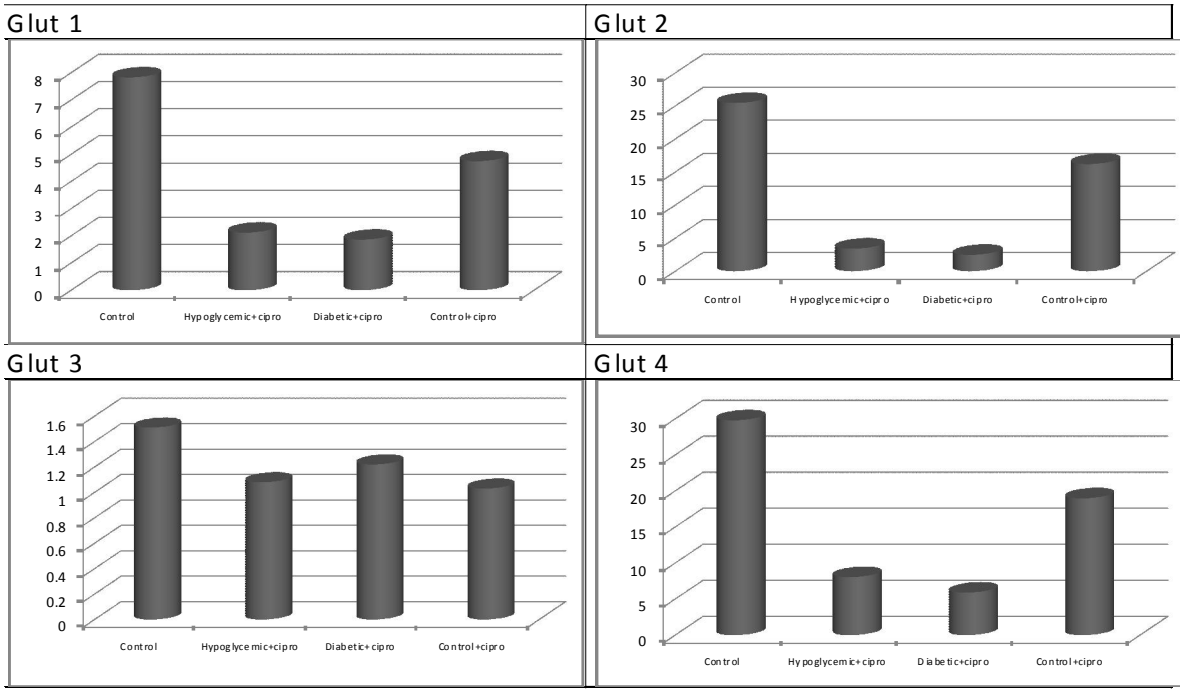

Figure (3); Glucose transporters concentration (ng / ml tissue extract) in hypoglycemic, diabetic and rats treated with ciprofloxacin (80 mg / kg body weight) daily for one week as compared with control

Regarding, the glucose transporters concentration as shown in table (3) and figure (3), this study showed a significant decrease. Similarly, Daniel et al., (2009) demonstrated that ciprofloxacin and levofloxacin disturb glucose transport into HePG2 cells and such inhibition is associated with inhibited glucose transporter type 1 (GLUT 1) function. Moreover GLUT 1protein expression and glucose uptake were significantly reduced.

In conclusion, this study revealed that disturbed glucose transporters function may be the cause of the dysglycemic effects of ciprofloxacin. Moreover, patients with diabetes should be aware in the use of ciprofloxacin.

References

1. Allana, J. M and Dana, A. B (2007): Safety concerns with fluoroquinolones. Annals of Pharmacotherapy 41(11): 1859-66.
2. Antonina, I. F and Kell, H. M (2011): Glucose transporters in the uterus; an analysis of tissue distribution and proposed physiological roles. Reproduction 142: 211-220.
3. Bell, G.; Kayano,T.; Buse, J; Burant, C; Takeda, J; Lin, D; Fukumoto, H and Seino, S (1990): molechular biology of mammalian glucose transporters. Diabetes Care 13 (3): 198-208.
4. Camps, M; Castello, A; Munoz, P; Monfar, M; Testar, X; Palacin, M and Zorzano, A (1992): Effect of diabetes and fasting on GLUT4 (muscle/ fat) glucose-transporter expression in insulin-sensitive tissues. Biochem J 282: 765-772.
5. Daniel, T. G; Pui, Y. P. L; Siu-kai, K and Yuan-Yuan, H (2009): Disturbance of cellular glucose transport by two prevalently used fluoroquinolone antibiotics ciprofloxacin and levofloxacin involves glucose transporter type 1. Toxicology Letters 184 (2): 81-84.
6. Davis, R.; Markham, A.; and Balfour, J. A. (1996): ciprofloxacin. An updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs. 51 (5): 1019-74.
7. Fitton, A (1992): the quinolones. An overview of their pharmacology. Clin Pharmacokinet 22 (suppl 1): 1-11.
8. Greenberg, A. I; Decerbo, M; and Fan, J (2005): Gatifloxacin therapy associated with hypoglycemia. Clin Infect Dis 40; 1210-1211.
9. Herman, M. A and Kahn, B. B (2006): Glucose transporter and sensing in the maintenance of glucose homeostasis and metabolic harmony. J Clin Invest 116:1767-75.

10. James, D. E; Brow, R.; Navarro, J. and Pilch, P. F (1988): Insulin-regulatable tissues express a unique insulin- sensitive glucose transport protein. Nature 333(6169):183-5.
11. Just, P. M. (1993): overview of the fluoroquinolones antibiotics. Pharmacotherapy. 13: 4s -17s.
12. Kellett, G. L; Brot-Laroche, E; Mace, Q. J and Leturque, A (2008): Sugar absorption in intestine : the role of Glut 2. Annu Rev Nutr 28-35.
13. Klepper, J and Leiondecker, B (2007): GLUT2 deficiency syndrome. Dev Med Child Neurol 49(9): 707-16.
14. Klepper, J; Willemsen, M; Verrips, A; Guertsen, E; Herrmann, R; Kutzick, C; Florcken, A and Voit, T (2001): Autosomal dominant transmission of GLUT1 deficiency. Hum. Mol Genet 10(1): 63-68.
15. Maeda, N; Tamagawa, T; Niki, I; Miura, H; Ozawa, K; Waranabe, G; Nonogaki, K; Hrmura, K and Iguch, A (1996): Increase in insulin release by rat pancreatic islets by quinolone antibiotics. Br J Pharmacol 117: 372-376.
16. Mays, H. V and Stephen, M. S (2011): Drug-induced glucose alterations paret 1: Drug-induced hypoglycemia. Diabetes Spectrum 24(3):171-177.
17. Mehlhorn, A. J and Brown, D. A (2007): safety concerns with fluoroquinolones. Ann Pharmacother 41: 1859-1866.
18. Simpson, I. A; Dwyer, D; Malide, D;Moley, K.H; Travis A. and Vannucci, S. J. (2008): The facilitative glucose transporter Glut 3! 20 years of distinction. Am J Physiol Endocrinol Metabo 295 (2): E242-53.
19. SPSS (2001): SPSS/PC+(2001) for the PC/XT-SPSS Inc.
20. Stahlmann, R and Lode, H (1999) : toxicity of quinolones. Drugs 58 (2):37-42
21. Stein, G. E and Ensberg, M. (1998): use of newer fluoroquinolones in the elderly. Clin Geriatr. 6 (8): 53-58.
22. Tayama, Y; Miyake, K.; Nagafuji, T.; Gouhara, T.; Morita, S; Arai, S.; Sato, E; Kitaura, T and Kihira, K. (2007): Influence of tacrolimus and ciprofloxacin on glucose metabolism. Yakugaku Zasshi 127(11) 1883:9
23. Trinder, P (1969): Enzymatic determination of glucose. Ann Clin Biochem, 6,24.
24. Uldryl, M; Ibberson, M; Horisberger, J.D; Chatton, J. Y; Riederer, B. M and Thorens, B (2001): Identification of mammalian H(+)myo-inositol transporter expressed predominantly in the brain. EBMBO J 20: 4467- 77.
25. Wolfson, J. S and Hooper, D. C. (1989): fluoroquinolone antimicrobial agents. Clin Microbiol Rev. 2; 378-424.

3/7/2015