Ciprofloxacin induced body weight and Serum Biochemical changes in rats and Anti-oxidant vitamin A, C and E as rescue agents

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Abstract

Ciprofloxacin is a 4 quinolone approved by the food and drug administration (FDA) in 1987 in oral formulation. This drug is indicated for the management of acute uncomplicated cystitis or uncomplicated pyelonephritis caused by *E.coli* and complicated Urinary Tract Infections (UTI) caused by a variety of pathogens (Schaeffer, 2002). In a series of tests evaluating the safety of ciprofloxacin in children, serum fluoride levels increased after 12 hours in 79% of the children, on day 7 and 24 hour urinary fluoride excretion was higher in 88.9% of children observed (Pradhan, 1995). The emergency of this "Antibiotic resistance" is a result of the over whelming use of antibiotics in human and veterinary medicine. High rates of fluroquinolone resistance have been reported in many countries (Cambau and Goodmann, 1993 and Denis and Moreau, 1993; Coronado *et al.*, 1995; Banerjee *et al.*, 1996). Mutations was correlated with resistance to quinolones (Cambau *et al.*, , 1994; Holf. 1994 and Alangaden *et al.*,). Since this fluroquinolone is used for prolonged periods it will be worth while to study its effect on serum biochemical changes in order to assess its toxic nature, if any, and the study is an attempt to investigate in detail **of total soluble proteins, of Blood Glucose, serum glutamate oxaloacetic transaminase (SGOT) and Serum GlutamatePyruvic Transaminase (SGPT)** also serve the purpose. To find out the level of the drug dosage at which it show its harmful / beneficial effects in the organs, two different doses were selected in accordance with the body weight of the experimental animal. The drug effects to the physiological system were found out by weighing the body weight of the animal. To verify if any rescue agents minimize / maximize the drug effect three excellent antioxidants vitamins, C, and E were given to animals of separate groups. To learn the recovery of the drug effect withdrawal group was maintained for each dose and durations. To observe the difference in drug effect for a short course and a long course, two different experimental periods were charted as short duration (7 days) and long duration (30 days). A control group was maintained to study the difference, variations and level of impact made by the drug in different experimental groups of rat. Ciprofloxacin alone and in co-administration with vitamin supplementations did not cause any marked changes in the body weight of rats at their dose and durational dependent administration. A dose dependent variations in serum biochemical parameters were seen after ciprofloxacin administration. Ciprofloxacin administration, though had no significant effect on the transaminases, protein and glucose in the short duration study it had significant effect when the drug was administered for a long duration.

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Introduction

Ciprofloxacin is widely distributed throughout the body through circulation. The volume of distribution of quinolones is high, with concentrations of quinolones in urine, kidney, lung, stool, bile, macrophages and neutrophil higher than serum levels. Studies have not been done in humans. Fluoroquinolones bring about their bactericidal action by selectively inhibiting the bacterial DNA synthesis, acting on the enzyme DNA gyrase. At concentrations above 25 mg/l, ciprofloxacin inhibit the production of the cytokines inter-leukin 1 (IL-1), IL-6 and tumour necrosis factor (TNF) by monocytes in response to endotoxin (Bailly *et al.,* 1991). At concentration above 50 mg/l, ciprofloxacin reduces extracellular IL-1 production (Shalit, 1991) but at a low concentration of 1-2.5 mg/l, ciprofloxacin increases IL-1 production (Rubinstein and Shalit, 1993). Currently, the most important benefit claimed for vitamins A, C and E is their role as antioxidants, which are scavengers of particles known as oxygen free radicals (or oxidants). Consuming 3-6mg of β carotene daily will maintain plasma beta-carotene blood levels in the range associated with a lower risk of chronic diseases (Institute of medicine, 2001). Vitamin C does not get stored in the body. Yeast is one of the richest sources of vitamin C (Chatwal, 1997). It strengthens and protect the immune system by stimulating the activity of antibodies and immune system cells (Masquelier, 1987). Vitamin E does not have a specific carrier protein in the blood stream. Over 90% of total body vitamin E is stored in the adipose tissue (Meydani, *et al.*, 1996 and Traber, 1999). The total protein concentration varies because of changes in the volume of plasma water or changes in the amount of individual proteins. Some drugs induce accumulation of protein in certain tissues as evidenced by Baraona *et al.*, (1977). The protein raise may be due to the excessive loss of body water due to the diuretic effect of caffeine (Robertson *et al.*, 1978). There are a number of drugs, which may influence the fasting level of glucose and glucose tolerance (Kennedy, 1951;Smith *et al.*, 1959; Bleiler and Sehedl, 1962). High levels of ALT in the blood stream mean that there may be liver inflammation and / or damage. The normal range of ALT / SGPT levels is between 5 IU/L to 60 IU/L (international units per litre). ALT is found only in the liver. High AST levels in the blood stream can be a sign of liver trouble. The normal range for AST/ SGOT levels in the blood stream are 5 IU/L to 43 IU/L. AST can be found in other organs besides the liver. Several workers have shown acetaminophen to cause a marked rise in serum levels of AST and ALT (Vijaya, 1996).

Methodology

Animals

Healthy, adult male albino rats of Wistar strain weighing 260-300 grams were used for the present investigation. The animals were housed in proper ventilated animal house with constant 12 ± 1 hour light and 12 ± 1 hour dark schedule. Experimental animals were provided with standard diet and clean drinking water *ad libitum.*

Experimental protocol

The animals were weighed and divided into three groups of five animals each.

Group I: Control:

The healthy rats were selected and treated as control and they received saline orally. A separate batch of five rats was maintained for vitamin supplementation groups and received gingelly oil orally.

Group II: Short duration

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

Group III: Long Duration

Here the animals were treated with ciprofloxacin at twelve hours interval for thirty consecutive days.

Group II and Group III were further sub-divided into six groups, each group consisting of five animals. The animals received the following regimen of treatment and all the treatments were designed on the basis of adult human dosage prescribed by the physicians and interpolated to the body weights of rats.

a. Low dose

The animals selected for short duration treatment were treated with ciprofloxacin at twelve hours interval for seven days.

b. High Dose

The animals received 400mg of ciprofloxacin / 60kg body weight as an oral dose.

c. High Dose + Vitamins A

The animals received 400mg. of ciprofloxacin followed by 7.5mg of vitamin A / 60kg body weight, as an oral dose.

d. High dose + Vitamin C

The animals received 400mg of ciprofloxacin followed by 500mg of vitamin C / 60kg body weight as an oral dose.

e. High dose + Vitamin E

The animals received 400mg of ciprofloxacin followed by 600mg of vitamin E / 60kg body weight, as an oral dose.

f. High dose withdrawal

The experimental animals received 400mg of ciprofloxacin as an oral dose and were allowed a withdrawal period of the drug for further seven days for short duration and one month for long duration. Suitable controls were maintained for each duration of

treatment. However, as there was no difference in any parameter among control group, a common control was employed in the present study.

Serum Biochemical parameters

1.Estimation of Total soluble proteins

The protein concentration of blood serum was estimated by the method of Lowry *et al.*, (1951).

Procedure:

For plotting the standard curve, a set of standards were run from 0.1, 0.2 0.3, 0.4, 0.5, 0.6, 0.7 to 1.0ml of standard solutions were taken in a series of test tubes. The volume in each test tube was made upto 1ml with distilled water. 5ml of alkaline copper reagent was added, mixed and then allowed to stand for 10 minutes at room temperature. 0.5ml of Folin – Ciocalteau phenol regent was then added to each tube and was shaken well. The blue colour developed was read at 720nm after 20 minutes against a reagent blank in a spectrophotometer. The standard graph was drawn by plotting the concentration of the standard solution on the Y – axis and the optical density on the X – axis.

For the estimation of blood protein 0.1ml of the serum was taken and made up to a final volume of 1ml with distilled water. The same procedure as described for the standard was followed. The amount of protein present in 0.1ml of the sample was calculated by referring to the standard curve obtained. The protein concentration was expressed as g/dL of serum.

2.Estimation of Blood Glucose

The blood glucose was estimated by the method of Ramnik Sood, (1994).

Procedure

Preparation of protein free blood filtrate

A test tube was taken and to that 0.1ml of blood and 9ml of 3.0% (w/v) trichloroacetic acid was added. It was mixed well and allowed to stand at least for 5 minutes. Then it was centrifuged for 10 minutes at 2500rpm.

Unknown

In a test tube 1.0ml of protein free blood filtrate was placed and 5.0ml O– toluidine reagent was added. It was mixed well by careful lateral shaking. The tube was capped with aluminum caps. It was then heated in a boiling water bath for 10 minutes, cooled in cold tap water for 4 minutes. This reading was taken in a colorimeter at a wavelength of 650nm.

Blank

1.0 ml of distilled water was taken in a tube in place of blood filtrate. 5.0ml of 0-toluidine reagent was added and heated in a boiling water bath for 10 minutes. Then it was cooled for 4 minutes and was transferred to a colorimeter tube for setting zero optical density.

Standard

1.0 ml of working standard solution was taken in a test tube instead of blood filtrate and the same procedure was followed as described above.

Calculation

$$\frac{\text{O.D of unknown}}{\text{O.D of standard}} \times 100 = \text{mg glucose \%}$$

3.Estimation of SerumGlutamateOxaloaceticTransaminase (SGOT) (EC.2.6.1.1)

The concentrations of serum glutamate oxaloacetic transaminase (SGOT) in the blood were estimated by the method of Reitman and Frankel (1957).

Procedure

Two-test tubes were marked as "T" and "B" corresponding to test and blank. 0.5 ml of buffer substrate was added in both the tubes and they were incubated at 37^0C for three minutes. 0.1ml of serum was added to the test tube "T" alone and shaken well. Both the tubes were incubated at 37^0C for 60 minutes. After 60 minutes, the tubes were removed from the incubator and 0.5ml of DNPH colour reagent was added and mixed well and allowed to stand at room temperature for 20 minutes to start the colour development. After 20 minutes, 0.1 ml of distilled water was added to blank alone and 5.0ml of working sodium hydroxide was added to both the test tubes, mixed well and allowed to stand at room temperature for 10 minutes. After 10 minutes the developed colour in both the test tubes was read at 505 nm against water blank. The enzyme activity was expressed as units/ml blood serum.

Calibration curve

Five test tubes were taken and numbered as 1, 2, 3, 4 and 5. To each test tube buffered substrate 0.5ml, 0.45ml, 0.40ml, 0.35ml, 0.30ml, respectively was added. Pyruvate standard was added in test tube 2, 3, 4, 5 as 0.05 ml, 0.10ml, 0.15 and 0.2ml excluding test tube 1. Now 0.10 ml distilled water was added to all the five test tubes followed by 0.5ml DNPH colour reagent. All the tubes were mixed well and allowed to stand at room temperature for 20 minutes.

Finally 5.0ml working sodium hydroxide solution was added mixed well and allowed to stand at room temperature for 10 minutes. The tubes from 2 to 5 were read against tube 1 (reagent blank) on spectrophotometer at 505nm. The graph was plotted with the absorbances of test tubes 2, 3, 4 and 5 on Y –axis versus corresponding enzyme activity on X – axis.

4.Estimation of Serum GlutamatePyruvic Transaminase (SGPT)(EC.2.6.1.2)

The concentration of serum glutamate pyruvic transaminase (SGPT) in the blood was estimated by the method of Reitman and Frankel (1957).

Procedure

The two test tubes were marked as 't' and 'b' corresponding to test and blank. 0.5ml of buffer substrate was added in both the tubes and incubated at 37^0C for three minutes. 0.1ml of serum was added to the test tube 't' alone and shaken well. Both the tubes were incubated at 37^0C for 30 minutes. After 30 minutes, the tubes were removed form the incubator and 0.5ml of DNPH colour reagent was added and mixed well and allowed to stand at room temperature for 20 minutes to start the colour development. After 20 minutes 0.1ml of distilled water was added to blank alone and 5.0

of working sodium hydroxide was added to both the test tubes. It was mixed well and allowed to stand at room temperature for 10 minutes and then both the tubes were read at 505nm against a water bath. The enzyme activity was expressed as units / ml blood serum.

Calibration Curve

Five test tubes were taken and numbered as 1, 2, 3, 4 and 5. To each test tube buffered substrate 0.5ml, 0.45ml, 0.40ml, 0.35ml and 0.30ml was added. Pyruvate standard was added in test tube 2, 3, 4, 5 as 0.05ml, 0.10ml, 0.15ml and 0.20ml excluding test tube. Now 0.10ml distilled water was added to all the five test tubes following 0.5ml DNPH colour reagent. All the tubes were mixed well and allowed to stand at room temperature for 20 minutes. Finally 5.0ml working sodium hydroxide solution was added and allowed to stand at room temperature for 10 minutes. The tubes 2 to 5 were read against tube 1 (reagent blank) on spectrophotometer at 505nm. The graph was plotted with the absorbance of test tubes 2, 3, 4 and 5 on Y – axis verse corresponding the enzyme activity on X- axis.

Result

Table-1. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on body weight of rats

Treatments		Body Weight (in grams)	
		Initial	Final
Group – I – Control		198.00±4.722^{c}	213.00 ± 5.466^{a}
Group - II - Short Duration			
1	*Low dose*	180.00±2.35^{**a}	199.00±2.84^{*a}
2	High dose	185.00±0.94^{*ab}	204.00±1.52^{a}
3	High dose + vitamin A supplementation	193.00±4.78^{bc}	202.00±1.7^{a}
4	High dose + vitamin C supplementation	191.00±1.7^{abc}	209.00±5.9^{a}
5	High dose + vitamin E supplementation	192.00±2.6^{abc}	208.00±0.9^{a}
6	High dose withdrawal	190.00±0.9^{abc}	202.00±1.2^{a}
Group - III - Long Duration			
1	Low dose	201.00±1.4^{c}	233.00±0.9^{**c}
2	High dose	189.00±1.8^{*a}	225.00±2.055^{*bc}
3	High dose + vitamin A supplementation	214.00±1.2^{**d}	24.00±1.2^{**d}
4	High dose + vitamin C supplementation	211.00±2.45^{**d}	245.00±1.8^{**d}
5	High dose + vitamin E supplementation	184.00±1.0^{**a}	215.00±0.9^{a}
6	High dose withdrawal	190.00±0.9^{ab}	222.00±0.9^{*ab}

Each value is the mean ± SE of five animals

* Control vs Treatment significant at 5% level by ANOVA

** Control vs Treatment significant at1% level by ANOVA

Mean± SE followed by a common letter are not significantly different at the 5% level by DMRT (a, b, c etc).

Effect on Body Weight (Table-1)

Ciprofloxacin administration had no significant effect on the body weight of the rats at the doses and duration of its treatments. Neither vitamins A, C and E supplementations nor withdrawal of the drug altered the body weights.

Effect on blood glucose(Figure- 13)

Both long and short durational drug treatment had a blood glucose lowering effect. Vitamins supplementations were more effective in the short duration group in partial restoration of this biochemical parameter. Withdrawal of the drug had positively affected the blood glucose level by raising it around to 74% to 76% irrespective of the duration of treatments.

Effect on serum total protein (Figure- 14)

Ciprofloxacin administration for a shorter duration nor vitamin supplementations to the high dose groups as well as withdrawal of the drug have no marked effect on the serum total protein concentrations. Unlike the short duration group a significant dose dependent increase in total protein concentrations was observed after ciprofloxacin administrations. Vitamins supplementations with A, C and E have no marked effect on the drug induced response except vitamin A, where a slight decrease (14%) was seen. Drug withdrawal also had no specific effect on these biochemical parameters.

Effect on serum transaminases (SGPT and SGOT) (Figure- 15a, 16a)

While the SGPT was elevated by a 10% to 12% after ciprofloxacin treatment, no change was observed in the SGOT enzymatic activity. Vitamin A administration to the high dose group has caused a decrease both of SGPT (26%) and SGOT (13%) respectively. Unlike vitamin A, vitamin C as well as vitamin E has no marked effect on the drug induced enzymatic changes. However drug withdrawal had raised the SGPT activity by 19% and SGOT by 15% respectively compared to the high dose groups.

Unlike the short-term treatments a varied response was observed in the ciprofloxacin treatment animals as far as the SGPT and SGOT enzymatic activities were observed. While SGPT activity showed an increase (20% - 30%) in its activity in a dose dependent manner, the SGOT enzymatic activity had shown a decline of the same magnitude (23% - 25%). Similar to the short duration groups both the SGOT (35%) and SGPT (27%) activity was lowered by vitamin A supplementation to the high dose drug treated groups, while vitamin C supplementations has no marked effect as in short duration groups. Vitamin E however, had raised the SGOT activity like short duration group, but not the SGPT activity. The drug withdrawal had raised markedly both the SGPT (63.5%) and SGOT (249%) activity, which is far higher than in short duration groups.

Figure-13. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on blood glucose concentration in rats

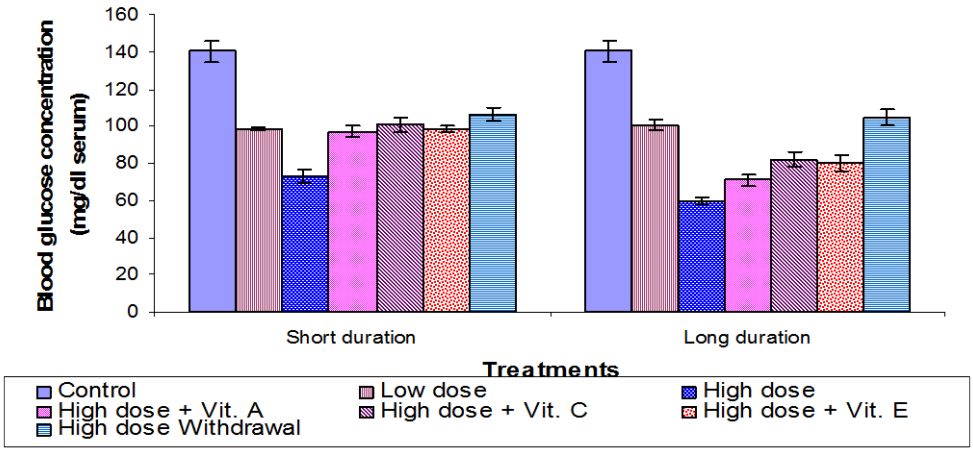


Figure-14. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on serum total protein concentration in rats

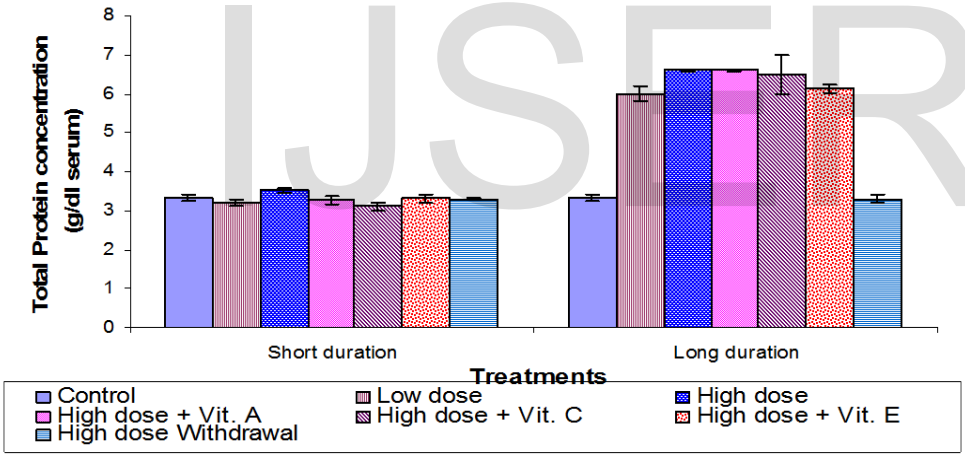


Figure-15a. Comparison of response of SGOT to ciprofloxacin

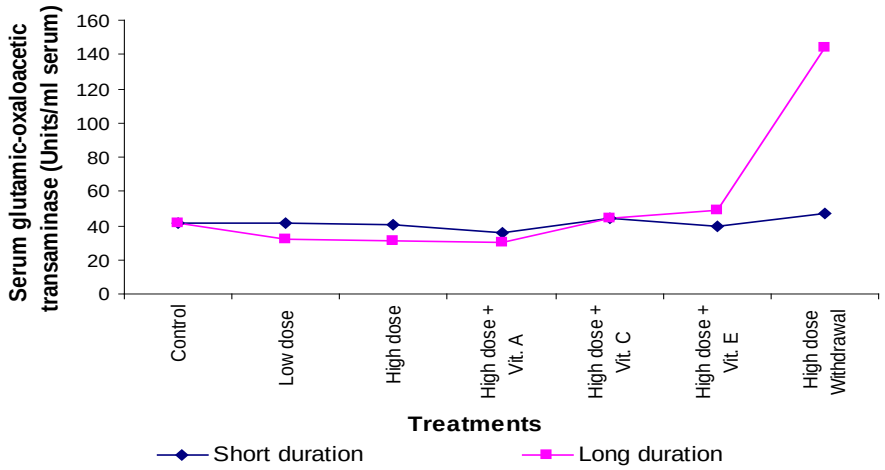
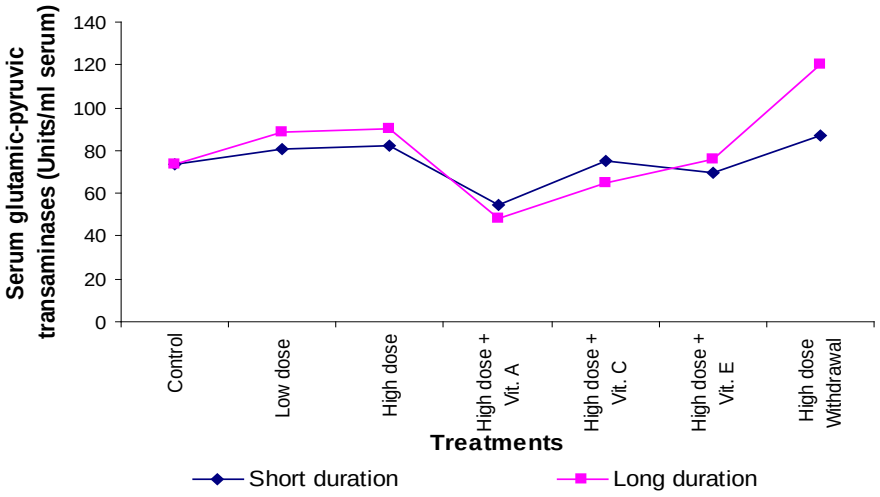


Figure-16a. Comparison of response of Serum SGPT concentration to ciprofloxacin



Each value is the mean ± SE of five animals
* Control Vs Treatment, Significant at 5% level by ANOVA
** Control Vs Treatment, Significant at 1% level by ANOVA
Means ± SE followed by a common letter are not significantly different at the 5% level by DMRT

Discussion

Effect on Body Weight

Ciprofloxacin kills a variety of bacteria both harmful and harmless types, and it is known to play on important role in toxicity of many organs (Friedman and Polifka, 2000). It is the inhibitor for the prostaglandin synthesis through the cyclo-oxygenase pathway, which is important for male reproductive function. . In view of that, the present study is focussed on the analysis of the effects of low and a high dose of ciprofloxacin for a short duration of 7 days and a long duration of 30 days. Drug withdrawal effects were seen in order to understand the permanent or transient effect of the drug. Further, vitamin A, C and E supplementations were studied so as to understand the antioxidant effects of these vitamins. The effects on body weight and serum parameters, like SGPT and SGOT, total protein, blood glucose were studied. The drug ciprofloxacin administration did not cause any significant alteration in the body weight of rats at the given doses used at shorter or longer durations. The food intake by the experimental animals was found to be absolutely normal and no significant change in the behaviour of the animals was observed. Vitamins A, C and E supplementations given with the drug and withdrawal of the drug also had no marked influence on the body weights of the rats. Similar findings were observed after methotrexate (Sampathraj, 1994) and diclofenac (Selvaraj, 2002), treatments in male rats. Exendin-4 reduced the weight of the experimental rats in a study conducted by Young *et al.*, (1999), while streptozotocin increased the body weight (Tourrrl *et al.*, 2001). The polybrominated diphenyl ethers (PBDE) did not exert significant changes in the body weight (Zhou *et al.*, 2001) similar to the present observation with ciprofloxacin. Vitamin E when

taken as a dietary dosage decreases the endogenous oxidative stress in healthy humans, who perform routine normal activities (Patrignani *et al.*, 2000). A study conducted in male rabbits with supplementing the anti – oxidants, vitamin C and E in their drinking water, was reported to increased food intake with no significant weight gain (Yousef *et al.*, 2003). In the present study, similar vitamin supplementations to the rats treated with high drug dosage did not cause any significant change in body weight. The antioxidant properties of these supplemented vitamins might have suppressed the consequent/adverse effect of ciprofloxacin.

Effect on Serum Biochemical Parameters

Effect on blood glucose

Glucose is the measure of sugar level in the blood. A number of drugs may influence the fasting level of glucose and glucose tolerance (Kennedy, 1951; Smith *et al.*, 1959 and Bleiler and Schedl, 1962). Cyfluthrin significantly reduced the blood glucose in rats (Federal Register, 2002). In the present study, reduction in glucose was observed when ciprofloxacin was administrated in a dose dependent manner. Withdrawal of the drug caused 74% - 76% recoveries in glucose. The glucose reduction might be due to increased utilization for energy process in order to overcome the stressful condition induced by the drug or due to decreased synthesis of glucose or due to the defect in cell secreting hormones. The antioxidant vitamins supplemented along with the drug partially restore glucose concentration (by 70% - 72% in short duration and by 14% to 30% in long duration). Sauberlich (1994) observed vitamin C supplementation resulted in improvement of glucose level. Supplementation with ascorbic acid may modulate insulin activity in diabetic patients (Harris, 1996) and improve the glycemic control and vascular health.

Effect on total protein

Total protein measures the amount of protein in the blood stream and some medications can interfere with the total protein level (www.atdn.org, 2002). Decrease in protein concentrations have been observed in many instances of liver damage due to various causes (Oscer, 1965). By excessive protein metabolism the protein may be lost through urine leading to hypoproteinaemia (Prakash and Arora, 1998). There were no significant changes observed in body weight, blood pressure, platelet count, fasting blood sugar, serum cholesterol, and total plasma protein level following administration of the injectable contraceptive norethisterone enanthate over the year and cyfluthrin significantly reduced the total protein in rats (FederalRegister, 2002). In the present study, the total protein concentration was raised by 45% - 50% in the treatment groups (drug alone and with vitamin supplementation) in long duration studies. The withdrawal of ciprofloxacin brought back to normalcy the protein levels. The antioxidant vitamins had no effective role in maintaining the protein concentration.

Effect on transaminases (SGPT and SGOT)

Hepatocytes are virtually the only cells with high ALT concentration, although kidney, heart and skeletal muscle contain moderate amounts. Hepatocytes contain three to four times more AST

than ALT (Widmann, 1984). The transaminases are enzymes that are involved in the transfer of an amino group from an ∝ - amino acid to an oxalo acid. SGOT (AST) is widely distributed with high concentrations in cytosol as well as mitochondria of heart, liver, skeletal muscle, kidney and erythrocytes whereas, SGPT (ALT) is present in high concentration in the cytosol of liver and to a lesser extent in skeletal muscle, kidney and heart (Zilva *et al.,* 1991). When liver cells are damaged or dying, ALT and AST leak into the blood stream. In the present study, the SGPT level in short duration treatment with ciprofloxacin brought about 10% – 12% increase in SGPT level in a dose dependent manner. Very high level of ALT suggests viral or severe drug induced hepatitis (QUEST 2001). In the vitamin supplemented groups, except vitamin A, vitamin C and E had no marked effect on the drug induced enzymatic changes. The drug withdrawal could effectively raise the enzyme activities, when compared to high dose drug treated rats. Thus from the present study, it was clear that the low and high dose of ciprofloxacin could exert a mild effect on SGPT an SGOT levels in the serum for a short period and it was high when given for a long duration. In fact transient elevation of serum aminotransferase have been observed with all fluoroquinolones. In the vast majority of the cases this alteration was self-limited and reversible and did not require withdrawal of the drug (Hoigne *et al.,* 1998). Vitamin A supplementation could improve the SGPT and SGOT activity only in short duration of ciprofloxacin treatment. But vitamin C and E restored the enzyme activity to more than normal. Ramnik Sood (1994) have shown that supplementation of vitamin A could cause the elevated levels of these enzymes.

CONCLUSION

Drug ciprofloxacin at the administered dosage had caused adverse effect on many of the haematological parameters as well as serum biochemical parameters, in a dose dependent manner. Ciprofloxacin administration, though had no significant effect on the transaminases, protein and glucose in the short duration study it had significant effect when the drug was administered for a long duration. Supplementations of vitamins have been seen to be effective in maintaining the drug-induced effect on various biochemical parameters. Durational dependent drug withdrawal could restore the drug effect only partially in very few parameters, whereas in many others, it was found inactive. Recommended doses of vitamin supplementations, especially for longer duration of drug administration is advisable for counteracting the ciprofloxacin induced toxicity and also a judicious use of the drug is safer.

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