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Ciprofloxacin induced body weight and haemotological changes in rats and anti-oxidant vitamin a, c and e as rescue agents

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ABSTRACT: *Prevention, diagnosis and treatment of diseases had led to the effective usage of drugs. Ciprofloxacin, a first generation fluoroquinolone was introduced recently, has broad antimicrobial activity. Vitamin A helps in part to regulate the immune system. Vitamin C strengthens and protect the immune system by stimulating the activity of antibodies and immune system cells (Masquelier, 1987). The antioxidant activity of α-tocopherol, which is the most active form of vitamin E in animals protect cell membranes against damaging effect of free radicals, this may contribute to the arrest of development of chronic diseases such as cancer (USDA, 2004), by enhancing immune function (Weitberg and Corvese, 1997). Antibiotics such as chloramphenicol brought a reversible suppression of erythrocyte formation (Keller and Follath, 1998). White blood cells are the cellular part of the immune system and are very important in surveying the body for infection. They find, trap, neutralize and kill invading pathogens. The Food and Drug Administration (FDA) had assured that ciprofloxacin is a powerful antibiotic taken only after consultation as it is associated with serious side effects. Since this fluroquinolone is used for prolonged periods it will be worth while to study its effect on hematological changes in order to assess its toxic nature, if any, and the study is an attempt to investigate in detail the complete blood count which comprises RBC count, WBC count, differential count and hemoglobin measurement. Packed cell volume and Red blood cell Indices 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 was 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). Hematology comprises RBC study on the oxygen carrier, WBC the cellular part of immune system and hemoglobin the oxygen transporter were analyzed. 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 reduction in red blood cell count was seen after ciprofloxacin administration. Vitamin A and E were more effective in preventing the drug induced adverse reaction than vitamin C at both the duration of treatments and withdrawal of the drug proved better in restoring the red blood counts.*

I. INTRODUCTION

Ciprofloxacin is widely distributed throughout the body through circulation.

The distribution phase half life of oral ciprofloxacin is 0.5 hours for 250 mg and 1.0 hour for 750 mg (Bergan *et al.,* 1987), that of intravenous ciprofloxacin is 0.18-0.29 hours. High concentrations of ciprofloxacin (150 mg/l) inhibit lymphocyte proliferation.

A three day course of intraperitonial ciprofloxacin given to mice inhibits IgG forming cells, delayed type of hypersensitivity responses and proliferation of lymphocytes in response to lipopolysaccharide or concanavalin A (Valera *et al.,* 1995). High concentrations of ciprofloxacin (150 mg/l) inhibit lymphocyte proliferation. Vitamins protect us against a variety of diseases such as heart disease, Alzheimer's diseases, respiratory diseases and infectious diseases by boosting our immunity. 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 A is an important nutrient in maintaining cell membrane integrity . Red blood cells are derived from precursor cells called stem cells. These stem cells are dependent on retinoids for normal differentiation into red blood cells. Additionally, vitamin A appears to facilitate the

mobilization of iron from storage sites to the developing red blood cells for incorporation into haemoglobin (Ross, 1999). It may activate neutrophils, increase production of lymphocytes and help to defend against infections. Red blood cells because of their high oxygen tension are particularly susceptible to oxidative damage and can be neutralized by vitamin E. Sickle cell anemia has been successfully treated with vitamin E (IBIS, 1998).

II. 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 was 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

Haematological Parameters

Haemoglobin estimation, Complete Blood Count (CBC) includes packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count and differential count.

1. Estimation of Haemoglobin

The haemoglobin concentration of blood was estimated by the method using Shali's haemoglobinometer (Samuel, 1986). The level of the fluid in the haemoglobin tube was read and expressed in gram percentage.

2. Red Blood Cell (RBC) Count

Erythrocytes / Red Blood Cells were counted by the method of Samuel (1986) using haemocytometer. The method involves an accurate dilution of a measured quantity of blood with a fluid which was isotonic with blood and which will prevent its coagulation. A dilution of 1:2000 was usually necessary. The dilute blood was

placed in a Neubauer counting chamber and the number of cells in a circumscribed volume was enumerated under a microscope.

$$\text{Number of erythrocytes} = \frac{\text{Number of cells counted x diluting factor x depth faces}}{\text{Area counted}}$$

Where

Number of cells counted	:	x
Depth of the square	:	1/10sq.mm
Area of small square	:	1/400sq.mm
Volume of the square	:	1/400 x 1/10
Dilution factor	:	200

III. WHITE BLOOD CELL (WBC) COUNT

Leukocytes / White Blood Cells were counted by the method of Samuel, (1986) using haemocytometer.Leukocytes were less numerous, a dilution of 1 in 20 was used and the diluent was usually the one which destroys the red blood corpuscles. The cells in the four corner blocks were counted.

$$\text{Number of leukocytes} = \frac{\text{Number of cells counted x Blood dilution x Chamber depth}}{\text{Area of chamber counted}}$$

Dilution	:	20
Area counted	:	4sq.mm
Depth	:	0.1mm

IV. DIFFERENTIAL COUNT

Differential count is to estimate the percentage distribution of white blood corpuscles in a blood smear (Merck Veterinary Manual, 1979). Clean slides, few drops of fresh blood samples, Leishman's stain, methanol, lancet, spirit, compound microscope. A drop of blood is kept on a slide. Touch the blood at one end of a slide, put the edge of other slide at a angle of 45^0 to touch blood. Move slide forward, so that a thin film of blood is spread on the slide. It was then observed under compound microscope, for different types of white blood corpuscles, like neutrophils, eosinophils, basophils, lymphocytes and monocytes.

5.Packed Cell Volume (PCV) (Merck Veterinary Manual, 1979)

The packed cell volume of blood is obtained by centrifuging a tube of blood and measuring the percentage of packed red blood cells (RBC's) in the total sample.

6. Mean Corpuscular Volume (MCV) (Merck Veterinary Manual, 1979)

The MCV denotes the average size of erythrocytes. The MCV is determined by multiplying the PCV (Packed cell volume) by 10 and dividing the product by the erythrocyte count per cubic millimeter

$$\text{MCV} = \frac{\text{PCV x 10}}{\text{RBC / cu mm}}$$

7. Mean Corpuscular Haemoglobin (MCH) (Merck Veterinary Manual, 1979)

The MCH denotes the average haemoglobin weight per red blood cell and is calculated by multiplying the haemoglobin in g/100 ml of blood by 10 and dividing the product by the erythrocyte count in millions per cubic millimeter.

$$\text{MCH} = \frac{\text{Hb (g/dL)}}{\text{RBC Count (1/L)}}$$

8.Mean Corpuscular Haemoglobin Concentration (MCHC) (Merck Veterinary Manual, 1979)

The MCHC is the average concentration of haemoglobin in red blood cells. The MCHC is determined by dividing the haemoglobin (Hb) in g/100 ml with the PCV and multiplying by 100. The result is a percentage

$$\text{MCHC} = \frac{\text{Hb (g/ml)}}{\text{PCV}} \times 100$$

Result

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.

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 red blood corpuscles (RBC) count (Table-2)

A dose dependent 34% to 27% reduction in red blood count was seen after ciprofloxacin administration for a short duration. Vitamins A and E were more effective (95% and 93% respectively) in preventing the drug induced adverse effect than vitamin C (79%). Withdrawal of drug could bring complete restoration of the RBC counts. Long duration of ciprofloxacin treatment simulated the short durational effect on RBC counts. But the percent reduction in RBC count was higher in low dose (31%) and high dose (49%) treatment groups. The vitamin A and E supplementations proved to be better in restoring the RBC counts than vitamin C and the withdrawal of drug restored this parameter to normalcy better than short duration group.

Table – 2. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on red blood cell counts of rats

Treatments		RBC Count (In millions / mm^3 of blood)			
		Short Duration	Percentage (%)	Long Duration	Percentage (%)
1	***Control***	**7.33±0.09**	**-**	**7.33±0.09**	**-**
2	**Low dose**	**5.22±0.1^{**f}**	**-29**	**4.85±0.1^{**e}**	**-34**
3	**High dose**	**4.81±0.1^{**g}**	**-9**	**3.87±0.06^{**f}**	**-47**
4	***High dose + vitamin A supplementation***	**6.82±0.07^{**c}**	**-19**	**6.97±0.02^{**b}**	**-5**
5	***High dose + vitamin C supplementation***	**5.96±0.001^{**e}**	**-7**	**6.07±0.09^{**d}**	**-17**
6	***High dose + vitamin E supplementation***	**6.66±0.1^{**d}**	**-34**	**6.82±0.05^{**c}**	**-7**
7	**High dose withdrawal**	**7.06±0.06^{b}**	**-29**	**7.28±0.07^{a}**	**-0.7**

Each value is the mean ± SE of five animals

** Control Vs Treatment significant at 1% level by ANOVA

Mean± SE followed by a common letter are not significantly different at the 5% level by DMRT (a, b, c etc).(Applicable for tables 2-8).

Effect on white blood corpuscles (WBC) counts (Table-3)

Like the RBC counts, the WBC counts were also decreased at both low dose (22%) and high dose (34%), respectively of ciprofloxacin treatment. All the three vitamins namely A, C and E could prevent the decrease caused by the high dose of the drug by 50%. However, drug withdrawal was effective in restoring these parameters to normalcy.About 23% to 29% reduction in WBC counts was observed after low and high doses of ciprofloxacin when given for a longer period. The vitamins A, C and E were less effective than short duration groups in preventing the high dose drug induced reduction of counts. Withdrawal of the drug was again effective in complete restoration of the WBC count to control level.

Table-3. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on white blood cell counts of rats

Treatment		WBC count (In Thousands / mm^3 of blood)			
		Short Duration	Percentage (%)	Long Duration	Percentage (%)
1	*Control*	8.88 ± 0.05^{a}	-	8.88 ± 0.05^{a}	-
2	*Low dose*	$6.4\pm0.17^{**d}$	-10	$6.2\pm0.2^{**d}$	-30
3	High dose	$5.13\pm0.2^{**e}$	-24	$5.5\pm0.2^{**c}$	-38
4	*High dose + vitamin A supplementation*	$6.81\pm0.03^{**c}$	-24	$6.2\pm0.07^{**d}$	-30
5	High dose + vitamin C supplementation	$6.71\pm0.04^{**c}$	-23	$6.5\pm0.04^{**c}$	-27
6	High dose + vitamin E supplementation	$6.97\pm0.03^{**c}$	-42	$6.3\pm0.4^{**cd}$	-29
7	High dose withdrawal	$7.98\pm0.04^{**b}$	-28	8.3 ± 0.2^{b}	-7

Effect on differential count - Effect on neutrophil counts (Table-4)

Only the high dose drug treatment induced 22% decrease in the neutrophil count at this duration of treatment. Supplementations with vitamins C and E were only partially effective in raising the neutrophil counts whereas, vitamins A effectively restored these parameters to normalcy. Similarly, drug withdrawal also effectively restored the neutrophil count. Unlike short duration group a dose dependent decrease in neutrophil count was seen in low dose (11%) and high dose (18%) drug treatment. All the three-vitamin supplementations could not prevent the drug induced further decrease in the neutrophil count. Drug withdrawal in these high dose groups was 50% effective in restoring the neutrophil counts to control values.

Effect on eosinophil counts (Table-5)

The eosinophil counts showed a decrease in both low (60%) and high (30%) dose ciprofloxacin treatment groups, while the vitamin supplementations effectively restored the eosinophil count to near normalcy, drug withdrawal could exert such an effect only by 87%.After the drug was given for a longer duration, ciprofloxacin caused a similar decrease in the eosinophil counts like the short duration group, only at low doses. However, the drug had raised the eosinophil counts when given at higher doses. The vitamin supplementations had maintained the drug-induced response, though to a lesser degree. Vitamin E was more effective than vitamin A and C. Drug withdrawal in these high dose groups was not able to prevent the decrease in this parameter.

Table –4. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on differential count -neutrophil counts of rats

Treatments		Neutrophil counts (In %)			
		Short Duration	Percentage (%)	Long Duration	Percentage (%)
1	*Control*	73.3 ± 1.8^{a}	-	73.3 ± 1.8^{a}	-
2	Low dose	$70.3\pm1.0^{**ab}$	-4	$65.6\pm1.9^{**bc}$	-10.5
3	High dose	$57.0\pm1.7^{**c}$	-22	$60.0\pm0.8^{**c}$	-18
4	*High dose + vitamin A supplementation*	73.3 ± 2.1^{a}	0	$54.3\pm2.4^{**d}$	-26
5	High dose + vitamin C supplementation	$62.6\pm1.5^{**e}$	-15	$53.7\pm4.4^{**d}$	-27
6	High dose + vitamin E supplementation	$64.6\pm2.4^{**d}$	-12	$56.0\pm2.9^{**d}$	-24
7	High dose withdrawal	71.6 ± 0.7^{b}	-2	$66.7\pm1.4^{**b}$	-9

Effect on basophil counts (Table-6)

The basophils were decreased in a dose dependent manner. While the low dose administration caused 41%, the high dose caused a 78% decrease. Vitamin supplementations brought about a 50% restoration of the

basophilic counts, when given to the high dose drug treated animals. Similarly, drug withdrawal also could partially restore the basophilic counts.Unlike short duration groups ciprofloxacin at low dose only could induce a decrease in basophilic counts by 37%, whereas the high dose of the drug caused only a 15% of decrease of the basophil counts. Neither vitamins supplementations nor drug withdrawal could prevent the drug induced decrease in the basophil counts. Among the vitamin groups, vitamin C appears to exert a more adverse effect.

Table – 5.Effect of ciprofloxacin alone and in co-administration with vitamin supplements on differential count -eosinophil counts of rats

Treatments		Eosinophil counts (In %)			
		Short Duration	**Percentage (%)**	**Long Duration**	**Percentage (%)**
1	***Control***	**8.7 ± 0.3^{c}**	**-**	**8.7 ± 0.3^{c}**	**-**
2	**Low dose**	**$7.3\pm0.5^{**b}$**	**-16**	**$7.3\pm0.3^{**d}$**	**-16**
3	**High dose**	**$5.6\pm0.3^{**a}$**	**-36**	**$10.7\pm0.7^{**a}$**	**23**
4	**High dose + vitamin A supplementation**	**8.3 ± 0.9^{b}**	**-5**	**$9.7\pm1.7^{**b}$**	**11.5**
5	***High dose + vitamin C supplementation***	**8.6 ± 1.4^{b}**	**-1**	**$9.7\pm0.7^{**b}$**	**11.5**
6	**High dose + vitamin E supplementation**	**8.0 ± 0.9^{a}**	**-8**	**$10.7\pm0.9^{**a}$**	**23**
7	**High dose withdrawal**	**$7.6\pm0.2^{**d}$**	**-16**	**$7.3\pm1.4^{**d}$**	**-16**

Table – 6. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on differential count -basophil counts of rats

Treatments		Basophil count (In%)			
		Short Duration	**Percentage (%)**	**Long Duration**	***Percentage (%)***
1	***Control***	**2.7 ± 0.7^{d}**	**-**	**2.7 ± 0.7^{a}**	**-**
2	**Low dose**	**$1.6\pm0.3^{**b}$**	**-41**	**$1.7\pm0.3^{**d}$**	**-37**
3	***High dose***	**$0.6\pm0.1^{**a}$**	**-78**	**$2.3\pm0.3^{**b}$**	**-14**
4	***High dose + vitamin A supplementation***	**$1.6\pm0.5^{**b}$**	**-41**	**$1.7\pm0.8^{**d}$**	**-37**
5	**High dose + vitamin C supplementation**	**$1.6\pm0.2^{**b}$**	**-41**	**$1.3\pm0.3^{**e}$**	**-52**
6	**High dose + vitamin E supplementation**	**$1.6\pm0.8^{**b}$**	**-41**	**2.0 ± 0.9^{c}**	**-26**
7	**High dose withdrawal**	**2.0 ± 0.4^{c}**	**-26**	**$1.7\pm0.3^{**d}$**	**-37**

Effect on lymphocyte counts (Table-7)

Nearly 16% decrease in lymphocytes was observed after low dose ciprofloxacin administration for a shorter duration. High dose of the drug had decreased the same by 32%. Vitamins supplementations as well as drug withdrawal could partially (50%) raise the lymphocyte counts like control. Like the shorter duration groups both low and high doses of drug had brought similar reduction in lymphocyte counts. However, the three vitamins were unable to exert an impact on the lymphocytes,whereas drug withdrawal was able to partially restore the lymphocyte counts.

Table – 7.Effect of ciprofloxacin alone and in co-administration with vitamin supplements on differential count -lymphocyte counts of rats

Treatments		Lymphocyte counts (In%)			
		Short Duration	**Percentage (%)**	**Long Duration**	**Percentage (%)**
1	***Control***	**25.3 ± 0.5^{a}**	**-**	**25.3 ± 0.5^{a}**	**-**
2	***Low dose***	**$21.3\pm1.1^{**bc}$**	**-16**	**$21.3\pm1.0^{**b}$**	**-16**
3	**High dose**	**$17.3\pm1.4^{**d}$**	**-32**	**$16.3\pm0.7^{**e}$**	**-36**
4	**High dose + vitamin A supplementation**	**$21.0\pm1.2^{**bc}$**	**-17**	**$16.3\pm1.8^{**c}$**	**-36**
5	**High dose + vitamin C supplementation**	**$20.6\pm1.1^{**c}$**	**-19**	**$15.7\pm0.5^{**d}$**	**-38**
6	**High dose + vitamin E supplementation**	**$22.0\pm1.2^{**b}$**	**-13**	**$16.3\pm0.7^{**c}$**	**-36**
7	**High dose withdrawal**	**$21.3\pm1.0^{**bc}$**	**-16**	**$18.7\pm3.0^{**b}$**	**-27**

Effect on monocyte counts (Table-8)

The low dose drug treatment brought a 16% decrease in monocyte counts, whereas, high dose treatment had no marked effect on the monocyte counts. Among the three vitamins, vitamin C was very effective in restoring the monocyte counts (16%) than vitamin A, whereas, vitamin E, supplementation and withdrawal of the drug had brought a decrease in the monocyte count (21% and 27%, respectively) compared to the high dose drug treated group. The low dose drug treatment as well as drug withdrawal animal, had brought a significant 70% decrease in the monocyte counts.

Table – 8. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on differential count -monocyte counts of rats

Treatments		Monocyte count (In%)			
		Short Duration	Percentage (%)	Long Duration	Percentage (%)
1	Control	6.3 ± 0.5^{b}	-	6.3 ± 1.0^{c}	-
2	Low dose	$5.3\pm0.5^{**cd}$	-16	$5.3\pm1.4^{**d}$	70
3	High dose	6.0 ± 1.4^{bc}	-5	$10.7\pm0.5^{**a}$	70
4	High dose + vitamin A supplementation	$6.6\pm1.4_{ab}$	-4.8	$10.7\pm1.4^{**a}$	70
5	High dose + vitamin C supplementation	$7.3\pm0.5^{**a}$	16	$9.7\pm0.3^{**b}$	54
6	High dose + vitamin E supplementation	$4.6\pm0.7^{**e}$	-27	$10.7\pm1.4^{**a}$	70
7	High dose withdrawal	$5.0\pm0.4^{**d}$	-16	$4.7\pm0.5^{**e}$	-25

Effect on haemoglobin concentration (HB) (Figure-1)

About 23% to 30% reduction respectively was brought about by the ciprofloxacin treatment in a dose dependent manner. Neither vitamins A, C and E supplementations nor the drug withdrawal could completely restore the haemoglobin concentration. Except for the high dose drug treatment all the other experimental groups including the withdrawal group had exhibited a near normal haemoglobin concentration. The high dose of ciprofloxacin had caused a decrease in the haemoglobin concentration by 21%.

Effect on packed cell volume (PCV)(Figure-9)

Ciprofloxacin at high doses induced a 31% decrease in the PCV. Vitamin C and E could only partially restore the PCV while, vitamin A could bring in a 84% recovery of the same. Withdrawal of the drug completely restored the PCV to normalcy. Unlike short duration study, a dose dependent decrease in PCV was observed. All the vitamins were effective in partial restoration (71% to 80%) of this parameter. Drug withdrawal brought a 90% recovery of the PCV.

Effect on mean corpuscular volume (MCV)(Figure- 10)

While the low dose of ciprofloxacin could elevate the MCV by 87%, high dose drug treatment had only negligible effects. Vitamins supplementations appear to cause a small decrease (11% to 19%), drug withdrawal had no marked effect on this parameter. Unlike the short duration group the MCV was elevated in a dose dependent manner. The vitamin supplementation especially A and E caused a decrease similar to short duration group but slightly more in percentage. Here, the drug withdrawal appears to induce a negligible (12%) decrease in the MCV.

Effect on mean corpuscular haemoglobin (MCH) (Figure-11)

The MCH was not affected by ciprofloxacin treatment. However, supplementations with vitamin A and E as well as drug withdrawal to the high dose drug treated groups, was seen to induce a 16% to 21% reduction of this parameter. Vitamin C maintained the MCH at the control values. Unlike short duration groups, ciprofloxacin administration caused an elevation of MCH in a dose dependent manner. Vitamin C appeared to reduce the MCH (26%), but vitamin supplementations with vitamin A and E as well as drug withdrawal in the high dose group had maintained the MCH in near normal values.

Effect on mean corpuscular haemoglobin concentration (MCHC)(Figure-12)

Ciprofloxacin at low dose only had caused a negligible decrease (15%) in MCHC. Vitamin A supplementation alone brought a 13% decrease in MCHC in high dose drug treated group and a similar response was elicited by drug withdrawal. Unlike short duration response long duration of drug treatment had caused an elevation (15% to 18%) in MCHC. Vitamins supplementations appeared to further increase the MCHC and

vitamin E has more effect when compared to A and C. Drug withdrawal appeared to restore the MCHC to control values.

V. DISCUSSION

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), diclofenac (Selvaraj, 2002) treatments in male rats.

The high dose of ciprofloxacin had caused a decrease in the haemoglobin concentration in both the duration of its treatments. All the three vitamins (A, C and E) and drug withdrawal was seen to be very effective, especially in the long duration treatments in restoring the haemoglobin concentration.

A dose dependent reduction in red blood cell count was seen after ciprofloxacin administration. Vitamin A and E were more effective in preventing the drug induced adverse reaction than vitamin C at both the duration of treatments and withdrawal of the drug proved better in restoring the red blood counts.

White blood cell counts were reduced by ciprofloxacin administration, Vitamins (A, C and E) could not prevent their reduction, but withdrawal of the drug was very effective and it could completely restore this parameter to normalcy.

Differential count sub classifies each component of the white cell population. Low WBC could be a sign of the result of various chronic diseases and also be a side effect of various drugs, particularly chemotherapeutic drugs for cancer treatment (NHL Cyberfamily, 2005).

Neutrophils act as first line of defense against invading pathogens by destroying them and by stopping them from multiplying in the body (Mike Gleeson, 2004). In the present study, there was an 18 to 22% decrease in neutrophil count after high dose of ciprofloxacin was administered at both the duration of its treatments. The withdrawal group of short duration brought effective recovery than that of long duration group. Chloramphenicol reduced the neutrophil count (Saba *et al.*, 2002). Similar to ciprofloxacin some drugs (eg.carbimazole; suiphonamides) may increase the neutrophil counts and they might be destroyed by neutrophil antibodies and reduced B_{12} manufacture and finally bone marrow failure (CWS, 2002).

Eosinophils protect the body against allergic reactions and parasites. Elevated levels indicate an allergic response and low count is normal (CWS, 2002). In the present study, all treatment groups of rats in long duration treatment have shown an elevated eosinophil count except low dose drug treated and withdrawal group. This indicated a development of allergic response to ciprofloxacin by long-term administration of this drug and the condition was reversed in short duration treatment groups. The drug withdrawal also brought only partial recovery, suggestive of persistent effect of ciprofloxacin on the eosinophils.

Basophils are the least common of the WBCs and their count at zero is quite normal. They are involved in fighting bacteria and their increased number reflect a possibility of parasitic activity in the body (NHL Cyberfamily, 2005).

In the present study, the basophil count was seen to be raised in the high dose ciprofloxacin treated groups at long duration only and the same was very low in the short duration treatment. This may be due to the development of allergy by the body against ciprofloxacin when used continuously for a longer period. . However, all the three vitamins acted effectively and equally in the short duration treatment period by lowering the basophil counts.

Lymphocytes are involved in protecting the body from viral infections and an elevated level of lymphocytes indicated an active viral infection and a depressed level indicated an exhausted immune system (CWS, 2002). Diclofenac alone and its combination with vitamin E did not produce any consistent effect on whole blood (Fuller, 2000).

Monocyte cells are helpful in fighting severe infections and are considered the body's second line of defense against infection. Their elevated levels are seen in tissue breakdown and indicate chronic infections. Their low level are indicative of a good state of health (CWS, 2002). In the present investigation, the monocyte count was raised markedly by the high dose ciprofloxacin administration for long duration with vitamins supplementation. However a reverse process was observed, in short-term study with this drug. So by long term administration of ciprofloxacin there might be some tissue breakdown and this might in turn lead to elevated monocyte counts in the experimental animals. Neither the supplemented vitamins nor the withdrawal of drug in long term study groups could stop the tissue damage caused by the drug effectively.

Haematocrit is the measurement of the percentage of red blood cells in whole blood (CWS 2002). A decrease in PVC is always seen with a decrease in the hemoglobin-indicating anemia.

In the present study, the high dose ciprofloxacin reduced the PCV value both in long and short duration of its treatment, as well as haemoglobin. Vitamin C could bring some improvement in PCV value than vitamin A and E. The

absorption of iron is increased by Vitamin C (Mike Gleeson, 2004). The withdrawal of ciprofloxacin effectively restored the PCV value. The low PCV can be due to blood loss or destruction of blood cells, which might have occurred internally (bloodbook.com).

MCV is a measure of the average RBC size. In persons taking AZT, the MCV will always be elevated and Vitamin B_{12} deficiencies also causes increase in MCV (CWS, 2002). In the present study the MCV value increased in a dose dependent manner in ciprofloxacin treated rats. The vitamins, such as A, C and E had brought significant recovery of MCV value caused by the drug. The drug withdrawal restored the MCV to the normalcy more rapidly in short duration groups than in the long duration groups. High MCV causes haemolysis, hypothyroidism and iron deficiency anemia (CWS, 2002). Since vitamins increase iron absorption, animals with vitamin supplementation along with the drug showed normal MCV. In long duration of drug administration, vitamin C was observed to be more effective than vitamin A and E in its restorative action (QUEST, 2001).

MCH provides the average weight of hemoglobin in the red blood cell. Both, its increased and decreased levels, are associated with macrocytic and microcytic anemia, respectively (CWS, 2002). In the present investigation, ciprofloxacin has exerted a positive influence on MCH in both short and long durational studies. The antioxidant vitamins and drug withdrawal proved effective in retaining the normal MCH value indicating the anti oxidant action of vitamins and transient nature of drug effect.

MCHC measures the average concentration of haemoglobin in red blood cells. It is very important to diagnose anemia, because haemoglobin and haematocrit only are used as effective measures and not the RBC in the circulation. Low MCHC denotes less haemoglobin and high MCHC means more haemoglobin in a unit of RBCs. The decreased level indicate iron deficiency and blood loss (CWS, 2002). In the present study, the observed MCHC value was in an increased level in both the drugs as well as vitamin-supplemented groups in the long duration study. Withdrawal of the drug has brought back the MCHC value to normalcy. Thus, ciprofloxacin when administrated in high dose for a long duration might increase the average concentration of HB in the blood.

VI. CONCLUSION

An ideal drug would be one that enhances the salutary effects of inflammation, yet controls it's harmful sequelae. Supplementations of vitamins have been seen to be effective in maintaining the drug-induced effect on various haematological parameters. Vitamin A and E supplementation appears to be effective in restoring the effects of the drug but vitamin C has comparatively more effect in limiting the drug induced ill effects. This was mainly by their antioxidant properties. Thus, neither proliferation nor degeneration process was encouraged by the vitamin supplementations.

FIGURES

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

Figure-1. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on haemoglobin concentration in rats

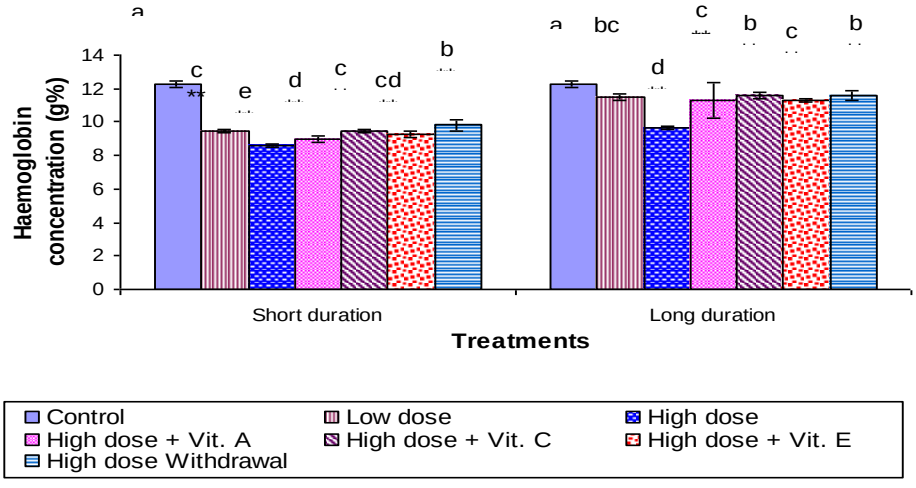


Figure-9. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on PCV in the blood of rats

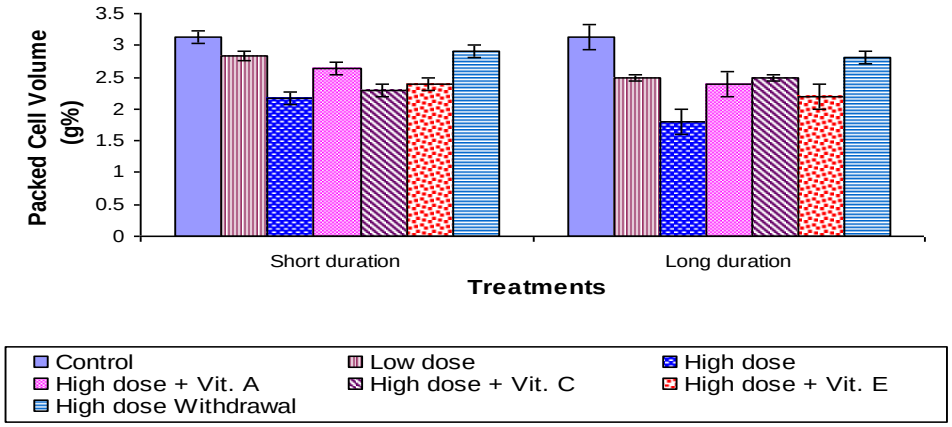


Figure-10. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on MCV in the blood of rats

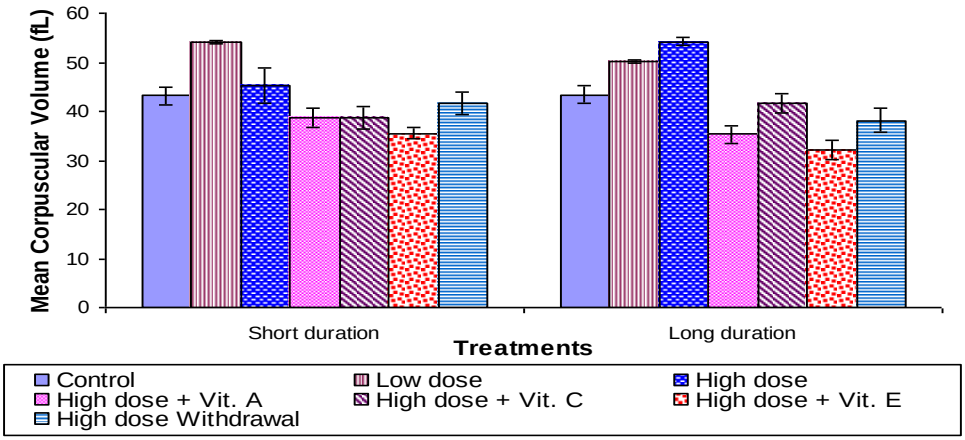


Figure-11. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on MCH in the blood of rats

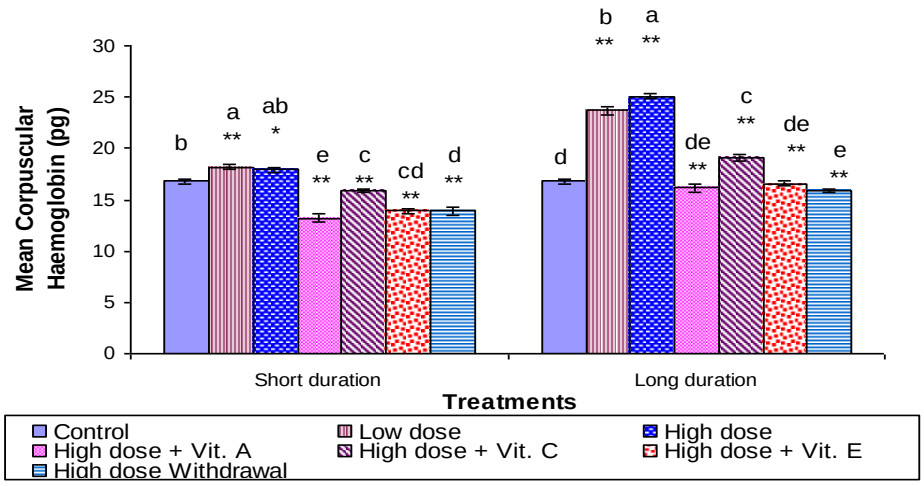
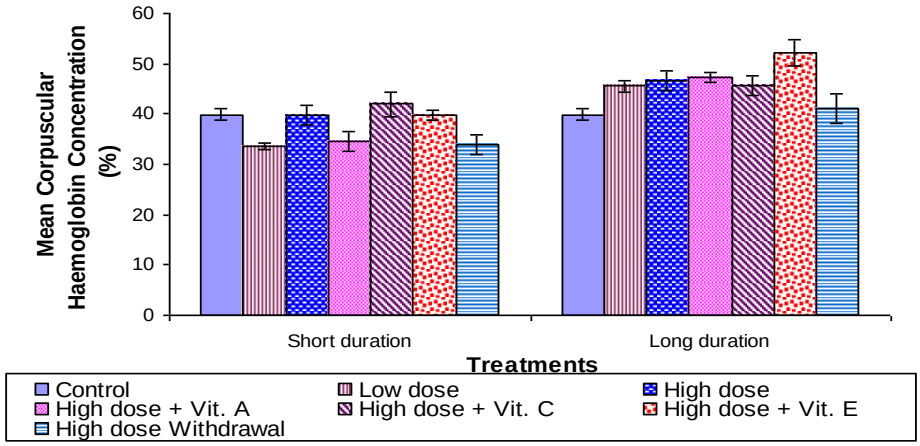


Figure-12. Effect of ciprofloxacin alone and in co-administration with vitamin supplements on MCHC in the blood of rats



REFERENCES

[1]. Bergan, T., Thorsteinsson S.B., Solberg, R., Bjornskau, L., Kolstad, I.M. and Johnson, S.(1987). Pharmacokinetics of ciprofloxacin: intravenous and increasing oral doses. *Am J. Med*. 82 (Supply 4a): 97-102.

[2]. CWS.(2002).CellMate Wellness System ™ Blood Chemistry Definitions, Haematology. webmaster@carbonbased.com.

[3]. Fuller, C.J., May, M.A. and Martin, K.J. (2000). The effect of vitamin C and vitamin E supplementation on LDL oxidizability and blood cells in young smokers. *J.Am.Coll. Nutr*., 19(3): 361-369.

[4]. IBIS, (1998). Integrative body mind information system. Your Authoritative Source for Integrative Medicine. Vitamin E, Integrative Medical Arts Group, Inc.

[5]. Institute of Medicine, Food and Nutrition Board. (2001). Dietary Reference Intakes. Vitamin A, Vitamin E, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nichol, Silicon, Vanadium and Zinc. National Academy press, Washington, USA Keller, H. and Follath, F. (1988) . Misscellaneous antibiotics, In : Mayler's side effects of drugs .Dukes, M.N.G.(eds).11th ed. Elsevier Science Publishers , NewYork. pp . 546-556.

[6]. Masquelier J. (1987). Pychealth. Plant extract with a proanthocyanidins content as a therapeutic agent having radical scavenging effect and use there of. (Rev) – 20031 – 888-764-2209. (757) E mail488-5627.info@pycheath.com .

[7]. Merck Veterinary Manual. (1979). Hematology and blood values for cats. 5th edn., Merck and Co. Inc. Rahway NJ. pp. 21, 1472. *Copywrite © 2001-2005 felidae world*.

[8]. Mike Gleeson. (2004). What Blood Tests can tell you about possible causes of under performance. www.medicdirect sport.com.

[9]. Non Hodgkin's Lymphomic Cyber family.(2005). Complete blood count. In: Blood definitions. *Copywrite © 2005 NHL Cyberfamily.*

[10]. QUEST, (2001). Simply stated--- Elevated Enzymes, Muscular Dystrophy Association Publications, and Vol-8.

[11]. Ross A.C. (1999). Vitamin A and Retinoids. In: Shils, M. (eds.). Nutrition in health and disease, 9th edn., Baltimore Williams and Wilkins, pp. 305-327.

[12]. Saba, A.B., Awe, O.E., Akinloye, A.K. and Oladele, G.M.(2002). Haematologicl changes accompanying prolonged ocular chloramphenicol administration in laboratory rabbits. *Arf. J. Biomed. Res*: vol 5; 131-135. Sampathraj, R (1994). Studies on an antineoplastic drug-methotrexate: histological and biochemical responses of epididymis and hormonal changes in adult albino rats, Ph. D. Thesis, Bharathiar University, Coimbatore, India.
Samuel, R.M. (1986). Haematology In: Notes on Clinical lab techniques 4th ed. Tyyer and Sons, Madras.pp.23-27.

[13]. Selvaraj, M. (2002). Studies on Diclofenac Sodium: Endocrine changes, epididymal biochemsitry and histoarchitecture in rats. Ph.D. Thesis, Bharathiar University, Coimbatore, India.

[14]. U.S Department of Agriculture, Agriculture Research Service.(2004). USDA National Nutrient Database for standard Reference, Release 16-1. Nutrient Data Laboratory. *http: //www.nal.usda.gov/fnic food comp.*

[15]. Valera, M.J., Sampedro, A., Moxeno, E. and Ruizbraw, A. (1995). Modification of immune response in mice by ciprofloxacin. *Antimicrob Agents chemother*, 39: 150-154.

[16]. Weitberg, A.B. and Corvese, D.(1997). Effect of vitamin E and beta-carotene on DNA Stand breakage induced by to bacio – specific nitrosamines and stimulated human phagocytes. J.*Exp. Clin.Cancer. Res*: 16:11-4.

[17]. http://www.bloodbook.com.