ejpmr, 2016,3(8), 142-149

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

DRAWBACK OF CORTICOSTEROID TREATMENT AND SUBSEQUENT ALTERATION OF THE HEMATOLOGICAL PROFILES WITH HEPATIC TEXTURE IN RAT

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Article Received on 09/06/2016 **Article Revised on 29/06/2016** **Article Accepted on 19/07/2016**

ABSTRACT

An investigation was conducted on the effect of two corticosteroid drugs such as dexamethasone (Oradexon®) and predinisolone (Deltasone®) on hematological profiles (blood glucose, absolute number of various granulocytes) and histopathology of liver in rats for a period of 60 days. A total of 25 apparently healthy rats were randomly divided into 5 groups and each group contained 5 rats. Group-A (control) and was given only basal ration. Other treatment groups (B, C, D and E) were given dexamethasone (Oradexon®, @ 0.5 mg/kg b.wt.) and predinsolone (Deltason @ 2 mg/kg b.wt.) mixed with drinking water. The blood glucose level was increased significantly ($P<0.01$) in all four treatment groups (B, C, D and E). Corticosteroid drugs did not alter the feed intake and water consumption in treated rats. All treated rats showed a significant ($P<0.05$ and $P<0.01$) decrease in bodyweight, where Oradexon @ decreased body weight very significantly ($P<0.01$) from day 30 to 60. Absolute count of Neutrophil increased significantly ($P<0.01$). On the other hand, absolute count of Lymphocyte and Eosinophil were decreased significantly ($P<0.01$). Grossly, Rats in group B and D showed slight congestion in the liver. Thigh muscles were atrophied in all treatment groups (B, C and D). Hemorrhage and ulceration were found in all the treated rats. Swollen joints and softening long bones were observed in group B, C and E.

KEYWORDS: Dexamethasone, Prednisolone, Rat, Lymphocyte, Liver. Hepatocytes.

INTRODUCTION

Corticosteroid drugs are among the most widely used and misused class of drugs in veterinary medicine. Despite this, scientific information on corticosteroid therapy in most domestic species is scarce, particularly with respect to optimal dosages and dosage intervals, physical and endocrine side effects and efficacy in clinical applications. Much has been adopted from clinical use in humans and much more is known about the finding points of corticosteroid therapy in dogs and cats than in other species. It is important to recognize that corticosteroids rarely cure diseases, with the possible exception of spontaneous corticosteroid deficiency. Corticosteroids are used to try to suppress clinical signs long enough for a condition to run its natural course. Corticosteroids are generally only palliative and do not provide a true cure of any disease.

Many strategic treatment programs have been developed for rational use of corticosteroids. There are a few contra-indications to the use of corticosteroids such as late pregnancy, corneal ulcer, diabetes mellitus, hypertension, renal insufficiency and decreased cardiac reserve. Infectious conditions required special consideration because of dissemination of micro-organisms throughout the body which may cause a fulminating infectious condition. Muscle wasting in weak thin animals and growth inhibition in young animal must be considered. A single dose would not be contra-indicated any time. In Bangladesh, there is very few data regarding the corticosteroids on the laboratory

animals. The present study has been conducted to investigate the effect of corticosteroids on clinical parameters like body weight and food intake and some hematological parameters. The study was further extended to check the lesion in the liver.

MATERIALS AND METHODS

The experiment was carried out in the Department of Physiology, Bangladesh Agricultural University, Mymensingh-2202 for a period of 60 days between 31st August, 2012 to 29th October, 2012.

Experimental animals

A total of twenty five apparently healthy 7 weeks old Long Evans rats weighing between 110-115 g were purchased from Bangabandhu Sheikh Mujib Medical University, Bangladesh.

After procurement, all the 25 rats were kept under close observation in order to acclimatize for a period of one week prior to commencement of the experiment. All the rats were maintained under good housing conditions and were provided with HI-PRO-VITE Feed (C.P. Bangladesh Co. Ltd.).

Chemicals and reagents

Dexamethasone micronised BP (Oradexon®, Organon Bangladesh Ltd) and Prednisolone USP (Deltasone®, Renata Ltd) were collected from local market.

Experimental design

The rats were randomly divided into 5 equal groups and each group contained 5 rats. The groups were designated as group A, B, C, D and E of which group A was kept as control. Rats of group A were fed control diet or basal ration. Rat of group B and C were fed basal ration along with dexamethasone. Rat of group D and E were fed basal ration along with prednisolone. The ration and fresh water were supplied ad libitum during 60 days of experimental period. Weights and blood glucose level were taken on a regular interval like day 1, 30 and 60. Hematological parameters were recorded on day 1, 30 and 60. Post-mortem findings were obtained after the animals were sacrificed at the end of experiment.

Determination of blood glucose level in rats

A small drop of blood was collected directly from the tail of all rats during the research work and was placed on the glucose strips at automatic Glucotrend (Glucometer). The reading of blood glucose level from the Glucotrend® (Glucometer) was carefully observed on the day 1, 30 and 60.

Examination the clinical parameters of rats

The effects of dexamethasone (Oradexon®, Organon Bangladesh Ltd.) and predinisolone (Deltasone®, Renata Ltd.) were studied on the following clinical parameters; Feed intake and water, consumption, Body weight. During the experimental period, the rats were under close observation and they were examined very carefully. Their feed consumption and water intake were carefully noted. Their body weight was measured on day 1, 30 and 60 recorded.

Post-mortem examination of rats

At the end of the experiment (60 days), post-mortem examination of the rat was performed. The vital organs such as liver, stomach, bones and muscles were examined. The gross post-mortem findings of those organs were recorded. The bones and muscles were also examined carefully and recorded. Liver was collected for histopathology and was preserved in 10% buffered formalin. Fixed tissue sections were processed, paraffin-embedded, sectioned & were routinely stained with Hematoxylin & Eosin stain as per standard procedure. The tissues were examined & photomicrography was taken at the Pahology Laboratory, BAU, Mymensingh.

Statistical analysis

The data were analyzed between the control and treated values by using SPSS version 18.0 for windows (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Differences in mean values were tested using ANOVA, followed by a multiple pairwise comparison using a student *t*-test. In all cases, $P \leq 0.05$ was considered significant and the graphical presentation was done by the Sigma Plot version 12.

RESULTS AND DISCUSSION

Effects on blood glucose level

The results of effect of oral administration of dexamethasone and prednisolone on blood glucose level are showed in the Table I. A significant ($P<0.01$) increase of the blood glucose level was found on day 30 and 60 of group B, C,D and E. Blood glucose level of group A was not altered significantly on day 30 and 60. During the experimental period (60 days) blood glucose level was measured on day 1, 30 and 60. The blood glucose level of all treatment groups (B, C, D and E) were highly significant ($P<0.01$) in comparison to that of the pretreatment values. The blood glucose level was increased significantly ($P<0.01$) following oral administration of corticosteroids (Dexamethasone and Prednisolone) in the treatment groups (B, C, D and E). In conformity to the present findings, Furll and Knobloch[16] reported that prednisolone increased blood glucose levels in cows. The increase of blood glucose level may be due to increase gluconeogenesis, decrease protein synthesis, reduce peripheral glucose utilization and activated lipolysis following dexamethasone, prednisolone administration Adams.[15]

Examination of the clinical parameters

The effect of oral administration of dexamethasone and prednisolone on body weight of different groups of rats is represented in the Table II. The mean initial weight

of rats for all groups were almost similar, which was higher than 110 g, was recorded on day 1. The highly significant (P<0.01) reduction of body weight was recorded on day 1,30 and 60 following administration of dexamethasone in group B, C and administration of prednisolone in group D, E respectively. Significant (P<0.01) reduction of body weight was recorded in day 1, 30 and 60 following administration of dexamethasone in group B and C respectively.

During the whole experimental period (60 days) all the rats of treatment groups (B, C, D and E) showed same feeding efficiency and water consumption as observed in control group (A). Oral administration of dexamethasone and prednisolone in the treatment groups did not significantly alter the amount of feed intake and water consumption. During the experimental period body weight of rats of all groups were recorded on day 1, 30, 60. The results showed that body weight were decreased significantly (P<0.05 and P<0.01) on treatment groups (B, C, D and E). The finding is similar to the reports of Bray[2] and Knecht *et al.*[8] The body weight decreased may due to excessive catabolism, muscle atrophy and mobilization of free fatty acids from adipose store Adams.[15]

Effect on corticosteroids on absolute count of Lymphocyte, Neutrophil and Eosinophil

Following dexamethasone and prednisolone treatment Lymphocyte and Eosinophil counts were decreased significantly (P<0.01) which are shown in Table III and IV. Dietary steroids resulted in rising absolute Neutrophil count in the treated group B, C, D and E that are represented in Table 5. The increases of neutrophil count was highly significant (P<0.01) in all treatment groups (B, C, D and E). The increase number of neutrophils may be due to increase rate of entrance of neutrophils in to the blood from the bone marrow and a diminish rate of their removal (Bishop *et a1.*[1] Luna *et* al.[11] reported that prednisolone caused neutrophilia in lactating buffaloes. Similar findings also reported by several workers (Lan *et al.*[9]; Wasfi *et al.*[14]; Kemppainen[7]; and Targowski[13] The lymphocyte count was significantly (P<0.01) decreased in all the treatment groups (B, C, D and E). The decrease in lymphocyte may be a relative one, because when the neutrophil count increases the lymphocyte count decreases respectively. Similar results have been observed by many workers (Lan *et a1.*[9]; Wasfi *et al.*[14]; Kemppainen[7] and Targowski[13] The increases of monocyte count was highly significant (P<0.01) in the treatment groups (B, C and D). Similar reports have been stated by Jain *et al.*[6] and Kemppainen[7]. The increase occurred may be due to its immunosuppresent effects of steroids. The eosinophil count was significantly (P<0.01) decreased in the treatment groups (B, C and D). Similar results have also been stated by some researchers (Luna *et al.*[11]; Jain *et al*.[6] and Kemppainen.[7] The decrease number of eosinophil may be due to immunosuppressive activities of corticosteroid drugs. The changes in the basophil count were not statistically significant.

The gross post mortem findings in rats

At the end of the experimental period (60 days), the rats were subjected to post-mortem examination with a view to study the gross pathological changes in rats. All vital organs were apparently normal in group A. Slight congestion of liver was found in rats receiving either dexamethasone or prednisolone of group B, C, D and E. Muscles atrophy, hemorrhage and ulceration of the stomach were found in all treatment groups (B, C, D and E). Swollen joint and softening of the long bone were found in receiving dexamethasone of the rats group B and C.

Following administration of dexamethasone and prednisolone for *60* days, post-mortem examination was performed. Slight congestion in the liver was found in rat of group B and D. Similar findings have also been reported by Dillon *et al*[3] Congestion may be due to micronodular cirrhosis (Adams, *1995).*Hemorrhage and ulceration in the stomach of rat was found in all treatment groups (B, C, D and E). The findings may be due to corticosteroids stimulation which cause excessive production of acids and pepsin and tends to reduce the gastric mucosal protective barrier Diplma.[4]

Muscle atrophy of rats was found in treatment groups (B, C, D and E). Similar observations were stated by Adams.[15] This observation may be due to excessive catabolism and gluconeogenesis. Swollen joint and softening of the long bone were found in rats received both dexamethasone and prednisolone. Similar observations were stated by Samad.[12] This is thought to be due to antagonistic effect of corticosteroids to vitamin-D on calcium absorption (Aron and Tyrrell, 1994).

Histopathology

The histopathological texture of liver has been shown in the fig. 1 and 2. All the rats in treatment group B and D showed slight congestion in the liver. Thigh muscle was atrophied in all treatment groups (B, C and D). Hemorrhage and ulceration were found in all the treated rats. Swollen joints and softening long bones were observed in group B, C and E.

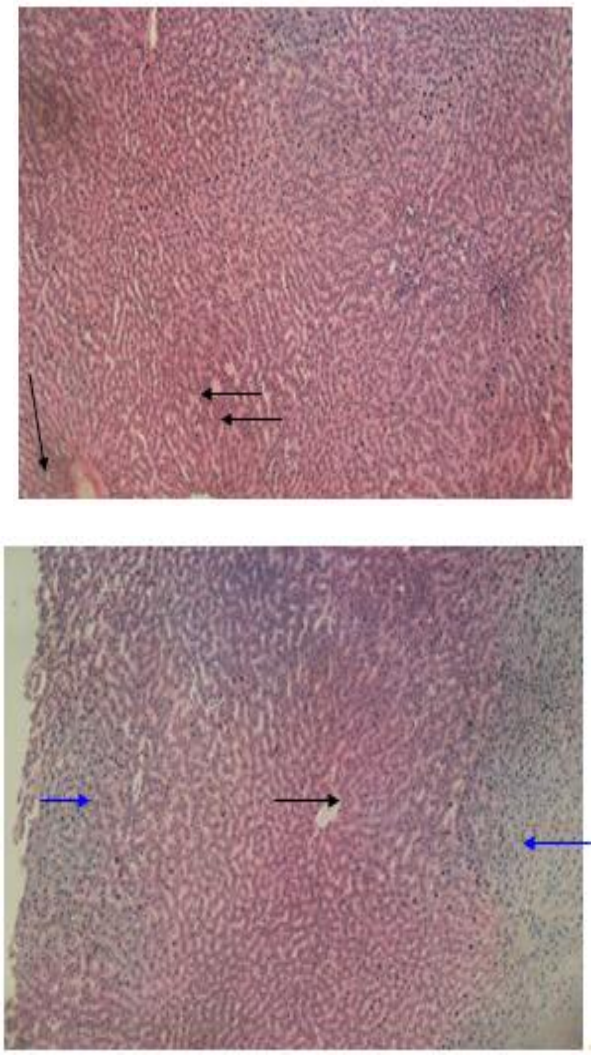

Fig-1 (A& B). The microscopic section of liver of experimental rat (A & B): The cytoplasm of hepatocytes are swollen, enlarged, necrotic, hemorrhagic lesions and nucleus is pyknotic (H & E stainX10).

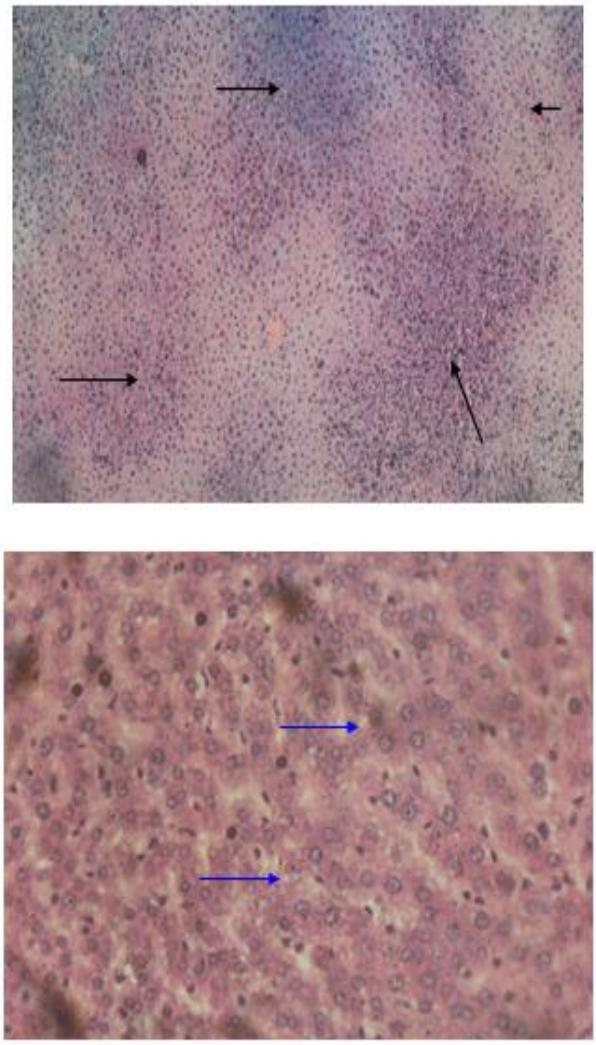

Fig-2 (C & D). The microscopic section of liver of experimental rat (c & d): The cytoplasm of hepatocytes foamy, vacuolated, condensation of nuclear chromatin in few cases (H & E stainX10).

Table 1. Effect of dexamethasone and prednisolone on the blood glucose level (mg/dl).

Groups of rats	Blood glucose (mg/dl)		
	Pre treatment	Post treatment	
	Day 1	Day 30	Day 60
A Control	125.0±1.789	126.0±3.162	125.0±1.949
B Dexamethasone	122.0±3.050	130.0±2.470**	135.0±1.844**
C Dexamethasone	123.0±0.894	128.0±2.214**	130.0±1.378**
D Prednisolone	127.0±1.549	135.0±1.703**	140.0±1.703**
E Prednisolone	121.8±1.068	130.0±1.673**	135.0±2.720**

The above values represent the mean ± standard error (SE) of the blood glucose level of 5 rats.
****=**Significant at 1 percent level (P<0.01);***=**Significant at 5 percent level (P<0.05).
b= mean ± standard error (SE) of 5 rats.

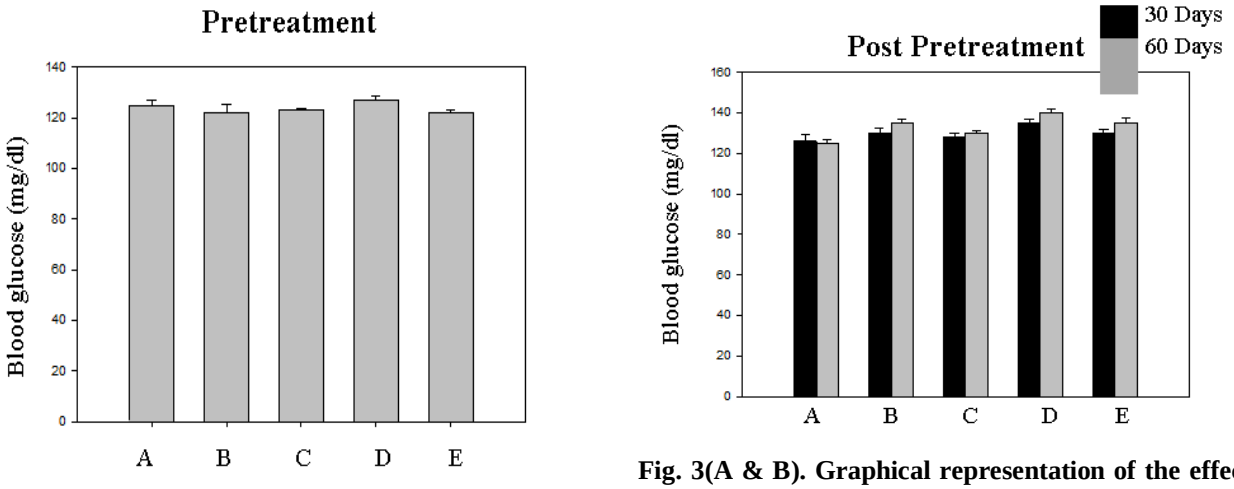

Fig. 3(A & B). Graphical representation of the effect of dexamethasone and prednisolone on the blood glucose level (mg/dl).

Table 2. Effect of dexamethasone and prednisolone on body weight (g).

Groups of rats	Body weight (g)		
	Pre treatment	Post treatment	
	Day 1	Day 30	Day 60
A Control	110.98±1.967	130.53±1.590	144.50±2.102
B Dexamethasone	110.58±1.147	107.28±1.472**	90.54±1.743**
C Dexamethasone	110.75±0.917	109.85±1.305**	95.57±1.500**
D Prednisolone	110.49±0.414	109.45±0.377**	85.45±1.605**
E Prednisolone	110.60±1.367	108.65±0.273**	90.98±0.615**

The above values represent the mean ± standard error (SE) of the body weight of 5 rats.
******=Significant at 1 percent level (P<0.01);*****=Significant at 5 percent level (P<0.05).
b =mean ± standard error (SE) of 5 rats.

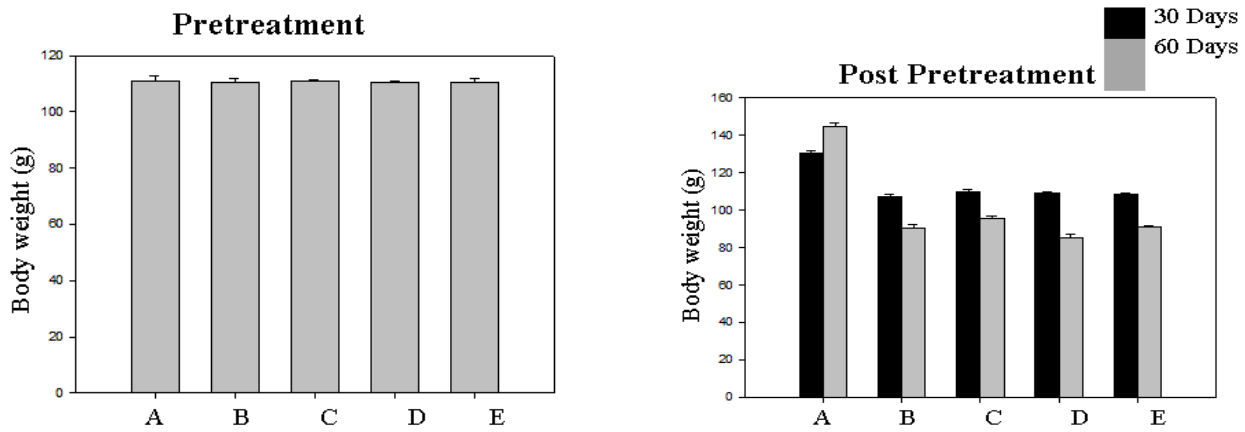

Fig 4 (A & B). Graphical representation of the effect of dexamethasone and prednisolone on body weight (g).

Table 3. Effect of dexamethasone and prednisolone on absolute number of lymphocyte.

Group of rats	Absolute number of Lymphocyte /cu.mm		
	Pre treatment	Post treatment	
	Day 1	Day 30	Day 60
A Control	6794.4±0.040	6881.04±0.100	6794.4±0.009
B	6712.5±0.058	6465.5±0.032**	6336.0±0.087**
C	6452.6±0.111	6460.0±0.009**	6457.0±0.029**
D	6733.6±0.030	6679.2±0.061**	5929.0±0.013**
E	6675.0±0.046	6514.4±0.007**	5978.5±0.006**

The above values represent the mean ± standard error (SE) of the leukocyte count of 5 rats.
**=Significant at 1 percent level (P<0.01).

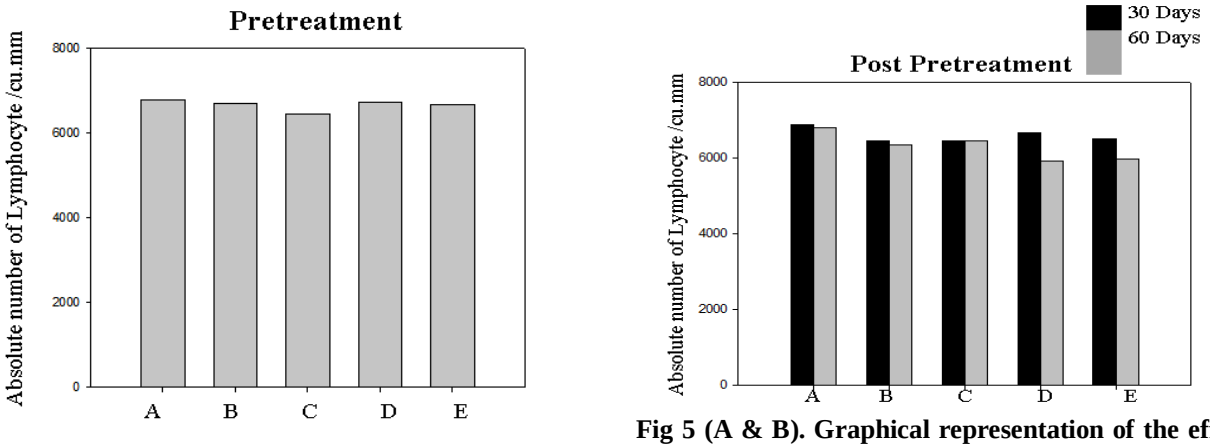

Fig 5 (A & B). Graphical representation of the effect of dexamethasone and prednisolone on absolute number of lymphocyte.

Table 4. Effect of dexamethasone and prednisolone on absolute number of eosinophil

Groups of rats	Absolute number of Eosinophil /cu.mm		
	Pre treatment	Post treatment	
	Day 1	Day 30	Day 60
A control	447.0±0.16	452.0±0.002	447.0±0.003
B	447.5±0.002	193.0±0.002**	105.6±0.002**
C	442.5±0.002	190.0±0.004	102.5±0.01
D	443.0±0.002	193.6±0.002	107.8±0.001**
E	445.0±0.002	191.6±0.003	108.7±0.00*

The above values represent the mean ± standard error (SE) of the Eosinophil count of 5 rats.
**=Significant at 1 percent level (P<0.01);*=Significant at 5 percent level (P<0.05).

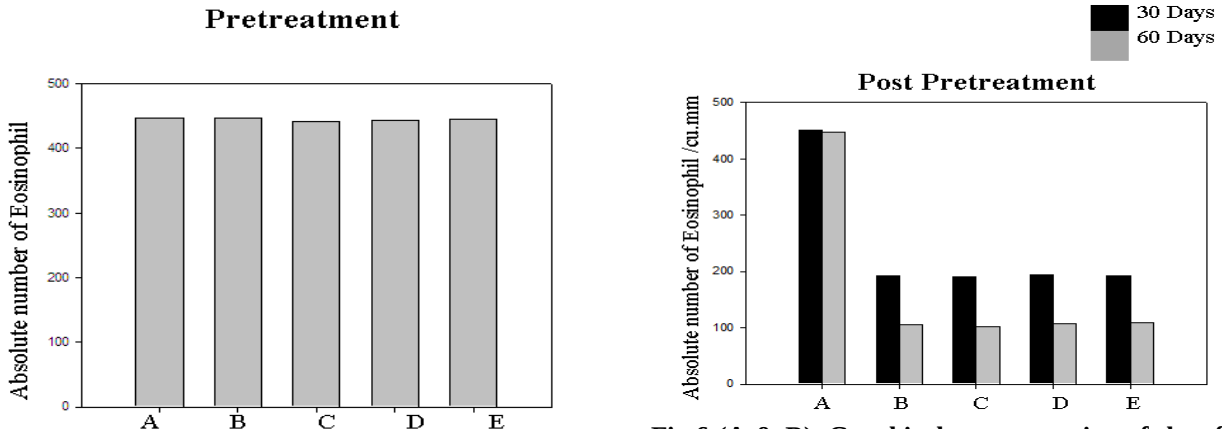

Fig.6 (A & B). Graphical representation of the effect of dexamethasone and prednisolone on absolute number of eosinophil

Table 5. Effect of dexamethasone and prednisolone on absolute number of neutrophil.

Group of rats	Absolute number of Neutrophil /cu.mm		
	Pre treatment	Post treatment	
	Day 1	Day 30	Day 60
A Control	1072.8±0.000	1086.4±0.000	1072.8±0.00
B	1074.0±0.003	1930.0±0.019**	3062.0±0.004**
C	1062.0±0.005	1805.0±0.084**	3075.0±0.004**
D	1063.2±0.109	1936.0±0.007**	3665.0±0.003**
E	1068.0±0.005	1916.0±0.015**	3478.0±0.001**

The above values represent the mean ± standard error (SE) of the neutrophil count of 5 rats.
**=Significant at 1 percent level (P<0.01).
*=Significant at 5 percent level (P<0.05).
*=Significant at 5 percent level (P<0.05).

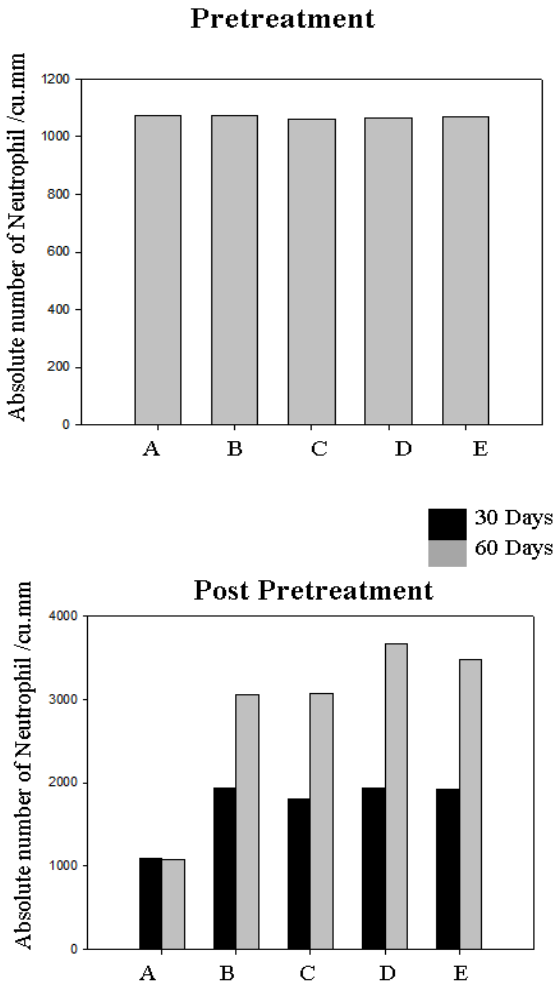

Fig 7 (A & B). Graphical representation of the effect of dexamethasone and prednisolone on a absolute number of neutrophil.

CONCLUSION

Although the treatment with corticosteroid drugs causes dramatic action against inflammation but prolonged use of these drugs consequences alteration in the normal physiological parameter with various types of chronic diseases. Our data demonstrated that, oral administration of corticosteroid drugs increases the blood glucose level, decreases body weight and changes the formed elements of blood. It also increases the TLC, neutrophil count and decreases the lymphocyte and eosinophil count. Further study is needed for the investigation of corticosteroid at cellular level with molecular mechanism.

Conflict of interest statement

The authors have no competing interests.

ACKNOWLEDGMENTS

The author is grateful to the Post graduate studies faculty of Patuakhali Science and Technology University for financial assistance in the research work, Dept of Pathology, Bangladesh Agricultural University and Dept of Physiology, Bangladesh Agricultural University for their cooperation in conducting the research work.

Author's contribution

M. M. R. Chowdhury designed the experiment, Fahmida Afrin performed the experiment, D. Das performed the hematological studies S. S. Saha helps in histopathology of the samples, Md. Ali Asgar and Md. Ataur Rahman analyzes the data and Professor M. K. Islam PhD supervises and revises the manuscript.

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