

# Effect of Prednisolone on the Lymphoid Tissue of Small Intestine of Adult Wistar Rats

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## Abstract

Peyers patches are central to mucosal immunity as they play a major role in the gastrointestinal tract; they modulate intestinal immune and inflammatory responses or tolerance and they are subject to other factors that influence immunity. This study aims at assessing the immunosuppressive effect of prednisolone on the histology of peyers patches of adult wistar rats. Twenty eight adult albino wistar rats weighing between 190g to 300g were randomly assigned seven groups A, B, C, D, E, F & G of four rats each. Group A animals served as the control and were given distilled water only; Group B received 2mg/kg of prednisolone for two weeks, Group C received 4mg/kg of prednisolone for two weeks, Group D received 6mg/kg of prednisolone for two weeks, Group E received 2mg/kg of prednisolone for four weeks, Group F received 4mg/kg of prednisolone for four weeks and Group G received 6mg/kg of prednisolone for four weeks. The oral administration was in two phases; short term (two weeks) and long term (four weeks) which lasted for twenty eight days. Body weight result showed general increase in body weight in all the groups. Histological examination revealed significantly increased influx of lymphocytes in peyers patches into the lumen in groups F & G compared with the control while other groups B, C, D, & E were not affected when compared with the control group. The groups given prednisolone for a longer period showed reduction in numbers of peyers patches and lymphoid follicles, suggesting that higher doses of prednisolone tend to lower the number of peyers patches.

**Keywords:** Prednisolone, Lymphoid tissue, Small intestine, Peyers patches, Wistar rats

## 1. Introduction

Immunosuppression can be defined as a loss in the ability of the immune system to respond to a challenge at a level that is considered normal, whether or not clinical diseases ensue. Over the past decades, immunosuppression therapy has undergone striking changes in the scale and pace by which new immunosuppressive molecule and antibodies have become incorporated into daily transplant media (Bob *et al.*, 2004).

Immune response may be globally attenuated by drugs such as steroids or more specifically by Prednisolone, Cyclosporine A and Tacrolimus, which inhibit T lymphocyte proliferation by inhibiting expression of interleukin (Bradyden *et al.*, 2005). Such immunosuppression may cause persistence of normally mild infections such as cytomegalovirus or cryptosporidium and permit an increase in commensal organisms. In transplantation, intestinal graft-versus-host disease (GVHD) is a risk when the mass of the donor lymphocytes received is comparable with that of the recipient's as is the case in allogeneic bone marrow transplantation or even small bowel transplantation. Immunosuppressors like corticosteroids are synthetic glucocorticoids whose anti-inflammatory and/or immunosuppressive properties are widely used for the symptomatic treatment of many disorders, including asthma and arthritis. Glucocorticoids reduce inflammation and are used to treat a wide range of inflammatory and autoimmune conditions (Hooks, 1994; Walsh *et al.*, 1996).

Availability of new immunosuppressive agents has provided the opportunity for investigators to formulate novel and distinctly tailored strategies that employ combination therapies with the goal of decreasing doses of individual agents and minimizing their toxicities. While the non-sensitized patient undergoing first transplant is expected to require only modest immunosuppression, the immunologically high-risk patient requires more intensive immunosuppression. Prednisolone, a glucocorticoid remains a vital component of the maintenance immunosuppressive regimen of transplant recipients for the prevention of allograft rejection (Jusko *et al.*, 1992).

Prednisolone dosage is currently adjusted on empirical grounds alone, and protocols are primarily designed to reduce doses to a maintenance level as soon as possible, while taking into consideration differences in the pharmacokinetics of each transplant recipient. Consequently, there is considerable variation in the response to fixed doses of prednisolone between patients (Ulrich *et al.*, 1992).

The gut is a major component of the human immune system with a total lymphoid mass which is comparable with bone marrow (Dobbins, 1982). The gut is also a site of synthesis and release of a specialized form of immunoglobulin A (secretory IgA) which is resistant to digestion. These immunological mechanisms are important because the gut has a huge surface area which interacts with the numerous potentially noxious agents including micro-organisms and dietary antigens (Walker *et al.*, 1985; Webster, 1987).

The intestinal tract is also one of the most metabolically active tissues in the body, with mucosal renewal taking place every three to five days, it is not surprising therefore that the gut is often the target organ for pathological processes in the immunosuppressed patients (Papadopoulou *et al.*, 1996; Engelhard *et al.*, 1986).

The lymphoid organs are involved with the immune system and they affect growth, development and the release of lymphocytes. One of the lymphoid organs is the Peyer's patches which is the principal lymphoid tissue in the small intestine. The Peyer's patches contain high concentrations of white blood cells that help protect the body from infection and diseases. They detect antigens such as bacteria and toxins and mobilize highly specialized white blood cells (B cells) to produce protein structures called antibodies that are designed to attack foreign entities. These cells are important in the normal immune response to infection and tumors, but also mediate transplant rejection and autoimmunity (Fauci *et al.*, 1976).

Therapy with steroids such as prednisolone and hydrocortisone are major factors that compromise the mucosal barrier and have been shown to increase gut permeability with moderate use. Long term steroid use causes stomach and duodenal ulcers and immune suppression contributing significantly to gut hyper permeability and its complications (Bloemena *et al.*, 1990).

Therefore this study is aimed at investigating the immunosuppressive action of prednisolone using the peyer's patches as a surrogate marker and examine the gut mucosa for any adverse effect due to prednisolone administration.

## **2. Materials and Methods**

### **2.1. Location and Duration of Experiment**

This study was conducted in the Animal House of the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Anambra State. The animals were acclimatized for two weeks and the administration of prednisolone lasted for 28 days.

### **2.2. Breeding of Animals**

Twenty eight (28) apparently healthy male wistar rats weighing between 190g to 300g were used for this study. The rats were purchased from the Animal House, of the Department of Anatomy, College of Medical Science, Nnamdi Azikiwe University, Nnewi. The animals were fed ad libitum with grower's mash and distilled water for a period of twenty eight days.

### **2.3. Drug Preparation**

Prednisolone tablets produced by Shijiazhuang Pharma Group Zhongnuo Pharmaceutical Co., Ltd. were purchased from Nnamdi Azikiwe Pharmaceutical Science Centre Agulu. Six (6) tablets of 5mg prednisolone equivalent to 30mg prednisolone were dissolved in 12.52mls of distilled water daily and given orally based on the animal's individual body weight.

### **2.4. Experimental Protocol**

The twenty eight animals were randomly selected and divided into seven groups (A-G) of six animals each. Group A served as the control and received distilled water; while groups B, C and D received prednisolone orally for two weeks usually between the hours of 9am and 10am at concentrations of 2mg, 4mg, and 6mg/kg body weight respectively while groups E, F and G received prednisolone orally for four weeks between the hours of 9am and 10am at concentrations of 2mg, 4mg and 6mg/kg body weight respectively daily.

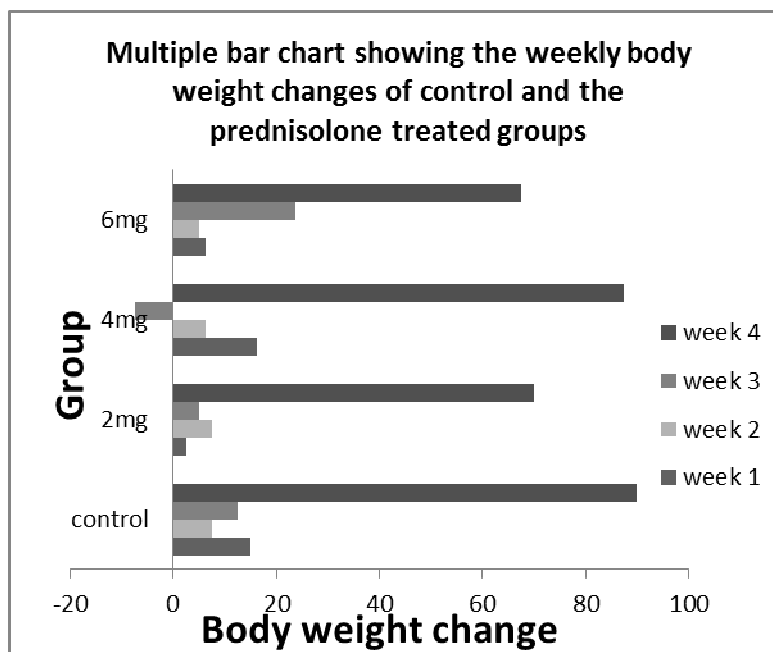
### **2.5. Data Analysis**

Data obtained from this experiment was analyzed using Statistical Programme for Social Sciences SPSS (Version 16) software package and the one way ANOVA test was used to compare the mean number of peyer's patches and lymphoid follicles of the control group and prednisolone treated groups. The Pearson's correlation coefficient test was used to compare the weekly body weight changes.

### 3. Results and Discussion

**Table 3.0** Indicates weekly body weight changes for the control group A, 2mg/kg, 4mg/kg and 6mg/kg prednisolone treated groups

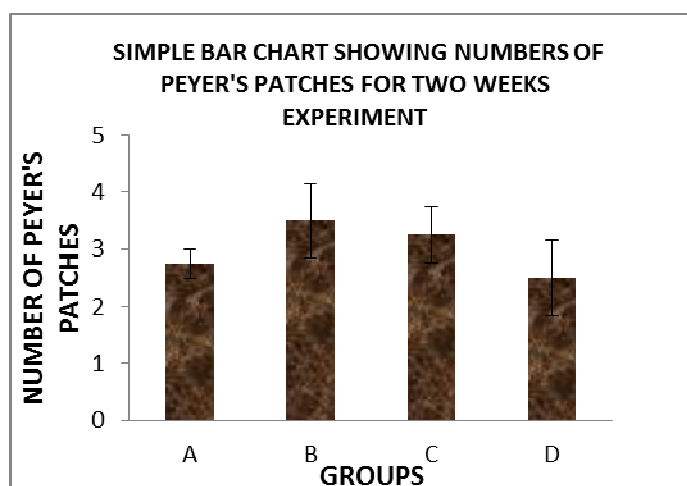
Groups	Control		2mg pred		4mg pred		6mg pred	
	weight	w.change	Weight	w.change	Weight	w.change	weight	w.change
<b>Acclimatization</b>	200	0	210	0	212.5	0	215	0
<b>week 1</b>	215	15	212.5	2.5	228.75	16.25	221.25	6.25
<b>week 2</b>	222.5	7.5	220	7.5	235	6.25	226.25	5
<b>week 3</b>	235	12.5	225	5	227.5	-7.5	250	23.75
<b>week 4</b>	305	90	295	70	315	87.5	317.5	67.5



**Fig 3.1** Multiple bar chart showing the weekly body weight changes for control, 2mg, 4mg and 6mg prednisolone treated groups.

**Table 3.1:** shows the mean values and the standard error of mean (SEM) of numbers of peyer’s patches in the control and prednisolone treated groups for the period of two weeks (Mean ± SEM).

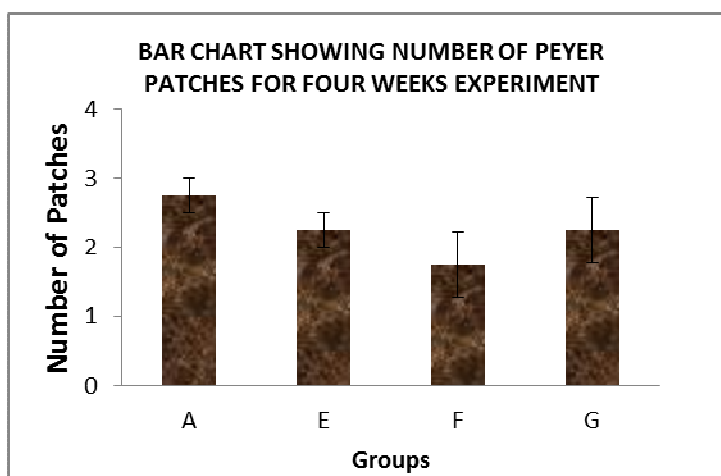
GROUP	SAMPLE SIZE	NUMBER OF PEYER’S PATCHES	F-RATIO	P-VALUE
<b>A (Control)</b>	4	2.75±0.25	1.582	0.202
<b>B (2mg/kg b. weight)</b>	4	3.5±0.65	1.582	0.202
<b>C (4mg/kg b. weight)</b>	4	3.25±0.48	1.582	0.202
<b>D (6mg/kg b. weight)</b>	4	2.5±0.65	1.582	0.202



**Fig 3.1** Bar chart showing the numbers of peyer's patches in prednisolone treated and control groups (Mean ± SEM)

**Table 3.2** shows the mean values and the standard error of mean (SEM) of numbers of peyer's patches in the control and prednisolone treated groups for the period of four weeks (Mean ± SEM).

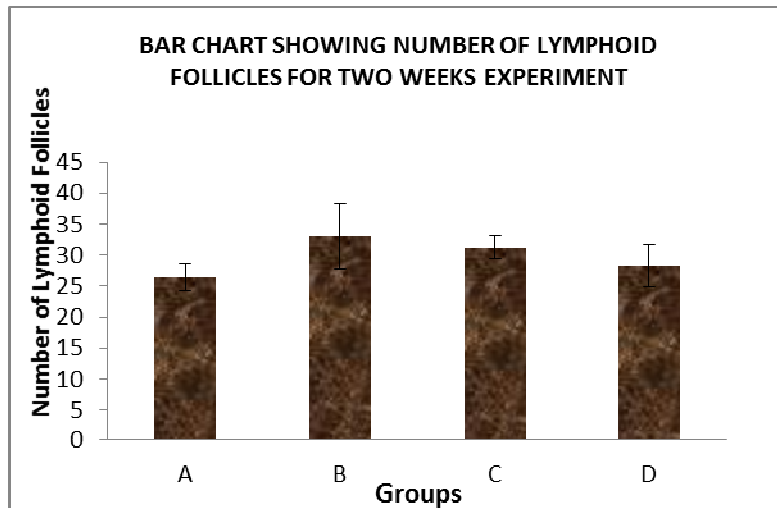
GROUP	SAMPLE SIZE	NUMBER OF PEYER'S PATCHES	F-RATIO	P-VALUE
A (Control)	4	2.75±0.25	1.582	0.202
E (2mg/kg b. weight)	4	2.25±0.25	1.582	0.202
F (4mg/kg b. weight)	4	1.75±0.48	1.582	0.202
G (6mg/kg b. weight)	4	2.25±0.48	1.582	0.202



**Fig 3.2** Bar chart showing the numbers of peyer's patches in prednisolone treated and control groups (Mean ± SEM)

**Table 3.3** shows the mean values and the standard error of mean (SEM) of numbers of lymphoid follicles in the control and prednisolone treated groups for the period of two weeks (Mean ± SEM).

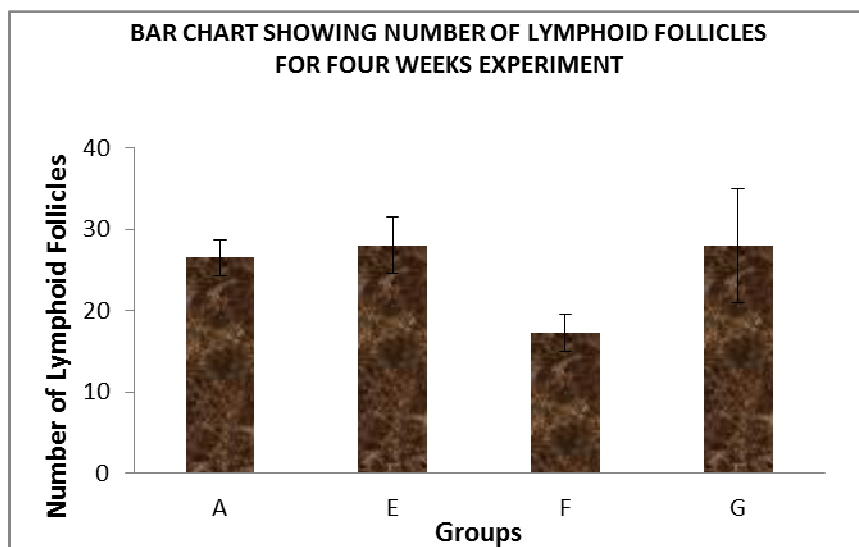
GROUP	SAMPLE SIZE	NUMBER OF LYMPHOID FOLLICLES	F-RATIO	P-VALUE
A (Control)	4	26.5±2.18	1.571	0.205
B (2mg/kg b. weight)	4	33±5.21	1.571	0.205
C (4mg/kg b. weight)	4	31.25±1.80	1.571	0.205
D (6mg/kg b. weight)	4	28.25±3.35	1.571	0.205



**Fig 3.3** Bar chart showing the numbers of lymphoid follicles in prednisolone treated and control groups (Mean ± SEM)

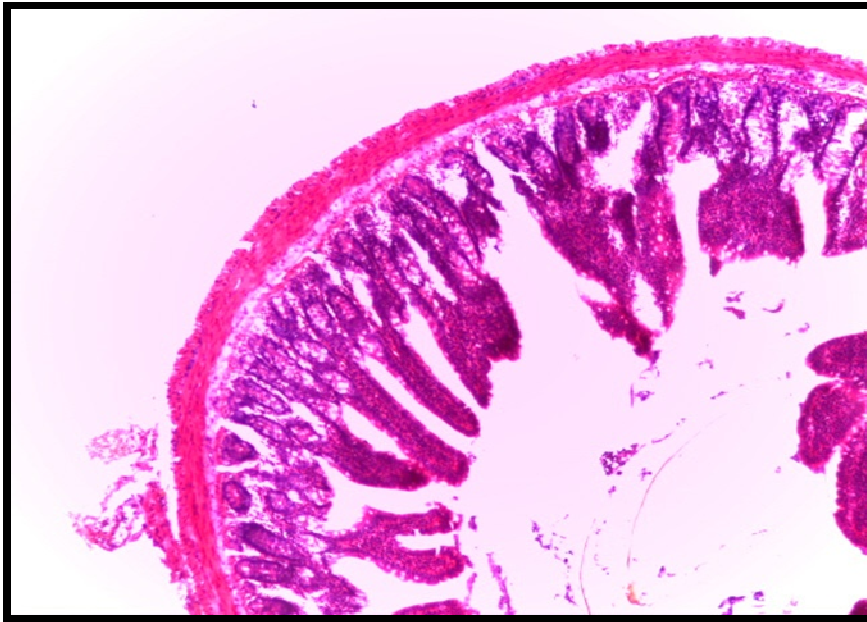
**Table 3.4** shows the mean values and the standard error of mean (SEM) of numbers of lymphoid follicles in the control and prednisolone treated groups for the period of four weeks (Mean ± SEM).

GROUP	SAMPLE SIZE	NUMBER OF LYMPHOID FOLLICLES	F-RATIO	P-VALUE
<b>A (Control)</b>	4	26.5±2.18	1.571	0.205
<b>E (2mg/kg b. weight)</b>	4	28±3.49	1.571	0.205
<b>F (4mg/kg b. weight)</b>	4	17.25±2.32	1.571	0.205
<b>G (6mg/kg b. weight)</b>	4	28±6.96	1.571	0.205



**Fig 3.4** Bar chart showing the numbers of lymphoid follicles in prednisolone treated and control groups (Mean ± SEM)

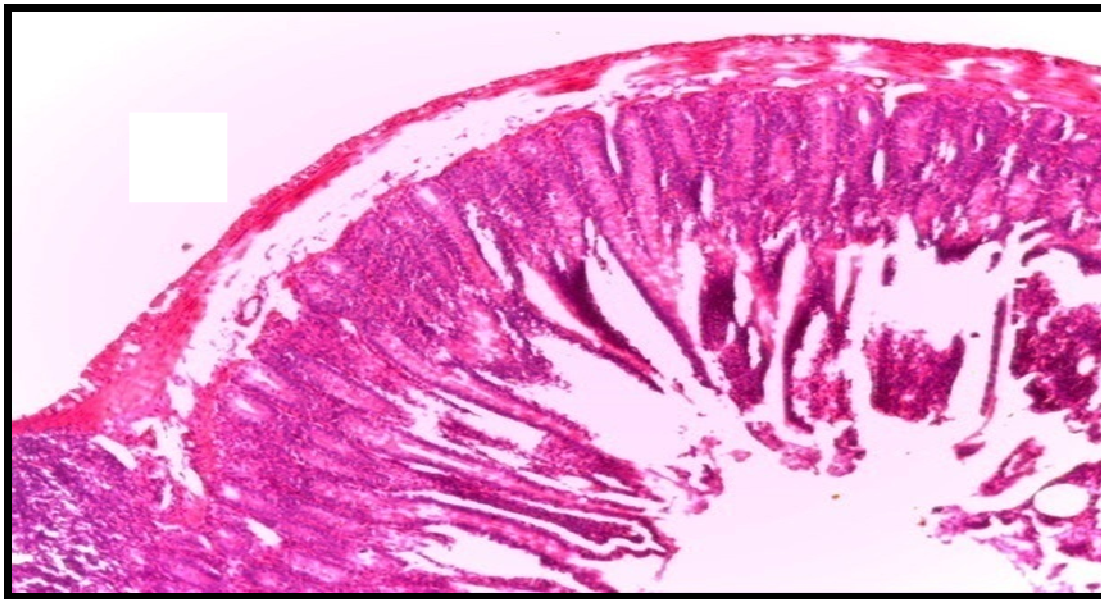
### 3.1. Histological Results



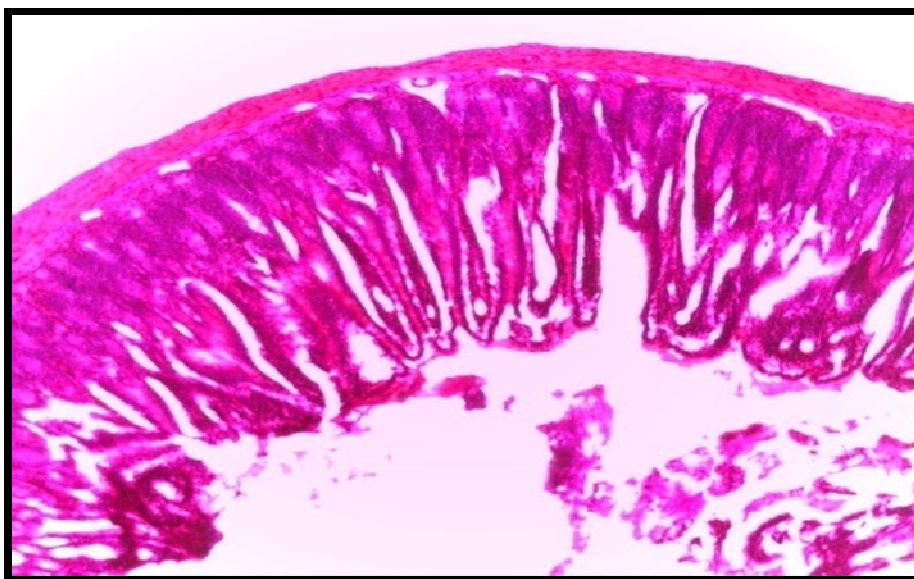
**Plate 1:** Photomicrograph of Peyer's patches in the small intestine of group A (control) showing normal histology x 200



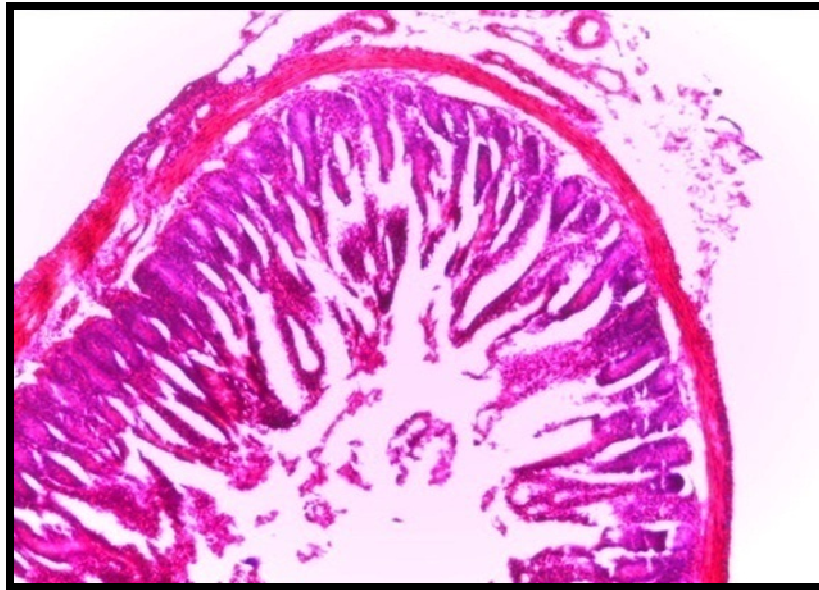
**Plate 2:** Photomicrograph of Peyer's patches in the small intestine of group B (2mg/kg prednisolone for 2 weeks) showing normal histology x 200.



**Plate 3:** Photomicrograph of Peyer's patches in the small intestine of group C (4mg/kg predniolone for 2 weeks) showing normal histology x 200.



**Plate 4:** Photomicrograph of Peyer's patches in the small intestine of group D (6mg/kg prednisolone for 2 weeks) showing normal histology x 200.

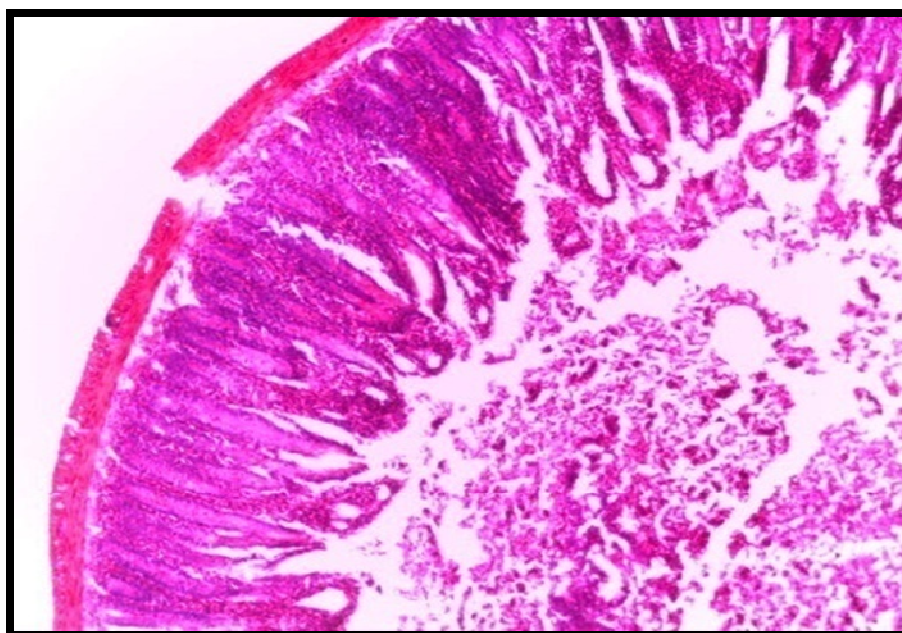


**Plate 5:** Photomicrograph of Peyer's patches in the small intestine of group E (2mg/kg prednisolone for 4 weeks) showing normal histology x 200.



**Plate 6:** Photomicrograph of small intestine showing increasing lymphocytic aggregation in the Peyer's patches of group F (4mg/kg prednisolone for 4 weeks) x 200.





**Plate 7:** Photomicrograph of small intestine with peyer's patches showing influx of lymphocytes and irregular lymphocytic aggregates of group G (6mg/kg prednisolone for 4 weeks) x 200.

Peyer's patches play an important role in the local immunity of the small intestines of mammals. Their numbers vary distinctly between species, from about 100–300 in man to 5–9 in rabbits (Cornes, 1965).

In this present study, 2 to 3 peyers patches were counted in the distal 10cm of the ileum of 11 to 13-week-old untreated or control wistar rats. Cyclophosphamide given orally to young adult wistar rats at doses of 5 and 10 mg/kg bw during 4 weeks or given by single intravenous injection of 50 mg/kg body weight reduced the number and size of peyer's patches and also reduced the number of lymphocytes in all compartments of peyer's patches (Fauci *et al.*, 1974), but in this study prednisolone given orally to young wistar rats at doses of 2, 4 and 6mg/kg body weight during 4 weeks reduced the number and size of peyers patches but increased the number of lymphocytes in peyers patches.

At the end of short term administration of prednisolone, groups B, C and D, showed a decrease in peyers patches while at long term administration of prednisolone, groups E, F and G, there was also reduction in the number of peyers patches. The reduction of peyer's patches was seen significantly in the rats treated with 4mg/kg body weight of prednisolone for 4 weeks. This observation was similar to the number of lymphoid follicles, therefore it can be deduced from this study that increased doses of prednisolone administered for a long duration may lower the number of peyer's patches and lymphoid follicles in rats.

Comparable effects of cyclosporin on the B-lymphocyte compartment of peyers patches have been described by previous works on reduction of B cells in mouse, reduced follicle cellularity and disappearance of germinal center in rat, reduction of height of domes and height and width of follicles in rat (Fauci, 1975).

In this present study, it was documented that prednisolone showed a powerful immunosuppressive effect in the peyers patches of the group F wistar rats administered 4mg/kg prednisolone orally as seen in Plate 6 where the PPs were seen to have increased lymphocytes aggregations. This is in line with previous studies in man and in experimental animals, in which it was demonstrated that glucocorticoids induce a decrease of all lymphocyte (sub)populations, namely T, B and null cells (Walzer *et al.*, 1974), although the effect on T cells, especially CD4<sup>+</sup> lymphocytes, is somewhat more pronounced in blood [18].

The most striking feature of prednisolone is the induction of a dramatic increase in number of lymphocytes in the peyer's patches. Oral administration of prednisolone at 4mg/kg or 6mg/kg selectively increased the number of lymphocytes to extremely high levels in peyer's patches within 2 to 4 weeks after administration thereby reducing the lymphocytes in blood.

It was also reported at the end of the 4 weeks experiment that group D rats treated with 6mg/kg body weight prednisolone had gained 38.5% of their original weight

#### 4. Conclusion

The result of this study has shown that administration of a glucocorticoid (e.g. prednisolone) reduces the size of the lymphoid tissue of the small intestine and at the same time increases the lymphocyte aggregations at 4mg/kg prednisolone and at higher doses such as 6mg/kg prednisolone given for four weeks, influx of lymphocytes and irregular lymphocytic aggregations occurred maybe due to excessive immune suppression. Steroids are first line

immunosuppressive therapy for both solid organ and haematopoietic stem cell transplant recipients High doses, e.g., 4mg/kg /d of prednisolone can be used to treat organ rejection or graft-versus-host disease without fear of marrow toxicity. The toxicity of high dose, long term prednisolone therapy can be severe.

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