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# Evaluation of Immunomodulatory Effects of Silkworm (*Bombyxmori* L.) Cocoon Extracts 0n Methylprednisolone Induced Rats

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***Abstract*-**The present study aimed to evaluate the immunomodulatory effects of cocoon extracts of Silkworm on methylprednisolone drug induced immunosuppression in rats. Silkworm cocoons were collected and extracts were prepared. A total of 24 Wistar Albino rats divided into four groups were used in the study. Group-I rats were treated with distilled water. Group-II rats received Methyl prednisolone (20mg/kg), Group-III rats given cocoon extract of Silkworm (300mg/kg) + Methyl prednisolone(20mg/kg) and Group-IV received cocoon extract of Silkworm (500 mg/kg) + Methyl prednisolone (20mg/kg). Physical and immunological parameters were measured on $6^{th}$ and $12^{th}$ day. At the end of study rats were sacrificed under anesthesia. Spleen was collected , weighed and examined for histopathology. The results and findings of the study suggest the immune enhancement activity of cocoon extacts of Silkworm on methylprednisolone induced immunosuppression.

***Keywords*:** Cocoon extracts, immunomodulatory, methylprednisolone, immunosuppression, , immunological, histopathology

## I. INTRODUCTION

Immune system plays a pivot role in the survival of an organism. If it becomes abnormal, either over abundance or over diminished it may lead to death. Defects in the regulation of the immune system are implicated in the pathogenesis of chronic inflammatory diseases and even dreadful diseases such as Cancer [1]. Alleviation or management of diseases by immunomodulation is attaining prominence and has been areas of research interest for years. Currently, majority of research and development focuses on Natural products and folklore medicines which are the main contributors in the design and development of potential drugs[2].

Methylprednisolone is an immunosuppressor drug used in the present study . It is used to treat conditions such as, low corticosteroid levels, arthritis, allergic reactions and certain types of cancers. It acts by various pathways to decrease the inflammatory cycle by dampening the inflammatory cytokine cascade [3]. During the short-term use, these immune suppressor medications interfere and suppress the immune cell function. Long term effects of these medications may have adverse drug reactions associated with side effects.

Recent years Insect extracts has gained importance in the scientific research. Insects and their extracts have been used in Folk Medicine to treat huge range of conditions including arthritis. These are among the oldest group of terrestrial organisms on earth. They have not only been used as food, but also played important roles in the treatment of diseases and other dysfunctions [4]. Recent years scientists are using knowledge acquired from exponents of folk Medicine to develop novel potential medicines for treating intractable diseases such as cancer and the problems associated with newly emerging antibiotic-resistant bacteria.

Among insects, *Bombyx mori* L. is the main lepidopteron used in scientific research. *Bombyx mori* a major player in sericulture has gained importance as a powerful biological model system for screening human drugs [5]. Recent studies reveal the importance of silkworm products in improving immunity, hypoglycemic effect [6], hepatoprotective, neuroprotective role and in treatment of cognitive disorders such as Alzheimer's disease [7]. Considering these facts the present study conducted to evaluate the immunomodulatory actions of cocoon extracts of silkworm on methylprednisolone induced immunosuppression in rats.

## II. MATERIALS AND METHODS

### Collection of Silk worms and Cocoons

Silkworms and cocoons were collected from local area at Regional Sericulture Research Station (RSRS) Rapthadu, Anantapuramu, Andhra Pradesh. Test samples were prepared from cocoons of *Bombyx mori*. Cocoons were boiled in 500 ml of deionised water and filtered through a

membrane. The filtrate was stored at -20˚C and used for studies.

**Animals**

Albino rats (Wistar strain) weighing 150-200 g were obtained from authorized animal house (Sri Raghavendra Enterprises, Bangalore). The animals were housed in cages under controlled conditions of temperature (25˚C) and alternating 12 hr cycle of light and darkness. The animals have free access to standard rat pellet diet and tap water *ad libitum*. The University Ethics Committee (1889/GO/RE/S16/CPCSEA-30.05.2016) has approved the experimental protocol at Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh.

| Groups | Dose/Route of administration | Number of rats |
|---|---|---|
| Group-I | Distilled water (Control) | 6 |
| Group-II | MethylPrednisolone (20mg/kg) (Inducing agent) | 6 |
| Group-III | Silkworm Cocoon extract (300 mg/kg)+ Methyl prednisolone (20 mg/kg/) (Test-1) | 6 |
| Group-IV | Silkworm cocoon extract (500 mg/kg)+ Methyl prednisolone (20 mg/kg/) (Test-2) | 6 |

**Procedure**

The 24 rats were used in this study which were divided into 4 groups with 6 each. Group-I was treated with distilled water. Group-II received Methylprednisolone (20 mg/kg), Group-III given Silkworm cocoon extract (300 mg/kg)+ Methyl prednisolone (20 mg/kg) (Test-1) and Group-IV received Silkworm cocoon extract (500 mg/kg)+ Methylprednisolone (20 mg/kg) (Test-2). Methylprednisolone and test drugs were administered to their respective groups for 12 days. Blood was collected on $6^{th}$ and $12^{th}$ day and used for the estimation of physical, immunological and biochemical parameters.

## III. OBSERVATIONS

**Physical parameters**

Body weight and isolated Spleen weight (gm**)** was recorded using digital electronic balance.

Food intake (gm) is calculated as "The weight of food kept in the cage - The food remaining in the cage after 24h".

**Immunology parameters**

**Estimation of humoral antibody response to SRBC (Hemagglutination antibody titer test) (%)**

Blood was collected from healthy sheep, mixed with Alsever's solution (1:1) and then centrifuged at 1609.92×*g* for 5 min to enable RBCs to settle at the bottom of the test tube. The supernatant was discarded, leaving sheep red blood cells (SRBC) pellets, that were washed three times with pyrogen-free phosphate buffered saline (pH 7.2) and kept under refrigeration for further immunization and challenge studies. Rats were immunized by injecting 0.5ml of SRBCs at the end of the $12^{th}$ day. Blood samples were collected by cardiac puncture and was centrifuged at 1609.92×*g* to get serum. Antibody titers were then determined by the hemagglutination technique. Serum was diluted two fold times serially using normal saline in microtiter plates of 96-well capacity and then SRBCs (25 µL of 1% SRBC prepared in normal saline) was added to each of these dilutions and incubated at 37 °C for 1 h for hemagglutination examination. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the hemagglutination antibody titer in percentage (%).

## IV. HEMATOLOGICAL STUDIES

**RBC Count**

RBC count was done with a Neubauer counting chamber. The blood was collected in a vial containing 1% EDTA as an anticoagulant. Blood was drawn up to 0.5 mark in RBC pipette and then immediately the RBC diluting fluid was drawn up to the mark 101. It was then shaken gently and allowed to stand for 2 to 3 min. The counting chamber and the cover glass were well cleaned and cover glass was placed over the ruled area. Again the solution was expelled and a drop of fluid was allowed to flow under the cover slip holding the pipette at an angle 40 degrees. It was allowed to stand for 2-3 min to settle RBC. It was then focused under the microscope and the number of RBC was counted on 5 small squares of the RBC column under 100 x magnifications. The number of RBC per cubic mm was calculated.

**WBC Count**

The blood was collected in vials containing 1% EDTA as an anticoagulant. The blood was drawn up to 0.5 mark of WBC pipette and immediately WBC diluting fluid was drawn up to the 11 mark. It was allowed to stand for 2-3 min. The counting chamber and cover glass were cleaned and the cover glass was placed over the ruled area. Again the solution was expelled and a drop of fluid was allowed to flow under slip holding the pipette at an angle 40 degrees. It was allowed to stand for 2-3 min to settle WBC. It was then focused under the microscope and the number of WBC was counted in 4 corner square millimeters. The number of WBC per cubic mm was calculated.

**Estimation of Haemoglobin (Hb)**

Sahli's haemglobinometer was used for determining the hemoglobin content by acid haematin method. The haemoglobin tube was filled with 0.1N HCl upto the 20 mark. Blood is drawn up to 20µl mark of hemoglobin pipette and was dispensed into haemoglobin tube containing 0.1N HCl. Tube was placed at room temperature for 10 minutes for complete conversion of hemoglobin into acid hematin. After completion of the reaction Hb tube was placed in the column in Sahli's Comparision box and the dark brown coloured compound formed in the Hb tube was diluted using the 0.1N HCl or water by adding drop by drop into the solution and mixed with the help of stirrer after each addition.This process is done until the endpoint comes matching the colour of standard with the colour of the test.

## V. RESULTS AND DISCUSSION

**Effect of silkworm cocoon extracts on physical parameters**

Body weight, food intake, water intake, spleen weight were taken as physical parameters in the study. Body weight, food intake and water intake were measured on day zero, day six and day twelve and compared. Group-II rats showed small decrease in body weight, food intake and spleen weight compared to control. Co-administration of cocoon extracts of silkworm significantly prevented methylprednisolone induced changes in body weight , food intake and spleen weight.

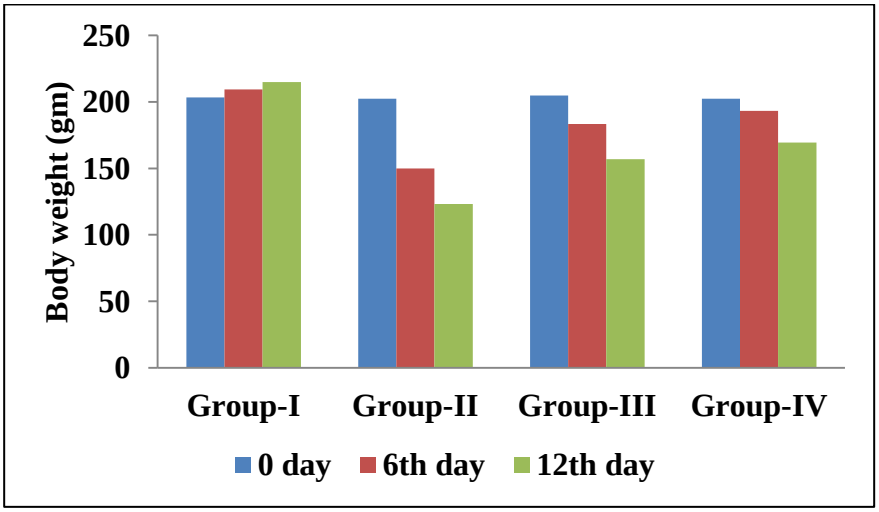
Fang Jet al., 2011 [8] studied the influence of methylprednisolone (MPL) on food consumption and body weight (BW), and the effects of MPL on glycemic control in normal male Wistar rats. Similar results were obtained in our study which caused reduction of food, water consumption and body weight during methylprednisolone treatment.

Mishina et al., 1994 [9] investigated the effect of a liposomal formulation of methylprednisolone (MPL) on the inhibition of lymphocyte proliferation in spleen of rats. Results demonstrate enhanced efficacy of local immunosuppression by targeting spleen with liposomal methylprednisolone. Kubeck et al., 2006 [10] analyzed the histologic effects of high-dose human equivalent methylprednisolone on hepatic, and splenic tissues in a spinal cord injury rat model. Results indicate Lymphocytic depletion in spleen of methylprednisolone treated rats.

**Table-1: Effect of Cocoon extracts of Silkworm on body weight**

| Groups | Body weight (gm) (MEAN±SD) | | |
|---|---|---|---|
| | 0 day | 6th day | 12th day |
| Group-I | 203.45±1.34 | 209.34±1.45 | 214.98±1.45 |
| Group-II | 202.34±2.13 | 150.02±2.21* | 123.12±1.34* |
| Group-III | 204.84±1.12 | 183.45±1.12*,# | 156.90±1.65*,# |
| Group-IV | 202.45±1.03 | 193.12±1.84*,# | 169.45±1.03*,# |

**(*p<0.05significant compared Group-I with other groups, #p<0.05significant compared Group-II with other groups)**



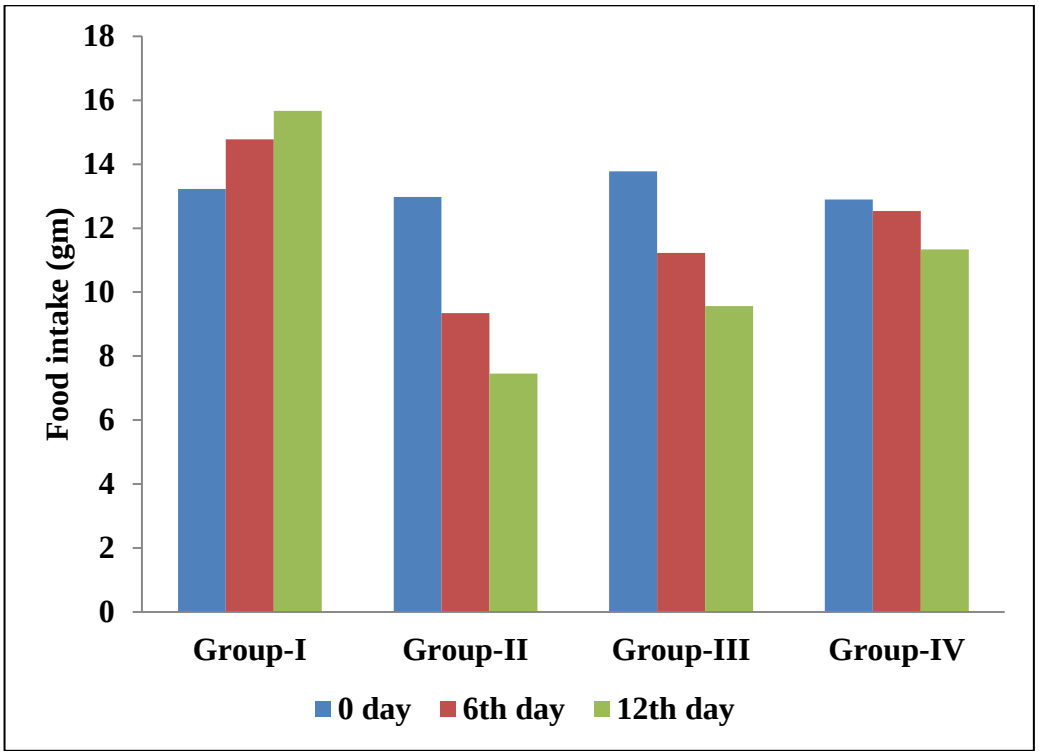

**Graph-1: Effect of Cocoon extracts of Silkworm on body weight**

**Table-2: Effect of Cocoon extracts of Silkworm on food intake**

| Groups | Food intake (gm) (MEAN±SD) | | |
|---|---|---|---|
| | 0 day | 6th day | 12th day |
| Group-I | 13.23±1.19 | 14.78±2.16 | 15.67±2.67 |
| Group-II | 12.98±2.13 | 9.34±1.03* | 7.45±1.94* |
| Group-III | 13.78±1.12 | 11.23±1.92*,# | 9.56±0.93*,# |
| Group-IV | 12.90±1.10 | 12.54±1.34*,# | 11.34±1.45*,# |

**(*p<0.05 significant compared Group-I with other groups,**
**#p<0.05 significant compared Group-II with other groups)**



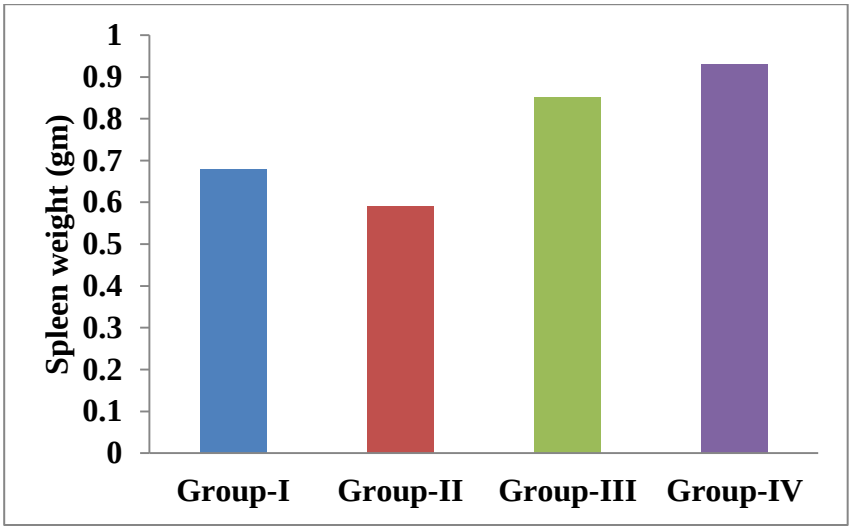

**Graph-2: Effect of Cocoon extracts of Silkworm on food intake**

**Table-3: Effect of Cocoon extracts of silkworm on spleen weight**

| Groups | Spleen weight (gm) (MEAN±SD) |
|---|---|
| Group-I | 0.68±2.56 |
| Group-II | 0.59±1.23* |
| Group-III | 0.85±2.56*,# |
| Group-IV | 0.93±1.13*,# |

**(*p<0.01 significant compared Group-I with other groups,**
**#p<0.01 significant compared Group-II with other groups)**




**Graph-3: Effect of Cocoon extracts of silkworm on spleen weight**

## VI. IMMUNOLOGICAL PARAMETERS

**Effect of Cocoon extracts of Silkworm on Humoral antibody titter**

In SRBC challenge test Group-II showed significant ($p<0.05$) decrease in the AB titer values compared to control group. Rats treated with the cocoon extracts of silkworm significantly prevented the methylprednisolone induced changes in SRBC challenge. Gluco corticoids are important in anti-inflammatory and immunosuppressive therapies. Intravenous methylprednisolone is used to treat acute relapses of multiple sclerosis as it is known to suppress the immunological activation. In this study also rats treated with methylprednisolone showed decrease in titer value on $12^{th}$ day. The decrease in the titer value could be dueto the effect of methylprednisolone on immune system.

Studies on the Gamma irradiated silk Fibroin for anti tumor activity and non specific immune response indicate that it augmented the important elements of innate and adaptive immune response by enhanced NK cell activity ,T-Lymphocyte proliferation, NO production and by the cytokine levels[11]. Promphet et al 2014[12] also studied Silk Lutein extracts prepared from *Bombyx mori* cocoons for boosting immunity. In-Vivo Studies reveal that feeding with silk lutein extract increased the populations of CD3+ and CD4 + CD3 + cells.

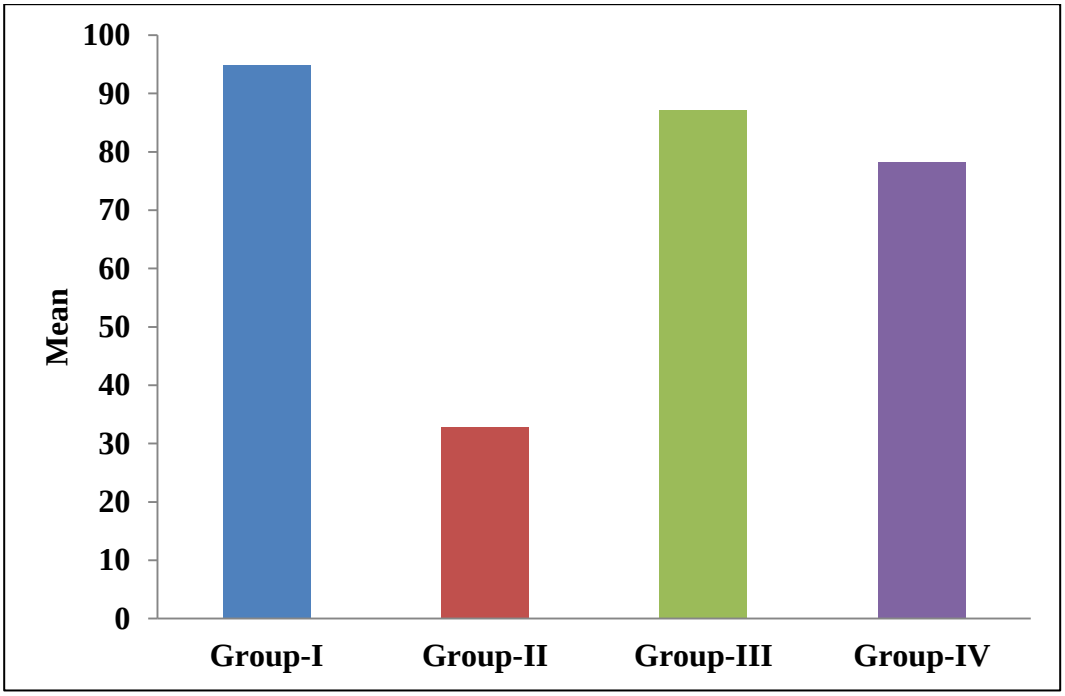
In the present study also co-administration of Silkworm cocoon extracts increase the titer value compared to methylprednisolone induced group. This effect may be due to stimulation of the immune system with SRBC. Upon stimulation with sheep RBC antigen, the levels of anti-RBC antibodies in plasma were elevated in rats treated with cocoon extracts. The increased concentration of antigen-specific antibodies would thus indicate the enhancement activity of the silkworm cocoon extract on humoral immune system responding to pathogens and other foreign particles.

**Table-4: Effect of Cocoon extracts of Silkworm on Humoral antibody titter**

| Groups | Humoral antibody (SRBC challenge) (MEAN±SD) |
|---|---|
| Group-I | 94.90±1.89 |
| Group-II | 32.90±2.02* |
| Group-III | 87.12±1.34*,# |
| Group-IV | 78.34±2.19*,# |

**(*p<0.05 significant compared Group-I with other groups,**
**#p<0.05 significant compared Group-II with other groups)**




**Graph-4: Effect of Cocoon extracts of Silkworm on Humoral antibody titter**

## VII. HEMATOLOGICAL PARAMETERS

**Effect of Cocoon extracts of Silkworm on Blood parameters**

Group II showed significant ($p<0.05$) decrease in RBC count and hemoglobin levels compared to control group. Rats treated with Silkworm cocoon extracts showed changes in RBC count and hemoglobin content compared to control and methylprednisolone treated group.

Airla et al., 2004 conducted a study on cDNA microarray for 448 genes to identify the target genes in methylprednisolone therapy. Results indicate methylprednisolone significantly reduced mRNA levels for T-cell-specific transcription factors. Fitzpatricket al., 1987 [13] studied the effect of several steroids on the regenerating thymus in male rats. Histologically, much of the thymus remained poorly organized and contained a much lower density of thymocytes. The total white blood cell count especially lymphocytes was significantly reduced in those animals,

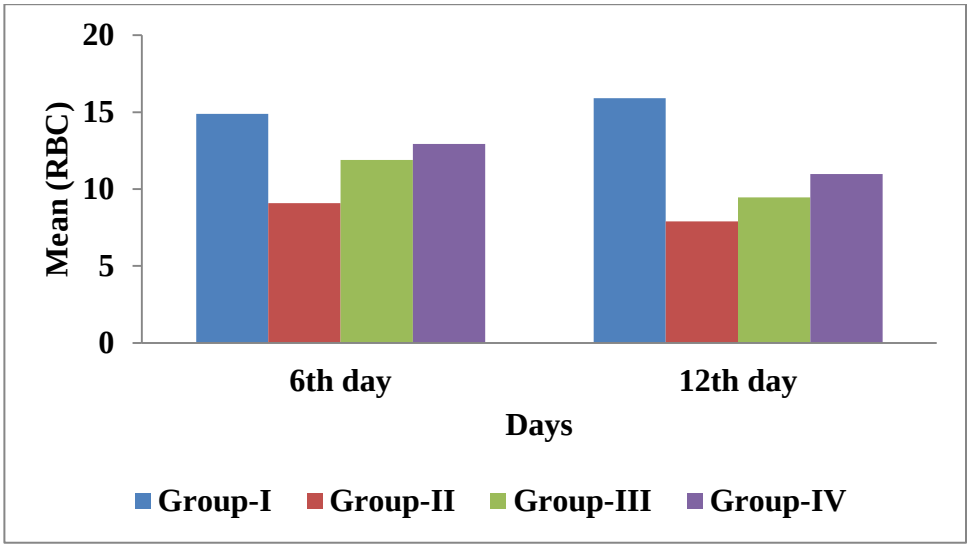
Similar effects were also observed in the present study.In the present studytotal white blood cell count and percentage of lymphocytes were significantly ($p<0.05$) decreased in Group-II compared to Group-I. G-III and IV showed difference ($p<0.05$) compared to G-II and the difference was significant.

**Table-5: Effect of Cocoon extracts of Silkworm on RBC**

| Groups | RBC ($10^6$ cells/$mm^3$) (MEAN±SD) | |
|---|---|---|
| | $6^{th}$ day | $12^{th}$ day |
| Group-I | 14.89±1.34 | 15.90±1.90 |
| Group-II | 9.09±2.78* | 7.89±2.12* |
| Group-III | 11.89±3.23$^{*,\#}$ | 9.45±3.14$^{*,\#}$ |
| Group-IV | 12.93±1.12$^{*,\#}$ | 10.98±2.90$^{*,\#}$ |

**(*p<0.05 significant compared Group-I with other groups,**
**$^{\#}$p<0.05 significant compared Group-II with other groups)**

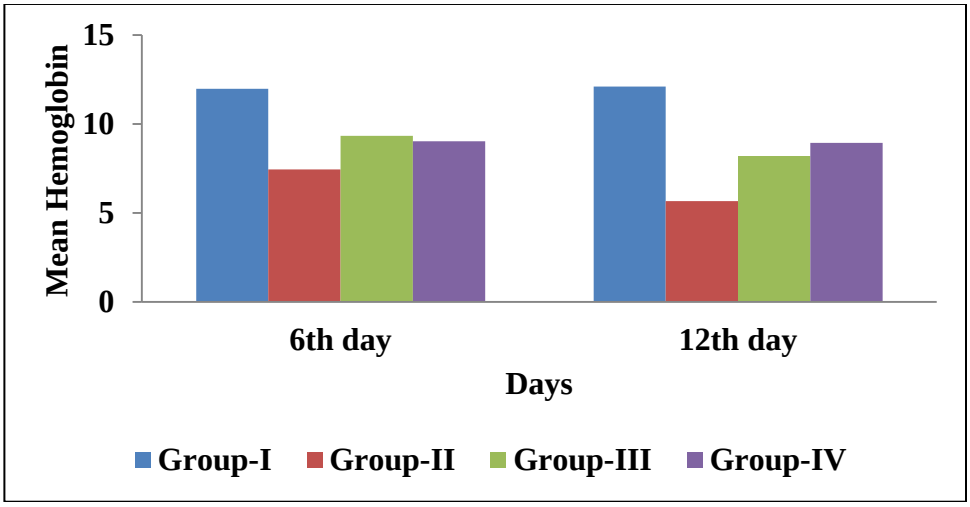



**Graph-5: Effect of Cocoon extracts of Silkworm on RBC**

**Table-6:Effect of Cocoon extracts of Silkworm on hemoglobin**

| Groups | Hemoglobin (g $dL^{-1}$) (MEAN±SD) | |
|---|---|---|
| | $6^{th}$ day | $12^{th}$ day |
| Group-I | 11.98±1.45 | 12.10±1.67 |
| Group-II | 7.45±1.91* | 5.67±2.90* |
| Group-III | 9.34±2.78*,# | 8.21±1.12*,# |
| Group-IV | 9.03±1.83*,# | 8.94±2.67*,# |

**(*p<0.05 significant compared Group-I with other groups,**
**#p<0.05 significant compared Group-II with other groups)**




**Graph-6: Effect of Cocoon extracts of Silkworm on hemoglobin**

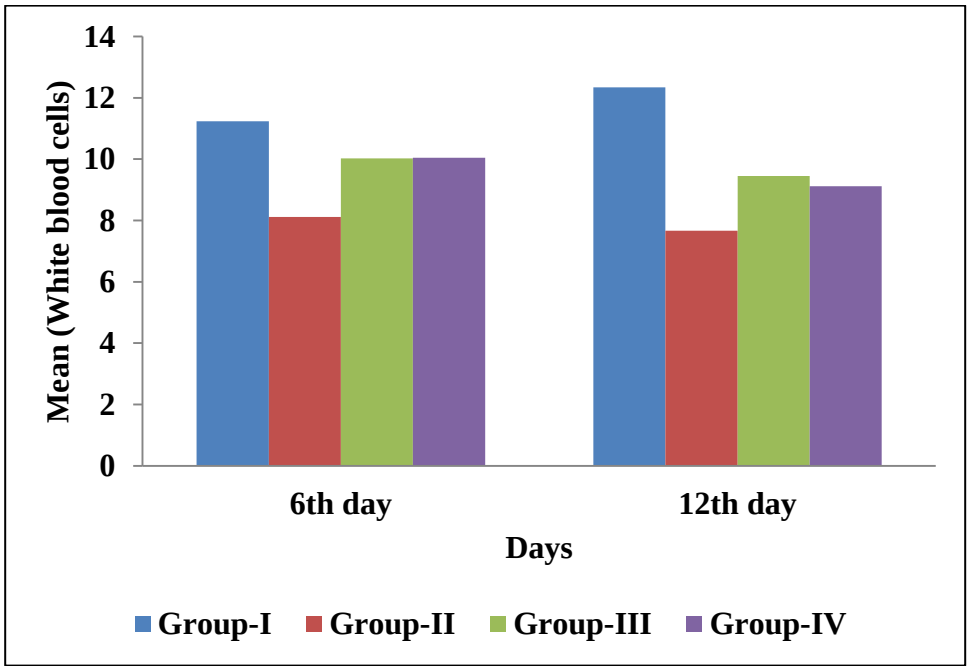
**Table-7: Effect of Cocoon extracts of Silkworm on WBC**

| Groups | White Blood Cells (x$10^3$ cells/$mm^3$) (MEAN±SD) | |
|---|---|---|
| | $6^{th}$ day | $12^{th}$ day |
| Group-I | 11.23±1.93 | 12.34±2.90 |

| | | |
|---|---|---|
| Group-II | 8.11±2.94* | 7.67±1.09* |
| Group-III | 10.02±2.34*,# | 9.45±2.09*,# |
| Group-IV | 10.04±1.23*,# | 9.12±3.91*,# |

**(*p<0.05 significant compared Group-I with other groups,**
**#p<0.05 significant compared Group-II with other groups)**




**Graph-7: Effect of Cocoon extracts of Silkworm on WBC**

## VIII. CONCLUSION

Cocoon extract of Silkworm enhanced the antibody production against sRBC and increased the WBC count in methylprednisolone treated rats. From the above results, it can be concluded that cocoon extracts of Silkworm has potential immunomodulatory activity. This finding is of clinical significance and provides scientific understanding to further investigate other pharmaco-therapeutic properties of Silkworm extracts and their products.

## IX. ACKNOWLEDGEMENT

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