



Amelioration of Endosulfan Induced Hematological Alterations in Mice by *Phyllanthus emblica* and *Withania somnifera*

Pryuttma^{1,*}, Parimal K. Khan²

¹Department of Zoology, GMRD College Mohanpur Samastipur, LNMU, Darbhanga, India

²Department of Zoology, Patna Science College, Patna University, Patna, India

Email address:

pryuttma@gmail.com (Pryuttma)

*Corresponding author

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Abstract: Endosulfan is a man made insecticide which is highly controversial agrochemical due to its acute toxicity potential for bioaccumulation but still used in large scale in India even though it is being phased out globally. Endosulfan plays a great role in alteration of Hematological status of human beings. The present study was carried out to investigate the acute effects of endosulfan on Hematological parameters such as Erythrocyte count (RBC), Leucocyte count (WBC), percent Hemoglobin (Hb%), Packed cell volume (PCV%) and Mean Corpuscular Volume (MCV) on swiss albino mice. Adult male mice was segregated into four groups, each group with 6 animals. Group I, served as the control was administered with distilled water @0.1 ml/10gb.w; Group II, received endosulfan @3mg/kg b.w; Group III, concomitantly received *Phyllanthus emblica*; Group IV, concomitantly received *Withania somnifera* (Ashwagandha) 150 mg/kg b.w along with endosulfan through oral gavaging. All the treatments were continued for 3 months. The toxic effects observed upon endosulfan treatment include Erythrocyte count (RBC) gradually decreased, Leucocyte count (WBC) increased, percent Hemoglobin (Hb%) significantly decreased, Packed cell volume (PCV%) reduced significantly and Mean Corpuscular Volume (MCV) significantly increased. On contrast, treatment of extract of *Phyllanthus emblica* and *Withania somnifera* was found to alleviate the toxic consequences of endosulfan, thereby producing ameliorative effect.

Keywords: Endosulfan, *Phyllanthus emblica*, *Withania somnifera*, Erythrocyte Count, Leucocyte Count, Hemoglobin, Packed Cell Volume, Mean Corpuscular Volume

1. Introduction

India is the second largest populous country with nearby 1.33 billion people. Due to tremendous growth of population over a confined country's land there is the share of every individual. Thus to fulfil the growing demand there was need to increase production per unit area for which green revolution come into existence under guidance of M. S. Swaminathan from 1965, the increased uses of chemical fertilizers, pesticide and high yield variety (HYV) seeds, Initially it gave good result but as time passed it was found that chemical fertilizers further resulted to the degradation of soils natural fertility. Immense chemical pesticide use for long term lead to the death of insects which helps in

pollination.

The intensive chemicalisation of world's agriculture by indiscriminate and reckless use of chemical pesticide led to the large scale contamination of our living environment. Among these chlorinated pesticides, as they are long lived and fat soluble in the environment for very long periods, causing their bioaccumulation and biomagnification which in turn impact toxicity to non-target organisms including human beings. Accordingly manufacture and use of several chlorinated pesticides has either been banned or severely restricted.

Endosulfan is one of the man made insecticide with chemical formulae 6, 7, 8, 9, 10, 10-hexachloro-2,4,6,9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide is

a broad-spectrum organochlorine insecticide and acaricide. It is used for control of a number of insects and pests on a variety of food crop field, fruit, cashew, tea-coffee and vegetables crops [23] and on other cash crops like tobacco, cotton and timber crops. Endosulfan is applied to crops over 100 different pest (EPA, 1980 b).

Technically, Endosulfan is a 7:3 mixture of stereoisomers alpha and beta. Persistence of endosulfan in soil and water have been widely reported by several researchers different conditions [29]; even frequently found in the environment at considerable distance from the point of its original applications owing to its potential transport [22]. The chemical row has been banned worldwide by the Stockholm convention of persistent Organic pollutants (POPs) due to its unacceptable. Acute toxicity may lead to nausea, vomiting, skin irritation, redness and pain of eyes [18], chronic toxicity lead to immunosuppression, neurological disorders, congenital birth defects, chromosomal abnormalities, mental retardation, impaired learning and memory loss [31]. And reproductive risks to farm workers and wild life is still used in some developing nations including India.

The effect of Endosulfan are most pronounced in immature animals whose reproductive systems and brains are still developing [8]. Blood or hematological parameters are probably the most rapid and detectable variations under stress and are fuel in assessing different health conditions [32]. Hence, the significance of hematological parameters in clinical and experimental studies in life sciences cannot be overemphasized. Particularly literature reports have proved that the alterations in the hematological parameters, from normal state may be used as valuable indicators of disease, or stress in different animal species [34]. Assessment of hematological parameters can therefore be used to determine the extent of damage from foreign substances on the blood constituents of an animal [12].

The *Phyllanthus emblica* commonly known as Indian gooseberry or Amla (belonging to deciduous tree of the family phyllanthaceae). The branchlets are glabrous or finally pubescent 10-20 cm long, usually deciduous. The fruit is nearby spherical, light greenish yellow, quite smooth and hard with six vertical stripes a furrows [21]. This plant is of great medicinal importance. It has antiviral and antimicrobial properties [30]. There is preliminary evidence in Vitro that its extract induces apoptosis and modify gene expression in osteoclasts involved in rheumatoid arthritis and Osteoporosis [27]. It prove to have potential activity against some cancers also [24]. The earlier studies have demonstrated that the fruit in this plant has potent antimicrobial [3], antioxidant [5], adaptogenic [29], hepatoprotective [19] and anticulerogetic activities (*Phyllanthus emblica* leaf extracts have also been shown to possess anti-inflammatory activity).

Withania Somnifera known commonly as Ashwagandha, Indian ginseng, poison gooseberry or winter cherry. It is a low lying perennial shrub in the Solanaceae or night-shade family. It is used as herb in Ayurvedic medicine [13]. The plants long, brown, tuberous roots are used in traditional medicine [26]. The roots of the plant are categorized as rasayans (Store house

of chemicals), which is reported to promote health and longevity by augmenting defense against disease, arresting aging process and as an antioxidant also. Leaves and root of this plant are abortifacient, aphrodisiac, diuretic, nerving toxic, alliterative, narcotic, sedative, growth promoter and anthelmintic. The earlier studies have demonstrated that the root of this plant has potent acts as anti-oxidant activity [10], Anti-Carcinogenic activity [17], Anti inflammatory activity [1], Anti-aging activity [7], Hypothyroid activity [25] and Immunomodulatory activity [9].

In the present work, ameliorating effect of the fruit extract of *Phyllanthus emblica* and root extract of *Withania somnifera* is used to mitigate the effect of endosulfan on some of the blood parameters like RBC, WBC, Hb%, PCV% and MCV.

2. Material and Methods

Young and sexually mature Swiss Albino mice (*Mus musculus*), each with body weight 2.57 gm, (Obtained from DRL Lucknow) were maintained in the animal house of Department of Zoology, TM Bhagalpur University, Bhagalpur. A total of 24 mice segregated into 4 experimental group were kept separately in cages at 24°C temperature and humidity with 12±1 hr light/dark cycle. Body water was made available to the animal ad libitum. All the animals were maintained according to the accepted principal for laboratory animal use and care as per guidelines of CPCSEA. Initially, the mice were acclimatized for two weeks before the start of experiment.

2.1. Test Substance as Used

The pesticide used for treatment of animals were endosulfan, purchased from the market under trade name of enclosed 35EC (Excel industries Pvt. Ltd; Mumbai). It is a dark brown liquid consisting of 35% w/w endosulfan technical (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a hexahydro-6, 9-methano-2,4,3-benzo dioxathiepin-3-oxide) widely used as broad spectrum organochlorine insecticide and acaricide.

2.2. Treatment Protocol

The 1/8th of the LD50 dose of endosulfan (3.0 mg/kg b.w.) was administered orally for chronic toxicity study. Once daily, for 30 days by gavage method.

Similarly, maximum tolerable dose (MTD) of *Phyllanthusemblica* (100 mg/kg b.w.) and *Withania somnifera* (150 mg/kg b.w.) was administered for amelioration study at regular interval of 28 days by gavage method.

2.3. Experimental Design

a) The animals were divided into 4 experimental groups with 6 animals in each group.

Group - I: Animals were maintained as untreated ones (Control);

Group - II: Animals were treated with endosulfan only;

Group - III: Animals were treated with endosulfan

followed by fed with fruit extract of *Phyllanthus emblica*;

Group - IV: Animals were treated with endosulfan followed by fed with root extract of *Withania somnifera*.

b) Fruit extract of Amla (Phyllanthus emblica):

Ripe fruits of Amla (*Phyllanthus emblica*) were collected from healthy plants during the winter season in plastic bags at Bhagalpur and were subsequently washed in running tap water. Collected fruits were dried in hot air over at 60°C [14] for 5-8 days and then powdered coarsely with the help of glass mortar and pestle. The powder so obtained was sexhlated with ethanol (80% v/v) [20] at 60-70°C [14] for 50-90 hours till the return of the usual shine of ethanol (full extraction).

c) Extract prepration of Withania Somnifera (Ashwagandha):

The roots from healthy plants of *W. somnifera* were collected from Bhagalpur. The mature roots were collected in plastic bags and were washed immediately in running tap water to remove adhered dust and other extraneous materials. The roots were dried in hot air over at 60C [14] for 5-8 days and then powdered coarsely with the help of glass mortar and pestle. The powder was sexhlated with ethanol (80% v/v) [20] at 60-70°C [14] for 50-90 hours till the return of the usual shine of ethanol (full extraction). The extract solvent was concentrated under reduced pressure with the help of rotatory evaporator and finally dried on water bath at 60°C [15].

3. Results and Discussion

Hematological parameters are probably the more rapid and detectable variations under stress [16, 12]. Hence, the significance of hematological parameter in clinical and experimental studies in life sciences cannot be ignored. Literatures have proved that the alterations in the hematological parameters from normal state may be used as valuable indicators of disease or stress in different animal species [34].

3.1. Erythrocyte Count

The Erythrocyte count of control mice (Group I) were recorded after each incubation period and the values ranged from 5.96 million per cubic mm at days 1 to 28. There was a significant decrease of 3.96±0.08 million per cubic mm of blood was noticed in the Erythrocyte count at day 28 in the endosulfan treated mice (Group II). The gradual decrease in Erythrocyte count in (Group II) was corrected by supplementing fruit extract of *Phyllanthus emblica* and root extract of *Withania somnifera* mixed with their food. It was found that at day 28, Erythrocyte count increases significantly from 3.99 million per cubic mm to 5.16 million per cubic mm in Group III and 5.77 million per cubic mm in Group IV mice *Phyllanthus emblica* and *Withania somnifera* extract treated mice group when compared with Endosulfan treated mice.

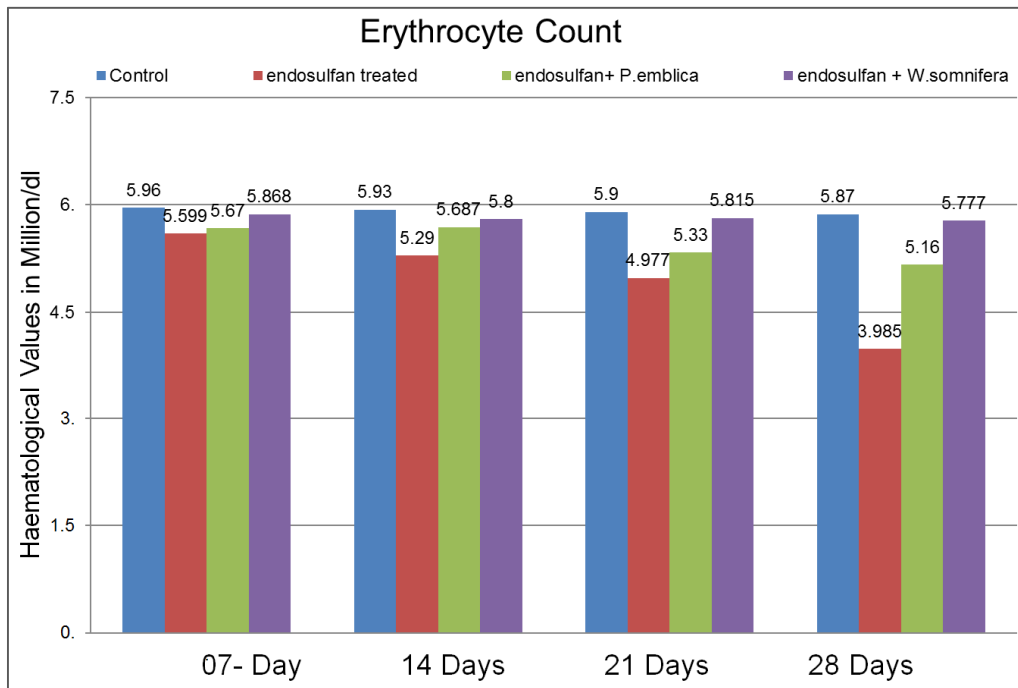


Figure 1. Ashwagandha- Withania somnifera extract for Four weeks on RBC Count (10⁶/mm³).

3.2. Leucocyte Count

The Leucocyte count (WBC) of control mice were recorded after incubation period and the values ranged from 6.20 thousand per cubic mm to 6.36 thousands per cubic mm at days 1 to 28.

There was significant increase in number of WBC to 8.11 thousand per cubic mm in endosulfan treated (Group II)

when compared to control mice.

The gradual recovery in Leucocyte count in Group II (endosulfan treated mice) was noticed by application of Amla and Ashwagandha extract. It was found that at days 28, the Leucocyte count significantly decreased from 8.11 to 6.93 thousand per cubic mm in Group III mice and 6.42 thousand per cubic mm in Group IV mice when compared with endosulfan treated mice.

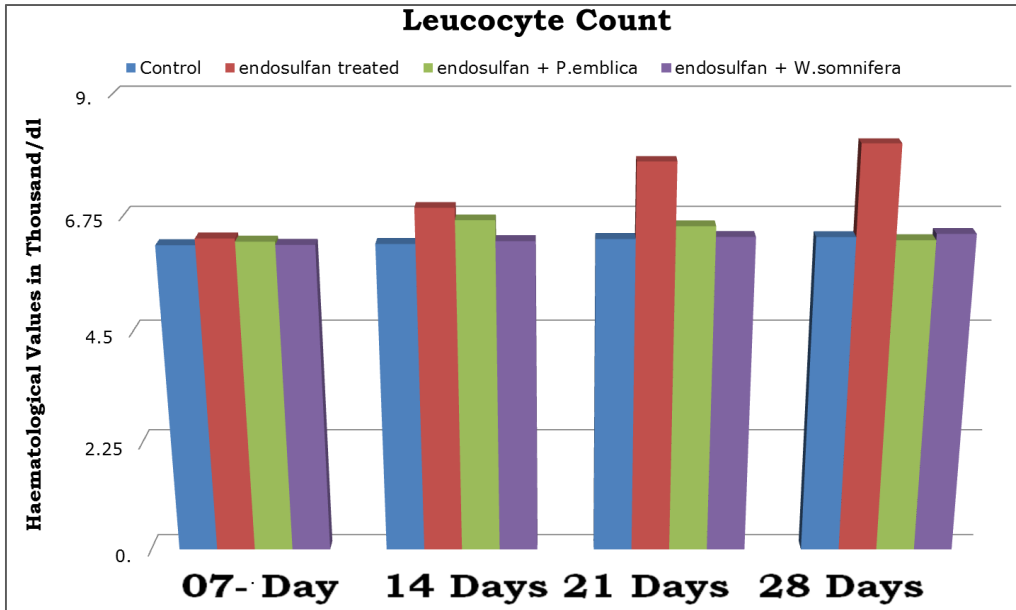


Figure 2. Endosulfan Induced Haematological Changes and Their Treatment with Amla-*Phyllanthus emblica* and Ashwagandha- *Withania somnifera* extract for Four weeks on WBC Count (10⁴/mm³).

3.3. Percent Hemoglobin (Hb%)

The control group showed 9.85 to 9.86 gram/100 ml of Hemoglobin from day 1 to days 28. Significant decrease in Hemoglobin (Hb%) was observed in Group - 2 to 7.13 gm/100 ml of Hemoglobin in endosulfan treated (Group 2) when compared to control mice.

The gradual increase in Hemoglobin (Hb%) content from 7.13 gm/100 ml to 8.98 gm/100 ml in mice fed with Amla (Group III) and 7.13 gm/100 ml to 9.39 gm/100 ml of Hemoglobin in Ashwagandha fed mice (Group IV) was observed.

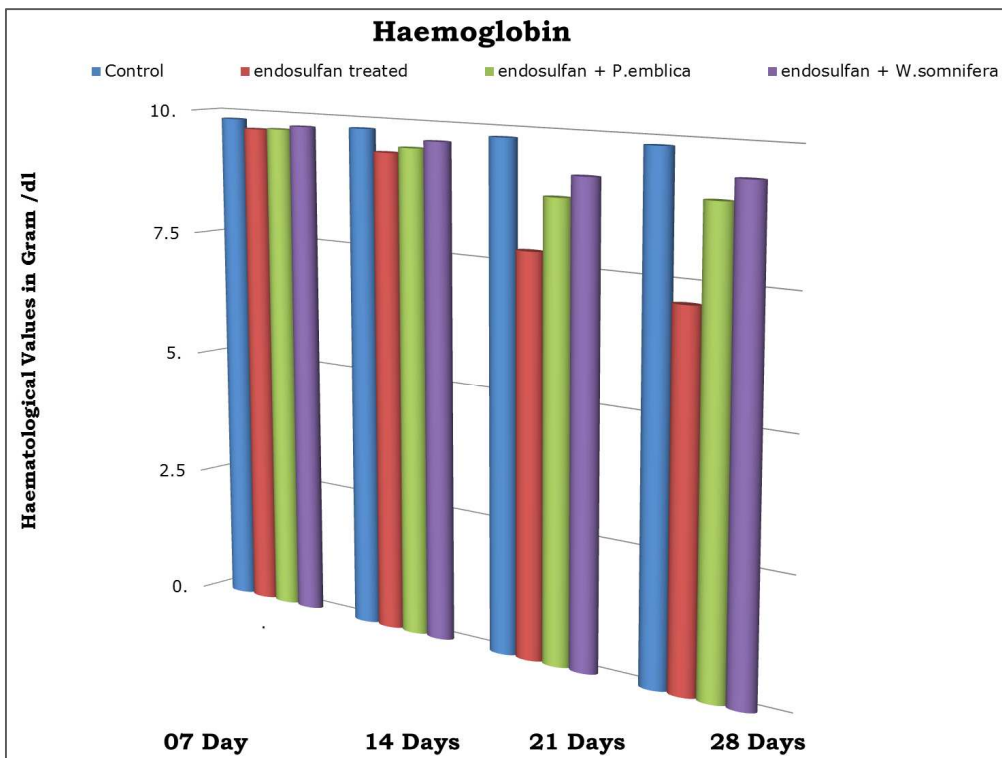


Figure 3. Endosulfan Induced Haematological Changes and Their Treatment with Amla-*Phyllanthus emblica* and Ashwagandha- *Withania somnifera* extract for Four weeks on Hb Count (Gram/100ml).

3.4. Packed Cell Volume (PCV%)

The PCV% of control animal (Group I) ranged from 39.47 pico-gram to 39.54 pico-gram. The packed cell volume (%) was found to be reduced significantly with the increase of exposure period from days 7 to 28th day of endosulfan treatment, the PCV% decreased to 33.10 but when

Endosulfan treated mice was treated with Amla and Ashwagandha extract, showed increased value of Packed Cell Volume (PCV%). It was found that at days 28, the Amla treated Group III, the elevation of PCV% to 36.19 and Ashwagandha treated Group IV, showed slightly increased PCV% to 39.09.

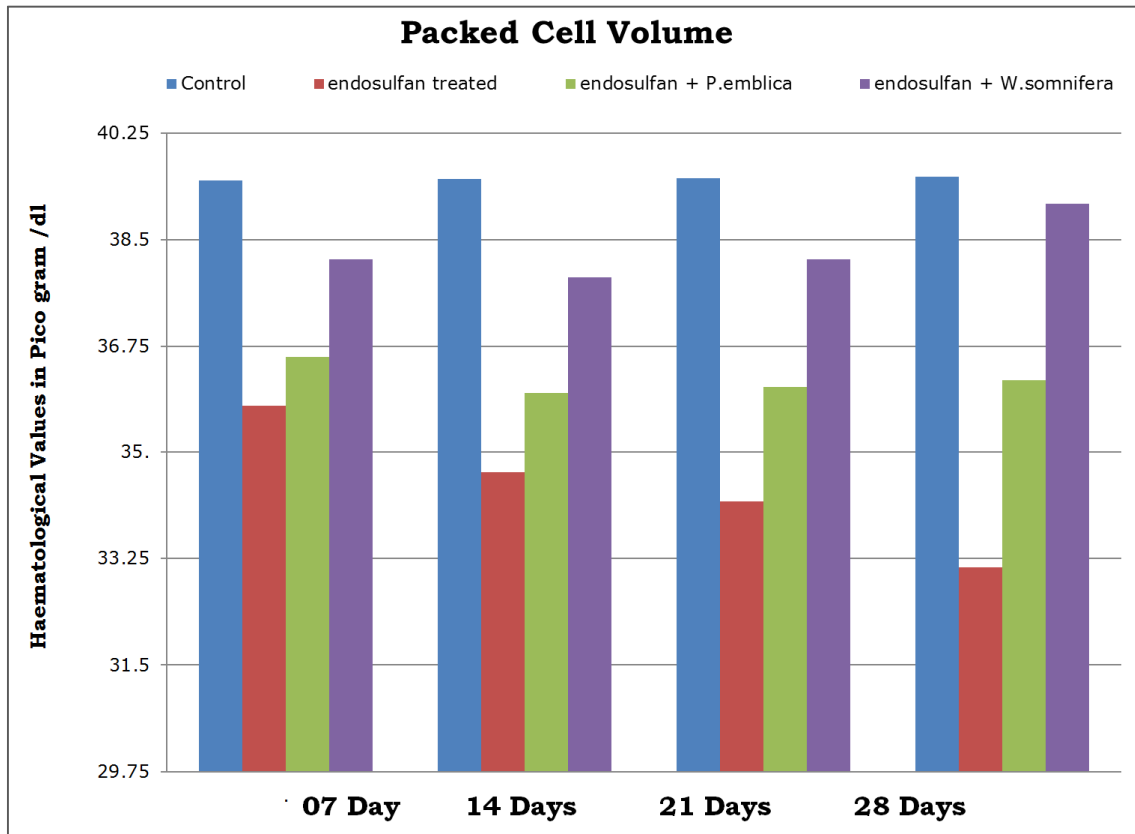


Figure 4. Endosulfan Induced Haematological Changes and Their Treatment with Amla-Phyllanthus emblica and Ashwagandha- Withania somnifera extract for Four weeks on PCV (Picogram/100ml).

3.5. Mean Corpuscular Volume (MCV)

The mean corpuscular volume of control animal showed 64.31 to 67.38 $\mu\text{m}^3/100\text{ ml}$ at days 7 to 28 respectively.

Significantly increase in Mean Corpuscular Volume was

observed in Group II at days 28 was 83.38 $\mu\text{m}^3/100\text{ ml}$. A gradual decreases in MCV was noticed in Group III (Amla treated) was 70.84 $\mu\text{m}^3/100\text{ ml}$. Ashwagandha treated Group IV showed even more better result of 67.78 $\mu\text{m}^3/100\text{ ml}$ of MCV.

Table 1. Endosulfan Induced Hematological changes and their treatment with Phyllanthus emblica and Withania somnifera extract for 28 days on Erythrocyte count, Leucocyte count, percent Hemoglobin (Hb%), Packed Cell Volume (PCV%) and Mean Corpuscular Volume (MCV).

Hematological Parameters	Group of Mice			
	Group I	Group II	Group III	Group IV
Erythrocyte Count	5.96 million cubic m to 5.87 million cubic mm	3.96+- 0.8 million per cubic mm	5.16 million cubic per mm	5.37 million cubic per mm
Leucocyte Count	6.20 thousand/cubic mm to 6.36 thousand per cubic mm	8.11 thousand per cubic mm	6.93 thousand per cubic mm	6.42 thousand per cubic mm
Percent Hemoglobin (Hb%)	9.85 to 9.86 gm/100 ml	7.13 gm/100 ml	8.98 gm/100 ml	9.39 gm/100 ml
Packed Cell Volume (PCV)	39.47 to 39.54 pico gram	33.10 pico gm	36.19 pico gm	39.09 pico gm
Mean Corpuscular Volume (MCV)	64.31-67.38 $\mu\text{m}^3/100\text{ ml}$	83.38 $\mu\text{m}^3/100\text{ ml}$	70.84 $\mu\text{m}^3/100\text{ ml}$	67.78 $\mu\text{m}^3/100\text{ ml}$

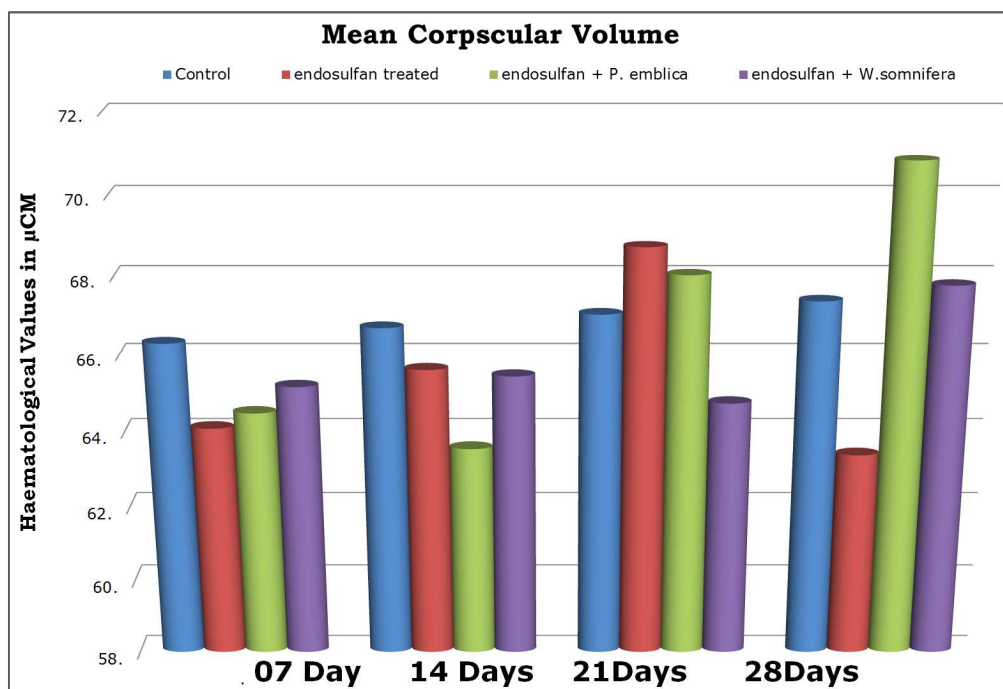


Figure 5. Endosulfan Induced Haematological Changes and Their Treatment with Amla-*Phyllanthus emblica* and Ashwagandha- *Withania somnifera* extract for Four weeks on MCV ($\mu\text{m}^3/100\text{ml}$).

The probable reason for the increase in RBC count and Hemoglobin concentration in mice administered with endosulfan would be due to prevailing hypoxic conditions in the test animals. It has been reported that dehydration or loss of fluid from circulation could also contribute to increase in RBC count [4]. It is observed that PVC decreases when RBC count decreases [2]. MCV indicates the average size of red blood cell in the sample of blood. In the present study the MCV exhibited a significant increase, which is generally associated with corresponding decreases in RBC, Hb% and PCV [33].

The disturbed hematological alterations of RBC and its relative was improved by Amla and Ashwagandha fruit and root extract supplementation, it may be because of preventing hemolysis of erythrocytes caused by lipid peroxidation through its antioxidant property [6].

There is an increased level of total WBC in endosulfan treated mice may be due to exposure of mice to pathogenic infections or due to chronic exposure to insecticides [28]. Increase in WBC count suggests stepped up defensive capability of mice during endosulfan induced Pathological stress.

The significant increase of Lymphocyte level was corrected by Amla and Ashwagandha in the present study, it may be due to the property of plant Amla and Ashwagandha containing bioactive compound with ability to improve the impairment committed by stem cells responsible for WBC production.

4. Conclusion

In the present investigation it was revealed that the endosulfan alters all the hematological parameters in

Laboratory mice *Mus musculus* such as erythrocyte count, Leucocyte count, Hemoglobin percent (Hb%), Packed Cell Volume (PCV) % and MCV of blood. On the basis of various data generated after several analysis the endosulfan was found harmful and toxic for the life supporting system and is suggested that acute contact with endosulfan may have deleterious effect on hematological functions in the animals and man.

The results of this study supported the report about *P. emblica* (100 mg/kg bwt) and *Withania somnifera* (150 mg/kg bwt) is having medicinal effect in using endosulfan exposed problems associated with hematological alterations in the experimental animals. The present work indicates improvement recorded in hematological parameters were successfully ameliorated by *P. emblica* and *W. somnifera*.

Therefore, *P. emblica* (100 mg/kg bwt) and *W. somnifera* (150 mg/kg bwt) can be effectively exposed to patients for therapeutic purpose.

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