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ELEVATION OF WHITE BLOOD CELL (COUNT) DUE TO THE EFFECT OF ENDOSULFAN INDUCED SWISS ALBINO MICE (*MUS MUSCULUS)* AND ITS AMELIORATION BY *WITHANIA SOMNIFERA*.

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ABSTRACT

Various forms of insecticides, pesticides and fungicides are used in agricultural fields to protect agricultural products from pests insects and fungus Endosulfan is an off-patent insecticide cum acaricide that is being phased out globally but is still used in large scale. It has become a highly controversial agro-chemical due to its acute toxicity potential for bioaccumulation and role as leukocyte (WBC) elevator. The present study was carried out to investigate the acute effect of Endosulfan on leukocyte parameters like neutrophils, Endosulfan, basophils monocytes and lymphocytes on Swiss albino mice *(Mus musculus).* Adult mice were segregated into three groups each group with six animals. Group I served as the control was administered with distilled water @ 0.1 ml /10 gm body weight Group II received Endosulfan @ 3 mg/ kg body weight, Group III concomitartly received *Withania somnifera* (Aswagandha) @ 150 mg/ kg body weight along with Endosulfan through oral gavaging. All the treatments were continued for three months. The toxic effects observed upon Endosulfan treatment include elevation of neutrophils count, eosinophils count, basophils count, monocyte count and lymphocyte count, which may be linked to various organ dysfunctions. On contrast, treatment of root extract of *Withania somnifera* was found to alleviate the toxic consequences of Endosulfan, thereby producing ameliorative effect.

KEYWORD: Endosulfan, *Withania somnifera*, neutrophil, eosinophil, basophil, acute chronic.

INTRODUCTION

The intensive chemicalization of world's agriculture by indiscriminate and reckless use of chemical pesticides led to the large scale contamination of our living environment (Miller, 2004). 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. Some of the commonly used insecticides used in India are Endosulfan, Phorate, Methyl, Parathion, Monocrotophos, cypermethrin, Malathion, and Dichlorvos. Among these Endosulfan is a widely used chlorinated hydrocarbon insecticide and acaricides of cyclodien subgroup. Endosulfan is a derivative of hexa-chloro-cyclo-pentadine and is chemically similar to aldrin, chlordane and heptachlor. Specifically, it is produced by Diels and Alder reactions of hexa-chloro-cyclo-pentadine with cis-butene-1, 4-diol and subsequent reaction of the adduct with thionyl chloride. In India during 2005-2010, it is commonly used against a variety of agricultural pest about 15537 metric tonnes was manufactured in India and was next only to sulphur 16424 metric tonnes. (Anonymous 2001), pest control chemicals are poisons and they may present immediate danger to user if used improperly. Some of them highly toxic and may cause serious metabolic disorders and even death if inhaled or ingested through oral route (Frank and Brown, 1984, Zhou and Hu 1984).

Widespread use of Endosulfan in agriculture, results to exposure of humans by eating food and water contaminated with endosulfan. Population that are usually susceptible to Endosalfan include the unborn and neonates, the elderly and people with liver, kidney, immunological, haematological defects (Yourself et al. 2003) or or neurological disease (ATSTR, 2000) the Endosulfan may be found in many food particles such as oil and fats, fruits and vegetables (Mitchell, 1976; Pokharkar and Dethe, 1981) fish, milk and milk products (Nag and Raikwar, 2008, Company et al. 2001).

Endosulfan can have adverse effect on the immune system at low levels of exposure and (Anonymous,

2002). Blood or haematological parameters are probably the more rapid and detectable variations under stress and are fuel in assessing different health conditions (Singh et al. 2008). Hence, the significance of haematological parameters in clinical and experimental studies in life science it cannot be overemphasised. Particularly, literature reports has proved that the alterations in the haematological parameters, from normal state may be used as valuable indicators of disease or stress in different animal species (Yakuba, et al. 2007). Assessment of haematological parameters can therefore be used to determine the extent of damage from foreign substances on the blood constituents of an animal (Friday et al. 2012). *Withania somnifera* known commonly as Ashwagandha, Indian ginseng, poison gooseberry or winter cherry (GRIN, 2011). It is used as a herb in Ayurvedic medicine (Gupta et al. , 2011). Leaves and roots of this plant are abortifacient, aphrodisiac, diuretic, nerving tonic (Bahr and Hansel, 1982), alternative, narcotic, sedative, astringent, growth promoter and anthelmintic. It has anti-arthritic, anti-bacterial, anti-dote for scorpion sting, anti-stress, anti-tumour anti-cancer activities. It is used in toning of uterus, constipation, dropsy, leucoderma, impotence, rheumatism, debility from old age, ulcer, sexual and genital weakness assumption, rheumatic swelling, less of memory, loss of muscular energy, spermatorrhoea, syphilis, sterility of women, blood discharge, leucorrhoea, anaemia with emaciation, nervous exhaustion, multiple sclerosis, neoplasia, cancer and fatigue.

Laboratory analysis has revealed over 35 chemical constituents contained in the roots of *Withinia somnifera* (Staba, 1980). The biologically active chemical constituents are alkaloids (Isopollertierine, anferine) steroidal lactones (withanoides, withaferin) saponins containing an additional acyl group (sitoindoside VII and VIII), and withinaloids with a glucose at carbon 27 (sitoindoside XI and X). *Withania somnifera* is also rich in Iron (Rastogi and Mehrotra 1998). Ashwagandha has been found to provide potent antioxidant protection (Panda and Kar, 1997 and Abou-Doub, 2002) stimulate the activation of immune system cells, such as lymphocytes and phagocytes and work against the effects of stress and generally promote wellness (Singh et al. 2003).

In the present work, ameliorating effect of the root extract of *Withania somnifera* is used to mitigate the elevated number of WBC included by Endosulfan treatment in Swiss Albino mice has been studied as a remedial measure.

MATERIAL AND METHODS

Test animal

Young and sexually mature swiss albino mice *(Mus muscules)*, each with body weight of 2.57gm, (obtained from CDRI Lucknow) were maintained in the animal house of Department of Zoology, T.M.B. University, Bhagalpur (India - 812007). A total of 18 mice segregated into 3 experimental groups and kept separately in cages at 24°C temperature and humidity with 12(±)1 hour light/dark cycle. Food and water were 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 the guidelines of CPCSA. Initially, the mice were acclimatised for two weeks before the start of experiment.

Test Substance Used

The pesticide used for treatment of animals were Endosulfan, purchased from the market under trade name of endocoel 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-banzo dioxathiepin-3-oxid) widely used as broad spectrum organochlorine insecticide and acaricide.

Treatment Protocol

The 1/8th of the LD 50 dose of Endosulfan (3.0mg/kg b.w.) was administered orally for chronic toxicity study. Once daily, for 28 days by garage method (oakberg, 1957).

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

Experiment Design

a. The animals are divided into 3 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 feedings with root extract of *Withania somnifera*.

b. Root extract of *Withania somnifera* (Ashwagandha)
The roots from healthy plants of *Withania somnifera* were collected from Bhagalpur (India-812007). The mature roots were collected in the polythene bags and were washed immediately in running tap water to remove the adhered dust and other extraneous materials. These materials were dried in hot air oven at 60°C (Handa et al. 2008) for 5-8 days and then powdered coarsely with the help of glass mortar and pestle. The powder were soxhlated with ethanol (80% V/V) (Jones and Kingkorn, 2006) at 60-70°C (Handa et al. 2008) for 50 to 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, (Hansen 2007).

RESULT AND DISCUSSION

The effect of Endosulfan and its treatment with *Withania somnifera* root extract 150 mg kg/ b.w. for four weeks in

different groups of mice (Group II and Group III) along with control (Group I) at all incubation period (7,14,21 and 28) have been shown in table (1-6).

The leucocyte count of control mice were recorded after each incubation period and the values ranged from 6.20 (±) 0.02 thousand mm³ to 6.36 (±) 0.03 thousand mm³ at days 1 to 28.

There was a significant increase in the Leucocyte count i.e 6.33(±)0.06, 6.91(±)0.02, 7.77(±)0.01 and 8.11(±)0.06 thousand mm³ at days 7, days 14, days 21 and days 28 respectively in Endosulfan treated (Group II) when compared to control mice ($P \geq 0.001$).

The gradual recovery in Leucocyte count in Group II was noticed in Endosulfan treated mice by supplementing root extract of *Withania somnifera* treatment. It was found that at days 28, the Leucocyte count significantly decreased from 8.11(±) 0.06 thousand mm(cube) to 6.42(±)0.09 thousand group III.

The Biostatistical analysis show that the effect of treatment and incubation period were found to be insignificant in control and Endosulfan + *Withania somnifera* but highly significant in Endosulfan treated group at 0.01 significant level of confidence ($P \geq 0.05$, $P \geq 0.01$).

There is an increased level of total WBC and its functional indices in Endosulfan treated mice and similar result have been reported by Paul (2002). The increment of these parameters may have a significant effect against the animals exposing them to pathogenic infections (Adedapo et al. 2007).

Several authors have noticed similar increase in WBC in animals upon chronic exposure to insecticides (Phillip et al. 1989). Increase in WBC count suggests stepped up defensive capability of mice during Endosulfan induced pathological stress. *Withanina somnifera* root extract supplementation makes the count in the approximately normal (Oyedemi et al. 2011) into Endosulfan rats.

The significant increase of lymphocyte levels reflect possible immunomodulatory effects of *Withanina somnifera* on Endosulfan treated mice. It could be inferred from the study that *Withanina somnifera* may contain bioactive compound with ability to improve the impaired production of WBC by stimulating maturation of committed stem cells responsible for WBC production.

Table-1: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on WBC Count (10^4/mm^3).

Group of Mice	WBC (10^4/mm^3)			
	Day 07	Day 14	Day 21	Day 28
Group-I	6.201±0.023	6.226±0.028	6.321±0.041	6.357±0.032
Group-II	6.33±0.565	6.91±0.188	7.77±0.149 [a]	8.105±0.058 [b]
Group-III	6.209±0.033	6.28±0.062	6.359±0.77 [a]	6.418±0.092 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statistically
[a] significant ($p<0.01$); [b] highly significant ($p<0.001$).

Table-2: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on on Lymphocyte (%/100ml).

Group of Mice	Lymphocyte(%/100ml)			
	Day 07	Day 14	Day 21	Day 28
Group-I	35.5±0.521	35.6±0.706	35.6±0.625	35.7±0.662
Group-II	35.2±0.741	39.7±1.805	45.8±0.687 [a]	53.5±1.044 [b]
Group-III	35.5±0.573	35.5±0.651	35.3±2.183 [a]	35.5±2.535 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statisticall
[a] significant ($p<0.01$); [b] highly significant ($p<0.001$).

Table-3: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on Neutrophil (%/100ml).

Group of Mice	Neutrophil (%/100ml)			
	Day 07	Day 14	Day 21	Day 28
Group-I	58.5±0.483	58.4±0.928	58.2±0.790	57.7±0.829
Group-II	58.7±0.663	52.7±1.388	44.4±1.422 [a]	34.8±0.753 [b]
Group-III	58.4±0.635	58.2±0.674	58.3±2.501 [a]	57.8±2.461 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statistically
[a]significant ($p<0.01$); [b] highly significant ($p<0.001$).

Table-4: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on Eosinophil (%/100ml).

Group of Mice	Eosinophil (%/100ml)			
	Day 07	Day 14	Day 21	Day 28
Group-I	2.1±0.216	2.2±0.269	2.2±0.231	2.0±0.308
Group-II	2.2±0.269	2.3±0.391	2.5±0.284 [a]	2.9±0.376 [b]
Group-III	2.2±0.332	2.2±0.332	2.4±0.314 [a]	2.3±0.310 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statistically [a]significant ($p<0.01$); [b] highly significant ($p<0.001$).

Table-5: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on Basophil (%/100ml).

Group of Mice	Basophil (%/100ml)			
	Day 07	Day 14	Day 21	Day 28
Group-I	0.9±0.166	0.9±0.256	0.9±0.256	1.1±0.322
Group-II	0.8±0.185	0.7±0.241	0.7±0.241 [a]	0.6±0.247 [b]
Group-III	0.9±0.216	0.9±0.256	0.8±0.269 [a]	0.8±0.269 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statistically [a]significant ($p<0.01$); [b] highly significant ($p<0.001$).

Table-6: Endosulfan Induced Haematological Changes and Their Treatment with *Ashwagandha- Withania somnifera extract* for Four weeks on Monocyte (%/100ml).

Group of Mice	Monocyte (%/100ml)			
	Day 07	Day 14	Day 21	Day 28
Group-I	3.0±0.586	2.9±0.941	3.1±0.593	3.5±0.831
Group-II	3.1±0.889	4.6±0.720	6.6±1.340[a]	8.2±0.766 [b]
Group-III	3±0.730	3.2±0.629	3.2±0.474 [a]	3.6±0.635 [b]

N=10 Values are given as mean ±SEM for groups of ten mice. Values are statistically [a]significant ($p<0.01$); [b] highly significant ($p<0.001$).

In the present study, there is an increase in the concentration of lymphocytes following exercise due to the recruitment of Lymphocytes NK, Cells T & B from the periphery of the body (Bruunsguard and Pederson, 2000). The administration of root extract of *Withanina somnifera* (150mg kg/b.w.) (Group II) in dose body weight/day for 28 days to Endosulfan treated mice led to significant decrease in the count of lymphocytes versus test group. The changes were statistically significant ($P \leq 0.001$). The treated group animals showed significantly raised lymphocytes activity as compared to the control group ($P \leq 0.01$).

In our study, Endosulfan toxicity decreased neutrophils count. The neutrophils count of Endosulfan treated mice Group II do not increase their bacterial activity in response to the same intensity of infection as compared to control (Moss et al. 2000). The Endosulfan generated toxicity decreases the affinity of the neutrophils with the endothelial cells. This effect, on the other hand, protects the lungs from neutrophils migration, thus decreasing its oxidative production. The increase in blood neutrophils count was recorded after *Withanina somnifera* root extract administration may be a result of minor adherence of cell in target tissues. Simultaneously the count of eosinophils was significantly increased ($P \leq 0.001$) after administration of once daily for 28 days (Group II) compared to the control group I. Eosinophils are also altered in stress conditions was observed a huge increase in the number of eosinophils during the course Endosulfan toxicity. Elevation of inflammation markers is frequently reported in Endosulfan organisms with increase in sub-population of monocyte which may contribute to the development of atherosclerosis (Patino et al. 2000). On the other hand, the exercise usually produced a some sub-populations of monocytes count. Nevertheless, in our study, Endosulfan didn't cause alteration in the monocytes percentage.

CONCLUSION

The results of the study supported the reports about *Withanina somnifera* (150 mg/kg b.w.) is having medicinal effect in using Endosalfan exposed problems associated with Leucocyte parameters which is important in determining the actual physiological condition of animals. To function properly, an organism must keep its Leucocyte composition and constituent relatively constant under natural conditions (Rodrigues and Mc. Neil, 1992). The present work indicates improvement in leucocyte count were successfully ameliorated by *Withania somnifera*.

Therefore, *Withania somnifera* (150 mg/kg b.wt.) can be effectively Endosulfan exposed patient for therapeutic purpose.

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