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Study on the Effects of Ethanolic Extract of *Withania somnifera* on Hematological Parameters in Male Albino Rats

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ABSTRACT

The plant *Withania somnifera has* been used for centuries in traditional medicine in India. However, like many medicinal plants, the belief that things of natural origin are safe may not be entirely true. So present study has planned to find the acute and chronic effects of Ethanolic Extracts of *Withania somnifera* (EEWS), on haematology indices of Wistar albino rats. The animals were divided into three groups. Two groups served as experimental, which will receive dose at a level of 250 mg/kg and second group act as control which received vehicle only. In experimental groups one receives acute dose and other one receive the chronic dose. The animals were kept in same environmental condition and after 0, 7, 14, 21 and 28 days the haematological parameters were studied**.** It was noted that during acute administration of EEWS the RBC, WBC, Hb, PCV, ESR, MCH and MCHC have increased at shorter duration but recapitulates to its normal level as compared to the control group. While during chronic administration EEWS these parameters increased significantly to its control group. On the other hand it was noted that on single dose the MCV and Color index have decreased and recouped to the normal value. While during daily administration of the dose these parameters decreased significantly as compared to the control group. In conclusion the present study of EEWS shows control over the hematological parameters without producing any toxicity in albino rats and thus it is safe to use.

Keywords : *Withania Somnifera*, Haematology, Color Index, Ethanolic Extract.

I. INTRODUCTION

Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. The revival of interest with herbal-based medicine is due to the increasing realization of the health hazards associated with the indiscriminate use of modern medicine. According to data of the Food and Agricultural Organization (FAO), more than 50,000 plant species are being used in the traditional folk medicine throughout the world (Schippmann *et al*., 2002). The highest percentage of native flora species used for medication was observed in countries of south East Asia such as India (20%) and China (19%). In the US and Russia, slightly more than 10% of plant species are used for therapeutic purposes. According to World Health Organization (Schippmann *et al*.,2002) more than 90% of therapeutic classes derived from a natural product prototype and roughly two-third to three quarters of world's population relies upon medicinal plants for its primary pharmaceutical care.

In today`s era of medicine engineering, plants play an equally important role in drug discovery and development. They have the great medicinal properties and are used to cure so many diseases. Only few of the plants/herbs used in herbal medicine herbs have been scientifically validated for the claimed medicinal effects, hence slowing down the pace of drug discovery from such plants. Among the factors responsible for this is the myth surrounding herbal medicine especially in developing nations as well as dosages administered. To this end, fewer side effects and high therapeutic values are two key criteria used to scientifically evaluate plants as adjunct or sole pharmaceuticals.

This approach is both scientific and logical. For instance, research outcomes have faulted the popular belief that things of natural origin are safe. Thus, it is reasonable

and profitable to human safety that certain data be generated through toxicological investigations to show the safety profile of herbal preparations. Such data is often derived from experiments carried out using non-human primates and rodents. The results are to a large extent reliable, can be extrapolated to human and serve as guide in clinical studies in human since the animals and humans have biological similarities.

India is one of the 12 mega biodiversity centers having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000-18,000 flowering plant, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million microorganisms. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. In this vast array of medicinal plants, *Withania somnifera* holds an important place in Indian medicinal system and thus has been selected for the current study.

Withania somnifera Dunal belongs to the family Solanaceae. It is a xerophytic plant, found in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions, the Canaries and Cape of Good Hope. It is found in high altitude ascending to 5,500 feet in the Himalayas. This shrub is common in Bombay and Western India, occasionally met within Bengal. It grows wildly throughout India particularly in hotter parts, on waste places and on road sides. It is also cultivated for medicinal purposes in fields and open grounds throughout India. (Dey et al., 1973; Dymock et al., 1976; Kirtikar et al., 1980; Nadkarni, 1982 ; Anonymous, 2007).

Medicinal properties of *Withania somnifera* are very well known.It is anxiolytic-anti-depressive (Bhattacharya et al., 2000), Anti-atherogenic (Andullu& Radhika 2000), antifungal (Girish *et al.,* 2006), antimalarial (Dikasso *et al.,* 2006), apoptotic (Senthil *et al.,* 2007), chondroprotective (Sumantran *et al.,* 2007), cardioprotective (Hamza *et al.,* 2008), immunomodulatory (Davis *et al.,* 2000), neuroprotective (Bhattacharya et al., 1995), inhibition of cox-enzyme (Jayaprakasan *et al.,* 2003), promoter of learning and memory in Alzheimer's disease (Bhattachrya *et a*l., 1995), anti-inflammatory (Kaileh *et al.,* 2007), antioxidant (Gupta *et al.,* 2003), anti-aging (Bone 1996), anti-cancer(Ichikawa *et al.,* 2006), chemopreventive (Prakash *et al.,* 2002), anti-ulcer (Bhatnager *et al.,* 2005), an adaptogen (Bhattacharya *et al.,* 200l; Bhattacharya & Muruganandam, 2003), hypolipidemic (Udaykumar *et al.,* 2009), hypoglycemic (udaykumar *et al.,* 2009), Hepatoprotectant (Mohanty *et al*., 2008).Several bioactive molecules like withanolides, indosides, withaferin-A, others are responsible for these properties.

Blood is considered good indicator to determine the health of an organism and is also a good pathological mirror of the entire body. Cellular component of blood is valuable in immune toxicology to evaluate immune toxic potential of a compound. Hence hematological parameters are important in establishing the body's functional status to exposure to toxicants. Keeping this in view and lack of toxicity studies, *Withania somnifera* has been selected to study the effect of its EEWS on various hematological parameters of male albino rats and detect its toxic effects.

II. METHODS AND MATERIAL

The present study was carried out at Department of Zoology, Institute of Basic Science Bundelkhand University Campus Jhansi. The study was conducted in sexually mature, male Albino rats of Wistar strain (200±10gm), purchased from DRDE (Defence Research Development Establishment) Gwalior. Prior to study, the ethical clearance was obtained from the animal Ethical Committee (CPCSEA, MOEF, and Government of India) Proposal No. Bu/Pharm/IAEC/12/037

Maintenance of animals

Before starting any experimentation it is necessary to maintain the animals properly. The animals were housed in animal house having standard conditions, at a temperature of 25^0C to 30^0C and 12 hours light and 12 hours darkness. They were fed with rat pelleted diet and water *ad-libtum*. For experimentation animals were randomly distributed into three groups, two serves as experimental and other as control. The rats weighing about 200±10gm were used for study. Experimental

group will receive test material and control will receive vehicle only.

Preparation of plant extract

Medicinal principle is present in different parts of the plant like root, stem, leaf, flower, fruits, or plant exudates. These medicinal principles are separated by different process, the most common being extraction. Extraction is the separation of the required constituents from plant materials using a solvent.

Preparation of dose

Doses were prepared in gum acacia in distilled water. 1ml of chronic and acute dose of EEWS plant was prepared according to 250mg/kg body wt.
The rats were divided in 3 groups of 6 each as per treatment schedule given below.

Group I	Normal Control
Group II	Acute treatment EEWS 250mg/kgbw
Group III	Chronic treatment EEWS 250mg/kgbw

Dose was given orally for 28 days. Acute study of haematological parameters was done in single dose administration of EEWS. The effect was studied after 0, 7, 14, 21 and 28 days of treatment, whereas the chronic study was done by same procedure after 28 days administration of plant extract.

Route of administration

The dosages were given to the animals via oral route through gastric feeding needle. The entry normally obtained without anesthesia. Feeding needle with a ball tip was used to prevent introduction of the needle into the trachea and prevent trauma to the oral cavity.

Plan of work

Present study has been planned to study the combined effect of EEWS on two different aspects, acute and chronic. The animals were divided into 3 groups. Two served as experimental, which will receive dose at a level of 250mg/kgbw and 3 group acts as control which received vehicle only. The animals were kept in same environmental condition and after 0, 7, 14, 21and 28 days the hematological parameters were studied**.**

Collection of blood samples for analysis

Blood samples were collected through the orbital plexus of the rates, under anesthesia into anticoagulant bottles for hematological analysis. Hematological parameters were then evaluated on whole blood.

Statistical analysis

The results were expressed as Mean±S.E. significance of differences compared to the control was determined using student's t-test.

III. RESULTS AND DISCUSSION

RBC COUNT: In the present study the acute administration of EEWS at a dose level of 250mg/kgbw was studied, the RBC count significantly increased at 7 days and gradually recouped at normal level on compared to control group Table 1. The chronic administration EEWS significantly increases the RBC count at all days. The dose dependent increase in the number of RBC is due to immunomodulatory constituents present in *Withania somnifera*. Root extract of *Withania somnifera* was tested for immunomodulatory effects in three myclosuppression models in mice: cyclophosphamide, azathioprin, or prednisolone (Ziauddin *et al.*, 1996). Significant increases ($p<0.05$) in hemoglobin concentration, red blood cell count and white blood cell count were observed in *Withania somnifera* treated mice compared to untreated control mice.

Similarly, Uboh *et al*., (2010) reported the effect of the aqueous extract of *Psidium Guajava* leaves which significantly increased the RBC count in both experimental male and female rats as compared to *their control rats.* Balaji *et al., (2004) reported that pretreatment with alcoholic extract of Sargassum polyeystum* reduced the toxic of acetaminophen by improving the altered RBC and WBC count suggesting a free radical scavenging property. Also, this may be due to immunomodulatory property as reported in *Tridax procumbens* plant which is also having the capacity of

restoration of RBC content during presence of any type of infection (Ogwumike, 2002). However, Thisoda *et al*., (2006) reported that a 50% ethanolic extract of *Amaranthus spinosus* leaves administered orally to growing pigs elicited significant reduction in RBC while at 7 days post treatment. Azza *et al*., (2009) reported that those rats receiving the methanolic extract of *Guiera Senegalensis* significantly decreased the mean value of RBC while Wannag (2007) revealed that when group treated rats received the dose of *cucumis mituliferus* fruits at a low concentration of 500mg/kg) significantly decreased the RBC countt (<0.05) but at (1000mg/kg) significantly increase in RBC count was observed as compared to the control. This has indicated that the rats were not anemic while decreased level is a sign of anemia.

Table 1 : Effect of single and daily administration of EEWS on RBC count of albino rats (million/Cu mm).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	9.0± 0.3	8.9± 0.4	9.0± 0.5	8.9±0.1	8.8± 0.2
II	**Acute dose**	8.9±0.5	10.7±0.5*	9.6±0.2	9.0±0.1	8.8± 0.1
III	**Chronic dose**	8.8±0.5	10.9±0.4*	12.1±0.7*	13.3±0.9*	14.5±1.0*

*** Statistical analysis:** P Vs respective control, P<0.05

WBC COUNT: In the present study effect of *Withania somnifera* at a dose level of 250mg/kg b.wt. was studied for 0, 7, 14, 21 and 28 days of duration on WBC count in albino rates on single administration, WBC count significantly increased at an early time, which gradually drops to normal value. The chronic administration of EEWS the WBC count significantly increased at all the durations. The dose dependent increase in the number of WBC is due to due to certain constituents having immunomodulatory activity found in *Withania somnifera* Table 2. A series of animal studies have demonstrated that *Withania somnifera* has profound effects on the healthy production of white blood cells, which indicates that it is an effective immuno-regulatory agent (Kuttan 1996; Ziauddin *et al.,* 1996).

Similarly, Yakuba *et al*.,(2005) have studied haematological parameter in male albimo rats; following chronic administration of aqueous extract of *Fadogia agrestes* stem also reported that WBC were significantly altered which indicate pathological conditions which may imply challenge on the immune system by plants by plants extract. Azza *et al.,* (1999 reported that chronic administration of extract of *Gulera senegalensis* at a dose level of 250mg/kg significantly increases WBC count. Also Srikumar *et al., (*2005) reported that oral administration of Trepala stimulates the neutrophil functions. Orafidiya *et al., (*2004) reported that chronic administration of the essential oil of *Ocimum gratissimum* Linn. increased white blood cells count. However, significant reduction in WBC occurred in whole blood of rat due to effect of *Cucumis menullferus* fruits and *Bacillus thuringiensis* in pesticide treated rats at the higher dose as teported by Wannang et al., (2007) and Eissa *et al., (*2009) respectively. This may be related to suppression of the WBC resulting from toxic reactions to substances while no significant changes were observed in WBC count during administration of aqueous extract of *Psidium guajava* leaves and *Sorghum Micaloron* in Albino rats (Uboh *et al.,* 2010; Akande *et al*., 2010).

Table 2 : Effect of single and daily administration of EEWS on WBC count of albino rats (million/Cu mm).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	8.5± 0.3	8.3± 0.4	8.6± 0.1	8.6±0.3	8.5±0.2
II	**Acute dose**	8.6±0.1	9.6±0.3	9.4±0.2	8.8±0.3	8.3±0.4
III	**Chronic dose**	8.8±0.2	10±0.4	10.6±0.3	11.0±0.2*	12.1±0.3*

*** Statistical analysis:** P Vs respective control, P<0.05

HAEMOGLOBIN PERCENTAGE : Haemoglobin percentage is a chromo protein and is the coloring matter of RBC. The function of haemoglobin is to carry the respiratory gases, oxygen and carbon dioxide. In the present study effect of *Withania somnifeara* on haemoglobin at a single dose were studied for 0, 7, 14, 21 and 18 days. During acute administration, percent of haemoglobin significantly increased at an early duration which gradually returns to its normal position. On chromic administration percent of haemoglobin significantly ($p<0.05$) increased at all the durations (Table 3). A series of animal studies show Ashwagandha to have profound effects on the hematopoietic system, acting as an immunoregulatory (Kuttan and Ziauddin, 2006). Consequently increase in percent of haemoglobin was observed.

Similarly, aqueous extract of Sorghum bicolor significantly increased the Hb concentration in rats (Akande *et al.*, 2010). Also, fruits of *psidium gusfave* and *Cucumis netuliferus* significantly increased the Hb percent in treated albino rats (Uboh *et al*., 2010).

However, Ahmad *et al.*, (1999) reported that chronic administration of extract of *Guiera senigulensis* at a dose level of 259mg/kg significantly decreases haemoglobin. Similarly, *Thislda et al*., (2006) reported that a 50% ethanolic extract of *Amaranthus spinosus* leaves administered orally to growing pigs solicited significant reduction in WBC level at 7 days post treatment. Joshi *et al*., (2004) reported that there were on significant changes observed in percent haemoglobin of rat during acute and sub acute toxicity due to polyherbal antidiabetc formulations. Even, Yakuba *et al*., (2007) studied the non-significant effect of Hb due to stem extract of *Fadogia agresis* at various doses of chronic administration in male albino rats.

Table 3: Effect of single and daily administration of EEWS on Haemoglobin percentage count of albino rats (Hb% = gm/dl).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	13.2± 0.3	12.9± 0.1	13.0± 0.4	13.1±0.3	12.89±0.3
II	**Acute dose**	13.1±0.4	15.5±0.7	14.7±0.5	13.9±0.3	13.0±0.1
III	**Chronic dose**	12.9±0.2	15.4±0.4	16.9±0.7	17.6±0.9*	18.5±1.0*

*** Statistical analysis:** P Vs respective control, $P<0.05$

PACKED CELL VOLUME : It determines the percentage of RBC in plasma. In the present study effect the EEWS decreases the packed cell volume at an early duration, which gradually recouped at normal, but during chronic administration, packed cell volume increased at all the durations. Similarly, garlic extract increased the PVC significantly in blood of treated rats as compared to their respective control group (Iranloye *et al*., 2002). Similarly, Wannang *et al., (*2007) also noticed that *cucumis metulifrus* fruits significantly ($p<0.05$) increased the PVC in blood of treated rats Table 4. The extract of *Mangifera indica* stem bark had some positive effect on the haemopoietic system of the test rats. This was manifested by an increase in the PCV following administration of plant extract to the rats (Nwinuka *et al*., 2007).

However, Azza *et al.,*(2009) have observe the significant decrease in mean value PCV at the dose level of 250mg/kg/day due to administration of aqueous extract of *Guiera senegalensis*. Similarly, Olukunle *et al.,* (2008) studied that the effect of aqueous leaf extract of *Tridax poprocumbers* on the haematological and serum biochemical parameter of Wistar Albino ratsand reported that there was a decrease in packed cell volume (PCV). But in case of Fadogiaagrestis, stem extract did not produce any significant changes in the PCV of blood (Yakuba *et al.,* 2007).

Table 4 : Effect of single and daily administration of EEWS on Packed Cell Volume of albino rats (PCV=%).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	40.3± 0.4	39.4± 0.1	40.0± 0.3	41.0±0.1	39.8±0.2

II	**Acute dose**	39.2±0.8	44.2±0.7	42.0±0.9	40.8±1.0	38.9±1.3
III	**Chronic dose**	41.1±0.8	47.5±0.9	49.9±1.0	50.9±1.5*	51.8±1.2*

*** Statistical analysis:** P Vs respective control, P<0.05

ESR, MCH, MCHC, MCV and CI values: It was noted that during single administration of EEWS the ESR (Erthrocyte sedimentation rate) table 5, MCH(mean corpuscular haemoglobin) table 6 and MCHC(mean corpuscular haemoglobin concentration) table 7 have increased at shorter duration and recouped to the normal value as compared to the control group. While during daily administration of the dose these parameters increased both at shorter as well as longer durations. On the other hand it was noted that during single of the dose MCV and CI (Colour index) have decreased at shorter duration but at longer duration they recouped to the normal as compared to the control group. While during daily administration of the dose these parameters decreased both at shorter as well as longer durations. These results may be due to the presence of different constituents present in the plants. Mishra *et al*., (2012) reported that the crude extract of *Bougainvillea spectabilis* leaves produced significant decline in level of MCH, MCHC AND MCV. Ofem *et al*., (2009) observed reduced ESR and no significant alteration of MCV, MCH, MCHC and CI by extract of *viscum album* in high salt- fed rats.

Table 5: Effect of single and daily administration of EEWS on Erythrocyte Sedimentation Rate of albino rats ERS (mm/hr).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	1.9± 0.1	1.8± 0.4	1.81± 0.3	1.75±0.1	1.85±0.2
II	**Acute dose**	1.89±0.2	2.7±0.4	2.4±0.3	2.1±0.1	1.91±0.1
III	**Chronic dose**	1.7±0.1	2.6±0.3	3.1±0.2*	3.7±0.3*	3.9±0.1*

*** Statistical analysis:** P Vs respective control, P<0.05

Table 6 : Effect of single and daily administration of EEWS on Mean Corpuscular Hemoglobin of albino rats MCH (Pg).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	30.1±1.0	29.8± 0.3	30.0± 1.0	29.94±0.4	30.1±0.1
II	**Acute dose**	29.9±0.3	31.3±0.8	31.3±0.5	30.6±0.3	30.0±0.2
III	**Chronic dose**	30.2±0.4	34.1±0.9	34.1±1.0	35.0±1.2	36.2±1.5*

*** Statistical analysis:** P Vs respective control, P<0.05

Table 7 : Effect of single and daily administration of EEWS on Mean Corpuscular Hemoglobin Concentration of albino rats MCHC (%).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	33.2±1.0	35.1± 1.2	34.5± 1.0	33.8.0±0.1	34.6±0.4
II	**Acute dose**	34.5±2.0	38.3±1.0	36.4±1.2	35.0±1.1	33.4±1.5
III	**Chronic dose**	35.2±1.0	42.1±1.3	43.5±1.5	44.7±1.7	45.1±1.8*

*** Statistical analysis:** P Vs respective control, P<0.05

Table 8: Effect of single and daily administration of EEWS on Mean Cell Volume of albino rats MCV (Cu.μ).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	95.5±1.0	96.1± 0.2	95.9± 2.0	96.0±0.7	96.2±0.5
II	**Acute dose**	96.59±2.2	93.1±0.2	94.8±0.2	96.0±1.1	96.5±1.3
III	**Chronic dose**	96.0±2.4	92.5±1.9	90.7±1.7	89.4±1.3	88.1±1.1*

*** Statistical analysis:** P Vs respective control, P<0.05

Table 8 : Effect of single and daily administration of EEWS on Color Index of albino rats C.I (%).

Group	Dose 250mg/kgbw	0 day	7 days	14 days	21 days	28 days
I	**Normal control**	1.5±0.01	1.46± 0.1	1.51± 2.1	1.41±0.1	1.49±0.03
II	**Acute dose**	1.45±0.2	1.1±0.02	1.2±0.01	1.3±0.04	1.4±0.01
III	**Chronic dose**	1.4±0.03	1.1±0.02	1.0±0.021	0.9±0.01*	0.7±0.02*

*** Statistical analysis:** P Vs respective control, P<0.05

IV. CONCLUSION

On the basis of this study it has been established that *Withania somnifera* possess many pharmacological properties and it's single and daily administration at a dose of 250mg/kg of body weight for 0, 7, 14, 21 and 28 days shows the control over haematological parameters but does not lead to any severe ailments in albino rats. Hence the drug does not produce any toxicity and is safe to use. Further various biochemical parameters and histological studies at cellular level are to be performed to support the present study.

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