

Research Article



Efficacy of *Eclipta alba* (L.) Against Sub Lethal Dose of Endosulfan Induced Biochemical and Haematological Alterations in Swiss Albino Mice

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ABSTRACT

Eclipta alba (L.) Hassk has important role in the traditional systems of herbal medicine. Its free radical scavenging and antioxidant activity has been reported due to the presence of phenolic and flavonoids compounds. Endosulfan is a chlorinated hydrocarbon pesticide widely used in the field of agriculture and reported to have toxicity to liver, kidneys and blood chemistry of non target organisms including humans. The present study aims to assess the potential of medicinal plant *E. alba* to counter pesticide endosulfan induced biochemical and haematological alterations in Swiss albino mice. The biochemical analysis showed that serum levels of SGPT, ALP, total protein, albumin, urea, creatinine, total cholesterol and triglycerides, statistically significant increase ($P < 0.01$) in the endosulfan treated groups (groups-II and III) as compared to the control group (group-I). The RBC count and Hb concentrations showed a statistically very significant decrease ($P < 0.01$) in mice that were orally administered Endosulfan (3 mg/Kg) for two weeks (group-II) and four weeks (group-III). The values of HCT, MCV, MCH and MCHC decreased and were statistically significant ($P < 0.05$) as compared to the control group (group- I). Statistically a very significant ($P < 0.01$) increase in the white blood corpuscles (WBC) count was observed in group-II and group-III. Where Endosulfan treated mice which were post-treated with aqueous leaf extract of *E. alba* (group-IV) showed recovery as indicated by the values of biochemical and hematological indices. The results indicate the potential of the aqueous leaf extract of *E. alba* as a therapeutic agent which may be used against Endosulfan induced hematological and biochemical alterations in swiss albino mice.

Keywords: *Eclipta alba*, Endosulfan, Haematology, Swiss albino mice.

INTRODUCTION

Pesticides are widely used in agriculture and horticulture and reported to cause adverse effects on animal and human health. Endosulfan, a chlorinated hydrocarbon pesticide is effective against a wide range of insects and mites and has also shown significant toxicity to non target organism including humans.¹ The World Health Organization (WHO) classifies Endosulfan in Category II (moderately hazardous) and US Environmental Protection Agency (US EPA) classifies it as a Category 1b (highly hazardous) pesticide.² Endosulfan is easily absorbed by the organism from the areas where it has been sprayed, contaminated water and food. It has been reported that pesticide is most likely to affect liver, kidney, lipids profiles and blood chemistry³ and can also induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity.⁴

Previous experimental studies have shown that Endosulfan may cause inhibition of microsomal enzymes and lipid peroxidations with regard to acute and sub acute toxicity in liver and kidney.^{5,6} Hematological parameters also may be used as valuable indicators of disease or stress in animals and it has been reported that Endosulfan exposure results in significant decrease in the RBC number, hemoglobin amount, and mean corpuscular volume.^{7,8}

The use of medicinal plants which have antioxidative properties can represent the best solution against the alterations caused by pesticides because phytochemicals are less toxic or non-toxic compared to the synthetic drugs⁹ and anti-oxidants prevent cell damage from free radicals or lowers free radicals damaging effects. One of these medicinal plants, *Eclipta alba* (L.) Hassk. (Asteraceae, commonly known as Bhringaraja) which has important role in the traditional medicine.¹⁰ The main constituents of the plant are wedelolactone, dimethyl wedelolactone, β amyryn, ecliptine, ecliptal, α -terthienyl methanol, stigmaterol, 2-formyl- α -terthenyl and luteolin-7-O-glucoside.¹¹

It has analgesic, antihaemorrhagic¹² immunomodulatory and potent antihepatotoxic activity.^{13,14} It has antioxidant and free radical scavenging action¹⁵, and the antioxidant activity of *E. alba* can be attributed to the presence of phenolic and flavonoids compounds.¹⁶ Plant extracts with antioxidant activity are traditionally used to strengthen the natural immune defenses. One of the toxic mechanisms of Endosulfan toxicity is due to oxidative stress and *E. alba* act as potent antioxidant. So the aim of present study is to evaluate the beneficial effects of orally administered aqueous leaf extract of *E. alba* against sub lethal dose of pesticide Endosulfan induced biochemical as well as hematological alterations in Swiss albino mice.



MATERIALS AND METHODS

Animal model

Healthy adult Swiss albino mice of same age group and weight ($30 \pm 2g$) were used in this study. They were housed in groups of six per cage, maintained under standard laboratory conditions (temperature $22 \pm 1^\circ C$, 12:12 h light: dark cycle) and offered balanced standard maintenance diet with free access to water. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA).

Chemical

Pesticide Endosulfan was obtained from Excel India Pvt. Ltd., Mumbai with EC 35% and utilized for the experiment.

Toxicity studies of aqueous solution of Endosulfan and dose selection

Endosulfan was diluted in distilled water for obtaining the experimental concentrations of 10mg/Kg, 7.5 mg/Kg, 6.0 mg/Kg, 5 mg/Kg, 3 mg/Kg and 1mg/ Kg b. wt. and were orally administered to six groups of 6 mice each. Based on the experimental observations the acute oral LD₅₀ of Endosulfan was 7.5mg/kg b. wt., calculated using software for probit analysis (EPA PROBIT ANALYSIS PROGRAM, Version 1.5) and the sub lethal dose of Endosulfan at 3mg/kg b. wt was selected for the present investigation.

Preparation of aqueous leaf extract

E. alba was collected from the campus of B.M.D College and its identification was confirmed by Prof. S. Bedi, Department of Botany, Patna Women's College, Bihar. The voucher specimen was kept in the herbarium of the laboratory of Department of Botany under the reference number B.M.D/ BOT/03/10. The leaves were cleaned, dried and powdered in an electrical grinder and stored at 5 °C until further use and mixed in 10 ml of distilled water to obtain the therapeutic concentration to be used for the experiment.

Phytochemical screening

Freshly prepared crude aqueous extract of the leaves of *E. alba* was subjected to preliminary phytochemical screening.¹⁷

Toxicity studies of aqueous extract of *E. alba* and dose selection

Six groups of female mice of after acclimatization were treated as follows: the control group received food and tap water *ad libitum*, while the experimental groups received orally in addition different doses of 500mg/Kg, 1750mg/Kg, 2000mg/Kg, 2500mg/Kg and 3000mg/Kg b. wt. of aqueous leaf extract of *E. alba*. The mice were then observed for gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity, lethality and mortality at various intervals. Based on the experimental observations the acute oral LD₅₀ of the

extract was calculated as 2316.626 mg/Kg b.wt using software for probit analysis (Environmental Protection Agency PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, Version 1.5)¹⁸ and the treatment dose was thus selected as 250mg/kg/ b. wt. which was about 1/10th of LD₅₀.

Experimental Design

Mice were divided into four groups, each group consisting of 6 animals. Mice of group - I received food and distilled water *ad libitum*, group- II received orally a daily dose of Endosulfan at 3mg/Kg b. wt. for two weeks, group-III received orally a daily dose of Endosulfan at 3mg/Kg b. wt. for four weeks, while mice of group-IV received a daily dose of Endosulfan at 3mg/Kg b. wt. for four weeks and then were post-treated with aqueous leaf extract of *E. alba* at 250mg/Kg/b. wt. for four consecutive weeks.

Biochemical Parameters analysis

Mice of different groups were weighed, blood samples were collected, left 10 minutes to clot and centrifuged at 3000 r.p.m for serum separation which was stored at - 20°C for further determinations of liver function tests, kidney function test and lipid profile. The Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP), total protein, albumin, serum urea, creatinine, cholesterol, High Density Lipoprotein (HDL) and Triglyceride were determined by the use of standard kit methods¹⁹ and were measured using fully Automated Biochemistry Analyzer (Model No-SELECTRA-"E", VITALAB BY MERCK) in the Biochemistry Department of Mahavir Cancer Sansthan and Research Centre, Patna.

Hematological Parameters analysis

Blood samples were collected by orbital sinus puncture from different experimental groups in EDTA rinsed vials for analyzing hematological parameters including red blood cell count (RBC), mean corpuscular volume (MCV), white blood cell count (WBC), haemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and were estimated by standard procedures using Cell Counter (Medonic M- Series) in the Department Of Hematology, Mahavir Cancer Sansthan, Patna.

Statistical analysis

The data obtained in each group were represented as mean + SEM and *P* value was calculated using one way analysis of variance (ANOVA). *P*<0.05 was considered statistically significant.

RESULTS

The preliminary phytochemical investigation of the *E. alba* aqueous leaf extract showed that it contained carbohydrates, tannins, flavonoids, saponins, alkaloids, oils, amino acids and quinines.

The biochemical analysis (Table-1) showed that the level of SGPT in the Endosulfan treated groups (groups-II and



III) showed significant increase ($P < 0.01$) as compared to the control group (group-I). Highest level of SGPT was recorded in mice orally administered with 3mg/Kg b. wt. Endosulfan for four weeks (group-III), followed by mice orally administered with 3mg/Kg b.wt. Endosulfan for two weeks (group-II). Significant increase ($P < 0.01$) in the mean levels of ALP was noticed in all the Endosulfan treated groups as compared to the control group (group-I). The increase in level of ALP in the group treated with 3 mg/Kg b. wt. Endosulfan for four weeks (group-III) exceeded the level of ALP in the group treated with the same dose for two weeks (group-II). While non significant ($P > 0.05$) changes were noticed after *E. alba* treatment (group-IV) in Endosulfan treated groups as compared to normal. A significant ($P < 0.01$) decrease in time dependent manner in serum total proteins and albumin levels were detected in groups treated with Endosulfan alone (group-II and III), accompanied with a slight amelioration ($P > 0.05$) in the group treated with *E. alba* (group-IV) as compared with the control group.

In the case of urea and creatinine levels, statistically significant ($P < 0.01$) increase were recorded in Endosulfan treated groups in time dependent manner and continued through the experimental period. But after the oral treatment with aqueous leaf extract of *E. alba* (group-IV) the levels of urea and creatinine showed a trend towards value of normal levels. No significant changes ($P > 0.05$) in

the levels of urea and creatinine after *E. alba* treatment (group-IV) in mice were observed.

Concerning lipid profile, statistically a highly significant ($P < 0.01$) increase in total cholesterol and triglycerides level in Endosulfan treated groups (groups II and III) were found in time dependent manner as compared to control group (group-I). After the treatment with *E. alba* the increased levels of cholesterol and triglyceride decreases and only a significant ($P < 0.05$) increase was found as compared to normal group (group-I).

Data of hematological parameters as recorded in Table-2 showed that in experimental groups of mice treated with Endosulfan (3 mg/Kg b.wt) for two (group-II) and four weeks (group-III) the RBC count and Hb concentrations decreased and were statistically very significantly ($P < 0.01$) while HCT, MCV, MCH and MCHC values decreased significantly ($P < 0.05$) in comparison to the control group (group I). The white blood corpuscles (WBC) count showed statistically very significantly increase in group-II and group-III ($P < 0.01$). Count of WBC decreased after four week treatment with aqueous leaf extract of *E. alba*, however the values did not come up to those of normal. So in mice that were orally administered Endosulfan dose for four weeks and then treated with aqueous leaf extract of *E. alba* (Group-IV) for four weeks, a recovery pattern was observed as recorded in their biochemical and hematological parameters.

Table 1: Changes in Biochemical parameters of different experimental groups of Swiss albino mice

Biochemical parameters	Normal mice (Group-I)	Endosulfan(2W) (Group-II)	Endosulfan (4W) (Group-III)	Endosulfan (4W) + <i>Eclipta alba</i> (4W) (Group-IV)
SGPT (IU/L)	37.0 ± 0.5	60.0** ± 0.23	68.0** ± 0.12	38.5 [#] ± 0.112
ALP (IU/L)	136.6 ± 0.044	312.0** ± 0.032	324.0** ± 0.65	134.0 [#] ± 0.324
Total Protein (gm/dl)	6.04 ± 0.44	5.86* ± 0.35	5.25** ± 0.45	5.89 [#] ± 0.56
Albumin (gm/dl)	3.7 ± 0.55	3.2** ± 0.24	3.16** ± 0.51	3.56 [#] ± 0.18
Urea (mg/dl)	41.0 ± 1.44	41.65 [#] ± 1.02	44.0** ± 0.76	41.32 [#] ± 0.23
Creatinine (mg/dl)	0.5 ± 0.047	0.6* ± 0.032	0.84** ± 0.054	0.57 [#] ± 0.13
Triglyceride (mg/dl)	95.6 ± 0.326	99.6** ± 0.42	109.0** ± 0.34	100.2* ± 0.36
Cholesterol (mg/dl)	134.6 ± 0.263	162.8** ± 0.132	192.0** ± 0.32	141.33* ± 0.534

Values are given as mean ± SEM for groups of six animals each. Values are statistically significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, [#] $P > 0.05$.

Table 2: Changes in hematological parameters of different experimental groups of Swiss albino mice

Hematological parameters	Normal mice (Group-I)	Endosulfan(2W) (Group-II)	Endosulfan (4W) (Group-III)	Endosulfan (4W) + <i>Eclipta alba</i> (4W) (Group-IV)
RBC ($10^6/\text{mm}^3$)	8.98 ± 0.095	8.26** ± 0.116	7.68** ± 0.824	8.37 [#] ± 0.041
HGB (gm/dl)	14.43 ± 0.176	14.28 [#] ± 0.23	13.1** ± 0.442	14.42* ± 0.081
PCV (%)	38.29 ± 0.012	36.2** ± 0.054	35.23** ± 0.076	38.0* ± 0.116
MCV (μm^3)	40.18 ± 0.608	39.21* ± 0.216	39.73** ± 0.866	40.40 [#] ± 0.087
MCH (Pg)	14.69 ± 0.086	14.23* ± 0.094	14.1* ± 0.668	14.8 [#] ± 0.056
MCHC (gm/dl)	36.63 ± 0.522	36.2 [#] ± 0.035	35.0** ± 0.080	36.0 [#] ± 0.065
WBC ($10^3/\text{mm}^3$)	6.91 ± 0.142	14.5** ± 0.023	16.5** ± 1.021	12.7** ± 0.090

Values are given as mean ± SEM for groups of six animals each. Values are statistically significant at *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, [#] $P > 0.05$.



DISCUSSION

Pesticides are commonly used agricultural chemicals and in addition to its primary target, pesticides can also affect human and animal in the vicinity of sprayed area. Once the pesticides enter the body, it is transported to different parts of it through the blood and effects the biochemical environment of vital organs and blood.²⁰

In the case of liver function test profile, statistically a highly significant increase in the levels of SGPT and ALP were recorded in case of Endosulfan administered groups, while no significant changes were noticed in all other groups. The pesticide Endosulfan when administered orally reaches the liver first through the hepatic portal vein and the elevated level of these enzymes may be due to Endosulfan induced alterations in the hepatic cells. It has also been reported that sub acute Endosulfan toxicity can cause bile ducts proliferations, hemorrhage, necrosis and inflammation in rabbit's liver which may be attributed to the increased level of SGPT and ALP. In our study the levels of serum total protein and albumin in Endosulfan treated groups (group II and group III) showed statistically significant decrease. The decrease in total protein level might be due to catabolism of protein and/ or malfunction of liver.²¹ Our result correlated with the experiment of Arshad et al.²² where mice fed on Endosulfan showed a statistically significant decrease in levels of serum protein and albumin. A recovery pattern in the serum levels of SGPT, ALP, total protein and albumin were observed after four weeks treatment with aqueous leaf extract of *E. alba* (group-IV). Various reported studies have showed that hepatoprotective activity of *E. alba* is by regulating the levels of hepatic microsomal drug metabolizing enzymes.^{14,23} In the present study serum urea and creatinine levels were found to be elevated in Endosulfan administered groups (group-II and III), indicating the stress on kidney due to Endosulfan toxicity. Such elevation in the serum urea and creatinine induced by pesticides may be due to the increase of plasma membranes permeability of the renal cells or damages induced by insecticide in the kidney which cause insufficient excretion of wastes leading to more urea and creatinine accumulation in the blood. Furthermore, the increase of urea and creatinine level in this study is convincingly supported by the findings of Choudhary et al.²⁴ in rat after Endosulfan treatment. In our study treatment with aqueous leaf extract of *E. alba* (250 mg/Kg b. wt.) significantly reduced the levels of urea and creatinine in the serum of mice administered with Endosulfan for four weeks as compared to only Endosulfan administered mice (group-II and III). A number of literature reported that the plant extracts containing phenolic compounds and flavonoids have the ability to remove excess urea, uric acid and creatinine during various nephrotoxic conditions.^{25,26}

In our study it has been found that the increase in the duration of exposure to Endosulfan caused marked

increase in serum cholesterol level and triglyceride level. The increased level of cholesterol may be due to increased synthesis and accumulation of it in the liver and kidney or impaired biliary function and the increased level of triglycerides probably may be due to the cells apoptosis because the triglycerides are the main components of cell membrane.²⁷ These results are in accordance with a previous report where blood cholesterol and triglyceride levels show significant increase due to Endosulfan induced stress.²⁸ The levels of serum cholesterol and triglyceride decreased significantly in group-IV that was treated with aqueous leaf extract of *E. alba* for four weeks. Our results are corroborative with Dhandapani²⁹ who reported that the aqueous extract of *E. prostrata* have tendency to decrease serum cholesterol and triglyceride levels in the case of atherogenic diet induced hyperlipidemic rats. The hypolipidemic effects of *E. alba* may rely on the presence of polypeptides, steroids, and flavonoids.³⁰

Hematological constituents provide information for evaluating the bio-toxicity of certain compounds.³¹ Therefore, in the present study the changes in hematological parameters due to Endosulfan toxicity in mice was recorded and the potential of medicinal plant *E. alba* known for its antioxidative properties was evaluated to counter the changes. The results of the present study showed statistically significant decrease in the values of RBC count, MCV, MCH, MCHC and HCT. This indicates injurious action of the pesticide Endosulfan on the hematological parameters of the experimental animals. Reduction in RBC count and hemoglobin concentration may be due to increased rate of breakdown of red cells and/or reduction in the rate of RBC formation.³² Moreover, the hepatic heme biosynthesis has already been reported to be affected by insecticidal exposure which also contributes to decreased RBC count and hemoglobin concentration.³³ In a similar study by Morowati³⁴ in mice, a statistically significant decrease in haemoglobin concentration (Hb conc.), red blood corpuscle count (RBC) count and packed cell volume percent (PCV%) were observed from the 6th week exposure of Endosulfan.

The MCV relates to the average size of the red blood cell. The amount of hemoglobin in a single red blood cell is indicated by the MCH. In the present study, the levels of both MCV and MCH decreased in the mice of group-II and it may be due to decrease in size of RBC, destruction of number of RBC or impaired biosynthesis of heme. In another study decrease in MCV, MCH and MCHC were observed in rats treated with various insecticides such as Endosulfan, malathion, methyl parathion, phosphomidon, monocrotophos and fenvalerate.³⁵

Total leukocyte count (WBC count) showed a statistically significant rise in the second and fourth week of exposure to Endosulfan which is in agreement with a similar report of Azhar Baig³⁶ where increased value of WBC was observed in rats treated with Endosulfan. The results of



our study also indicated that oral treatment with aqueous leaf extract of *E. alba* for four weeks had the potential to reverse the extensive changes in the hematological parameters of Endosulfan administered mice (group-IV) and it could bring the values near to the normal levels for all the hematological parameters such as RBC, WBC, MCV, HCT, HGB, MCH and MCHC.

Our study and previously reported studies have shown that *E. alba* has high quantity of flavonoids, ascorbic acid and phenolic content^{16, 18} and that can probably explain its stronger free radical scavenging activity. Pesticide Endosulfan induced oxidative stress which generates free radicals and *E. alba* has free radical scavenging activity and due to this property it protect the biochemical and hematological alterations induced by exposure of Endosulfan. Considering these factors we suggest that the biological activity of plant extract against Endosulfan toxicity might be due to the presence of these compounds in the aqueous extract of leaf of *E. alba*.

CONCLUSION

The results obtained from the present study indicated that the aqueous leaf extract of *E. alba* may protect severe alterations induced by exposure to Endosulfan, therefore, it may be inferred that the aqueous leaf extract of *E. alba* may be considered as a promising therapeutic agent against Endosulfan induced toxicity. However, in order to understand the exact mechanism by which *E. alba* exerts its protective effect against Endosulfan induced biochemical hematological alterations, further in depth investigations are required.

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