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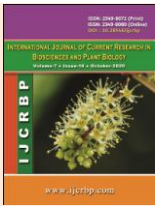

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Role of newly developed novel multi herbal formulation (AKSS16-LIV01) in ameliorating carbon tetrachloride induced haemato-toxicity in Swiss albino mice

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ABSTRACT

Haematological disorders and related complications are very common phenomenon against hazardous chemicals. Alteration of hematologic parameters disrupts the body's normal homeostasis. There is a worldwide need to develop a safe and symptomatic medication which controls the haematological complications. Healthy adult Swiss albino mice were assigned to four groups of six mice each according to their weights. Group-I serve as control, Group-II received multi herbal formulation (AKSS16-LIV01) 400 mg/kg/day, Group-III received carbon tetrachloride (CCl_4) 1 ml/kg-bw and Group-IV received CCl_4 along with AKSS16-LIV01 (400 mg/kg). Blood samples were collected from the retro orbital plexus of each animal to determine various blood parameters and liver transaminase. Administration of carbon tetrachloride (CCl_4) showed decline body weight, food consumption and water intake in mice whereas treatment with multi herbal formulation (AKSS16-LIV01) normalized the same as compared with untreated animals. Treatment with CCl_4 (Group-III) decline the packed cell volume (PCV), haemoglobin (Hb), means cell volume (MCV), means cell hemoglobin (MCH) and greater the white blood cell (WBC) compared with control. Pre-treatment with AKSS16-LIV01 significantly ($p<0.001$) increased the PCV, Hb, MCH, MCH and decreased WBC count in experimental animals as compared with CCl_4 treated group. On the other hand elevated liver transaminase enzymes i.e. AST and ALP by CCl_4 was restored with administration of multi herbal formulation (AKSS16-LIV01). Chronic administration of CCl_4 indicated adverse effects on haematologic parameters upon experimental animals. Simultaneous administration with newly developed novel multi herbal formulation (AKSS16-LIV01) was able to ameliorate these adverse effects and may be a potent drug in future which controls the blood related medical complications against the toxicants.

Introduction

Last few decades in various industrial processes carbon tetrachloride (CCl_4) is extensively used as a solvent (Arindkar et al., 2012). Due to its solvent property, this hazardous chemical used as refrigerator fluids, as a propellant for aerosol cans, as a dry-cleaning agent in industry, as a household spot remover, as grain fumigant and as intermediate in the synthesis of chlorofluorocarbons. As a result CCl_4 can easily found in the water bodies and contaminant the ground and surface water. Exposure and consumption of excessive CCl_4 disrupt body's homeostasis and make liver and kidney damage (Essawy et al., 2010; Gupta et al., 2004). Within the body CCl_4 can generate reactive oxygen species (ROS) like peroxides, superoxide, hydroxyl radical, singlet oxygen, and alpha-oxygen caused oxidative damage. Hepatotoxicity is very common when people exposed with CCl_4 (Mandal et al., 1998).

Adverse effect of carbon tetrachloride (CCl_4) in blood is well established. A recent study depicted that administration of CCl_4 reduced red blood cell (RBC), packed cell volume (PCV) and Haemoglobin (Hb) that disturbed the haematopoiesis (Travlos et al., 1996; Uchechukwu et al., 2018).

Various ultra-structural abnormalities in the leukocytes in the blood were visible under electron microscopy of mice those treated with CCl_4, clearly demonstrated that this notorious chemical makes the structural deformities in blood (Parasuraman et al., 2014).

Multi herbal formulations mean a dosage form consisting of one or more herbs or processed herbs in specified quantities which have potent therapeutic efficacy without adverse effects (Hasan et al., 2009; Srivastava et al., 2012). Scientific study revealed that this plant based formulation is very effective to cure anaemia and control the blood (Darbar et al., 2020; Thyagarajan et al., 2002).

Here we developed a multi herbal formulation (AKSS16-LIV01) based on six Indian medicinal plants and three Indian spices. Our previous study established that the formulation is completely safe in various doses upon experimental animals (Abdel- Wahhab and Aly, 2005; Adhikari et al., 2018). With view of the above, there is need to developed and safe and symptomatic medication that controls all haematological parameters in the body when system exposed with toxicant.

Materials and methods

Chemicals

Carbon tetrachloride (CCl_4) and TRIS buffer were obtained from Merck, India. PBS pH 7.4 was procured from Sigma-Aldrich. Biochemical determination kits i.e. ALT and AST were procured from Thermo Scientific, USA. All others reagents used in this study are laboratory grade.

Preparation of plant extract

All the medicinal plant and spice ingredients were collected from registered local herbal suppliers and authenticated by pharmacognosist. Plants parts were cleaned and dry with normal temperature. The dried plant parts were used for preparation of multi herbal formulation as per standard validated protocol (Adhikari et al., 2018). The plants and plant parts used in preparation of the extract are listed in Table 1.

Animals

Twenty four young, healthy Swiss albino mice weighing 25g ± 5g have been randomly included for the study. The animals have been housed in healthy atmospheric conditions (12 h light and dark cycles, at 25±2 ^{0}C and 50-60% humidity), normal feeding, drinking, and medical care based on the CPCSEA guidelines. Mice were kept under observation for one week before the onset of the experiment for acclimatization and to exclude any unsercurrent infection. The experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. 261/JU/s/IAEC/Pharma/2018).

Experimental procedure

The mice were randomly assigned to four major groups of six mice each according to their body weights such that each group was made up of mice within the close range of body weight. The groups are as follows: Group-I serve as control, Group-II

received multi herbal formulation (AKSS16-LIV01) 400 mg/kg/day, Group-III received carbon tetrachloride (CCl_4) 1 ml/kg-bw and Group-IV received CCl_4 along with AKSS16-LIV01 (400 mg/kg).

Body weight, food consumption and water intake

Body weights were measured on weekly basis from the initial day to the final day of experiment to calculate body weight alteration. Feed intake was determined by measuring feed residue on weekly basis since the beginning of the experiment. Feed conversion was obtained by dividing total feed intake by body weight gain. Water intake was determined by subtracts the remaining of water found in the drinking bottle from the initial water given to the animals.

Blood collection and serum preparation

At the end of the respective fasting period, blood was collected from each mouse by retro orbital venous puncture. 200 µL of blood sample were collected into micro-centrifuge tubes with and without EDTA (2%). Collected bloods were placed in slanting position at room temperature for 2 hrs. Then, they were centrifuged at 3500 g for 10 min. Clear light yellow colour serum was separated and used for further analyses.

Evaluation of haematological parameters

Complete blood count includes hemoglobin (Hb), packed cell volume (PCV), total red blood corpuscles (TCRBC), total count of white blood cells (TCWBC), differential count (DC), platelets count, RBC indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were analysed by Sysmex KX-21, TRANSASIA, a fully automated 3-part differential hematology analyzer.

Determination of biochemical parameters

Liver function enzymes such as AST and ALT were used as biochemical markers for hepatotoxicity and assayed by the standard (14).

Statistical analysis

Data are presented as mean ±SE. Statistical analysis of the data was carried out using two way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. Statistical significance was acceptable to a level of $p< 0.05$.

Results

Effect of multi herbal formulation (AKSS16-LIV01) on Body weight, Food Consumption and Water Intake

Gross body weights and relative changes, food consumption and water intake was presented in table 2. Administration of carbon tetrachloride (CCl_4) (1 ml/kg-bw) significantly reduced ($p<0.001$) the body weight, food intake and water intake capacity as compared with control animals. Treatment with multi herbal formulation (AKSS16-LIV01) 400mg/kg/day normalized the body weight, daily food intake and water intake capacity and reduced the liver weight as compared with control animals. Administration of AKSS16-LIV01 did not show any abnormal changes as compared with control animals.

Effect of multi herbal formulation (AKSS16-LIV01) on Haematological parameters

Haematological parameters of control and experimental groups are shown in Table 2 as well as in figures (Fig. 1 to Fig. 5). 28days treatment with newly developed novel multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day did not showed significant differences in PCV, haemoglobin (Hb), WBC, mean cell volume (MCV), and mean cell hemoglobin (MCH) compared with the control (figure 1-5). Significant reduction in Hb ($p<0.001$), PCV ($p<0.001$), MCV ($p = 0.001$), and MCH ($p< 0.001$) was noticed in CCl_4 intoxicated mice when compared with the control. The WBC count was significantly ($p< 0.001$) greater in Group C compared with the control. In contrast, no significant differences were observed in RBC and MCHC between the control and Group C. Administration of multi herbal formulation (AKSS16-LIV01) along with CCl_4 significantly increased Hb ($p<0.001$), PCV ($p<0.001$), MCV ($p< 0.001$), and MCH ($p< 0.001$) when compared with the CCl_4 intoxicated animals.

On the other hand WBC count was significantly reduced in Group D CCl_4 intoxicated animals. Others haematological parameters (table 3) like Read Blood corpuscle (RBC); Reticulocyte (RT); Haematocrit (HCT); Mean corpuscular haemoglobin concentration (MCHC) did not show any significant changes upon all the experimental groups.

Table 1. Details of ingredient(s) present in the newly developed novel multi herbal formulation (AKSS16-LIV01).

Botanical name	Common name	Family	Part used	Quantity used in extract
Tinospora cordifolia	Guduchi	Menispermaceae	Stem	20 mg
Terminalia chebula	Haritaki	Combretaceae	Fruit	20 mg
Azadirachta indica	Neem	Meliaceae	Leaves	50 mg
Andrographis paniculata	Kalmegh	Acanthaceae	Leaves & Steam	50 mg
Aloe barbadensis miller	Aloe vera	Liliaceae	Leaves & Steam	50 mg
Curcuma longa	Curcuma, Haldi	Zingiberales	Rhizome	20 mg
Trigonella foenum-graecum	Methi	Fabaceae	Seed	10 mg
Piper nigrum	Black pepper	Piperaceae	Seed	10 mg
Elettaria cardamomum	Cardamom	Zingiberaceae	Seed	10 mg

* Amount required for preparation of 5 ml extract.

Table 2. Effect of multi herbal formulation (AKSS16-LIV01) on body weight, food consumption and water intake.

Parameters	Mice			
	Group-I	Group-II	Group-III	Group-IV
Body weight (g) Initial	26.35±1.91	26.51±2.35	26.71±4.2	26.68±5.1
Body weight (g) Final	37.84±2.03	36.94±1.69	21.81±2.41$^{\#}$	36.97±1.67*
Body weight (g) gain or loss	11.49±0.06	10.43±0.04	4.90±0.006	10.29±0.03
Food consumption (g)	4.52±0.05	4.37±0.07	2.94±0.06$^{\#}$	5.11±0.04*
Water intake (ml)	4.01±0.04	4.25±0.04	3.01±0.02$^{\#}$	4.31±0.06*

Table 3. Effect of novel multi herbal formulation (AKSS16-LIV01) on haematological parameters.

Parameters	Mice			
	Group-I	Group-II	Group-III	Group-IV
RBC (x10^{6} µL^{-1})	10.8±4.1	10.2±5.3	10.1±4.2	10.6±5.1
RT (%)	2.8±1.1	2.4±1.6	2.8±2.4	2.9±1.6
HCT (%)	34.8±1.3	32.8±2.1	32.8±2.1	35.1±3.1
MCHC (%)	41.4±7.6	41.7±2.4	40.4±1.4	41.4±1.4
Platelets (x10^{3} µL^{-1})	6.6±2.0	6.9±1.2	6.3±1.2	6.5±2.6
Lymphocyte (%)	76±6.3	72±3.3	73±5.4	73±3.4
Neutrophil (%)	25±6.2	22±4.3	21±5.1	25±6.9
Monocyte (%)	2.3±0.01	2.6±0.01	1.1±0.02	2.4±0.01
Eosinophil (%)	9.6±2.6	9.3±4.1	9.4±2.5	9.2±3.6
Basophil (%)	1.2±0.05	1.5±0.02	1.4±0.02	1.2±0.04

Data are expressed as mean ± standard deviation (N=6); RBC: Read Blood corpuscle; RT: Reticulocyte; HCT: Haematocrit; MCHC: Mean corpuscular haemoglobin concentration.

Table 4. Effect of novel multi herbal formulation (AKSS16-LIV01) on serum biochemical parameters

Groups	AST (Unit/L)	ALT (Unit/L)
Group-I	54.25±6.31	27.88±4.58
Group-II	56.92±7.06	29.58±4.64
Group-III	108.95±9.17$^{\#}$	68.57±7.91$^{\#}$
Group-IV	61.28±5.21*	31.09±5.45*

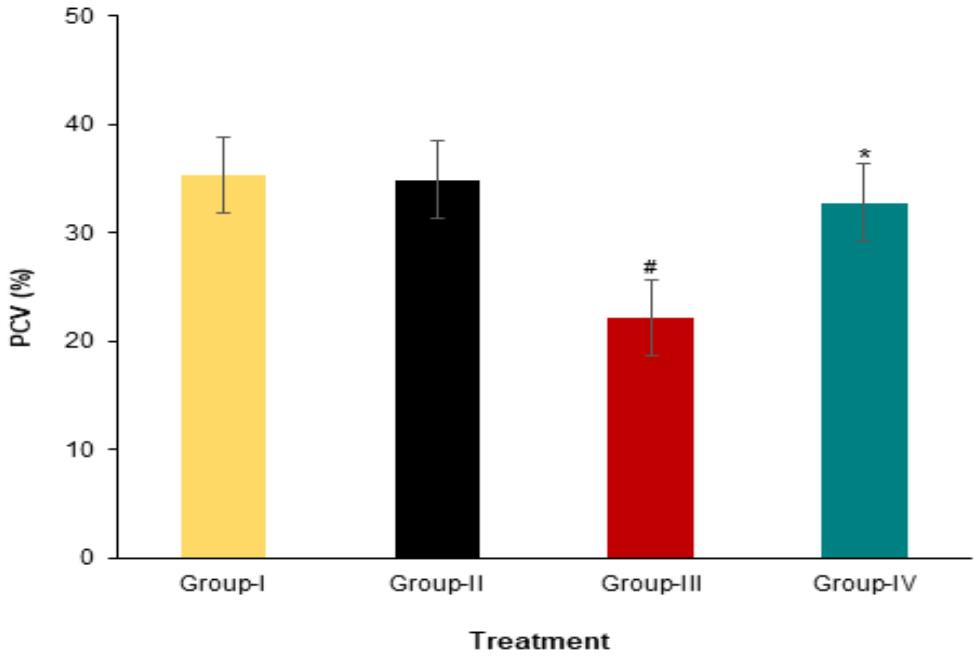

Fig. 1: Effect of multi herbal formulation (AKSS16-LIV01) on packed cell volume (PCV) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *Significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

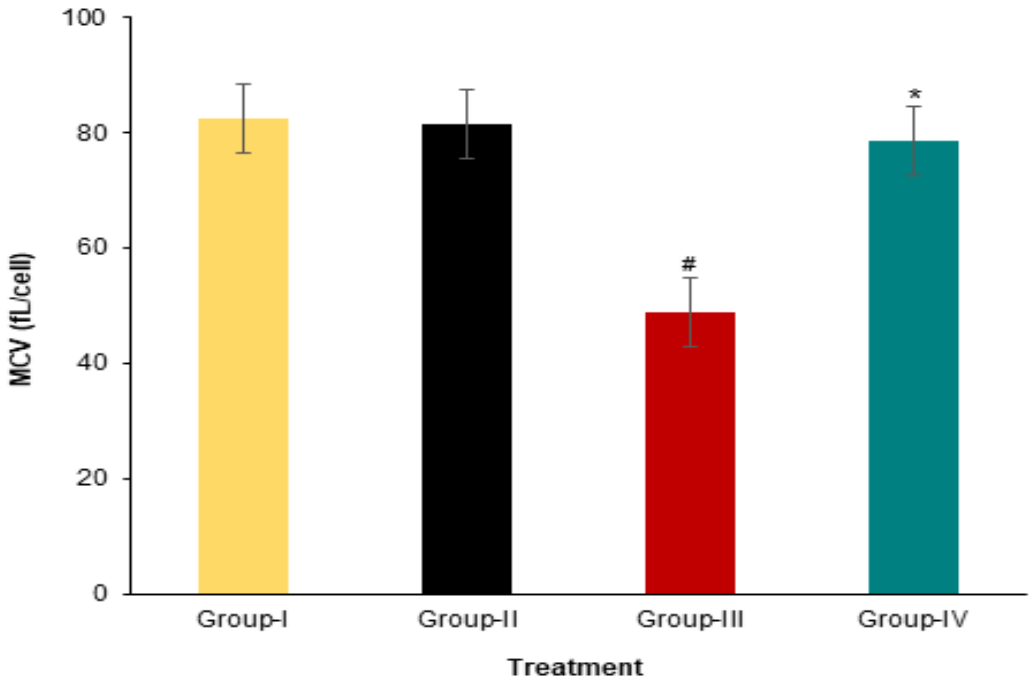

Fig. 2: Effect of multi herbal formulation (AKSS16-LIV01) on mean cell volume (MCV) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

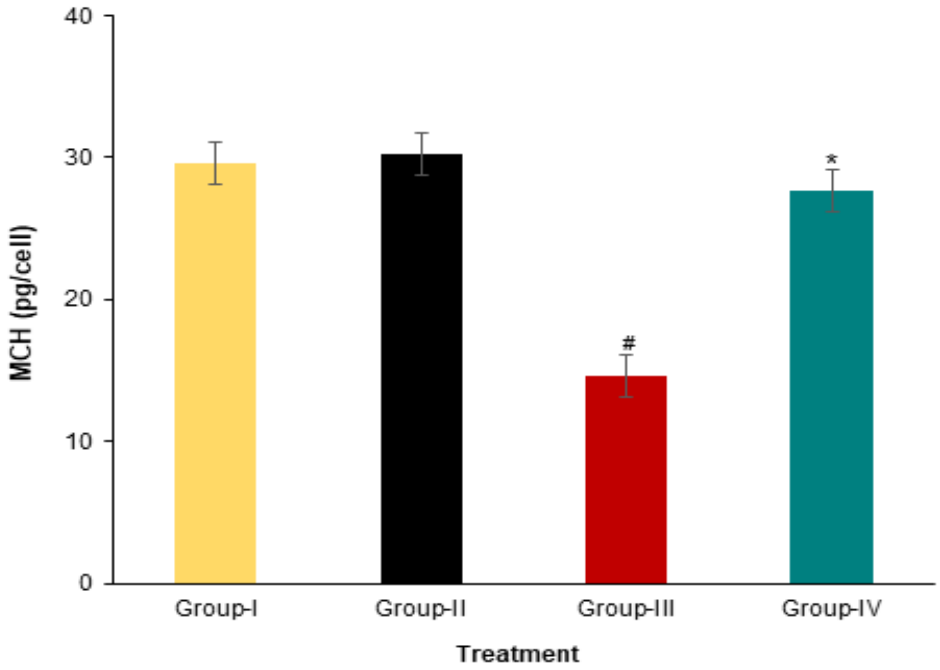

Fig. 3: Effect of multi herbal formulation (AKSS16-LIV01) on mean cell hemoglobin (MCH) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

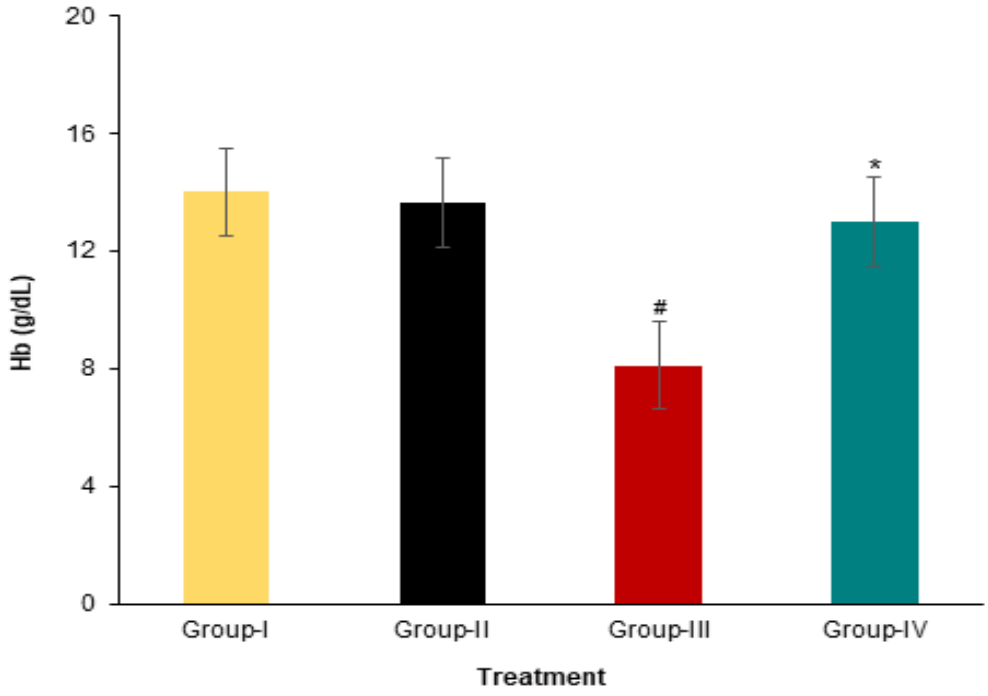

Fig. 4: Effect of multi herbal formulation (AKSS16-LIV01) on haemoglobin (Hb) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *Significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

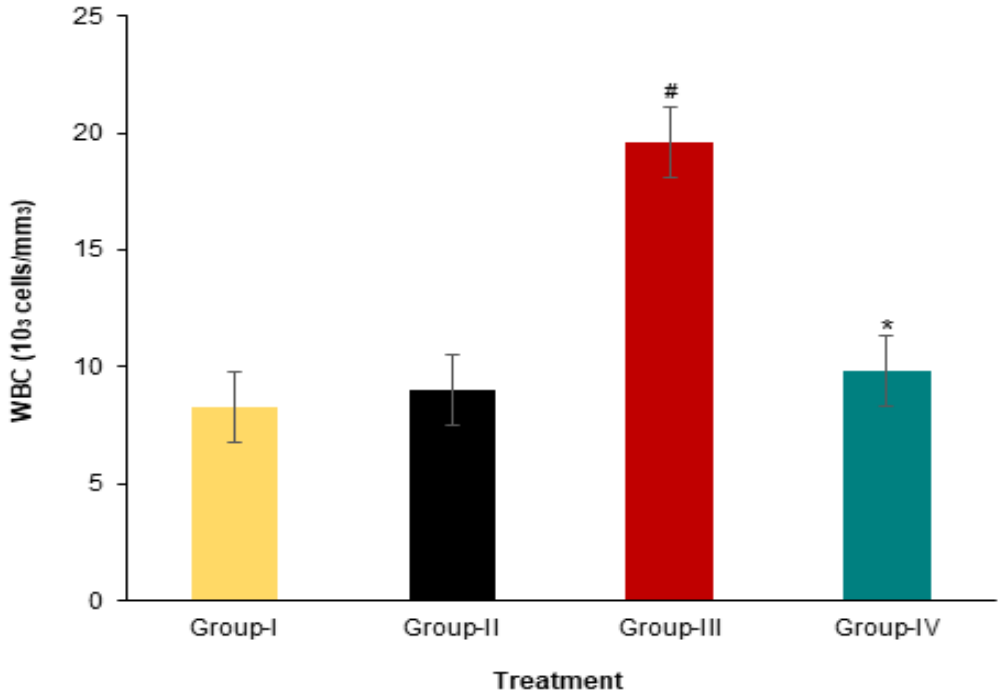

Fig. 5: Effect of multi herbal formulation (AKSS16-LIV01) on white blood cell (WBC) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at p<0.001 and *Significantly different from (CCl_4) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.

Effect of multi herbal formulation (AKSS16-LIV01) on Biochemical parameters

Table 4 shows the mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in control and experimental groups of mice. Data indicate that CCl_4 intoxicated mice had significantly greater mean AST and ALT compared with the control (p<0.001). Pre-treatment with multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day normalized the elevated AST and ALT levels when compared with CCl_4 treated mice. 28days treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not shows significant differences in AST and ALT when compared with control group.

Discussion

Hazardous toxicants and chemicals leads to various haematological parameters and developed medical complications. Carbon tetrachloride (CCl_4) one of the very common solvent used in various industrial processes traded as an environmental pollutant (Manthorpe et al., 1977). It is reported that treated with CCl_4 at a dose of 0.05 ml on mice reduced the haemoglobin (Hb), packed cell volume (PCV), and mean corpuscular volume (MCV) values (Tung, Cook et al., 1975). Another report depict that lower haemoglobin (Hb) value leads to iron deficiency anaemia which is characterized by a microcytic hypochromic blood picture (Thapa and Walia, 2007). In the present study our result also confirm that administration of CCl_4 (1 ml per kg body weight) decline Hb, PCV, MCH and MCV values could be attributed to disturbed hematopoiesis, destruction of erythrocytes. The low PCV and Hb concentration and the abnormally low values of MCV and MCH are indications of microcytic anaemia. Medicinal plants enrich with various compounds capable to control and maintained the various blood parameters. Pre-treatment with newly developed multi herbal formulation (AKSS16-LIV01) along with CCl_4 elevate Hb, PCV, MCH and MCV values may indirectly protect the body from the anaemia.

Elevated aspartate transaminase (AST) and alanine transaminase (ALT) levels are strong indicators of inflammatory conditions and injury to the liver (Singh, 2013), while increased white blood cells (WBC) level is generally recognized as an inflammatory response. Inflammatory conditions may induce malnutrition in the body. It is reported that inflammatory conditions can interfere with the body's ability to use stored iron and absorb iron from the diet (Gkamprela and Pectasides, 2017; Gonzalez-Casas and Moreno-Otero, 2009). The results of the present study clearly showed that treatment with CCl_4 (1 ml per kg body weight) abruptly increased serum aspartate transaminase (AST) and alanine transaminase (ALT) levels as well as elevate white blood cells (WBC) count indicate CCl_4 produce inflammatory response and affects liver cell, disturbed homeostasis. On the other hand administration with newly developed multi herbal formulation (AKSS16-LIV01) along with CCl_4 decline the AST, ALT value and WBC count protect the liver against CCl_4 induced inflammation. Thus our developed multi herbal formulation composed with six medicinal plants and three medicinal spices may be able to protect haematological disturbance caused by CCl_4.

Conclusion

This investigation shows that the developed novel multi herbal formulation (AKSS16-LIV01) has the ability to protect the haematopoietic cells from the damaging effects of exposure to CCl_4 and this protection might be attributed to the anti-oxidative power of multi herbal formulation (AKSS16-LIV01). Thus, the developed formulation composed of medicinal herbs and medicinal spices might be a therapeutic medicine in future for the prevention of haematological dysfunction.

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Authors' contribution

Soumendra Darbar and Atiskumar Chattapadhyay conceived and designed the experiment. Soumendra Darbar and Srimoyee Saha conducted the animal and biochemical experiments. Soumendra Darbar, Atiskumar Chattapadhyay and Kaushikisankar Pramanik wrote and revised the manuscript.

Conflicts of Interest

All authors report no conflicts of interest regarding this manuscript.

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