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Research Article

## Ameliorative efficacy of novel multi herbal formulation (AKSS16-LIV01) upon Haematological modulations induced by fixed dose combination of tramadol hydrochloride/paracetamol (THP)

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### ABSTRACT

**Background:** Tramadol hydrochloride/paracetamol (THP) a fixed dose combination (FDC) is widely spread analgesic used to treat moderate to moderately severe pain. Over dose or chronic use of this fixed dose combination produce serious adverse effects. An acute Tramadol hydrochloride/paracetamol (THP) overdose can lead to a fatal liver damage.

**Objectives:** There is a worldwide need to develop a safe and symptomatic medication which controls the different medical complications.

**Materials and Methods:** 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 Tramadol hydrochloride/paracetamol (THP) 1.68 g / 300ml water and Group-IV received THP 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. **Results:** Administration of THP 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 THP (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. On the other hand elevated liver transaminase enzymes i.e. AST and ALP by THP was restored with administration of Multi herbal formulation (AKSS16-LIV01).

**Conclusion:** Chronic administration of THP indicated adverse effects on haematologic parameters upon experimental animals. Simultaneous administration with newly developed multi herbal formulation (AKSS16-LIV01) was ameliorate these adverse effects and may be potent drug in the future which controls the blood related medical complications against the toxicants.

**Keywords:** Tramadol hydrochloride/paracetamol; Fixed dose combination; Multi herbal formulation; Hematologic parameters; Liver transaminase; Swiss albino mice

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### INTRODUCTION

Tramadol hydrochloride/paracetamol is a fixed dose combination (FDC) used to treat moderate to moderately severe pain. This fixed dose combination (FDC) contains 37.5 mg of tramadol hydrochloride and 325 mg of paracetamol <sup>1</sup>. Immediate release (IR) formulation orally relief pain within

an hour. Tramadol has a central acting mechanism via serotonin receptors and acts by binding  $\mu$ -opioid receptors and neurons, and it is also a serotonin-norepinephrine reuptake inhibitor (SNRI) <sup>2</sup>. Over dose and chronic consumption of this combination produce constipation, itchiness and nausea <sup>3</sup>. Some times more serious adverse

effects like insomnia, drug dependency and a high risk of serotonin syndrome may occur <sup>4</sup>.

Intake of low dose of tramadol hydrochloride/paracetamol can acts as an effective analgesic but at high dosage and over a prolonged period the combination may cause various complications and disrupt body's homeostasis <sup>5, 6</sup>. Recent study showed that application of tramadol hydrochloride/paracetamol (THP) alters normal value of the various haematological parameters in animals <sup>7</sup>. Apart from this prolonged or chronic administration of THP may cause severe thrombocytopenia, leading to failure of the immune system, anemia and a very low erythrocyte count <sup>8,9</sup>.

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 <sup>10, 11</sup>. Scientific study revealed that this plant based formulation is very effective to cure anaemia and control the blood <sup>12</sup>. 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 <sup>13</sup>. 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 fixed dose combination.

## MATERIALS AND METHODS

### Chemicals

Tramadol hydrochloride and paracetamol were obtained from Dey's Medical Stores (Mfg.) Ltd., Kolkata as a gift sample. Ethanol, sodium chloride, sodium hydroxide 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 <sup>14</sup>. The plants and plant parts used in preparation of the extract are listed in Table 1.

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

Sl. No.	Botanical Name	Common Name	Quantity used in extract
<b>Indian Medicinal Herbs</b>			
1.	<i>Tinospora cordifolia</i>	Guduchi	20 mg
2.	<i>Terminalia chebula</i>	Haritaki	20 mg
3.	<i>Azadirachta indica</i>	Neem	50 mg
4.	<i>Andrographis paniculata</i>	Kalmegh	50 mg
5.	<i>Aloe barbadensis miller</i>	Aloe vera	50 mg
6.	<i>Curcuma longa</i>	Curcuma, Haldi	20 mg
<b>Indian Medicinal Spices</b>			
7.	<i>Trigonella foenum-graecum</i>	Methi	10 mg
8.	<i>Piper nigrum</i>	Black pepper	10 mg
9.	<i>Elettaria cardamomum</i>	Cardamom	10 mg

\* Amount required for preparation of 5 ml extract.

### 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 °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 uncurrent 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 Tramadol hydrochloride/paracetamol (THP) daily at dosage of 1.68 g/300 ml of water and Group-IV received THP (1.68 g/300 ml of water) 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.

### Hematological Parameters

For hematological studies, the blood was collected in heparinized tubes. Blood-cell count was done using blood smears in Sysmax-K1000 Cell Counter. Parameters studied were hemoglobin, total red blood cell, reticulocyte, hematocrit, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, total white blood cell and differential count.

### Determination of biochemical parameters

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

### Statistical analysis

Data are presented as mean  $\pm$  SE. Statistical analysis of the data was carried out using two way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. 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 Tramadol hydrochloride/paracetamol (THP) 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 as compared with Tramadol hydrochloride/paracetamol (THP) treated animals. Administration of AKSS16-LIV01 did not show any abnormal changes as compared with control animals.

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

Parameters	Groups			
	Control	AKSS16-LIV01	THP	THP + AKSS16-LIV01
Body weight (g) Initial	25.16 $\pm$ 2.57	25.84 $\pm$ 2.44	26.01 $\pm$ 5.21	25.11 $\pm$ 4.85
Body weight (g) Final	36.98 $\pm$ 2.55	36.91 $\pm$ 2.69	21.22 $\pm$ 2.61 <sup>#</sup>	36.67 $\pm$ 1.47 <sup>*</sup>
Body weight (g) gain or loss	11.82 $\pm$ 0.06	11.07 $\pm$ 0.04	4.79 $\pm$ 0.006	11.56 $\pm$ 0.03
Food consumption (g)	4.81 $\pm$ 0.05	4.39 $\pm$ 0.07	2.97 $\pm$ 0.05 <sup>#</sup>	5.18 $\pm$ 0.04 <sup>*</sup>
Water intake (ml)	4.18 $\pm$ 0.04	4.27 $\pm$ 0.04	2.98 $\pm$ 0.02 <sup>#</sup>	4.55 $\pm$ 0.04 <sup>*</sup>

All data were expressed as mean  $\pm$  SE (n=6/group). Data comparison was performed using two way ANOVA followed by Tukey's Multiple Comparison Test. <sup>#</sup>Significantly different from the control group at  $p < 0.001$  and <sup>\*</sup>Significantly different from (THP) group values at  $p < 0.001$

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

Haematological parameters of control and experimental groups are shown in table 3 and figure 1 to 5. Four weeks treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day did not showed significant differences in PCV, haemoglobin (Hb), WBC, RBC, mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH) compared with the control. Significant reduction in Hb ( $p < 0.001$ ), PCV ( $p < 0.001$ ), MCV ( $p < 0.001$ ), and MCH ( $p < 0.001$ ) was noticed in THP intoxicated mice when compared with the Control (Figure 1-4). The WBC count

(Figure 5) 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 THP significantly increased Hb ( $p < 0.001$ ), PCV ( $p < 0.001$ ), MCV ( $p < 0.001$ ), and MCH ( $p < 0.001$ ) when compared with the THP intoxicated animals. On the other hand WBC count was significantly reduced in Group D THP + AKSS16-LIV01 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-3:** Effect of multi herbal formulation (AKSS16-LIV01) on haematological Parameters

Parameters	Groups			
	Control	AKSS16-LIV01	THP	THP + AKSS16-LIV01
RBC ( $\times 10^6 \mu\text{L}^{-1}$ )	10.8 $\pm$ 4.1	10.2 $\pm$ 5.3	10.1 $\pm$ 4.2	10.6 $\pm$ 5.1
RT (%)	2.8 $\pm$ 1.1	2.4 $\pm$ 1.6	2.8 $\pm$ 2.4	2.9 $\pm$ 1.6
HCT (%)	34.8 $\pm$ 1.3	32.8 $\pm$ 2.1	32.8 $\pm$ 2.1	35.1 $\pm$ 3.1
MCHC (%)	41.4 $\pm$ 7.6	41.7 $\pm$ 2.4	40.4 $\pm$ 1.4	41.4 $\pm$ 1.4
Platelets ( $\times 10^3 \mu\text{L}^{-1}$ )	6.6 $\pm$ 2.0	6.9 $\pm$ 1.2	6.3 $\pm$ 1.2	6.5 $\pm$ 2.6

Data are expressed as mean  $\pm$  standard deviation (N=6)

RBC: Read Blood corpuscle; RT: Reticulocyte; HCT: Haematocrit; MCHC: Mean corpuscular haemoglobin concentration;

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

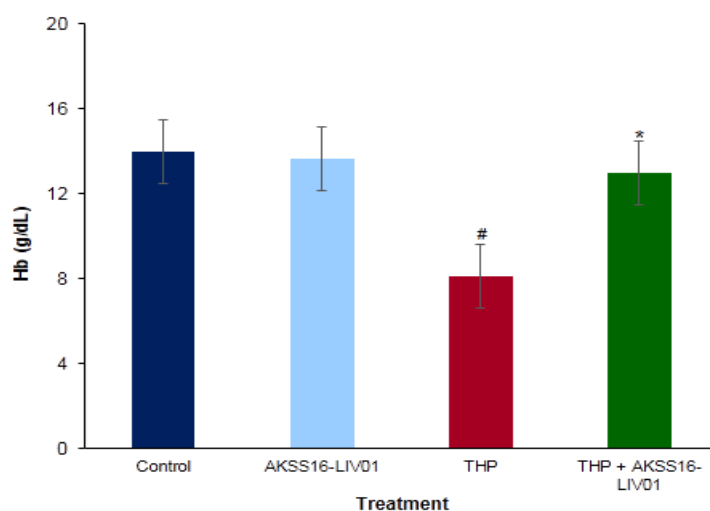
Table 4 shows the mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in control and experimental groups of mice. Data indicate that THP intoxicated mice had significantly greater mean AST and ALT compared with the control ( $p < 0.001$ ). Pre-treatment with

multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day normalized the elevated AST and ALT levels when compared with THP treated mice. Four weeks treatment with newly developed multi herbal formulation (AKSS16-LIV01) at a dose of 400 mg/kg/day alone did not show significant differences in AST and ALT when compared with control group.

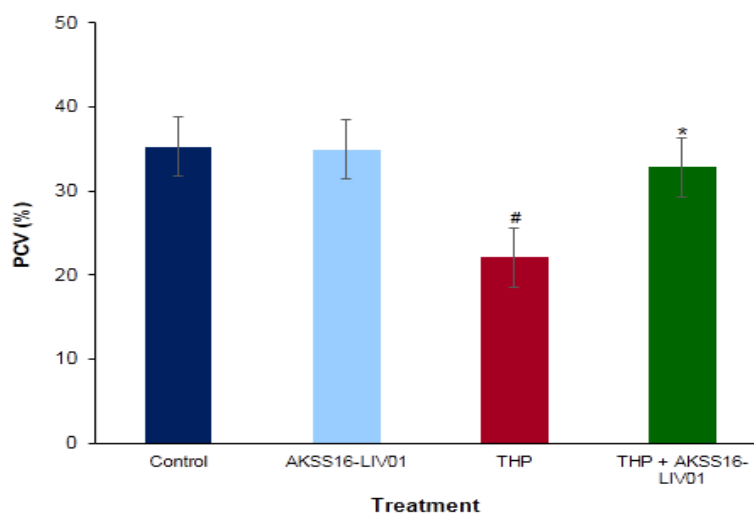
**Table-4:** Effect of multi herbal formulation (AKSS16-LIV01) on serum biochemical parameters

Groups	AST (Unit/L)	ALT (Unit/L)
Control	55.28±6.34	26.82±4.11
AKSS16-LIV01	54.91±5.81	27.54±4.62
THP	106.28±8.17#	67.59±6.01#
THP + AKSS16-LIV01	62.99±5.44*	31.83±5.18*

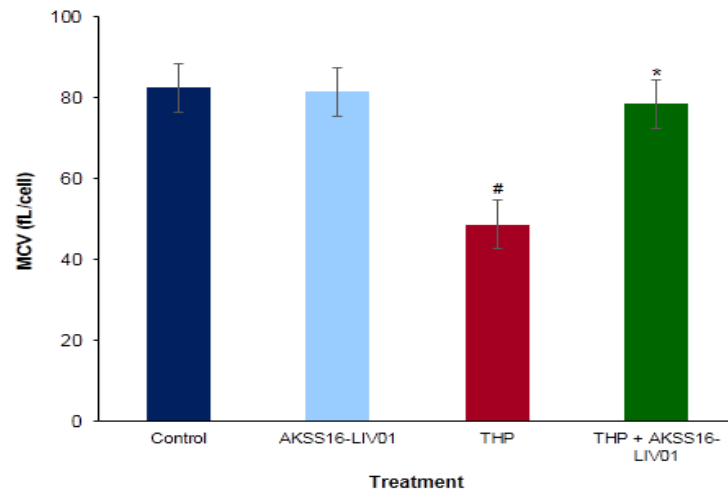
All data were expressed as means± SE (n=6/group). Data comparison was performed using two way ANOVA followed by Tukey's Multiple Comparison Test. #Significantly different from the control group at  $p < 0.001$  and \*Significantly different from (THP) group values at  $p < 0.001$



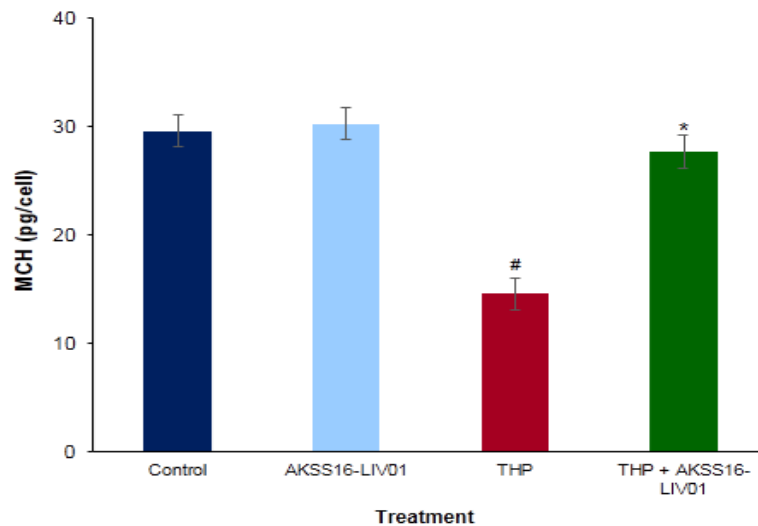
**Figure 1:** Effect of multi herbal formulation (AKSS16-LIV01) on haemoglobin (Hb) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at  $p < 0.001$  and \*significantly different from (THP) group values at  $p < 0.001$ . Data comparison was performed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



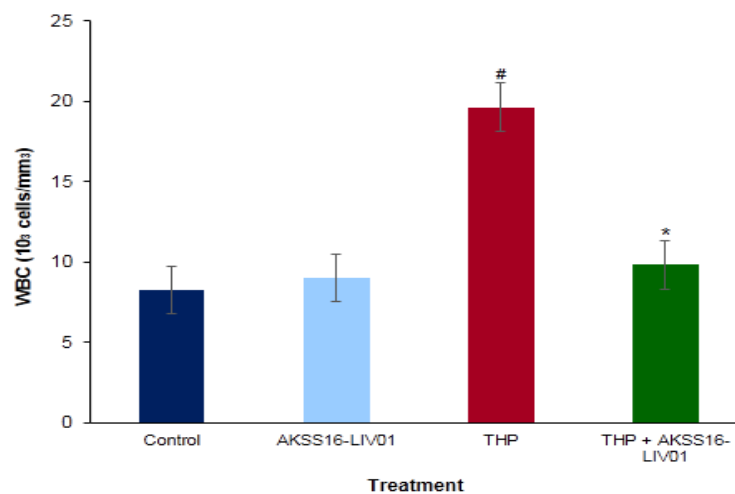
**Figure 2:** Effect of multi herbal formulation (AKSS16-LIV01) on packed cell volume (PCV) in mice. All data were expressed as means± SE (n=6/group). #significantly different from the control group at  $p < 0.001$  and \*significantly different from (THP) group values at  $p < 0.001$ . Data comparison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



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



**Figure 4:** Effect of multi herbal formulation (AKSS16-LIV01) on mean cell hemoglobin (MCH) in mice. All data were expressed as means $\pm$  SE (n=6/group). <sup>#</sup>significantly different from the control group at p<0.001 and <sup>\*</sup>significantly different from (THP) group values at p<0.001. Data comparison was performed using one way ANOVA followed by Tukey's Multiple Comparison Test.



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

## DISCUSSION

Analgesics as fixed dose combination are very useful for fast pain relief. Tramadol hydrochloride/paracetamol (THP) is a fixed dose combination consists of two analgesics tramadol and paracetamol used for treats moderate to severe pain<sup>15</sup>. It is well established that overdose or chronic use of analgesics specially fixed doses form developed mild to severe adverse effects and sometimes damage various organs like liver, kidney and brain<sup>16</sup>. In very recent study confirm that administration of THP upon animal model severely disturbed hematological and biochemical parameters<sup>17</sup>. To prevent these deleterious effects we simultaneously administered our newly developed multi herbal formulation (AKSS1-LIV01) in mice. It is reported that treated with THP at a dose of 1.68 g/300 ml of water on mice reduced the haemoglobin (Hb), packed cell volume (PCV), and mean corpuscular volume (MCV) values. Another report depict that lower haemoglobin (Hb) value leads to iron deficiency anaemia which is characterized by a microcytic hypochromic blood picture. In the present study our result also confirm that administration of THP (1.68 g/300 ml of water) 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 THP 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, while increased white blood cells (WBC) level is generally recognized as an inflammatory response<sup>18, 19</sup>. Inflammatory conditions may induce malnutrition in the body<sup>20</sup>. It is reported that inflammatory conditions can interfere with the body's ability to use stored iron and absorb iron from the diet<sup>21</sup>. Our result clearly showed that treatment with THP abruptly increased serum aspartate transaminase (AST) and alanine transaminase (ALT) levels as well as elevate white blood cells (WBC) count indicate THP produce inflammatory response and affects liver cell, disturbed homeostasis. On the other hand administration with newly developed multi herbal formulation (AKSS16-LIV01) along with THP decline the AST, ALT value and WBC count protect the liver against THP 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 THP.

## CONCLUSION

This investigation shows that multi herbal formulation (AKSS16-LIV01) has the ability to protect the haematopoietic cells from the damaging effects of exposure to Tramadol hydrochloride/paracetamol (THP) and this protection might be attributed to the anti-oxidative power of multi herbal formulation (AKSS16-LIV01). Thus, we believe that 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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