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Research Article

SANATIVE EFFECT OF MULTIHERBAL FORMULATION – AKSS16-LIV01 ON CCL_4-INDUCED HEPATIC DYSFUNCTION IN MICE

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ABSTRACT

Objectives: Hepatic dysfunction is a critical public health problem affecting the global population, characterized by excessive deposition of extracellular matrix components due to increased matrix production and decreased matrix degradation. The present work was aimed to evaluate hepatoprotective effect of AKSS16-LIV01 a newly developed multiherbal formulation against carbon tetrachloride (CCl_4)-induced liver dysfunction in Swiss albino mice to establish it as a bench to bedside formulation catering to the various facets of hepatic malfunction.

Methods: Thirty-six Swiss adult albino Wister mice divided into six groups. Group-I control untreated animals, Group-II received AKSS16-LIV01 (400 mg/kg), Group-III received CCl_4 (1 ml/kg-bw), Group-IV received AKSS16-LIV01 (200 mg/kg) after 2 weeks CCl_4 induction, Group-V received AKSS16-LIV01 (400 mg/kg) after 2 weeks CCl_4 induction, and Group-VI received standard drug silymarin (100 mg/kg). At the end of the experimental period, all the animals were fasted overnight and blood was collected through retro-orbital plexus for preparation of serum and was analyzed for biochemical parameters, lipid profile, and total plasma protein. Liver tissue was collected for histological study.

Results: The combined plant extract including six Indian medicinal herbs and three medicinal spices (AKSS16-LIV01) showed significant hepatoprotective effect by controlling the various essential biochemical parameters in serum. Moreover, treatment with AKSS16-LIV01 raised the level of serum total protein, normalizes the serum biochemical and lipid profiles parameters. Pre-treatment with AKSS16-LIV01 in mice also restored the alteration of various liver parameters such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, blood urea nitrogen, total bilirubin, and direct bilirubin on CCl_4-induced liver damage. Gross liver morphology and normal histological examination of the liver also supported hepatic protection by AKSS16-LIV01.

Conclusion: Taken together, these results suggest that AKSS16-LIV01 may induce remarkable protective effects against hepatic injury induced by CCl_4 treatment.

Keywords: Hepatoprotective, Natural therapy, Herbal formulation, Liver function test, Histopathological studies.

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INTRODUCTION

Hepatocytes, sinusoidal cells, Kupffer cells, and hepatic stellate cells (HSCs) are the main cells those are involved in normal liver functions. Several pathogens when attack the liver cells they alter the normal liver functions and damage the liver cells produce liver fibrosis [1,2]. Inflammatory reactions take place when hepatocytes cause damage which lead to the activation of HSCs [3]. During the abnormal situation, activated Kupffer cells release a number of soluble agents, including cytokines, such as platelet-derived growth factor, transforming growth factor-β, and tumor necrosis factor-α, generate reactive oxygen species (ROS), and other factors causing inflammation and damage to hepatocytes leads to liver fibrosis [4,5]. In an experimental setting, carbon tetrachloride (CCl_4) is able to induce hepatic fibrosis by stimulating the formation and generation of various free radicals, ROS, and lipid peroxidation products [6] and thus is widely used to generate hepatic fibrosis in model animals [7,8].

At present, global attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities [9]. Herbal extracts could significantly contribute to recovery processes of the intoxicated liver and kidney [10-14]. The World Health Organization defines traditional medicine as "diverse health practices, approaches, knowledge, and beliefs incorporating plant, animal and/or mineral-based medicines, spiritual therapies, manual technique, and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness" [15,16]. In third world countries including India, up to 90% of the populace still relies entirely on plants as a resource of medicines [17].

Herbal medicines have progressively become prevalent due to rising costs of treatments with synthetic western medicine, numerous side effects of allopathic drugs, drug resistance, unregulated purchase options for consumers on most herbal drugs, and easy availability of these medicines [18]. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Natural remedies from medicinal plant are considered to be effective and alternative treatment for hepatotoxicity [19].

In the present study, we formulated a new phytomedicine composed of nine indigenous medicinal and dietary herbs which were mentioned in Ayurveda. These herbs are natural resources of antioxidants that serve as the first line of defense against free radical damage and are considered to be important in maintaining optimum health and happiness. The results of the present study are expected to provide a clear picture about the role of our newly formulated AKSS16-LIV01 in CCl_4-induced hepatic damage, and may shed light on an achievable ethnobotany driven solution to the serious liver problems.

METHODS

Chemicals

CCl_4, trichloroacetic acids, and TRIS buffer were obtained from Merck, India. Phosphate-buffered saline pH 7.4 was procured from

Sigma-Aldrich. Biochemical determination kits, that is, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and lipid profile kits were procured from Thermo Scientific, USA. All others reagents used in this study are laboratory grade.

Collection and authentication of the herbs

All the medicinal plant and spice ingredients were collected from registered local herbal suppliers and authenticated by pharmacognosist. They were further identified by taxonomists of the Department of Botany, Uluberia College, University of Calcutta, India, and kept as voucher specimen. Ayurvedic identification parameters such as Varna (color), Gandha (odor), Ruchi (taste), Akriti (shape), and Parimana (size). The plants and plant parts used in preparation of the extract are listed in Table 1.

Preparation of extract

At first, collected plant parts were air dried and then clean with double distilled water and kept in a hot air oven at 80°C for 10 min and 60°C for 30 min. All the plants and spices were placed in a blade mill to obtained fine powder. Aqueous extract of the polar fraction was performed according to the method of Adhikari *et al.* (2018) with slight modifications. Five grams of dry plant parts were taken and dissolved it using 10 ml of methanol. The extract then sonicated at room temperature for 30 min using an ultrasonic bath, centrifuged at 4000 rpm for 20 min, and finally, the supernatant was removed. This procedure was repeated 4 times, collecting all the supernatants, which were finally evaporated in a rotary evaporator under reduced pressure at 35°C. Finally, the residue was reconstituted in 3 ml of methanol, filtered using Whatman filter papers (GE Healthcare and Life Sciences, MA, USA) and kept at 4°C for further use [20]. The composition of the formulation is presented in Table 1.

Animals

Healthy adult Swiss albino mice weighing 28±5 g taken from our registered animal house were divided into six experimental groups with six animals per group. The animals were maintained at 12 h light/dark cycle, at constant temperature (22±2°C) and humidity (55±5%). Mice were feed standard pellet diet (Purchase from Hind Unilever India Limited, Mumbai) containing 19.4% protein, 5.5% fiber, 11.1% water, 54.6% carbohydrates, 6.7% essential mineral mixture, and 2.6% by weight of lipids and water *ad libitum*. Mice were kept under observation for 1 week before the onset of the experiment for acclimatization and to exclude any insercurrent infection. All the experimental procedures were carried out according to the guidelines of CPCSEA, Government of India, New Delhi, and approved by the Institutional Animal Ethics Committee (IAEC) of Jadavpur University having approval number 261/JU/s/IAEC/Pharma/2018.

Acute toxicity studies

The acute toxicity studies were carried out for AKSS16-LIV01 following the general principles of OECD guideline 423. Overnight fasted healthy female mice were divided into four (one control and three test) groups and orally given the extract at doses up to 2000 mg/kg body weight

Table 1: Composition of ingredient(s) present in AKSS16-LIV01

S. No.	Botanical name	Common name	Family	Part used	Quantity used in extract
1.	*Tinospora cordifolia*	Guduchi	Menispermaceae	Stem	20 mg
2.	*Terminalia chebula*	Haritaki	Combretaceae	Fruit	20 mg
3.	*Azadirachta indica*	Neem	Meliaceae	Leaves	50 mg
4.	*Andrographis paniculata*	Kalmegh	Acanthaceae	Leaves and steam	50 mg
5.	*Aloe barbadensis* Miller	Aloe vera	Liliaceae	Leaves and steam	50 mg
6.	*Curcuma longa*	Curcuma, Haldi	Zingiberales	Rhizome	20 mg
7.	*Trigonella foenum-graecum*	Methi	Fabaceae	Seed	10 mg
8.	*Piper nigrum*	Black pepper	Piperaceae	Seed	10 mg
9.	*Elettaria cardamomum*	Cardamom	Zingiberaceae	Seed	10 mg

*Amount required for preparation of 5 ml extract

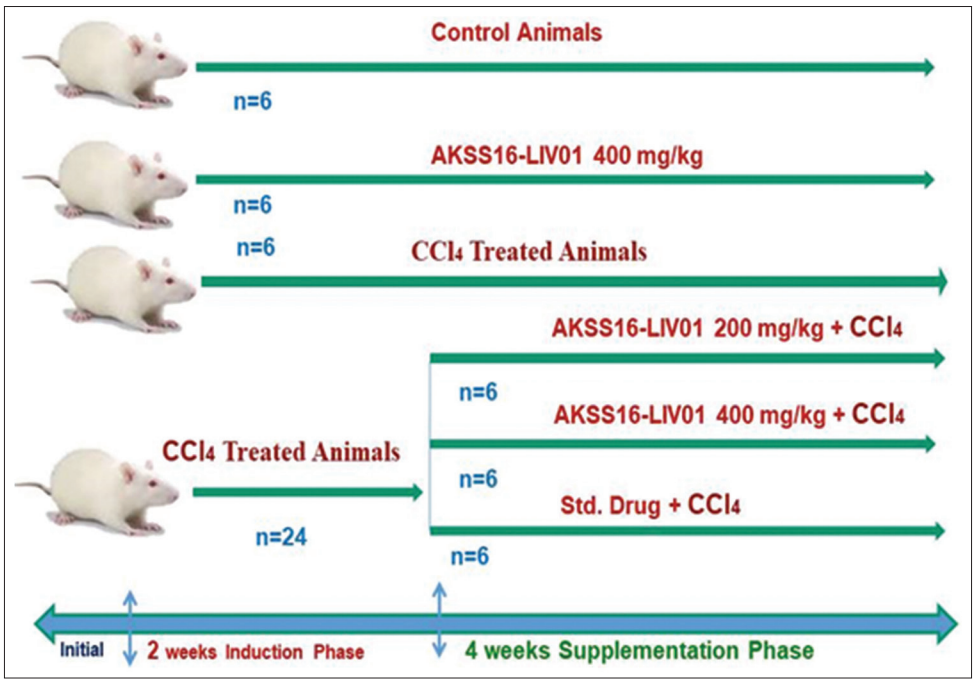

Fig. 1: Experimental design

(BW). They were observed continuously for 14 days for behavioral changes and mortality.

CC_{l4} induction and treatment

Male adult mice were divided into six groups as follows: Group I served as normal control received only the vehicle (1 ml/kg olive oil twice a week for 6 weeks), Group II treated with multi herbal formulation (MHF) AKSS16-LIV01 (400 mg/kg bw/day all over the experiment), Group III received 1 ml/kg bw of CCl_4 diluted 20% in olive oil twice a week for 6 weeks, Group IV pre-treated with herbal formulation AKSS16-LIV01 (200 mg/kg bw/day) low dose (MHF) for 4 weeks after 2 weeks CCl_4 induction and ensure occurrence of liver injury, Group V pre-treated with MHF AKSS16-LIV01 high dose (400 mg/kg bw) for 4 weeks after 2 weeks CCl_4 induction and ensure occurrence of liver injury, and Group VI pre-treated with silymarin standard hepatoprotective drug at a dose (100 mg/kg bw) for 2 weeks after CCl_4 induction and ensure occurrence of injury (Fig. 1).

BW gains and feed efficiency

BWs were measured on weekly basis from the initial day to the final day of experiment to calculate BW 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 BW 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

At the end of the respective fasting period, blood was collected from each mouse by retro orbital venous puncture. Two hundred microliters of blood sample were collected into microcentrifuge tubes with and without ethylenediaminetetraacetic acid (2%). Collected bloods were placed in slanting position at room temperature for 2 h. Then, they were centrifuged at 3500 g for 10 min. Clear light yellow color 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 Sysmex K1000 Cell Counter. Parameters studied were hemoglobin, total red blood cell, reticulocyte (RT), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, total white blood cell, and differential count.

Assessment of liver function parameters

The biochemical parameters such as serum enzymes: AST, ALT, serum ALP, GGT, albumin, globulin, total and direct bilirubin, and blood urea nitrogen (BUN) along were assayed using assay kits (Thermo Scientific, USA) following the protocol prescribed by manufacturers. Total protein concentration was determined in the serum by the method of Lowry *et al*. [21].

Lipid profile

Serum levels of total cholesterol (TC), triglycerides (TGs), phospholipids, free fatty acids (FFA), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were assayed using assay kits (ELITech Diagnostic, France) following the protocol prescribed by manufacturers.

Hematoxylin and eosin staining

Five micron paraffin-embedded liver sections were deparaffinized and washed with water. Hydrated tissue sections were incubated with Mayer's hematoxylin for 5 min followed by vigorous washing in running tap water. Sections were counter stained with 1% eosin for 2 min. Eosin stained sections were washed with water and dehydrated with alcohol. Dehydrated sections were washed with xylene. Images were taken with a microscope (Olympus BX51 fluorescence microscope).

Statistical analysis

All quantitative data are expressed as mean±standard deviation (SD) unless otherwise stated. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests was executed for comparison of different parameters between the groups using a computer program GraphPad Prism (version 5.00 for Windows), GraphPad Software, California, USA. p and lt; 0.05 was considered statistically significant.

RESULTS

BW, liver weight, liver index, food consumption, and water intake

In this study, we determined the BW, liver weight, liver index, food consumption, and water intake of mice treated with CCl_4 and pretreated with MHF-AKSS16-LIV01 (Fig. 2). BW and liver index of CCl_4-treated

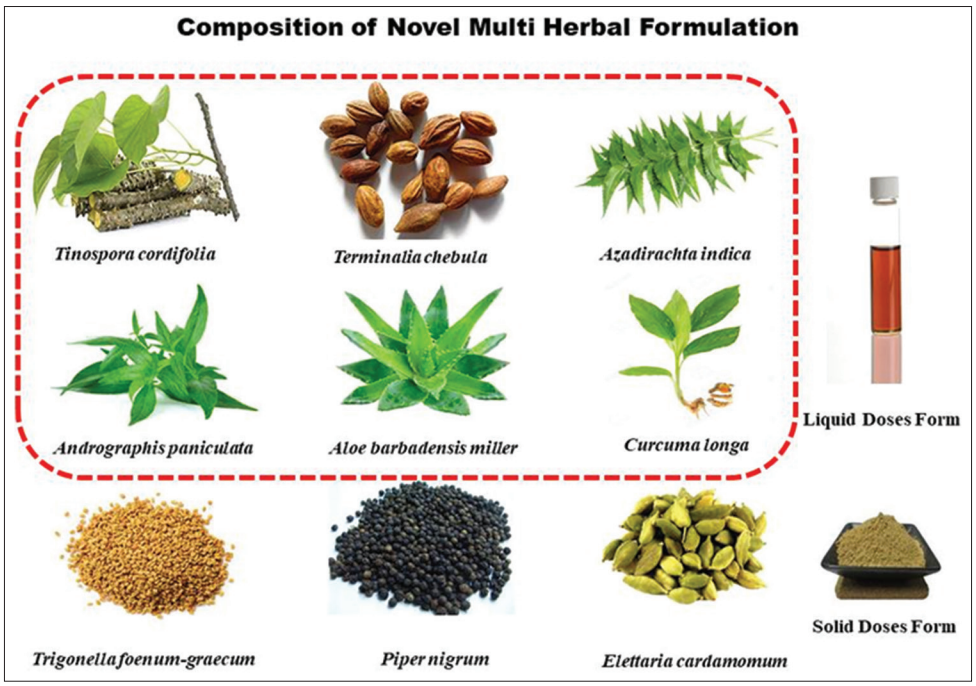

Fig. 2: Composition of multiherbal formulation (AKSS16-LIV01)

animals were significantly reduced (p<0.001) when compared with normal control animals. Liver weight of CCl_4-treated animals was significantly elevated (p<0.001) when compared with normal control animals. Treatment with AKSS16-LIV01 both 200 mg/kg and 400 mg/kg significantly increased (p<0.05 and p<0.001) BW and liver index whereas significantly decrease the liver weight (p<0.001) when compared with CCl_4-treated animals. Silymarin showed less positive effect in comparison with MHF. On the other hand, food consumption and water intake capacity of CCl_4-treated animals were significantly reduced (p<0.001) when compared with normal control animals. Treatment with AKSS16-LIV01 both 200 mg/kg and 400 mg/kg significantly increased (p<0.05 and p<0.001) the food consumption and water intake capacity when compared with CCl_4-treated animals. Treatment with MHF – AKSS16-LIV01 at a dose 400 mg/kg showed optimum protective capacity (Table 2).

Liver function test

Liver function parameters such as serum AST, ALT, GGT, ALP, BUN, total bilirubin, direct bilirubin, total protein, albumin, and albumin-globulin ratio were measured in mice treated with CCl_4 and pretreated with AKSS16-LIV01 after CCl_4 induction which are presented in Table 3. AST, ALT, GGT, ALP, BUN, total bilirubin, and direct bilirubin of CCl_4-treated animals were significantly elevated (p<0.001) when compared with normal control animals. Serum total protein, albumin, and albumin globulin ratio of CCl_4-treated animals were significantly reduced (p<0.001) when compared with normal control animals. The Pre-treatment with AKSS16-LIV01 both 200 mg/kg and 400 mg/kg significantly reduced (p<0.05 and p<0.001) AST, ALT, GGT, ALP, BUN, total bilirubin, and direct bilirubin whereas significantly increased (p<0.05 and p<0.001) the serum total protein, albumin, and albumin-globulin ratio when compared with CCl_4-treated animals. Silymarin showed less positive effect in comparison with MHF. Results of the biochemical study stated that treatment with MHF – AKSS16-LIV01 at a dose 400 mg/kg showed optimum protective capacity.

Lipid profile test

Lipid profile parameters such as serum TC, total TG, phospholipids, FFA, LDL, and HDL were measured in mice treated with CCl_4 and pretreated with AKSS16-LIV01 which are presented in Table 4. Cholesterol triglyceride, phospholipids, FFA, and LDL of CCl_4-treated animals were significantly elevated (p<0.001) when compared with normal control animals. Serum HDL level of CCl_4-treated animals was significantly reduced (p<0.001) when compared with normal control animals. The pre-treatment with AKSS16-LIV01 both 200 mg/kg and 400 mg/kg significantly reduced (p<0.05 and p<0.001) serum cholesterol triglyceride, phospholipids, FFA, and LDL level and significantly increased (p<0.05 and p<0.001) the serum HDL when compared with CCl_4-treated animals. Silymarin showed less positive effect in comparison with MHF. Results of the serum lipid profile study

Table 2: Summary of the body weight (BW), liver weight, and liver index of mice

Parameters	Normal	AKSS16-LIV01 (400)	CCl_4	CCl_4 + AKSS16-LIV01 (200)	CCl_4 + AKSS16-LIV01 (400)	CCl_4 + silymarin 100
BW (g)	36.52±1.18	35.01±1.34	26.94±1.22#	34.62±1.26*	38.01±2.21**	33.26±2.17*
Liver weight (g)	1.94±0.82	1.98±0.18	3.50±0.28#	1.99±0.52*	1.86±0.64**	2.03±0.64*
Liver index	4.02±0.12	5.65±0.17	7.72±0.15#	5.51±0.24*	4.95±0.17**	5.22±0.18*
Food consumption (g)	3.98±0.05	3.95±0.04	2.91±0.05#	3.74±0.06*	3.99±0.02**	3.92±0.05*
Water intake (ml)	4.11±0.02	4.02±0.04	2.95±0.05#	3.99±0.02*	4.04±0.05**	3.91±0.03*

CCl_4: Carbon tetrachloride. Values are mean of six individual observations in each group±SD. *Significantly different from control # (p<0.001) and significantly different from CCl_4 *(p<0.05) **(p<0.001) using ANOVA followed by.

Table 3: Summary of the liver function test parameters studied across the groups

Parameters	Normal	AKSS16-LIV01 (400)	CCl_4	CCl_4 + AKSS16-LIV01 (200)	CCl_4 + AKSS16-LIV01 (400)	CCl_4 + silymarin 100
AST (IU/l/min/mg protein)	137.25±2.62	135.25±3.02	255.49±1.98#	166.27±2.19*	140.27±2.02**	164.02±1.44*
ALT (IU/l/min/mg protein)	55.16±2.22	58.61±1.81	118.03±3.16#	82.58±1.02*	61.29±1.63**	72.58±1.85*
GGT (IU/l/min/mg protein)	0.26±0.02	0.26±0.09	2.11±0.51#	1.06±0.08*	0.34±0.12**	0.41±0.02*
ALP (IU/l/min/mg protein)	232.05±3.11	237.84±2.91	461.27±4.96#	279.55±2.06*	250.44±2.71**	262.11±2.04*
BUN (mg/dl)	0.41±0.02	0.46±0.03	0.72±0.04#	0.67±0.02*	0.46±0.02**	0.55±0.03*
Total bilirubin (mg/dl)	0.12±0.2	0.19±0.11	0.62±0.11#	0.34±0.08*	0.18±0.09**	0.25±0.08*
Direct bilirubin (mg/dl)	0.06±0.001	0.07±0.002	0.33±0.07#	0.09±0.003*	0.09±0.004**	0.24±0.005*
Total protein	6.51±0.65	627±0.21	2.56±0.32#	4.58±0.41*	6.22±0.27**	5.39±0.21*
Alb (gr/dL)	3.48±0.186	3.16±0.13	1.97±0.036#	3.70±0.11*	4.12±0.12**	3.30±0.15*
Alb/globulin	1.18±0.141	0.98±0.135	0.54±0.013#	0.91±0.096*	1.23±0.95**	1.06±0.091*

CCl_4: Carbon tetrachloride, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, BUN: Blood urea nitrogen, Alb: Albumin. Values are mean of six individual observations in each group±SD. *Significantly different from control #(p<0.001) and significantly different from CCl_4 *(p<0.05) **(p<0.001) using ANOVA followed by Dunnett's multiple comparison test.

Table 4: Summary of the lipid profile parameters studied across the groups

Parameters	Normal	AKSS16-LIV01 (400)	CCl_4	CCl_4 + AKSS16-LIV01 (200)	CCl_4 + AKSS16-LIV01 (400)	CCl_4 + silymarin 100
Cholesterol (mg/dL)	81.03±5.02	88.05±3.16	135.69±6.15#	91.47±2.37*	79.36±4.35**	85.11±2.98*
Triglyceride (mg/dL)	40.58±2.05	51.23±3.01	72.58±3.28#	47.98±1.97*	37.25±1.87**	41.75±3.25*
Phospholipids (mg/dL)	76.59±6.28	92.37±2.66	142.97±4.69#	98.51±3.28*	82.69±4.87**	88.03±2.84*
Free fatty acids (mg/dL)	15.97±0.58	20.14±1.96	31.87±1.67#	21.22±1.69*	14.09±1.22**	16.27±2.01*
LDL cholesterol (mg/dL)	39.65±1.96	43.61±1.88	76.94 ±1.77#	45.78±1.65*	36.85±1.25**	44.52±2.2*
HDL cholesterol (mg/dL)	19.58±0.69	15.28±0.55	10.28±0.28#	16.24±0.41*	10.28±0.28**	22.67±0.9*

Values are mean of six individual observations in each group±SD. *Significantly different from control # (p<0.001) and significantly different from CCl_4 *(p<0.05) **(p<0.001) using ANOVA followed by Dunnett's multiple comparison test

stated that treatment with MHF – AKSS16-LIV01 at a dose 400 mg/kg showed optimum protective capacity.

Hematological study

All the different hematological parameters such as hemoglobin (Hb): Hb, red blood corpuscle, RT, HCT, MCV, MCH, MCHC, and white blood corpuscle were studied in mice treated with CCl_4 and pretreated with AKSS16-LIV01. Hemoglobin level was significantly reduced ($p<0.05$) and WBC count was significantly elevated ($p<0.05$) in CCl_4 group as compared with normal control group. The pre-treatment with AKSS16-LIV01 (400 mg/kg) was recovered these alteration when compared with CCl_4 group (Table 5).

Histology

H and E staining

Fig. 3 shows histological photographs of the liver tissue both control and different experimental groups. The normal control group animals showed the typical architecture of liver tissue with a central vein and chords of hepatocytes radiating, whereas CCl_4 treatment produced extensive necrosis of hepatocytes which was more pronounced in the centrizonal (zone 3) area. The fatty changes were of macrovesicular type which was evident in centrizonal and portal areas with inflammatory reactions (Fig. 3). Partial hepatic protection with reduction in the extent of hepatic necrotic areas, fatty infiltration, and mild portal inflammation was visualized in the liver section of AKSS16-LIV01 (200 mg/kg) treated animals. On the other hand administered with AKSS16-LIV01 (400 mg/kg) almost completely protected the liver as evidenced by restoration of a normal histoarchitecture of the liver.

DISCUSSION

CCl_4 is a well-known hepatotoxin which is widely used to induce toxic liver injury and to study the cellular mechanisms behind oxidative damage in laboratory animals [22]. Fight against various liver dysfunctions such as liver fibrosis, fatty liver, and liver cirrhosis through safe and symptomatic medicine is a new challenge. At present, there is no effective treatment for hepatic dysfunctions. To overcome this worldwide health complication, we formulated a novel herbal drug composed of nine indigenous medicinal and dietary herbs those were mentioned in Ayurveda. These herbs are natural resources of antioxidants that serve as the first line of defense against free radical damage and are considered to be important in maintaining optimum health and happiness. Our previous reports stated that the formulation has no adverse side effect and no toxicity in mice [23,24].

AST and ALT are the most sensitive indicators for the diagnosis of liver cell damage. During amino acid synthesis and catabolism, AST and ALT play vital roles as endoenzymes in hepatocytes. Under the circumstance of the normal working condition of the body, ALT and AST levels in the blood are very low and, thus, the activity of these two enzymes in

Table 5: Summary of the hematological parameters studied across the groups

Parameters	Normal	AKSS16-LIV01 (400)	CCl_4	CCl_4 + AKSS16-LIV01 (200)	CCl_4 + AKSS16-LIV01 (400)	CCl_4 + silymarin 100
Hb (g %)	12.1±1.05	11.21±0.82	9.03±0.89#	11.05±0.99	12.51±0.95*	10.96±0.74
RBC ($x10^6$ cm^2)	10.8±0.82	9.62±0.84	9.1±0.71	9.44±0.71	10.02±0.85	9.85±0.79
RT (%)	2.7±0.12	3.6±0.16	4.9±0.26	3.1±0.14	2.8±0.15	3.0±0.12
HCT (%)	34.6±0.48	35.1±0.77	39.4±0.55	35.8±0.51	34.9±0.56	34.4±0.51
MCV (μm^3)	37.8±0.32	35.5±0.36	31.0±0.68	36.5±0.44	35.9±0.79	36.2±0.43
MCH (pg)	21.2±0.15	21.1±0.12	22.2±0.14	21.1±0.12	21.4±0.11	21.2±0.14
MCHC (%)	41.2±1.06	36.2±0.91	32.4±0.95	37.1±0.92	39.6±0.87	38.6±0.99
Platelets	6.5±0.02	5.4±0.06	5.5±0.03	5.8±0.05	6.1±0.07	5.5±0.05
WBC ($x10^5$ $cm^{2)}$)	9.2±0.09	10.7±0.11	12.4±0.11#	10.8±0.12	9.2±0.11*	10.1±0.13
Lymphocyte	74±2.98	71±3.11	79±3.04	73±3.06	71±2.58	72±3.08
Neutrophil	26±1.12	21±0.55	15±0.49	20±0.56	25±0.69	24±0.51

Data are expressed as mean±standard deviation (N=6). Hb: Hemoglobin, RBC: Red blood corpuscle, RT: Reticulocyte, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood corpuscle. Values are mean of six individual observations in each group±SD. *Significantly different from control group #($p<0.05$) and significantly different from CCl_4 group *($p<0.05$) using ANOVA followed by Dunnett's multiple comparison test

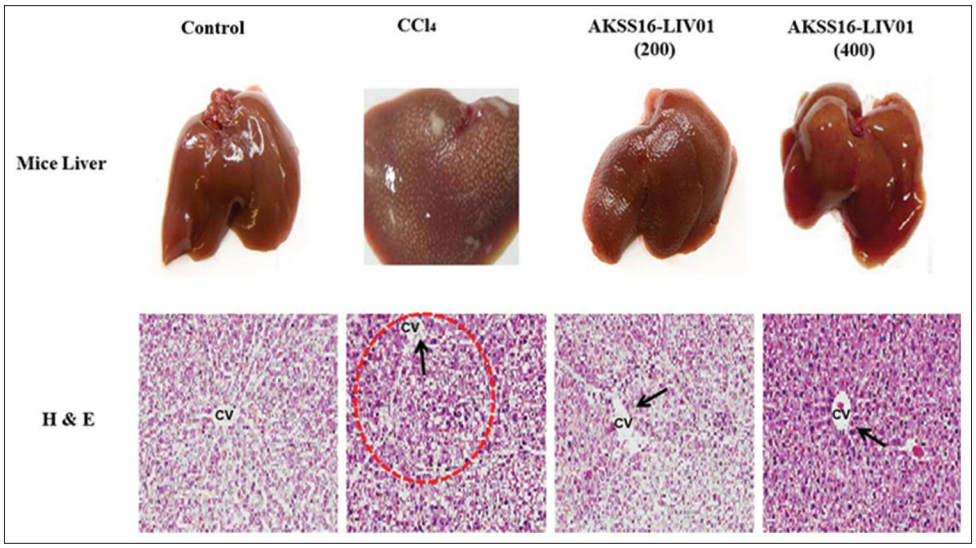

Fig. 3: Morphological and histopathological photographs of liver tissue. Lane 1: Morphological photographs of whole liver tissue; lane 2: H and E staining section of liver tissue

normal serum is very low. When the liver tissue is damaged and the cell membrane permeability increases, these two enzymes penetrate into the blood in large quantities, leading to a significant increase in the activity of the enzymes in the sera. Therefore, an increase in serum AST and ALT can reflect the extent of liver cell damage [25]. When CCl_4 enters the animal body, liver microsomal lipids and hepatocyte membrane phospholipid molecules are attacked by free radicals generated by CCl_4, which, in turn, trigger changes in the TC and TG levels in the liver [26,27]. The increase in the AST, ALT, ALP, GGT, and bilirubin levels indicates an exaggeration of liver damage. The experimental data from this study also confirmed that CCl_4 resulted in an increase in the AST, ALT, ALP, GGT, and bilirubin levels in mice. On the other hand, serum lipid profile, that is, total cholesterol, triglyceride, FFA, phospholipids, and LDL significantly elevated by the deleterious action of CCl_4.

The damage to the hepatic parenchymal cells due to accumulated toxins acts on the liver leading to the formation of reactive oxygen species which further leads to oxidative stress augmenting hepatic damage and dysfunction altering the liver transaminase enzyme parameters [28,29].

Therapeutic application of novel MHF AKSS16-LIV01 significantly reduced the serum biochemical and lipid profile levels in the serum and thus exerted a preventive effect on liver damage. This is further confirmed by our histopathological analysis of liver tissues.

CONCLUSION

The protective effect of MHF (AKSS16-LIV01) in CCl_4-induced liver injury was established in this study in mice. The newly developed MHF AKSS16-LIV01 normalized biochemical enzymes levels, lipid profile parameters, and blood parameters from CCl_4-induced liver injury. Liver histology strongly supported that AKSS16-LIV01 (44 mg/kg) able to protect liver cell form CCl_4-induced liver damage. 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 liver dysfunction.

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AUTHORS' CONTRIBUTIONS

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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