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Research Article

A novel Multi Herbal Formulation (AKSS16-LIV01) protects Obesity and associated Non-alcoholic Fatty Liver Disease

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Abstract

Obesity has become a very common symptom in present day world. It also causing fatty liver which is non-alcoholic in nature. The unhealthy food habit and sedentary lifestyle are the main causes behind it. The aim of the study was to develop a multiherbal formulation for protection from these kinds of metabolic phenomena develops from dyslipidemia. For that, combination of nine (9) Indian herbs and spices were chosen based on extensive literature survey and research. The formulation AKSS16-LIV01 was prepared on the basis of experiments, and the effects of its administration on seven (7) experimental groups of mice were studied. Results showed that administration of AKSS-16-LIV-01 at a dose of 300mg /kg/day showed a significant ($p<0.001$) reduction in TC, TG, LDL-C and VLDL-C levels and increase in HDL-C level in high fat diet (HFD) treated mice. Our study also indicated that obese mice showed marked decline in the SOD, catalase, GSH and GPx essential first and second order antioxidant enzymes activities, which reflected in the normalization of the body weight of the treated animals. From the histological studies it was observed that normal tissue microstructure of the liver was regained. As a whole the results showed a great potential of the novel multiherbal formulation in protecting obesity and non-alcoholic fatty liver disease (NAFLD).

Keywords: Multi herbal formulation; Obesity; High fat diet (HFD); Dyslipidemia; NAFLD

Introduction

Overweight and Obesity is a serious health problem throughout the world, and is reaching pandemic levels [1]. According to World Health Organization's (WHO) report more than one billion people are overweight out of which three hundred million meeting the criteria for obesity [2-4]. Due to the excessive fat accumulation in the body, people of both developed and developing countries are suffering from various overweight related complications, such as type 2 diabetes, cardiovascular disease, coronary artery disease, stroke, myocardial infarction, dyslipidemia, anxiety, hypertension, mental stress, hepatic disorder and various types of cancers [5,6]. Another report stated that 26% nonpregnant woman ages between 20 to 39 years are overweight and out of which 29% are obese. World statistics stated that 35% men and 37% woman are overweight and 40% men and 30% women are obese [7,8].

Abnormal accumulation of free fatty acids (FFA) in the liver is one of the prime causes of obesity related liver diseases, which gradually produce fatty liver or hepatic steatosis. Accumulation of

more than 5% triglycerides in hepatocytes is commonly observed in fatty liver related to alcohol consumption, metabolic syndrome and drug abuse [9].The primary cause of metabolic disorders is insulin resistance which accumulating fat in the adipocyte by suppressing the enzymes, hormone-sensitive lipases (HSLs), resulting released of triglycerides into the blood in the form of fatty acids. Deposition of free fatty acids (FFA) in the liver increases fat accumulation in the hepatic cell developed non-alcoholic fatty liver [10-12].

Intake of high fat enrich diet, especially junk foods and street foods developed obesity and increased body fat. Excessive consumption of fatty food generates intracellular reactive oxygen species (ROS), that disrupt the balance between oxidant and antioxidant agents. Redox imbalance is the main cause of hepatic mitochondrial dysfunction which produced inflammatory responses, and a breakdown of lipid metabolism [12,13]. Mitochondria are the major organs involved in ROS production, generates various types of free radicals such as superoxide (O_2^-), peroxyl (RO_2^-), hydroxyl (OH^-), lipid peroxyl (ROO^-) radicals and non-free radicals such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2^{-1}), ozone (O_3) and lipid peroxide (LOOH) those are actively involved in cellular damage. Study revealed that in non-alcoholic fatty liver disease (NAFLD) ROS can induce damaged mitochondrial membrane potentials and changes in mitochondrial structure causing a breakdown of lipid homeostasis [14].Medicinal plants are enriched with polyphenols, flavonoids, alkaloids, and tannins which takes part in prevention of many non-communicable diseases through inhibition of ROS production [15]. Regular consumption of plant polyphenols protects the liver cell from external chemicals and foreign injury and save the hepatic cell from fat deposition. During oxidative stress our body required sufficient amount of antioxidant compounds which protects the hepatic cell against the deleterious effects of ROS [16-20]. Scientific study revealed that combination therapy produces enormous positive results as herb-herb interaction produce synergism, which could be pharmacokinetic synergism or pharmacodynamic synergism. Recently scientist and medical practitioners are interested for combination therapy to obtain the better therapeutic efficacy in comparison to single one [16,21,22].

Prevention of obesity and obesity induced complications is a new challenge throughout the globe. With view of this in the present study we formulated a new novel phytomedicine (AKSS16-LIV01) containing six indigenous medicinal herbs and three medicinal spices which were mentioned in Ayurveda. These herbs are natural resources of antioxidants that serve as the first line of defence against free radical damage and are considered to be important in maintaining optimum health and hygiene. Phytochemicals present in these herbs have potent lipid lowering capacity make a barrier against excessive body growth. Our previous reports stated that the formulation have no adverse side effect and no toxicity in mice and medicinally safe for medication [19,20,23,24]. Therefore, the present study was undertaken to evaluate the anti-obesity effects and reduction of tissue oxidative stress and its deleterious effects by the application of multi herbal formulation (AKSS16-LIV01). We therefore used mice with high-fat diet (HFD)-induced obesity to evaluate changes in fat accumulation, liver function enzymes, MDA content and lipid profiles, as well as examined the redox mechanisms.

Materials and Methods

Chemicals and reagents

Standard biochemical kits i.e., AST, ALT, GGT, ALP, total protein, albumin etc. were procured from Merck (Germany). Triglyceride, cholesterol, phospholipids, free fatty acids, HDL and LDL were purchased from Thermo Scientific. Antioxidant enzymes determination kits such as SOD, CAT, GSH and GPx were obtained from Boehringer, USA. All other laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India).

Collection of Plants and Preparation of Extract

All the medicinal plants and spices were collected from the registered supplier, Kolkata. Plants were authenticated by pharmacognosist of Department of Pharmacy, Jadavpur University for preliminary identification and verification and further identified based on Ayurvedic parameters by a renowned taxonomist of Department of Botany, Uluberia College, University of Calcutta, India and properly kept as voucher specimen. Ingredients used in the formulation are listed in Table 1 and Figure 1. For preparation of extract the plants and spices were cleaned with double distilled water air dried until dry. Then the plants were kept in hot air oven at 75ºC for 10 min and 55ºC for 30 min. then all the plants were grinded by a blade mill to obtained the fine powder. Then 5g of dry plant powder was taken and dissolved into 10 ml methanol followed by sonication for 30 minutes using an ultrasonic bath. Centrifuged the material at 4000 rpm for 15 minutes and collected the supernatants. The procedure was repeated four times and collects all the supernatant. The supernatant was finally evaporated in a rotary evaporator under reduced pressure at 35°C. Finally, the residue was re-constituted in 3 ml of methanol, filtered using Whatman filter papers (GE Healthcare and Life Sciences, MA, USA) and kept at 4ºC for further use [21,25].

Sl. 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 & Steam	50 mg
5.	*Aloe barbadensis miller*	Aloe vera	Liliaceae	Leaves & 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 5ml extract.					

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

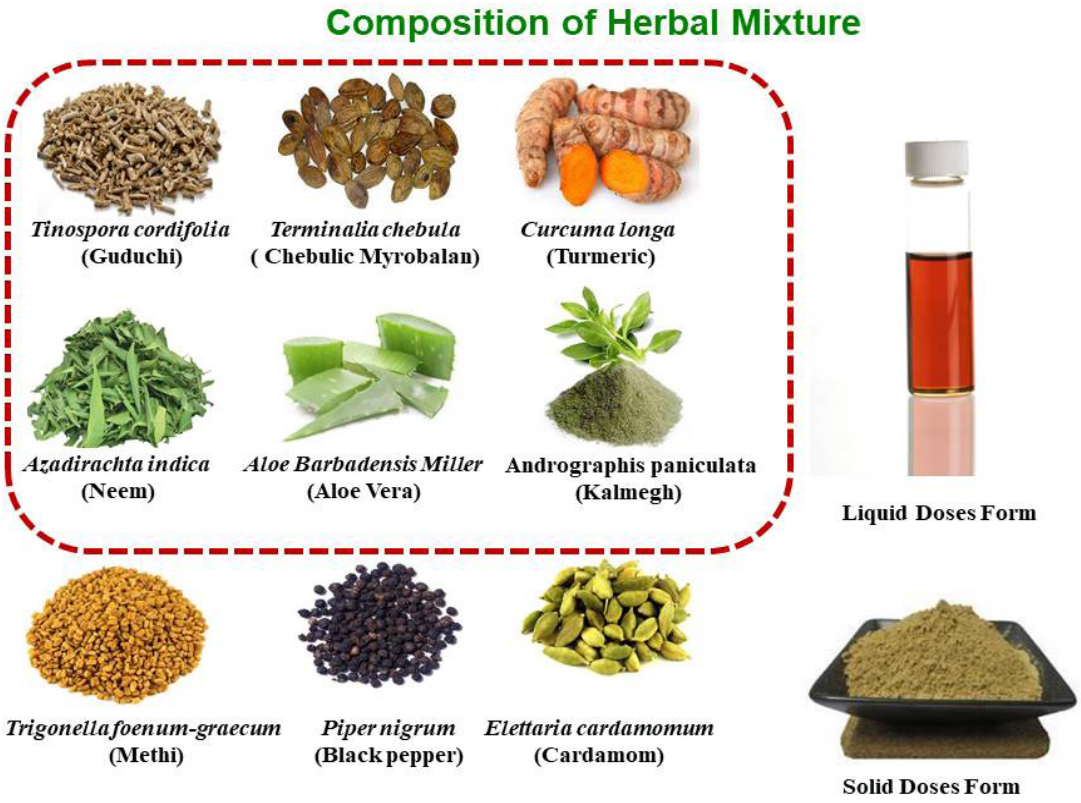

Figure 1: Composition of multi herbal formulation (ASKK16-LIV01) containing six medicinal herbs and three medicinal spices.

Animals and treatment

Experimental animals were taken from registered vendor. Adult mice, C57BL/6J weighing 25 g ± 2g were used to conduct the preclinical study. Before experiment the animals acclimatized for 7 days in an air ventilated room with constant temperature (25±2°C) and humidity (55±5%). The animals were maintained 12 hours light and dark cycle. Freshly prepared healthy diet was given to the animals with water *ad libitum*. Animal received human care in compliance with the revised guidelines 2018 of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Animal Husbandry, Govt. of India.

Acute Toxicity Studies

As per the drug development protocol we conduct the acute toxicity study for novel formulation AKSS16-LIV01 followed by the general principles of OECD guideline 423 [22,26]. Overnight fasted healthy twenty-four male 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 (BW). They were observed continuously for 14 days for morbidity and mortality.

Diets

Healthy standard laboratory diet was used under supervision of the animal care committee of this Institution. Control mice (n=10) were fed standard laboratory diet (7-10% fat, 68-70% carbohydrate, 18-20% protein, 1-2% vitamins and minerals; 210 kcal/1000 g/day). High fat (HF) diet was prepared according to the standard method. High fat diet consists of 30% calories from animal fat (30% fat, 50-52% carbohydrate, 18-20% protein 1-2% vitamins and minerals; 210 kcal/1000 g/day), the diet was prepared, Table 2, and necessary vitamins and minerals are added.

Ingredients	Normal diet	High Fat diet
1. Corn Starch	400	200
2. Sucrose	250	200
3. Cellulose	50	50
4. Protein (Casein)	200	200
5. Fat (ground nut oil and butter)	50	300
6. Salt mixture	35	35
4. Vitamin Mixture	15	15
5. Calories (kcal/100g/day)	210	210

Table 2: Composition of experimental diets (g/kg diet).

Treatment Protocol

After acclimatization, animals were randomly divided into four groups (N=10/group) and treated according to the following protocol. All doses were calculated from earlier reports and pilot experiments.

I. Control Group: Received normal food and water on daily basis.

II. HFD Group: High fat diet (30%) given to the animals regularly.

III. AKSS-75 Group: Animal received high fat diet along with multi herbal mixture (AKSS-16-LIV-01) at a dose of 75 mg/kg/day.

IV. AKSS-150 Group: Animal received high fat diet (30%) along with multi herbal mixture (AKSS-16-LIV-01) at a dose of 150 mg/kg/day.

V. AKSS-300 Group: Animal received high fat diet (30 %) along with multi herbal mixture (AKSS-16-LIV-01) at a dose of 300 mg/kg/day.

VI. Std. drug Group: Animal received Lovastatin (50 mg/kg/day) along with high fat diet (30%).

VII. AKSS-300 Group: Animal received only multi herbal mixture (AKSS-16-LIV-01) at a dose of 300 mg/kg/day without high fat diet.

The duration of the study is six weeks.

Measurement of body weight and food intake

Routine food intake and body weight of each animal was recoded on daily basis using a sensitive digital balance. To obtain the actual food intake subtract the remaining food from the initial food given to the animals. Container of the food were removed at 10.00 a.m. and returned to animals with fresh food at 2:00 p.m. every day.

Blood Collection

At the end of the experimental period 200 µL of blood sample were collected into micro-centrifuge tubes with and without EDTA (2%) from the retro orbital plexus of the mice. Blood collected from animals were placed in slanting position (45°) at room temperature for 2.5 hrs. Then, the blood samples were centrifuged at 4000 g for 10 min. Serum was separated and used for further analyses.

Biochemical estimation

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) were analysed using standard commercial biochemical kits (Span Diagnostic, Surat, India) following the protocol prescribed by manufacturer. Total protein concentration was determined in the serum by the method of Lowry et al. [23,28]. Serum lipid profile like Cholesterol, Triglyceride, Phospholipids, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol (ELITech Diagnostic, France) were measured using enzymatic calorimetric kits according to manufacture instructions.

Preparation of tissue homogenate

For determination of liver lipid profile, the tissue sample was rinsed in ice-cold saline and blotted carefully. The liver tissue was chopped with a scissor and placed in phosphate buffer (at pH 7.4) containing glass tube for homogenization at 6000 g for 10 minutes. Clear supernatant was discarded and collected with a sterilized container for determination of cholesterol, triglyceride, LDL, VLDL and HDL by standard biochemical kit obtained from ELITech Diagnostic, France.

Measurement of liver malondialdehyde (MDA)

Serum and tissue MDA content were measured spectrophotometrically as Thiobarbituric acid (TBA) reactive substances. In brief TBA reacts with MDA and is formed TBA reactant substances (TBARs) which are basically biomarkers of oxidative damage to polyunsaturated fatty acids. MDA content was measured at 532 nm by spectrophotometer [24,29].

Determination of Antioxidant Enzymes activities

Serum antioxidant markers such as total ROS content, SOD, CAT, GSH and GPx were determined through Elisa. For determination of liver antioxidant enzymes contents, we follow the following standard protocol . After the experimental period liver tissue was taken for determination of various antioxidant enzymes activity. Liver tissue was chopped into small pieces and homogenized in ice-cold phosphate buffer (pH 7.2) at a concentration of 15% (weight by volume). Homogenised tissue was centrifuge at 4°C (Hettich Zentrifugen, Germany) at 980 g for 10 minutes. The supernatant was carefully separated and further centrifuged at 7680 g for 30 min at 4°C to obtain the final clear supernatant for evaluation of SOD, CAT, GPx and GSH by standard methods [25-28,30-33].

Histopathological examination

A small portion of the liver tissues from all the experimental groups were excised immediately after sacrifice. Tissues were fixed in 10% formalin saline solution (pH 7.0) for 24hr at room temperature for histopathology. After twenty-four hours the tissues were embedded in paraffin wax (melting point 45°C) and sections were cut at 3-5 µm slices and were stained with haematoxylin and eosin (H&E). The mounted stained section was observed under light microscope [34].

Massion's Trichrome and Sirius red staining

Massion's Trichrome and Sirius red staining were carried out according to the manufacture's standard protocol.

Oil-Red O Staining

Intracellularly accumulated triglyceride was measured using the Oil-Red O staining assay [29,35].

Statistical analysis

All quantitative data are expressed as mean ± standard deviation (SD) unless otherwise stated. One-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test were 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 < 0.05 was considered significant.

Results

Effect of AKSS-16-LIV-01 on body weight and lipid profile

Anti-obesity effects of the newly developed novel formulation (AKSS-16-LIV-01) were evaluated against high fat diet (HFD) induce obese mice. In this study we analyzed the changes in body weight, food consumption, water intake, body fat, liver weight, and liver body weight ratio (Figure 2) as well as serum lipid profile (Table 3) of HFD-induced hyperlipidemic mice treated with AKSS-16-LIV-01 for 6 weeks. Marked significant differences were detected in the body weight food consumption, water intake, body fat, liver weight, and liver body weight ratio of HFD treated group and HFD + AKSS-16-LIV-01 (150 & 300 mg/kg) treated group as compared with standard lipid lowering drug.

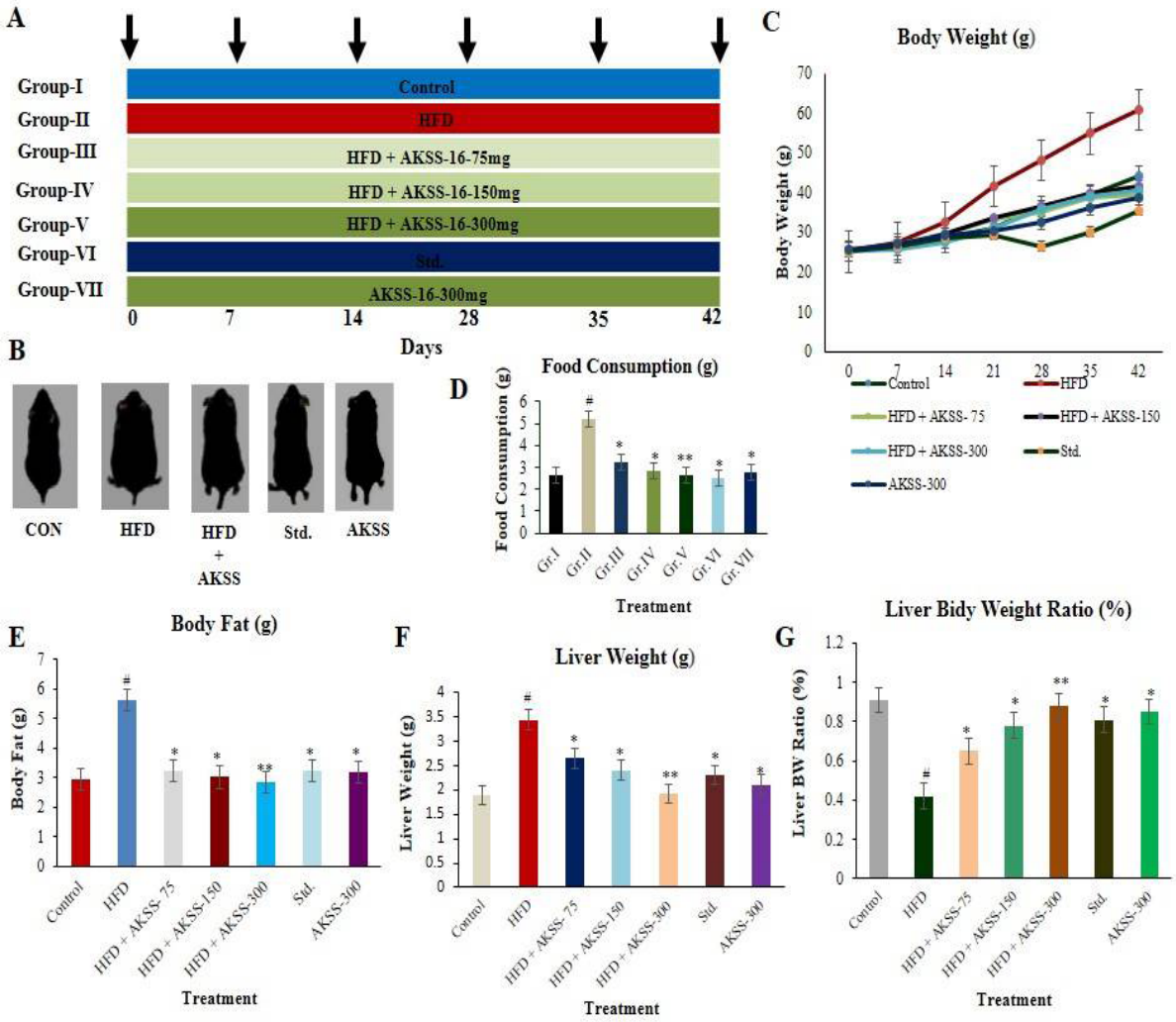

Figure 2: Effect of multi herbal formulation against high fat diet (HFD) induced obesity in mice. **A)** Experimental design **B)** Morphological of the mice in different groups **C)** Measurement of body weight **D)** Determination of food consumption **E)** Determination of body fat **F)** Assessment of liver weight **G)** Determination of Liver body weight ratio.

Table 3 showed serum lipid profile abruptly elevated in the HFD group and differed after AKSS-16-LIV-01 treatment as compared with standard drug. As shown in Figure 6A.3, all liver tissue lipid profile factors were 80-120% higher in the HFD treated group than in the untreated control group. However, the concentration of LDL, VLDL, TG and TC significantly decreased and HDL concentration was significantly increased in the HFD+ AKSS-16-LIV-01 (150 & 300 mg/kg) treated group as compared to the HFD treated group ($P<0.005$, $P<0.001$). These results suggest that AKSS-16-LIV-01 promotes recovery of the serum and tissue lipid profile in HFD-induced obese mice.

Parameters	TC (mg/dL)	TG (mg/dL)	Phospholipids (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control	81.03 ± 5.02	40.58 ± 2.05	76.59 ± 6.28	25.61±1.91	39.65 ± 1.96	19.58 ± 0.69
HFD (30% fat of total diet)	135.69 ± 6.15$^{\#}$	72.58 ± 3.28$^{\#}$	142.97 ± 4.69$^{\#}$	36.25±2.15$^{\#}$	76.94 ± 1.77$^{\#}$	10.28 ± 0.28$^{\#}$

HFD + AKSS-16 (75 mg/kg)	86.39±4.29*	60.12±2.15*	88.21±3.24*	31.24±1.98*	44.27±2.01*	16.11±0.62*
HFD + AKSS-16 (150 mg/kg)	88.05±3.16*	51.23±3.01*	92.37±2.66*	28.67±2.36*	43.61±1.88*	15.28±0.55*
HFD + AKSS-16 (300 mg/kg)	79.36 ± 4.35**	37.25 ± 1.87**	77.25 ± 1.87**	24.15±2.47**	36.85 ± 1.25**	10.28 ± 0.28**
Std Drug	91.47 ± 2.37**	41.75±3.25**	88.03±2.84**	28.14±1.95**	44.52 ± 2.28**	22.67 ± 0.99**
AKSS-16 (300 mg/kg)	85.11±2.98	39.64±3.51**	91.51 ± 3.28**	25.11±2.02**	45.78±1.65**	16.24±0.41**
Values are mean of six individual *(observations in each group ± S.D. Significantly different from control #(p<0.001), and significantly different from HFD group * (p<0.05), ** (p<0.001) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.						

Table 3: Effect of AKSS16-LIV01 on serum lipid profile in high fat diet (HFD) induced obesity in mice.

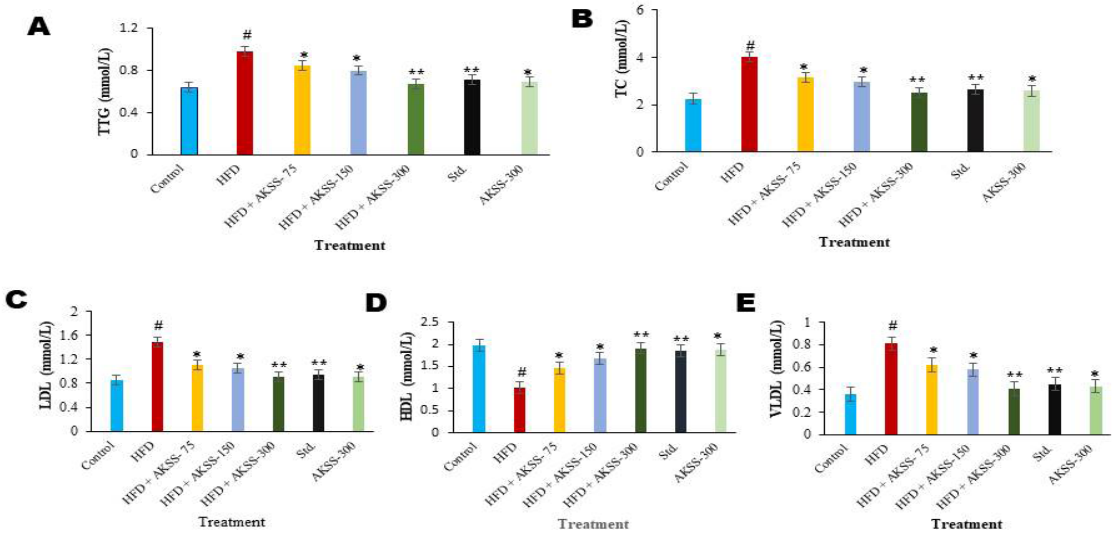

Figure 3: Ameliorative effect of AKSS-16-LIV01 on the activity of Total triglycerides, Total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol under HFD induced Obesity in mice. Values are expressed as Mean ± SD (n = 6 per group). *Significantly different from control # (p<0.001) and significantly different from HFD *(p<0.05) ** (p<0.001) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. TTG: Total Triglycerides, TC: Total Cholesterol, LDL: Low-density lipoproteins, HDL: High-density lipoproteins, VLDL: Very-Low-density lipoproteins.

Effect of AKSS-16-LIV-01 on haematological parameters

Various haematological parameters were presented in Table 4. High fat diet (HFD) significantly reduced (P<0.05, P<0.001) haemoglobin, mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) levels and significantly elevate reticulocyte (RT) and WBC count. Pre-treatment with AKSS-16-LIV-01 150 mg/kg/day and 300 mg/kg/day significantly increased

(P<0.05, P<0.001) haemoglobin, mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) levels and significantly decreased reticulocyte (RT) and WBC count. Application of standard drug partially controls the hyperlipidemic effects by the application of high fat diet upon blood parameters. Multi herbal formulation (AKSS-16-LIV-01) at a dose of 300 mg/kg/day provides the optimum therapeutic results as compared with standard modern drug.

Parameters	Normal	HFD (30% fat of total diet)	HFD + AKSS16-LIV01 (75)	HFD + AKSS16-LIV01 (150)	HFD + AKSS16-LIV01 (300)	HFD + Std. drug	AKSS16-LIV01 (300)
Hb (g %)	13.6± 2.3	9.03± 0.59$^{\#}$	11.0±1.02**	11.05±0.99*	14.01±1.95*	12.96±0.74*	11.21±0.82*
RBC ($x10^6$ cm^2)	10.8±1.9	9.2±0.96	10.5±0.77	9.44±0.71	10.02±0.85	9.85±0.79	9.62±0.84
RT (%)	2.8±0.15	4.9±0.26$^{\#\#}$	2.6±0.14*	3.1±0.14**	2.8±0.15*	3.0±0.12*	3.6±0.16*
HCT (%)	34.1±2.48	36.4±0.66	34.1±0.44	35.8±1.51	34.9±1.56	34.4±1.51	35.1±0.77
MCV (μm^3)	37.2±1.3	31.0±1.68$^{\#}$	36.7±0.29**	36.5±0.44*	35.9±0.72*	36.2±0.43*	35.5±0.36*
MCH (pg)	21.4±0.85	22.2±0.14	22.8±0.23	21.1±0.12	21.4±0.11	21.2±0.14	22.1±0.12
MCHC (%)	41.2±1.06	32.4±0.95$^{\#\#}$	40.2±1.07*	37.1±0.92*	39.8±0.87*	38.6±0.99*	36.2±0.91*
Platelets	6.5±0.02	5.5±0.03	6.5±0.04	5.8±0.05	6.1±0.07	5.5±0.05	5.4±0.06
WBC ($x10^5$ cm^2)	9.2±0.09	12.4±0.15$^{\#}$	9.1±0.08*	10.8±0.12*	9.2±0.11*	10.1±0.13*	10.7±0.11*
Lymphocyte	72±2.98	79±3.04$^{\#}$	72±2.54*	73±3.06*	71±2.58*	72±3.08*	71±3.11*
Neutrophil	26±1.12	16±0.38$^{\#}$	24±1.09*	20±0.56*	25±0.69*	24±0.51*	21.52±2.09*

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

Values are mean of six individual *(observations in each group ± S.D. Significantly different from control $^{\#\#}$(p<0.001), $^{\#}$(p<0.05) and significantly different from HFD group ** (p<0.001), *(p<0.05) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

Table 4: Effect of AKSS16-LIV01 on haematological parameters in high fat diet (HFD) induced obesity in mice.

Effect of AKSS-16-LIV-01 on serum biochemical parameters

Anti-obesity effects of the newly developed novel formulation (AKSS-16-LIV-01) on all the biochemical parameters were depicted in Table 3 and Fig. 4. A significant increase in serum transaminase (ALT and AST), alkaline phosphatase (ALP), γ- glutamyl transferase activity were observed in HFD-intoxicated mice compared to normal control. Treatment with AKSS-16-LIV-01 both 150 mg/kg/day and 300 mg/kg/day for 42 days significantly (P < 0.05, P < 0.001) reduced ALT, AST, ALP and GGT levels as compared to HFD group. Application of standard drug also showed significant effect but more positive effect observed in AKSS-16-LIV-01 (300 mg/kg/day) group due to presence of medicinal spices in the extracts. Treatment with only AKSS-16-LIV-01 has no significant effect on serum biochemical parameters. On the other hand, significantly reduced (P<0.001) serum total protein in HFD group was normalized by the treatment with AKSS-16-LIV-01 (Table 5).

Treatment	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	ALP (IU/L)	TP (g/L)
Control	14.26±1.36	27.16±1.36	5.12±0.04	313.02±11.54	6.95±0.91
HFD (30% fat of total diet)	52.19±2.09$^{\#}$	88.02±1.97$^{\#}$	10.16±0.06$^{\#}$	546.74±10.65$^{\#}$	3.25±0.95$^{\#}$
HFD + AKSS-16 (75 mg/kg)	36.59±1.39*	51.22±3.62*	8.01±0.08*	416.91±12.37*	4.12±1.02*
HFD + AKSS-16 (150 mg/kg)	28.91±2.11*	35.11±2.18*	6.11±0.09*	347.84±14.25*	5.78±1.14*
HFD + AKSS-16 (300 mg/kg)	16.06±1.98**	28.35±3.01**	5.29±0.07**	312.05±11.69**	6.58±0.94**
Std Drug	26.02±1.12**	32.06±1.99**	6.01±0.06**	351.74±13.25**	6.01±1.04**
AKSS-16 (300 mg/kg)	22.19±2.06**	30.16±2.15**	5.35±0.04**	330.03±14.05**	6.33±1.11**
Values are mean of six individual *(observations in each group ± S.D. Significantly different from control $^{\#}$(p<0.001), and significantly different from HFD group * (p<0.05), ** (p<0.001) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.					

Table 5: Effect of AKSS16-LIV01 on serum biochemical parameters in high fat diet (HFD) induced obesity in mice.

Effect of AKSS-16-LIV-01 on liver transaminase levels

Figure 4 represented the liver aspartate and alanine transaminase activity. Daily intake of high fat diet (HFD) significantly elevated (P<0.001) liver AST and ALT levels as compared with control untreated animals. Treatment with AKSS-16-LIV-01 both 150 mg/kg/day and 300 mg/kg/day for 42 days significantly (P < 0.05, P < 0.001) reduced tissue ALT and AST levels as compared to HFD group. Administration of standard drug also showed significant effect but optimum effects observed in AKSS-16-LIV-01 (300 mg/kg/day) group due to presence of medicinal spices in the extracts.

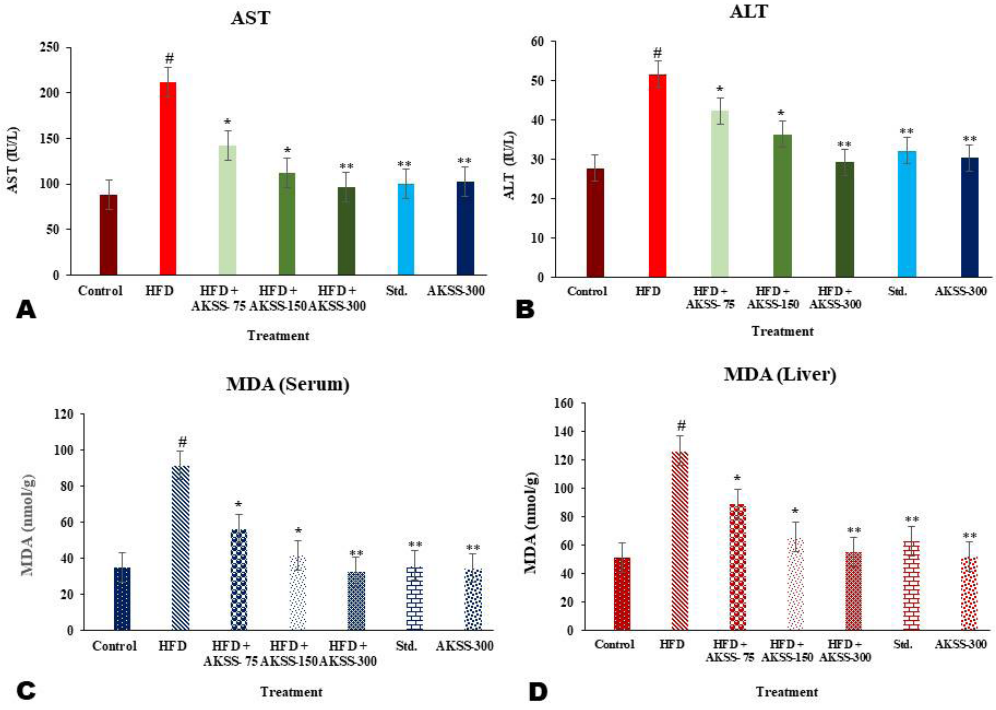

Figure 4: Ameliorative effect of AKSS-16-LIV01 on the activity of Aspartate amino transferase Alanine amino transferase, MDA content in Serum & MDA content in Liver under HFD induced Obesity in mice.

Effect of AKSS-16-LIV-01 on Lipid peroxidation

Administration of High fat diet (HFD) showed elevated level of MDA both serum and liver when compared to normal control (Figure 4). In comparison with HFD control animals, treatment with AKSS-16-LIV-01 both 150 mg/kg/day and 300 mg/kg/day for 6 weeks has significantly decreased the MDA level in serum and liver. Application of standard drug also showed significant effect but more positive effect observed in AKSS-16-LIV-01 (300 mg/kg/day) group. Treatment with only AKSS-16-LIV-01 has no significant effect on tissue lipid peroxidation.

Effect of AKSS-16-LIV-01 on serum antioxidant status

The activities of the antioxidant enzymes total ROS content, levels of SOD, CAT, GSH and GPx were altered prominently in serum of HFD group when compared with normal untreated group. Administration of AKSS-16-LIV-01 both 150 mg/kg/day and 300 mg/kg/day for 42 days significantly increased the serum antioxidant enzyme activities such as SOD, CAT, GSH and GPx in liver tissue (Table 6). Pre-treatment with multi herbal formulation (AKSS-16-LIV-01) significantly reduced the elevated ROS content caused by high fat diet (HFD).

Parameters	ROS (FIU)	SOD (U/mg)	CAT (U/mg)	GSH (mmoles/mg Protein)	Gpx (mmoles/mg Protein)
Control	412.59±14.3	5.53±0.62	14.85±1.02	35.14±3.36	20.09±0.95
HFD (30% fat of total diet)	736.13±17.8$^{\#}$	2.23±0.95$^{\#}$	6.71±1.25$^{\#}$	19.91±4.15$^{\#}$	10.27±2.25$^{\#}$
HFD + AKSS-16 (75 mg/kg)	510.27±14.71*	4.52 ±0.32*	8.02±0.96*	25.47±4.69*	15.62±1.48*
HFD + AKSS-16 (150 mg/kg)	492.81±11.24*	4.96 ±0.75*	10.58±0.94*	29.02±3.48*	18.56±0.98*
HFD + AKSS-16 (300 mg/kg)	434.51±9.65**	5.28±0.85**	14.12±1.12**	37.31±3.78**	22.69±1.48**
Std Drug	478.64±12.69**	4.99 ±0.91**	12.95±1.03**	28.94±5.94**	19.65±0.96**
AKSS-16 (300 mg/kg)	450.27±11.28**	5.21 ±0.54**	13.65±1.67**	35.47±4.85**	20.36±1.25**

Values are mean of six individual *****(observations in each group ± S.D. Significantly different from control $^{\#}$($p<0.001$), and significantly different from HFD group ***** ($p<0.05$), ****** ($p<0.001$) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

Table 6: Effect of AKSS16-LIV01 on serum antioxidant parameters in high fat diet (HFD) induced obesity in mice.

Effect of AKSS-16-LIV-01 on tissue antioxidant status

The activities of the antioxidant enzymes SOD, CAT, GSH and GPx were decreased prominently in liver tissue of HFD control when compared with normal group. Administration of AKSS-16-LIV-01 both 150 mg/kg/day and 300 mg/kg/day for 42 days significantly increased the antioxidant enzyme activities such as SOD, CAT, GSH and GPx in liver tissue (Figure 5) . Application of standard drug also showed significant effect but more positive effect observed in AKSS-16-LIV-01 (300 mg/kg/day) group. Treatment with only AKSS-16-LIV-01 300 mg/kg/day alone has no significant effect on tissue lipid peroxidation.

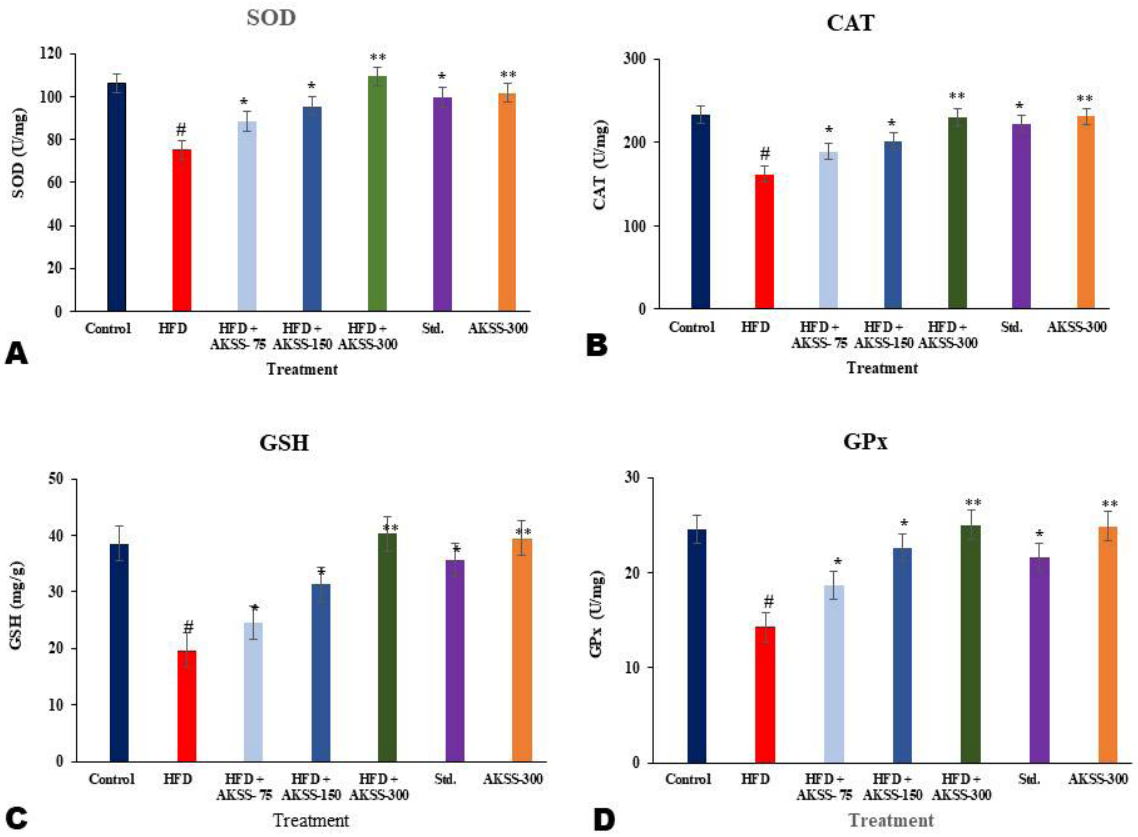

Figure 5: Free radical scavenging activity (SOD, CAT, and GSH & GPx). Values are expressed as Mean ± SD (n = 6 per group). *Significantly different from control $^{\#}$($p<0.001$) and significantly different from HFD *($p<0.05$) ** ($p<0.001$) using analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. SOD: Super oxide dismutase, CAT: Catalase, GSH: Glutathione, GPx: Glutathione Peroxidase.

Effect of AKSS-16-LIV-01 on Histopathology of liver

The liver of the High fat diet group was significantly enlarged and the colour of liver becomes pale. The physical appearance, i.e., colour, size and smoothness of the liver of AKSS-16-LIV-01 supplemented with HFD group remain unaltered comparing those of the normal control group. Figure 6, shows the microscopic appearance of the liver tissues. Steatosis (fatty change) clearly existed in the liver tissue of the HFD group as shown in Fig. 6. Lipid accumulation in the hepatocytes as vacuoles, and large droplets were observed in most hepatocytes, in particular in the periportal regions. In contrast administration of AKSS-16-LIV-01, protected the liver tissue from high fat diet, little or no fatty droplets were observed in the AKSS-16-LIV-01 treated group, and decreased the number of vacuoles appeared (Figure 6).

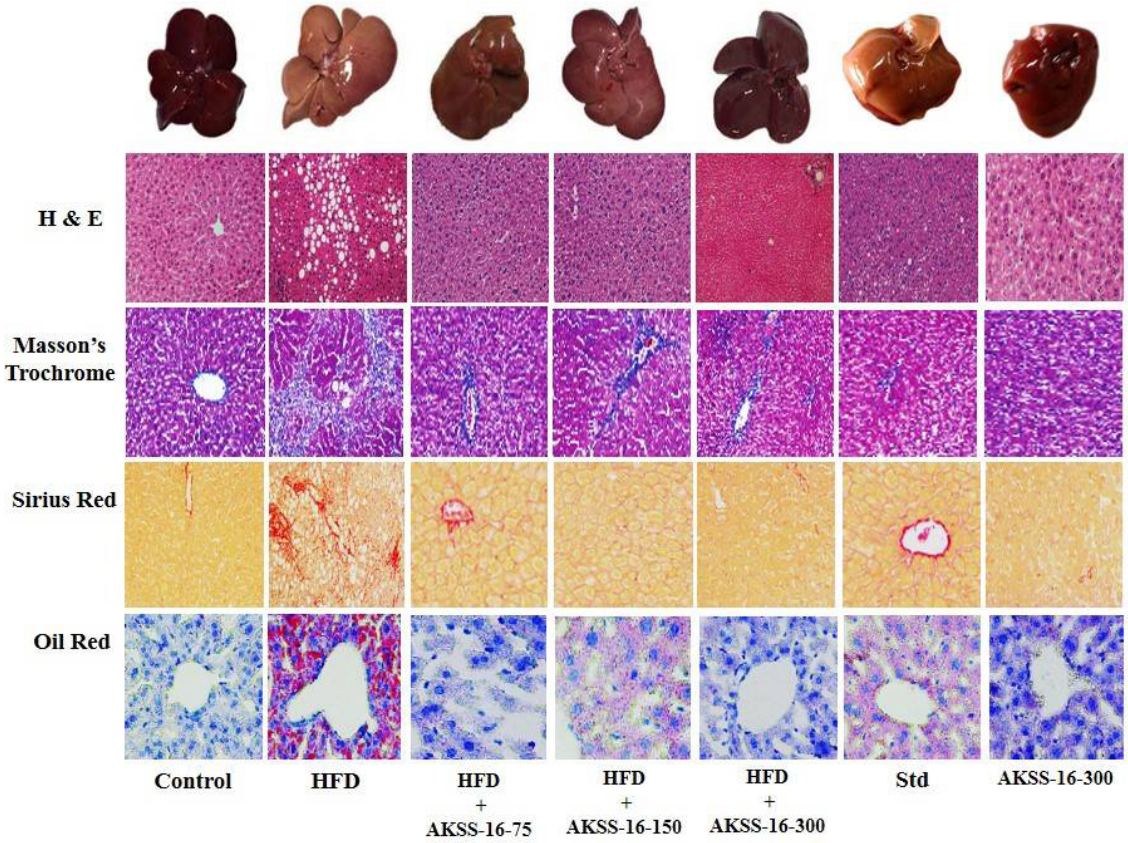

Figure 6: Ameliorative effect of AKSS-16-LIV01 on different morphology & Histology under HFD induced Obesity in mice.

No fatty infiltration was observed in the Normal group. Mice in the HFD group developed macrovesicular steatosis, steatohepatitis changes, inflammation, and massive infiltration of inflammatory cells. Multi herbal formulation ameliorated these morphological features in the HFD+ AKSS-16-LIV-01 groups. Moreover, compared with the HFD group, hepatocyte lipid accumulation, especially in the HFD+ AKSS-16-LIV-01 groups, was significantly decreased (Figure 6).

Groups	Injury of scores				
Treatment	Fatty degeneration	necrosis	Cell swelling	Inflammation	Total score
Group-I	0	0	0	0	0
Group-II	4	4	3	3	14
Group-III	1	0	1	1	3
Group-IV	1	1	0	0	2
Group-V	0	0	0	0	0
Group-VI	1	0	0	1	2
Group-VII	0	0	0	0	0

Tables 7: Effect of AKSS16-LIV01 on Histopathological scoring of liver section both HFD and treatment groups.

Discussions

Obesity is closely associated with liver abnormalities, including liver fibrosis, steatohepatitis, fatty liver, non-alcoholic fatty liver disease (NAFLD) [30,31,36,37]. People all over the globe are really worried about that. To overcome this situation presently scientists and researchers are engaged in research to find out a permanent safe and symptomatic anti-obesity solution. Unfortunately, different indirect strategies like diet, exercise & yoga, behavioural adaptation modifications not only make a solution to prevent obesity but also showed several health complications. Therefore, world need a novel, effective, and safe anti-obesity interventions which not only prevent obesity but also minimise the related complications. High-fat diet (HFD) is a major cause of obesity which is closely linked to a variety of health issues, including coronary heart disease, high blood [32] pressure, stroke, liver diseases, diabetes and cancer [33,38]. Reactive Oxygen Species (ROS) are closely associated with obesity leads to numerous degenerative diseases such as atherosclerosis, ischemic heart disease and aging, etc. In the obese condition ROS generally suppressed the antioxidant enzymes activity which disrupt the antioxidant defence mechanism produce cellular imbalance and ultimately cell apoptosis [32,34,35,39-41]. So, oxidative stress is one of the causative factors that link hypercholesterolemia with the pathogenesis of linked diseases [36,37,42,43]. In the present study, administration of high fat diet (HFD) showed an increase in TC, TG, LDL-C and VLDL-C level and decrease HDL-C level in mice. Furthermore, administration of AKSS-16-LIV-01 at a dose of 300mg /kg/day showed a significant ($p<0.001$) reduction in TC, TG, LDL-C and VLDL-C levels and increase in HDL-C level in HFD treated mice. Deviations from cholesterol transport increases LDL levels or decreases HDL cholesterol flux, which may result in accumulation of cholesterol in extra hepatic tissues. Therefore, from the present findings it is presumed that the prevention of hyperlipidaemia may be due to the inhibition of biosynthesis of cholesterol and triglycerides by the developed novel formulation.

Scientific study revealed that oxidative stress is a causal factor that associates hyperlipidaemia with the pathogenesis of obesity. HFD disrupt body's homeostasis and generate free radicals which produce oxidative stress [38-41,44-47]. In the present study appears to validate the view that the HFD group mice have shown a marked increase in MDA levels in both serum and liver tissues. Our study showed that treatment with the novel multi herbal formulation AKSS-16-LIV-01 at a dose of 300mg /kg/day prevented the lipid peroxidation in the serum and liver tissues of hyperlipidaemic obese mice. Our study also indicate that obese mice showed marked decline in the SOD, catalase, GSH and GPx essential first and second order antioxidant enzymes activities when compared with the normal control, and the treatment with AKSS-16-LIV-01 at a dose of 300mg /kg/day has significantly ($p<0.001$) restored these enzymes in a dose-dependent manner when compared with the HFD group animals. Our study also established that AKSS-16-LIV-01 with the therapeutic optimum dose control the obesity as compared to standard marketed drug.

Microscopic and histologic examinations revealed reduction in the mass of adipose tissue and adipocyte cell sizes of the HFD + AKSS-16-LIV-01 treated groups as compared to the HFD group. The liver weight and number of lipid droplets also decreased in the HFD+ AKSS-16-LIV-01 treated organ. Scientific study established that adipose tissue is widely distributed in the body and acts as a key energy reservoir [45]. The microscopic images have shown marked reduction of lipid accumulation in adipocyte of visceral adipose tissue of HFD group animals on the administration of AKSS-16-LIV-01 at a dose of 300mg /kg/day. Moreover, histological scoring of the liver tissue clearly showed that fatty degeneration, necrosis, cell swelling and inflammation were in control as compared with HFD group.

Conclusion

Our developed novel multi herbal formulation (AKSS-16-LIV-01) composed of six Indian medicinal herbs and three Indian medicinal spices have a potent anti-obesity activity in experimental animals. AKSS-16-LIV-01showed optimum hypolipidemic and antioxidant properties at a dose of 300mg /kg/day against HFD induced obese mice. In addition, the AKSS-16-LIV-01 significantly protected the liver tissues against oxidative stress triggered by HFD, ultimately causing NAFLD , as a new therapeutic option. The present study also successfully demonstrated a modified method for lipid staining using Oil Red O dye which helps observing the lipid droplets in the cells drawn from in vivo system.

Conflict of Interest:

No conflict of interest.

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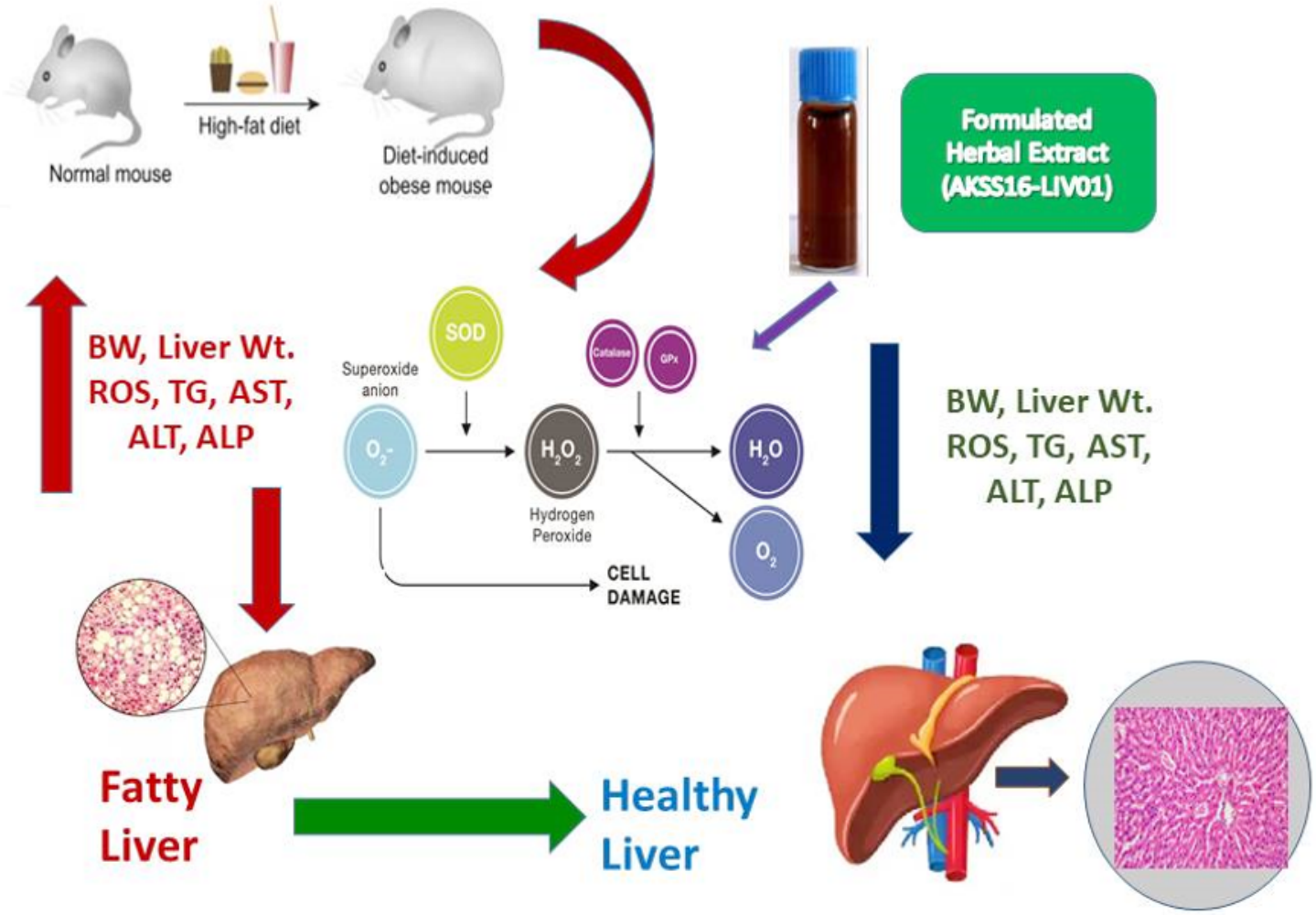
Summary



References

1. Choe D, Jung HH, Kim D, Shin CS, Johnstona VT, et al., (2020) *In vivo* evaluation of the anti-obesity effects of combinations of Monascus pigment derivatives. RSC Adv 10:1456-1462.
2. Vasanth Rao VRB, Candasamy M, Bhattamisra SK (2019) Diabetes. Metab Syndr Obes: Targets Ther. 13: 2112-2120.
3. Rabeea N, Eldabi T, Kamel DM (2019) Obesity Review in GCC and New Evaluation Strategy. KnE soc sci 3:78-89.
4. Chien MY, Ku YH, Chang JM, Yang CM, Chen CH (2016) Effects of herbal mixture extracts on obesity in rats fed a high-fat diet. J Food Drug Anal 24:594-601.
5. Bessesen DH, Van Gaal LF (2018) Progress and challenges in anti-obesity pharmacotherapy. Lancet Diabetes Endocrinol 6:237-248.
6. Glass CK, Witztum JL (2001) Atherosclerosis: the road ahead. Cell 104:503-516.
7. McKenney JM (2001) Pharmacotherapy of dyslipidemia. Cardiovasc Drugs Ther 15:413-422.
8. Chisolm GM, Steinberg D (2000) The oxidative modification hypothesis of atherogenesis: an overview. Free Radic Biol Med 28:1815-1826.
9. Qureshi K, Abrams GA (2007) Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol 13:3540-3553.
10. Tamura S, Shimomura I (2005) Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. J Clin Invest 115:1139-1142.
11. Lambert JE, Ramos-Roman MA, Browning JD, Parks EJ (2014) Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology 146:726-735.
12. Wang LL, Yu QL, Han L, Ma XL, Song RD, et al., (2018) Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. Food Chemistry 244:394-402.
13. Lai XX, Li YP (2016) Antitumor effect and mechanism of action of polysaccharides extracted from Polygonum perfoliatum L whole plant in human lung carcinoma A549 cell line. Tropical Journal of Pharmaceutical Research 15:1243-1249.
14. Liu YM FWC (2011) Pharmacological action and clinical application research progress of the *Polygonum perfoliatum* L. Asia-Pacific Traditional Medicine 7:161-162.
15. Perez-Carreras M, Del Hoyo P, Martín MA, Rubio JC, Martín A, et al., (2003) Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. Hepatology 38:999-1007.
16. Darbar S, Chattopadhyay S (2019) Single dose acute oral toxicity of Livina, a polyferbal formulation in mice model. European Journal of Pharmaceutical and Medical Research 6:492-495.
17. Bello IA, Ndukwe GI, Audu OT, Habila JD (2011) A bioactive flavonoid from Pavetta crassipes *K. Schum*. Org Med Chem Lett 1:14.
18. Thompson M, Jaiswal Y, Wang I, Williams L (2017) Hepatotoxicity: Treatment, causes and applications of medicinal plants as therapeutic agents. The Journal of Phytopharmacology 6:186-193.
19. Shravan KN, Nischal Y, Sarika M, Sri GSS (2014) Hypolipidemic activity of ethanolic extracts of Cassia angustifolia in triton- x 100 induced hyperlipidemia in rats. Asian J Pharm Clin Res 7:189-191.
20. Garcia-Ruiz C, Kaplowitz N, Fernandez-Checa JC (2013) Role of mitochondria in alcoholic liver disease. Curr Pathobiol Report 1:159-168.
21. Thompson M, Jaiswal Y, Wang I, Williams L (2017) Hepatotoxicity: Treatment, causes and applications of medicinal plants as therapeutic agents. JPHYTO 6:186-193.
22. De S, Suresh R, Sri Sudhakar Babu MA, Aneela S (2017) In-vivo Hepatoprotective Activity of Methanolic Extracts of Sphaeranthus amaranthoides and Oldenlandia umbellate. Pharmacogn J 9:98-101.
23. Darbar S, Saha S, Pramanik KS and Chattopadhyay A (2018) Preliminary Acute Oral Toxicity Study of a Newly Developed Herbal Formulation. World Journal of Pharmaceutical Research 7:924-930.
24. Darbar S, Saha S, Pramanik KS and Chattopadhyay A (2019) Preliminary Assessment of Acute and 28-Day Repeated Dose Oral Toxicity of a Newly Developed Herbal Mixture on Experimental Animal. Indian Journal of Pharmaceutical Education and Research 53(Suppl): 669-676.
25. Adhikari A, Darbar S, Chatterjee T, Das M, Polley N, et al., (2018) Spectroscopic Studies on Dual Role of Natural Flavonoids in Detoxification of Lead Poisoning: Bench-to-Bedside Preclinical Trial. ACS Omega 23 :15975-15987.
26. Asharani PV, Hande MP, Valiyaveettil S (2009) Anti-proliferative activity of silver nanoparticles. BMC Cell Biol 10:1-14.
27. Darbar S, Saha S, Pramanik KS, Chattopadhyay A (2019) Toxicological Assessment of Silver Nanoparticles Synthesized through Green Route using Andrographis paniculata. J Nanosci Tech 5:619-621.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.
29. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351-358.
30. Mishra PH, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170-3175.
31. Abei H (1984) Catalase in vitro. Methods Enzymol 105:121-126.
32. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77.
33. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, et al., (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588-590.
34. Kim HL, Park J, Park H, Jung Y, Youn DH, et al., (2015) Platycodon grandiflorum A. De candolle ethanolic extract inhibits adipogenic regulators in 3T3-L1 cells and induces mitochondrial biogenesis in primary brown preadipocytes. J Agric Food Chem 63:7721-7730.
35. Zhang L, Zhang HY, Ma CC, Wei W, Jia LH (2014) Increased body weight induced by perinatal exposure to bisphenol A was associated with downregulation zinc-alpha2-glycoprotein expression in offspring female rats. Mol Cell Toxicol 10: 207-213.
36. Marchesini G, Moscatiello S, Di Domizio S, Forlani G (2008) Obesity-associated liver disease. J Clin Endocrinol Metab 93:S74-80.

37. Perla FM, Prelati M, Lavorato M, Visicchio D, Anania C (2017) The role of lipid and lipoprotein metabolism in non-alcoholic fatty liver disease. Children (Basel) 4: 46.

38. Kim MH, Park SJ, Kim JH, Seong JB, Kim KM, et al., (2018) Peroxiredoxin 5 regulates adipogenesis-attenuating oxidative stress in obese mouse models induced by a high-fat diet. Free Radical Biol Med 123: 27-38.

39. Gomez-Lechon MJ, Donato MT, Martinez-Romero A, Jimenez N, Castell JV, et al., (2007) A human hepatocellular in vitro model to investigate steatosis. Chem Biol Interact 165:106-116.

40. Hanschmann EM, Godoy JR, Berndt C, Hudemann C, Lillig CH (2013) Thioredoxins, glutaredoxins, and peroxiredoxins–molecular mechanisms and health significance: from cofactors to antioxidants to redox signalling. Antioxidants Redox Signal 19:1539-1605.

41. Ito R, Takahashi M, Ihara H, Tsukamoto H, Fujii J, et al., (2012) Measurement of peroxiredoxin-4 serum levels in rat tissue and its use as a potential marker for hepatic disease. Mol Med Rep 6:379-384.

42. Nabeshima A, Yamada S, Guo X, Tanimoto A, Wang KY, et al., (2013) Peroxiredoxin 4 protects against nonalcoholic steatohepatitis and type 2 diabetes in a nongenetic mouse model. Antioxid Redox Signal 19: 1983-1998.

43. Nawata A, Noguchi H, Mazaki Y, Kurahashi T, Izumi H, et al., (2016) Overexpression of peroxiredoxin 4 affects intestinal function in a dietary mouse model of nonalcoholic fatty liver disease. PLoS One 11: e0152549.

44. Young IS, Mceneny J (2001) Lipoprotein oxidation and atherosclerosis. Biochem Soc Trans 29:358-362.

45. Marzyieh Z, Alireza OR, Reza M, Mohammed N, Morteza VJ, et al., (2007) Assessment of anti-hyperlipidemic effect of *Citrullus colocynthis*. Braz J Pharmacogn 17:492-496.

46. Lehninger AL, Nelson DL, Cox MM (2008) Lehninger Principles of Biochemistry. 5th ed. New York: W.H. Freeman 827-897.

47. Darbar S, Garai A, Ghosh B, Chattapadhyay S, Chakraborty MR (2010) Antilipidemic effect of Livina, a polyherbal liquid formulation on high fat diet induced obesity in swiss albino mice. Journal of Pharmaceutical Research 7:186-191.