ORIGINAL ARTICLE

Antiobesity activity of methanolic extract of *Lagerstroemia parviflora* Roxb. (leaves) on Wistar albino rat model

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Abstract

Objective: The objective of research paper is to evaluate the antiobesity potential of methanolic extract of *Lagerstroemia parviflora* Roxb. (MELPR) (leaves) in obese Wistar albino rats induced with high-calorie diet (HCD). **Materials and Methods:** The plant extract of *L. parviflora* Roxb. (leaves) with different solvents was evaluated for the lipase inhibitory activity. The MELPR at different concentrations (200 and 300 mg/kg body weight [b.w.]) was administered orally and evaluated for the estimation of biochemical parameters (serum glutamate pyruvate transaminase [SGPT] and alkaline phosphatase), skin and behavioral activity, oral glucose tolerance test (OGTT) and very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol (VLDL-C and LDL-C), and antiobesity potential. Histopathological evaluation was also performed. **Results and Discussion:** The pancreatic lipase (PL) inhibitory activity result indicated that hexane extract did not inhibit the PL, whereas methanolic extract showed 75% inhibitory activity. Hematological study indicates that hemoglobin concentration was increased in treated group compared to control group. Biochemical study results indicate that creatinine and urea levels were found to be little lowering in treated groups compared to control group. OGTT result data indicate a significant ($P < 0.05$) elevation in plasma glucose, insulin, and insulin resistance in HCD control obese rats when compared to normal control rats. The antiobesity activities data indicate that HCD has substantially altered physiological and biochemical aspects. Administration of MELPR reduced significantly, b.w., total fat, fat percentage, blood glucose, insulin resistance, and lipid profile in a dose-dependent manner (200 and 300 mg/kg b.w.). **Conclusion:** The MELPR is non-toxic and safe up to 3000 mg/kg bodyweight in rats. Treatment with MELPR has dose dependently and significantly alleviated HCD-induced obesity, hyperlipidemia, as supported by other studies. This study demonstrates the antihyperlipidemic and antiobesity potential of MELPR and offers scientific validation and basis to develop antiobesity drugs.

Key words: Lagerstroemia parviflora, Antiobesity, serum glutamate pyruvate transaminase [SGPT] and alkaline phosphatase, oral glucose tolerance test, low-density lipoprotein (LDL) cholesterol (VLDL-C and LDL-C)

INTRODUCTION

Herbal medicines are being used increasingly as dietary supplements to fight or prevent common maladies such as cancer, heart attacks, and depression. When added to foods as supplements, herbs have also been termed as nutraceuticals. Herbal remedies are unpurified plant extracts containing several constituents, which often work together synergistically.

Traditionally medicines include diverse type of health practices, approaches, knowledge and beliefs to incorporate plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises. And these were applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness in humans. In Africa, up to 80% of the populations use traditional medicines for primary health care.

In industrialized countries, adaptations of traditional medicine retermed Complementary or Alternative Medicine.

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In the United States, 158 million of the adult population use complementary medicine, US$ 17 billion was spent on traditional remedies in 2000. In the United Kingdom, annual expenditure an alternative medicine was US $ 230 million. The global market for herbal medicines currently stands at over US $ 60 billion annual and is growing steadily 25% of modern medicines are made from plants first use traditionally.

Over one-third of the population in developing countries lack access to essential medicines. The provision of safe effective traditional medicine therapies could become a critical tool to increase access to health care. Seventy countries have a national regulation on herbal medicines but the legislative control of medicinal plants has not evolved around a structured model.

This is because medicinal products or herbs are defined differently in different countries and diverse approaches have been adopted with regard to licensing, dispensing, manufacturing, and trading.[1]

Indian subcontinent is a rich source of plant and animal wealth, which is due to its varied geographical and agro-climatic regions. Besides it is varied biodiversity, it has a diverse cultural heritage too. Although at present, Indian health-care delivery consists of both modern systems of medicines and traditional system of medicine such as Ayurveda, Siddha, Unani, and unorganized system like folk medicine has been flourishing well. Ayurveda and Siddha are of Indian origin and accounted for about 60% health-care delivery in general and 75% of rural Indian population depends on these traditional systems.

Obesity can be defined as excess accumulation of body fat arising from a sustained or a periodic positive energy balance which happens when energy intake exceeds energy expenditure. Obesity can be observed as a defined cluster of non-transmissible diseases called "New World Syndrome," creating a massive socioeconomic and civil health burden in poorer countries. The WHO has described obesity as one of most neglected public health problems, touching every section of the globe.[2]

Prevalence

In recent years, the prevalence of obesity has increased reaching epidemic levels. Worldwide, an estimated number of overweight and obese people increased from 857 million in 1980 to 2.1 billion in 2013. This is one-third of the world's population. Globally, an increase in the section of adults with a body mass index (BMI) of 25 kg/m^2 or greater was found between 1980 and 2013 from 28.8% to 36.9% in men and from 29.8% to 38.0% in women. In the same period, the number of kids and teenagers who are overweight and obese has increased significantly in developed countries by 23.8% in boys and 22.6% in girls. It has also increased in kids and teenagers in developing countries, from 8.1% to 12.9% in boys and from 8.4% to 13.4% in girls. In 2010, overweight and obesity were estimated to cause 3–4 million deaths, that is, 3.9% of years of life lost and 3.8% of disability-adjusted life-years worldwide (Ng *et al*., 2014). According to a recent study, India is just behind the US and China in the global hazard list of top 10 countries with highest number of obese people. Furthermore, it was observed that the BMI values were similar in males and females; however, there were more overweight or obese (BMI ≥25 kg/m^2) females (6.6%) than males (3.5%). In few areas, obesity and its subsequent diseases are posing a massive public health problem.[3]

MATERIALS AND METHODS

Assay of Lipase Inhibitory Activity of Plant Extract

Lipase inhibitory activity of diabetic rats received methanolic extract of *Lagerstroemia parviflora* Roxb. (MELPR) leaves extracts (hexane, ethy1 acetate, ethanolic, and aqueous extracts) was determined using a modified assay method.[4] Briefly, a suspension containing 1% (v/v) triolein and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (PH8) was prepared and emulsified. Assay was then initiated by adding 800 µl of the triolein emulsion to 200 µl of porcine pancreatic lipase (PL) (0.5 g pancreatin in 15 ml of 0.1 M phosphate buffer at pH 8.0) and 200 µl of plant extract.[5]

Acute Oral Toxicity Studies

Before testing a plant extract/compound for its biological activity, the acute toxicity studies are carried out to find toxic effects or mortality or lethal dose, and in the present study, 3000 mg/kg b.wt of MELPR was administered (one dose) and observed initially for 72 h, and then, the same dose was given on alternative days for 14 days to observe any toxic or behavioral changes and to optimize concentration of plant extract for further studies.[6]

Animals and experimental design

Rats weighing 180–220 g were randomly divided into two groups, normal control (NC) group and treated group (n = 6).

Group 1: NC group.
Group 2: MELPR treated group (3000 mg/kg b.wt).

Biochemical Parameters

Combined with hematology parameters, biochemical parameters are major indicators for most diagnostic investigations. Many biochemical parameters tend to have specificity for an organ and/or a limited range of the pathological processes. Interpretation of diagnostic biochemical patterns requires an understanding of the pathological implications of each abnormal result.[7]

Hematological tests

Blood samples were collected from all groups of rats in heparinized and non-heparinized tubes and used for estimating hemoglobin percent and total blood cell counts. Non-heparinized samples centrifuged and plasma was alienated for further analysis.

Estimation of hemoglobin

Hemoglobin content of blood was estimated by the cyanomethemoglobin method of Drabkin and Austin (1932). When blood is diluted with an alkaline solution of potassium cyanide and potassium ferricyanide, hemoglobin is oxidized to methemoglobin and combines with cyanide to form cyanomethemoglobin, which is measured calorimetrically at 540 nm.[8]

Liver and renal function tests

Liver and kidney tests are very important to diagnose the disease or status of disease. Below-mentioned parameters are major diagnostic profiles in liver and renal function tests. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP) levels were estimated by the following methods.[8]

Assay of SGPT

SGPT activity was determined by the method of Reitman *et al*., 1957. Transamination is the process in which an amino group transferred from amino acid to an α-ketoacid. The enzymes responsible for transamination are called transaminases.[8]

Assay of SGOT

SGOT activity was determined by the method of Reitman *et al*., 1957.[8]

Assay of serum ALP

Activity of serum ALP was determined by p-nitropheny1 phosphate method (Bessey *et al*., 1946).[3]

Para-nitropheny1 phosphate, which is colorless, is hydrolyzed by ALP (pH 10.5) and at 37.0 to form free paranitrophenol, which is colored yellow. The addition of NaOH stops the enzyme activity and the final color shows maximum absorbance at 410 nm.

Estimation of protein

Protein estimation was done to the tissue homogenate by the method of Leary *et al*., 1998.[9]

Estimation of serum urea

Estimation of serum urea was done by diacetyl monoxime method (Wybenga *et al*., 1971).[10]

Urea reacts directly with diacetyl monoxime under strong acidic conditions to give a yellow condensation product. The reaction is intensified by the presence of ferric ions and thiosemicarbazide. The intense red color formed is measured at 540 nm/yellow-green filter.

Estimation of serum creatinine

The serum creatinine was measured by the Jaffe's method (Slot *et al*., 1965).[11]

Creatinine present in serum or plasma directly reacts with alkaline picrate resulting in the formation of a red color, the intensity of which is measured at 505 nm/green filter. Protein interference is eliminated using sodium lauryl sulfate. A second absorbance reading after acidifying with 30% acetic acid corrects for non-specific chromogens in the samples.

Estimation of bilirubin in serum

Serum bilirubin levels were estimated by the method of Dangerfield *et al*., 1953.[12]

Bilirubin estimation in serum is based on the van den Bergh reaction in which bilirubin couples with diazotized sulfanilic acid to give red "azobilirubin" which absorbs at 570 nm. Caffeine and a surfactant are used as reaction accelerators. The absorbance at 570 / 660 nm is proportional to the bilirubin concentration in the serum.

Induction of Obesity with High-fat Diet (HFD) and Supplementation of Plant Extract

HFD fed to the rats for inducing obesity and after getting obesity, plant extracts were orally administered to the rats to know the activity of plant against the obesity.

Animals and diets

Male Wistar albino rats, normal diet, and HFD were obtained from animals the Animal House of College of Veterinary Science and Animal Husbandry, Mhow, Madhya Pradesh, India. Normal diet contained pellet chow of standard composition containing all the recommended macro and micronutrients (56% carbohydrate, 18.5% protein, 8% fat, 12% fiber, and adequate levels of minerals and vitamins). HFD contained 29.5% beef tallow, 22.0% casein, 23.0% starch, 17.9% cellulose, 4.0% L-cystine, 0.3% choline chloride, 1.8% vitamin mixture (AIN-93 ViX), and 1.5% salt mixture [Table 1]. During the course of the experimental period (20 weeks), rats were fed with either normal diet or freshly prepared HFD (15 g/rat/day) as mentioned below and water *ad libitum*. Experimental animals were maintained under standard laboratory conditions (temperature: 22 ± 2°C; humidity: 40–60%). Rats initially weighing 180–220 g were randomly divided into six groups of six each ($n = 6$). After induction of obesity, to test the activity of MELPR, rats were treated with different doses of MELPR (200 or 300 mg kg/b.wt), suspended

Table 1: Composition of high-fat diet

Ingredients	Weight in %
Beef tallow	29.5%
Casein	22.0%
Starch	23.0%
Cellulose	17.9%
L-cystine	4.0%
Choline chloride	0.3%
Vitamin mixture (AIN-93 Vix)	1.8%
Salt mixture	1.5%

in 0.5% carboxymethylcellulose (CMC), for 42 days (15th–20th weeks), using an intragastric tube.

Experimental design

Group 1: Normal diet control (normal diet).
Group 2: HFD control.
Group 3: HFD + Orlistat (5 mg kg^{-1} b. wt.).
Group 4: HFD + MELPR (200 mg kg^{-1} b. wt.).
Group 5: HFD + MELPR (300 mg kg^{-1} b. wt.).

Determination of body weight (b.w.) and food intake

Throughout the experimental period, the weight gain of rats was monitored weekly and the food intake was monitored every 2nd day. The average weight of the animals in a group is represented in the results. Initial weight means weight of the animals at the end of 14th week and final weight means, the weight of the animals at the end of 20 weeks. Total amount of food consumed by a rat was measured as follows:

Consumption of feed = Total quantity of feed given to rat - left over feed.

Estimation of fasting blood glucose

Rats were fasted overnight and blood was drawn by retro-orbital puncture method. Blood glucose levels were measured by dextrostix (glucose oxidase peroxidase method) with a basic touch Accu-Chek glucometer (Johnson *et al*., 1998).[13]

Oral glucose tolerance test (OGTT)

To test glucose tolerance, OGTT was performed. At the end of the experiment, after overnight fasting, glucose was administered orogastically at a dose of 2.0 g/kg b.wt to rats and blood samples were collected from supra orbital sinus at 0, 30, 60, and 120 min and glucose level was estimated.

Estimation of leptin and adiponectin

Plasma leptin and adiponectin levels were measured using enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, IL, USA), performed in duplicate, as per the manufacturer's guidelines and were expressed in ng/ml.

Assay of PL and amylase activity

PL and amylase activities were determined by kinetic method using commercial kits (Bioclin, Minas Gerais, Brazil) following the guidelines of the manufacturer.

Estimation of plasma lipid profiles

Estimation of lipid profiles was placed major role in obesity condition. Usually, in obese condition, the levels of lipids were higher than normal. So that, to know the activity of plant extract, lipid profiles were studied.

Estimation of cholesterol

Estimation of cholesterol was carried out by the method of Zlatkis *et al*., 1953.[14]

Estimation of triglycerides (TG)

Plasma TGs were measured by the method of Foster *et al*., 1973.[15]

Estimation of HDL cholesterol (HDL-C)

Determination of plasma HDL-C was carried out by the method of Burstein *et al*. (1970).[16]

Estimation of VLDL and LDL Cholesterol (VLDL-C AND LDL-C)

Using Friedewald formula, the concentration of VLDL and LDL cholesterol in serum was calculated.

$$\frac{\text{VLDL} - \text{C}}{\text{LDL} - \text{C}} = \frac{\text{Triglycerides}}{(\text{Total Cholesterol} - \text{VLDL} - \text{C}) - (\text{HDL} - \text{C})}$$

Fecal Lipid Extraction and Estimation

Fecal matter was collected from the experimental rats at the end of 14th week and at the end of the 20th week, dried, and powdered for further analysis. Fecal lipids were extracted with chloroform and methanol (2:1 v/v), then dissolved in 1% triton ×100, and estimated by the method of Folch *et al*. (1957).[17]

Organs and adipose tissue (fat pad) weights

At the end of the experimental period, animals were anesthetized with isoflurane and sacrificed. Organs such as liver, spleen, kidney, testis, and adipose tissue (fat pads) from each rat were surgically removed, wet weights were measured with experimental electrical balance (Shimadzu) and stored at −80°C for further studies.

Estimation of liver lipid profile

Liver tissues were washed in ice-cold buffer NaCl (0.9%), blotted on absorbent paper, and weighed. Hepatic lipids were extracted from 1 g liver with chloroform and methanol (2:1 v/v) according to the procedure of Folch *et al*., 1957.[17] Hepatic

cholesterol, TGs, and free fatty acids (FFAs) were estimated by commercially available kits (Gupta Diagnostic, Bhopal).

Catalase (CAT)

CAT activity was measured by a modified method of Aebi (1984). Hydrogen peroxide (H_2O_2) decomposition by CAT enzyme was measured spectrophotometrically at 240 nm. The molar extinction coefficient of 0.043 mM/cm was used to determine CAT activity. One unit of enzyme activity is equal to the micromole of H_2O_2 degraded per minute per milligram of protein (min/mg).

Lipid peroxidation

Lipid peroxidation was estimated by modified method of Buege *et al*., 1978. Briefly, the malondialdehyde (MDA) levels were estimated by measuring thiobarbituric acid reactive substances (TBARSs) and expressed in terms of MDA content. Before the assay, liver tissues were washed in ice-cold buffer NaCl 0.9%, blotted on absorbent paper, and weighed. Each sample was minced in a Tri-HCl buffer (pH 7.4) and homogenized. After centrifugation at 3000 g for 10 min at 4°C, the clear homogenate was used for biochemical assay. One hundred and twenty-five microliters of 20% trichloroacetic acid containing 1% butylhydroxytoluene and centrifuged (1000 g, 10 min, 4°C). Then, 200 µl of supernatant (S2) was mixed with 40 µl of HCl (0.6 M) and 160 µl of Tris-thiobarbituric acid (120 mM) and the mixture was heated at 80–85°C for 10–12 min. The absorbance was measured spectrophotometrically at 530 nm. The MDA levels were expressed as µmol/L/mg protein/mg tissue.

Histopathological Examination of Adipose and Liver Tissue

Adipose and liver tissues from all groups of rats were removed and kept in 10% formalin buffer solution. A small piece of tissue was sectioned with microtome, fixed on slides, and stained using hematoxylin and eosin (H and E) staining procedures and observed under optical microscope [Figure 1].

Statistical Analysis

All the results were expressed as the mean ± S.D for six animals in each group. All the grouped data were statistically evaluated with SPSS/10.0 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. Significance level at $P < 0.05$ and 0.001 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Effect on Lipase Activity

Hexane, ethyl acetate, ethanol, and aqueous extracts of *L. parviflora* Roxb. leaves were tested for PL inhibitory activity. Hexane extract did not inhibit the PL whereas ethyl acetate showed 19%, methanolic extract showed 75%, and aqueous extract exhibits 12% PL inhibitory active [Table 2].

Toxicity Studies

Skin and behavioral analysis

In the present study toxic effects of *L. parviflora* Roxb. leaves extract observed on rats and found that even at a dose of 3000 mg/kg b.wt, no toxic symptoms or mortality rate in treated group. All the animals in treated group were alive up to 14 days after administration of the plant extract. The behavioural changes were observed from the 1st day to 14th day in control and treated groups. Both the groups were normal and no changes were observed in behaviour, sleep, eyes, salivation, diarrhea like problems, skin, hair loss, lethargy, food consumption, and water intake. Moreover, b.w. was also very similar in NC and test group [Table 3].

Analysis of Hematological Parameters

Blood samples were collected from all animals on the 15th day and hematological parameters were observed. Hemoglobin concentration was increased in treated group compared to control group. No significant variations were observed in remaining parameters between treated and NC groups [Table 4].

Liver and Renal Function Tests

Biochemical parameters such as SGOT, SGPT and ALP, total proteins, albumins, total bilirubin, serum creatinine,

Table 2: Effect on lipase activity

Plant name/ drug	Family	Solvent extract	Inhibition (%)
Lagerstroemia parviflora Roxb. leaves	Leguminosae	Hexane	0.00
		Ethyl acetate	19.02
		Ethanolic	75.10
		Aqueous	12.04
Orlistat	-	-	89.14

Table 3: General appearance and behavioral observations in control and treated groups

Observation	Control group	Treated group
Behaviors	Normal	Normal
Skin and fur	Normal	Normal
Sleep	Normal	Normal
Eyes	Normal	Normal
Salivation	Normal	Normal
Diarrhea	Normal	Normal
Lethargy	Normal	Normal

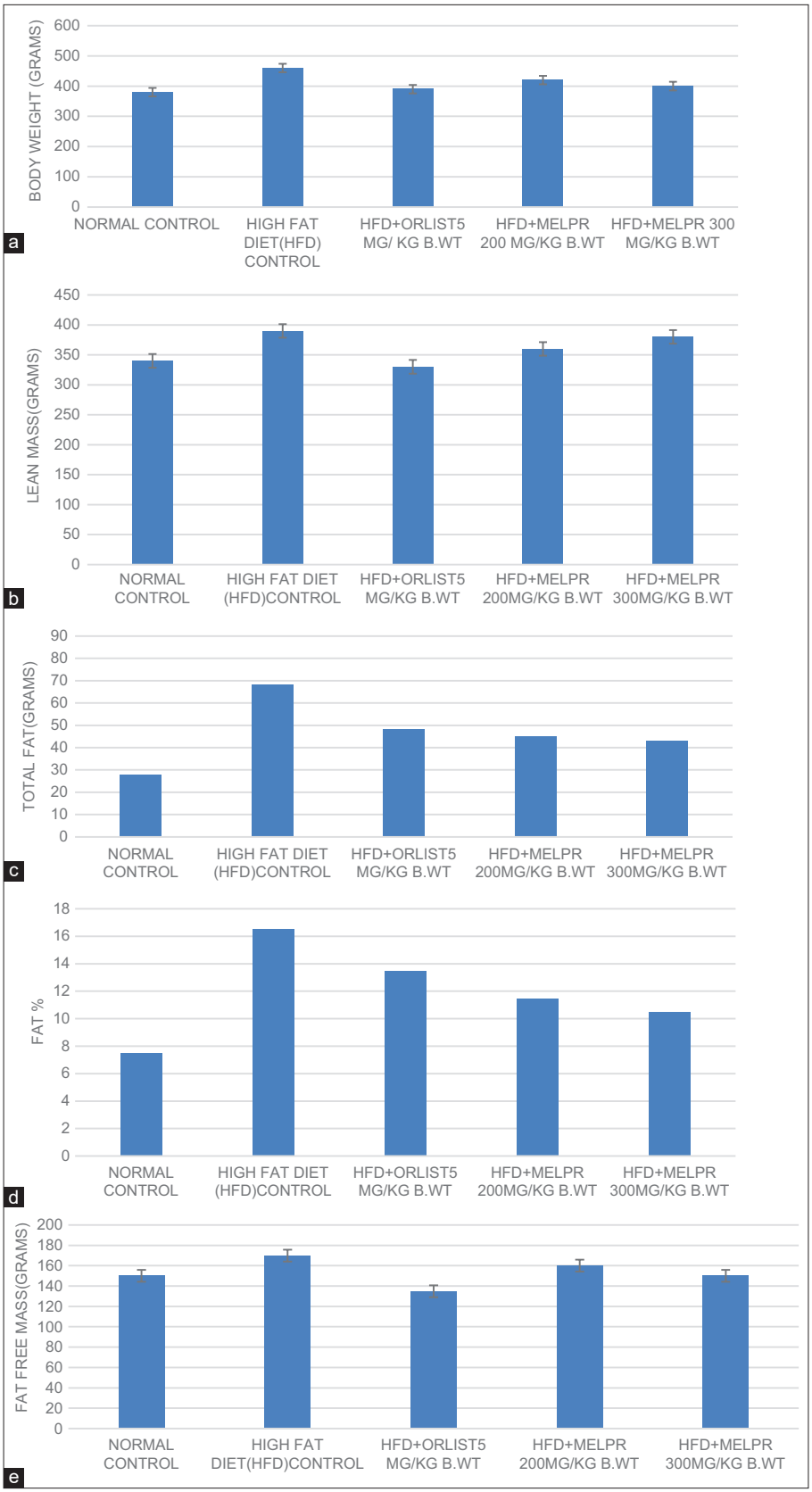

Figure 1: (a-e) Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on body composition of different groups of experimental rats. Values are statistically significant at *P<0.05. a* Significantly different from normal control. b* Significantly different from high-fat diet control

and urea levels were studied in control and treated groups. Biochemical parameters such as SGOT, SGPT and ALP, total proteins, albumins, total bilirubin, serum creatinine, and urea levels were studied in control and treated groups.

Like creatinine and urea levels were found to be little lowering in treated groups compared to control group, whereas total proteins, albumin levels, and total bilirubin were almost same in both the groups. Administration of MELPR had showed effective antioxidant and protective character, which is supported by the improvements of biochemical parameters [Table 5].

Results are expressed as mean ± SD. The statistical analysis was carried out using one-way analysis (ANOVA) [Table 6].

Effect of MELPR on Body Composition and Food Intake

The changes in body composition and food consumption of experimental rats was studied and found that the consumption of HFD for 20 weeks produced a substantial increase in b.w. (468 ± 8.3 g), total fat (75.9 ± 9.4 g), fat % (16.1 ± 3.8), and fat-free mass (173.1 ± 2.8 g) in HFD control group when compared to NC group of rats whose b.w., total fat, fat %, and fat free mass were 367 ± 11.6 g, 26.9 ± 4.8 g, 7.3 ± 1.2%, and 153.4 ± 6.6 g, respectively. Oral administration of MELPR (200 and 300 mg kg b.wt) for 42 days (from 15th to 20th weeks) considerably reduced b.w. and body composition in a dose-dependent manner. Among the three doses administered, MELPR at a dose of 300 mg kg/b.wt showed significant ($P < 0.05$) therapeutic effect. At 300 mg kg/b.wt of MELPR, the b.w., total fat, fat %, and fat free mass were 387.1 ± 6.7 g, 40.2 ± 3.3 g, 10.97 ± 2.6%, and 155 ± 4.9 g, respectively.

Effect of MELMW on Plasma Lipid Profile

The levels of total cholesterol (TC), FFAs, TGs, phospholipids (PLs), HDL, LDL, and VLDL were measured in plasma and are depicted. The concentrations of TC, FFAs, TGs, PLs, LDL, and VLDL were markedly elevated while HDL level decreased in experimental obese rats when compared to NC rats. Oral administration of MELPR has reversed these alterations in a dose-dependent manner, the most profound effect being noted at a dose of 300 mg kg^{-1} b.wt.

Effect of MELPR on Liver Lipid Profile

Liver tissue lipid profiles (TC, FFAs, TGs, and PLs) of control and HFD-induced obese rats was studied and the Supplementation of HFD led to profound increase in tissue levels of TC, FFAs, TGs, and PLs. Treatment with MELPR (300 mg kg−1 b.wt) or orlistat significantly (P < 0.05) reduced liver tissue lipids in obese rats [Table 7 and Figures 1-3].

(a) Body weight
(b) Lean mass
(c) Total fat
(d) Fat percentage
(e) Fat-free mass

Effect of MELPR on OGTT

The levels of plasma glucose, insulin and insulin resistance in control and experimental obese rats was studied and it was observed that there was a significant ($P < 0.05$) elevation in plasma glucose, insulin, and insulin resistance in HFD control

Table 4: Oral administration of MELPR on hematological changes in rats

Observation	Control group	Treated group
Hemoglobin (g/dL)	12.7±0.8	14.5±0.3
Total WBC (cells/cumm)	1185±0.4	12240±0.52
RBC (106/mm^3)	6.7±0.42	6.2±0.22
Platelet count (%)	3.8±0.5	4.2±0.42
Neutrophils (%)	20.2±2.2	23.4±5.8
Lymphocytes (%)	78±4.2	68.5±6.2
Monocytes (%)	2.2±0.5	1.5±0.2
Eosinophils (%)	3.8±0.82	2.9±0.72
HCT (%)	39.6±1.6	42.1±0.64
MCV (%)	56.8±2.2	54.6±0.43
MCH (%)	19.8±1.2	19.3±0.22
MCHC (%)	36.8±1.6	36.2±0.32
MPV (%)	9.2±0.82	8.2±0.61
PCT (%)	0.41±0.06	0.41±0.05

Results are expressed as mean±SD. The statistical analysis was carried out using one-way analysis (ANOVA). MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb, WBC: White blood cells, RBC: Red blood cells, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MPV: Mean platelet volume, PCT: Plateletcrit

Table 5: Effect of MELPR on liver function tests

Observation	Control group	Treated group
SGOT (U/L)	22.6±4.2	19.6±4.3
SGPT (U/L)	34.2±1.63	25.6±4.2
ALP (U/L)	250.6±4.65	215±4.6
Total proteins (g/dL)	9.2±0.48	7.8±0.44
Albumins (g/dL)	5±0.32	4.8±0.32
Total bilirubin (mg/dL)	0.92±0.12	0.98±0.89

MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb., SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, SGOT: Serum glutamate oxaloacetate transaminase

Table 6: Effect of MELPR on renal function tests

Observation	Control group	Treated group
Serum creatinine. (mg/dL)	0.96±0.05	0.94±0.05
Urea (mg/dL)	46.6±4.82	43.6±4.8

Results are expressed as mean±SD. The statistical analysis was carried out using one-way analysis (ANOVA). MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb.

obese rats when compared to NC rats. Oral administration of different doses of MELPR could bring these changes to near normalcy in a dose-dependent manner, the most significant effect being observed at 200 mg/kg b.wt [Table 8].

Effect of MELPR on OGTT

In the NC group of rats, blood glucose level reached its maximum value at 60 min after glucose load and declined to near basal level at 120 min, whereas, in HFDinduced obese rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Administration of MELPR (300 mg/kg b wt.) or orlistat to obese rats elicited a significant decrease in blood AQ4 AQ4 glucose level at 60 min and beyond when compared with HFD control rats [Figure 4].

Leptin and Adiponectin Levels

Leptin and adiponectin are two major adipocytokines of adipose tissue. The levels two major adipocytokines of adipose tissue i.e. leptin and adiponectin in control and experimental obese rats was observed and a marked elevation in leptin and decrease in adiponectin levels in HFD-fed obese rats over their NC rats found. Interestingly, treatment with MELPR (300 mg/kg b.wt) or orlistat has significantly ($P < 0.05$) restored their levels to normalcy [Figure 5].

Assay of Amylase and Lipase

The activities of amylase and PL of normal and experimental obese rats studied and 2-fold increase in the activities of lipase and amylase in HFD-fed control rats was observed when compared to NC group of rats. Administration of MELPR has brought down their activities in a dose-dependent manner. A significant ($P < 0.05$) reduction in their activities was noted at 300 mg kg/b.wt of MELPR [Figure 6].

Activity of Liver Antioxidant Enzymes

To determine the *in vivo* antioxidant activity, the levels of superoxide dismutase (SOD), CAT, and MDA content were

Table 7: Effect of MELPR on body weight, food intake, and adipose tissue weight in rats fed with high-fat diet

Physical parameters	NC	HFD	Orlistat	MELPR (200) mg/kg b.wt	MELPR (300) mg/kg b.wt
Initial body weight	200±2.26	180±3.24	184±3.24	191±2.36	188±2.16
Final body weight	367±11.6	468±8.3	378±2.44*	410±1.48	387.1±6.7*
Weight gain	167±9.34	288±5.06	194±0.8*	219±0.88	199.1±4.54*
Food intake	121±1.1	14.2±1.3	13.8±1.3*	13.9±0.1	14.2±0.2*
Retroperitoneal fat (g)	2.2±0.4	4.4±0.6	2.6±0.3*	3.4±0.5	4.1±0.2*
Epididymal fat (g)	1.4±0.3	3.1±0.7	1.7±0.9*	2.4±0.1	1.4±0.2*

The data are given as mean±S.D (n=6). *P<0.05 compared with HFD control group. NC: Normal control group, high-fat diet group, MELPR: Methanolic extract of *Lagerstroemia Parviflora* Roxb. leaves (200 and 300 mg/kg/day).

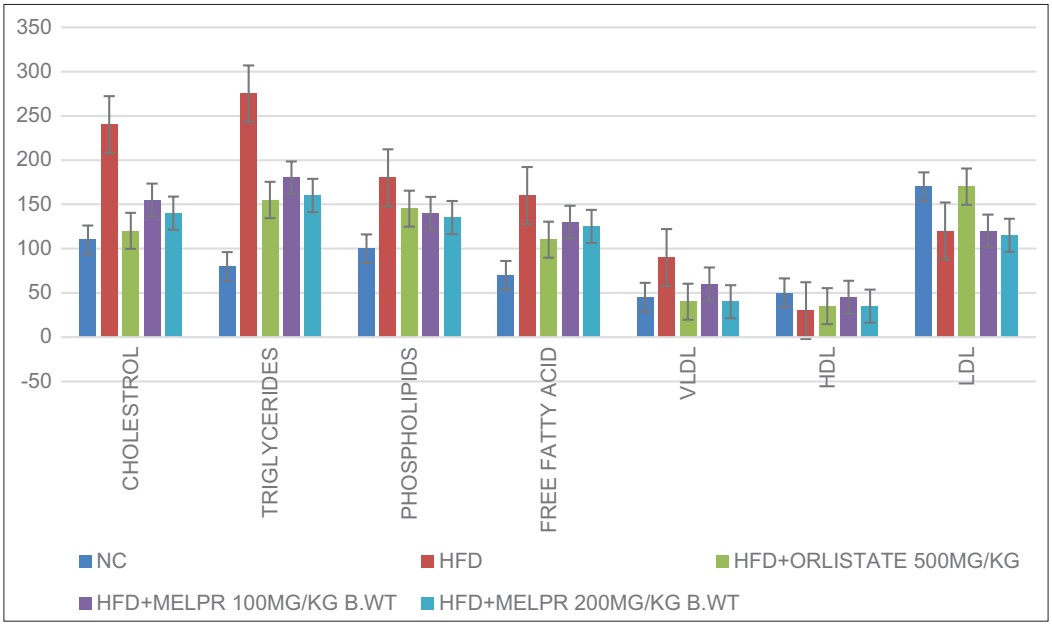

Figure 2: Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on plasma lipid profiles in normal and experimental obese rats

estimated from the liver tissue. There was a substantial decrease in the activity of SOD and CAT but raise in MDA content in HFD-fed groups. However, MELPR (200 and 300 mg/kg b.wt) administration has significantly reversed these alterations in SOD, CAT, and MDA levels.

At a dose of 30 mg/kg, b.wt. MELPR increased the activity of SOD and CAT by 66% and 46%, respectively, but decreased MDA content by 30% [Figure 7].

Effect of MELPR on Fecal Lipid Content

Fecal mat was collected from the rectum and wet weight of the feces was measured. Decreased fecal matter weights were noticed in HFD + MELPR administered groups when compared to HFD group. Further, the extracted lipids from dry feces were analyzed to find the metabolic fat of unabsorbed TG. Increased excretion of fecal TG was observed in orlistat and MELPR treated HFD groups, indicating that MELPR might interfere in lipid absorption and transportation.

Effect MELMW on Fat Pads and Adipose Tissue

Adipose tissue is a dynamic organ the mass of which changes during lifetime in response to metabolic requirements of the animal, and thus, plays an important role in energy balance.

Extensive morphological changes in fat pad deposition were observed in retroperitoneal and epididymal tissues among NC, HFD, and MELPR treated groups. The weight of retroperitoneal, epididymal adipose tissues was markedly increased in HFD-fed group. However, administration

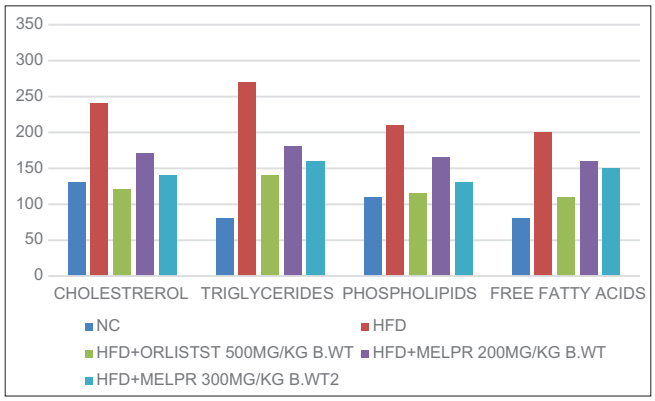

Figure 3: Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on liver tissue lipid profiles in normal and experimental obese rats. Values are mean ± S.D, *n* = 6. Values are statistically significant at **P* <0.05. a* Significantly different from normal control. b* Significantly different from high-fat diet control

Table 8: Effect of MELPR on plasma glucose, insulin, and insulin resistance in normal and experimental obese rats

Groups	Glucose (mg.dl^{-1})	Insulin (μU.ml^{-1})	Insulin resistance
Control	81.9±4.3	5.5±0.7	3.3±0.07
HFD control	160.9±12.2a***	14.8±1.5a*	5.1±1.6a*
HFD+Orlistat	128.5±13.1b***	7.8±1.2b*	3.1±0.8b*
HFD+MELPR (200 mg.kg^{-1} bw)	132.1±2.6b**	9.5±0.8b*	3.8±1.2b*
HFD+MELPR (300 mg.kg^{-1} bw)	129.7±15.3b**	8.1±0.7b*	3.2±0.6b*

Values mean±S.D, *n*=6. Values are statistically significant at **P*<0.05. a* Significantly different from normal control. b* Significantly different from HFD control, HFD: High-fat diet, MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb

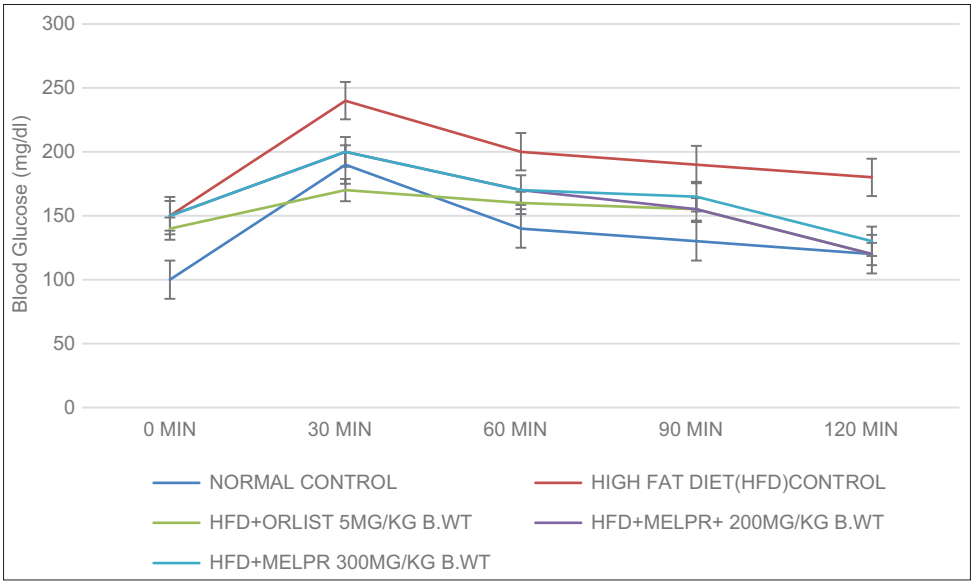

Figure 4: Effect of *Lagerstroemia parviflora* Roxb. on glucose tolerance in control and experimental obese rats. Values are mean ± S.D., *n* = 6. Values are statistically significant at **P* <0.05. a* Significantly different from normal control; b* Significantly different from high-fat diet control.

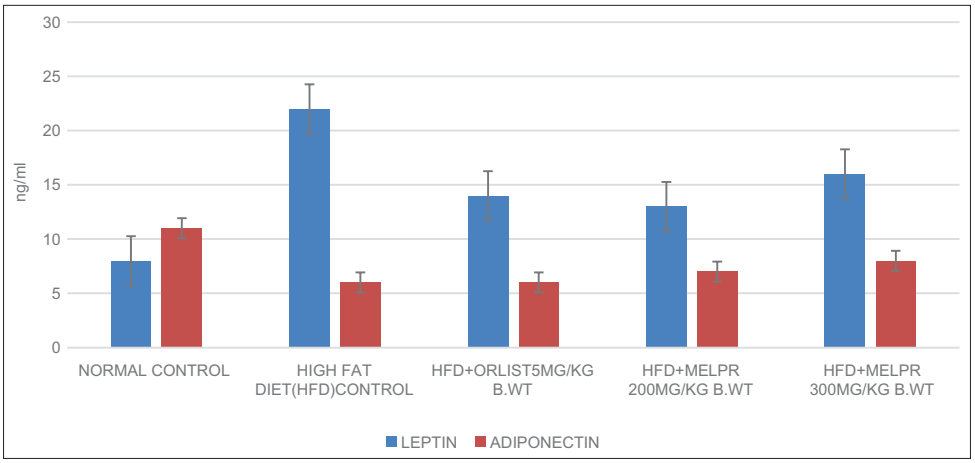

Figure 5: Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on leptin and adiponectin levels. Values are mean ± S.D, $n = 6$. Values are statistically significant at $P < 0.05$. a Significantly different from normal control. b Significantly different from high-fat diet control

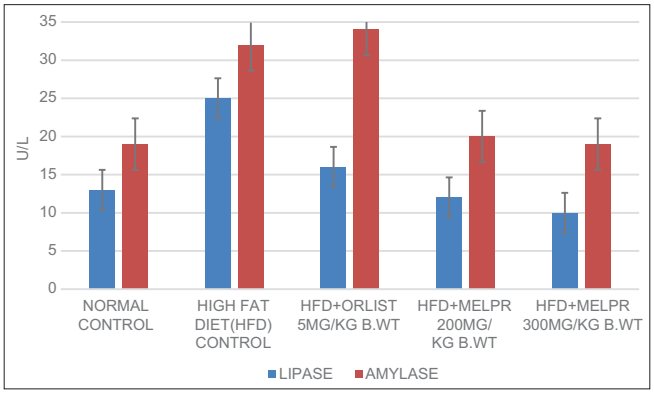

Figure 6: Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on lipase and amylase activities in experimental rats. Values are mean ± S.D, $n = 6$. Values are statistically significant at $^*P < 0.05$. a* Significantly different from normal control. b* Significantly different from high-fat diet control

of MELPR reduced in a dose-dependent manner the accumulation of fat pads and adipose tissue weight [Table 9].

Effect of MELPR on Organ Weights

After the end of the experimental period, rats were anesthetized with isoflurane and sacrificed, weighed all organs such as liver, kidney, spleen, and testis [Figure 8]. The liver and kidney weights of HFD-fed rats increased considerably, which were significantly ($P < 0.05$), and dose dependently reduced by MELPR treatment (200 and 300 mg/kg b.wt), whereas spleen and testis of MELPR treated groups did not show any significant variation compared to HFD control.

Picture showing dissection of rat

Table 10 shows the effect of MELPR on organ weights.

Histopathology Studies

To further confirm the antiobesity activity of MELPR on liver and adipose tissue, we carried out histopathology studies to know the deposition of fat, droplets, and architecture of tissue.

Liver histopathology examination

The hematoxylin and eosin stained liver microtome sections were observed under microscope. HFD-fed control group rats showed higher accumulation of lipid droplets, loss of nucleus, inflammatory cells, and severe swelling of hepatocytes indicating steatosis. However, MELPR treated groups showed, decreased lipid accumulation, lesser damage, and near-normal hepatocytes [Figure 8].

Adipose tissue histopathology

To evaluate the active role of MELPR, we have examined the hematoxylin and eosin stained adipose tissue microtome sections under light microscope at ×10. The size of adipocytes enlarged considerably and more fat deposits were noticed in HFD-fed groups when compared to NC group.

Administration of MELPR (200 and 300 mg/Kg b.w.) has substantially reduced the size of adipocytes and normalized the architecture of adipocytes in a dose-dependent manner [Figure 9].

DISCUSSION

Overweight and obesity are commonly neglected health issues. In recent decades, obesity has reached to high levels in both the developed and developing world. The present work was aimed to evaluate the antiobesity activity of MELPR (leaves). MELPR showed more phytochemicals with highest DPPH scavenging activity and ABTS inhibitory activity.

In an acute toxicity study, MELPR extract was orally administered (up to 3000 mg/kg b.w.) to rats and observed for 72 h for any toxic symptoms. No behavioral changes and toxic symptoms or mortality rates were observed in treated groups after either within 72 h or during 14 days, and it proved that

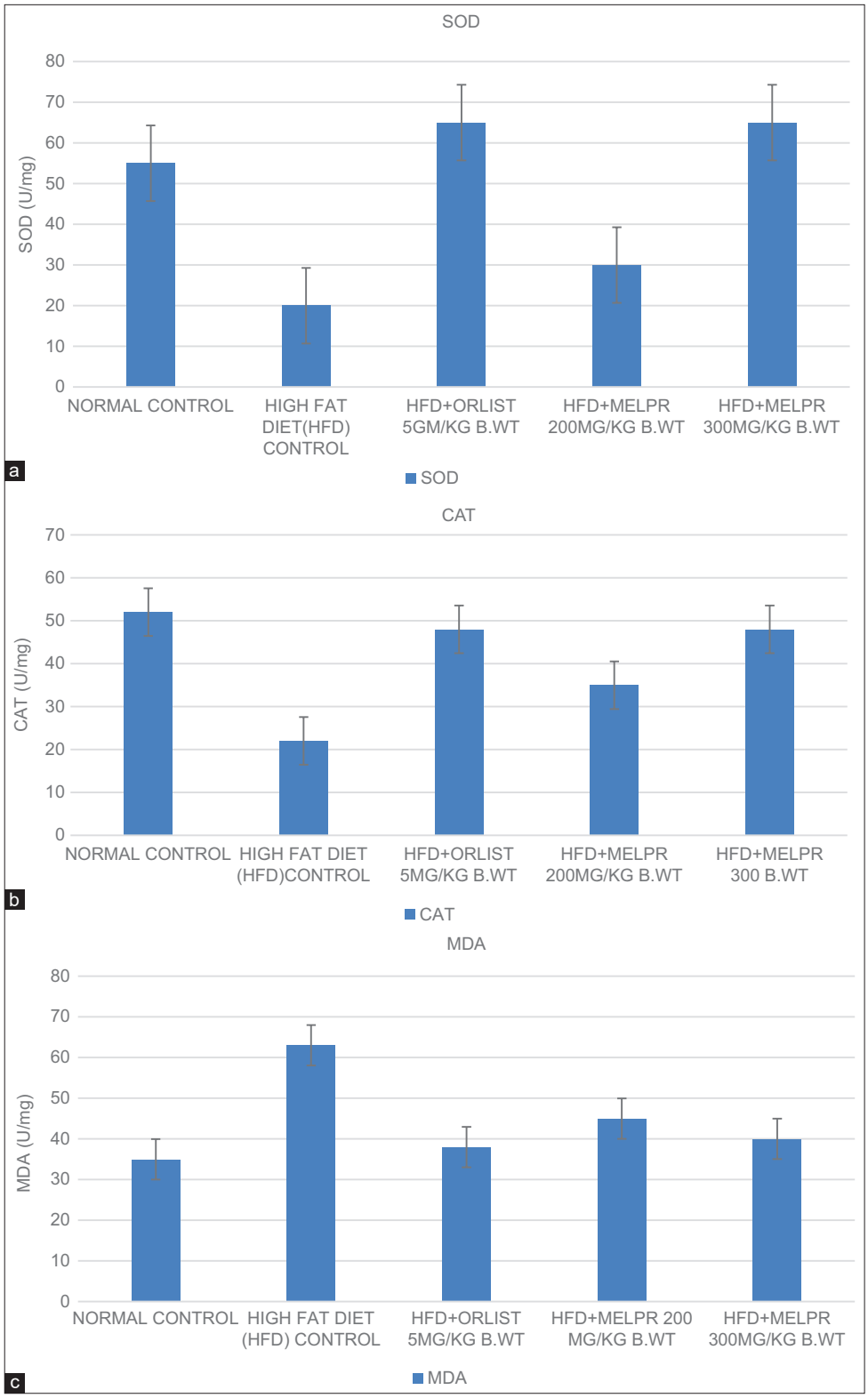

Figure 7: (a-c) Effect of methanolic extract of *Lagerstroemia parviflora* Roxb. on liver superoxide dismutase, catalase activities, and malondialdehyde content in control and high-fat diet–fed rats

the MELPR (leaves) is safer and non-toxic. On the 15th day, rats were sacrificed and blood samples were collected from control and test animals and analyzed for some biochemical parameters. We did not observe any behavioral changes in test groups in comparison with their controls and also there were no significant alterations in biochemical, hematological levels and liver function parameters between MELPR treated and NC groups.

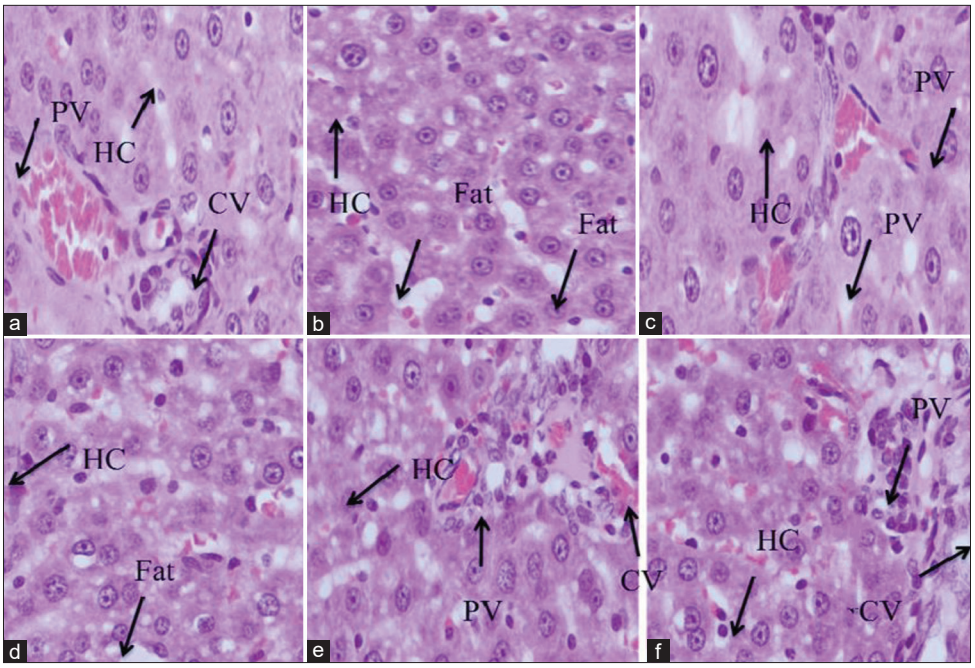

Figure 8: Liver histopathology. (a) NC: Normal control group, (b) HFD: High-fat diet, (c) Orlistat, (d) MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb. 200 mg/kg/b.wt, (f) 300 mg/kg/b.wt

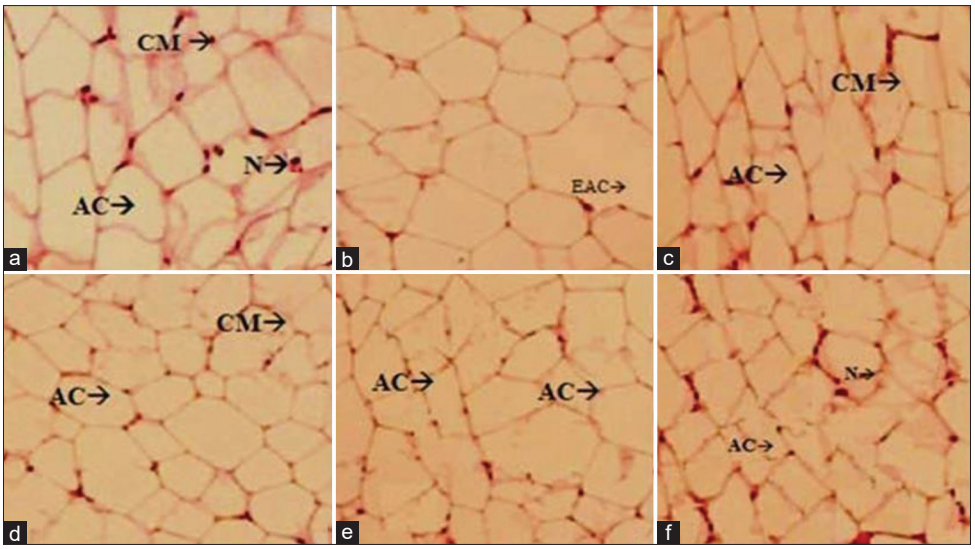

Figure 9: Histopathology slides of adipose tissue. (a) NC: Normal control group, (b) HFD: High-fat diet, (c) Orlistat, (d) MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb. 200 mg/kg/b.wt, (f) 300 mg/kg/(b.wt) CM-Cytoplasmic Membrane, N-Nucleus, AC-Adipose cell, EAC- Enlarged adipose cell

Table 9: Effect of MELPR on fecal lipids of normal and HFD-fed obese rats

Parameters	NC	HFD	Orlistat	MELMW (200)	MELMW (300)
Initial weight	1.2±0.02	1.8±0.02	1.8±0.02	1.8±0.4	1.8±1.30
Final weight	1.8±0.34	2.4±0.021	1.2±0.52	1.4±0.022	1.3±0.9
Initial level	11.0±3.25	16.0±1.32	16.5±1.2	17.4±0.51	17.0±0.02
Final level	10.8±0.18	16.±1.3	19.4±0.2	18.2±2	6.8±1.6

NC: Normal control, HFD: High-fat diet

Our results demonstrate that the MELPR has potent antioxidant activity and it was safer and non-toxic to rats and hence could be well considered for further investigations for its medicinal and therapeutic efficacy. For studying antiobesity

Table 10: Effect of MELPR on organ weights

Groups	Liver	Kidney	Spleen	Testis
NC	9.5±0.8	1.9±0.1	0.4±0.01	2.9±0.1
HFD	13.6±0.7	4±0.2	0.6±0.02	1.7±0.2
ORL	10.3±0.2	1.9±0.3	0.4±0.03	2.7±0.3
MELPR (200)	12.2±0.3	2.2±0.1	0.5±0.01	2.2±0.4
MELPR (300)	11.1±0.2	2.0±0.2	0.4±0.02	2.6±0.3

NC: Normal control, HFD: High-fat diet, MELPR: Methanolic extract of *Lagerstroemia parviflora* Roxb, ORL: Orlistat

activities, rats were fed with high-calorie diet (HCD) and the MELPR was administered. HCD has substantially altered physiological and biochemical aspects. Administration of MELPR reduced significantly ($P < 0.05$), b.w., total fat, fat percentage, blood glucose, insulin resistance, lipid profile, and regulated liver antioxidant enzymes in HCD-fed groups in a dose-dependent manner (200 and 300 mg/kg b.w.).

The elevation of fecal lipids clearly indicates the active role of MELPR on lipase inhibitory activity. HCD significantly increased liver TC, TG, FFA, and MDA but decreased the activities of SOD and CAT which were subsequently reversed by the administration of MELPR in a dose-dependent manner and most profound activity was shown by MELPR at a dose of 300 mg/kg b.w. Similarly, HCD-induced alterations in plasma lipid profiles were also alleviated by MELPR administration. Furthermore, histopathological examination of adipose tissue revealed that HCD caused enlargement of adipocyte with more fat drops. Administration of MELPR along with HCD resulted in reduced size of adipocytes. Histopathological examinations of liver sections have clearly demonstrated the appearance of normal hepatocytes with reduced lipid droplets in HCD+ MELPR treated groups.

CONCLUSION

Based on acute toxicity studies, we present that MELPR is non-toxic and safe up to 3000 mg/kg b.w. in rats. Treatment with MELPR has dose dependently and significantly alleviated HCD-induced obesity, hyperlipidemia, as supported by physiological, biochemical, histological, and molecular studies. This study demonstrates the antihyperlipidemic and antiobesity potential of MELPR and offers scientific validation and basis to develop antiobesity drugs.

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