

Antioxidant and hypercholesterolemia effects of *Lagerstroemia speciosa* ethanolic green and red leaf extracts against Lauric acid and ketogenic diets in female albino rats

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Abstract

The study investigates the therapeutic value of *Lagerstroemia speciosa* ethanolic green and red leaf extracts against Lauric acid (LA) and a Ketogenic diet (KGD) in Albino rats. Lauric acid is an important constituent in breast milk, forming nutritional factors and having various medicinal values. It is present in 6.2% of human breast milk, 3.1% of goat milk, and 2.9% in cow milk. It is also found in plant sources like coconut, cohune, and palm kernel oil. The Ketogenic diet uses fat, protein, and low carbohydrate for energy. The experimental groups were fed a Ketogenic diet for 21 days, with the treated groups showing a significant increase in blood cell count and Hg levels. The phytochemical profile reveals abundant phytoconstituents, including corosolic acid, lagerstroemin, and anthocyanin, at high levels. The study compared experimental groups fed with LELE Low Dose and High Dose (250 and 500 mg/kg body weight) and standard groups treated with KGD and Lauric acid. The treated groups showed significant elevations in biochemical parameters and increased blood cells and Hg levels. LELE's anti-hypercholesterolemia activity in KGD significantly reduced body weight, total lipid profile, and blood glucose in a dose-dependent manner. The study found that LELE is safe and non-toxic, with the treated group demonstrating dose dependence and decreased obesity.

Keywords: Obesity; hypercholesterolemia; *Lagerstroemia speciosa* Ethanolic Green Leaf Extract (LEGLE); *Lagerstroemia speciosa* Ethanolic Red Leaf Extract (LERLE); Lauric Acid (LA); and Ketogenic Diet (KGD).

1. Introduction

Hypercholesterolemia is global disease-causing hypertension and type 2 diabetes mellitus. It began in the 1990s due to processed foods high in fat and sugar, particularly fructose [1]. Obesity is exacerbated by reduced physical activity and sedentary behaviour [2]. Excessive fat accumulation in specialized fat cells leads to various illnesses, including diabetes, heart disease, osteoarthritis, and cancer, with a rapidly increasing number [3]. High-fat diets with lard, or saturated oil, added to diet takes 3 weeks to develop obesity. The most commonly used model KGD contains 32.6% Protein, 33% Fat, 30% carbohydrate, normal chow, lard, casein, cholesterol, vitamins, minerals, yeast powder, methionine, and NaCl [4].

The ketogenic diet is a high-fat, low-carbohydrate diet that meets daily caloric needs, with 10% of 2,000 kcal diet meeting carbohydrates [5]. It improves fat oxidative metabolism, decreases body weight, and aids appetite control. However, understanding the physiological mechanisms is challenging, and Hans Krebs developed the term "physiological ketosis" to describe the metabolic condition [6].

Animal studies have shown that various herbs, including fenugreek, cayenne pepper, ginger, oregano, and ginseng, aid in weight loss and fat reduction [7]. These herbs reduce appetite, increase metabolism, and alter fat synthesis while also

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stimulating weight loss and modifying fat formation [8]. Natural antioxidants gain popularity as complementary and alternative drugs due to less side effects compared to synthetic drugs in the cosmetic, pharmaceutical, and food industries [9].

Lagerstroemia speciosa, a native to Southeast Asia, has medicinal and horticultural properties, with bioactive phytochemicals showing hypoglycemic, antibacterial, anti-inflammatory, antioxidant, and hepatoprotective properties [10]. *L. speciosa* is popularly called as “Jarul” in West Bengal, India, and it belongs to the family Lythraceae. It is known as Pride of India, and is also called Queen’s Flowers or Queen Crape Myrtle in English. This plant is widely distributed in the Southeast Asian countries, Philippine and India [11]. The GC-MS analysis shows the presence of Phytocompounds.

Gymnemic acid (from *Gymnema sylvestri*), oleanolic acid (from *Panax ginseng*), and corosolic acid from *L. speciosa* have potential action on obesity [12]. The Corosolic acid was identified as an effective component of the Banaba extract responsible for the anti-obesity activity [13]. The objective of the study is to evaluate the anti-hypercholesterolemia potential of *L. speciosa* ethanolic green and red leaf extract in Albino rats induced with a Ketogenic diet (KD).

2. Materials and methods

2.1. Collection and Authentication of plant samples

The leaves of *L. speciosa* were collected from the PG Girls Hostel, Government Arts College (Autonomous), Coimbatore District, Tamil Nadu, India. The identification and authentication of *L. speciosa* are done by the Botanical Survey of India, Coimbatore, and the voucher specimens numbered BSI/SRC/5/23/2020/Tech/50 were placed in the Department of Zoology, Government Arts College (Autonomous), Coimbatore.

2.2. Plant extracts preparation

L. speciosa leaves were collected, washed, and sun-dried for 2 weeks. The leaves were ground to powder (100g) and soaked in ethanol (1000ml). The powder was solubilized and mixed well with intermittent stirring for 4 days. After that, the extract was filtered using Whatman No. 1 filter paper and kept in a plastic tray to dry at room temperature [14].

2.3. Quality Control Analysis

Qualitative phytochemical analysis of the green and red leaves of *L. speciosa* ethanolic extracts were carried out according to the methodology of Harborne [15], Trease and Evans [16]. The GC-MS analysis at The South Indian Textile Research Association in Coimbatore identified important compounds in *L. speciosa* ethanolic extracts of green and red leaves. The analysis used a Thermo GC-Trace Ultra ver. 5.0, Thermo MS DSQ 11 chromatography [17].

2.4. Phytochemical Assay

Phytochemical analysis involves the study of plant-derived compounds to identify and quantify various bioactive components.

2.4.1. Determination of Corosolic acid

It includes of plant material preparation. Extraction of corosolic acid, concentration and evaporation fractionation, identification of corosolic acid, data analysis, and reporting [18].

2.4.2. Determination of Lagerstroemin

A naturally occurring substance, also known as corosolic acid, is present in several plants, most notably *L. speciosa*. Chromatography on a thin layer (TLC): TLC is a simple and reliable technique for qualitative analysis. Its presence in the sample can be determined by comparing the migration distance of the sample with that of a standard Lagerstroemin [19].

2.4.3. Determination of Tannin

Various techniques can be used to determine the presence of tannins in a sample, depending on the kind of tannins and the goal of the analysis. Using high-performance liquid chromatography (HPLC), it is possible to separate and quantify the various tannin components in a sample. The identification and measurement of various tannin components are made possible by its great specificity and precision [20]. In order to synthesize 4,6-O-(S)-hexahydroxydiphenoyl gluconic acid (20 mg), fraction 1, which was previously obtained by Sephadex LH-20 Chromatography from the 70%

aqueous acetone extract of the dried leaves was chromatographed on Sephadex-20 ethanol and then MCI-gel CHP 20P with water containing an increasing amount of methanol. Lagerstanin C (8) (40 mg) and 7 (100 mg) were synthesized by repeated chromatography of fraction 23a on MCI-gel CHP 20P, Cosmosil 75 C18-OPN, Toyopearl HW-40F, and Sephadex LH-20 with water and methanol [21].

2.4.4. Determination of Anthocyanin

The vanillin-HCL method is specifically used to identify condensed tannins (proanthocyanins and anthocyanins). A complex that is pink to red in colour forms after the material reacts with the vanillin-HCL reagent [22]. An established standard curve with known tannin concentrations is used to calculate the complex's absorbance at a certain wavelength, and the tannin content is then calculated. The spectrophotometric approach is one of various techniques for determining anthocyanins, and it is frequently employed [23].

2.5. Feed Composition

Ketogenic diet (KGD) cocktail combines high fats, moderate proteins, and very low carbohydrates. The dietary macronutrients are divided into approximately 55% to 60% fat, 30% to 35% protein, and 5% to 10% carbohydrates. Specifically, in a 2000 kcal per day diet, carbohydrates amount up to 20 to 50 g per day [24 and 25].

2.6. Acute Oral Toxicity Studies

Behavioural alterations observed in experimental groups after oral administration of ethanolic leaf extract; toxicological analysis confirmed mortality, trial continued [26].

2.7. Experimental Groups

Animals were divided into three major groups: the control group fed with a normal diet, and the standard laboratory chow (STD) fed with KGD, and Lauric acid with KGD and the experimental groups fed with a ketogenic diet (KGD) along with leaf extract. The animals in the experimental group were fed an KGD containing 55% to 60% fat, 30% to 35% protein, and 5% to 10% carbohydrates for 3 weeks.

2.8. Experimental Design

Female Albino Wistar rats aged 10-12 weeks weighing between 200 and 250 g were used in this study. They were raised in the animal house of the Department of Pharmacy, KMCH, Coimbatore, Tamil Nadu. All the animals were placed in polypropylene cages at room temperature with a 12/12 h cycle (light/dark). They had free access to water and standard diet. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. This study was approved by the Institutional Animal Ethics Committee of KMCH College of Pharmacy in Coimbatore, Tamil Nadu (Approval No: KMCRET/ReRc/Ph. D/24/2021). The animal protocol was accomplished in accordance with the guidelines of Institutional Animal Ethics Committee of KMCH College of Pharmacy and Use of Laboratory Animals Manual (8th Edition). Forty-two (42) rats were randomly divided into 6 groups (n = 6) and treated orally daily for 21 days. Group I (normal control): Rats received normal diet and 10 mL/kg of distilled water; Group II (obesity standard): Rats received KGD and 10 mL/kg of distilled water; Group III (positive control): Rats received KGD and Lauric acid (10mL/ 100mg/kg); Groups IV, V, VI, and VII: Rats received KGD, and LELE's extracts (250 and 500 mg/kg) respectively. Body weight, Blood sample, food and water consumption of the animals were evaluated on the 1st, 21st and 42nd days of the experiment.

2.9. Biochemical Analysis

The rats were sacrificed, and their blood was collected for haematological studies. Blood was drawn from the medial cantus and placed in EDTA bottles for biochemical analysis. The serum was separated by centrifugation at 2500 rpm for 15 minutes at 37°C, and the LDL, and VLDL levels were determined [27]. Antioxidant enzymes, viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), and Lipid peroxidation (LPO), were determined in all the liver tissues of all the tested rats [28].

2.10. Histological Slides

Histological examinations of adipose tissues and kidney specimens were performed by the method described by Lillie and Fulman [29]. The tissue samples were embedded in paraffin wax after being fixed with 10% formalin. The paraffin-embedded tissues were sliced into sequential sections that were 5 micrometers thick. Using an Olympus BX51 light microscope, the tissues were stained with hematoxylin and eosin. Evaluations were made of the epithelization, necrosis, and ulcerations.

2.11. Statistical Analysis

Each group (n=6), each value represents Mean \pm SEM. One-way ANOVA, followed by Dunnett comparison was performed. (** $P < 0.001$) control group was compared with std group-II. (** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) treated groups III, IV, V, VI, and VII was compared with Group I. GraphPad Prism 8.02 (California, USA) was the statistical software used for the analysis of biodata obtained.

3. Results

3.1. Result: Analysis of Quality Control

Phytochemical analysis involves the study of plant-derived compounds to identify and quantify various bioactive components (Table 1). The qualitative and quantitative analysis shows the presences of Corosolic acid, Lagerstroemin, Tannin, and Anthocyanin.

3.2. Result: Acute Oral Toxicity

Acute toxicity testing with LS at a dose of 2000 mg/kg on rats revealed no toxicity, mortality, or morbidity, and there were also no noticeable alterations in behaviour or gait.

3.3. Result: Body Weight

The initial and final body weight differences between the control group and Group II were 7.38% and 41.02% at the end of the experiment after 42 days, respectively. The groups VI and V are compared with the positive control. The initial and final body weight differences between the LEGLE low dose and the positive control are 8.81% and 10.76%, respectively. The initial and final body weight differences between the LEGLE high dose and the positive control are 14.59% and 11.74%, respectively. The initial and final body weight differences between the LERLE low dose and the positive control are 26.10% and 11.13%, respectively. The initial and final body weight differences between the LERLE high dose and the positive control are 7.99% and 10.52%, respectively.

3.4. Result: Biochemical Analysis

The treatment of 250 and 500 mg/ 1ml/100 g BW ethanolic extracts of *L. speciosa* showed Group-VII is 143 \pm 3.54 and Group - V is 191 \pm 1.3 significant differences in LDL, VLDL in Group - VII is 47.8 \pm 3.14 and Group - V 49.8 \pm 3.78. The results of Lipid profile analysis are shown in the table 2.

3.5. Result: Antioxidant Activity

The control group is compared with group III positive control. The SOD (0.146 \pm 0.0100 and 0.1808 \pm 0.0046), CAT (0.3 \pm 0.0111 and 0.363 \pm 0.00857), GPx (0.039 \pm 0.006 and 0.075 \pm 0.0049), GSH (0.18 \pm 0.0052 and 0.12 \pm 0.021), The LPO (0.076 \pm 0.001 and 0.15 \pm 0.0061^{***}) is significant to the control group. The Negative control is comparted with the experimental groups (Table 3).

3.6. Result: Histological Analysis

Obese white adipose tissue and kidneys show increased adipocytes, stem cell differentiation, and fat cell suppression in the supplementation group. The control group shows the normal structure of adipose tissue with cytoplasmic lipids covered by a thin membrane. The kidney cross-section shows the normal structure of cells. Group II shows the nucleus is pushed to one side by the lipid and the concentric salts, and the lobules are appearing in the thin section of the kidney. The lauric acid group shows regenerative cells with slight modifications to the normal structure of the kidney. The supplementation groups VI and VII treated with LERLE and LERLE reduced the fat cells to a significant level (Figure 1).

4. Discussion

Ketone bodies provide energy to tissues, with glycerol producing 38% of new glucose in lean and 79% obese individuals [30]. Abnormalities in lipogenesis and lipolysis can cause adipose tissue toxicity [31]. Ketogenic treatment involves incorporating meat and green vegetables in butter or mayonnaise for a satisfying meal. Maintain a high fat-to-carbohydrate ratio for effective nutrition. Traditional medicinal herbs, including *L. speciosa* leaf extracts, have shown anti-obesity benefits in rats, reducing body weight and cholesterol levels [10].

The experimental animal reveals the significant impact of KGD treatment on body weight gain there are (41.02%) differences between the control group and group II for the animals from group II, at the final stage of the experiment after 42nd day of the 2nd week after the administration of KGD, the body weight had significantly increased in control group. BMI measures obesity, a metabolic disorder characterized by excess fat accumulation due to energy intake exceeding expenditure.

Herbal plants exhibit anti-oxidative role in managing diseases like diabetes, obesity, and hyperlipidemia, with phenolic compounds like quercetin reducing body weight and decursin improving glucose tolerance [32]. The phytochemistry of the experimental plant *L. speciosa* shows the list of phytochemicals in the Table 1. Significant reduction of body weight and parametrial adipose tissue weight was observed in obese female KK-AY mice when fed with a hot water *L. speciosa* leaf extract [33].

The mechanisms by which pigments such as the flavonoids are directed to the correct subcellular compartment are poorly defined, although some of the steps for anthocyanins have been elucidated [34]. Anthocyanins have been demonstrated to play a very important role in plant physiology and are important to the food industry and in human health [35]. *Nelumbo nucifera* contains catechins with anti-obesity properties, including sitosterol, *Citrus aurantium* p-synephrine, and flavonoids, which reduce appetite, increase energy expenditure, and adipocyte differentiation [36].

The Group III, no difference revealed for the body weight between control and obesity model. Gallotannins appeared to be more efficient than ellagitannins in insulin receptor binding, insulin receptor activation and glucose transport induction [37]. Hence in our present experiment conditions, KGD treated IV, V, VI and VII groups are failed to gain much higher body weight than control. The experimental plant *L. speciosa* ethanolic leaf extract contains corosolic, lagerstroemin, tannins and anthocyanin. Corosolic acid (CRA), a constituent of Banaba leaves, has been reported to exert anti-hypertension, anti-hyperinsulinemia, anti-hyperglycemia, and anti-hyperlipidemia effects [17 and 38].

In normal physiology, catalase functions in the peroxisome to breakdown the H₂O₂ generated by peroxisomal β -oxidation of long-chain fatty acids. Catalase activity was increased in the visceral adipose tissue of centrally obese men but not in the subcutaneous depot. Glutathione plays a crucial role in modifying obesity responses. The lower secretion of GSH increases energy metabolism and reduces adipose accretion [39]. The level of GSH is significantly lower in Group VII 0.051 ± 0.013 and 0.098 ± 0.029 in Group V. The depletion of GSH increases energy metabolism and reduces adipose accretion, so experimental groups VII and V show the depletion of GSH secretion. LPO levels were increased in overweight people. In all groups, there is a decreased level of LPO, except in Group II.

The experimental Group IV&V shows the antilipidemic activity at moderate level. The green leaves of *L. speciosa* constitute abundant amount of corosolic acid [40]. The table. 2 shows the moderate reduced amount of LDL, and VLDL in higher dose of LEGLE. The anthocyanin are condensed tannins present in the red leaf of *L. speciosa* is responsible for higher rate of anti-obesity effect in the experimental group VI & VII. Natural sources have been actively researched to develop effective medicines for obesity treatment. Corosolic acid, Lagerstroemin, tannin, and anthocyanin are found in *L. speciosa*, an ornamental plant with potential for medicinal use. Obese white adipose tissue (WAT) and Kidney showed increased adipocyte quantity due to hyperplasia, indicating stem cell differentiation.

5. Conclusion

Traditional medicinal plants natural constituents have traditionally opened the path for the creation of novel medicines. Terpenoids, flavonoids, tannins, alkaloids, sterols, cardiac glycosides, and saponins were discovered in *L. speciosa* during investigations on phytochemical analysis. It has been determined that phenolic chemicals and lipase inhibitory properties are related and that *L. speciosa* may have therapeutic promise for the treatment of obesity. The results showed that administration of the ethanol extract of *L. speciosa* red leaf can lower the levels of LDL, and VLDL in the blood serum of Albino rats. It concluded that the ethanol extracts of red and green leaves could potentially be used as a supplement for the prevention of hypercholesteremia.

Table 1 Phytochemical analysis of Ethanolic Green and Red leaf extracts of *L. speciosa*

Sl No	Phytoconstituents	Green Leaf	Red Leaf
1	Carbohydrates	+	+
2	Tannins	+	+
3	Saponins	+	+
4	Flavonoids	+	+
5	Alkaloids	+	+
6	Quinones	+	-
7	Glycosides	+	+
8	Terpenoids	+	+
9	Triterpenoids	+	+
10	Phenols	+	+
11	Coumarins	+	+
12	Steroids	+	+

'+' indicates the presence of Phytoconstituents; '-' indicates the absence of Phytoconstituents

Table 2 Lipid profile analysis (LDL, and VLDL) of *Lagerstroemia speciosa* ethanolic green and red leaf extract against KGD

	Initial Lipid Profile		Lipid Profile After 21 st Days KGD		Lipid Profile After 42 nd Days	
	LDL	VLDL	LDL	VLDL	LDL	VLDL
Control	29.3±2.78	18.7±1.55	133±0.482	29.8±0.248	161±1.73	50.2±1.07
Only KGD	33.5±1.2	17.2±0.167	329±9.91***	44.2±0.346***	380±2.27***	113±1.8***
KGD + Lauric acid	35±1.09	18.3±0.676	293±2.39 ^{ns}	37.2±0.993***	245±3.53***	57.3±0.159 ^{ns}
KGD + LEGLE L.D	31.8±3.41	15±0.439	132±62.7 ^{ns}	33±0.0953**	251±3.15***	87.9±0.222***
KGD + LEGLE H.D	35±5.95	15±0.502	194±3.46 ^{ns}	27.1±0.173*	191±1.3***	49.8±3.78 ^{ns}
KGD + LERLE L.D	31.9±0.548	17.1±0.473	226±5.47**	27.3±0.537*	320±1.03***	73±2.38***
KGD + LERLE L.D	30.3±2.84	16.2±0.257	251±3.19*	23.3±0.895***	143±3.54***	47.8±3.14 ^{ns}

5.1. Statistical comparison

Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett comparison was performed. (***) $P < 0.001$ control group was compared with std group-III. (***) $P < 0.001$ -** $P < 0.01$, * $P < 0.05$) treated groups IV, V, VI and VII was compared with Group I.

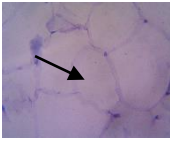
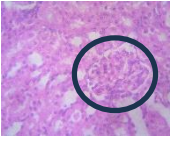
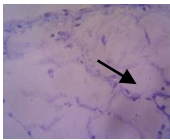
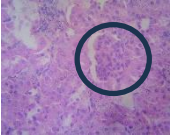
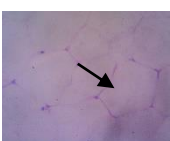
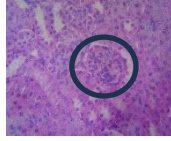
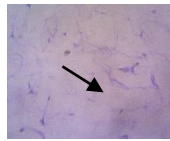
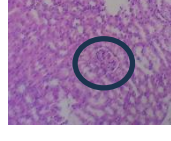
Table 3 Antioxidant effects of *Lagerstroemia speciosa* ethanolic green and red leaf extract against KGD

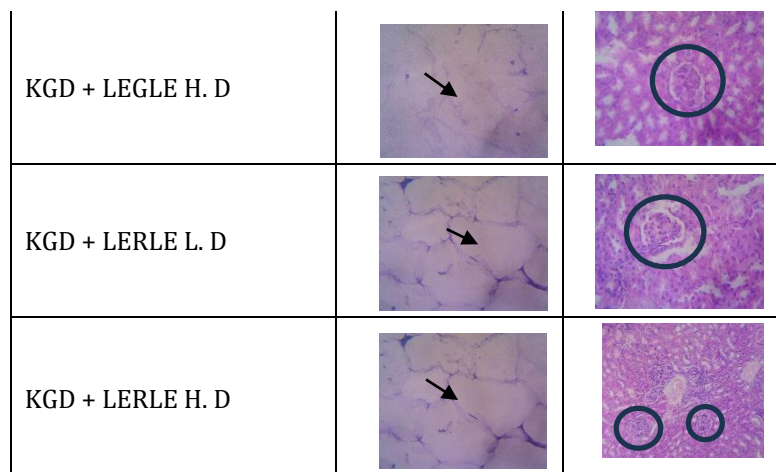
Groups	Control	Only KGD	KGD + Lauric acid	KGD + LEGLE L.D	KGD + LEGLE H.D	KGD + LERLE L.D	KGD + LERLE H.D
SOD (unit/min/mg protein)	0.146± 0.0100	0.124± 0.0117	0.1808± 0.0046	0.109± 0.0020	0.117± 0.014	0.1658± 0.036	0.205± 0.022
CATALASE (µmole of H2O2 consumed/min/mg protein)	0.3± 0.0111	0.898± 0.0806***	0.363± 0.00857	0.307± 0.104	0.238± 0.0216	0.143± 0.0229	0.138± 0.0036
GPx (µmole of glutathione oxidized/min/mg protein)	0.039± 0.006	0.22± 0.023***	0.075± 0.0049	0.093± 0.016	0.083± 0.021	0.13± 0.0091**	0.12± 0.007**
GSH (µmol/mg of protein)	0.18± 0.0052	0.32±0.063	0.12± 0.021	0.16± 0.025	0.098± 0.029	0.1± 0.015	0.051± 0.013
LPO (µmol/mg of MDA nmol/gm)	0.076± 0.001	0.15± 0.0061***	0.076± 0.0014	0.1± 0.0032***	0.091± 0.0024*	0.095± 0.0058**	0.073± 0.002 ^{ns}

5.2. Statistical comparison

Each group (n=6), each value represents Mean ± SEM. One way ANOVA, followed by Dunnett comparison was performed. (***)P<0.001) control group was compared with std group-III. (***)P<0.001-**)P<0.01, *)P<0.05) treated groups IV, V, VI and VII was compared With Group I.

Figure 1 Histology (40×) of white adipose tissue and kidney of rat

Groups/ Organs	Adipose Tissue	Kidney
Control		
Only KGD		
KGD + Lauric acid		
KGD + LEGLE L. D		



Adipose tissues are shown by Black arrows. Control group shows normal adipose tissue, Only KGD group shows varying sizes of adipose tissue with scattered inflammatory infiltrate's, Standard group shows adipocytes shows small in size, The experimental groups show normal adipose tissue with adipocytes; **Kidney-Glomeruli are shown by black Circle.** Control group shows normal glomeruli and tubules, Only KGD group glomeruli shows mild mesangial hypercellularity shows the nucleus is pushed to one side by the lipid and the concentric salts, Standard group shows normal glomeruli and tubules, The experimental groups show normal glomeruli and tubules.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained.

References

- [1] Tappy L, Le K-A. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev.* 2010; 90:23-46.
- [2] Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev.* 2012;70(1):3-21.
- [3] Mela DJ. Implications of fat replacement for nutrition and food intake. *J Am Diet Assoc.* 1996; 98:50–55.
- [4] Sampathkumar MT, Kasetti RB, Nabi SA, Renuka A. Antihyperlipidemic and antiatherogenic activities of *Terminalia pallida* fruits in high fat diet-induced hyperlipidemic rats. *J Pharm Bioallies Sci.* 2011;3: 449-452.
- [5] Matthew C, Debra LS, Laura R. Saslow, Tro K, Ashley E. Mason, Eric C. Westman, Shebani SD. Treating binge eating and food addiction symptoms with low-carbohydrate Ketogenic diets: a case series. *Journal of Eating Disorders.* 2020; 8(2): 1-7.
- [6] Paoli A, Rubini, A, Volek, JS, Grimaldi KA. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal of Clinical Nutrition.* 2013; 67(8): 789-796.
- [7] Mathern JR, Raatz SK, Thomas W, Slavin JL. Effect of fenugreek fiber on satiety, blood glucose and insulin response and energy intake in obese subjects. *Phytother. Res.* 2009 Nov; 23(11):1543-8.
- [8] Han LK, Gong XJ, Kawano S, Saito M, Kimura Y, Okuda H. Antiobesity actions of *Zingiber officinale* Roscoe. *The pharmaceutical society of Japan.* 2005 Feb;125(2):213-217.
- [9] Alok Sharma, Shanker, Lalit Kumar Tyagi, Mahendra Singh, Rao V. Herbal Medicine for Market Potential in India: An Overview. *Academic Journal of Plant Sciences.* 2008; 1(2): 26-36.
- [10] Eric Wei Chiang Chan, Lea Ngar Tan, Siu Kuin Wong. Phytochemistry and Pharmacology of *Lagerstroemia speciosa*: A Natural Remedy for Diabetes. *International Journal of Herbal Medicine.* 2014; 2 (1): 81-87.
- [11] Chaitanya R, Sandhya S, David B, Vinod KR, Murali S. HRBC Membrane Stabilizing Property of Root, Stem and Leaf of *Glochidion velutinum*. *Int J Res Pharmaceut Biomed Sci.* 2011; 2(1):256–259.

- [12] Rohit Kumar Verma, Thomas Paraidathathu. Herbal medicines used in the traditional Indian medicinal system as a therapeutic treatment option for overweight and obesity management: a review. 2014; 6(2):40-47.
- [13] Guy Klein, Jaekyung Kim, Klaus Himmeldirk, Yanyan Cao, Xiaozhuo Chen. Antidiabetes and Anti-obesity Activity of *Lagerstroemia speciosa*. Advance Access Publication. 2007; 4(4):401–407.
- [14] Kongkathip N. Chemistry and extraction method of neem Bangkok: 3rd Workshop in the using neem leaf extracts for control and eradicate in insects, 1994.
- [15] Harborne JB. Phytochemical Methods; A guide to modern techniques of plant Analysis. 2nd Edition, London New York, 1984.
- [16] Trease GE, Evans WC. Pharmacognosy. 11th ed, Brailliar Tiridel Can. Macmillian publishers, 1989.
- [17] Lakshmi KK, Akalanka D, Satyavathi K, Bhojaraju P. GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. Pharmacognosy Research. 2013; 6(1):58-61.
- [18] Manjeshwari P. Sonar, Virendra K. Rathod. Extraction of type II antidiabetic compound corosolic acid from *Lagerstroemia speciosa* by batch extraction and three phase partitioning. Biocatalysis and Agricultural Biotechnology. 2020;27(101694):1-7.
- [19] Kulkarni RN, Pandhare RB, Deshmukh VK, Mohite PB, Pawar AR. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Journal of Pharmaceutical and Biological Sciences. 2021;9(1):7–14.
- [20] Aude A. Watrelot, Delia L. Schulz, James A. Kennedy. Wine polysaccharides influence tannin-protein interactions. Food Hydrocolloids. 2017; 63:571-579.
- [21] Takashi Tanaka, Hui-Hong Tong, Ya Ming Xu, Kanji Ishimaru, Gen-ichiro Nonaka, Itsuom Nishioka. Tannins and Related Compounds. CXVII. Isolation and Characterization of Three New Ellagitannins, Lagerstannin A, B and C, Having a Gluconic acid core from *Lagerstroemia speciosa* (L.) Pers. Chem. Pharm. Bull. 1992;40(11);2975-2980.
- [22] Adelio Rigo, Fabio Vianello, and Giovanni Clementi. Contribution of Proanthocyanidins to the Peroxy Radical Scavenging Capacity of Some Italian Red Wines. J. Agric. Food Chem. 2000; 48:1996-2002.
- [23] Jeremy S. Barnes, Hien P. Nguyen, Sijia Shen, Kevin A. Schug. General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry. Journal of Chromatography A. 2009;1216(3);4728-4735.
- [24] Diabetes UK. Position Statement: Low-Carb Diets for People with Diabetes; Diabetes UK: London, UK, 2017.
- [25] Heart, U.K. Heart UK's very low fat eating plan. Available online: [https://www.heartuk.org.uk/very low-fat-diet/introduction](https://www.heartuk.org.uk/very-low-fat-diet/introduction) (accessed on 29 September 2019).
- [26] Saad Alkahtani, M Saquib Hasnain, Hamzah Algamdy, Nada H. Aljarba, Abdullah AlKahtane. Acute and sub-acute oral toxicity *Lagerstroemia speciosa* in Sprague Dawley rat. Saudi Journal of Biological Sciences. 2022; 29:1585-1591.
- [27] Camila M, Loyanne Francine Souza Magri, Marcela de Rezende Costa, Daniela CU Cavallini, Katia S. Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. Lipids in Health and Disease. 2012; 11(29):1-8.
- [28] Bergymer HU, Scheibe P, Wahlefeld AW. Methods for aspartate and alanine amino transferase. Clin. Chem. 1979; 125:129-152.
- [29] Lillie RD, Fulman HM. Histopathological Technique and Practical Histopathology. The Blauiston Division, New York and London, Acad Sci. 1967;111:789-792.
- [30] Antonio Paoli. Ketogenic Diet for Obesity: Friend or Foe? International Journal of Environmental Research and Public Health. 2014;11(2):2092-2107.
- [31] Moreno-Indias I, Tinahones FJ. Impaired Adipose Tissue Expandability and Lipogenic Capacities as Ones of the Main Causes of Metabolic Disorders. Journal of Diabetes Research. 2015; 1-12.
- [32] Fatima A, Agrawal P, Singh PP. Herbal option for diabetes: an overview. Asian Pacific Journal of Tropical Disease. 2012; 2: S536-S544.
- [33] Suzuki Y, Unno T, Ushitani M, Hayashi K, Kakuda T. Antiobesity activity of extracts from *Lagerstroemia speciosa* L. leaves on female KK-Ay mice. Journal of Nutritional Science and Vitaminology. 1999; 45:791-795.

- [34] Winefield C. The final steps in anthocyanin formation: A story of modification and sequestration. *Advances in Botanical Research*. 2002; 55–74.
- [35] Xianli WU, Ronald L. Prior. Systematic Identification and Characterization of Anthocyanins by HPLC-ESI-MS/MS in Common Foods in the United States: Fruits and Berries. *J. Agric. Food Chem.* 2005; 53(7):2589–2599.
- [36] Ohkoshi E, Miyazaki H, Shindo K, Watanabe H, Yoshida A, Yajima H. Constituents from the Leaves of *Nelumbo nucifera* Stimulate Lipolysis in the White Adipose Tissue of Mice. *Planta Medica*. 2007; 73(12):1255-1259.
- [37] Li Y, Kim J, Li J, Liu F, Liu X, Himmeldirk K, Chen X. Natural anti-diabetic compound 1,2,3,4,6-penta-O-galloyl-d-glucopyranose binds to insulin receptor and activates insulin-mediated glucose transport signaling pathway. *Biochemical and Biophysical Research Communications*. 2005; 336(2):430–437.
- [38] Yamada K, Hosokawa M, Yamada C, Watanabe R, Fujimoto S, Fujiwara H, Inagaki N. Dietary Corosolic Acid Ameliorates Obesity and Hepatic Steatosis in KK-Ay Mice. *Biological & Pharmaceutical Bulletin*. 2008;31(4):651-655.
- [39] Bjorndal B, Burri L, Staalesen V, Skorve J, Berge RK. Different Adipose Depots: Their Role in the Development of Metabolic Syndrome and Mitochondrial Response to Hypolipidemic Agents. *Journal of Obesity*. 2011;1-15.
- [40] Kaihei Koshio, Yoshinori Murai, Atsushi Sanada, Takaaki Taketomi, Masato Yamazaki, Tae-Soon Kim, Hee-Ock Boo, Mitsuru Obuchi, Tsukasa Iwashina. Positive Relationship between Anthocyanin and Corosolic acid contents in Leaves of *Lagerstroemia speciosa* L. *Pars. Trop. Agr. Develop.* 2012; 56(2): 49-52.