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Evaluation of the anti-oxidant and anti-obesity effects of ethanolic extract of *Ficus auriculata* Lour. fruit

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ABSTRACT: *Ficus auriculata* (*F. auriculata*), a deciduous tree in the *Moraceae* family, is a globally significant crop. Because of its abundance of phytochemicals and antioxidants, which are essential to human health, the fig tree is revered as a holy tree. The goal of this study was to determine if the ethanolic extract of *F. auriculata* fruit has any anti-oxidant or anti-obesity effects, both *in vitro* and *in vivo*. The DPPH free radical technique for the quantitative determination of free radical scavenging activity and the inhibitory action against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. Blood serum was collected for serum lipid profile determination, histopathological analysis using hematoxylin and eosin stain, and results were viewed using a microscope at x400 magnification. From the results obtained, *F. auriculata* fruit had a large number of phenolic compounds and possible antioxidant components. The IC_{50} value of the sample was found to be 49.99 µg/mL. The extract exhibited the highest lipase inhibitory action, with an IC_{50} value of 45.61 µg/mL. At a dosage of 300 mg/kg, the extract reduced serum lipid profile and body weight in a manner similar to that of the standard drug orlistat and exhibited a reduction in the crown-like structure (CLS/mm^3) to 4 CLS/mm3, while the control group had 34 CLS/mm^3. Thus, the anti-obesity properties of *F. auriculata* fruit were confirmed using an HCD-induced obesity rat model. It reduced sugar levels, improved lipid profiles, reduced hepatic steatosis, slowed weight gain, and altered the histological marker of local inflammation.

KEYWORDS: *Ficus auriculata*; anti-oxidant activity; anti-obesity activity; pancreatic lipase inhibition; hepatic steatosis.

1. INTRODUCTION

Ficus, a deciduous tree in the *Moraceae* family, is a globally significant crop with numerous medical benefits due to its phytochemicals and antioxidants. It is considered a holy tree, providing essential nutrients for human wellness. The Moraceae family comprises 50 genera and 1400 species [1]. *Ficus auriculata* (*F. auriculata*) fruits in particular are noted to be an effective therapy for diabetes. They are also used to treat menorrhea, haemoptysis, diarrhoea, iron deficiency, cancer, and constipation [2]. The genus "figs" is distinguished by its distinctive syconium and lactory latex. They are utilized by humans in tropical and subtropical climates for food, medicine, fuel, hedges, fences, lac hosts, ornamental trees, and devotional plants. [3]. Genetic variation in fig types reveals their pharmacological properties, vital to the fig industry. Ficus medicinal plants contain triterpenes, flavonoids, polyphenols, alkaloids, sterols, and coumarins [4].

Oxidative stress (OS) is a physiological phenomenon that induces direct or indirect harm to several organs, hence playing a role in the pathogenesis of numerous diseases, including cancer, diabetes, cardiovascular disease, neurodegenerative disorders, atherosclerosis, and obesity [5].

The "New world syndrome" of obesity has been alarmingly rising. There are relatively few alternatives available for treating obesity. Long-term, diet and lifestyle changes are ineffective. Furthermore, unreliable as a class of anti-obesity medications are appetite suppressants and metabolism boosters [6-8]. Therefore, one possible therapy option for obesity management might be to suppress the pancreatic lipase enzyme, which would decrease the digestion and absorption of dietary fats [9]. Currently on the market as a pancreatic lipase inhibitor, orlistat is not highly recommended because of its gastrointestinal discomforts and other adverse effects [10]. The compounds that can successfully neutralize free radicals are called antioxidants. Plants are a high-end source of antioxidants known as polyphenolic chemicals [11]. Ficus species are reported to be abundant in flavonoids and phenolic acid, which equips them to fend against

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oxidative stress-related illnesses [12]. Therefore, the purpose of this research was to examine the potential anti-oxidant and anti-obesity properties of *Ficus auriculata* fruit ethanolic extract *in vitro* and *in vivo*, respectively.

2. RESULTS DISCUSSION

Obesity and overweight are global health crises because they raise the odds of several diseases and health problems, such as dyslipidaemia, type 2 diabetes, cancer, heart disease, and hypertension [13]. Two primary classes of obesity treatment drugs are now available: anorectic sibutramine and orlistat, the former of which inhibits pancreatic lipase to reduce fat absorption in the intestines. However, they may be costly and come with potential negative side effects. The use of herbal remedies for weight loss is, nevertheless, a topic of continuing investigation [14,15]. One new approach to treating obesity involves blocking pancreatic lipase, the enzyme responsible for absorbing dietary lipids, which is the main source of excess calories [16]. *F. auriculata* contains phenolic compounds that have several bioactive properties, one of which is antioxidant. The radical scavenging activity is mainly due to the redox properties of the hydroxyls present in phenols [17,18]. This research set out to determine whether or not *F. auriculata* fruit has any anti-oxidant or anti-obesity properties.

The ethanolic extracts and their fractions were evaluated for their anti-oxidant properties by DPPH (2,2-diphenyl-1pyrylhydrazyl) radical scavenging activity. Research has shown that phenolic compounds have a significant impact on the free radical scavenging activity of a sample [19]. Figure 1 shows the free radical scavenging activity of the ethanolic extract of *F. auriculata* fruit at different concentrations. The extract showed more activity than the standard drug, ascorbic acid. This suggested that the *F. auriculata* fruit had a large number of phenolic compounds and possible antioxidant components. The IC_{50} value of the sample was found to be 49.99 µg/mL.

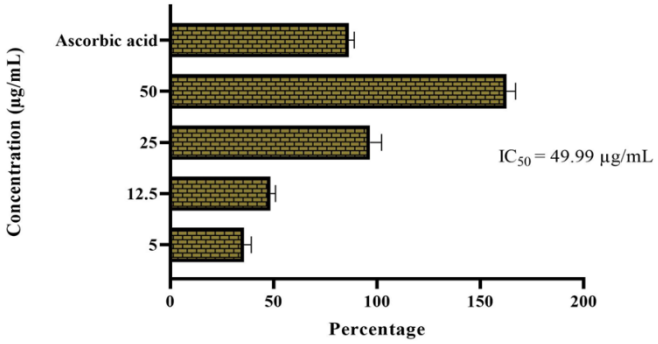

Figure 1. Percentage change in the *F. auriculata* fruit extract concentration and its DPPH scavenging activity. Each test was run three times, and the IC_{50} value was given as the mean ± SD of the three runs.

The discovery of phytochemicals in conventionally used medical plants offers a promising prospect for the creation of novel treatments. The antilipase activity of several plants has been investigated in an attempt to develop naturally occurring herbal resources that may provide physiologically useful anti-obesity medications [16]. The percentage and IC_{50} value (the concentration required to inhibit lipase activity by 50%) of the *F. auriculata* extract-induced pancreatic lipase inhibition are shown in table 1. The extract exhibited the highest lipase inhibitory action, with an IC_{50} value of 45.61 µg/mL. Serious adverse effects of orlistat include headaches, stomach discomfort, irregular menstrual cycles, and gas with oily spotting [20].

When comparing the HCD-fed animals to the control group, which was given a regular diet, there was a substantial increase in body weight (87.57%) (p<0.05). Rats were split into two groups and given different doses of ethanolic extract: 300 mg/kg and 600 mg/kg. Following the end of therapy, the rats' body weight decreased by 19.55 and 13.91%, respectively (table 2). At a dosage of 300 mg/kg, the extract reduced body weight in a manner similar to that of the standard drug orlistat (13.14%). Furthermore, the group treated with extract at a dosage of 300 mg/kg exhibited a reduction in the crown-like structure (CLS/mm3), microscopic clusters of dead adipocytes that are encircled by macrophages, to 4 CLS/mm3, while the control group had 34 CLS/mm3.

Table 1. The inhibitory activity of pancreatic lipase by *F. auriculata* fruit.

Concentration (µg/mL)	Inhibition (%)	Orlistat (Positive control)
100	10.87 ± 0.30	16.50 ± 0.20
200	26.72 ± 0.50	35.10 ± 0.10
300	38.83 ± 0.50	49.67 ± 0.50
400	52.85 ± 0.10	66.93 ± 0.45
500	72.30 ± 0.50	80.30 ± 0.55
IC_{50}	45.61 µg/mL	50.02 µg/mL

The values are the mean ± SD (n=3).

Table 2. Effect of extract on body weight of animals, percentage body weight gain and score of crown like structure (CLS/mm2) in animals fed with HCD.

Groups	Body weight in gm	% gain	Crown like structure
Control	182.6 ± 11.92	-	3
HCD control	342.5 ± 10.96	87.57	34
HCD + Orlistat (30 mg/kg b.wt)	206.6 ± 14.35	13.14	8
HCD + extract dose 1 (300 mg/kg b.wt)	218.3 ± 12.33	19.55	4
HCD + extract dose 2 (600mg/kg b.wt)	208 ± 9.76	13.91	10

The data is shown as mean ± SD. (n=6).

Treatments with *F. auriculata* extracts effectively changed the lipid profile in a way that was similar to the effects of orlistat. The 300 mg/kg body weight extract was found to have TC, TG, and LDC-c levels that were almost normal (Table 3). In comparison to the normal control group, the HCD group had elevated risk levels for several cholesterol measures.

Histological analysis revealed that the livers of the HCD group had micro- and macro-steatosis, giving them a distinct appearance, in contrast to the groups administered *F. auriculata* fruit extract, which exhibited essentially no such lesions (Figure 2). This group of animals had a reduced density of crown-like structures in their adipose tissue sections (4 and 10 CLS/mm2) compared to the group that received just HCD (34 CLS/mm2).

Table 3. Effect of *F. auriculata* extract on serum lipid profile and glucose level.

Parameters	Normal Control	HCD	Standard (orlistat)	Extract (300 mg/kg b.wt)	Extract (600mg/kg b.wt)
TC (mg/dl)	105.16± 0.47*	213.3±1.17	111.6±0.88*	110.6±1.88*	112±1.98*
TG (mg/dl)	74±0.96	253.83±1.62*	72±0.89	75.33±1.14*	77.3±1.3
HDL-c (mg/dl)	33±0.81*	26.5±0.91	36.83±0.60	41.66±1.05*	42.55±1.09*
LDL-c (mg/dl)	22.66±0.75	166.33±2.02*	60.83±0.65	54.16±1.88	56.67±1.9
Glucose(mg/dl)	126.16±1.35*	197±0.51*	126.83±1.73	120.16±0.6*	122.98±0.7*
AST (U/L)	52.16±1.04*	98±1.06*	61.5±1.22	63.5±0.98	65.54±1.9*
ALT(U/L)	22.83±1.44	70.16±1.49	30.6±0.88*	35.5±0.42	38.4±0.65*

All the data were expressed as mean ± SD (n=6). * p<0.005.

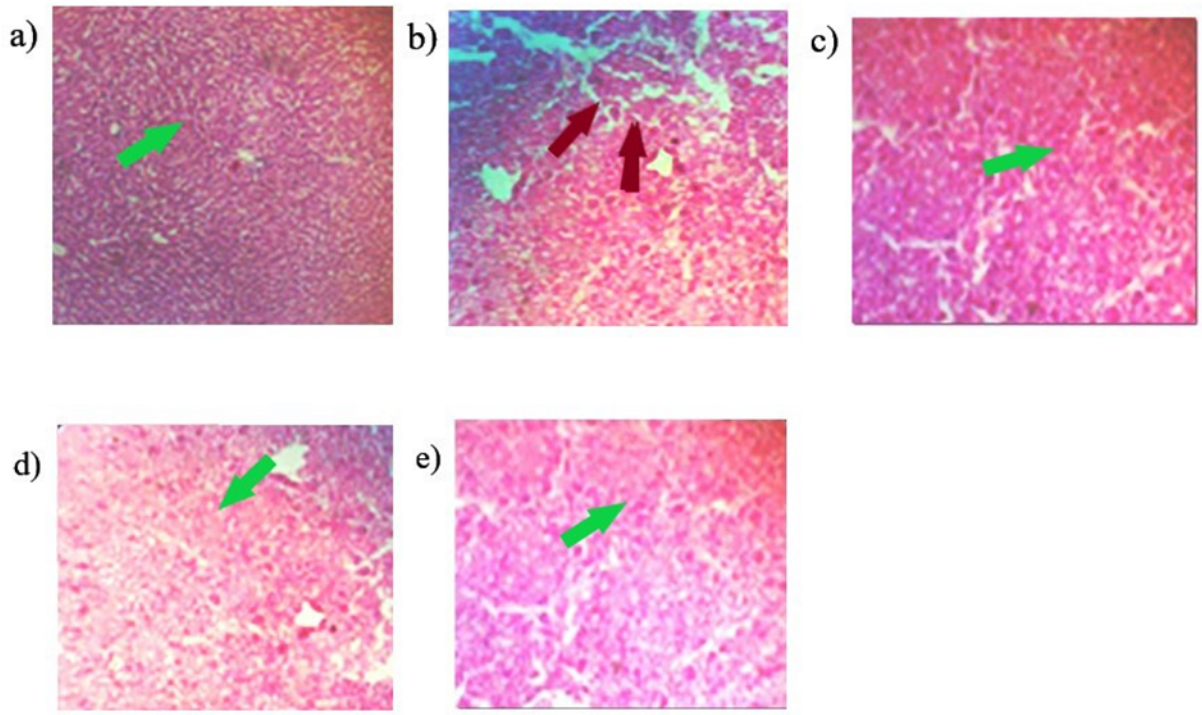

Figure 2. Histopathology of the liver sections (H&E, x400). a) normal control, b) HCD, c) standard control (orlistat), d) extract dose 300 µg/mL, e) extract dose 600 µg/mL. Normal cells are shown by the green arrow, whereas micro- and macro-vesicular steatosis are indicated by the red arrow.

3. CONCLUSION

Given that obesity raises the risk of several major illnesses, the worrisome statistics on the condition clearly demonstrate that it poses a danger to the general health of the world's population. In this work, the anti-obesity properties of *F. auriculata* were confirmed using an HCD induced obesity rat model. *F. auriculata* reduced sugar levels, improved lipid profiles, reduced hepatic steatosis, slowed weight gain, and altered the histological marker of local inflammation. It is also proven that the plant extract posses' markable effect of pancreatic lipase enzyme. Consequently, it makes sense to look into this extract's possible pharmacological effects in metabolic diseases and to further explore its mechanisms against the metabolic effects of HC feeding in rats..

4. MATERIALS AND METHODS

4.1 Plant sample collection and authentication

The fruits of *F. auriculata* were gathered in the rural regions of Kerala, India, including Kozhikode, Malappuram, and Wayanad. A specimen was stored in the herbarium (No. 10012) after the fruits were inspected and verified by the Pharmacognosy Division, Centre for Medicinal Plant Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala (Figure 3)

Figure 3. Fruits of *F. auriculata*.

4.2 Preparation of sample

The gathered fruits were cleaned with running tap water to get rid of any sticky substances, then sliced into tiny pieces and sun-dried for five days. A mechanical grinder was then used to grind the fruits into a coarse powder, which was then sealed in an airtight container [21]. About 150 grams of powdered fruits were packed in a Soxhlet apparatus and extracted with 500 ml of ethyl acetate by soxhlation for 48 hours. The ethyl acetate extract was concentrated in a rotary evaporator (Superfit Rotary Flash Evaporator, 40 °C at 50 rpm). The extract was then measured in weight, and the percentage yield was determined based on the amount of air-dried crude material. (21.05%). The extracts were stored in a desiccator (Borosil 100mm Flenge 3082033) for further examination.

4.3 Animals and experimental protocol

For the intended investigation, 150–200g (3–4 months old) Wistar rats were used. These animals were purchased from the Small Animal Breeding Station (SABS) at the Agricultural University of Kerala's

College of Veterinary Medicine in Thrissur, Mannuthi, Kerala. A 12-hour light/dark cycle, temperature range of 22-28°C, and relative humidity of 60-70% are some of the regulated conditions they may inhabit in the Animal House of Al Shifa College of Pharmacy. Before the trial, all of the animals were given a week to acclimate and were split up into five groups, totalling six. The remaining groups will be administered a high-calorie diet (HCD) to develop obesity and water ad libetum for 16 weeks, whereas group 1 and group 2 were provided a regular control pellet diet and HCD [22]. Rats who gained 20% or more of their body weight above the usual control group were designated as obese [23]. Group 3 received standard Orlistat (30 mg/kg), group 4 and 5 received extract of doses 300 and 600 mg/kg body weight. The doses were selected as per available toxicological data in previous literatures [24,25]. All pharmaceutical treatments, including those involving HF in the diet, began at the start of week eight and lasted until week sixteen, when the trial ended. Weekly, the body weight was recorded. All animal study, which followed the rules set forth by the Committee for Control and Supervision of Research on Animals (CCSEA), was permitted by the Institutional Animal Ethics Committee (IAEC) at Al Shifa College of Pharmacy in Perinthalmanna, Kerala, India (IAEC/071/20).

4.4 DPPH radical scavenging activity

We used the DPPH free radical technique (2,2-diphenyl-1pyrylhydrazyl) for the quantitative determination of free radical scavenging activity [26]. Using a no-cost to achieve final concentrations of 5, 12, 25, and 50 µg/mL, each sample of the stock solution (1 mg/mL) was diluted. Afterwards, three and a half millilitres of a 50 µM DPPH methanolic solution (1 mg/50 mL) from Sigma Aldrich in the USA was combined with two millilitres of each sample solution and left to react for half an hour at room temperature. The absorbance at 520 nm was measured using a Shimadzu UV 1900i UV spectrophotometer. For the purpose of creating a blank solution, absolute ethanol was substituted for the DPPH solution. The standard is ascorbic acid. With the control's absorbance value (A blank) and the sample's absorbance value (A sample) subtracted by the blank, and then multiplied by 100, we get the percentage inhibition. By graphing inhibition percentages versus sample concentration, we were able to determine the sample concentration that produced 50% inhibition, also known as the IC_{50}.

4.5 Pancreatic lipase inhibition assay

The inhibitory action against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate [27]. In a 96-well plate, 10 mL of extracts were pipetted into each well at doses of 100, 200, 300, 400, and 500 mg/mL. A positive control was also added, consisting of 100 mM of Orlistat. The negative control medium was DMSO. In both the positive and negative controls, 40 mL of freshly produced swine pancreatic lipase was added, resulting in a total volume four times that of the test samples. The first incubation period for the plates was 15 minutes at 37 °C. After that, each well received 170 mL of substrate solution. Following a 25-minute incubation period at 37 °C, the microplate reader (Allsheng AMR-100 ELISA reader) was used to measure absorbance at 405 nm. The percentage of lipase inhibition was determined by dividing the absorbance of the test by the absorbance of the control, and then multiplying the result by 100.

4.6 Estimation of serum lipid profile

After the experiment was finished, a fully autoanalyzer (Fujifilm DRI-CHEM NX500) was used to measure the serum levels of various enzymes, including alkaline phosphatase (ALP), alanine transaminase (ALT), aspartic transaminase (AST), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), triglyceride (TG), low density lipoprotein cholesterol (LDL-c), glucose, and enzymes other than cholesterol.

4.7 Histopathological analysis

The animals' liver tissues were preserved in 10% phosphate buffered formalin for one day before being cut into paraffin slices that were 3-5 µm thick. These slices were then stained with hematoxylin and eosin (H&E), and the results were seen using a microscope set at a magnification of x400. A conventional light microscope (Zeiss, Axioplan 2) was used to count the number of crown-like structures (CLS) among the four groups in the adipose sections. The objective lens and eyepiece of the microscope included an ocular grid reticle. CLS were counted at X400 magnification inside the ocular reticle squares.

4.8 Statistical analysis

For these data, we used IBM SPSS Statistics 21 with the Graph Pad Prism trial version, as well as one-way analysis of variance (ANOVA) and Tukey's test for multiple comparison. The data's mean ± SD is provided. Changes were seen at a significance level of $p<0.05$.

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Conflict of interest statement: The authors declare no conflict of interest in the manuscript.

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