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Anti-obesity effects of *Ficus benghalensis* Linn. bark extract in a progesterone-induced model: A biochemical and histological study

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Obesity, particularly in women, has become a growing global concern, often linked to hormonal imbalances such as elevated progesterone levels. Current pharmacological interventions for hormone-induced obesity may pose limitations due to side effects or inconsistent outcomes. Therefore, there is an increasing interest in exploring natural and plant-based alternatives with potential anti-obesity effects. To study the progesterone-linked obesity, this study was conducted in female Swiss albino mice. This study evaluated the anti-obesity potential of *Ficus benghalensis* bark extract in female Swiss albino mice using a progesterone-induced obesity model. Twenty-five mice were divided into five groups (n=5) and treated for 29 days. Group A (Normal Control) received saline. Group B (Obese Control) received progesterone (10 mg/kg, s.c.). Group C received progesterone with Orlistat (10 mg/kg, oral). Groups D and E were administered 200 mg/kg and 400 mg/kg of *Ficus benghalensis* extract, respectively, alongside progesterone. Parameters such as food intake, body weight, liver weight, adipose tissue, blood glucose, and lipid profile were monitored. Histological analyses of liver and fat pads were also performed. Mice treated with the extract showed reduced food intake, body weight, fat accumulation, blood sugar, and improved lipid profiles compared to the obese group. Phytochemical analysis confirmed the presence of quercetin, flavonoids, and saponins. Histology showed reduced fat buildup and healthier liver and kidney structures. These findings suggest *Ficus benghalensis* extract may offer protective and therapeutic effects against hormone-related obesity.

Keywords: BMI, Atherogenic index, Cardiac risk factor, Lipid glucose metabolism, Liver function test

Obesity is a leading global health issue characterized by accumulation of excessive fat, increasing the risk of metabolic disorders such as cardiovascular disease, diabetes, kidney disease and fatty liver disease. By 2035, over half of the world′s population could be overweight or obese, according to the World Obesity Federation[1]. The World Health Organization (WHO) defines obesity on basis of Body Mass Index (BMI), where a BMI of 25–29.9 kg/m² is categorized as overweight and ≥30 kg/m² as obese[2]. The prevalence of obesity among Indian women has been rising due to urbanization, dietary shifts, and reduced physical activity, with nearly 24% classified as overweight or obese according to the National Family Health Survey (NFHS-5)[3]. Obesity can lead to problems with glucose metabolism, increasing the risk of insulin resistance and type II diabetes[4]. It also elevates blood lipid profile causing risk to Cardio vascular Diseaseas well as leptin level[5]. Additionally, obesity can put stress on the liver, as seen in elevated SGOT and SGPT levels, increasing risk to fatty liver disease[6]. Progesterone a natural sex hormone that have a crucial role in female reproductive part. Apart from its reproductive function, it also affects metabolism and fat storage[7]. It is commonly used as contraceptives pill and in hormone replacement therapy (HRT) in menopausal case, but long-term use can cause weight gain[8]

Ficus benghalensis, commonly known as the banyan tree, is widely grown across India and neighbouring countries, especially in regions with moderate rainfall and fertile, well-draining soil[9,10]. A 20-year-old banyan tree annually provides roughly 200 kg of green fodder, 100 kg of fuelwood and about 0.85 m³ of timber, with a combined market value around ₹7,500. Its leaf litter also enriches dryland soils, supplying up to 77% of needed nitrogen, 20% of phosphorus and 68% of potassium[11].

The literature showed that the selected plant is rich in steroids, flavonoids, phenols, tannins, saponins, ß-sitosterol, a-D-glucose and meso-inositol etc[12]. This plant is reported to possess many useful pharmacological activities also viz. antihyperglycemic, antidiabetic, antihyperlipidemic, antimutagenic, hypocholesterolemia, anti-inflammatory, analgesic, antibacterial, antiallergic, antifungal, larvicidal, anti-diarrheal, antioxidant, cytotoxic, hepatoprotective, anti-arthritic, and immunostimulatory[13-16].

Botanical sources offer safer, more cost-effective alternatives to synthetic drugs, which often carry significant side effects. This study evaluates *Ficus benghalensis* bark extract for mitigating progesterone-induced obesity by monitoring body weight, food intake, glycemic and lipid parameters, and tissue histology.

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Materials and Methods

Plant material and extraction

The Botanical Survey of India (Kolkata) authenticated the *Ficus benghalensis* Linn stem bark via HPLC (Ref. No. BSI/PLANT CHEM/0003-2022). The coarse powder was defatted with petroleum ether, macerated in chloroform and methanol for seven days, filtered, concentrated under reduced pressure, dried, and stored and phytochemical screening confirmed the presence of alkaloids, carbohydrates, glycosides, tannins, phenolics, flavonoids, saponins, terpenoids, coumarins, sterols, phlobatannins, and quinones through standard qualitative tests. Total phenolic and flavonoid content were estimated using Folin-Ciocalteu and aluminum chloride methods, with absorbance measured at 765 nm and 415 nm, respectively. All the drug and chemical used in this experiment of analytical grade.

Dose selection

An acute oral toxicity study was carried out for the methanolic extract of *Ficus benghalensis* bark (MEFB) following OECD Guideline 420. The extract was found to be non-toxic at a limit dose of 2000 mg/kg body weight, with no observable signs of toxicity or mortality during the 14-day observation period. Furthermore, previously reported studies suggest that the LD_{50} of *Ficus benghalensis* bark extract is greater than 5000 mg/kg body weight, indicating a high safety margin[17]. Considering these findings, two therapeutic doses were selected based on one-tenth of the reported safe limit: Low dose: 200 mg/kg body weight; High dose: 400 mg/kg body weight. These doses were chosen to evaluate the dose-dependent anti-obesity potential of MEFB. The selected dose range is consistent with effective and safe concentrations used in previous *in vivo* studies involving herbal extracts.

Experimental animals and study design

All experimental procedures were approved by the Institutional Animal Ethical Committee (Reg No:-1972/PH/BIT/56/23/IAEC. Swiss albino female mice (20–25g) were used for the study and placed in the animal house of BIT Mesra, Ranchi. The animals were maintained under standard condition with a temperature of 23 ± 2°C, relative humidity of 55 ± 10%, and a 12-hour dark/light cycle. They were provided with a standard pellet diet) and water *ad libitum* throughout the experiment. A total of 25 Swiss albino female mice were randomly divided into five groups (n=5) and treated daily for 29 days. Group A (Normal Control): Received no treatment, only saline; Group B (Progesterone PG): 10 mg/kg body weight, administered subcutaneously in the dorsal neck region; Group C (Standard Drug, STD+PG): Orlistat (10 mg/kg body weight, oral) + progesterone; Group D (Low-Dose Extract):*Ficus benghalensis* linn bark extract (200 mg/kg body weight, oral) + progesterone; Group E (High Dose Extract): *Ficus benghalensislinn* bark extract (400 mg/kg body weight, oral) + progesterone[18]. Progesterone (10 mg/kg body weight) was dissolved in arachis oil and injected subcutaneously into the dorsal neck region for 29 days. The control group received progesterone alone, without any plant extract. The test groups were given the plant extract orally 30 minutes before progesterone administration using a calibrated oral cannula and syringe (gavage). Treatment continued daily for 29 days, with the low- and high-dose groups receiving the extract, while the control group received only the saline[19].

Food intake

Effect on food intake due to plant extract was recorded on 1st day, 15thday & 28th day was recorded. All mice were fastened overnight. On the experimental day, 10g of boiled sweet corn was given to very group and the food consumption was recorded at time interval of 2-hour. Result was recorded as an average of all days[20].

Comparison of body weight

Body weight was recorded every week for entire period of experiment using electronic balance and then mean changes in body weight was calculated and anthropometric parameter such as BMI and LEE Index was calculated. Atherogenic index and cardiac risk factor measurement was also calculated[21].

$$\text{BMI} = \frac{\text{Body weight (g)}}{\text{Nasal to anal length (cm)}^2}$$

$$\text{Lee index} = \frac{\text{(Body Weight in grams) ^ (1/3)}}{\text{Naso} - \text{anal Length in cm} \times 1000}$$

$$\text{AI} = \frac{(\text{TC} - \text{HDL})}{\text{HDL}}$$

$$\text{Cardiac risk factor} = \text{Cardiac risk factor} = \text{TC/HDL}$$

Biochemical analysis

On the 30th day, blood samples were collected using the heart puncture method. Before collection, the mice were fasted overnight but had continuous access to water. The blood samples were then centrifuged at 2000 rpm for 20 minutes, allowing for the separation of serum, which was subsequently stored under deep refrigeration for further analysis[22].

Biochemical assessments included blood glucose levels, lipid profile analysis, and liver function tests (SGOT and SGPT).

Histological examination

At the end of the experiment, all mice were humanely sacrificed using the cervical dislocation method. Liver and adipose tissues were carefully isolated and preserved in a 10% formalin solution. Thin tissue sections of both the liver and adipose tissue were then prepared, stained with hematoxylin and eosin, and examined under a Leica DME microscope (40X magnification) integrated with a Canon camera for imaging[23].

Statistical analysis

The data were expressed as mean ± SD (n = 5). Statistical analysis was carried out using one-way ANOVA and two-way ANOVA, followed by Tukey's multiple comparisons test. A *P*-value of less than 0.05 ($P < 0.05$) was considered statistically significant. All analyses were performed using GraphPad Prism version 10.4.1.

Results and Discussion

Food Consumption

Excessive food intake contributes to obesity by promoting fat accumulation. In the treated groups (Fig. 1), administration of the plant extract led to a noticeable reduction in food consumption compared to the control group. This decrease suggests that the extract may help regulate appetite and caloric intake, potentially aiding in obesity management.

Body weight changes

Body weight is most important parameter for assessing obesity and treatment effectiveness. Progesterone induced group showed highly significant increase in weight at 4th week as compared to 1st week (Fig. 2). However, on comparing low dose extract as well as high dose extract there is no significant rise in weight between 1st week and 4th week. The higher dose (400 mg/kg body weight) demonstrated the most effective weight reduction, suggesting the potential anti-obesity effects of the extract.

BMI, atherogenic index and cardiac risk factor

Body Mass Index (BMI) is a key indicator for assessing obesity and body fat accumulation. In the treated groups, BMI was significantly lower compared to the control group, suggesting that the plant extract helped regulate weight gain and fat deposition (Fig. 3A). This reduction indicates the extract's potential in managing obesity and improving overall metabolic health. Similarly at atherogenic and cardiac risk factor was found lower in comparison to obese control (Fig. 3B) showing similar with standard treated group.

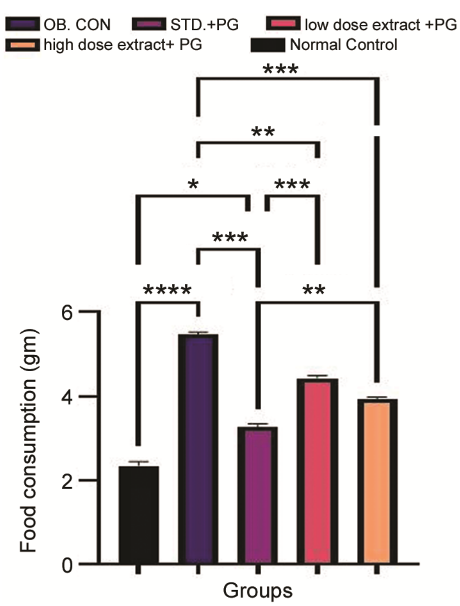

Fig. 1 — Food consumption. [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents *P*<0.0001, ****P*<0.0005 and ***P* value 0.0025 whereas * represents *P*<0.01].

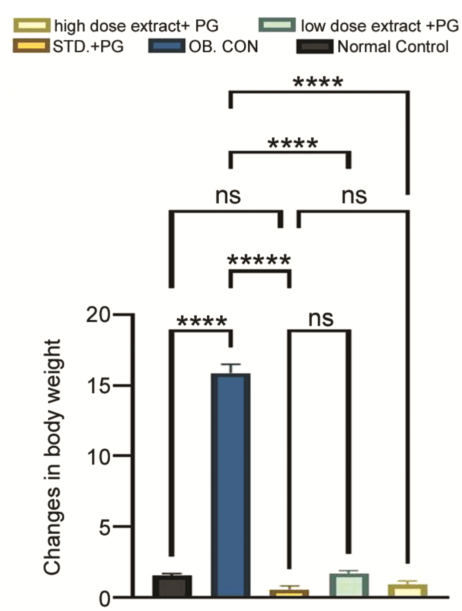

Fig. 2 — Changes in body weight of various group. [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents *P*<0.0001, ****P*<0.0005 and ***P* value 0.0025 whereas * represents *P*<0.01].

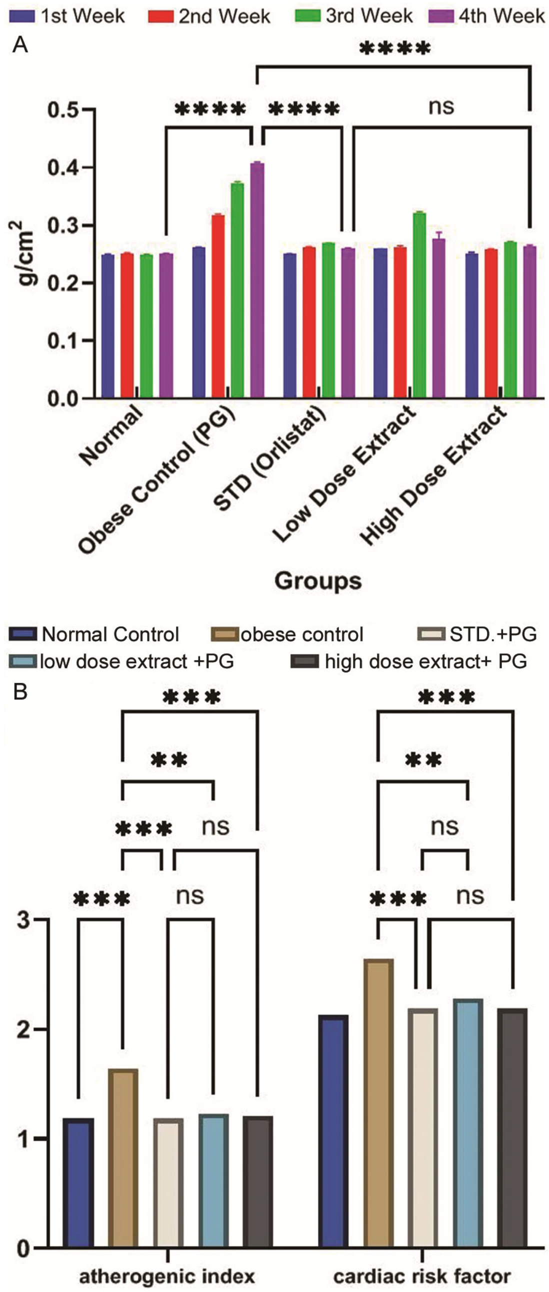

Fig. 3 — Basic metabolic rate of various group (A); Atherogenic index and cardiac risk factor (B). [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents P<0.0001, ***P<0.0005 and **P value 0.0025 whereas * represents P<0.01].

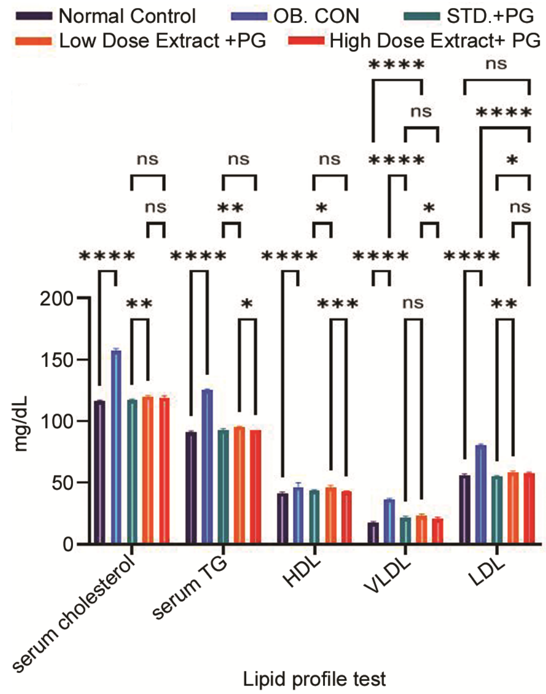

Fig. 4 — Graph of lipid profile test. [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents P<0.0001, ***P<0.0005 and **P value 0.0025 whereas * represents P<0.01].

Lipid profile

The obese Control group showed significant dyslipidemia, with elevated cholesterol, TG, VLDL, LDL, and reduced HDL levels (***$P < 0.001$ to ****$P < 0.0001$) (Fig. 4). Treatment with Orlistat and both extract doses significantly improved lipid parameters. The high-dose extract group showed lipid levels comparable to the Normal group (ns), indicating near normalization. These findings suggest the extract′s hypolipidemic and cardioprotective potential in obesity management. This imbalance in the lipid profile suggests that progesterone contributes to metabolic disturbances, potentially increasing the risk of cardiovascular complications.

Liver function test

Obesity leads to excessive lipid accumulation, causing liver damage and triggering the release of liver enzymes such as SGOT and SGPT. Elevated levels of these enzymes indicate liver stress and dysfunction. In obese Control group (Fig. 5) there is significant increase in SGPT and SGOT level as compared to other group. In SGPT, high dose extract treated group slight difference as compared to standard treated group, while in SGOT, there is significant difference between standard and high dose extract treated group.

Blood glucose

Fasting blood glucose levels were checked with the help of glucometer before the mice were sacrificed. The obese Control group that is given progesterone for 28 days showed elevated glucose levels, whereas

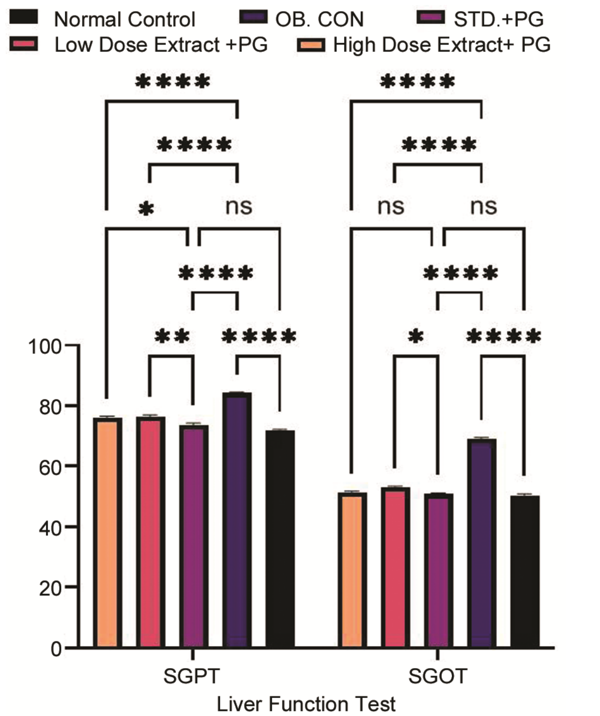

Fig. 5 — Liver function test. [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents P<0.0001, ***P<0.0005 and **P value 0.0025 whereas * represents P<0.01].

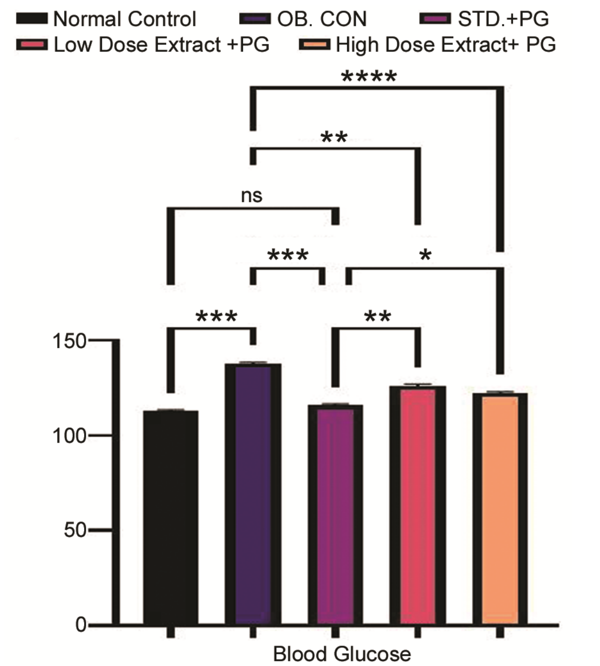

Fig. 6 — Blood Glucose test of various groups. [Statistically analyzed through one-way ANOVA followed by Tukey′s multiple comparison test where ****represents P<0.0001, ***P<0.0005 and **P value 0.0025 whereas * represents P<0.01].

co-administration of the plant extract helped lower them significantly (Fig. 6). The higher dose (400 mg/kg body weight) was the most effective in reducing glucose levels compared to other treatment groups.

Morphological and histological analysis of liver

Histological analysis of liver sections revealed severe lipid droplet accumulation in hepatocytes, leading to liver damage (Fig. 7). The normal control group showed normal liver cells with intact cytoplasm, whereas the obese control group exhibited significant fatty infiltration and liver cell atrophy. Treatment with the standard drug and *Ficus benghalensis* extract effectively reduced fat accumulation, with the higher dose restoring liver architecture closer to normal. Also the morphological representation of liver show liver damage (B) in obese control group, Low dose extract (D) shows some heal damage whereas high dose extract (E) showed a kind of normal liver with less sign of damage.

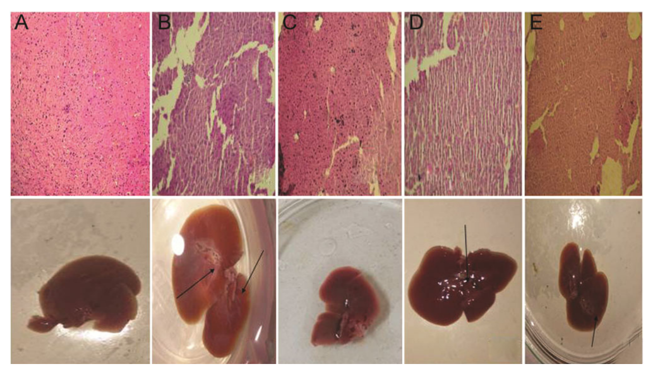

Fig. 7 — Histopathological and morphological representation of liver cells of different groups of mice. Normal control (A); Obese control (B); Standard treated (C); Low dose extract treated (D) and High dose extract treated group (E).

Histological analysis of adipose tissue

Histological examination of adipose tissue showed distinct differences between the normal Control, obese Control, standard treated and bark extract treated Group (Fig. 8.). The normal control group (A) as well as standard treated group (C) had absolute well-structured adipocytes, while the obese control group (received Progesterone) (B) showed enlarged adipocytes (hypertrophy) and oil droplets as well as increased fat accumulation. Treatment with *Ficus benghalensis* extract (D & E) significantly reduced both the size and number of adipocytes, along with a noticeable decrease in macrophage infiltration in fatty tissues. This suggests that the extract may help regulate fat storage and reduce inflammation

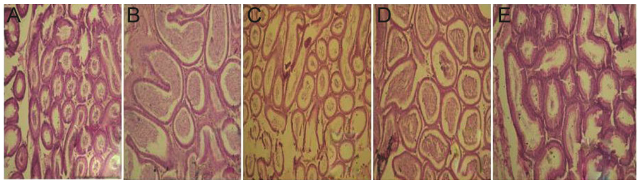

Fig. 8 — Histopathological representation of adipose tissue. Normal control (A); Obese control (B); Standard treated (C); Low dose extract treated (D) and High dose extract treated group (E).

associated with obesity. Obesity, mostly in females is mainly due to hormonal dysfunction. Women taking contraceptives and going through hormone replacement therapy have most common side effects which are fat accumulation or obesity. It increases the risk of serious conditions like heart disease, diabetes, and liver problems. In this study, progesterone was used to induce obesity in Swiss albino female mice, and the effects of *Ficus benghalensis* bark extract were evaluated. The results suggest that the plant extract has strong potential in managing obesity by controlling weight gain, improving lipid and glucose levels, and protecting liver function.

Progesterone is one of the major known cause to disrupt fat and carbohydrate metabolism, leading to alterations in biochemical parameters such as blood glucose and lipid profile [24,25]. Its elevated levels during pregnancy are linked to gestational diabetes due to increased food intake (hyperphagia)[26]. In the study, it was found that progesterone administration resulted in significant differences in HDL, LDL, and triglyceride (TG) levels between the control (progesterone-alone) and treated groups. Progesterone influences lipid metabolism by stimulating lipoprotein lipase, an enzyme that hydrolyzes dietary fats, promoting fat storage and elevating lipid levels in the body. This happens because progesterone increases appetite and fat storage, leading to metabolic imbalances. Its effect on fat metabolism is a major reason why women using contraceptives or postmenopausal hormone therapy may experience weight gain. Because of its influence on metabolism, progesterone is a key focus in obesity research, particularly in understanding how hormones contribute to weight gain in women. As a progesterone-induced obesity model mimics the hormonal weight gain seen in women, it serves as a useful model for studying obesity and potential herbal treatments. Progesterone-induced mice gained significant weight, leading to an increase in BMI, a key marker for obesity. However, mice treated with *Ficus benghalensis* extract showed a notable reduction in both body weight and BMI, especially in the higher dose group (400 mg/kg body weight). This suggests that the extract may help regulate fat metabolism or suppress appetite, leading to better weight management.

Obesity is often associated with dyslipidemia or elevated lipid profile mainly increased levels of total cholesterol, total triglycerides, LDL, and VLDL, along with a reduction in beneficial HDL cholesterol. These lipid imbalances significantly increase the risk of cardiovascular diseases. In this study, treatment with *Ficus benghalensis* extract resulted in a notable improvement in lipid profiles, reducing harmful fats, which suggests its potential in maintaining healthy fat metabolism and lowering cardiovascular risks. A high cardiac risk factor is found in progesterone-induced mice compared to the treated group.

Another major concern in obesity is impaired glucose regulation, which predisposes individuals to diabetes. The progesterone-treated mice exhibited increased fasting blood glucose levels, indicating a higher risk of insulin resistance. However, treatment with *Ficus benghalensis* extract significantly lowered blood glucose levels, with the higher dose showing the most pronounced effect. These findings suggest that the extract may enhance insulin sensitivity, making it a promising candidate for preventing obesity-related diabetes.

Liver health is also negatively impacted by weight gain, as excess fat accumulation or fatty liver leads to liver dysfunction, which is mainly reflected by elevated SGOT and SGPT enzyme levels. Main function of SGOT or SGPT is amino acid metabolism and energy production within in the cell. However, alterations or elevations in both can disrupt the metabolism of amino acids. The progesterone-treated group showed a marked increase in these enzymes, signaling potential liver damage or injury. However, administration of *Ficus benghalensis* extract resulted in a significant reduction in enzyme levels, indicating hepatoprotective properties. This suggests that the extract may help prevent liver stress and inflammation, potentially by reducing fat accumulation in the liver.

Excessive food intake is a major contributor to obesity, leading to increased fat storage. Interestingly, in this study, mice treated with *Ficus benghalensis* extract exhibited lower food consumption compared to the control group. This suggests that the extract may have appetite-suppressing effects, which could aid in weight management. The reduction in caloric intake likely contributed to the observed improvements in body weight and metabolism.

Conclusion

The study highlights the potential of *Ficus benghalensis* bark extract in managing obesity and its related complications, including elevated blood glucose levels, lipid imbalances, and impaired liver function. Notably, the extract, particularly at a higher dose, significantly reduced body weight, improved metabolic parameters, and supported liver health in progesterone-induced obese mice. The most pronounced effects were observed at 400 mg/kg body weight, indicating a possible dose-dependent relationship. These results suggest that *Ficus benghalensis* bark could be a promising natural alternative for obesity management. However, further studies are necessary to identify its active compounds, explore the underlying mechanisms, and assess its long-term safety and efficacy in human subjects. This research serves as a foundation for developing plant-based strategies to combat obesity and its associated metabolic disorders.

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Conflict of interest

The authors have no conflict of interest to declare.

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