



METHANOLIC LEAF EXTRACT OF *Abutilon indicum* ATTENUATES ADIPOSITY AND DYSLIPIDAEMIA IN HIGH- FAT DIET-INDUCED OBESE SWISS ALBINO MICE: BIOCHEMICAL AND HISTOPATHOLOGICAL EVIDENCE

Sharandeep Kaur¹, Sarbjot Singh^{2*}, Aaditya Thakur², Anita Devi², Vasundhra², Anchel Sharma², Anish Verma³, Sonika Rattan⁴

¹Department of Pharmacy, Aakash Institute of Medical Sciences, Nalagarh, Distt. Solan, Himachal Pradesh-India, ORCID ID-0009-0003-6707-432X

^{2*,2}Department of Pharmacy, Himachal Pharmacy College, Nalagarh (HP)-India

³Department of Pharmacy, Amar Shaheed Baba Ajeet Singh Jhujhar Singh Memorial College, Bela(Ropar)-India

⁴Department of Pharmacy, Institute of Pharmaceutical Sciences, IET Bhaddal, Ropar-India

***Corresponding Author:** Sarbjot Singh

^{*}Department of Pharmacy, Himachal Pharmacy College, Nalagarh (HP)-India, ORCID ID-0009-0005-8547-2043, Email- sarbjots771@gmail.com, Contact- +91-9816374847

ABSTRACT

Background: Obesity is a multifactorial metabolic disorder that rarely travels alone; it is usually accompanied by abnormal blood lipids and disturbed glucose regulation. This study asked whether a methanolic leaf extract of *Abutilon indicum* (MEAI) could limit weight gain and metabolic disturbance in mice made obese with a high-fat diet (HFD), with orlistat serving as the reference drug.

Methods: Leaves of *A. indicum* were extracted with methanol in a Soxhlet apparatus. The extract was screened phytochemically and its total flavonoid and total phenolic contents were measured. Swiss albino mice were divided into five groups (n = 6 per group): normal control, HFD control, orlistat (10 mg/kg), MEAI (200 mg/kg) and MEAI (400 mg/kg). Treatments were given orally from day 22 to day 49 of a 49-day HFD protocol. We followed body weight, feed intake, body mass index (BMI), Lee index, serum biochemistry, organ weights and histopathology.

Results: The extract yield was 22% w/w. Preliminary screening showed alkaloids, flavonoids, tannins, phenols, terpenoids, saponins, steroids and glycosides. Total flavonoid and phenolic contents were 34.92 ± 1.161 mg QE/g and 207.40 ± 2.347 mg GAE/g of crude extract, respectively. At week 7, MEAI-treated mice weighed 28% (200 mg/kg) and 37% (400 mg/kg) less than HFD controls, and the lipid profile moved in the same favourable direction. In general, the 400 mg/kg dose outperformed the 200 mg/kg dose. Liver and mesenteric fat-pad histology also showed less marked HFD-related change in treated animals.

Conclusion: MEAI appears to have anti-obesity and lipid-modulating activity in this mouse model. Before any claim about therapeutic value can be made, the findings need confirmation with fully documented statistics, a chemically standardized extract and a proper safety assessment.

Key words: *Abutilon indicum*; obesity; high-fat diet; methanolic extract; lipid profile; orlistat

1. INTRODUCTION

Obesity has moved from being a problem of wealthy societies to one of the defining health challenges of the century. Excess adipose tissue is not a passive store of energy. It is metabolically active, and when it expands beyond a certain point it changes how the body handles lipids, glucose and inflammation, which is why obesity sits at the root of dyslipidaemia, insulin resistance, type 2 diabetes, cardiovascular disease and fatty liver disease [1,2]. The numbers are striking. Between 1980 and 2013 the proportion of adults with a BMI of 25 kg/m² or more rose from roughly 29% to between 37% and 38% worldwide, and the rise has not been confined to high-income countries [3]. The way obesity develops is rarely simple. Genetic susceptibility, early-life environment, diet quality, physical inactivity and the biology of appetite all contribute, and their relative weight differs from one person to the next [4,5]. One consequence is that no single intervention works for everyone. Another is that the metabolic fallout of excess fat often outlasts the excess weight itself. Fat that accumulates in the liver, for instance, is closely linked to insulin resistance and the metabolic syndrome, and in some people it progresses from simple steatosis to steatohepatitis [6,7]. Lifestyle change, meaning a lower energy intake and more physical activity, remains the foundation of obesity management. When that is not enough, drug treatment may be added [8]. Choices are limited, though. Orlistat, one of the longest-established agents, inhibits gastric and pancreatic lipases and so reduces the absorption of dietary fat [9], but the weight loss it produces over and above placebo is modest, in the order of a few kilograms over a year, and gastrointestinal side effects lead many patients to stop taking it [10]. Other agents have been withdrawn or restricted because of safety concerns [11]. It is this gap between what patients need and what current drugs deliver that keeps drawing researchers back to natural products.

Plants have supplied medicine for most of human history, and they continue to supply chemical scaffolds that synthetic chemistry would not easily imagine. A number of plant extracts and isolated compounds have been reported to act on obesity through several routes: blocking the digestion and absorption of dietary fat, suppressing appetite, increasing energy expenditure, or interfering with the differentiation and growth of fat cells [12,13]. Pancreatic lipase inhibition alone has attracted considerable interest, with polyphenols, saponins and terpenoids among the compound classes repeatedly implicated [14]. Dietary polyphenols have also been linked to better lipid handling and lower adipose inflammation in experimental models [15]. At the same time, the clinical evidence for most herbal anti-obesity preparations is thin, and the quality of the trials is uneven [16]. Plant-derived does not mean safe or effective, and each candidate has to earn its place through careful testing.

Abutilon indicum (L.) Sweet, known in English as Indian mallow, is a member of the Malvaceae that grows widely across the tropical and subtropical belts of Asia. It has a long history in traditional medicine, where different parts of the plant are used for inflammatory conditions, urinary complaints, fever, and as a general tonic [17]. Laboratory work has since begun to catch up with the folklore. Leaf extracts have been reported to lower blood glucose in rats [18], and extracts of the plant have shown protective effects in experimental liver injury [19]. Phytochemical investigations describe flavonoids, alkaloids, tannins and glycosides among its constituents [17,18]. Given that these are the very classes of compounds associated with lipid-lowering and insulin-sensitizing activity elsewhere, it seemed reasonable to ask whether the leaf extract could also influence obesity. Reports that test this directly in a diet-induced model are, as far as we are aware, scarce.

The present study therefore set out to evaluate the anti-obesity potential of the methanolic leaf extract of *A. indicum* (MEAI) in Swiss albino mice fed a high-fat diet. We characterized the extract phytochemically, treated established obesity (rather than preventing it) at two dose levels, and

compared the outcome with orlistat. The endpoints were body weight, feed intake, BMI, Lee index, serum lipids, glucose, insulin and liver enzymes, organ weights, and liver and mesenteric fat histology.

2. MATERIALS AND METHODS

2.1 Plant collection, authentication, and extraction

Leaves of *A. indicum* were collected locally from Rupnagar, Punjab, India, during January–February 2019. The plant was authenticated by the Herbarium Assistant, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, India (reference no. 1924; 25 February 2019). The leaves were washed, shade-dried for 5–10 days and ground to a coarse powder. Fifty grams of the powder were extracted with 250 mL of methanol in a Soxhlet apparatus at approximately 55–60°C for 15–18 h, which corresponds to about eight siphon cycles. Methanol was chosen because it dissolves a wide range of polar and moderately polar constituents, including phenolics, flavonoid glycosides and alkaloids, and Soxhlet extraction is a well-established way of exhausting plant material with it [20]. The solvent was then removed and the extract concentrated on a rotary evaporator. The dried extract was kept in an airtight container under refrigeration until use. The crude extract yield was 22% w/w.

2.2 Preliminary phytochemical screening and phenolic/flavonoid estimation

Qualitative tests were carried out by standard procedures [21,22] for alkaloids, flavonoids, tannins, phenols, terpenoids, steroids, saponins, glycosides, proteins and carbohydrates. Thin-layer chromatography (TLC) was run on silica gel-G plates with a chloroform:methanol solvent system, and the spots were visualized with iodine vapour. Total flavonoid content was estimated by the aluminium chloride colorimetric approach, which is the usual method for this purpose [23], using quercetin as the reference standard and reading absorbance at 510 nm. Total phenolic content was measured with the Folin–Ciocalteu reagent [24], using gallic acid as the standard and reading absorbance at 650 nm. Results are given as mg quercetin equivalents (QE)/g and mg gallic acid equivalents (GAE)/g of crude extract, respectively.

2.3 Experimental animals and ethical considerations

Swiss albino mice of either sex, weighing 25–30 g, were housed in the departmental animal facility under a 12 h light/dark cycle and controlled conditions (temperature 25°C). Standard chow or HFD and water were available ad libitum. The animal work followed the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with the registration identifier 874/CPCSEA/Po/Pe/2005. [Authors to insert the specific Institutional Animal Ethics Committee (IAEC) approval number and date.] Reporting of the animal experiments was organized along the lines recommended in the ARRIVE guidelines [25].

2.4 High-fat diet and experimental design

Rodents fed a diet rich in fat gain weight, accumulate visceral fat, and develop dyslipidaemia and impaired glucose handling, which makes the HFD model a reasonable, widely used (if imperfect) approximation of human obesity [26,27]. The study lasted 49 days, and the five groups, each of six mice, were organized as follows. Group I (normal control) received 0.9% sodium chloride and a standard diet. Group II (HFD control) received the high-fat diet throughout. Groups III to V received the HFD together with orlistat (10 mg/kg), MEAI 200 mg/kg or MEAI 400 mg/kg, respectively, by the oral route from day 22 to day 49. In other words, the animals were allowed three weeks of HFD to establish obesity, and treatment was then started while the diet continued. This is closer to the clinical situation than giving the test drug from the first day. Two dose levels of MEAI, one twofold higher than the other, were used so that dose dependence could be assessed. The composition of the HFD and the group allocation are summarized in Table 1.

Table 1. High-fat diet composition and experimental groups

Section / group	Composition or treatment
HFD: fat	58%
HFD: protein	12.4%
HFD: carbohydrate	17%
HFD: lard	11%
HFD: cholesterol	1%
HFD: vitamins and minerals	0.6%
Group I: normal control	0.9% NaCl, 10 mL/kg orally; standard diet for 49 days
Group II: HFD control	HFD throughout the 49-day study
Group III: reference treatment	HFD + orlistat 10 mg/kg orally, days 22–49
Group IV: test treatment	HFD + MEAI 200 mg/kg orally, days 22–49
Group V: test treatment	HFD + MEAI 400 mg/kg orally, days 22–49

2.5 Outcome measures

Body weight was recorded weekly, and feed intake was measured and expressed as g/week. BMI and Lee index were calculated over the study period. In rodents, BMI is conventionally derived from body weight and naso-anal length (g/cm²) [28], whereas the Lee index relates the cube root of body weight to naso-anal length and correlates with carcass fat content [29]. Rectal body temperature was measured with a digital tele-thermometer. Serum total cholesterol, triglycerides, HDL, LDL, VLDL, glucose, fasting insulin, SGOT/AST and SGPT/ALT were measured with commercial diagnostic kits. Liver, kidney and mesenteric fat-pad weights were recorded, and liver and mesenteric fat tissue was examined histologically.

2.6 Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM), generally with $n = 6$ per group. Group comparisons were made by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test, in which each treated group is compared with a single control group [30]. Exact P values and the significance annotations from the original statistical output were not available for reproduction here, so the tables report descriptive values and the text describes direction and size of change rather than formal significance.

3. RESULTS AND DISCUSSION

3.1 Extract yield and phytochemical profile

The methanolic extract yield was 22% w/w, which is a fairly generous return from dried leaf material and is consistent with the polar nature of the solvent. Qualitative screening showed alkaloids, flavonoids, tannins, phenols, terpenoids, saponins, steroids and glycosides, whereas proteins and carbohydrates were absent (Table 2). The measured total flavonoid content was 34.92 ± 1.161 mg QE/g and the total phenolic content was 207.40 ± 2.347 mg GAE/g of crude extract.

Two things stand out. First, the phenolic content is roughly six times the flavonoid content, so a

large share of the phenolic fraction must come from compounds other than flavonoids, plausibly tannins and phenolic acids, which the screening also detected. Second, a phenolic content above 200 mg GAE/g makes this a polyphenol-rich extract, and polyphenols are the compound class most often invoked to explain the anti-obesity activity of plant preparations, whether through inhibition of digestive lipases, effects on adipocyte differentiation, or reduction of inflammation [13–15]. That said, qualitative colour tests are non-specific and tell us only that a class is present [20]. These results confirm the presence of phenolic and flavonoid constituents in MEAI, but they cannot tell us which compounds, if any, are responsible for the biological effects described below.

Table 2. Phytochemical screening and total phenolic/flavonoid content

Parameter	Finding
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Phenols	Present
Terpenoids	Present
Saponins	Present
Steroids	Present
Glycosides	Present
Proteins	Absent
Carbohydrates	Absent
Total flavonoid content	34.92 ± 1.161 mg QE/g crude extract
Total phenolic content	207.40 ± 2.347 mg GAE/g crude extract

3.2 Body weight

Mice in the normal control group gained weight slowly and steadily, from 25.70 ± 0.295 g to 27.25 ± 0.147 g over seven weeks, a rise of about 6%. The HFD group followed a very different course. Its mean body weight climbed from 25.43 ± 0.221 g at baseline to 37.03 ± 0.302 g at week 7, a gain of about 11.6 g or 46%, which confirms that the diet produced marked obesity (Table 3).

Once treatment began, the picture changed. In the MEAI 200 mg/kg group, body weight fell from 30.05 ± 0.172 g in week 3 to 26.78 ± 0.199 g in week 7, a drop of about 11%. In the MEAI 400 mg/kg group the fall was steeper, from 29.71 ± 0.113 g to 23.33 ± 0.156 g, or about 21%. Relative to the HFD controls at week 7, that is 28% and 37% lower body weight for the two doses. The orlistat group also declined after week 3, from 30.10 ± 0.165 g to 20.48 ± 0.221 g.

A few things about these numbers are worth pointing out. The weight loss in the MEAI 400 mg/kg and orlistat groups did not simply return the animals to their pre-treatment trajectory; by week 7 both groups were lighter than at the start of the experiment (23.33 g against 25.65 g, and 20.48 g against 25.55 g). For animals that were still on a high-fat diet this is a large effect. It could reflect a genuine loss of fat mass, but it could equally reflect reduced food intake, malabsorption, or some degree of general toxicity, and body weight alone cannot separate these possibilities. The orlistat pattern in particular is steeper than one would normally expect from a lipase inhibitor in mice, which is one reason the underlying records are worth rechecking.

Table 3. Weekly body weight of mice (g; mean $\pm$ SEM, n = 6)

Group	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Normal control	25.70 $\pm$ 0.295	25.55 $\pm$ 0.184	25.96 $\pm$ 0.261	26.11 $\pm$ 0.229	26.36 $\pm$ 0.206	26.60 $\pm$ 0.194	26.81 $\pm$ 0.177	27.25 $\pm$ 0.147
HFD	25.43 $\pm$ 0.221	26.70 $\pm$ 0.188	28.03 $\pm$ 0.196	29.81 $\pm$ 0.215	31.16 $\pm$ 0.183	33.20 $\pm$ 0.196	35.16 $\pm$ 0.423	37.03 $\pm$ 0.302
Orlistat	25.55 $\pm$ 0.184	26.85 $\pm$ 0.204	28.41 $\pm$ 0.164	30.10 $\pm$ 0.165	27.31 $\pm$ 0.147	24.93 $\pm$ 0.197	22.80 $\pm$ 0.195	20.48 $\pm$ 0.221
MEAI 200 mg/kg	25.50 $\pm$ 0.157	26.83 $\pm$ 0.204	28.40 $\pm$ 0.139	30.05 $\pm$ 0.172	29.28 $\pm$ 0.164	28.53 $\pm$ 0.174	28.23 $\pm$ 0.350	26.78 $\pm$ 0.199
MEAI 400 mg/kg	25.65 $\pm$ 0.266	26.78 $\pm$ 0.199	28.03 $\pm$ 0.142	29.71 $\pm$ 0.113	28.08 $\pm$ 0.149	26.55 $\pm$ 0.168	24.95 $\pm$ 0.199	23.33 $\pm$ 0.156

The week-3 value for the MEAI 200 mg/kg group appeared as “30..05” in the source table and has been read as 30.05.

3.3 Feed intake, BMI, and Lee index

The HFD group ate more than the normal control group throughout the study, between roughly 26 and 28 g/week against 22 to 24 g/week for controls. Among the treated groups, feed intake in the MEAI 400 mg/kg animals fell gradually over the treatment period and reached 24.00 $\pm$ 0.22 g/week by week 7, about 14% below the HFD group. The 200 mg/kg group showed a smaller decline (25.60 $\pm$ 0.52 g/week, about 8% below HFD), and the orlistat group the largest (22.10 $\pm$ 0.32 g/week, about 20% below HFD).

BMI and Lee index told a consistent story. Both rose progressively in the HFD group, with BMI reaching 1.026 $\pm$ 0.008 and Lee index 0.392 $\pm$ 0.001 at week 7. Both MEAI doses held these values below the HFD group, with the larger reduction at 400 mg/kg: week-7 BMI was about 28% lower than HFD at 200 mg/kg and about 38% lower at 400 mg/kg, and the Lee index was about 10% and 15% lower, respectively (Table 4). The Lee index is generally regarded as a reasonable surrogate for adiposity in rodents [29], so its fall in treated animals fits the lower organ and fat-pad weights reported below.

It would be tempting to attribute the weight response entirely to reduced eating. The data cannot settle the question either way. Intake fell in the treated groups, yet the drop in body weight (28–37%) was much larger than the drop in intake (8–14%), which suggests that something more than appetite is involved. Resolving this properly would require a pair-feeding design or energy-balance measurements, neither of which was part of this study.

Table 4. Feed intake, BMI, and Lee index during the study (mean $\pm$ SEM, n = 6)

Measure	Week	Control	HFD	Orlistat	MEAI 200 mg/kg	MEAI 400 mg/kg
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Measure	Week	Control	HFD	Orlistat	MEAI 200 mg/kg	MEAI 400 mg/kg
Feed intake (g/week)	1	23.15±0.68	27.34±0.41	25.97±0.21	26.00±0.72	27.05±0.37
Feed intake (g/week)	2	23.84±0.64	26.04±0.51	26.94±0.40	27.05±0.25	26.68±0.32
Feed intake (g/week)	3	23.57±0.61	26.90±0.38	27.63±0.28	27.38±0.39	27.73±0.25
Feed intake (g/week)	4	22.52±0.51	27.85±0.45	24.00±0.44	26.73±0.33	25.75±0.15
Feed intake (g/week)	5	23.95±0.37	28.14±0.31	23.60±0.43	26.01±0.49	25.10±0.34
Feed intake (g/week)	6	22.64±0.64	27.71±0.30	23.45±0.31	26.36±0.20	24.60±0.35
Feed intake (g/week)	7	23.01±0.66	27.77±0.33	22.10±0.32	25.60±0.52	24.00±0.22
BMI	0	0.708±0.006	0.706±0.006	0.704±0.005	0.703±0.004	0.704±0.005
BMI	1	0.715±0.006	0.744±0.008	0.742±0.005	0.742±0.005	0.739±0.005
BMI	2	0.719±0.006	0.778±0.006	0.787±0.004	0.785±0.004	0.776±0.003
BMI	3	0.724±0.005	0.824±0.006	0.833±0.004	0.831±0.004	0.822±0.003
BMI	4	0.720±0.005	0.865±0.0067	0.756±0.003	0.809±0.005	0.778±0.003
BMI	5	0.735±0.004	0.923±0.006	0.688±0.004	0.786±0.005	0.734±0.005
BMI	6	0.740±0.004	0.978±0.010	0.629±0.005	0.767±0.005	0.689±0.005
BMI	7	0.749±0.004	1.026±0.008	0.565±0.006	0.740±0.005	0.638±0.004
Lee index	0	0.347±0.009	0.344±0.001	0.346±0.009	0.345±0.007	0.346±0.009
Lee index	1	0.347±0.007	0.352±0.001	0.360±0.008	0.352±0.008	0.360±0.007
Lee index	2	0.348±0.008	0.357±0.009	0.358±0.007	0.358±0.007	0.356±0.006
Lee index	3	0.349±0.009	0.364±0.009	0.365±0.007	0.365±0.008	0.362±0.009
Lee index	4	0.349±0.001	0.370±0.009	0.354±0.006	0.362±0.008	0.357±0.0012
Lee index	5	0.351±0.001	0.378±0.008	0.343±0.007	0.358±0.008	0.349±0.007
Lee index	6	0.351±0.001	0.385±0.001	0.333±0.009	0.355±0.008	0.342±0.009
Lee index	7	0.353±0.009	0.392±0.001	0.314±0.004	0.352±0.008	0.333±0.008

Note: BMI was reported in the original records with the unit g/cm², and the same values are reproduced here. Statistical annotations were not available for these outcomes.

3.4 Serum biochemical parameters and liver enzymes

Feeding the HFD produced the metabolic disturbance we expected (Table 5). Compared with normal controls, HFD mice had about 57% higher total cholesterol, 108% higher triglycerides, 78% higher VLDL and a more than five-fold higher LDL (125.33 versus 24.33 mg/dL), while HDL was cut by more than half (26.66 versus 58.33 mg/dL). Fasting glucose rose by about 52% and fasting insulin more than tripled (2.50 versus 0.80 µg/L), indicating a degree of insulin resistance, a combination that mirrors the human metabolic syndrome [2]. The liver enzymes SGOT/AST and SGPT/ALT were also higher by roughly 79% and 100%, a sign of hepatocellular stress in line with fat accumulation in the liver [6].

MEAI moved every one of these variables in the right direction. Relative to the HFD group, MEAI at 400 mg/kg reduced total cholesterol by about 23%, triglycerides by 35%, LDL by 56% and VLDL by 30%, and raised HDL by roughly 85%. The 200 mg/kg dose produced smaller changes of about 13%, 16%, 41% and 11% for the same four lipid measures, with HDL up by 46%. For comparison, orlistat lowered total cholesterol by 29%, triglycerides by 39% and LDL by 68%. On the lipid profile, then, the higher dose of MEAI came reasonably close to the reference drug, notably for HDL (49.33 against 51.66 mg/dL) and triglycerides (136 against 126 mg/dL).

The glucose and insulin results were more uneven. Glucose fell by about 22% at 200 mg/kg and 24% at 400 mg/kg, a very small difference between doses, while orlistat lowered it by 50%. Insulin showed a clearer dose response, with reductions of about 22% and 43% for the two MEAI doses and 57% for orlistat. The glucose-lowering tendency is in keeping with the earlier report of hypoglycaemic activity in leaf extracts of this plant [18]. Liver enzymes also improved: SGOT/AST fell by 14% (200 mg/kg) and 31% (400 mg/kg), and SGPT/ALT by 12% and 29%, which fits with the hepatoprotective activity previously described for the plant [19]. One of the table entries, the SEM of the orlistat LDL value, is clearly mistyped in the source (see the note under Table 5) and should be confirmed from the raw data.

Table 5. Serum biochemical and liver enzyme parameters (mean $\pm$ SEM)

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Glucose (mg/dL)	Insulin (μ g/L)	SGOT/ AST (U/L)	SGPT/A LT (U/L)
Contro l	75 $\pm$ 1.732	100 $\pm$ 1.764	58.33 $\pm$ 1.202	24.33 $\pm$ 1.667	24.66 $\pm$ 2.404	70 $\pm$ 2.887	0.80 $\pm$ 0.057 7	38.34 $\pm$ 1.202	32.32 $\pm$ 1.202
HFD	118 $\pm$ 1.732	208 $\pm$ 2.082	26.66 $\pm$ 1.453	125.33 $\pm$ 0.881	44 $\pm$ 2.082	106.66 $\pm$ 4.910	2.50 $\pm$ 0.115 5	68.67 $\pm$ 2.028	64.66 $\pm$ 1.764
Orlistat	84.33 $\pm$ 1.764	126 $\pm$ 2.082	51.66 $\pm$ 2.186	39.66 $\pm$ 1856†	29.33 $\pm$ 1.453	53 $\pm$ 3.464	1.066 $\pm$ 0.185 6	43.66 $\pm$ 2.333	42.66 $\pm$ 0.881
MEAI 200 mg/kg	102.66 $\pm$ 1.856	175 $\pm$ 1.732	39 $\pm$ 2.082	74.33 $\pm$ 0.881	39 $\pm$ 1.528	83.66 $\pm$ 1.453	1.96 $\pm$ 0.120 2	59.33 $\pm$ 1.453	57 $\pm$ 0.577
MEAI 400 mg/kg	91.33 $\pm$ 1.453	136 $\pm$ 2.309	49.33 $\pm$ 1.453	55.33 $\pm$ 1.764	31 $\pm$ 2.082	81 $\pm$ 2.082	1.43 $\pm$ 0.176 4	47.66 $\pm$ 1.202	46 $\pm$ 1.732

† The source lists the orlistat LDL result as “39.66 $\pm$ 1856”; this is probably a typographical error and is reproduced as printed. TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; SGOT/AST, aspartate aminotransferase; SGPT/ALT, alanine aminotransferase.

3.5 Organ weights and histopathological observations

HFD feeding was accompanied by heavy organs. Compared with normal controls, liver weight was about 166% higher (7.26 versus 2.73), mesenteric fat-pad weight about 185% higher (2.05 versus 0.72), and kidney weights about 150% higher (Table 6). Treatment brought these values down. MEAI at 400 mg/kg reduced liver weight by about 36% and fat-pad weight by about 33% relative to HFD, against reductions of about 11% and 15% at 200 mg/kg, while orlistat reduced both by about 47%.

Kidney weight was the exception to the dose-response pattern. The right kidney weighed 0.65 in the 200 mg/kg group but 0.80 in the 400 mg/kg group, and the left kidney followed suit (0.66 and 0.82). So the lower dose reduced kidney weight more than the higher one. Because no renal function markers such as serum creatinine or urea were measured, it is not possible to say whether this reflects chance, a genuine difference in renal response, or an early sign of dose-related stress on the kidney. This deserves follow-up before the higher dose is pursued any further. The units for organ weights were also not stated in the source records.

Table 6. Organ and mesenteric fat-pad weights (mean $\pm$ SEM; units not specified in source)

Parameter	Control	HFD	Orlistat	MEAI 200 mg/kg	MEAI 400 mg/kg
Liver	2.73 $\pm$ 0.10	7.26 $\pm$ 0.136	3.82 $\pm$ 0.155	6.50 $\pm$ 0.164	4.64 $\pm$ 0.180
Right kidney	0.387 $\pm$ 0.01	0.964 $\pm$ 0.058	0.51 $\pm$ 0.01	0.65 $\pm$ 0.03	0.80 $\pm$ 0.03
Left kidney	0.397 $\pm$ 0.02	0.97 $\pm$ 0.02	0.52 $\pm$ 0.01	0.66 $\pm$ 0.01	0.82 $\pm$ 0.04
Mesenteric fat pad	0.72 $\pm$ 0.02	2.05 $\pm$ 0.06	1.08 $\pm$ 0.08	1.74 $\pm$ 0.03	1.37 $\pm$ 0.12

The histology agreed with the organ weights. In the liver of HFD-fed mice, the sections showed microvesicular fatty change and cellular injury (Figure 1). Orlistat-treated mice showed marked protection, and MEAI at 400 mg/kg showed a more pronounced improvement than MEAI at 200 mg/kg. Microvesicular steatosis of this kind is a characteristic early feature of diet-induced fatty liver, driven by the excess delivery of fatty acids to hepatocytes and their incomplete disposal [7]. Its partial reversal here supports the idea that the extract acts on the underlying metabolic disturbance and not just on body weight.

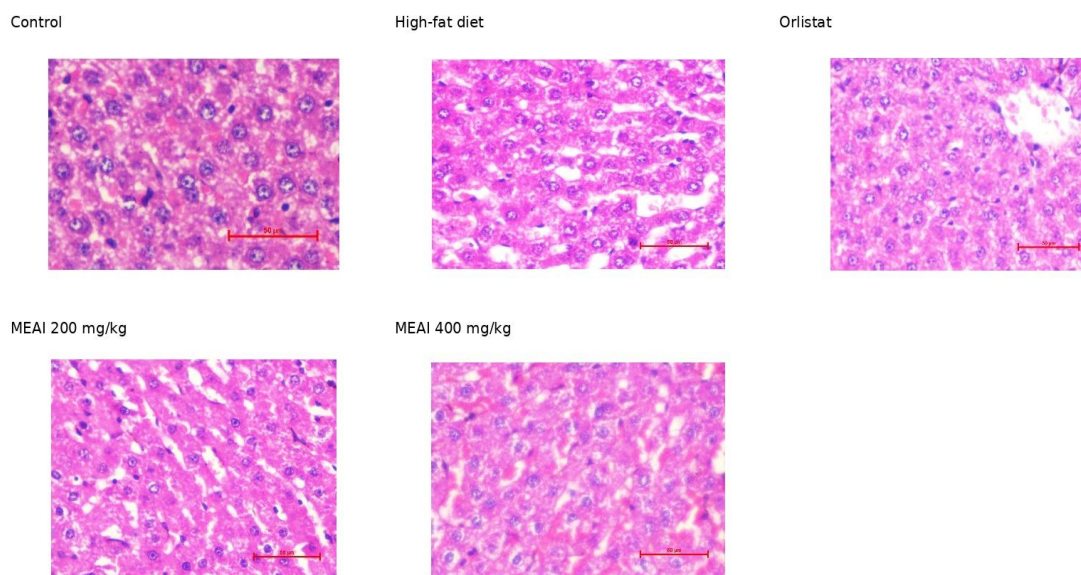


Figure 1. Representative liver histology panels: normal control, HFD control, orlistat, MEAI 200 mg/kg and MEAI 400 mg/kg. Panels, left to right and top to bottom: normal control, HFD control, orlistat, MEAI 200 mg/kg, MEAI 400 mg/kg.

In mesenteric fat, HFD feeding was associated with enlarged lipid droplets and reduced membrane integrity, whereas the treated groups showed smaller cells and less fat accumulation (Figure 2). Mesenteric depots are of particular interest because visceral fat, more than subcutaneous fat, tracks with insulin resistance and cardiometabolic risk [2]. A reduction in the size of adipocytes and in the

weight of this depot is therefore a meaningful finding, provided it is confirmed by quantitative morphometry rather than description alone.

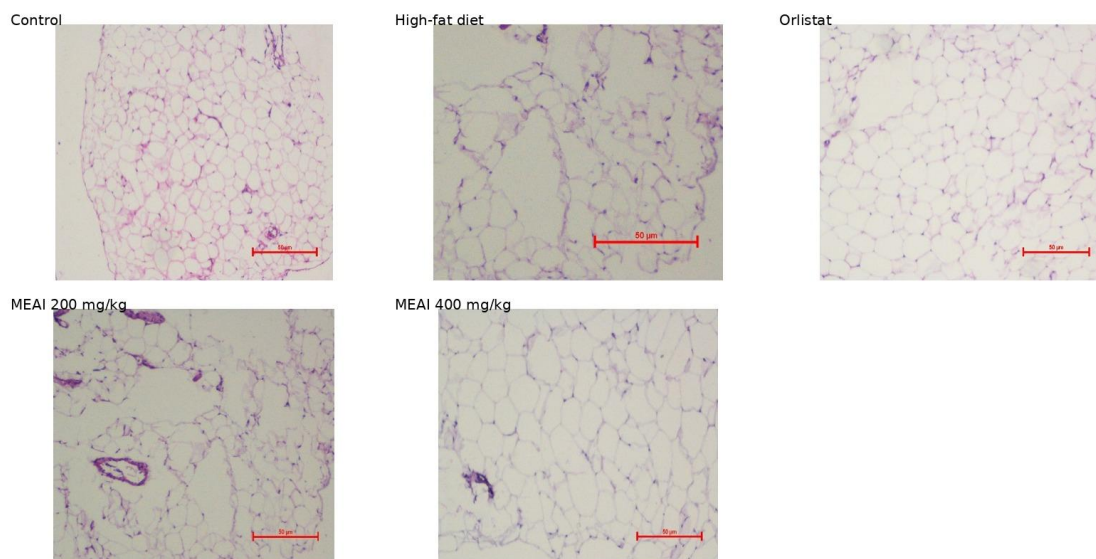


Figure 2. Representative mesenteric fat-pad histology panels: normal control, HFD control, orlistat, MEAI 200 mg/kg and MEAI 400 mg/kg. Panels, left to right and top to bottom: normal control, HFD control, orlistat, MEAI 200 mg/kg, MEAI 400 mg/kg.

3.6 Possible mechanisms of action

This study was designed to ask whether MEAI works, not how it works, so what follows is informed speculation rather than a conclusion. Still, the pattern of results points toward several plausible mechanisms that are not mutually exclusive.

The first is interference with fat digestion and absorption. Orlistat acts by blocking lipases in the gut lumen [9], and many plant polyphenols, tannins and saponins have been shown to inhibit pancreatic lipase in vitro [14]. MEAI is rich in phenolics and contains tannins and saponins, so a lipase-inhibitory component is a reasonable hypothesis. The fact that MEAI and orlistat moved the lipid profile in the same direction, and that both reduced weight, is compatible with this idea, though it does not prove it.

A second route is through effects on adipose tissue and lipid metabolism. Dietary polyphenols have been reported to restrain adipocyte differentiation, enhance fatty-acid oxidation and dampen the low-grade inflammation that accompanies fat expansion [13,15]. The smaller adipocytes and lighter mesenteric fat pads in MEAI-treated mice would fit with such effects.

A third is improvement of insulin sensitivity. Fasting insulin was lowered in a dose-dependent way, and glucose also fell. Since obesity-related insulin resistance is a major driver of dyslipidaemia and hepatic fat accumulation [2], an improvement here could account for part of the favourable lipid and liver findings. Finally, the plant has already been shown to protect the liver against chemical injury [19], so a direct hepatoprotective contribution cannot be ruled out. Each of these hypotheses is testable, through lipase inhibition assays, gene and protein expression in adipose tissue and liver, glucose and insulin tolerance tests, and measurement of faecal fat.

3.7 Dose dependence and comparison with orlistat

For most endpoints, 400 mg/kg did better than 200 mg/kg. This was true for body weight, BMI, Lee index, cholesterol, triglycerides, HDL, LDL, VLDL, insulin, liver enzymes, liver weight and fat-pad weight. A graded response of this kind is one of the more persuasive features of a pharmacological

effect, because it is hard to explain by chance or by a non-specific artefact. The exceptions, namely glucose, which was almost the same at both doses, and kidney weight, which ran the other way, are worth noting and should not be glossed over.

Orlistat, as might be expected of an established drug, was more potent than MEAI on most measures, and the difference was largest for glucose, insulin and body weight. The comparison is not entirely like for like, however. Orlistat is a purified single compound given at 10 mg/kg, while MEAI is a crude mixture whose active constituents are unknown. A crude extract that approaches the activity of a pure drug on several lipid measures, even at a considerably higher dose by mass, is interesting enough to justify fractionation and the search for the responsible compounds. Equally, the very large weight reduction in the orlistat group relative to what lipase inhibition alone would be expected to achieve is a reminder that the underlying data should be rechecked before anyone reads too much into them.

3.8 Strengths and limitations

The study has several strengths. It used a recognized diet-induced model, included both a vehicle-treated obese group and a reference drug, tested two doses, started treatment after obesity was established, and collected a broad panel of outcomes, from body weight through serum biochemistry to tissue histology. Agreement among these independent measures makes the overall finding more credible than any single endpoint would.

The study also has limitations that readers should keep in mind. Group sizes were small ($n = 6$), mice of either sex were used without separate analysis, and the source records describe the number of animals per group slightly differently in different places, so $n = 6$ has been adopted throughout. The exact P values were not available, and the tables do not carry significance markers. The extract was characterized only by colour tests and two colorimetric assays, with no chromatographic fingerprint or marker compound, so batch-to-batch reproducibility cannot be assured. Energy intake was recorded as mass of feed rather than calories, there was no pair-fed group, and body composition was assessed indirectly through organ weights and indices rather than by imaging or carcass analysis. Rectal temperature was measured according to the protocol, but the values were not available for presentation. Safety was assessed only through liver enzymes and organ weights, with no renal function tests, haematology or acute and sub-chronic toxicity study. Finally, units were missing for organ weights, and a few table entries contained evident typographical errors, which have been flagged where they occur and should be corrected against the raw laboratory records.

Perhaps most importantly, these are results in mice. Mouse models of diet-induced obesity are useful for screening, but they differ from human obesity in physiology, in the time scale of weight gain and in how drugs are handled by the body, and many compounds that work in rodents fail in people [8,27]. These findings cannot simply be extrapolated to humans.

3.9 Translational outlook and future directions

For MEAI to move from an interesting extract to a credible lead, several steps are needed. The first is chemical: standardizing the extract against marker compounds by HPLC or LC-MS, and ideally fractionating it by bioassay-guided methods to find out which constituents carry the activity. The second is mechanistic: lipase inhibition assays, studies of adipogenesis in cell culture, and analysis of lipid and glucose metabolism genes in liver and fat. The third is safety: acute and repeated-dose toxicity studies, with renal and haematological end points, are essential, particularly in view of the kidney observation and the very large weight reduction in the higher dose group. The fourth is design: larger groups, single-sex cohorts or sex-stratified analysis, pair-feeding, direct energy-expenditure and body-composition measurements, and full reporting of the statistics in the manner recommended by the ARRIVE guidelines [25].

It is also worth keeping in mind the benchmark that any anti-obesity candidate will eventually face. Approved drugs produce modest but sustained weight loss with an acceptable safety profile [8,10]. A plant extract that causes rapid, large weight loss in mice is therefore not automatically a good sign; what matters is whether the effect is real, durable and safe. Used with that caution, the present results offer a useful starting point for more rigorous work on *A. indicum*.

4. CONCLUSION

The methanolic leaf extract of *Abutilon indicum* showed anti-obesity and lipid-modulating effects in mice exposed to a high-fat diet. Treatment with MEAI, especially at 400 mg/kg, was associated with lower body weight, BMI and Lee index, better serum lipid, glucose, insulin and liver enzyme values, lighter liver and mesenteric fat pads, and less marked histological change in liver and fat tissue compared with untreated HFD mice. The extract is rich in phenolic compounds, which may contribute to these effects. The results are encouraging but preliminary. Confirmation with complete statistics, a standardized extract, mechanistic studies and a thorough safety evaluation is needed before the therapeutic relevance of *A. indicum* in obesity can be judged.

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Conflict of Interest

The authors declare no conflict of interest. [To be confirmed by all authors.]

Ethics Statement

The animal experiments followed CPCSEA guidelines (identifier 874/CPCSEA/Po/Pe/2005). [The specific IAEC approval number and date should be inserted, and the authors should confirm that the approval covered the protocol described here.]

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