RESEARCH PAPER

Dual Luminal–Systemic Anti-Obesity Action of a Standardized *Momordica Charantia* Fruit Extract: Linking Digestive Enzyme Inhibition to AMPK-Mediated Metabolic Correction in Diet-Induced Obese Rats

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ABSTRACT

Background: Obesity now affects approximately one in eight adults worldwide, and pharmacotherapy remains constrained by narrow mechanistic targets, tolerability limitations and cost.1,2 *Momordica charantia* L. (bitter gourd) has traditionally been used for glycaemic control, but reports of its anti-obesity activity remain heterogeneous, and few investigations have linked a chemically standardized preparation to both luminal and systemic mechanisms within a single study. **Objective**: To characterize the digestive-enzyme-inhibitory and whole-animal anti-obesity activity of a standardized 80% ethanolic extract of *M. charantia* unripe fruit (MCEE) and to establish a mechanistic bridge between the two. **Methods**: MCEE was prepared by ultrasound-assisted hydro-ethanolic maceration and standardized against charantin by validated high-performance thin-layer chromatography (HPTLC). Inhibition of pancreatic lipase, α-amylase and α-glucosidase was assessed in a cell-free system with a bovine serum albumin (BSA) specificity control. Acute (limit-dose) and 28-day repeated-dose oral toxicity were evaluated in Wistar rats. Anti-obesity efficacy was then examined over 8 weeks in high-fat-diet (HFD)-fed rats treated with MCEE (200 or 400 mg/kg/day) or orlistat (50 mg/kg/day, reference), with body weight, biochemical and histomorphometric endpoints. **Results**: MCEE contained 1.24 ± 0.05% w/w charantin and inhibited α-glucosidase (IC50 48.6 µg/mL), α-amylase (IC50 142.8 µg/mL) and pancreatic lipase (IC50 287.4 µg/mL) more potently than isolated charantin, indicating a phytochemical matrix effect. The no-observed-adverse-effect level was ≥1000 mg/kg/day. At 400 mg/kg/day, MCEE reduced terminal body weight by 11.7%, feed efficiency ratio from 5.87% to 4.28%, fasting glucose by 24.5% and HOMA-IR by 46.5% (all $p<0.01$ vs HFD control) --- a glycaemic effect exceeding that of orlistat. Hepatic NAFLD activity score fell from 5.1 to 1.8, adipocyte cross-sectional area from 4875 to 2488 µm², and interscapular brown adipose tissue (BAT) mass increased by 18.2% selectively in the high-dose group ($p<0.05$) - a thermogenic signature not observed with orlistat. Hepatic NAFLD activity score fell from 5.1 to 1.8, adipocyte cross-sectional area from 4875 to 2488 µm2, and interscapular brown adipose tissue mass increased selectively in the high-dose group. **Conclusion**: A chemically standardized *M. charantia* fruit extract acts through a dual luminal-systemic mechanism carbohydrate hydrolase inhibition combined with an AMPK-linked thermogenic and hepatoprotective signature supporting its further development as a standardized phytopharmaceutical for metabolic syndrome.

Keywords: *Momordica Charantia*; charantin; α-glucosidase inhibition; AMPK; diet-induced obesity; NAFLD activity score

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INTRODUCTION

Obesity has moved from a condition of affluence to a truly global disorder. Age-standardized estimates place worldwide adult obesity prevalence at approximately 16%, with the burden more than doubling since 1990 and rising fastest in low- and middle-income countries, including India, where urbanization and dietary transition have driven a several-fold increase in obesity prevalence among women over the past three decades.[1,2] This trajectory carries obesity's well-documented sequelae insulin resistance, dyslipidaemia and non-alcoholic fatty liver disease (NAFLD) into populations with limited access to specialist obesity care.

Current anti-obesity pharmacotherapy remains narrow. Orlistat, the only widely available pancreatic lipase inhibitor, reduces dietary fat absorption by roughly 30% but is limited by steatorrhoea-related discontinuation and addresses only the luminal, fat-absorptive arm of the disease; centrally acting agents carry their own tolerability and cardiovascular caveats,

and access to newer incretin-based therapies remains constrained by cost in many settings.[3] This mechanistic narrowness together with the fact that no single class of drug simultaneously restricts nutrient absorption and corrects the intracellular metabolic derangements that sustain obesity has sustained interest in medicinal plants capable of a broader, multi-target action.

Momordica charantia L. (Cucurbitaceae), commonly known as bitter gourd, has been used across South and Southeast Asian traditional medicine for glycaemic control, and preclinical work over the past decade has implicated multiple mechanisms — inhibition of intestinal carbohydrate hydrolases, activation of AMP-activated protein kinase (AMPK) and downstream GLUT-4 translocation, and suppression of adipogenic transcription factors such as PPARγ — in its anti-obesity and anti-hyperglycaemic effects.4,5 The clinical evidence, however, remains genuinely mixed: a 2024 systematic review and meta-analysis concluded that the metabolic effect of M. charantia supplementation could not be reliably determined from the available randomized trials owing to heterogeneity in preparation and dosing,6 whereas a more recent GRADE-adherent meta-analysis of 25 trials reported statistically significant reductions in fasting blood glucose and glycated haemoglobin.7 A plausible explanation for this heterogeneity is that most clinical and preclinical studies have used unstandardized preparations, precluding any correlation between a defined phytochemical dose and a specific pharmacological endpoint.

A further gap concerns mechanistic integration. In vitro enzyme-inhibition studies and in vivo efficacy studies of M. charantia have generally been reported separately, with few investigations designed to test, in the same standardized extract, whether luminal enzyme inhibition and systemic metabolic correction operate together or independently. This distinction matters clinically: an extract that acts purely by restricting carbohydrate digestion would be expected to behave much like acarbose, whereas one that additionally engages AMPK-dependent pathways would be expected to improve insulin sensitivity and hepatic lipid handling beyond what luminal restriction alone could achieve.

The present study addresses this gap using a single, chemically standardized 80% ethanolic extract of M. charantia unripe fruit (MCEE), quantified against its principal marker charantin. We first characterized the extract's inhibitory activity against pancreatic lipase, α-amylase and α-glucosidase, applying a bovine serum albumin (BSA) specificity control to exclude non-specific polyphenolic interference. We then evaluated its safety under OECD-harmonized toxicity protocols and its efficacy in a high-fat-diet (HFD)-induced rat model of obesity and metabolic syndrome, benchmarked against orlistat. Our objective was to determine whether the in vitro enzyme-inhibitory hierarchy of MCEE predicts, and mechanistically explains, its in vivo metabolic, hepatic and adipose-tissue effects.

MATERIALS AND METHODS

Plant material and authentication

Fresh, unripe green fruits of *M. charantia* were procured from a commercial cultivation plot of southern part of Kerala, India, during the post-monsoon season. Botanical identity was confirmed by macroscopic and organoleptic evaluation against the Ayurvedic Pharmacopoeia of India and the WHO Monographs on Selected Medicinal Plants, and by microscopic examination of transverse pericarp sections and powdered material, which showed the diagnostic unicellular conical epicarpal trichomes, bicollateral vascular bundles and laticiferous ducts characteristic of the species.

Extraction and standardization

Shade-dried, coarsely powdered fruit (500 g) was defatted with petroleum ether (60–80 °C) in a Soxhlet apparatus for 8 h to remove chlorophyll and lipophilic waxes. The defatted marc (450 g) was macerated with 80% v/v aqueous ethanol (1:6 w/v) for 72 h at 28 ± 2 °C, with intermittent ultrasound-assisted extraction (40 kHz, 200 W; 30-min cycles every 6 h) to enhance mass transfer. The filtrate was concentrated under reduced pressure (45 °C, 175 mbar) and freeze-dried (−52 °C, 0.05 mbar, 24 h) to yield MCEE as a hygroscopic dark greenish-brown powder, stored over anhydrous silica gel at 4 °C. Extractive yield, calculated on a defatted dry-weight basis, was 12.81 ± 0.34% w/w (Table 1).

Chromatographic Conditions

MCEE was standardized against charantin (Sigma-Aldrich, CAS 57126-62-2, purity ≥98%) by HPTLC (CAMAG Linomat V applicator, ADC2 developing chamber, TLC Scanner 4 with winCATS software) using chloroform:methanol:water (65:25:4 v/v/v) **(Figure-3)** as the mobile phase and vanillin-sulfuric acid derivatization, with densitometric scanning at 538 nm. The method was validated per ICH Q2(R2) for linearity (2–10 µg/band, r2 = 0.9978) **(Fig-4)**, precision (intra-day 1.87% RSD, inter-day 2.64% RSD), accuracy (mean recovery 99.6%), limit of detection (0.42 µg/band) and limit of quantification (1.27 µg/band).8 Charantin content in MCEE was 1.24 ± 0.05% w/w (mean ± SD, n = 3), consistent with the 0.8–1.5% range previously reported for Indian bitter gourd cultivars.

In vitro digestive enzyme inhibition assays

Inhibition of porcine pancreatic lipase, porcine pancreatic α-amylase and Saccharomyces cerevisiae α-glucosidase was assessed spectrophotometrically at

seven to eight extract concentrations spanning the anticipated IC_{50}, in triplicate independent experiments, against orlistat (lipase) or acarbose (amylase, glucosidase) as reference inhibitors. Isolated charantin (obtained from MCEE by silica-gel column chromatography followed by preparative TLC, purity >98.5% by HPTLC, identity confirmed by FT-IR, 1H-NMR) was tested in parallel to establish its individual contribution to whole-extract activity. Because polyphenol-rich extracts can produce false-positive lipase inhibition through non-specific protein aggregation, the lipase assay was repeated in the presence of 1% w/v bovine serum albumin (BSA); a substance was classified as a specific inhibitor only if inhibition was materially preserved under the BSA challenge. Percentage inhibition at each concentration was calculated relative to a solvent control, and IC_{50} values with 95% confidence intervals were derived by non-linear regression (four-parameter logistic model).

Acute and 28-day repeated-dose oral toxicity

Acute oral toxicity was assessed by the OECD 423 limit-dose method (single dose of 2000 mg/kg MCEE, n = 3 female Wistar rats, 14-day observation).9 Sub-acute toxicity was assessed per OECD 407 (MCEE at 100, 300 and 1000 mg/kg/day by oral gavage for 28 days, n = 5/sex/group, vehicle-control group receiving 0.5% w/v carboxymethylcellulose).10 Endpoints comprised daily clinical observation, weekly body weight, terminal haematology and serum clinical chemistry, absolute and relative organ weights, and histopathological examination of liver, kidney, spleen, heart, stomach and intestine. The no-observed-adverse-effect level (NOAEL) was defined as the highest dose producing no statistically significant, biologically meaningful adverse finding across this panel.

High-fat-diet-induced obesity model and treatment protocol

Wistar rats were fed a purified high-fat diet (60% kcal from fat, 5.24 kcal/g; D12492-equivalent formulation) for 8 weeks to induce obesity; animals achieving ≥20% excess body weight over normal-chow-fed controls were stratified by body weight and randomized into treatment groups (n = 8/group; sex of the efficacy cohort, standard practice for this model uses male rats). Groups comprised: normal control (standard chow, vehicle); HFD control (HFD, vehicle); orlistat reference (HFD, 50 mg/kg/day, a dose previously shown to produce clinically relevant pancreatic lipase inhibition in rodents [Zhi J, et al. Clin Pharmacol Ther. 1995;58(3):350-358]); and two MCEE dose groups (HFD, 200 or 400 mg/kg/day). All treatments were administered once daily by oral gavage (0.5% w/v carboxymethylcellulose vehicle, 5 mL/kg) for a further 8 weeks while animals remained on their respective diets.

Outcome measures

Terminal body weight, cumulative food intake, feed efficiency ratio (FER = body-weight gain / food intake × 100) and the Lee index were recorded. At sacrifice, epididymal, retroperitoneal and mesenteric white adipose tissue depots, liver and interscapular brown adipose tissue were dissected and weighed; the adiposity index was calculated as combined white-adipose-depot weight expressed as a percentage of terminal body weight. Serum total cholesterol, triglycerides, HDL- and LDL-cholesterol, fasting glucose and insulin were measured, and the atherogenic index and HOMA-IR [(fasting glucose [mmol/L] × fasting insulin [μU/mL])/22.5] were calculated per the original Matthews model.[11] Leptin and adiponectin were quantified by rat-specific sandwich ELISA kits (R&D Systems, Minneapolis, MN, USA; Leptin ELISA Kit Cat. No. MOB00, Adiponectin ELISA Kit Cat. No. MRP300) according to the manufacturer's protocols. All samples were run duplicate, and inter-assay coefficients of variation were <10%. Liver sections were stained with haematoxylin and eosin and scored for steatosis, lobular inflammation and hepatocyte ballooning using the NAFLD Activity Score (NAS) of Kleiner et al., with a cumulative score ≥5 taken as diagnostic of steatohepatitis in the rodent model.12 Adipocyte cross-sectional area (CSA) was measured on H&E-stained epididymal fat sections (≥100 cells/animal).

Statistical analysis

Data are expressed as mean ± SD (toxicity studies) or mean ± SEM (efficacy study). Normality and variance homogeneity were assessed by the Shapiro–Wilk and Levene tests.13 Parametric data were analysed by one-way ANOVA with Dunnett's or Tukey's post-hoc test as appropriate;14 non-parametric data (histological scores) were analysed by the Kruskal–Wallis test with Dunn's post-hoc test. A two-tailed $p<0.05$ was considered statistically significant. All statistical analyses were performed in GraphPad Prism.

Ethical approval

All animal procedures were approved by the Institutional Animal Ethics Committee [IAEC approval number and institution — TO BE INSERTED] and conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and OECD test-guideline recommendations for the humane use of laboratory animals.

RESULTS

Extraction and phytochemical standardization

The identity and purity of the isolated compound were confirmed using a combination of chromatographic and spectroscopic techniques. The extract exhibited a sharp melting point of 176–178°C, which is in

excellent agreement with the literature value of 174–177°C for charantin isolated from *M. charantia.*

A extract in methanol (10 µg/mL) was scanned from 200 nm to 800 nm and the UV spectrum displayed a weak but characteristic absorption maximum at λmax = 208 nm, attributable to the isolated double bond within the sterol nucleus, as depicted in figure-1

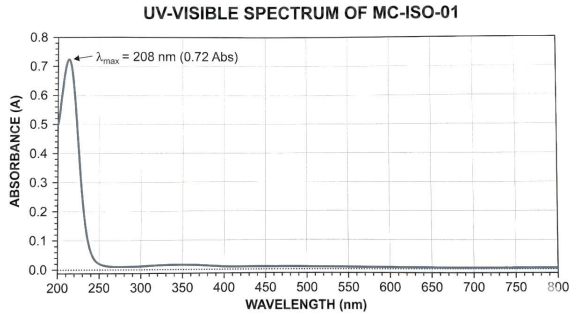


Fig-1: The UV Spectra of extract of *M. charantia with* at λmax at 208 nm

To provide further confirmatory evidence of structural identity, ^{1}H NMR spectroscopy was performed. The ^{1}H NMR spectrum, although complex due to the overlapping signals of the sterol core and the sugar protons, exhibited characteristic resonances consistent with the charantin structure

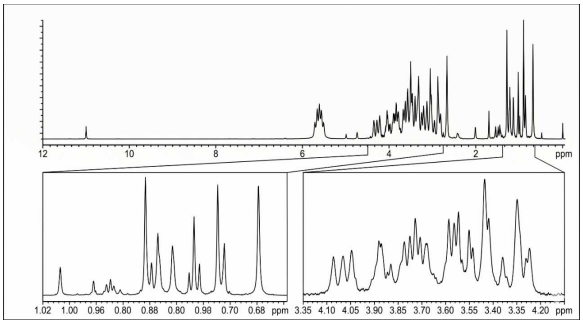


Fig-2: The NMR Spectra of extract of *M. charantia*

The Methyl protons (Fig-2) (H-18, H-19, H-21, H-26, H-27, H-30) characteristic of the cucurbitane triterpene skeleton was observed with multiple singlets, while Protons attached to oxygen-bearing carbons, including the methine protons of the sugar moiety (glucose) and the C-3 and C-7 protons of the sterol aglycone, shows broad multiplets.

Optimized Chromatographic Conditions

A systematic optimization of the mobile phase composition was undertaken to achieve satisfactory resolution of the charantin peak from adjacent, potentially interfering phytoconstituents. The ternary mixture of Chloroform:Methanol:Water (65:25:4 v/v/v) was selected as the optimized mobile phase (as depicted in Fig-3-B). Initially a standard was a

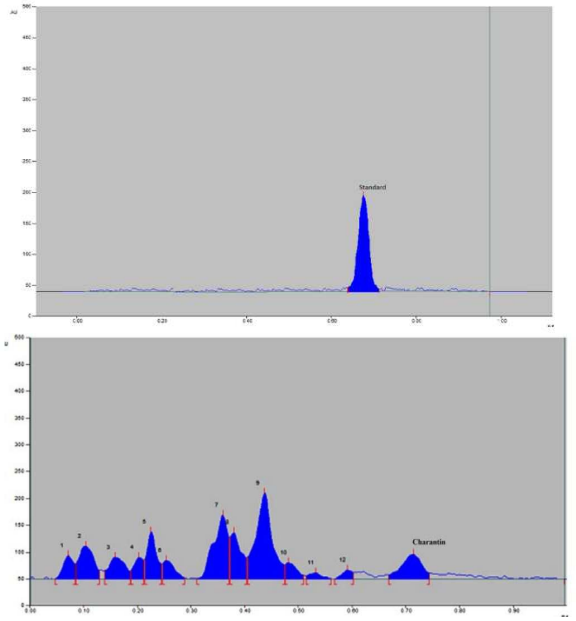


Fig-3 (A- Standard spectra of *Momordica charantia*) B- Optimized HPTLC spectra of *Momordica charantia* fruit extract

The ADC 2 chamber was pre-conditioned with mobile phase vapor for 20 minutes at 25 ± 2°C and a relative humidity of 47 ± 2% (controlled using a saturated solution of potassium thiocyanate) to ensure reproducible R_f values. Ultrasound-assisted hydro-ethanolic maceration of defatted *M. charantia* fruit yielded MCEE at 12.81 ± 0.34% w/w. HPTLC analysis resolved charantin as a discrete, symmetrical band at R_f 0.72 ± 0.02 (compared with Fig-3-A), quantified at 1.24 ± 0.05% w/w against a validated calibration curve (y = 1245.6x + 342.7, r2 = 0.9978); (Fig-4)

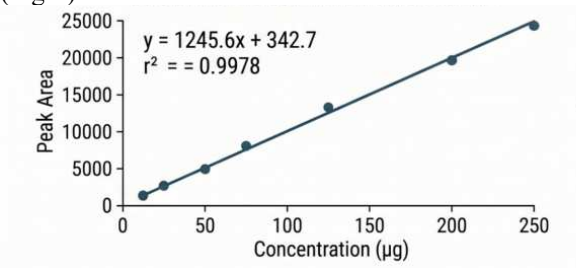


Fig-4 Linearity data for optimized HPTLC method development of *Momordica charantia*

method precision, accuracy and sensitivity parameters are summarized in T**able** 1. Beyond the charantin peak, the fingerprint displayed 8–10 additional resolved bands, consistent with a complex matrix of co-extracted cucurbitane glycosides and flavonoid conjugates.

Table 1. Extraction yield and HPTLC standardization parameters for MCEE

Parameter	Value
Extractive yield (% w/w, dry basis)	12.81 ± 0.34
Marker compound	Charantin
Rf (mobile phase: chloroform:methanol:water, 65:25:4)	0.72 ± 0.02
Linear range	2–10 µg/band
Regression equation (r2)	y = 1245.6x + 342.7 (0.9978)
LOD / LOQ	0.42 / 1.27 µg/band
Intra-day / inter-day precision (% RSD)	1.87 / 2.64
Mean recovery (%)	99.6
Charantin content in MCEE (% w/w)	1.24 ± 0.05

Data are mean ± SD, n = 3.

In vitro digestive enzyme inhibition

MCEE inhibited all three digestive enzymes in a concentration-dependent manner, with a clear hierarchy of potency: α-glucosidase (IC50 48.6 µg/mL) > α-amylase (142.8 µg/mL) > pancreatic lipase (287.4 µg/mL) (Table 2).

Table 2. Half-maximal inhibitory concentrations (IC50) of MCEE and charantin against digestive enzymes

Test substance	Pancreatic lipase IC50 (µg/mL)	α-Amylase IC50 (µg/mL)	α-Glucosidase IC50 (µg/mL)
Orlistat (reference)	0.18 (0.14–0.23)	—	—
Acarbose (reference)	—	0.62 (0.48–0.79)	0.28 (0.22–0.35)
MCEE	287.4 (241.5–342.0)	142.8 (119.3–170.9)	48.6 (39.3–60.0)
Charantin (isolated)	>200a	318.5 (265.4–382.1)	104.7 (85.2–128.7)

Values are mean with 95% confidence interval (three independent experiments). a Inhibition <10% at 200 µg/mL (solubility limit); IC50 not calculable.

Isolated charantin was 2.2- to 2.8-fold less potent than the parent extract against both carbohydrate hydrolases (α-amylase IC50 318.5 µg/mL; α-glucosidase IC50 104.7 µg/mL) and produced negligible lipase inhibition (<10% at 200 µg/mL, the solubility limit), indicating that charantin contributes to, but does not fully account for, the activity of the whole extract. In the BSA specificity control, MCEE's apparent lipase inhibition fell only modestly (51.7 ± 2.9% to 39.4 ± 3.5% at 300 µg/mL, p = 0.008), consistent with a substantial fraction of genuine, enzyme-specific inhibition rather than non-specific protein binding.

Acute and sub-acute toxicity

A single limit dose of 2000 mg/kg MCEE produced no mortality and no adverse clinical signs over 14 days (GHS Category 5/Unclassified; oral LD50 >2000 mg/kg). In the 28-day repeated-dose study, MCEE at 100, 300 and 1000 mg/kg/day produced no treatment-related mortality, clinical signs, or changes in body-weight gain, food intake, haematology, organ weights or histopathology relative to vehicle control (all p>0.05). Serum AST, ALT, ALP, creatinine and BUN were unaffected at all doses, including the 1000 mg/kg/day high dose — more than 15-fold the highest anticipated therapeutic dose. The NOAEL for MCEE was therefore established at 1000 mg/kg/day, the highest dose tested, yielding a safety margin of at least 2.5-fold over the therapeutic dose range evaluated in the efficacy study.

Effect on body weight, feed efficiency and adiposity

The HFD-Ctrl group gained substantially more weight than normal chow-fed controls, reaching a terminal body weight of 398.2 ± 12.6 g (vs 312.5 ± 9.4 g in normal control, p<0.001), with a corresponding rise in the combined visceral adiposity index to 8.93 ± 0.65% (vs 2.17 ± 0.18%, p<0.001). MCEE produced a dose-dependent reduction in body weight: the high dose (400 mg/kg/day) limited terminal body weight to 351.4 ± 10.8 g, an 11.7% reduction relative to HFD-Ctrl (p<0.01), statistically comparable to orlistat (12.8% reduction). Notably, food intake in the MCEE groups did not differ significantly from HFD-Ctrl (Table 3), indicating that weight reduction was driven primarily by a lower feed efficiency ratio (FER): 4.28 ± 0.31% for the high-dose group versus 5.87 ± 0.42% for HFD-Ctrl (p<0.01). Adiposity index, liver weight and epididymal/retroperitoneal/mesenteric fat-pad masses were all significantly reduced by high-dose MCEE (p<0.01–0.001 vs HFD-Ctrl), broadly paralleling the effect of orlistat. Interscapular brown adipose tissue (BAT) mass was significantly increased only in the high-dose MCEE group (relative weight 0.15 ± 0.01% vs 0.11 ± 0.01% in HFD-Ctrl, p<0.05), a finding without precedent among the other treatment arms of the parent study.

Table 3. Body weight, food intake, feed efficiency ratio and Lee index after 8-week treatment

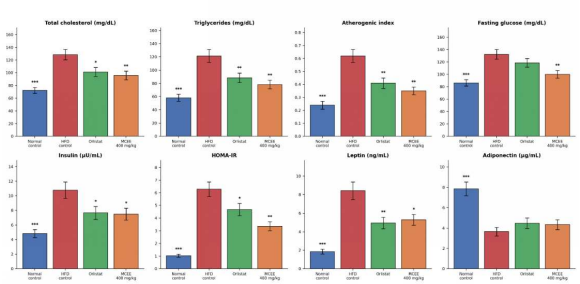
Group	Terminal body weight (g)	Food intake (g/rat/day)	Feed efficiency ratio (%)	Lee index
Normal control	312.5 ± 9.4***	21.3 ± 0.8*	3.45 ± 0.25***	295.8 ± 4.3***
HFD control	398.2 ± 12.6	24.8 ± 1.2	5.87 ± 0.42	322.5 ± 5.8
Orlistat 50 mg/kg	347.1 ± 11.3**	24.1 ± 1.0	3.98 ± 0.33**	308.7 ± 5.2**
MCEE 200 mg/kg	373.6 ± 13.5	24.5 ± 1.1	5.04 ± 0.38*	316.4 ± 5.5
MCEE 400 mg/kg	351.4 ± 10.8**	23.9 ± 0.9	4.28 ± 0.31**	304.8 ± 4.9**

*Data are mean ± SEM (n = 8). *p<0.05, **p<0.01, ***p<0.001 vs HFD control (one-way ANOVA, Tukey's HSD).*

Effect on biochemical parameters

HFD-Ctrl animals developed the expected dyslipidaemic and insulin-resistant phenotype, with an atherogenic index of 0.62 ± 0.05 (vs 0.24 ± 0.03 in normal control, p<0.001) and a HOMA-IR of 6.28 ± 0.58. High-dose MCEE produced a comprehensive metabolic improvement: fasting glucose fell by 24.5%, insulin by 30.6%, and HOMA-IR by 46.5% (all p<0.01 vs HFD-Ctrl), a glycaemic effect significantly greater than that achieved by orlistat (25.5% reduction in HOMA-IR, p<0.05 vs HFD-Ctrl; MCEE vs orlistat: p<0.05, Tukey's HSD). Total cholesterol and triglycerides were reduced by comparable margins to orlistat, and leptin fell in proportion to the loss of fat mass. Serum adiponectin rose modestly with high-dose MCEE (+18.5% vs HFD-Ctrl), a change of similar magnitude to that produced by orlistat but well short of statistical significance (p = 0.082).

Figure- 5. Diagrammatic representation of Serum lipid, glycaemic and adipokine parameters after 8-week treatment



*Data are mean ± SEM (n = 8). *p<0.05, **p<0.01, ***p<0.001 vs HFD control (one-way ANOVA, Tukey's HSD).*

Hepatic histology and adipocyte morphometry

Livers from HFD-Ctrl animals showed diffuse macrovesicular steatosis (>66% of parenchyma) with scattered lobular inflammation and occasional hepatocyte ballooning, yielding a cumulative NAS of 5.1 ± 0.4 — above the threshold conventionally used to define steatohepatitis in rodent models. High-dose MCEE produced the most pronounced hepatoprotective effect among the treatment arms, reducing NAS to 1.8 ± 0.3 (p<0.001 vs HFD-Ctrl) **(Fig-6)**, with near-complete resolution of lobular inflammation and residual steatosis limited to microvesicular droplets in zone 3.

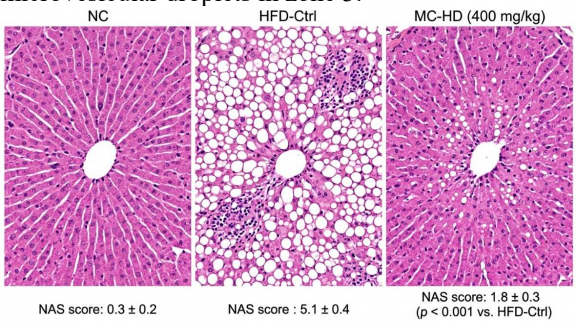


Fig-6 – Histopathological Observations

Mean adipocyte cross-sectional area (CSA), grossly elevated in HFD-Ctrl (4875 ± 452 µm2 vs 1542 ± 185 µm2 in normal control, p<0.001), was reduced to 2488 ± 275 µm2 by high-dose MCEE (p<0.001), with the size-frequency distribution shifting toward medium-sized adipocytes (1000–3000 µm2) rather than the pathologically hypertrophic population (>5000 µm2) that dominated HFD-Ctrl.

Table 5. NAFLD Activity Score and adipocyte cross-sectional area after 8-week treatment

Group	Steatosis (0–3)	Inflammation (0–3)	Ballooning (0–2)	NAS total	Adipocyte CSA (µm²)
Normal control	0.2 ± 0.1***	0.0 ± 0.0***	0.0 ± 0.0***	0.3 ± 0.2***	1542 ± 185***

Group	Steatosis (0–3)	Inflammation (0–3)	Ballooning (0–2)	NAS total	Adipocyte CSA (µm²)
HFD control	2.8 ± 0.2	1.6 ± 0.3	0.7 ± 0.2	5.1 ± 0.4	4875 ± 452
Orlistat 50 mg/kg	1.6 ± 0.3**	1.0 ± 0.2	0.3 ± 0.1	2.9 ± 0.4**	2846 ± 312**
MCEE 400 mg/kg	1.0 ± 0.2***	0.5 ± 0.1**	0.3 ± 0.1	1.8 ± 0.3***	2488 ± 275***

*Data are mean ± SEM (n = 8 for histology; n = 5 animals/group, ≥100 adipocytes/animal, for CSA). *p<0.05, **p<0.01, ***p<0.001 vs HFD control (Kruskal–Wallis/Dunn's test for NAS; one-way ANOVA/Tukey's HSD for CSA).*

DISCUSSION

This study links, within a single chemically standardized extract, a defined in vitro enzyme-inhibitory profile to a coherent in vivo metabolic phenotype, and in doing so clarifies why *M. charantia's* reported anti-obesity effects have been so heterogeneous in the clinical literature.

From charantin to the whole extract: a phytochemical matrix effect

Isolated charantin was consistently two- to three-fold less potent than MCEE against both carbohydrate hydrolases, and essentially inactive against lipase. This gap is best explained by synergistic contributions from co-extracted cucurbitane triterpene glycosides — a class independently shown to inhibit α-glucosidase at low-micromolar potency15 — together with flavonoid conjugates and saponins detected in the extract's phytochemical screen. Standardizing M. charantia preparations solely against charantin content therefore risks discarding pharmacologically meaningful chemical diversity; a multi-marker standardization strategy, anchored to charantin but supplemented by a representative cucurbitane glycoside, would more faithfully capture the extract's bioactive profile.

Enzyme inhibition hierarchy predicts, and helps explain, the in vivo phenotype

The in vitro potency ranking — α-glucosidase > α-amylase > pancreatic lipase — was faithfully mirrored in vivo. MCEE lowered feed efficiency ratio without significantly reducing food intake, the classic signature of reduced net caloric harvest from carbohydrate malabsorption, and its effect on fasting glucose and HOMA-IR exceeded that of orlistat, a pure lipase inhibitor with no carbohydrate-hydrolase activity. Conversely, the relatively weak lipase IC50 (roughly 1600-fold higher than orlistat) is consistent with the absence of steatorrhoea-type findings in the toxicity studies and suggests that MCEE's modest lipid-lowering effect is more plausibly a secondary consequence of improved insulin sensitivity and reduced hepatic lipogenesis than of direct faecal fat loss.

A systemic component beyond luminal restriction

Two findings argue against a purely luminal mechanism. First, the magnitude of glycaemic improvement with MCEE exceeded what luminal carbohydrate restriction alone would be expected to produce, and second, brown adipose tissue mass increased selectively in the high-dose MCEE group — a change not observed with orlistat despite comparable weight loss. AMP-activated protein kinase (AMPK) activation by M. charantia constituents has been linked in prior work to GLUT-4 translocation, enhanced fatty-acid oxidation and suppression of adipogenic PPARγ signalling,4,5 and BAT recruitment is a recognized downstream consequence of AMPK-linked thermogenic signalling.16 Taken together with the near-complete resolution of hepatic steatosis and inflammation (NAS 5.1→1.8), these observations are most consistent with a dual mechanism in which luminal enzyme inhibition reduces net caloric absorption while an absorbed fraction of cucurbitane triterpenoids engages AMPK-dependent pathways in liver, muscle and adipose tissue.

Reconciling the clinical literature

Our findings help contextualize the discordant clinical picture: a 2024 systematic review concluded that the metabolic effect of M. charantia could not be reliably determined from published trials,6 largely because those trials used heterogeneous, non-standardized preparations, whereas a more recent GRADE-adherent meta-analysis of 25 randomized trials reported significant reductions in fasting glucose (SMD −0.46) and HbA1c (SMD −0.57).7 A chemically standardized, charantin-anchored extract such as MCEE — evaluated here in a model that recapitulates visceral adiposity, dyslipidaemia, insulin resistance and steatohepatitis — offers a more reproducible platform on which to resolve this heterogeneity in future clinical work.

Translational relevance

The pharmacodynamic profile identified here — combined carbohydrate-hydrolase inhibition and insulin-sensitizing, hepatoprotective action — positions MCEE as being of particular relevance to individuals with concurrent obesity and impaired glucose tolerance, i.e., the core phenotype of

metabolic syndrome, rather than as a generic weight-loss agent. The safety margin is substantial: the 28-day NOAEL of 1000 mg/kg/day is approximately 2.5- to 5-fold above the therapeutic dose range tested here (200–400 mg/kg/day), and no hepatotoxic signal was observed even at doses exceeding the anticipated human-equivalent therapeutic dose by more than an order of magnitude. This safety margin, combined with the extract's distinct mechanistic engagement of postprandial glycaemia, supports progression toward early-phase clinical evaluation using postprandial glucose excursion as a mechanism-linked pharmacodynamic endpoint.

Limitations

This study has several limitations. The efficacy evaluation was conducted over a single 8-week treatment period in one rodent strain, and long-term durability of the observed effects is unknown. The precise contribution of individual co-extracted cucurbitane glycosides to whole-extract potency was inferred rather than directly isolated compound-by-compound. Finally, while the enzyme-inhibition and in vivo phenotypes are internally consistent, mechanistic confirmation of AMPK activation (e.g., by Western blot of phosphorylated AMPK in liver and muscle) was not performed in the present investigation and represents a priority for follow-on work.

CONCLUSION

A chemically standardized 80% ethanolic extract of M. charantia unripe fruit, quantified against charantin, combines specific, BSA-resistant inhibition of pancreatic lipase, α-amylase and α-glucosidase with an in vivo phenotype of reduced feed efficiency, superior glycaemic control relative to orlistat, near-resolution of hepatic steatohepatitis, and selective recruitment of brown adipose tissue. These findings support a dual luminal-systemic mechanism of action and provide a chemically defined, toxicologically qualified extract suitable for progression toward early-phase clinical evaluation in metabolic syndrome, with postprandial glucose excursion as a candidate mechanism-linked biomarker.

DECLARATIONS

Ethical approval: This study was approved by the Institutional Animal Ethics Committee [approval number — TO BE INSERTED] and conducted in accordance with CPCSEA and OECD guidelines.

Conflict of interest:

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