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Anti-obesity potential of a latex-derived bioactive peptide fraction from Calotropis procera: Depot-selective fat reduction and systemic metabolic improvement in high-fat diet-fed mice

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Abstract

BACKGROUND: This study investigated the effects of the latex protein fraction (LP) from *Calotropis procera* (Ait.) R.Br. on body weight, food intake, fat deposition, organ weight, and plasma inflammatory and metabolic markers in male Swiss mice fed a high-fat diet (HFD).

METHODS: Thirty mice were divided into four groups: normal diet (ND), HFD, HFD plus LP 5 mg/kg (LP5), and HFD plus LP 50 mg/kg (LP50), administered intraperitoneally every 4 days during the final 6 weeks of a 27-week protocol.

RESULTS: LP50 prevented the HFD-induced increase in final body weight (46.50±2.06 g vs. 53.60±2.15 g in HFD) and showed a non-significant trend toward reduced subcutaneous fat accumulation, without affecting food intake or kidney, heart, spleen, or liver weight. LP50 also significantly reduced HFD-induced elevations in plasma TNF-α, IL-6, triglycerides, and glucose.

CONCLUSIONS: These findings suggest that LP exerts a dose-dependent, depot-specific anti-obesity effect, together with systemic anti-inflammatory and metabolic benefits, independent of appetite suppression.

INTRODUCTION

The global prevalence of obesity has become a major public health concern, with a marked increase observed in most nations since the 1980s. Current estimates suggest that 39–49% of the world's population, approximately 2.8–3.5 billion individuals, are affected by overweight or obesity [1]. Obesity is characterized by abnormal fat accumulation in adipose tissue, primarily resulting from an imbalance between energy intake and energy expenditure, and is closely associated with chronic low-grade inflammation and metabolic dysfunction, including dyslipidemia and impaired glycemic control [1, 2]. Initial recommendations for preventing or managing obesity emphasize lifestyle changes, reduced energy intake, and physical exercise [2]. Consequently, anti-obesity drugs are used to treat severe cases, typically targeting increased metabolism and/or appetite suppression, among other strategies [3]. However, these pharmacological interventions often cause side effects such as insomnia, vomiting, hyperpyrexia, and constipation, frequently occurring in combination [3]. These adverse effects often discourage patients from continuing treatment. Moreover, given the multifactorial nature of obesity, involving metabolic, inflammatory, and psychological factors, identifying a universal drug or standard treatment remains challenging. Consequently, there is a growing need for novel and safer alternative therapies, including food-derived bioactive peptides and medicinal plant fractions for obesity prevention or management [3, 4, 5]. Bioactive peptides, whether released by enzymatic hydrolysis of food proteins or occurring naturally within plant tissues, have attracted growing interest as multifunctional agents capable of simultaneously targeting several of the interconnected metabolic and inflammatory pathways that drive obesity and its comorbidities, rather than acting on a single mechanism as most conventional anti-obesity drugs do.

Among plant sources, the Apocynaceae family includes several species, such as *Caralluma fimbriata*, *Ichnocarpus frutescens*, *Hoodia gordonii*, and *Gymnema sylvestre*, that have demonstrated antiobesogenic effects in scientific studies [6–9]. Comprising tropical shrubs, trees, and vines, the Apocynaceae family is renowned for its medicinal diversity, with approximately 4,600 species across 415 genera [10]. Different parts of these plants have traditionally been used to treat gastrointestinal disorders, fever, malaria, pain, obesity, diabetes, migraine, and skin damage [10–12]. *Calotropis procera* (Ait.) R.Br. is a member of the Apocynaceae family commonly found in arid to semi-arid regions of Africa, the Americas, and Asia [13, 14]. Its leaves, flowers, roots, and latex are rich sources of bioactive compounds, including alkaloids, flavonoids, glycosides, phenols, terpenoids, tannins, saponins, and steroids [13]. Known as "Rakta arka" in ancient Ayurvedic literature, *C. procera* has been used in Afghan, Ayurvedic, and Unani medicine to treat asthma, hemorrhoids, tumors, fever, leprosy, dysentery, diarrhea, and ulcers [14–16].

Several studies have described the diverse beneficial properties of *C. procera* (Ait.) R.Br. latex, including antinociceptive, anti-inflammatory, hypoglycemic, antioxidant, and anticancer activities [17–21]. Within this complex secretion, the lyophilized protein fraction (LP), comprising mainly cysteine peptidases and osmotin isoforms, has emerged as the component chiefly responsible for many of these bioactivities, having shown promising effects in protecting against septic shock, promoting glycemic control *in vivo*, suppressing pro-inflammatory cytokine release, and restoring blood-coagulation homeostasis during acute *Salmonella* infection [22–25]. The glycemic-control activity of LP has been attributed specifically to its peptidase and osmotin components, which also enhance ATP-linked oxygen consumption and mitochondrial uncoupling [22]. This multifunctional profile, spanning metabolic, anti-inflammatory, and antinociceptive activities within a single, well-characterized peptide/protein fraction, positions LP as a particularly attractive candidate among plant-derived bioactive peptides for translational applications in functional foods and nutraceuticals.

Despite this growing body of evidence on the metabolic and anti-inflammatory properties of LP, including its capacity to improve glycemic control and mitochondrial function in hepatic cells [22] and to downregulate pro-inflammatory cytokines such as TNF-α and IL-6 in other inflammatory models [24, 25], whether this same protein fraction can directly modulate body weight, fat accumulation, systemic inflammation, and lipid/glucose homeostasis under diet-induced obesity had not previously been examined as an integrated outcome. This gap is particularly relevant because the anti-obesity activity already described for other Apocynaceae species has been attributed mainly to crude extracts or isolated small molecules, such as the steroidal glycosides of *Caralluma fimbriata* and *Hoodia gordonii* [8, 26] or, within *C. procera* itself, osmotin and α,β-amyrin [27, 28], rather than to the latex protein fraction as a whole. Because LP is, by definition, free of these small non-proteinaceous metabolites, its anti-obesity potential cannot be inferred from previous studies using crude extracts or isolated compounds and must be tested directly, as a discrete, intelligently characterized peptide fraction rather than an undefined botanical extract.

Given the increasing global prevalence of obesity, the side effects associated with current pharmacological treatments, and the largely unexplored anti-obesity potential of this multifunctional, protein/peptide-based latex fraction, the present study investigated, for the first time, the effects of LP on body weight, food intake, abdominal fat deposits, and organ weight, as well as on plasma pro-inflammatory cytokines (TNF-α and IL-6) and lipid and glucose homeostasis, in male Swiss mice subjected to a high-fat diet (HFD).

Materials and methods

Plant material

Leaves and fresh latex of healthy plants of *Calotropis procera* (Ait.) R.Br. growing in the city of Fortaleza, Ceará, Brazil (3°44'45.9"S 38°34'21.5'W) were collected between August and September 2017 (dry season). The plant material was identified by a taxonomist, and a voucher specimen (No. 32663) was deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará (UFC). Access to and use of Brazilian genetic biodiversity complied with national legislation, under registration code A689147.

Calotropis procera (Ait.) R.Br. latex protein fraction

The latex protein (LP) fraction of *C. procera* (Ait.) R.Br. was obtained following the protocol established by Ramos et al. [29]. Latex was collected from the aerial parts of the plant and diluted in distilled water at a ratio of 1:2 (v/v). The resulting suspension was subjected to centrifugation (5000 × g, 10 min, 10 °C), a step that allowed removal of the rubber-enriched pellet. The supernatant thus obtained was subsequently dialyzed against distilled water using a dialysis membrane with a molecular weight cut-off of 8000 Da. After 1 h of dialysis, the dialyzable fraction (DF) was collected and freeze-dried. The material retained within the membrane (non-dialyzable fraction), recovered after dialysis, was centrifuged once more, and the resulting supernatant, corresponding to the protein fraction of interest (LP), was lyophilized and used in subsequent experiments. This soluble phase containing the latex proteins was freeze-dried and stored at 25 °C until further use (Fig. 1).

Ethical statement

All experimental procedures followed the guidelines of the Guide for the Care and Use of Laboratory Animals of the Federal University of Ceará, and the experimental design was approved by the Institutional Animal Ethics Committee (License number 62/2012). Male Swiss mice (25–30 g, n = 30) were provided by the Central Animal House of the Federal University of Ceará. The mice were housed in polypropylene cages (41 × 34 × 30 cm) under controlled conditions (24 ± 2 °C, 12-h light/dark cycle) with free access to food and water. At the end of the experiment, the mice were euthanized by intraperitoneal injection of sodium thiopental (Cristália, São Paulo, SP, Brazil).

Diets

The high-fat diet (HFD) was prepared according to the methodology described by Pérez et al. [30]. It consisted of a specific combination of ingredients: 10 g of normal diet (ND; Evialis do Brasil Nutrição Animal LTDA, Descalvado, São Paulo), 10 g of roasted ground peanuts (Santa Helena Indústria de Alimentos S/A, Ribeirão Preto, São Paulo), 0.3 g of butter (Itambé Alimentos LTDA, São Paulo), 5 g of sweet wheat biscuit (Fortaleza®), 5 g of milk chocolate, and 5 g of white chocolate (Mondelez Lacta Alimentos LTDA, Curitiba, Paraná). These ingredients were precisely measured, processed, and combined into pellets, which were stored according to an established protocol and prepared fresh weekly. The proximate composition of the HFD and ND differed in protein, fat, moisture, ash, carbohydrate, and caloric content, as described by Pérez et al. [30]. Specifically, the HFD and ND contained, respectively, 16.15% and 18.49% protein, 22.52% and 4.73% fat, 9.10% and 8.23% water, 3.42% and 9.16% ash, 48.81% and 59.39% carbohydrates, and 452.52 and 354.09 kcal/100 g.

In vivo experiments

Thirty male Swiss mice (25–30 g), bred in the Central Animal House of the Federal University of Ceará, were divided into four groups according to diet and treatment: normal diet (ND, n = 12); high-fat diet (HFD, n = 10); HFD plus *C. procera* (Ait.) R.Br. latex protein fraction, 5 mg/kg i.p. (LP5, n = 4); and HFD plus *C. procera* (Ait.) R.Br. latex protein fraction, 50 mg/kg i.p. (LP50, n = 4). After 2 weeks of acclimatization, the animals received the HFD for 25 weeks; during the final 6 weeks, the LP fractions (5 or 50 mg/kg i.p.) were administered every 4 days. Total food intake (g/week) and water consumption (mL/week) were recorded weekly, and individual body weight was measured once a week (Figure 1) [30].

Plasma total cholesterol, triglycerides, glucose, cytokines, organs, and body fat.

At the end of the experiment, the animals were fasted overnight and anesthetized for blood collection from the retrobulbar venous plexus [31]. Animals were then euthanized by intraperitoneal injection of sodium thiopental (Cristália, São Paulo, SP, Brazil), a barbiturate acid derivative classified as an acceptable method of euthanasia by the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition [32]. Serum was separated by centrifugation at 1500g and 4 °C for 15 min and stored at −72 °C until analysis. Serum levels of total cholesterol (TC), triglycerides (TG), and glucose were measured using commercial enzymatic-colorimetric kits from Labtest® Diagnóstica S.A. (Lagoa Santa, MG, Brazil). Serum levels of the pro-inflammatory cytokines TNF-α and IL-6 were measured by ELISA using commercial kits (Mouse TNF-α ELISA Kit, RAB0477; Mouse IL-6 ELISA Kit, RAB0308) purchased from Sigma-Aldrich (St. Louis, MO, USA), according to the manufacturer's instructions. The liver, spleen, heart, kidney, retroperitoneal fat, periepididymal fat, and subcutaneous fat were excised and weighed on the day of euthanasia (Figure 1).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) and were analyzed using GraphPad Prism version 9.3.1 (GraphPad Software, CA, USA). One-way ANOVA followed by Tukey's test was used to determine statistical significance; $p < 0.05$ was considered significant.

RESULTS

Effects of LP on food intake and body weight

Baseline food intake was similar among the ND, HFD, LP5, and LP50 groups (24.92±0.86, 23.50±0.85, 25.83±1.25, and 26.67±0.56 g, respectively) (Fig. 2A). From week 2 onward, all HFD-fed groups showed reduced food intake relative to ND, a trend sustained throughout the experiment. Final food intake was lower in the HFD, LP5, and LP50 groups (26.10±1.49, 29.67±1.76, and 22.20±3.79 g, respectively) than in the ND group (35.50±1.43 g). Food intake in the LP50 group did not differ from the HFD group, indicating that the LP-induced effect on body weight is independent of appetite suppression. Baseline body weight was comparable among the ND, HFD, LP5, and LP50 groups (30.83±0.55, 30.30±0.33, 30.00±0.82, and 31.25±0.25 g, respectively) (Fig. 2B). From week 18 onward, the HFD, LP5, and LP50 groups showed increased body weight (50.70±2.32, 48.50±3.95, and 48.55±3.46 g, respectively) relative to ND (45.33±0.98 g). Final body weight was comparable between the ND (47.33±1.00 g) and LP50 (46.50±2.06 g) groups, both lower than the HFD (53.60±2.15 g) and LP5 (53.00±4.97 g) groups. LP at 50 mg/kg i.p., but not at 5 mg/kg i.p., reduced body weight from week 22 onward relative to HFD, an effect sustained through week 27, confirming a dose-dependent anti-obesity effect rather than a nonspecific consequence of protein administration.

Effects of LP on body fat depots

In addition to the increase in body weight, fat depots were differentially affected by HFD feeding (Fig. 3A–C). Subcutaneous fat mass was significantly increased in the HFD (1.38±0.62 g) and LP5 (1.67±0.85 g) groups compared with the ND group (0.59±0.19 g), as indicated in Fig. 3A; no significant difference was observed between the LP50 group (0.88±0.34 g) and the ND group, nor between LP50 and the HFD or LP5 groups. Periepididymal fat mass was significantly increased in the HFD group (1.87±0.22 g) compared with ND (1.02±0.10 g); the LP5 (1.83±0.12 g) and LP50 (1.78±0.41 g) groups did not differ significantly from ND, HFD, or each other, indicating no clear modulation of this fat depot by LP at either dose (Fig. 3B). Retroperitoneal fat mass did not differ significantly among the ND (0.21±0.16 g), HFD (0.22±0.08 g), LP5 (0.38±0.23 g), and LP50 (0.22±0.06 g) groups, indicating that this depot was not significantly affected by either HFD feeding or LP treatment (Fig. 3C).

Taken together, these findings show that HFD feeding increased subcutaneous fat accumulation (significant vs. ND for the HFD and LP5 groups) and periepididymal fat accumulation (significant vs. ND for the HFD group), while retroperitoneal fat was unaffected; LP treatment did not produce a statistically significant change relative to the HFD group in any of the three fat depots examined.

Effects of LP on plasma total cholesterol, triglycerides, and glucose

The protein fraction of *C. procera* latex (LP) affected HFD-induced alterations in plasma lipid and glucose levels (Fig. 4A,B). Plasma total cholesterol and triglyceride levels were both significantly higher

in the HFD group compared with the ND group (marked "&" vs. "#" in Fig. 4A). In the LP50 group, triglyceride levels were significantly reduced relative to HFD, returning to values comparable to those observed in the ND group (shared "#" symbol), whereas total cholesterol remained statistically similar to the HFD group (shared "&" symbol), indicating that this reduction did not reach statistical significance (Fig. 4A). Similarly, HFD feeding significantly increased plasma glucose levels compared with ND, and treatment with LP at 50 mg/kg significantly reduced glucose levels relative to the HFD group, with no significant difference remaining between LP50 and ND (Fig. 4B), indicating that LP improves glycemic control in HFD-fed mice.

Effects of LP on plasma pro-inflammatory cytokines

To evaluate whether LP modulates the low-grade inflammatory state induced by HFD feeding, plasma levels of TNF-α and IL-6 were measured (Fig. 5A,B). HFD feeding significantly increased plasma TNF-α levels compared with the ND group (Fig. 5A). Treatment with LP at 50 mg/kg significantly reduced TNF-α levels relative to the HFD group, restoring values to a level not significantly different from ND. A similar pattern was observed for IL-6: HFD-fed mice showed significantly higher plasma IL-6 levels than ND mice, and LP50 treatment significantly reduced IL-6 levels relative to HFD, with no significant difference remaining between the LP50 and ND groups (Fig. 5B). These results indicate that LP attenuates the HFD-induced systemic pro-inflammatory response.

Effects on organ weight and safety

LP administration did not significantly alter the weights of the kidneys, heart, spleen, or liver (all $p > 0.05$, one-way ANOVA followed by Tukey's post hoc test; Fig. 6A–E), and treated animals showed no behavioral alterations or other signs of toxicity throughout the experiment.

DISCUSSION

Food intake in the LP50 group did not differ from that of the HFD group, indicating that the LP-induced reduction in body weight is independent of appetite suppression, a mechanism distinct from that reported for most conventional anti-obesity drugs and plant extracts [33]. This dissociation between reduced body weight and unchanged food intake contrasts with the findings of Neto et al. [34], who reported that a hydroalcoholic extract of C. procera leaves reduced food intake without affecting body weight in rats with diabetes, essentially the inverse pattern observed here. This discrepancy likely reflects compositional differences between the two preparations: whole latex contains non-proteinaceous secondary metabolites, some of which are bioactive or even toxic [35], whereas LP is a protein-only fraction. This distinction points to a mechanism centered on energy expenditure or fat metabolism rather than on intake regulation, consistent with reports that osmotin, an adiponectin homolog present in LP, enhances mitochondrial uncoupling and energy expenditure independently of appetite [27], and that other Apocynaceae-derived triterpenoids modulate adipogenic and lipolytic pathways through non-appetite-related mechanisms [28].

Beyond its effects on adiposity, LP also modulated the systemic inflammatory state associated with HFD feeding. The reduction in circulating TNF-α and IL-6 observed here in LP50-treated mice is consistent with a substantial body of evidence attributing anti-inflammatory properties specifically to the protein fraction of C. procera latex, rather than to the whole latex or its non-proteinaceous constituents. Soluble laticifer proteins from C. procera have been shown to downregulate TNF-α and IL-1β expression and to abolish myeloperoxidase activity in a hamster model of 5-fluorouracil-induced oral mucositis [36], an effect later attributed to a highly homogeneous protein cocktail (PII-IAA), composed mainly of cysteine peptidases and osmotin isoforms, that eliminated oral mucositis and reduced TNF-α release while relieving oxidative stress [24]. Similarly, cysteine peptidases purified from the latex were shown to impair pro-inflammatory cytokine expression, including IL-1β, TNF-α, and IL-6, in LPS-stimulated macrophages [36], reinforcing that the anti-inflammatory activity of LP resides largely within its proteinaceous components. Earlier work using dialyzed latex fractions had already demonstrated that both pro- and anti-inflammatory activities of C. procera latex could be pharmacologically dissociated and that the pro-inflammatory component involved TNF-α-mediated mechanisms [17], while the antinociceptive and anti-inflammatory potential of whole latex had been documented in classical rodent models of paw edema and peritonitis [20]. Taken together, these findings support the interpretation that the reduction in TNF-α and IL-6 observed in the present study reflects a direct immunomodulatory action of the LP protein fraction on the low-grade inflammatory state associated with obesity, rather than a secondary consequence of weight loss alone.

Within the Apocynaceae family more broadly, anti-inflammatory and cytokine-modulating effects have also been reported for other genera, although rarely in the context of diet-induced obesity. Hoodia gordonii extract, for instance, was shown to influence both adipose and muscle tissue metabolism during weight loss in rats (Smith & Krygsman, 2014), while triterpenoid-rich fractions from related Apocynaceae species (e.g., α,β-amyrin) have been linked to downregulation of adipose tissue inflammatory markers alongside PPARγ and lipoprotein lipase expression [28]. However, direct evidence of TNF-α/IL-6 modulation by a purified plant protein fraction in an HFD-induced obesity model, as shown here for LP, remains scarce in the literature, underscoring the novelty of the present findings.

In addition to its anti-inflammatory action, LP also improved glycemic and lipid parameters altered by HFD feeding. The significant reduction in plasma glucose observed after LP50 treatment is in line with previous reports that phytomodulatory proteins isolated from C. procera latex promote glycemic control by inhibiting hepatic glucose production via AMPK activation and by improving hepatic mitochondrial function in HepG2 cells [23]. This protein-mediated glycemic effect complements earlier observations that whole C. procera latex exerts antioxidant and protective actions against alloxan-induced diabetes in rats [19], and is further corroborated by studies using other parts of the plant: aqueous extracts of C. procera flowers significantly reduced fasting glucose in rats with streptozotocin-induced diabetes, alongside a marked improvement in the lipid profile (decreased total cholesterol, triglycerides, and LDL, with increased HDL) [37]. Similarly, aqueous leaf extracts corrected alloxan-induced dyslipidemia, significantly lowering total cholesterol, LDL-cholesterol, and triacylglycerol levels toward control values from animals without diabetes [38], and a comparative study of leaf extracts and latex from C. procera in

rats with streptozotocin-induced diabetes confirmed that both preparations reduced blood glucose and normalized total cholesterol, triglycerides, and LDL while restoring HDL levels, with the latex itself among the most effective treatments [39]. Together with the present results, this body of evidence indicates that both the whole latex and its isolated protein fraction retain glucose- and lipid-lowering activity, reinforcing the therapeutic relevance of LP for metabolic dysfunction associated with obesity.

The partial dissociation observed here between the lipid-lowering effect of LP50, significant for triglycerides but not for total cholesterol, parallels findings in other Apocynaceae species, where the two parameters do not always respond with equal magnitude to a given treatment. In mice with obesity, Apocynum venetum-derived isoquercitrin significantly reduced fasting glucose, triglycerides, and total cholesterol by modulating the AMPK/SREBP-1c/FAS/CD36 signaling pathway [40], while Gymnema sylvestre extracts were reported to prevent triglyceride accumulation in muscle and liver and to lower circulating fatty acids, with additional antiatherosclerotic properties [6,9]. These differences across species and preparations suggest that lipid parameters may be regulated through partially distinct molecular targets, for example, triglyceride clearance via lipoprotein lipase or β-oxidation pathways versus cholesterol homeostasis via hepatic synthesis or biliary excretion, which could explain why LP50 normalized triglycerides but only partially affected total cholesterol in the present study. Future work directly assessing hepatic lipogenic and cholesterogenic gene expression will be needed to clarify the specific pathway(s) through which LP acts on lipid metabolism.

These metabolic effects of LP can also be placed in the broader context of anti-obesity agents described for other Apocynaceae species. Several succulent Apocynaceae species, including Caralluma fimbriata, Apocynum venetum, and Hoodia gordonii, have shown appetite-suppressing effects and reduced body weight gain after oral administration [8,26,40]. Apocynum venetum, for instance, significantly reduced fasting blood glucose and plasma triglyceride and total cholesterol levels in mice with obesity [40]. Likewise, Ichnocarpus frutescens (L.) R.Br. has demonstrated efficacy in managing obesity, diabetes, and hyperlipidemia [8,9], and Gymnema sylvestre has been reported to prevent triglyceride accumulation in muscle and liver while decreasing circulating fatty acid levels [6]. In most of these examples, however, the anti-obesity activity has been attributed to crude extracts or to isolated small molecules, particularly steroidal glycosides [8,26], rather than to a purified protein fraction, underscoring the novelty of the present findings with LP.

Within C. procera itself, two other bioactive substances, osmotin (the adiponectin homolog mentioned above) and α,β-amyrin (a triterpenoid mixture), have independently been identified as candidates against obesity [12,27,41,42]. Osmotin, administered at 5 µg/g three times weekly for two weeks, reduced HFD-induced body weight gain in mice [27]. Similarly, α,β-amyrin (10 or 20 mg/kg) lowered HFD-induced increases in body weight, visceral fat, and adipocyte surface area, downregulated PPARγ and lipoprotein lipase expression, and modulated leptin and ghrelin levels altered by HFD feeding [28].

Notably, the dose ranges effective for these two isolated compounds (5 µg/g and 10–20 mg/kg, respectively) are far lower than the 50 mg/kg dose of the whole LP fraction required here to elicit an anti-

obesity effect. Moreover, the lower LP dose (5 mg/kg) was not only ineffective but was associated with a further increase in subcutaneous fat. This non-monotonic, threshold-like pattern suggests that the active component(s) within LP may represent only a minor fraction of the total protein administered, and that a systematic dose–response study, including intermediate doses, will be needed to define the minimum effective dose and to clarify whether the apparent lack of effect at 5 mg/kg reflects insufficient exposure or a qualitatively different biological response.

Beyond dose–response considerations, translating these findings toward practical application also requires addressing the route of LP administration. It is important to note that LP was administered intraperitoneally in this study, a route that bypasses gastrointestinal digestion and is therefore not directly comparable to oral or dietary intake, the route most relevant to functional food applications. However, C. procera latex proteins are only partially degraded by digestive enzymes in vitro and are not detected immunologically in fecal material following oral exposure [43], suggesting that at least part of the bioactive fraction may retain structural and functional integrity after oral intake. Directly comparing oral and parenteral LP administration, together with identifying the specific peptide(s) responsible for the anti-obesity effect, will be necessary to determine whether this latex protein fraction can be developed into an orally active ingredient for functional foods or nutraceuticals targeting obesity.

These observations are consistent with previous reports of LP safety at the tested concentrations and dosing frequency [43], and with the absence of toxic signs after oral administration of C. procera leaf extract at doses up to 5 g/kg [34]. Although the precise mechanism underlying the present findings remains to be established, the absence of organ-weight changes or overt toxicity, combined with a clear anti-obesity effect at 50 mg/kg, supports the continued investigation of LP as a candidate bioactive peptide fraction.

CONCLUSION

This study provides the first evidence that a water-soluble latex protein fraction (LP) from *Calotropis procera* (Ait.) R.Br., administered intraperitoneally at 50 mg/kg, attenuates high-fat diet-induced obesity in mice, significantly reducing final body weight and showing a non-significant trend toward reduced subcutaneous fat accumulation, without affecting periepididymal fat, food intake, or major organ weights. This effect was dose-dependent, observed only at the higher of the two doses tested, and appeared unrelated to appetite suppression, distinguishing LP from many conventional anti-obesity strategies. The selective action on subcutaneous, rather than visceral, fat is consistent with, though not yet directly demonstrated to result from, the adiponectin-like signaling and mitochondrial-uncoupling activity previously attributed to osmotin-related proteins within this fraction.

Beyond its effects on adiposity, LP50 also improved the systemic metabolic and inflammatory profile associated with HFD feeding: it significantly lowered plasma TNF-α and IL-6, reduced triglycerides to levels comparable to normal-diet controls, and normalized plasma glucose, while total cholesterol showed only a non-significant reduction relative to the HFD group. In contrast, the lower dose (LP5) was

ineffective across all parameters and was even associated with a further increase in subcutaneous fat, revealing a non-monotonic, threshold-like dose-response relationship that warrants further investigation with intermediate doses.

These findings position LP as a novel, multifunctional, plant-derived bioactive peptide fraction with anti-obesity potential, complementing its previously reported anti-inflammatory, hypoglycemic, and antinociceptive activities. Because the fraction was administered parenterally in this proof-of-concept study, however, its translation into an orally active ingredient for functional foods or nutraceuticals will depend on future work establishing its oral bioavailability, defining a full dose-response relationship, identifying the specific peptide(s) responsible for the anti-obesity effect, and confirming efficacy and safety in larger, dose-balanced cohorts. With these steps, LP may represent a promising addition to the growing repertoire of food-derived bioactive peptides being explored for the prevention and management of obesity.

Declarations

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DATA AVAILABILITY

Data will be made available on request.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced this study.

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Figures

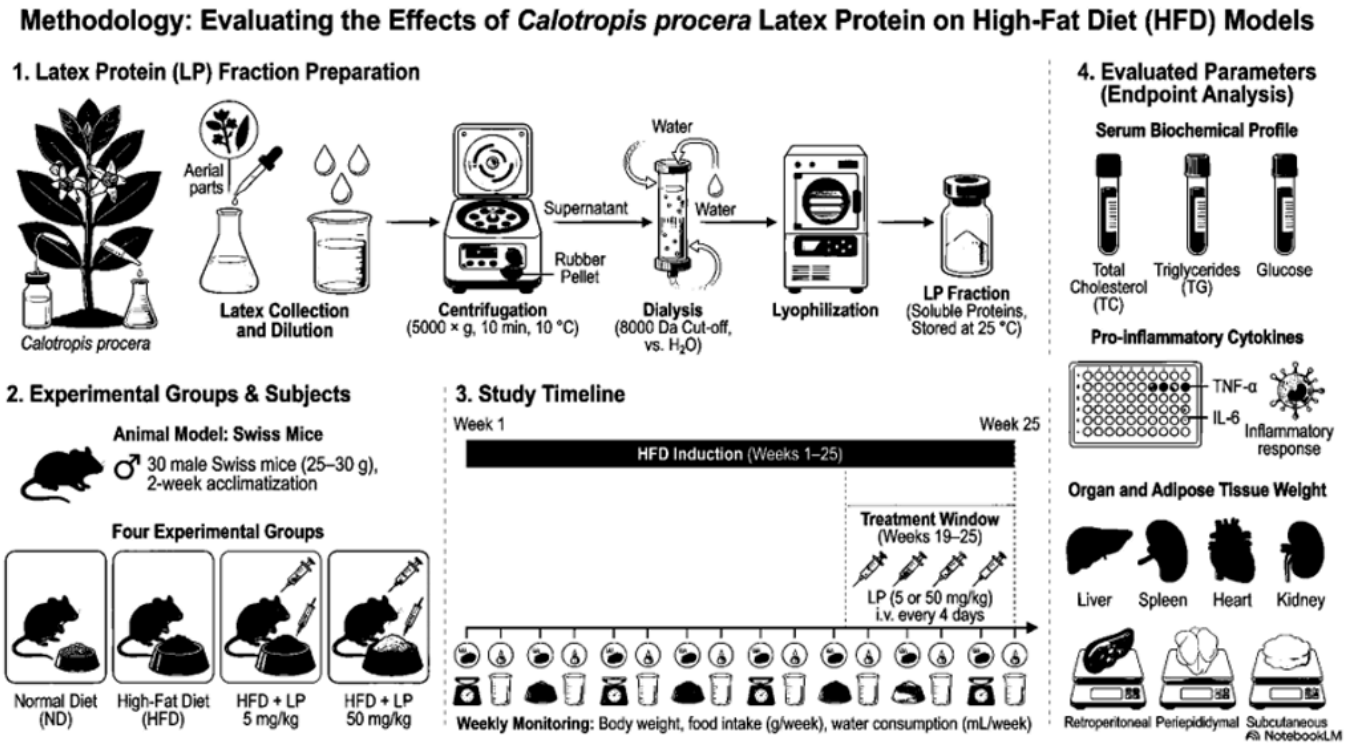
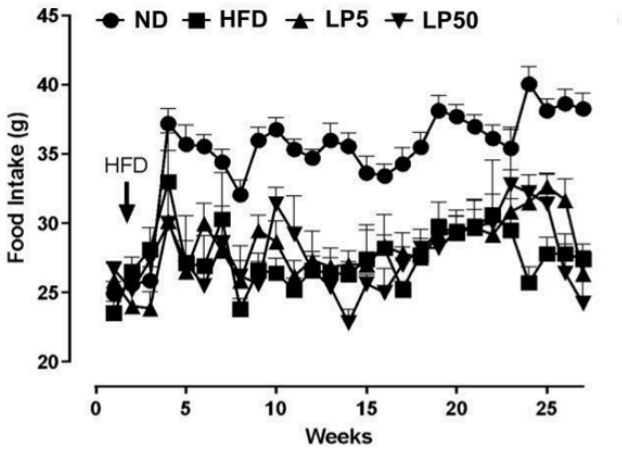


Figure 1

Methodology: Evaluating the effects of *C. procera* Latex Protein (LP) on High-Fat Diet (HFD) models.

A)



B)

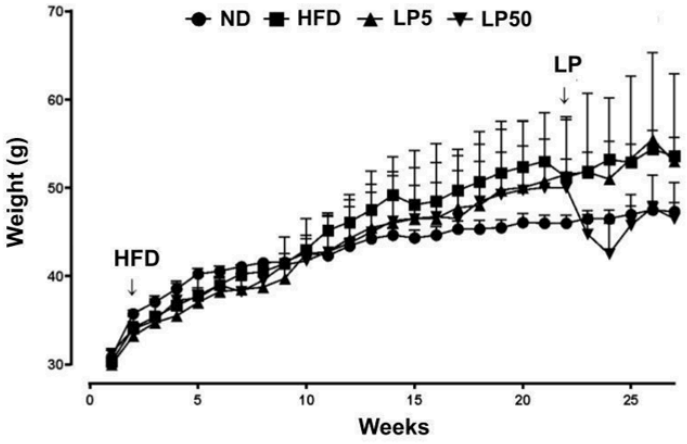


Figure 2

Food intake (A) and body weight (B) of mice fed a normal diet (ND), a high-fat diet (HFD), or HFD plus *C. procera* latex protein fraction at 5 mg/kg i.p. (LP5) or 50 mg/kg i.p. (LP50). Values are mean ± SEM, n =

4–12.

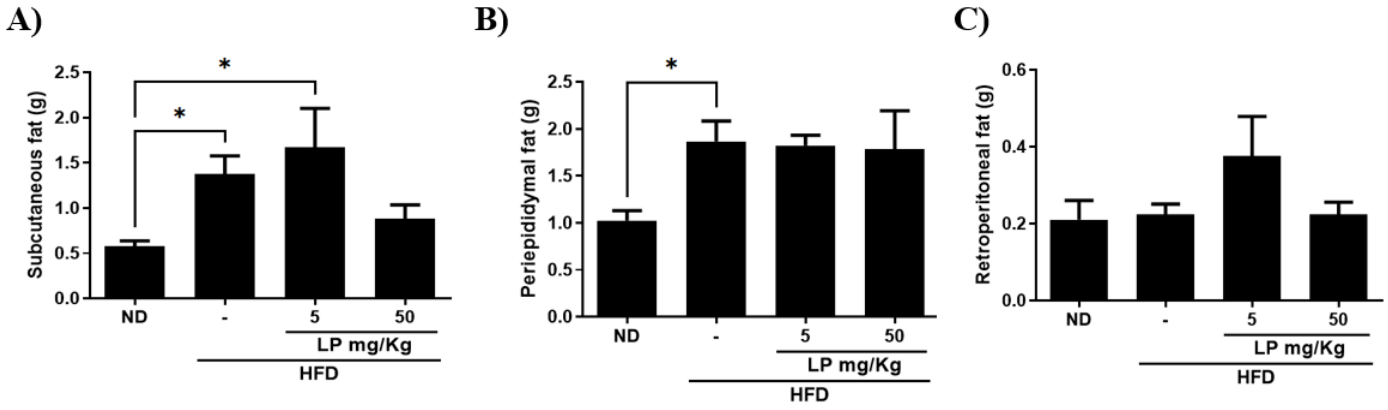


Figure 3

Subcutaneous **(A)**, periepididymal **(B)** and retroperitoneal **(C)** fat weights of mice fed a normal diet (ND), a high-fat diet (HFD), or HFD plus *C. procera*latex protein fraction at 5 mg/kg i.p. (LP5) or 50 mg/kg i.p. (LP50). Values are mean ± SEM, n = 4–12. *$p < 0.05$.

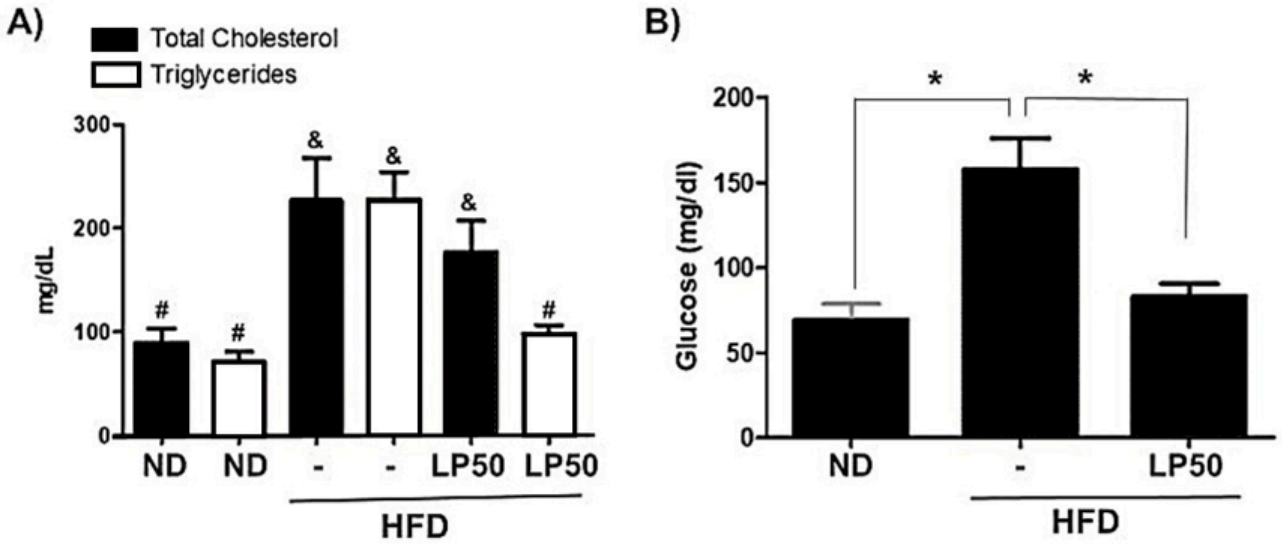


Figure 4

Effect of the protein fraction of *Calotropis procera* latex (LP, 50 mg/kg, i.p.) on total cholesterol and triglyceride levels (mg/dL) **(A)** and glucose levels **(B)** in Normal Diet (ND), High-Fat Diet (HFD), and HFD + LP50 groups. Data are expressed as mean ± SEM. Different symbols (# vs. &) indicate statistically significant differences between groups within each lipid parameter (panel A); *$p < 0.05$ (panel B) (one-way ANOVA followed by Tukey's post hoc test).

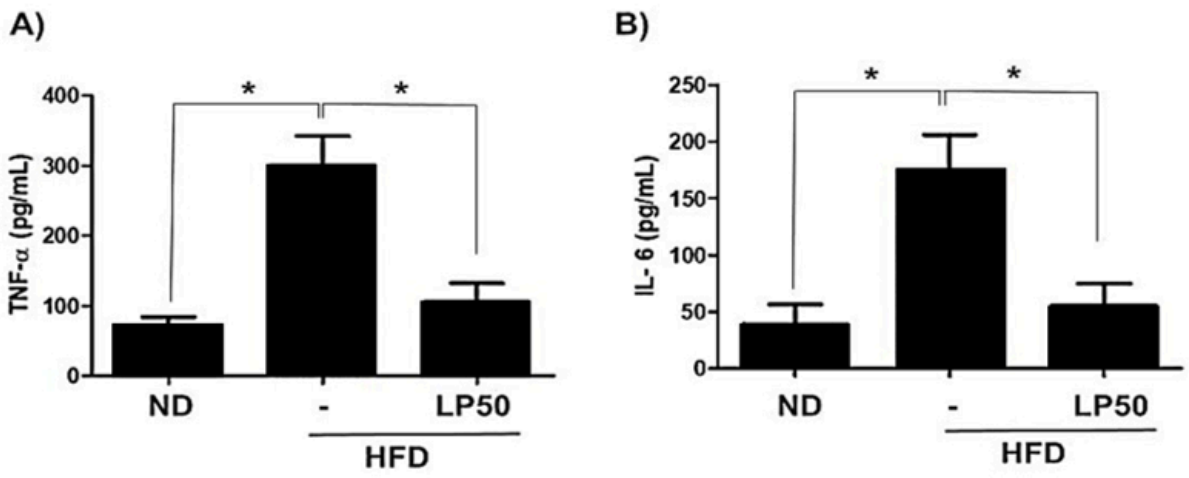


Figure 5

Effect of the protein fraction of *Calotropis procera* latex (LP, 50 mg/kg, i.p.) on TNF-α (pg/mL) **(A)** and IL-6 (pg/mL) **(B)** levels in mice fed a normal diet (ND), a high-fat diet (HFD), or HFD + LP50. Data are expressed as mean ± SEM. *$p < 0.05$ (one-way ANOVA followed by Tukey's post hoc test).

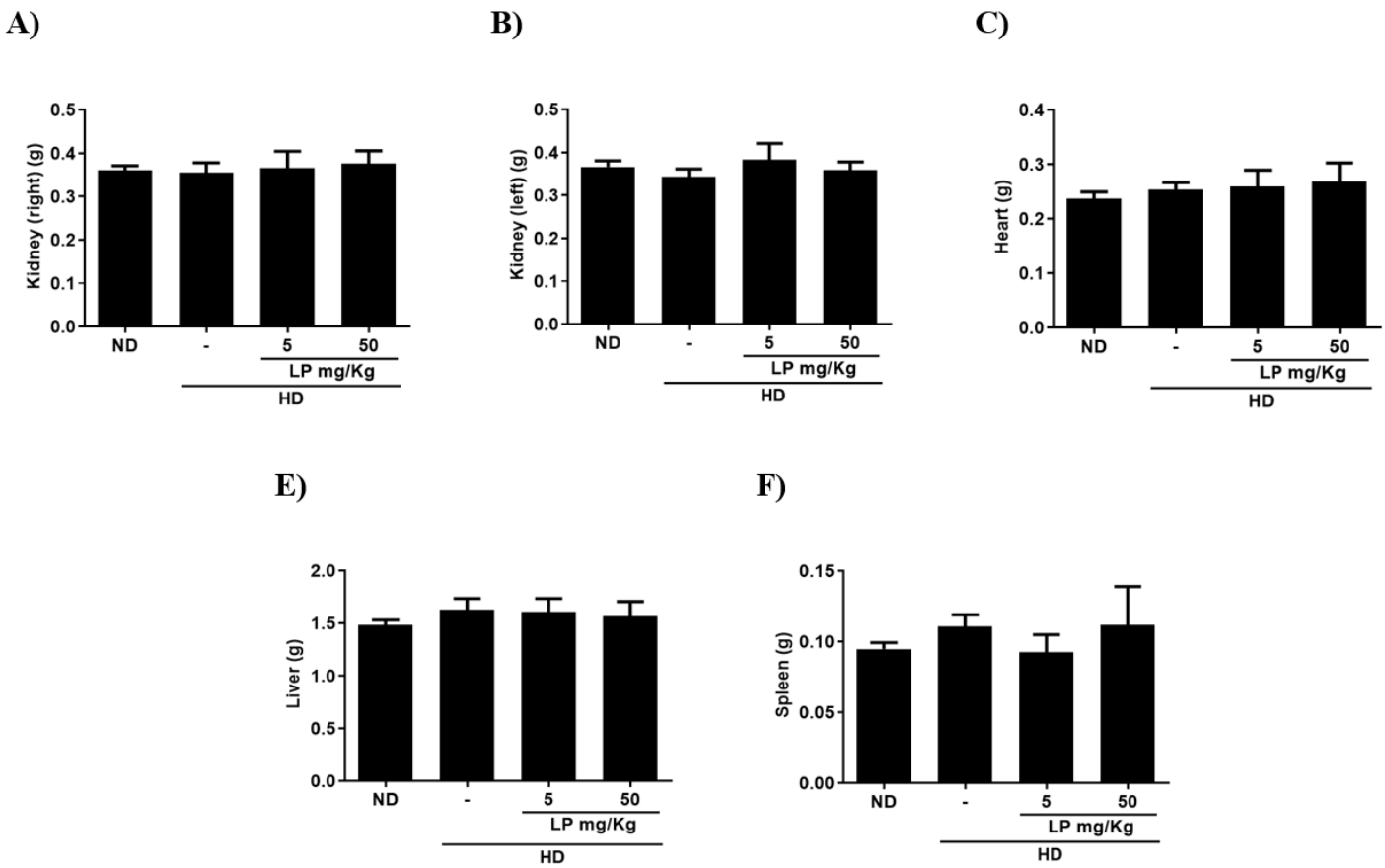


Figure 6

Right kidney **(A)**, left kidney **(B)**, heart **(C)**, liver **(D)**, and spleen **(E)** weights of mice fed a normal diet (ND), a high-fat diet (HFD), or HFD plus *C. procera* latex protein fraction at 5 mg/kg i.p. (LP5) or 50 mg/kg i.p. (LP50). Values are mean ± SEM, n = 4–12.