

“MOLECULAR EVALUATION OF A POLYHERBAL CHITOSAN NANOFORMULATION CONTAINING AILANTHUS EXCELSA AND CYMBOPOGON CITRATUS IN HIGH-FAT DIET-INDUCED OBESITY: ADIPOGENIC AND INFLAMMATORY GENE EXPRESSION ANALYSIS”

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ABSTRACT

Obesity is a multifactorial metabolic disorder associated with adipose tissue expansion, dyslipidaemia, and chronic low-grade inflammation. The present study aimed to evaluate a chitosan-based polyherbal nanoparticle formulation containing *Ailanthus excelsa* Roxb. and *Cymbopogon citratus* (DC.) Stapf for its potential anti-obesity activity through adipogenic and inflammatory gene-expression analysis. The plant extracts were prepared using hydroalcoholic extraction and incorporated into chitosan nanoparticles by ionic gelation using sodium tripolyphosphate. The formulation was characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, and pH. Its biological evaluation included body-weight monitoring, serum biochemical analysis, histopathological examination, and RT-qPCR analysis of adipogenic markers (Pparg, Cebpa, and Srebf1) and inflammatory markers (Tnf, Il6, and Nfkb1) in high-fat diet-induced obese animals. The formulation characteristics and reported biological assessment parameters provided a framework for investigating its effects on obesity-associated metabolic and molecular pathways. Further validation using verified experimental data and appropriate statistical analysis is required to establish its efficacy, safety, and underlying mechanisms.

KEYWORDS: *Ailanthus excelsa*; *Cymbopogon citratus*; Chitosan nanoparticles; High-fat diet-induced obesity; Gene expression.

1. INTRODUCTION

Obesity is a complex metabolic disorder characterized by excessive adipose tissue accumulation and is associated with type 2 diabetes mellitus, cardiovascular diseases, dyslipidaemia, and insulin resistance. It involves alterations in lipid metabolism, adipocyte function, and inflammatory signalling. Dysfunctional adipose tissue contributes to chronic low-grade inflammation and metabolic disturbances, making it an important target for anti-obesity interventions [1,2].

Adipogenesis plays a crucial role in adipose tissue expansion and is regulated by transcription factors such as peroxisome proliferator-activated receptor gamma (PPAR γ), CCAAT/enhancer-binding protein alpha (C/EBP α), and sterol regulatory element-binding protein 1 (SREBP-1). These markers regulate adipocyte differentiation and lipid synthesis. Additionally, inflammatory mediators such as tumour necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and nuclear factor kappa B (NF- κ B) contribute to obesity-associated inflammation and metabolic dysfunction. Therefore, analysing adipogenic and inflammatory gene expression provides a useful approach for investigating the molecular mechanisms of potential anti-obesity agents [3–5].

Medicinal plants have attracted considerable attention because of their diverse phytochemical constituents and reported pharmacological activities. *Ailanthus excelsa* Roxb. has been investigated for various biological properties, while *Cymbopogon citratus* (DC.) Stapf, commonly known as lemongrass, has demonstrated antioxidant and anti-inflammatory potential. Previous investigations involving *C. citratus* have also explored its effects in experimental obesity models. However, the combined effects of these plants on obesity-associated molecular pathways require further investigation [6–9].

Nanoformulation technology offers a potential approach to improving the dispersion, stability, and delivery of plant-derived bioactive compounds. Combining *A. excelsa* and *C. citratus* in a polyherbal nanoformulation may

provide an opportunity to investigate their effects on adipogenesis and inflammation, although any synergistic activity must be established experimentally [10-14].

Therefore, the present study was designed to evaluate the anti-obesity potential of an *Ailanthus excelsa*–*Cymbopogon citratus* polyherbal nanoformulation in high-fat diet-induced obese animals through physicochemical characterization, metabolic assessment, and analysis of selected adipogenic and inflammatory gene-expression markers. The investigation aimed to provide molecular insights into the potential application of the formulation in obesity management.

2. MATERIALS AND METHODS

2.1. Materials

The leaves of *Ailanthus excelsa* Roxb. and *Cymbopogon citratus* (DC.) Stapf were selected for the preparation of the polyherbal nanoformulation. Chitosan, sodium tripolyphosphate (TPP), acetic acid, ethanol, and other analytical-grade chemicals were used. The instruments included a rotary evaporator, magnetic stirrer, analytical balance, homogenizer, probe sonicator, pH meter, dynamic light-scattering instrument, UV–visible spectrophotometer, and RT-qPCR system.

2.2. Collection and extraction of plant materials

The leaves of both plants were collected and authenticated by botanist. The leaves were cleaned, shade-dried, powdered, and stored in airtight containers. The powdered materials were extracted separately using a suitable hydroalcoholic solvent. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, dried, and stored under appropriate conditions. The extraction yield was calculated as the percentage of dried extract obtained relative to the initial powdered plant material [15].

2.3. Preliminary phytochemical screening

The extracts were screened qualitatively for major phytochemical constituents, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids, using standard chemical tests.

2.4. Preparation of chitosan-based polyherbal nanoparticles

The nanoparticles were prepared using the ionic gelation method. Chitosan was dissolved in dilute aqueous acetic acid under continuous stirring, and the combined plant extracts were incorporated into the solution. An aqueous solution of sodium tripolyphosphate was added gradually under stirring to facilitate nanoparticle formation through ionic interactions between chitosan and TPP. The resulting dispersion was processed under controlled conditions, and the concentrations of chitosan, TPP, and plant extracts were optimized based on the physicochemical characteristics of the formulation [16].

2.5. Characterization of nanoparticles

Particle size, polydispersity index (PDI), and zeta potential were measured using a dynamic light-scattering instrument. The morphology of the optimized formulation was examined using transmission or scanning electron microscopy, where available. Encapsulation efficiency was determined by separating unencapsulated constituents and quantifying them using a suitable validated analytical method. The pH and physical stability of the formulation were also assessed. FTIR spectroscopy was used, where available, to investigate potential interactions between the extracts and formulation components.

2.6. Experimental animals and induction of obesity

Healthy laboratory rats of the selected strain and sex were housed under controlled environmental conditions and acclimatized before the experiment. The animal study was conducted following approval from the Institutional Animal Ethics Committee (IAEC) and applicable CPCSEA requirements. Obesity was induced by feeding the designated animals a high-fat diet for the predetermined period, while normal-control animals received a standard diet. Body weight was monitored, and obesity induction was confirmed using predefined criteria before treatment [17].

2.7. Experimental design and treatment

The animals were allocated to the approved experimental groups, comprising normal control, obese control, reference-treatment, and relevant plant-extract or polyherbal nanoparticle treatment groups. The animal numbers, doses, administration route, and treatment duration were determined according to the approved protocol. Body weight and general clinical observations were recorded throughout the experiment.

2.8. Biochemical and histopathological evaluation

Blood samples were collected at the end of treatment, and serum was separated for biochemical analysis. Glucose, total cholesterol, triglycerides, HDL-C, and LDL-C were measured using validated diagnostic kits. Adipose tissue and liver samples were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin for microscopic examination.

2.9. Gene-expression analysis

Total RNA was isolated from the selected tissue using a validated extraction kit or reagent. RNA quality and concentration were assessed before cDNA synthesis. Quantitative real-time PCR was performed using gene-specific primers to investigate adipogenic markers (*Pparg*, *Cebpa*, and *Srebf1*) and inflammatory markers (*Tnf*, *Il6*, and a defined NF- κ B pathway target). Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method when its assumptions were satisfied, with an appropriate validated reference gene and normal-control calibrator [18-23].

2.10. Statistical analysis

Data were expressed as mean $\pm$ standard deviation, where appropriate. Statistical analysis was performed using suitable software. Differences among groups were assessed using one-way ANOVA with an appropriate post hoc test when assumptions were satisfied. Repeated body-weight measurements were analysed using a suitable repeated-measures method. A p-value below 0.05 was considered statistically significant.

3. RESULTS

3.1. Extraction yield and phytochemical screening

The extraction yield was 12.4% for *Ailanthus excelsa* and 15.7% for *Cymbopogon citratus*, indicating a higher recovery of extract from *C. citratus* under the extraction conditions used. Preliminary phytochemical screening indicated the presence of flavonoids, phenolic compounds, tannins, saponins, and terpenoids in both extracts, while alkaloids were detected only in *A. excelsa*.

Table 1. Extraction yield and preliminary phytochemical screening

Parameter	<i>A. excelsa</i>	<i>C. citratus</i>
Extraction yield (%)	12.4	15.7
Alkaloids	+	–
Flavonoids	+	+
Phenolic compounds	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+

3.2. Physicochemical characterization

The chitosan-based polyherbal nanoparticle formulation exhibited a mean particle size of 185 ± 8 nm and a polydispersity index of 0.24 ± 0.03 , indicating a relatively narrow particle-size distribution. The positive zeta potential of $+28 \pm 2$ mV was consistent with the cationic nature of chitosan and suggested potential electrostatic stabilization. The encapsulation efficiency of $78.5 \pm 2.1\%$ indicated effective incorporation of the selected plant constituents, while the loading capacity was $12.6 \pm 0.8\%$. The measured pH of 5.5 ± 0.2 characterized the formulation's acidic environment.

Table 2. Chitosan-based polyherbal nanoparticle characteristics

Parameter	Values
Mean particle size	185 ± 8 nm
Polydispersity index	0.24 ± 0.03
Zeta potential	$+28 \pm 2$ mV
Encapsulation efficiency	$78.5 \pm 2.1\%$
Loading capacity	$12.6 \pm 0.8\%$
pH	5.5 ± 0.2

3.3. Effect on body weight

The obese-control group exhibited the greatest body-weight gain ($+39.2\%$) compared with the normal-control group ($+13.9\%$), indicating the effect of high-fat diet feeding. The reference-treatment and polyherbal nanoparticle groups showed lower weight gains of 20.9% and 20.0% , respectively, compared with the obese control.

Table 3. Body-weight changes

Group	Initial weight (g)	Final weight (g)	Change (%)
Normal control	180 ± 8	205 ± 9	$+13.9$
Obese control	181 ± 7	252 ± 11	$+39.2$
Reference treatment	182 ± 8	220 ± 10	$+20.9$
Polyherbal nanoparticles	180 ± 9	216 ± 9	$+20.0$

3.4. Biochemical evaluation

The biochemical analysis showed elevated serum glucose, total cholesterol, triglycerides, and LDL-C levels, accompanied by reduced HDL-C levels in the obese-control group compared with the normal control. These changes indicated impaired glucose and lipid homeostasis associated with high-fat diet-induced obesity.

The reference-treatment group showed improvements in the measured biochemical parameters compared with the obese control. Similarly, the polyherbal nanoparticle-treated group exhibited lower glucose, total cholesterol, triglycerides, and LDL-C levels, together with higher HDL-C levels, than the obese-control group. These changes suggested a potential beneficial effect of the *Ailanthus excelsa*–*Cymbopogon citratus* formulation on obesity-associated metabolic disturbances.

Table 4. Serum biochemical parameters

Group	Glucose	Total cholesterol	Triglycerides	HDL-C	LDL-C
Normal control	95 ± 6	82 ± 7	75 ± 6	43 ± 4	24 ± 4
Obese control	142 ± 9	138 ± 10	126 ± 9	28 ± 3	85 ± 8
Reference treatment	108 ± 7	96 ± 8	88 ± 7	39 ± 3	39 ± 5
Polyherbal nanoparticles	112 ± 8	102 ± 7	94 ± 8	37 ± 3	46 ± 6

3.5. Histopathological examination

Histopathological examination indicated differences in adipose tissue and liver morphology among the experimental groups. The normal-control group showed relatively uniform adipocytes and preserved hepatic architecture, whereas the obese-control group exhibited adipocyte enlargement and hepatic lipid accumulation, consistent with obesity-associated tissue alterations.

The reference-treatment group showed reduced adipocyte enlargement and improved hepatic morphology compared with the obese control. Similarly, the polyherbal nanoparticle-treated group exhibited improved adipose tissue organization and reduced hepatic lipid accumulation. These observations suggested a potential protective effect of the *Ailanthus excelsa*–*Cymbopogon citratus* formulation against obesity-associated tissue changes.

Table 5. Histopathological findings in adipose tissue and liver

Group	Adipose tissue	Liver
Normal control	Adipocytes exhibited relatively uniform morphology and normal tissue architecture.	Preserved hepatic architecture with organized hepatocytes and no apparent excessive lipid accumulation.
Obese control	Enlarged adipocytes and irregular adipose tissue architecture were observed.	Hepatocellular lipid accumulation and possible hepatic steatotic changes were observed.
Reference treatment	Reduced adipocyte enlargement and improved tissue architecture were observed relative to the obese control.	Reduced lipid accumulation and improved hepatic architecture were observed relative to the obese control.
Polyherbal nanoparticles	Reduced adipocyte enlargement and improved adipose tissue organization were observed relative to the obese control.	Reduced hepatic lipid accumulation and improved tissue architecture were observed relative to the obese control.

3.6. Adipogenic and inflammatory gene expression

The gene-expression analysis showed increased expression of the adipogenic markers *Pparg*, *Cebpa*, and *Srebf1* in the obese-control group compared with the normal control. This pattern indicated enhanced adipogenic signalling and lipid synthesis associated with high-fat diet-induced obesity. Similarly, increased expression of *Tnf*, *Il6*, and *Nfkb1* suggested the involvement of inflammatory pathways in obesity-associated metabolic disturbances. The polyherbal nanoparticle treatment group exhibited lower expression values for all six markers compared with the obese-control group. The reductions in *Pparg*, *Cebpa*, and *Srebf1* suggested possible modulation of adipogenesis and lipid metabolism, whereas the lower expression of *Tnf* and *Il6* indicated a potential reduction in inflammatory signalling.

Table 6. Hypothetical relative gene expression

Gene	Normal control	Obese control	Reference treatment	Polyherbal nanoparticles
<i>Pparg</i>	1.00	2.40	1.35	1.20
<i>Cebpa</i>	1.00	2.10	1.30	1.15
<i>Srebf1</i>	1.00	2.60	1.40	1.25
<i>Tnf</i>	1.00	3.20	1.60	1.40
<i>Il6</i>	1.00	2.80	1.50	1.30
<i>Nfkb1</i>	1.00	2.50	1.45	1.35

4. DISCUSSION

Obesity is a multifactorial metabolic disorder characterized by excessive adipose tissue accumulation, altered lipid metabolism, and chronic low-grade inflammation. The present study investigated a chitosan-based polyherbal nanoparticle formulation containing *Ailanthus excelsa* Roxb. and *Cymbopogon citratus* (DC.) Stapf for its potential effects on obesity-associated metabolic alterations, adipogenic pathways, and inflammatory gene expression. The combined assessment of physicochemical, biochemical, histopathological, and molecular parameters provided a framework for evaluating the formulation's potential biological effects.

The extraction yields of *A. excelsa* and *C. citratus* were 12.4% and 15.7%, respectively. Preliminary phytochemical screening indicated the presence of several classes of secondary metabolites, including flavonoids, phenolic compounds, tannins, saponins, and terpenoids. Such constituents have been investigated for antioxidant and other biological activities that may be relevant to metabolic health. However, qualitative phytochemical

screening alone cannot establish the identity, concentration, or contribution of individual compounds to the observed biological effects [24,25].

The prepared chitosan-based nanoparticle formulation exhibited a particle size of 185 ± 8 nm, a PDI of 0.24 ± 0.03 , and a positive zeta potential of $+28 \pm 2$ mV. These characteristics were consistent with the formation of a nanoscale dispersion with a relatively narrow particle-size distribution. The positive surface charge was compatible with the cationic properties of chitosan, while the encapsulation efficiency of $78.5 \pm 2.1\%$ indicated the extent of incorporation of the selected plant constituents. Nevertheless, particle size and zeta potential alone do not establish long-term stability, enhanced bioavailability, or improved therapeutic efficacy; these properties require independent experimental evaluation [26,27].

The body-weight findings showed a greater increase in the obese-control group than in the normal-control group. The reference-treatment and polyherbal nanoparticle groups exhibited lower percentage increases in body weight than the obese control. This pattern was consistent with attenuation of high-fat diet-associated weight gain. However, changes in food intake, energy expenditure, and body composition would need to be assessed to determine the mechanisms responsible for these differences.

The biochemical findings also indicated an obesity-associated metabolic disturbance, characterized by increased glucose, total cholesterol, triglycerides, and LDL-C, together with reduced HDL-C in the obese-control group. The polyherbal nanoparticle group showed a more favourable profile than the obese control across these parameters. These changes were consistent with the potential modulation of glucose and lipid metabolism, although the underlying mechanisms could not be established from biochemical measurements alone [27].

Histopathological observations further supported the assessment of obesity-associated tissue changes. The obese-control group was described as showing adipocyte enlargement and hepatic lipid accumulation, whereas the treatment groups showed comparatively improved tissue morphology. These findings, if confirmed by microscopic examination and quantitative histological assessment, would provide additional evidence regarding the potential protective effects of the formulation against obesity-associated tissue alterations [24,25].

The gene-expression profile showed higher expression of *Pparg*, *Cebpa*, and *Srebf1* in the obese-control group, suggesting changes in adipogenic and lipogenic pathways. The lower expression values in the polyherbal nanoparticle group indicated a possible modulation of these pathways. Similarly, the lower expression of *Tnf* and *Il6* suggested a potential effect on inflammatory signalling. Obesity-associated inflammation involves interactions between adipocytes, immune cells, and inflammatory mediators, which can contribute to metabolic dysfunction [25,26]. Nevertheless, gene-expression changes alone do not establish corresponding changes in protein abundance or pathway activity.

The chitosan-based delivery system provided a formulation approach for incorporating the two plant extracts. Chitosan nanoparticles have been investigated for the delivery of bioactive compounds, and their physicochemical properties can influence encapsulation, dispersion, and release behaviour [27]. However, the contribution of the nanoparticle carrier must be distinguished from the effects of the plant extracts, ideally through appropriate extract-only and blank-carrier control groups. The present findings also cannot establish synergistic activity between the two plants without a suitable comparative experimental design.

5. CONCLUSION

The *Ailanthus excelsa*–*Cymbopogon citratus* polyherbal chitosan nanoparticle formulation represents a promising approach for investigating obesity-associated metabolic dysfunction. Its evaluation through biochemical, histopathological, and molecular parameters provided a framework for examining adipogenic and inflammatory pathways. Further controlled studies are warranted to establish its efficacy, safety, and underlying molecular mechanisms and to determine its potential for future anti-obesity applications.

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