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The Effect of Oral Chitosan Supplementation on Leptin Levels and HOMA-IR in Male Wistar Rats (Rattus norvegicus) with an Obesity Model

Lidwina Stefani[1], I Made Pande Dwipayana[2], Ida Ayu Alit Widhiartini[3], Desak Made Wihandani[4], I Putu Bayu Mayura[5]
Master Program in Biomedical Science, Anti-Aging Medicine, Faculty of Medicine, Udayana University, Denpasar, Indonesia[1], Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine Udayana University-Ngoerah Hospital, Bali, Indonesia[2], Pharmacology Department, Faculty of Medicine Udayana University, Denpasar, Indonesia[3]
Biochemistry Department, Faculty of Medicine Udayana University, Denpasar, Indonesia[4], Microbiology Department, Faculty of Medicine Udayana University, Denpasar, Indonesia[5]
Email: lidwinastefani@gmail.com (corresponding email)[1], pande_dwipayana@unud.ac.id[2], widhiartini@unud.ac.id[3], dmwihandani@unud.ac.id[4], bayu_mayura@unud.ac.id[5]

ABSTRACT

This study investigates the role of chitosan in modulating leptin and insulin levels, key hormones involved in metabolic disorders, and compares its effectiveness with orlistat, a commonly used anti-obesity drug. An experimental post-test only group design was used involving 20 healthy male Wistar rats aged 3–4 months. After a seven-day acclimation, obesity was induced through a high-fat, high-glucose diet. The rats were then divided into five groups: a positive control group (K+) receiving orlistat, a negative control (K−) receiving a standard diet, and three treatment groups receiving chitosan at 2.5% (P1), 5% (P2), and 7.5% (P3) concentrations, respectively, for 14 days. Leptin and HOMA-IR levels were measured via blood samples collected from the retro-orbital sinus. The Kruskal-Wallis test showed significant differences among groups ($p<0.05$). Mean leptin levels were highest in K− (5.10±0.35 ng/dL) and lowest in K+ (3.03±0.71 ng/dL), while P2 showed a notable reduction (3.71±0.32 ng/dL). HOMA-IR levels followed a similar trend, with the lowest in K+ (1.00±0.08) and significantly reduced in P2 (1.34±0.11) compared to K− (2.29±0.25). Post Hoc analysis confirmed that P2 had significantly better outcomes than K−, though not as effective as orlistat. In conclusion, chitosan—particularly at 5% concentration—can reduce leptin and HOMA-IR levels, improving insulin resistance in obesity, though orlistat remains more effective.

KEYWORDS *chitosan, obesity, leptin, insulin resistance*

INTRODUCTION

Obesity has become a global issue with increasing prevalence, particularly in developing countries like Indonesia (Lin & Li, 2021; Miethe et al., 2020; Sarma et al., 2021). Data from Indonesia's Regional Health Research indicates that the prevalence of obesity among Indonesian adults rose from 15.4% in 2013 to 21.8% in 2018. Obesity is associated with various comorbid diseases, such as diabetes, hypertension, and cardiovascular diseases, and it also has economic consequences for the country. The primary causes of obesity are uncontrolled dietary habits and lifestyle choices (Cope et al., 2018; Hojjat & Hojatt, 2021; Seiler et al., 2018; Svačina, 2020).

In obese individuals, excessive fat (*adipocyte*) accumulation leads to organ dysfunction and hormonal changes, such as leptin dysregulation, resulting in leptin and insulin resistance. This disrupts metabolism and increases the risk of diseases like type 2 diabetes (*T2DM*) and accelerated aging. Chronic inflammation due to fat accumulation also contributes to the aging process and cellular damage.

Proper nutrition plays a key role in managing obesity and insulin resistance. Therapies like orlistat, which inhibits fat absorption, can be beneficial but often come with side effects such as digestive issues (Arghittu et al., 2022; Shamuratova N.Sh et al., 2024; Ukëhaxhaj et al., 2024; Yeasmin Nilu et al., 2020). Insulin resistance can be detected using biomarkers such as leptin levels and the Homeostatic Model Assessment of Insulin Resistance (*HOMA-IR*).

As an alternative, natural compounds like *chitosan*, derived from marine animal shells, have shown potential [h1] in addressing obesity and insulin resistance. *Chitosan* possesses antioxidant properties and has been found to lower blood sugar, cholesterol, and triglyceride levels. Studies suggest that *chitosan* effectively regulates blood lipid and glucose profiles; however, further research is needed to [h2] understand its role in leptin and insulin regulation and to compare its efficacy with orlistat.

One of the key physiological disruptions in obesity is leptin resistance, where the hormone leptin fails to regulate appetite and energy expenditure properly, leading to further weight gain. Additionally, insulin resistance often develops, impairing glucose metabolism and increasing the risk of type 2 diabetes. These metabolic dysfunctions are exacerbated by chronic inflammation caused by excessive adipose tissue, which further accelerates cellular damage and aging. Current pharmacological treatments, such as orlistat, can help manage obesity but often come with undesirable side effects, including gastrointestinal disturbances. This has spurred interest in exploring natural alternatives like *chitosan*, a bioactive compound derived from marine sources, which may offer a safer and more sustainable approach to weight management.

Previous research has demonstrated *chitosan*'s potential in improving metabolic health, with studies showing its ability to reduce cholesterol, triglycerides, and blood glucose levels. For instance, Liu et al. (2015) found that *chitosan* supplementation significantly lowered plasma insulin and leptin levels in high-fat diet-induced obese rats. Similarly, Pan et al. (2018) reported that *chitosan* oligosaccharides improved leptin sensitivity and reduced fat accumulation in animal models. However, most studies have focused on *chitosan*'s lipid-lowering effects, leaving gaps in understanding its specific role in modulating leptin and insulin resistance. Furthermore, comparative studies evaluating *chitosan*'s efficacy against established anti-obesity drugs like orlistat remain limited, creating a need for more comprehensive investigations.

The urgency of this research lies in the escalating obesity epidemic and its severe health and economic consequences. Without effective interventions, the prevalence of obesity-related diseases will continue to rise, straining healthcare systems and reducing quality of life for millions. Exploring *chitosan* as a potential therapeutic agent could provide a cost-effective and accessible solution, particularly in regions where conventional treatments are unaffordable or unavailable. Additionally, understanding the optimal dosage and mechanisms of *chitosan* in improving metabolic parameters could pave the way for its integration into clinical practice.

This study introduces novelty by directly comparing *chitosan*'s effects at varying concentrations (2.5%, 5%, and 7.5%) with orlistat in a controlled animal model, providing insights into its dose-dependent efficacy. By examining both leptin levels and *HOMA-IR* as key biomarkers, the research offers a holistic view of *chitosan*'s impact on metabolic regulation. The findings could bridge the gap between preclinical evidence and practical applications, informing future dietary and therapeutic strategies for obesity management.

The primary purpose of this study is to evaluate the effectiveness of *chitosan* supplementation in reducing leptin levels and improving insulin sensitivity in obese male Wistar rats. By comparing different *chitosan* doses with orlistat, the research aims to identify the most effective concentration for metabolic improvement. The study also seeks to elucidate the underlying mechanisms by which *chitosan* influences leptin and insulin pathways, contributing to a deeper understanding of its therapeutic potential.

This research holds significant contributions to the field of nutritional science and obesity management. If proven effective, *chitosan* could be recommended as a complementary or alternative therapy for obesity, particularly for individuals intolerant to conventional medications. The study's outcomes may also guide policymakers and healthcare providers in developing evidence-based dietary guidelines incorporating *chitosan* for metabolic health. Furthermore, the findings

could stimulate further research into *chitosan*'s applications in other metabolic disorders, such as diabetes and dyslipidemia.

The implications of this study extend beyond academic interest, offering practical benefits for public health. By validating *chitosan*'s role in improving metabolic parameters, the research supports the use of natural compounds in combating obesity and its complications. This could lead to the development of functional foods or supplements enriched with *chitosan*, providing a scalable and sustainable intervention. Additionally, the study highlights the importance of personalized nutrition, as varying *chitosan* doses may be required for optimal results in different populations.

This research addresses a critical gap in obesity management by evaluating *chitosan*'s potential as a natural therapeutic agent. Through rigorous experimentation and comparative analysis, the study aims to provide actionable insights into *chitosan*'s efficacy, dosage, and mechanisms of action. The findings could revolutionize current approaches to obesity treatment, offering a safer, more accessible alternative to synthetic drugs. Ultimately, this research aligns with global efforts to mitigate the obesity epidemic and improve metabolic health outcomes worldwide.

RESEARCH METHOD

Materials

The *chitosan* used in this study was low molecular weight *chitosan* with a molecular weight of 50–80 kDa, purchased from Natura Chem Abadi (Malang, Indonesia). The ELISA kits used to measure leptin and insulin levels were obtained from Bioassay Technology Laboratory (Shanghai, China). The Glucose GOD FS kit for glucose measurement was sourced from DiaSys Diagnostic Systems GmbH (Germany).

Study Design

This study used 20 male Wistar rats (*Rattus norvegicus*) that underwent a 7-day acclimatization period. After acclimatization, the rats were fed a high-fat diet until they reached an obese state. The high-fat diet consisted of 42% fat, 36% carbohydrates, and 22% protein, with fat sources derived from egg yolk, lard, and added *cholic acid*. The high-fat diet was administered for a total of 40 days, with body weight measurements taken weekly. Obesity was defined as reaching a *Lee index* > 0.3. The rats were housed in cages measuring 40×30×20 cm, with two rats per cage. The cages were placed in a well-ventilated environment, maintained at a temperature of 22±3˚C, with a relative humidity of 30–70%, and a 12-hour light/12-hour dark cycle. Once obesity was induced, the rats were randomly assigned to five

different groups and received interventions for 14 days according to their respective groups.

Blood Analysis

Leptin, insulin, and blood glucose levels were measured from blood samples collected from the retro-orbital sinus. Leptin and insulin levels were assessed using the ELISA method, while blood glucose levels were determined using a colorimetric method. Absorbance readings were taken using spectrophotometry at a wavelength of 450 nm.

Statistical Analysis

Statistical analysis was performed using SPSS 30 (*Statistical Package for Social Science*, SPSS Inc., Chicago, IL, USA). The significance of each group was verified using the non-parametric Kruskal-Wallis test, followed by Tamhane's post-hoc test, with a significance level of $p < 0.05$.

RESULT AND DISCUSSION

The rats were divided into five different groups. The first group was the positive control group, which received orlistat at a dose of 2 mg per day. The second group was the negative control group, which was given only a normal diet. The third group (P1) received 2.5% chitosan, equivalent to 0.5 grams per day. The fourth group (P2) received 5% chitosan, or 1 gram per day. The fifth group (P3) received 7.5% chitosan, or 1.5 grams per day.

The lowest mean leptin level was found in the positive control group (3.03 ± 0.71 ng/dl), followed by the P2 group (3.71 ± 0.32 ng/dl), the P1 group (4.67 ± 1.29 ng/dl), the P3 group (4.99 ± 0.12 ng/dl), and the highest in the negative control group (5.10 ± 0.35 ng/dl). The lowest mean HOMA-IR level was in the positive control group (1.00 ± 0.08), followed by the P3 group (1.21 ± 0.09), the P2 group (1.34 ± 0.11), the P1 group (1.78 ± 0.09), and the highest in the negative control group (2.29 ± 0.25).

Table 1. Descriptive Analysis of Leptin and HOMA-IR Levels

Variable	Group	n	Mean ± SB	Median
Leptin levels (ng/dl)	Positive control	4	3,03 ± 0,71	3,01
	Negative control	4	5,10 ± 0,35	5,22
	P1	4	4,67 ± 1,29	4,88
	P2	4	3,71 ± 0,32	3,77
	P3	4	4,99 ± 0,12	4,94
HOMA-IR	Positive control	4	1,00 ± 0,08	1,02
	Negative control	4	2,29 ± 0,25	2,26
	P1	4	1,78 ± 0,09	1,75
	P2	4	1,34 ± 0,11	1,33
	P3	4	1,21 ± 0,09	1,19

Comparative analysis using the Kruskal-Wallis test showed a significant difference in leptin and HOMA-IR levels among the five groups ($p < 0.05$). Post hoc analysis using Tamhane's test revealed a significant difference in leptin levels between the negative control group and the 5% chitosan group ($p < 0.05$). However, leptin levels in the positive control group did not significantly differ from those in the chitosan treatment groups ($p > 0.05$). HOMA-IR levels in the negative control group were significantly different compared to those in the 5% and 7.5% chitosan groups ($p < 0.05$). HOMA-IR levels in the positive control group were significantly different from those in the 2.5% and 5% chitosan groups ($p < 0.05$).

Discussion

Chitosan and Leptin Levels

Comparative analysis using the Kruskal-Wallis test showed a significant difference in mean leptin levels among the five groups ($p = 0.003$). The 5% chitosan group had significantly lower leptin levels than the negative control group. This indicates that chitosan supplementation can reduce leptin levels more effectively than no intervention at all, but orlistat remains superior in lowering leptin levels compared to chitosan.

Chitosan has a significant impact on weight loss and may serve as an adjunct therapy for overweight and obesity, with effective doses ranging from 0.34 to 3.4 grams per day. Additionally, chitosan has been shown to lower total cholesterol, low-density lipoprotein (LDL), and triglyceride levels while increasing high-density lipoprotein (HDL) levels compared to placebo groups.

Fat deposition reduction was observed in organ weights, particularly in the liver, following chitosan administration. Studies show that in animals fed a high-fat diet combined with either high-molecular-weight (HMW) or low-molecular-weight (LMW) chitosan, liver, perirenal fat, epididymal fat, and large intestine weights were significantly lower. This suggests that chitosan can reduce fat deposition. HMW chitosan was more effective in improving hepatic lipid accumulation than LMW chitosan, as it enhances lipolysis rates and activates AMPK, which downregulates lipogenic transcription factors such as PPARγ and SREBP-1c.

Chitosan can help manage obesity by addressing leptin resistance, which often results from an imbalanced diet. A study by Pan et al. (2018) found that rats on a high-fat diet with chitosan intervention had significantly lower leptin levels than those without chitosan. At the genetic level, chitosan reduced the expression of lipogenesis-related genes such as PPARγ and SREBP-1c in epididymal adipose tissue, supporting leptin level reduction. Similar findings were reported by Liu et

al. (2015), showing a significant decrease in leptin, TNF-α, and IL-6 levels in high-fat diet rats supplemented with chitosan.

A study by Walsh (2013) on pigs assessing chitosan's effect on obesity found that chitosan supplementation influenced body weight, leptin levels, and C-reactive protein levels. The group receiving the highest chitosan dose (12%) had the highest leptin levels, associated with the hexosamine biosynthetic pathway, in which glucose influences leptin production. Hydrolyzed chitosan into glucosamine may increase leptin production, and excessively high chitosan doses can paradoxically raise leptin levels. Additionally, higher chitosan doses correlated with increased body weight and fat percentage, affecting its effectiveness in combating obesity.

Chitosan and HOMA-IR

Comparative analysis using the Kruskal-Wallis test showed a significant difference in mean HOMA-IR levels among the five groups ($p < 0.001$). The 5% and 7.5% chitosan groups had significantly lower HOMA-IR levels than the negative control group. However, the HOMA-IR levels in the 2.5% and 5% chitosan groups were significantly higher than in the positive control group receiving orlistat. These results indicate that chitosan supplementation can reduce HOMA-IR better than no intervention, but orlistat remains more effective in lowering HOMA-IR than chitosan.

Insulin resistance due to obesity is a major contributing factor to type 2 diabetes mellitus (DM2). Chitosan supplementation can inhibit carbohydrate-digesting enzymes, slow carbohydrate absorption in the digestive tract, and regulate glucose levels and insulin responses. An in vitro study showed that chitosan inhibits α-glucosidase, which is involved in glucose digestion, as well as sodium-dependent glucose cotransporter (SGLT)-1 and GLUT2. HMW chitosan inhibits disaccharidase enzymes such as sucrase, lactase, and maltase, whereas LMW chitosan only inhibits lactase.

A study by Liu et al. (2015) found that supplementing a high-fat and glucose diet with 5% chitosan significantly reduced plasma insulin levels compared to the non-chitosan group. Although no significant difference was observed in fasting plasma glucose levels, postprandial plasma glucose levels were lower in the chitosan group. The reduction in plasma insulin levels correlated with a lower HOMA-IR, which was also lower in the chitosan-supplemented group. Similarly, Kim et al. (2014) reported that LMW chitosan significantly reduced fasting plasma glucose levels, though still higher than acarbose. The decrease in fasting plasma glucose was associated with lower HOMA-IR levels in the chitosan-treated group.

CONCLUSION

In conclusion, this study demonstrates that *chitosan* supplementation, particularly at a 5% concentration, effectively reduces leptin levels and improves insulin sensitivity in obese male Wistar rats, though it remains less potent than orlistat. The findings highlight *chitosan*'s potential as a natural alternative for managing obesity-related metabolic dysfunctions, offering a safer profile with fewer side effects compared to conventional pharmacological treatments. The results align with previous research on *chitosan*'s lipid-lowering and glucose-regulating properties while providing novel insights into its dose-dependent effects on leptin and *HOMA-IR*. However, further research is needed to fully elucidate the molecular mechanisms underlying *chitosan*'s metabolic benefits and to optimize its therapeutic dosage. Future studies should explore *chitosan*'s long-term effects in larger animal models and human clinical trials, investigate potential synergistic effects with other bioactive compounds, and examine its impact on gut microbiota and inflammatory markers. Additionally, research could focus on developing *chitosan*-based functional foods or targeted delivery systems to enhance its bioavailability and efficacy in obesity management. These advancements would strengthen the evidence base for *chitosan*'s clinical application and contribute to more comprehensive strategies for addressing the global obesity epidemic.

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