

## Research Article

## COMPARATIVE EFFECTS OF PLANT-BASED LOW-SODIUM SALTS AND SODIUM CHLORIDE ON OBESITY MARKERS IN WISTAR RATS

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### ABSTRACT

This study evaluated the comparative effects of plant-derived low-sodium salts (SHOV) and sodium chloride (NaCl) on obesity-related markers in Wistar rats. Sixty rats (30 males and 30 females) were randomly assigned to ten groups and orally administered SHOV or NaCl at doses of 70 mg/kg and 210 mg/kg for 28 consecutive days. Body weight, Lee index, and visceral adiposity index were assessed throughout the study. All groups exhibited progressive weight gain with no evidence of toxicity. Rats treated with 70 mg/kg SHOV showed growth patterns similar to the control group, whereas the 210 mg/kg dose produced slightly lower weight gain, suggesting a potential moderating effect on energy metabolism. The Lee index increased moderately during the experiment but remained statistically comparable to the control under SHOV, unlike NaCl, which significantly elevated this index on days 21 and 28. Visceral adiposity was lower in SHOV-treated rats, particularly at 210 mg/kg, and higher under NaCl at 70 mg/kg ( $p < 0.05$ ). A sex-dependent difference was also observed, with males exhibiting greater adiposity than females. Overall, SHOVs appear to promote improved water mineral and lipid homeostasis, thereby limiting fat accumulation and mitigating the deleterious effects of sodium. Plant-based low-sodium salts thus represent a promising nutritional alternative for the prevention of obesity and its associated metabolic complications.

**Keywords:** Plant-derived low-sodium salts, Sodium chloride, Obesity, Adiposity index, Wistar rats.

### INTRODUCTION

Obesity has emerged as one of the most pressing public health challenges worldwide. It is characterized by excessive accumulation of adipose tissue, often accompanied by low-grade chronic inflammation and metabolic disturbances that predispose individuals to cardiovascular diseases, type 2 diabetes, and chronic kidney disease (WHO, 2021 ; Ng *et al.*, 2014). Its global prevalence continues to rise across both industrialized and developing nations, largely driven by sedentary lifestyles and changing dietary patterns. Among the nutritional factors contributing to this multifactorial condition, excessive consumption of refined table salt primarily composed of sodium chloride (NaCl) plays a pivotal role. Numerous studies have demonstrated a positive association

between high sodium intake and increased body weight, visceral adiposity, and elevated risk of hypertension (He & MacGregor, 2009 ; Mozaffarian *et al.*, 2014 ; Grillo *et al.*, 2019). The underlying mechanisms involve sodium and water retention, stimulation of appetite, and alteration of hormonal pathways regulating satiety and energy balance (Lanaspa *et al.*, 2018). Experimental studies in Wistar rats have further shown that high-NaCl diets promote hypertrophy of visceral and subcutaneous adipose tissues, accompanied by enhanced lipogenic enzyme activity and elevated circulating lipid levels (Fonseca-Alaniz *et al.*, 2007).

However, the relationship between dietary sodium intake and lipid metabolism regulation remains complex. Although excessive sodium consumption promotes

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adiposity, severe sodium restriction may conversely disrupt energy homeostasis and stimulate compensatory fat storage over time. These observations underscore the sensitivity of metabolic regulation to sodium balance and highlight the need for suitable dietary alternatives. In this context, sodium reduction in food products has become a global public health priority (Dahl, 1960). The use of low-sodium salts of plant origin (LSHVOs) represents a promising nutritional strategy. These salts, derived from natural sources such as oil palm stalks, banana stems, and coconut husks, contain lower sodium levels while providing essential minerals including potassium, calcium, and magnesium (Ameyran *et al.*, 2014 ; Awah-Lekaka *et al.*, 2020). In Togo, their traditional use particularly in northern regions is believed to contribute to the prevention of hypertension and to promote overall mineral and nutritional balance.

Beyond their ethnonutritional relevance, these plant-based salts may exert beneficial effects on metabolic pathways involved in lipid storage and mobilization. Preliminary observations suggest that they could reduce visceral adipose tissue accumulation, improve the lean-to-fat mass ratio, and modulate key metabolic markers of obesity, while maintaining the organoleptic properties of foods (Fonseca-Alaniz *et al.*, 2007). However, scientific evidence supporting these effects remains limited, particularly regarding their comparative impact with NaCl on obesity-related indicators such as the Lee index, adiposity index, and visceral fat mass.

In the Togolese context, where hypertension and obesity are concurrently rising, the scientific evaluation of low-sodium plant-derived salts represents a public health priority. It is essential to experimentally assess their safety and efficacy on physiological markers of obesity before advocating their widespread use as nutritional substitutes for NaCl. The Wistar rat, a well-established experimental model for studying metabolic disorders, provides an appropriate framework for such an investigation. This study aimed to compare the effects of sodium chloride (NaCl) and low-sodium salts of plant origin on obesity-related markers in Wistar rats. Specifically, it sought to evaluate their influence on body weight evolution, food intake, and the distribution of visceral and subcutaneous adipose tissues, as well as on standardized metabolic indices such as the Lee index and adiposity index. The outcomes of this investigation are expected to provide insights into the potential regulatory effects of plant-based salts on energy

metabolism and to support the valorization of local natural resources for the nutritional prevention of obesity and its associated metabolic complications.

## MATERIALS AND METHODS

### Materials

#### Animal Material

The experimental material consisted of sixty healthy Wistar rats (30 males and 30 females) aged 6 to 14 weeks. Female rats had a mean age of  $8.51 \pm 0.46$  weeks and a body weight ranging from 150.38 g to 232.27 g (mean =  $171.34 \pm 7.73$  g), while males averaged  $8.23 \pm 0.42$  weeks of age with weights between 152.31 g and 223.16 g (mean =  $174.07 \pm 7.76$  g). These characteristics correspond to young adult rats with stable physiological functions and reduced mortality risk associated with growth or fragility, consistent with general recommendations for experimental studies in Wistar rats (6 – 8 weeks of age and 150 – 250 g body weight ; Hawk *et al.*, 2005).

All animals had free access to standard laboratory chow and drinking water and were acclimatized to laboratory conditions prior to experimentation. The rats were obtained from the Department of Animal Physiology, Faculty of Science, University of Lomé, and maintained in the department's animal facility under controlled ambient temperature ( $25 \pm 2$  °C) and relative humidity. The animals were maintained under natural light–dark cycles, corresponding to daytime and nighttime exposure. They were fed daily with pellets prepared from a mixture of corn flour, soybean meal, dried smoked fish, and wheat flour in proportions of 4 : 2 : 1 : 1 (w/w). The mixture was cooked at 45 °C for 24 hours to improve digestibility and stability. Rats were housed in metallic cages equipped with metal feeders and plastic drinking bottles. The cages were cleaned regularly to maintain optimal hygienic and experimental conditions.

#### Plant Material

The plant material used in this study consisted of salts of plant origin, traditionally produced from oil palm husks (*Elaeis guineensis*). These salts were obtained following a customary extraction and concentration process used in local food preparation (Figure 1).



**Figure 1.** Oil palm (*Elaeis guineensis*) fruit bunches (A) and plant-based low-sodium salts derived from these bunches, shown during the drying process (B) and in their final, ready-to-use form (C).

## Laboratory Equipment

The laboratory equipment used included a precision balance (sensitivity: 0.001 g), a dissection kit containing surgical scissors and forceps, a dissection tray, disposable gloves, and petroleum ether used for animal anesthesia.

## Methodology

### Formation of Experimental Groups

Sixty Wistar rats (30 males and 30 females) were randomly divided into ten groups of six animals each (five female groups and five male groups). The treatments were administered orally once daily for 28 consecutive days (D1–D28) as follows :

Group 1 (Control♀) : 6 females receiving 10 mL/kg of distilled water.

Group 2 (Control♂) : 6 males receiving 10 mL/kg of distilled water.

Group 3 (SV70♀) : 6 females receiving 70 mg/kg of plant-based salts.

Group 4 (SV70♂) : 6 males receiving 70 mg/kg of plant-based salts.

Group 5 (NaCl70♀) : 6 females receiving 70 mg/kg of sodium chloride.

Group 6 (NaCl70♂) : 6 males receiving 70 mg/kg of sodium chloride.

Group 7 (SV210♀) : 6 females receiving 210 mg/kg of plant-based salts.

Group 8 (SV210♂) : 6 males receiving 210 mg/kg of plant-based salts.

Group 9 (NaCl210♀) : 6 females receiving 210 mg/kg of sodium chloride.

Group 10 (NaCl210♂) : 6 males receiving 210 mg/kg of sodium chloride.

All solutions were freshly prepared and administered orally using an intragastric cannula once daily throughout the 28-day experimental period.

### Weight Monitoring

Body weight was monitored daily for all experimental groups over a 28-day period, following the protocol established for subchronic toxicity testing. Weighing was performed each morning between 7:00 and 8:00 a.m. using a precision balance (accuracy: 0.001 g). The variation in body weight, expressed as weight gain (WG), was calculated using the following formula:

$$WG = \text{Body weight on weighing day} - \text{Initial body weight}$$

## Evaluation of Obesity Markers

The effects of subchronic ingestion of SHOV and NaCl on obesity-related parameters were assessed through the determination of adiposity markers, including the adiposity index and Lee index.

On day 29, after euthanasia, visceral fat depots specifically mesenteric, perirenal, retroperitoneal, and omental (greater omentum) fat were carefully dissected, cleared of non-adipose tissues, blotted to remove excess moisture, and weighed fresh using a precision balance (0.001 g). In males, epididymal (gonadal) fat depots were also included. The total visceral fat mass was obtained by summing all dissected fat depots.

The adiposity index (AI) was then calculated according to the following formula :

$$\text{Adiposity index (\%)} = \frac{\text{Total visceral fat weight (g)}}{\text{Body weight (g)}} \times 100$$

### Determination of the Lee Index

The Lee index was determined for each rat on day 0 (D0) and day 29 (D29) based on body weight (g) and naso-anal length (cm), measured using a digital caliper without stretching the animal. It was calculated according to the following formula :

$$\text{Lee index} = \frac{\sqrt[3]{\text{Body weight (g)}}}{\text{Naso - anal length (cm)}} \times 1000$$

All measurements were taken in duplicate to ensure reproducibility and minimize experimental error.

### Statistical Analysis

Data were recorded using Microsoft Excel 2013 and analyzed with R (version 4.2.0) and GraphPad Prism (version 8.00). Continuous variables including the adiposity index, Lee index (D0 and D29), and total visceral fat mass were expressed as mean  $\pm$  standard error of the mean (SEM).

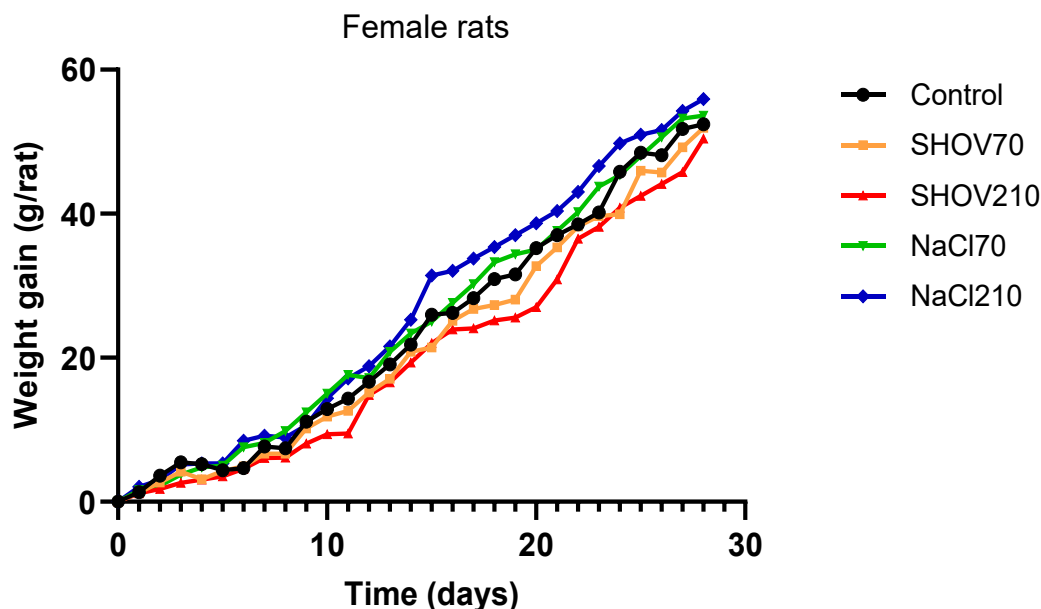
Group comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Correlations between the adiposity index, Lee index, and body weight were evaluated using Pearson's correlation coefficient (or Spearman's correlation coefficient in cases of non-normal data distribution). Statistical significance was set at  $p < 0.05$ .

## RESULTS AND DISCUSSION

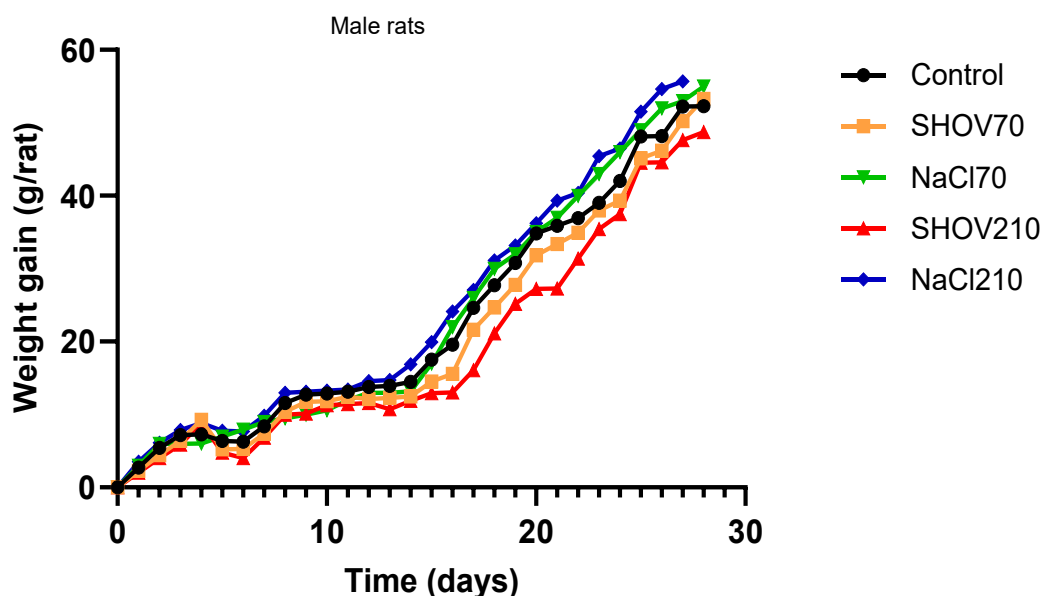
In both sexes, the body weight of rats increased steadily throughout the 28-day experimental period, reflecting normal physiological growth (Figures 2 and 3). The control group exhibited a linear and consistent weight gain, which served as a reference for comparison with the treated groups.

Rats administered plant-based low-sodium salts (SHOV) at 70 mg/kg displayed a weight gain pattern comparable to that of the controls, indicating good tolerance to this dose. Conversely, those receiving 210 mg/kg SHOV exhibited a slightly lower growth slope, suggesting a modest attenuation of weight gain. This effect was observed in both males and females, implying that higher SHOV concentrations may exert a mild inhibitory influence on weight accretion.

In contrast, rats treated with NaCl showed body weight evolution similar to or slightly above that of the controls. While the NaCl 70 mg/kg group did not differ significantly from the control, the NaCl 210 mg/kg group, particularly among males, exhibited a tendency toward higher body weight, indicating a possible dose-related stimulatory effect on growth.



**Figure 2.** Body weight evolution in female Wistar rats during 28 days of subchronic administration of plant-based hyposodium salts (SHOV) and NaCl.

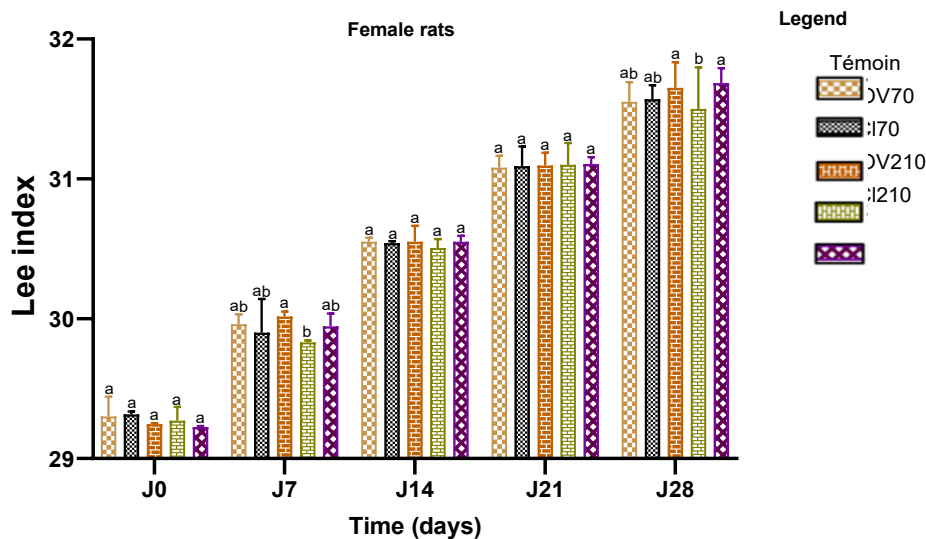


**Figure 3.** Body weight evolution in male Wistar rats during 28 days of subchronic administration of plant-based hyposodium salts (SHOV) and NaCl.

Analysis of the Lee index in female rats showed no statistically significant differences ( $p > 0.05$ ) between groups at baseline (D0) or on days 14 and 21. A significant difference ( $p < 0.05$ ) was observed on day 7, with the NaCl-treated group at 70 mg/kg exhibiting a higher Lee index (30.02%) compared to the SHOV-treated group at 210 mg/kg, which showed a lower index (29.83%). By day 28, the SHOV 210 mg/kg group displayed the lowest Lee index (31.50%), which was significantly different ( $p < 0.05$ ) from the NaCl-treated groups at 70 mg/kg and 210 mg/kg, with indices of 31.65% and 31.69%, respectively. Despite these isolated differences, no significant variations were detected between treated groups and the control throughout the 28-day period ( $p > 0.05$ ). Overall, the Lee index exhibited a gradual upward trend from D0 to D28 in

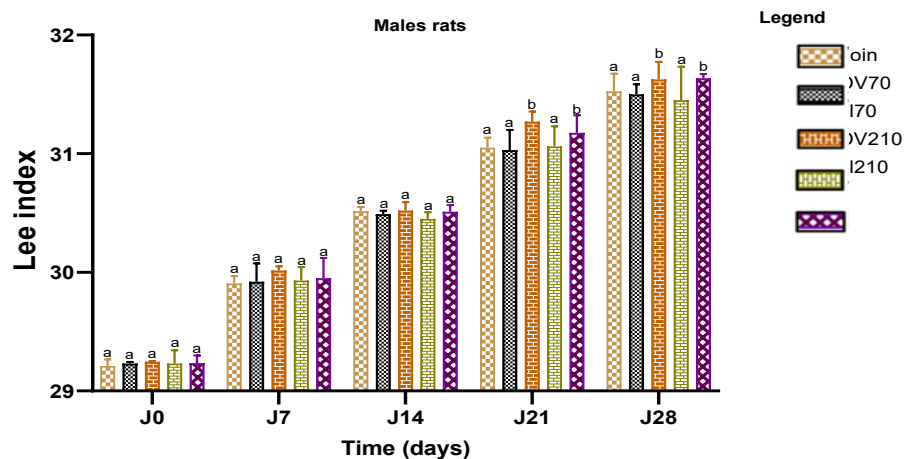
all groups, indicating normal growth progression (Figure 4).

In male rats, significant differences in the Lee index were observed on days 21 and 28. From day 0 to day 14, the Lee indices remained statistically comparable ( $p > 0.05$ ) across all groups. On day 21, the control group and the groups receiving SHOV at 70 and 210 mg/kg exhibited similar Lee indices, which were significantly lower ( $p < 0.05$ ) than those recorded in the NaCl-treated groups at 70 mg/kg (31.27%) and 210 mg/kg (31.18%). This pattern persisted on day 28, with the Lee indices of the control (31.53%) and SHOV 70 mg/kg (31.50%) and 210 mg/kg (31.45%) groups remaining statistically similar, yet significantly lower ( $p < 0.05$ ) than those of the NaCl 70 mg/kg (31.63%) and 210 mg/kg (31.64%) groups (Figure 5).



**Figure 4.** Changes in Lee index during subchronic toxicity testing in female rats according to treatment type.

Bars within the same time point sharing the same letter indicate Lee index values that are not significantly different, whereas bars with different letters represent statistically significant differences at the 5% level ( $p < 0.05$ ).

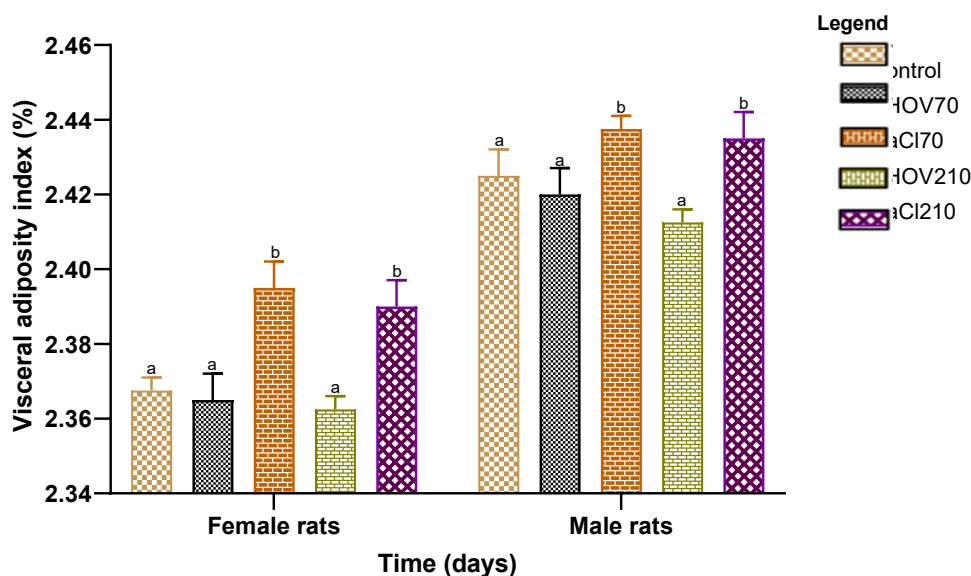


**Figure 5.** Changes in Lee index during subchronic toxicity testing in male rats according to treatment type.

Bars within the same time point that share the same letter indicate Lee index values that are not significantly different, whereas bars with different letters denote statistically significant differences at the 5% level ( $p < 0.05$ ).

The visceral adiposity index measured on day 29 varied according to both sex and administered dose (Figure 6). Male rats exhibited higher visceral adiposity than females, indicating a sex-dependent physiological response to the same treatment. In both sexes, rats receiving SHOV displayed lower adiposity indices, with no significant difference compared to controls ( $p > 0.05$ ), and a more pronounced reduction observed at the 210 mg/kg dose. In

contrast, NaCl-treated groups showed significantly higher visceral adiposity indices ( $p < 0.05$ ) compared to both controls and SHOV-exposed groups. Interestingly, this increase was slightly more marked at the 70 mg/kg dose than at 210 mg/kg. Overall, the visceral adiposity index was lowest in rats treated with SHOV at 210 mg/kg and highest in those receiving NaCl at 70 mg/kg, highlighting a treatment- and dose-dependent effect.



**Figure 6.** Changes in visceral adiposity index on day 29 following subchronic ingestion of SHOV and NaCl, by sex and dose.

Within the same sex, bars bearing different letters indicate statistically significant differences in adiposity index at the 5% level ( $p < 0.05$ ), whereas bars sharing the same letter represent values that are not significantly different.

Weight gain in both male and female rats remained stable throughout the 28-day experiment, indicating that SHOV and NaCl had no toxic effects at the doses tested. Doses of 70 mg/kg (SHOV and NaCl) showed no deleterious effect on weight gain, indicating good metabolic and physiological tolerance. These observations corroborate the work of N'Guessan *et al.* (2017) and Adebayo *et al.* (2020), who report that low concentrations of organic or mineral salts do not significantly disrupt the physical growth of laboratory rats. In females, the slight decrease in weight gain observed with the 210 mg/kg dose of SHOV could reflect a moderating effect on energy metabolism, probably linked to a reduction in appetite or nutritional assimilation efficiency. This observation is consistent with those reported by Oluwafemi *et al.* (2019), who indicated that an overload of phytochemicals can induce a metabolic adaptation aimed at limiting caloric intake or slowing basal metabolism.

The decrease in weight gain under SHOV210, observed in both sexes but more pronounced in females, could therefore result from a dose-dependent effect of the components of SHOV. These often contain ions that can interact with intestinal absorption processes or hormonal regulation of hunger, inhibiting food intake and weight gain (Kouadio *et*

*al.*, 2021; Rahman *et al.*, 2022). The results suggest that low doses (70 mg/kg) are well tolerated and do not affect weight gain, while high doses (210 mg/kg) of SHOVs induce a moderation of energy metabolism, which is more noticeable in females. These gender differences could be attributed to hormonal and metabolic factors: females have endocrine regulation that is more sensitive to variations in osmotic and electrolyte balance, particularly via estrogens, which influence water retention and lipid metabolism (Shi *et al.*, 2009). Overall, steady weight gain and tolerance observed at low doses confirm that the SHOVs analyzed do not cause overt systemic toxicity.

The Lee index and adiposity index are widely used morphometric indicators to assess changes in body composition and nutritional status during toxicological studies in rats. They reflect, respectively, alterations in the ratio between body weight and length, and the distribution of visceral fat deposits (Bernardis & Patterson, 1968; Novelli *et al.*, 2007). Overall, Lee index values showed a slight increase from the start (D0) to the end (D28) of the study, reflecting normal weight gain within the physiological limits expected for Wistar rats maintained on a standard diet (Novelli *et al.*, 2007). In female rats, no significant differences were observed between groups on

D0, D14, and D21, indicating stable weight-to-length ratios in most groups. However, significant differences observed on D7 and D28 suggest a transient effect of NaCl, particularly at the 70 mg/kg dose, which resulted in a higher Lee index compared to SHOV at 210 mg/kg. This finding implies that increased sodium intake may temporarily promote water and sodium retention or a slight weight gain (Nielsen *et al.*, 2008; Rahman *et al.*, 2022).

In males, significant differences appeared mainly on days 21 and 28, with higher Lee indices in the NaCl-treated groups compared to the SHOV groups. This result is consistent with the greater sensitivity of males to sodium load, often associated with different renal and hormonal activity, particularly in the regulation of aldosterone and vasopressin (Dronjak *et al.*, 2010 ; Gandhi *et al.*, 2015). Conversely, SHOV maintained indices comparable to controls, suggesting that their mineral composition (rich in potassium and magnesium, and lower in sodium) contributes to a better water-mineral balance and limits the effects of body hyperosmolality (Kouadio *et al.*, 2021).

The visceral adiposity index on day 29 varied significantly according to sex, dose, and type of salt administered. In both sexes, SHOVs led to a decrease in visceral adiposity compared to NaCl, with no significant difference compared to the controls, particularly at a dose of 210 mg/kg. This profile suggests a favorable effect of SHOVs on lipid regulation, probably related to their richness in minerals that regulate fat metabolism, such as potassium, magnesium, and calcium (Adroque & Madias, 2014 ; Kouadio *et al.*, 2021). Conversely, administration of NaCl, especially at 70 mg/kg, induced significantly higher adiposity indices, reflecting visceral fat accumulation. These observations are consistent with those of He & MacGregor (2010), according to whom increased sodium consumption not only promotes water retention but also disrupts lipid metabolism, leading to an increase in adipose tissue.

The difference observed between the sexes, with slightly higher adiposity in males, can be explained by sexual dimorphism in energy metabolism and fat distribution. Males generally have an androgenic profile that promotes visceral lipogenesis, unlike females, where estrogen promotes a more peripheral distribution of fat (Mayes & Watson, 2004 ; Palmer & Clegg, 2015). The trends observed indicate that SHOVs induce a more balanced physiological response than NaCl. The absence of significant variations in the Lee index and the reduction in adiposity under SHOV indicate better metabolic tolerability. In contrast, NaCl, even at low doses, tends to moderately increase water and sodium load and fat deposits, which could, in the long term, contribute to metabolic imbalance (He *et al.*, 2013 ; Rahman *et al.*, 2022). Partially replacing table salt with mineral-rich SHOVs could therefore be a promising nutritional approach to reducing the harmful effects of sodium on weight regulation and body composition.

## CONCLUSION

The findings of this study indicate that plant-based low-sodium salts (SHOV) exert more favorable metabolic effects than sodium chloride (NaCl) on obesity-related markers in Wistar rats. Subchronic administration of SHOV, particularly at 210 mg/kg, supported normal weight gain while reducing both visceral adiposity and the Lee index, without adversely affecting overall physiological status. In contrast, NaCl, especially at the lower dose of 70 mg/kg, induced a moderate increase in adiposity and weight-to-height ratio, suggesting a negative impact on lipid regulation. These differences are likely attributable to the mineral profile of SHOV, which combines low sodium content with elevated levels of potassium, magnesium, and calcium minerals known to contribute to water and electrolyte balance and to modulate fat metabolism.

Overall, SHOVs appear to be a promising nutritional alternative for partially replacing table salt, potentially contributing to the prevention of obesity and associated metabolic disorders. However, further studies, including biochemical and hormonal analyses, are needed to better elucidate the underlying physiological mechanisms and confirm the safety of these salts for large-scale nutritional use.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

The protocol of the present study was approved by the Bioethics Committee for Health Research of the Ministry of Health of Togo under the reference number 032/2022/CBRS, dated September 30, 2022.

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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