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PROTECTIVE EFFECT OF *CAMELLIA SINENSIS* L. AGAINST OXIDATIVE STRESS AND INTESTINAL DYSBIOSIS INDUCED BY A HIGH-FAT DIET IN WISTAR RATS

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Abstract: The purpose of this study is to assess the effect of green tea (*Camellia sinensis* L.) integrated into the diets of rats on the emergence of obesity and its nutritionally induced complications. 24 Wistar rats were divided into 4 groups. Groups 1 (control) and 2 (SGT) were subjected to a standard diet alone or supplemented with green tea. Group 3(HF) received a high-fat diet alone; however, Group 4 (HFGT) underwent the same diet supplemented with green tea. After a 16-week experiment, biochemical and oxidative analyses on plasma and tissue levels were performed, complemented by the enumeration of certain bacteria from the intestinal microbiota. The results revealed that the high-fat diet induced 19% and 156% increase in body weight and adipose tissue weight in rats, respectively. It also stimulated a significant increase in plasma blood glucose (53%) and triglycerides (27%). This diet affected the antioxidant status, inducing a decrease estimated at 48% in reducing power on the plasma level, at 38% of the hepatic level, and at 55% of the cardiac level. Thus, a reduction in thiol groups was observed at the renal (41%), cardiac (60%), and plasma (58%) levels. This diet promotes lipid accumulation in the liver, thus causing steatosis. Green tea supplementation restores all these metabolic imbalances. Indeed, the group of rats fed a high-fat diet supplemented with 2% tea showed a 16% and 48% reduction in their body weight and adipose tissue mass, respectively. Green tea restored blood sugar levels by 35% and triglycerides by 11%. It exerted an antioxidant effect by increasing the plasma reducing power by 37%, hepatic by 22%, and cardiac by 34%. The level of thiol groups in the heart and plasma has been restored, with an increase of 34% and 50%, respectively. This plant can inhibit the formation of lipid vacuoles induced by the high-fat diet. Also, the incorporation of green tea into the high-fat diet reduced the number of *E.coli* and *Bacteroides* spp. and increased the concentration of *Lactobacillus* spp. in the HFGT group compared to the SGT group. All these results indicated that green tea can be considered in new preventive nutritional approaches aimed at modulating biochemical and oxidative disorders and intestinal dysbiosis in the context of obesity.

Key words: obesity, green tea (*Camellia sinensis* L.), high-fat diet, oxidative stress, gut microbiota

INTRODUCTION

Obesity has been officially declared a chronic disease by the World Health Organisation

(WHO) (accessed on 3-5 June 1997). Today, it represents a real global public health problem,

even more significant than malnutrition and infectious diseases (Ahmed & Mohammed, 2025). Consequently, obesity has become the first non-infectious inflammatory disease in human history, affecting both industrialized and developing countries. It is defined by an abnormal or excessive accumulation of body fat and is characterized by a body mass index greater than 30 (WHO, 2003). Currently, this epidemic is considered a key element of metabolic syndrome and a risk factor involved in the development of numerous chronic diseases, such as cardiovascular diseases, diabetes, and certain cancers (Belladelli, Montorsi & Martini, 2022).

According to the latest WHO estimates (2024), 43% of adults worldwide were overweight (identical figures for men and women). This represents a total of 2.5 billion people, of whom 890 million were considered obese. In Algeria, the prevalence of obesity in 2018 was 48.3% among men and 63.3% among women (Zekri et al., 2022).

Obesity development is a complex process involving both genetic and environmental factors. It mainly results from the imbalance between energy intake and expenditure. Excessive lipid consumption (hyperlipid diet) is the most powerful nutritional factor for generating metabolic disorders. It has been clearly demonstrated that a high-fat diet exacerbates adipose tissue, the site of the genesis of biological disturbances such as inflammation and insulin resistance, closely associated with oxidative imbalance (Tang et al., 2024).

Some research highlights the involvement of a new crucial element in weight management and energy balance, namely the gut microbiota. In recent year, the human gut microbiota has been recognized as a true organ in its own right, heavily involved in the maintenance and well-being of human health. Indeed, modulating the composition of this microbiota could constitute a therapeutic and/or preventive approach likely to reduce the impact of an unbalanced diet on the metabolic complications of obesity (Augustynowicz et al., 2025).

The prevention and management of obesity have become a priority and a crucial health and socio-economic issue, concerning health authorities who are seeking to encourage all measures aimed at reducing its alarming prevalence. Furthermore, the scientific community has focused on iden-

tifying preventive and/or therapeutic tools and strategies for obesity and its consequences.

Green tea (*Camellia sinensis* L.), primarily epigallocatechin gallate, holds a place of excellence and is a choice to consider in this approach. Green tea and its phenolic compounds are widely described for their powerful antioxidant role, their insulin-potentiating effect, their modulating effect on the gut microbiota, and their anti-inflammatory activity (Hartley et al., 2013; Dey et al., 2020).

They should, therefore, have potential interest in the nutritional prevention of obesity and the metabolic pathologies it causes. In this context, the objective of this study was to induce obesity and associated metabolic disorders in Wistar rats through a diet rich in fat to evaluate the preventive effect of green tea on body weight gain and oxidative parameters, as well as its modulating effect on the composition of the intestinal microbiota in Wistar rats.

MATERIALS AND METHODS

Plant material

The green tea was purchased from a local herb store in Tiaret, Algeria. The leaves were finely pulverized using an electric grinder (Fritsch, Germany) and the resulting powder was then incorporated into the experimental diet.

Experimental design

The animals used during this experiment are adult Wistar strain rats with an initial weight of (215.38 ± 3.28 g), provided by the Pasteur Institute of Algiers. All animal procedures were performed in accordance with the internationally accepted guidelines of the European Union on Animal Care (European Union, 2010). The experimental protocol was approved by the Algerian Association of Sciences in Animal Experimentation (AASEA) under the Ethical Approval Number: 45/DGLPAG/DVA.SDA.14. All experiments were conducted at the Veterinary Institute of Tiaret, Algeria, under the direct supervision of qualified veterinarians. Upon their reception, the rats were randomly divided into 4 different groups (6 rats per group), placed in individual cages, and housed within the animal facility of the clinical autopsy laboratory of the Institute of Veterinary Sciences (University of Tiaret), where a constant temperature (24 ± 2 °C) and lighting that varies according to a circadian

rhythm (12h/12h light/dark cycle) are maintained. They had free access to food and drink. After a two-week acclimatization period, these rats were fed ad libitum for 16 weeks with either a standard diet or a high-fat diet containing 48% animal fat of ovine origin, 6.1% carbohydrates, and 20% proteins (591.93 kcal/100 g), supplemented or not with green tea powder. Thus, four experimental groups were formed:

- 1) The animals in the first group (**control**) received a standard diet (8% fat, 45% carbohydrates, and 19% proteins: 330.21 kcal/100g);
- 2) The animals in the second group (**SGT**) received the same diet supplemented with 2% (w/w) green tea (in powder incorporated into the diet);
- 3) The animals in the third group (**HF**) were subjected to a high-fat diet, a diet enriched with ovine fat 48% (w/w);

The animals in the fourth group (**HFGT**) received the previously mentioned hyper-fat diet supplemented with 2% (w/w) green tea (in powder form incorporated into the diet).

Study of biochemical parameters

The measurement of plasma levels of glucose, cholesterol, and triglycerides was determined by the enzymatic and colorimetric method using the Biomaghreb kit, Tunisia.

Determination of Ferric Reducing Antioxidant Power (FRAP) technique

This test is based on the measurement of the reduction by plasma or organ homogenate under acidic conditions (pH 3.6), of a solution including acetate buffer, a solution based on 2,4,6-Tripyridyl-s-triazine (TPTZ) (Sigma Aldrich Company, St. Louis, MO, USA), and a ferric salt, which causes the formation of the blue-colored TPTZ-Fe⁺⁺ complex. A calibration range is obtained from a stock solution of FeSO₄, 7H₂O at 1mM (31.25 µM to 500 µM). The FRAP solution was prepared from the three initial solutions: acetate buffer at pH 3.6, TPTZ at 10 mM, and FeCl₃, 6H₂O at 20 mM) and placed at 37°C for the duration of the analysis. 100 µL of either the sample or the standard solution was added to 900 µL of the FRAP solution. The samples were read after 30 minutes of incubation by spectrophotometry at 593 nm (Benzie & Strain, 1996).

Dosage of thiol groups

The evaluation of protein oxidation was carried out according to the method of Faure and Lafond

(1995). A volume of 375 µL of phosphate buffer (0.05 M) was added. 250 µL of plasma or homogenate, after gentle agitation, and 125 µL of Ellman's reagent were added, the resulting mixture was vortexed and incubated for 15 minutes at room temperature and protected from light.

The absorbance readings were taken at λ=412 nm. A range prepared from a 1mM N-acetylcysteine solution was carried out under the same experimental conditions as the samples.

Histological study

The liver tissue fragments were washed in ice cold saline and fixed in 10% neutral buffered formalin. Subsequently, samples were dehydrated through a graded series of ethanol using an automatic tissue processor (Leica TP1020, Germany). Then, the tissues were cleared in xylene and embedded in paraffin.

Formed blocks were sectioned at a thickness of 5 µm using a rotary microtome (Leica Biosystems, Germany). The sections were stained with hematoxylin and eosin (H&E) and examined under a trinocular light microscope (Zeiss, Germany) coupled with an AXIOCOMER C5S type camera.

Composition of the gut flora

We proceeded with the enumeration of certain bacteria involved in the development of this pathology, namely *Escherichia coli*, *Lactobacillus* spp., and *Bacteroides* spp. This research involved dissolving 1g of fresh fecal matter in 0.9% physiological saline. From the fecal solution, decimal dilutions were prepared, and surface spreads were performed on MacConkey agar for the enumeration of *E. coli*.

The Petri dishes thus prepared were incubated at 37 °C for 24 h. However, a pour plate on Man Rogosa and Sharpe (MRS) medium and *Bacteroides* Bile Esculin + kanamycin was performed for the enumeration of *Lactobacillus* spp. and *Bacteroides* spp., respectively. The inoculation was followed by anaerobic incubation for 24 h at 37 °C.

Statistical treatment

All the results obtained are presented in the form of mean ± SEM (Standard error of the mean). The statistical analysis of the data was conducted using the STATISTICA software (version 6.1, Statsoft, Tulsa, OK). The comparison of the

means was conducted using the one-way ANOVA test.

The latter was complemented by the Tukey test in order to compare the differences between means. The linear relationship between the studied parameters was quantified using Pearson's correlation coefficients (r). A p -value < 0.05 was considered a significance threshold.

RESULTS

Impact on energy intake, weight gain, and adipose tissue

During the present experiment, the two groups of rats on the HF or HFGT diet had an energy intake (508.54 ± 15.11 , 507.99 ± 16.02 kcal/week, respectively) significantly higher than that of the rats on the standard diet supplemented or not with green tea (458.87 ± 18.62 , 471.94 ± 23.21 kcal/week, respectively). At the same time, the supplementation of 2% (w/w) green tea promotes a significant reduction in weight gain in HFGT rats, evaluated at 16%, compared to the HF rat group.

On the other hand, the rats subjected to HF have a higher percentage of adipose tissue mass compared to the rats in the control, SGT, and HFGT groups, with a significant difference (Table 1).

Effect on plasma biochemical parameters

The results showed an increase in plasma glucose levels by 64% (0.64 ± 0.04 vs 0.39 ± 0.02 g/L, $p=0.0003$), triglycerides by 25% (0.15 ± 0.005 vs 0.12 ± 0.002 g/L, $p=0.0001$), and cholesterol by 43% (0.205 ± 0.008 vs 0.14 ± 0.003 g/L, $p=0.0001$)

in rats receiving the high-fat diet compared to control rats. Nevertheless, a significant decrease in these levels is revealed in rats receiving the high-fat diet supplemented with green tea compared to those receiving the high-fat diet alone, this decrease is estimated at 34%, 11%, and 23%, respectively (Table 2).

Total reducing power at the plasma and tissue level

The results of the reducing power are shown in Table 3. The rats receiving the high-fat diet exhibited a significantly lower reducing power, whether plasmatic or tissue-based, compared to the control rats ($P < 0.05$). This variation is marked by a 53% decrease in plasma reducing power (538.57 ± 94.87 vs 257.06 ± 25.2 $\mu\text{mol/L}$, $P < 0.05$), a 38% decrease in hepatic reducing power (1225 ± 88.34 vs 761.48 ± 67.95 $\mu\text{mol/g}$, $P < 0.05$), and a 55% decrease in cardiac reducing power (8723.6 ± 176.37 vs 3962.89 ± 232.5 $\mu\text{mol/g}$, $P < 0.001$). However, the reducing capacity of HFGT rats is enhanced by 85% at the plasma level (257.06 ± 25.2 vs 475.10 ± 32.77 $\mu\text{mol/L}$), 39% at the hepatic level (761.48 ± 67.95 vs 1061.83 ± 45.34 $\mu\text{mol/g}$), and 69% at the cardiac level (3962.89 ± 232.5 vs 5997.83 ± 116.38 $\mu\text{mol/g}$, $P < 0.001$) compared to the reducing potential of HF rats.

Thiol group at the plasma and tissue level

Plasma thiol group (SH) levels (table 4) vary significantly between the HF group and the control ($p < 0.001$). In parallel, HFGT rats showed a significant increase in thiol content compared to HF rats ($p < 0.05$).

Table 1.

Energy intake, weight gain, and adipose tissue mass in different groups of rats after 16 weeks

Parameters	Control	SGT	HF	HFGT
Weight gain (g)	79.33 ± 14.88	82.8 ± 11.8	$149.16 \pm 22.7^*$	$81.66 \pm 15.2^\#$
Energy intake (kcal/wk)	458.87 ± 18.62	471.94 ± 23.21	$508.54 \pm 15.11^*$	$507.99 \pm 16.02^*$
Adipose tissue (g)	3.76 ± 0.2	3.68 ± 0.16	$9.74 \pm 0.52^{***}$	$5.01 \pm 0.2^{***\#}$

The results are expressed as means $\pm$ SEM with ($n=6$), * significant difference control vs HF and HFGT; $^\#$ significant difference, HF vs HFGT. SGT: standard group + green tea, HF: high-fat group, HFGT high-fat group + green tea.

* $P < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2.

Plasma levels of cholesterol, triglycerides, and glucose in different groups of rats after 16 weeks

Parameters	Control	SGT	HF	HFGT
Glucose (g/L)	0.39 ± 0.02	0.41 ± 0.02	$0.64 \pm 0.04^{***}$	$0.42 \pm 0.02^{\#}$
Chol (g/L)	0.14 ± 0.003	0.17 ± 0.006	$0.205 \pm 0.008^{***}$	$0.16 \pm 0.004^*$
TG (g/L)	0.12 ± 0.002	0.118 ± 0.003	$0.15 \pm 0.005^{***}$	$0.13 \pm 0.003^{\#}$

The results are expressed as means $\pm$ SEM with ($n=6$); * significant difference control vs HF and HFGT; $^\#$ significant difference, HF vs HFGT. SGT: standard group + green tea, HF: high-fat group, HFGT: high-fat group + green tea.

Chol: cholesterol; TG: Triglycerides

* $P < 0.05$; *** $p < 0.001$

At the tissue level, our results indicated that thiol concentrations in the liver and heart of control rats are significantly higher than those of HF rats ($p < 0.001$).

Correlation of triglycerides and plasma oxidative parameters with adipose tissue weight

Plasma triglyceride levels show a positive correlation with the evolution of adipose tissue mass ($r = 0.764$, $p = 0.00001$) (Fig. 1A). Furthermore, it was observed that in obese rats, fat accumulation is negatively correlated with oxidative stress markers reflected by total reducing power ($r = -0.4719$, $p = 0.0199$) and thiol groups ($r = -0.6564$, $p = 0.0009$), (Fig. 1B,C).

Histological results

The rats in the control and SGT groups show a histological section of normal livers architecture

with normal sinusoidal morphology (Figure 2a and b). On the other hand, the livers of HF rats demonstrate a large number of lipid vacuoles in the hepatocytes (hepatic steatosis) with sinusoidal dilatation (Fig. 2c).

Rats subjected to the high-fat diet supplemented with green tea (Fig. 2d) indicate a reduction in the size and number of these lipid inclusions. Our results suggest that the high-fat diet induced an accumulation of lipid inclusions in the hepatocytes (Fig. 2c).

Intestinal microbiota

The results of the enumeration of *E.coli* and *Bacteroides* spp. (Fig. 3) in fecal matter reveal that the high-fat diet increases the number of *E.coli* by 17% and the concentration of *Bacteroides* spp. by 19% compared to the standard group.

Table 3.

Plasma reducing power and tissue in different groups of rats after 16 weeks

FRAP levels	Control	SGT	HF	HFGT
Plasma($\mu\text{mol/L}$)	538.57 $\pm$ 94.87	510.13 $\pm$ 72.84	257.06 $\pm$ 25.2*	475.10 $\pm$ 32.77
Liver ($\mu\text{mol/g}$)	1225 $\pm$ 88.34	1179.2 $\pm$ 134.8	761.48 $\pm$ 67.95*	1061.83 $\pm$ 45.34
Heart ($\mu\text{mol/g}$)	8723.6 $\pm$ 176.37	7229.02 $\pm$ 242.6	3962.89 $\pm$ 232.5***	5997.83 $\pm$ 116.38***##

The results are expressed as means $\pm$ SEM with($n=6$). * significant difference control vs HF and HFGT ; # significant difference, HF vs HFGT. SGT: standard group + green tea, HF: high-fat group, HFGT: high-fat group +green tea.

* $P < 0.05$; *** $p < 0.001$

Table 4.

Thiol levels in plasma and tissue in different groups of rats after 16 weeks

Thiol levels	Control	SGT	HF	HFGT
Plasma(mmol/L)	81.7 $\pm$ 12.01	81.825 $\pm$ 3.53	34.023 $\pm$ 4.27***	65.24 $\pm$ 4.04#
Liver (mmol/g)	669.87 $\pm$ 19.56	721.29 $\pm$ 29.13	333.99 $\pm$ 12.7***	369.28 $\pm$ 22.77***
Heart (mmol/g)	499.42 $\pm$ 33.23	418.30 $\pm$ 34.17	198.33 $\pm$ 16.73***	299.28 $\pm$ 30.4*

The results are expressed as means $\pm$ SEM with($n=6$). * significant difference control vs HF and HFGT ; # significant difference, HF vs HFGT. SGT: standard group + green tea, HF: high-fat group, HFGT: high-fat group +green tea.

* $P < 0.05$; ** $p < 0.00$

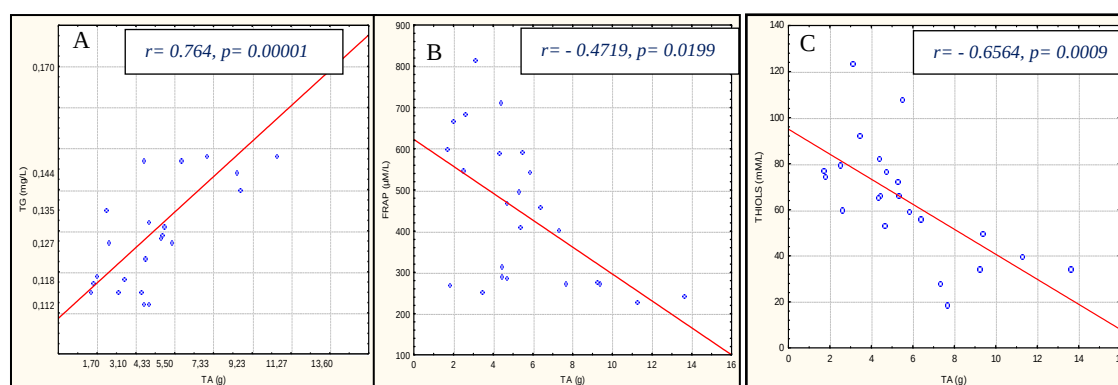


Figure 1. Correlation of triglycerides and plasma oxidative parameters with adipose tissue weight
TG: triglycerides; TA: adipose tissue

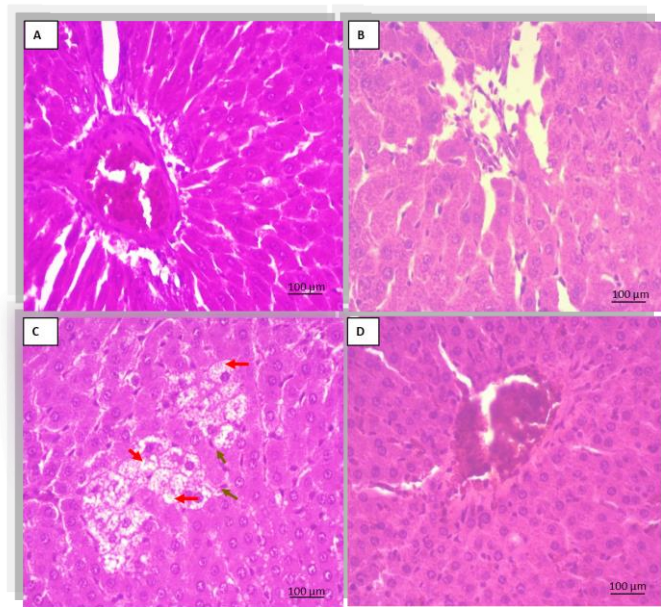


Figure 2. Microscopic observation (×400) of histological sections of rat livers (H&E). (A) Control group showing normal liver architecture with normal sinusoidal morphology; B) Green tea + Control group: normal liver morphology with absence of sinusoidal inflammation; C) Lipid vacuoles are present in the High-Fat (HF) group (hepatic steatosis) (red arrows) with sinusoidal dilatation (brown arrows); D) HF + Green Tea group: absence of lipid vacuoles

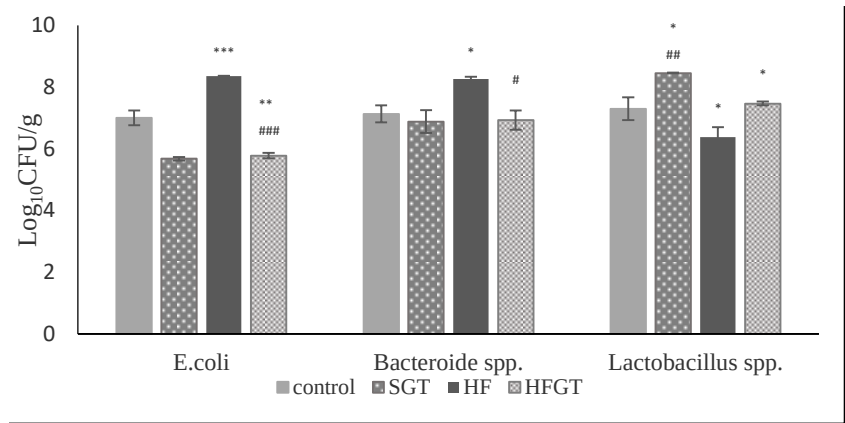


Figure 3. Enumeration of *E.coli*, *Lactobacillus* spp., *Bacteroides* spp. in rats subjected to different diets. The results are expressed as means ± SEM with (n=6); * significant difference control vs HF and HFGT; # significant difference, HF vs HFGT. SGT: standard group + green Tea, HF: high-fat group, HFGT: high-fat group +green Tea (* P< 0.05; ** p< 0.01; *** p< 0.001)

On the other hand, the incorporation of green tea into the high-fat diet influences the quantitative aspect of *E.coli* and *Bacteroides* spp. by inducing a significant decrease in the HFGT group compared to the high-fat diet group (7.77 ± 0.17 vs 8.34 ± 0.02 Log₁₀CFU/g) and (7.09 ± 0.85 vs 8.29 ± 0.006 Log₁₀CFU/g), respectively.

In parallel, the concentration of *Lactobacillus* spp. (Fig. 3) revealed a significant reduction in the number of these bacteria in the HF group compared to the control. This decrease goes from

(7.36 ± 1.15 to 6.30 ± 0.65 Log₁₀CFU/g). However, a significant increase in the number of this bacterium was observed in the group that was fed the high-fat diet supplemented with HFGT tea compared to the HF group (6.29 ± 0.65 vs 7.29 ± 0.04 Log₁₀CFU/g), respectively.

DISCUSSION

This study focuses on the induction of obesity in Wistar rats through a high-fat diet, as well as the associated metabolic and oxidative disorders. The objective is to evaluate the preventive effect

of green tea against oxidative stress and modulations in the composition of the intestinal microbiota. In this study, the high-fat diet administered induced an accumulation of adipose tissue and weight gain in the rats. This confirms that high caloric intake is linked to an increase in adipose tissue and remains the main factor in the onset of obesity. These results corroborate those indicated by Rosnah et al., (2022). The latter reported that Wistar rats subjected to a diet composed of lipids accounting for 60% of total calories for 4 weeks had a body mass 20% higher than that of rats subjected to a standard diet. Our results suggest that the accumulation of lipids in adipose tissue leads to the activation of certain transcription factors, causing an increase in the size of adipocytes (hypertrophy) as well as their number (hyperplasia), thus leading to the expansion of adipose mass.

However, the supplementation of green tea in the high-fat diet promotes a significant reduction in weight gain and adipose tissue. These results are consistent with the work conducted by Mei et al. (2023). The study demonstrated a decrease in weight gain and adipose tissue in mice that were subjected to a diet enriched with tea extract during 8 weeks of treatment. This observation can be attributed to the effect of catechins, particularly epigallocatechin gallate (EGCG) from green tea. Indeed, Peng et al. (2025) demonstrated that EGCG at 50 or 100 mg/kg significantly attenuated HFD-induced weight gain, fat accumulation, hyperlipidemia, and glucose intolerance in mice. It also improved autophagy and lipolysis in white adipose tissue. Another study conducted by Li et al. (2018) suggests that EGCG from green tea reduces fat mass by regulating genes involved in lipolysis in obese mice, and it is also involved in lowering cytokine production (TNF- α) (De Oliveira Assis, Vasconcellos, Lopes, De Macedo & Silva, 2024).

We also observed a reduction in blood glucose levels in rats on a high-fat diet supplemented with green tea compared to rats on a standard diet. These results are analogous to those of a study conducted by Park, Shin, Kim, Jin & Choi, (2020), who believe that green tea exerts a hypoglycemic, glucose-regulating effect by slowing down fat absorption. Several studies conducted on animal models demonstrate that green tea extracts and EGCG have significant insulin-like activity. Green tea improves insulin sensitivity in rats and activates the glucose transporter GLUT-4 (Erukainure et al., 2025).

Supplementation with green tea powder also leads to a decrease in serum cholesterol and triglyceride levels, which confirms the work conducted by Wang et al. (2018).

The results of the evaluation of oxidative parameters at the plasma and tissue levels resulting from our study show that the high-fat diet induces oxidative stress. This is reflected by a decrease in total reducing power and thiol groups at both plasma and tissue levels. It appears that the high-fat diet induces excessive production of Reactive Oxygen Species (ROS) on one hand, and a decrease in antioxidant defence activity on the other. The alteration of the oxidant/antioxidant balance during obesity causes molecular changes, resulting, among other things, in an increase in lipid peroxidation, protein oxidation, and DNA damage (Auberval et al., 2014). Moreover, green tea incorporated into the high-fat diet can improve reducing power, thereby restoring oxidative imbalance. This result is in line with that obtained by Sarhan and Sarhan (2020), who demonstrated that the administration of a green tea extract formulation increased the total antioxidant capacity measured by FRAP and decreased the level of lipid oxidative damage, evaluated by measuring thiobarbituric acid reactive substances in obese rats.

In parallel, in a study conducted by Shen et al., (2015) in mice, the administration of EGCG improved biliary-induced hepatic fibrosis through the modulation of oxidative stress and inflammation, involving various mechanisms: reduction of reactive nitrogen/oxygen species, pro-inflammatory cytokines (TNF α , IL1 β), and reduction of nuclear factor kappa B activity. Furthermore, this molecule inhibits cell death while improving the activity of antioxidant defense enzymes such as glutathione peroxidase and superoxide dismutase. Indeed, the antioxidant activity of green tea catechins is due to the acidic nature of the phenol function and their ability to form hydrogen bonds. This gives it the ability to complex metals and chelate free metal ions, particularly iron and copper, thanks to the catechol group present on ring B, which reduces the production of ROS, and consequently, the decrease in redox reactions (Yan, Zhong, Duan, Chen & Li, 2020). Moreover, catechins reduce hyperlipidemia and oxidative stress through the activation of transcription factors such as Nrf2, which is the main regulator of the cellular antioxidant defense system, thereby mitigating lipogenesis, steatosis, and inflammation induced

by ROS (Silva et al., 2025). The influence of green tea on liver morphology can be attributed to the property of EGCG to regulate lipid homeostasis through the activation of AMPK, which reduces macrovesicular steatosis and protects hepatocytes against radical damage by maintaining their structural integrity.

On the other hand, a high-fat diet can also alter the composition and type of bacteria present in the gut microbiota. This effect is widely documented in the literature, significantly contributing to the development of obesity and metabolic diseases such as type-2 diabetes and colon cancer (Benguiar, Benaraba, Hemida, Bouamar & Riazi, 2020). A study conducted by Fan, Chen and Lin (2023) revealed that a high-fat diet causes an increase in the permeability of the intestinal epithelial cells. This leads to the destruction of the intestinal barrier function, thereby promoting the proliferation of bacteria such as *Salmonella* and *E.coli*. Moreover, free fatty acids from a high-fat diet disrupt the intestinal immune system by increasing pro-inflammatory cytokines like TNF- α and IL-6, while reducing anti-inflammatory cytokines such as IL-10. This imbalance increases intestinal permeability, leading to pathological changes such as low-grade inflammation and a decrease in antimicrobial peptides, which impacts several systemic functions and contributes to obesity and its metabolic complications (Di Vincenzo, Del Gaudio, Petito, Lopetuso & Scaldaferri, 2024). However, the administration of green tea in rats on a high-fat diet influences the profile of their intestinal flora. This is manifested by a decrease in the number of Enterobacteria and an increase in *Lactobacillus* in these groups compared to rats on a high-fat diet. This reduction demonstrates the antibacterial effect of green tea catechins while the increase in the number of these beneficial bacteria confirms their prebiotic potential. This dual capacity to inhibit pathogenic bacteria and stimulate beneficial intestinal bacteria mentioned earlier is confirmed by several experimental studies showing that the phenolic compounds in green tea selectively influence the growth of *Bifidobacterium*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus bulgaricus* (Tabasco et al., 2011), and stimulate the number of *Bifidobacterium* spp. and *Lactobacillus* spp. to a value close to that observed in control animals, by reducing the permeability of the intestinal barrier and inflammation (Delzenne, Neyrinck, Bäckhed & Cani,

2011). In addition, a study conducted by Dong et al. (2024) reports that green tea polyphenols in obese ob/ob mice decreased Enterobacter, increased *Bifidobacterium*, short-chain fatty acids, and reduced TNF- α , IL-6, leptin, insulin resistance, and LPS in the plasma, thereby improving intestinal barrier function (Liu, Li & Shen., 2020).

CONCLUSIONS

This research confirms that green tea has a positive impact on reducing metabolic complications associated with obesity, favorably influencing body weight, adipose tissue mass proliferation, biochemical parameters, and oxidative balance. Thus, tea and its catechins play a crucial role in the prevention and fight against obesity through a nutritional strategy aimed at restoring biochemical and oxidative imbalances while regulating intestinal dysbiosis.

AUTHOR CONTRIBUTIONS

Conceptualization, R.B1. and R.B.2.; Methodology, J.J.O.T. and D.D.; Investigation, formal analysis, validation, N.F.,I.B. and H.H.; Writing-original draft preparation, R.B2 and D.D.; Writing-review and editing, R.B1 and R.B2; Supervision, R.B.1.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

- Ahmed, S. K., & Mohammed, R. A. (2025). Obesity: Prevalence, causes, consequences, management, preventive strategies and future research directions. *Metabolism Open*, 27, 100375. <https://doi.org/10.1016/j.metop.2025.100375>
- Augustynowicz, G., Lasocka, M., Szyller, H., Dziedziak, M., Mytych, A., Braksator, J., & Pytrus, T. (2025).

- The role of gut microbiota in the development and treatment of obesity and overweight: A literature review. *Journal of Clinical Medicine*, 14(14),4933. <https://doi.org/10.3390/jcm14144933>
- Auberval, N., Dal, S., Bietiger, W., Pinget, M., Jean-didier, N., Maillard-Pedracini, E., & Sigrist, S. (2014). Metabolic and oxidative stress markers in Wistar rats after 2 months on a high-fat diet. *Diabetology & Metabolic Syndrome*, 6(1), 130. <https://doi.org/10.1186/1758-5996-6-130>
- Belladelli, F., Montorsi, F., & Martini, A. (2022). Metabolic syndrome, obesity and cancer risk. *Current Opinion in Urology*, 32, 594-597. <https://doi.org/10.1097/mou.0000000000001041>
- Benguiar, R., Benaraba, R., Hemida, H., Bouamar, S., & Riazi, A. (2020). Pomegranate (*Punica granatum* L.) peel and probiotics modulate oxidative stress and intestinal microbiota associated with chemically induced colon cancer in high-fat-diet fed rats. *Journal of Applied Biotechnology Reports*, 7(4), 215-223. <https://doi.org/10.30491/jabr.2020.121479>
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Delzenne, N. M., Neyrinck, A. M., Bäckhed, F., & Cani, P. D. (2011). Targeting gut microbiota in obesity: effects of prebiotics and probiotics. *Nature Reviews Endocrinology*, 7(11), 639-646. <https://doi.org/10.1038/nrendo.2011.126>
- De Oliveira Assis, F., Vasconcellos, G., Lopes, D., De Macedo, L., & Silva, M. (2024). Effect of green tea supplementation on inflammatory markers among patients with metabolic syndrome and related disorders: a systematic review and meta-analysis. *Preventive Nutrition and Food Science*, 29, 106-117. <https://doi.org/10.3746/pnf.2024.29.2.106>
- Dey, P., Olmstead, B. D., Sasaki, G. Y., Vodovotz, Y., Yu, Z., & Bruno, R. S. (2020). Epigallocatechin gallate but not catechin prevents nonalcoholic steatohepatitis in mice similar to green tea extract while differentially affecting the gut microbiota. *The Journal of Nutritional Biochemistry*, 84, 108455. <https://doi.org/10.1016/j.jnutbio.2020.108455>
- Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L. R., & Scaldaferri, F. (2024). Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Internal and Emergency Medicine*, 19(2), 275-293. <https://doi.org/10.1007/s11739-023-03374-w>
- Dong, S., Wu, S., Li, L., Hao, F., Wu, J., Liao, Z., & Fang, X. (2024). Alleviation of lipid metabolic dysfunction through regulation of intestinal bacteriophages and bacteria by green tea polyphenols in Ob/Ob mice. *Food Chemistry*, 456, 139988. <https://doi.org/10.1016/j.foodchem.2024.139988>
- European Union. (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L276/33 <https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng>.
- Erukainure, O., Chukwuma, C., Nambooz, J., Tripathy, S., Salau, V., Olofinisan, K., Ogunlakin, A., Ebuehi, O., & Unuofin, J. (2025). Tea consumption and diabetes: a comprehensive pharmacological review of black, white, green, oolong, and puerh teas. *Plants*, 14, 1898. <https://doi.org/10.3390/plants14131898>
- Fan, S., Chen, S., & Lin, L. (2023). Research progress of gut microbiota and obesity caused by high-fat diet. *Frontiers in Cellular and Infection Microbiology*, 13, 1139800. <https://doi.org/10.3389/fcimb.2023.1139800>
- Faure, P., & Lafond, J. L. (1995). Measurement of plasma sulfhydryl and carbonyl groups as a possible indicator of protein oxidation. In A. E. Favier, J. Cadet, B. Kalyanaraman, M. Fontecave & J.-L. Pierre (Eds.), *Analysis of free radicals in biological systems* (pp. 237-248). Springer Nature Link.
- Hartley, L., Flowers, N., Holmes, J., Clarke, A., Stranges, S., Hooper, L., & Rees, K. (2013). Green and black tea for the primary prevention of cardiovascular disease. *Cochrane Database Systematic Reviews*, 2013(6), CD009934. <https://doi.org/10.1002/14651858.cd009934.pub2>
- Liu, Y. C., Li, X. Y., & Shen, L. (2020). Modulation effect of tea consumption on gut microbiota. *Applied Microbiology and Biotechnology*, 104(3), 981-987.
- Li, F., Gao, C., Yan, P., Zhang, M., Wang, Y., Hu, Y., & Sheng, J. (2018). EGCG reduces obesity and white adipose tissue gain partly through AMPK activation in mice. *Frontiers in Pharmacology*, 9, 389872. <https://doi.org/10.3389/fphar.2018.01366>
- Mei, H., Li, J., Liu, S., Jeyaraj, A., Zhuang, J., Wang, Y., & Li, X. (2023). The role of green tea on the regulation of gut microbes and prevention of high-fat diet-induced metabolic syndrome in mice. *Foods*, 12(15), 2953. <https://doi.org/10.3390/foods12152953>
- Park, J., Shin, Y., Kim, S., Jin, M., & Choi, J. (2020). Dietary epigallocatechin-3-gallate alters the gut microbiota of obese diabetic db/db mice: Lactobacillus is a putative target. *Journal of Medicinal Food*, 23(10), 1033-1042. <https://doi.org/10.1089/jmf.2020.4700>
- Peng, H., Cheng, Q., Chen, J., Wang, Y., Du, M., Lin, X., & Wang, X. (2025). Green tea epigallocatechin-3-gallate ameliorates lipid accumulation and obesity-associated metabolic syndrome via regulating autophagy and lipolysis in preadipocytes and adipose tissue. *Journal of Agricultural and Food Chemistry*, 73(20), 12272-12291. <https://doi.org/10.1021/acs.jafc.5c00973>
- Rosnah, R., Taslim, N. A., Aman, A. M., Idris, I., As'ad, S., Buchari, A., & Nugraha, G. I. (2022). The formulation and evaluation of high-fat pellet on lipid profiles and body mass index of male Wistar rats. *Iraqi Journal of Pharmaceutical Sciences*, 31(1), 285-292. <https://doi.org/10.31351/vol31iss1pp285-292>
- Tabasco, R., Sánchez-Patán, F., Monagas, M., Bar-tolomé, B., Moreno-Arribas, M. V., Peláez, C., & Requena, T. (2011). Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: Resistance and metabolism. *Food Microbiology*, 28(7), 1345-1352.

- <https://doi.org/10.1016/j.fm.2011.06.005>
- Sarhan, W., & Sarhan, O. (2020). Evaluation of ethosomal epigallocatechin-3-gallate gel formulations as an antioxidant and thermogenic agent. *International Journal of Pharmacy and Pharmaceutical Sciences*, 12, 122-130.
<https://doi.org/10.22159/ijpps.2020v12i11.39276>
- Shen, K., Feng, X., Su, R., Xie, H., Zhou, L., & Zheng, S. (2015). Epigallocatechin 3-gallate ameliorates bile duct ligation induced liver injury in mice by modulation of mitochondrial oxidative stress and inflammation. *PLoS One*, 10(5), e0126278.
<https://doi.org/10.1371/journal.pone.0126278>
- Silva, G., Da Cruz Monteiro Santana, T., Velozo, A., Macêdo, A., Gonçalves, M., Couto, R., Soares, M., Viana, M., & Villarreal, C. (2025). Green tea intake reduces high-fat diet-induced sensory neuropathy in mice by upregulating the anti-oxidant defense system in the spinal cord. *Antioxidants*, 14(4), 452.
<https://doi.org/10.3390/antiox14040452>
- Tang, C., Wang, Y., Xu, Z., Chen, D., Xu, J., Yang, D., & Kan, J. (2024). The relationships between high-fat diet and metabolic syndrome: Potential mechanisms. *Food Bioscience*, 59, 104261.
<https://doi.org/10.1016/j.fbio.2024.104261>
- please find attached : the revised manuscript
- Wang, L., Zeng, B., Liu, Z., Liao, Z., Zhong, Q., Gu, L., Wei, H., & Fang, X. (2018). Green tea polyphenols modulate colonic microbiota diversity and lipid metabolism in high-fat diet treated HFA mice. *Journal of Food Science*, 83(3), 864-873.
<https://doi.org/10.1111/1750-3841.14058>
- World Health Organization. (2003). Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation (Vol.916). Retrieved April 2025 from <https://www.who.int/publications/i/item/924120916X>.(accessed Avril, 2025).
- World Health Organization. (2024). Obesity and overweight. Retrieved April 2025 from <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>.
- Yan, Z., Zhong, Y., Duan, Y., Chen, Q., & Li, F. (2020). Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Animal Nutrition*, 6, 115-123.
<https://doi.org/10.1016/j.aninu.2020.01.001>
- Zekri, S. Tagzout, D. Hamrour, F. Mammeri, A. Ammi, M. Hadjene, L. Slimani, N. & Tebaibia A. (2022). Obésité et diabète: une équation à double sens. *Algerian Journal of Obesity and Metabolic Diseases*, 1, 6-8.

ZAŠTITNI EFEKAT *CAMELLIA SINENSIS* L. PROTIV OKSIDATIVNOG STRESA I INTESTINALNE DISBIOZE IZAZVANE ISHRANOM SA VISOKIM SADRŽAJEM MASTI KOD WISTAR PACOVA

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Sažetak: Svrha ove studije je bio da se proceni efekat zelenog čaja (*Camellia sinensis* L.) uključenog u ishranu pacova na pojavu gojaznosti i nutritivno izazvanih komplikacija. Grupa od 24 jedinki Wistar pacova su podeljena u 4 grupe. Grupa 1 (kontrolna) i Grupa 2 (SGT) bile su podvrgnute standardnoj ishrani samostalno ili uz dodatak zelenog čaja. Grupa 3 (HF) primala je samo hranu sa visokim sadržajem masti; međutim, Grupa 4 (HFGT) bila je na istoj dijeti uz dodatak zelenog čaja. Nakon 16 nedelja eksperimenta, sprovedene su biohemijske i oksidativne analize na nivou plazme i tkiva, dopunjene brojanjem određenih bakterija iz intestinalne mikrobiote. Rezultati su pokazali da je ishrana sa visokim sadržajem masti izazvala povećanje telesne mase za 19% i mase masnog tkiva za 156% kod pacova. Takođe je stimulisala značajno povećanje sadržaja glukoze u plazmi (53%) kao i triglicerida (27%). Ova ishrana je uticala na antioksidativni status, izazivajući smanjenje u redukcionoj moći na nivou plazme za 48%, 38% na hepatičkom nivou i 55% na kardijalnom nivou. Tako je primećeno smanjenje sadržaja tiolnih grupa na renalnom (41%), kardijalnom (60%) i plazmatskom (58%) nivou. Ova dijeta podstiče nakupljanje lipida u jetri, izazivajući steatozu. Suplementacija zelenim čajem vraća sve ove metaboličke disbalanse. Naime, grupa pacova hranjena hranom sa visokim sadržajem masti uz dodatak 2% čaja pokazala je smanjenje telesne mase od 16% i mase masnog tkiva od 48%. Zeleni čaj je normalizovao nivo glukoze u krvi za 35% i triglicerida za 11%. Zeleni čaj je pokazao antioksidativni efekat povećanjem redukcionog moći plazme za 37%, jetre za 22% i srca za 34%. Nivo tiolnih grupa u srcu i plazmi je obnovljen, sa povećanjem od 34% i 50%. Ova biljka može da inhibira formiranje lipidnih vakuola izazvanih ishranom sa visokim sadržajem masti. Takođe, uključivanje zelenog čaja u dijetu sa visokim sadržajem masti smanjilo je broj *E. coli* i *Bacteroides* spp., a povećalo koncentraciju *Lactobacillus* spp. u HFGT grupi u poređenju sa SGT grupom. Svi ovi rezultati ukazuju da se zeleni čaj može razmatrati u novim preventivnim nutritivnim pristupima usmerenim na modulaciju biohemijskih i oksidativnih poremećaja i intestinalne disbioze u kontekstu gojaznosti.

Ključne reči: gojaznost, zeleni čaj (*Camellia sinensis* L.), ishrana sa visokim sadržajem masti, oksidativni stres, crevna mikrobiota

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