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Lemon Juice Gavage Promoted Body Weight Loss and a Reduction of Visceral Fats and Blood Lipids in the Animal-Fat-Fed Wistar Rats

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Abstract

The increasing consumption of animal fat in developing countries encourages the need for exploration of natural products that can reduce associated cardiovascular risk factors in consumers.

This study assessed the effect of lemon juice on body weight, visceral fat, and blood lipid levels in Wistar rats under an animal fat diet. A total of 18 Wistar rats (8 months old) were randomly sorted into three groups of six rats each. Group 1, the control, received a basic diet. In addition to a basic diet, Group 2 received 6mg/g bw of animal fat, and Group 3 received 6mg/g bw of animal fat and 0.003ml/g bw of lemon juice by gastric gavage for 28 days. Assessments were carried out on the weight of the body, liver, and abdominal fat. Moreover, histology of abdominal fat, liver, and heart, size of fat cells, and plasma level of glucose and lipids.

The rat pre-treatment body weight varied insignificantly ($P>0.05$) among the studied groups. However, body weight gain as well as the weight of the liver and abdominal fat were significantly reduced in rats fed both the lemon juice and animal fat compared to rats fed a similar diet without the juice ($P<0.05$). The degree of fat infiltration in the liver parenchyma,

hepatocytes, cardiomyocytes, and coronary arteries, and the sizes of abdominal adipocytes were significantly reduced ($P<0.05$) in rats fed with animal fat and lemon juice compared to rats on the same diet without the juice. Moreover, while other cholesterol types remained unaffected, the triglyceride (TG) level was significantly reduced in rats fed with both the animal fat and lemon juice compared to rats eating a similar diet without the juice.

In this study, a treatment with lemon juice promoted a significant reduction in body weight, blood triglycerides, fat cell sizes, and fat levels in hepatocytes and coronary arteries in the animal fat-fed rats.

Keywords: lemon juice, animal model, animal fat, triglyceride, Wistar rats, cardiovascular risk factors

1. Introduction

Cardiovascular disease risk factors, including obesity, hyperglycemia, hyperlipidemia, and hypertension, are increasingly becoming a burden in developing countries (Njelekela *et al.,* 2001). Unhealthy eating increases the incidence of cardiovascular disease risk factors (Stanley, 2012; Sacks *et al*., 2017). Fat-rich diets, especially those from food animals, are among the significant risk factors increasing the prevalence of cardiovascular diseases (Skeaff, 2009; Sacks *et al*., 2017; Morison *et al*., 2023). Lifestyle changes, such as those associated with livelihood income gain, urbanization, or limited available food options, are the key drivers toward the increasing consumption of unhealthy, fat-rich animal products (Derek *et al*., 2016; Sseguya *et al*., 2020; Elihaika *et al*., 2021; Ping *et al*., 2022). Fat-loaded food products sourced from food animals include milk and milk products, also the cooked, barbequed, fried meat or visceral organs, broths, and eggs (Lukmanji *et al*., 2008; Mamiro *et al*., 2019; Morison *et al*., 2023). Moreover, there are cooking oils extracted from meat and tallow of food animals that are sold to food vendors and lower-income earners by unfaithful meat handlers, increasing the associated health risk. The unhealthy nature of animal fats is attributable to their high composition of saturated fatty acids (Salter, 2013; Perna and Hewlings, 2022). Regular consumption of saturated fatty acids may contribute to metabolic disorders and cardiovascular diseases (Salter, 2013; Perna and Hewlings, 2022; Morison *et al*., 2023). For instance, high intake of animal fats can significantly increase fat deposition in the body tissues (Poret *et al*., 2018; Morison *et al*., 2023). Excessive accumulation of fat in body tissues has been reported to impair insulin signaling mechanisms, leading to insulin resistance and hyperglycemia (Samwel and Shulman, 2012; Salter 2013; Wali *et al*., 2020; Jani *et al*., 2021). Untreated hyperglycemia may advance to type 2 Diabetes Mellitus (Jani *et al*., 2021). Also, excessive consumption of saturated fats may contribute to obesity, a significant cardiovascular risk factor for hyperglycemia, hypertension, hypercholesterolemia, and cardiovascular disease development (Njelekela *et al*., 2011; Morison *et al*., 2023). Moreover, overconsumption of saturated fats may lead to liver fat accumulation in consumers (Poret *et al*., 2018), leading to steatosis and Nonalcoholic Fatty Liver Disease (NAFLD) (Green and Hodson, 2014). Also, an excessive intake of animal fat may increase the synthesis and deposition in blood and blood vessels of bad cholesterol in consumers, increasing the risk for cardiovascular diseases such as coronary heart disease (Siri-Tarino *et al*., 2010). However,

despite all the available knowledge on the adverse effects of an animal-fat-rich diet in consumers, its consumption continues to prevail. Thus, needs are increasing need to search for natural products that can curb or reduce the adverse effects of saturated fats in consumers. Inclusion of certain vegetables, fruits, and other plant products in the diet during cooking or eating could probably help to lessen the adverse effects of animal fat in consumers. (Fukuchi *et al*., 2008). One of the food products highly suggested for that purpose is the extracts from Citrus or lemon fruits. Traditionally, lemon juice is willingly added to food during preparation and eating to increase flavour. However, lemon as a fruit is highly nutritious due to its high content of vitamins and polyphenols (mainly flavonoids) such as hesperidin, eriocitrin, naringin, neo hesperidin, rutin, quercetin, chlorogenic acid, luteolin, and kaempferol (González-Molina *et al.,* 2010). Moreover, some of the compounds contained in lemon have been revealed to ameliorate body weight, body fat, and blood cholesterol even in obese human individuals (Aslani *et al*., 2016; Fukuchi *et al*., 2008). Also, according to Fukuchi *et al*. (2008), the extract from lemon fruit peels prompted a reduction of body weight gain and a decrease of epididymal fat, mesenteric fat, and plasma and hepatic TG levels similar to the anti-obesity drug orlistat. Further exploration by Fukuchi *et al.* (2008) reported a significant reduction in body weight gain, fat pad accumulation, and the development of hyperlipidemia, hyperglycemia, and insulin resistance following treatment with lemon-extracted polyphenols in the high-fat diet-induced obese rats. Whether the lemon fruits found in the Tanzanian environment can be effective enough to counter the effects of fat fat-rich diet in consumers' needs to be studied. Therefore, this study assessed the effects of lemon juice consumption on body weight, blood lipids, and visceral fat using Wistar rats as a model animal.

2. Methodology

Study area

An experimental study was conducted in Morogoro urban (6° 50' 42.66" S, 37° 39' 29.14"E). Experiments setup and data collection were done in the College of Veterinary Medicine and Biomedical Science (CVMBS), at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Research clearance was granted by the Research Ethical Committee of the SUA (SUA/DPRTC/R/126/VET/3/2023/4) before the study commencement.

Collection and processing of lemons for lemon juice

The Lemon was bought from the common market in Morogoro urban, then peeled, and the inner flesh of the fruit was blended using an electric blender. The materials were then filtered using multiple folded gauze to obtain lemon juice.

Preparation of fat extract from beef tallow

The beef tallow was purchased from the meat shops (butchers) in Morogoro urban Tanzania. The meat shops sell beef, offal, beef tallow, and the fat extracted from those tissues. To extract fat from beef tallow, the materials were chopped by a knife into smaller pieces and then put in the saucepan, followed by gently heating on a stove to separate the fat from any remaining muscle or connective tissue. The liquid fat was then decanted into a different

container and left to cool and solidify at room temperature. The nutritional composition of beef tallow fat extracts was analyzed and found to be as follows: dry matter (DM) = 99.6 %, Ash = 0.01%, crude protein (CP) = 0.78 %, and ether extract (EE) = 98.7%.

Study animals

Wistar rats were model animals used during the experiments. The animals were collected from the small animal research unit of the CVMBS. Before treatment, the animals were left for 2 weeks in cages to adapt to the standard room temperature of 25°C to 37°C, relative humidity (80% and 80.5%), and day-night cycles (12/12 hours) while fed with broiler starter and ad lib water.

Experimental Setup and Animal Treatments

A total of 18 Wistar rats aged 8 months were used during the study. The animals were randomly sorted into three groups, with each group containing six animals. The first group of rats, labeled as control, received a basic diet and some tap water. The second group of rats received a basic diet, tap water, and 6mg/g body weight of beef tallow fat extract. The third group of rats received a basic diet, tap water, and 6mg/g body weight of beef tallow fat extract, followed immediately by lemon juice 0.003ml/g Body weight. Feeding was done by gastric gavage using a specific feeding needle for 28 days. Guidelines for the proper use of laboratory animals during research were adequately followed when handling and restraining the rats.

Data Collection

Assessment of body weights

The measurement of the body weight of the rats employed a digital weighing balance. Body weight recorded before treatment was labeled as initial or pre-treatment body weight. The second body weight was recorded at the end of treatment and labeled as post-treatment body weight. Bodyweight gain was calculated by subtracting the post-treatment body weight from the initial body weight.

Blood sample collection

After the measurement of the body weight, the animals were anesthetized with chloroform and then dissected. Blood samples were collected from the heart using a needle into a Vacutainer tube containing sodium fluoride as an anticoagulant to prepare plasma for glucose and lipid analysis.

Weights and histology of the abdominal fat and liver

Trimming of the rats' visceral fat and liver tissues was done to record their weight and for histological studies. The weights were measured using a weighing balance and then processed under the standard laboratory histological procedures involving fixing the tissues with 10% neutral buffered formalin, dehydration at increasing concentrations % of ethanol (75, 95, 95), clearing in xylene, embedding in paraffin wax, and tissue sectioning by a microtome before being assessed. Microscopic examination of the sectioned and

Haematoxyline & Eosin stained tissues employed a camera (MOTICAM PRO 205A, made in German, Christian-Kremp-Straβe 11, 35578 Wetzlar, Germany) mounted bright light microscope (OLYMPUS 2467, made by Olympus Life Science Solutions, 48 Woerd Ave, Waltham, MA, 02453, United States). Also, the micrometer of a microscope was employed for the size assessment of adipocytes. Microscopic examination was done at x 100 magnification.

Measurement of plasma glucose and lipid levels

The analysis of plasma glucose was done colorimetrically employing the Tinder method as per the kit manufacturer's instructions. Also, the analysis of plasma lipids was done colorimetrically employing the cholesterol assay kit (Erba Manheim, India, BLT 00035) for Total cholesterol (TCh) and the triglyceride assay kit (Erba Manheim, India, BLT 00059) for Triglycerides (TG) as per the kit's manufacturer's instructions. The analysis of High-Density Lipoprotein employed the Erba Manheim, India, BLT 00034 High-Density Lipoprotein Cholesterol (HDL-c) assay kit following the kit manufacturer's instructions. Then, the Friedewald Equation (Krishnaveni et al, 2015); LDL-c (mg/dL) = TCh (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5 was used to calculate the serum levels of Low-Density Lipoprotein Cholesterol (LDL-c).

Data analysis

Data analysis was done in the Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics were computed for the mean and standard errors of the mean of body weights and weights of visceral fat, liver, and heart, and the size of fat cells. Also, the mean and standard errors of the mean of blood glucose and lipids were analyzed. The Analysis of variance was used for the overall comparison of the means between the groups. Pairwise comparisons between the individual groups employed Tukey's HSD test. The level of significance difference between groups was considered at $P<0.05$.

3. Results

The average of rats' body weight recorded before treatment did not differ significantly ($P>0.05$) between the studied groups (Table 1). However, the body weight gained after 28 days of treatment was significantly lower ($P<0.05$) in the group of rats that consumed the animal fat and lemon juice compared to the negative control or to those consuming animal fats without lemon juice (Table 1). Rats eating animal fats without lemon juice had body weight gain comparable to the negative control (Table 1).

The weight of abdominal fat varied significantly between the groups ($P<0.05$). The group of rats eating animal fat and lemon juice had their abdominal fat weight significantly reduced compared to rats eating animal fat without lemon juice (Table 1). The amount of abdominal fat in rats that ate animal fat and lemon juice was comparable ($P>0.05$) to that of the negative control (Table 1). Also, while the weight of liver tissues did not differ significantly ($P>0.05$) between rats eating animal fat without lemon juice and the negative control, it was significantly reduced ($P<0.05$) in rats eating animal fat plus lemon juice (Table 1).

Results for histopathological studies of the liver, heart, and visceral adipocytes are presented in Figures 1, 2, 3, and 4, respectively.

The liver histopathology indicated normal parenchyma, sinusoids, and hepatocytes (Figure 1). However, the liver of rats consuming animal fat without lemon juice showed a high degree of fat infiltration in the liver parenchyma and hepatocytes, which was relatively reduced in rats fed with animal fat and lemon juice (Figure 1).

Histology of the heart indicated a marked infiltration of fat in the tunica intima of the rats' coronary arteries in the animal-fat-treated rats, which was significantly reduced following lemon juice treatment (Figure 2).

Cytometric studies of the abdominal fat cells revealed a significant enlargement in size in the group of rats exposed to animal fat without lemon juice treatment in comparison to the control and rats exposed to both animal fat and lemon juice (Figures 3 and 4).

Concerning blood lipids and glucose levels (Table 2), values of the plasma total cholesterol (TCh), HDL-c, and LDL-c, and glucose levels in rats fed with animal fat and lemon juice varied insignificantly from rats eating animal fat without the juice and the negative control rats ($p > 0.05$). However, the plasma level of triglyceride (TG) in rats that consumed animal fat and lemon was comparable to that of the control, and was significantly elevated in rats that consumed animal fat without lemon juice (Table 2).

Table 1. Effect of lemon juice treatment (28 days) on the body weight, and the weight of abdominal fat, liver, and heart, in rats (n=18) consuming beef tallow fat extract

Parameter	Control(n=6)	Treated with fat extract from beef tallow(n=6)	Treated with beef tallow fat extract + Lemon Juice(n=6)	P-value
Initial Body Weight (g)	182.75^{abc}±23.48	200.78^{abc}±23.13	165.3^{abc}±31	0.657
Post-treatment Body Weight (g)	218.23^{abc}±24.23	230.8^{abc}±24.02	174.35^{abc}±31.09	0.327
Mean Body weight gain (g)	35.48^{ab}±6.24	30.02^{ab}±5.43	9.05^{c}±1.87	p<0.001
Weight index of abdominal fat(g)	3.67^{a}±0.99	9.80^{b}±1.43	2.17^{ac}±0.75	p<0.001
Weight index of the Liver (g)	6.83^{ab}±0.48	7.60^{ab}±0.25	4.83^{c}±0.31	p<0.01
Weight index of the heart (g)	0.0044±0.001	0.0047±0.001	0.0049±0.001	0.867

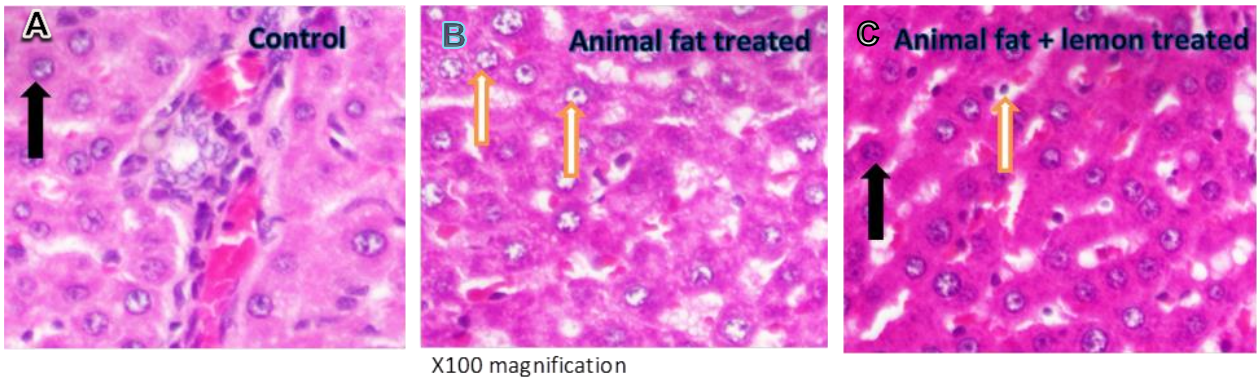


Figure 1. Effect of giving a mixture of beef tallow fat extract and lemon juice (for 28 days) on the Histopathological appearance of the liver sections in Wistar rats (n=18). Black arrows indicate hepatocytes. **A represents** the normal liver of the control rats. **B**-Reveals highly fat-infiltrated liver (shown by large white patches) in rats treated with a fat extract from beef tallow. **C**-Reveals a relatively low degree of fat-infiltrated liver tissues (indicated by small white patches) in rats treated with a mixture of beef tallow fat extract and lemon juice.

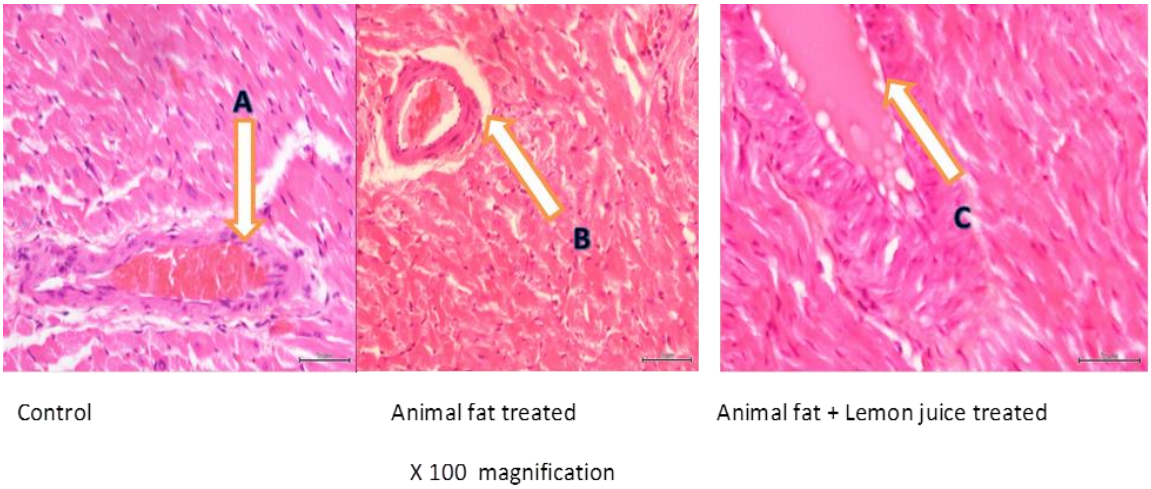
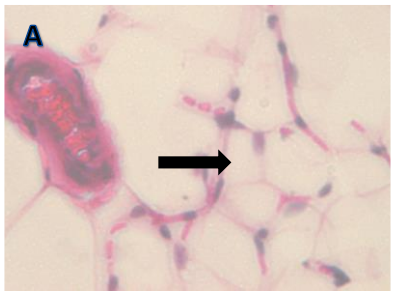
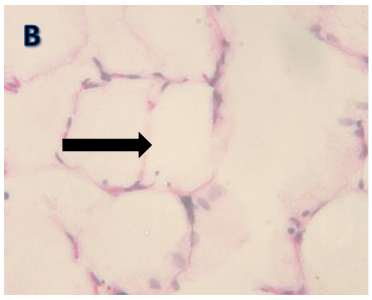


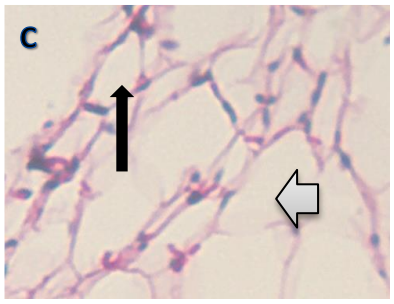
Figure 2. Effect of lemon juice gavage (28 days) on histology of the heart of animal fat-fed Wistar rats (n=18). **A**: indicates a normal coronary artery. **B:** Indicate the coronary artery with marked fat infiltration in the tunica intima. **C:** Indicate minor fat infiltrated tunica intima



Abdominal fat of control rat. X 200



Abdominal fat. Rat treated with beef tallow fat extract



Abdominal fat. Rat treated with mixture of beef tallow fat extract and lemon juice

*Figure 3. Effect of gavage of a mixture of beef tallow fat extract and lemon juice (for 28 days) on the Histopathological appearance of the abdominal fat in Wistar rats (n=18). In control rats (**A**), a black arrow indicates normal small-sized adipocytes. Enlarged adipocytes are dominant in rats treated with fat extract from beef tallow (**B**). There is a mixture of small-sized (black arrow) and enlarged (white arrow) adipocytes in rats treated with beef tallow fat extract, followed by lemon juice (**C**).*

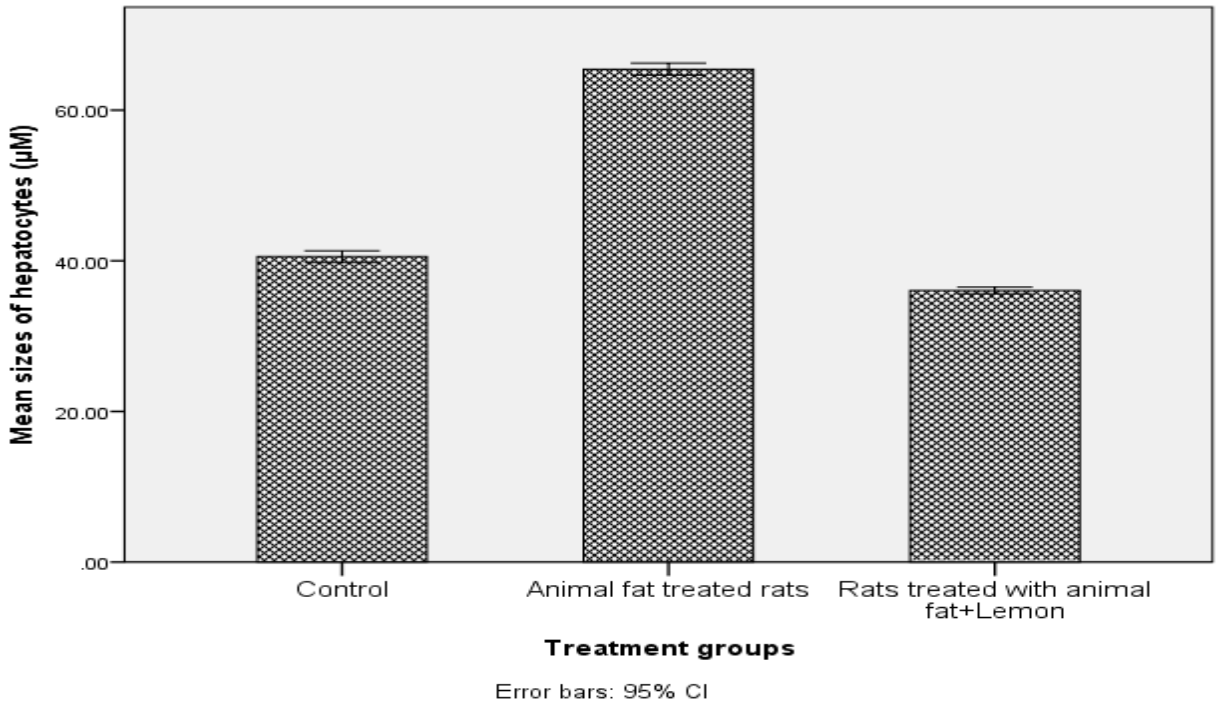


Figure 4. Comparison of abdominal fat cells' size among the rats (n=18) following various treatments (28 days): treated with animal fat only (n=6), treated with animal fat plus lemon juice (n=6), and the negative control (n=6).

Table 2. Effect of lemon juice treatment (28days) on plasma levels of Total cholesterol (TCh), Triglycerides (TG), High-Density Lipoprotein cholesterol (HDL-c), Low-Density Lipoprotein (LDL-c), and glucose in rats (n=18) in rats consuming beef tallow fat extract

Parameter	Control (n=6)	Treated with fat extract from beef tallow (n=6)	Treated with beef tallow fat extract + Lemon Juice (n=6)	P-value
TCh (mg/dl)	65.67^{abc}±7.135	85.8^{abc}±8.398	71.17^{abc}±11.065	0.268
TG (mg/dl)	21.40^{a}±4.895	56.20^{b}±13.695	28.40^{ac}±3.75	0.026
HDL-c (mg/dl)	55.22 ^{abc} ± 6.609	51.333 ^{abc} ±6.609	40.704 ^{abc} ±7.240	0.308
LDL-c (mg/dl)	21.596 ^{abc} ±5.588	23 ^{abc} ±5.588	30.833 ^{abc} ±5.101	0.434
Serum glucose (mg/dl)	164.52^{abc}±21.295	157.30^{abc}±5.521	163.43^{abc}±13.796	0.956

4. Discussion

This study assessed the potential of lemon juice inclusion in the diet to counteract the effect of an animal fat-rich diet in consumers using Wistar rats as model animals. We observed that gavage of Wistar rats with lemon after feeding them with high-fat diets significantly slowed down body weight gain. Moreover, this study revealed that the weights of abdominal fat and liver tissues were significantly reduced in rats that consumed animal fat and lemon juice compared to rats that consumed animal fat alone and the negative control rats. Further histological assessment revealed a significant reduction of fat distribution in hepatocytes of the rats that consumed both animal fat and lemon juice compared to rats eating animal fat without lemon juice supplementation. Also, the abdominal adipocytes of the rats eating animal fat and lemon juice were comparatively smaller in size relative to the counterpart fat cells from the rats eating animal fat without lemon juice.

The current findings corroborate the results of Fukuchi *et al*. (2008) and Garima and Amita (2010). Fukuchi *et al*. (2008) revealed a significant reduction in body weight and amount of body fat in mice exposed for 12 weeks to polyphenols extracted from lemon peel. More related results reported by Garima and Amita (2010) revealed a significant reduction in body weight in human participants supplemented with lemon juice compared to the counterpart non-supplemented group. However, contrary to the results of this study on body weight gain, Zakia *et al*. (2015) revealed a significant restoration in body weight of rats on green tea decoction and lemon juice supplementation compared to rats on green tea decoction without lemon juice. According to Fukuchi *et al*. (2008), polyphenols contained in lemon are responsible for slowing body weight gain and body fat accumulation. The mechanism is done by increasing peroxisomal β-oxidation through up-regulation of the mRNA level of acyl-CoA oxidase (ACO) in the liver and white adipose tissues, which is likely mediated via up-regulation of the messenger RNA (mRNA) levels of peroxisome proliferator-activated receptor-α (PPARα) (Fukuchi *et al*., 2008).

The levels of TCh, HDL-c, LDL-c, and glucose in blood did not vary significantly between the treatment groups of rats. Those results were contrary to the study of Zakia *et al*. (2015) and Aslani *et al*. (2016), which indicated a decrease in the measured parameters following lemon extract treatment in the experimental participants. Nevertheless, the levels of TG in the blood of rats eating animal fat and lemon juice were significantly reduced compared to those of the rats fed with animal fat without lemon juice. The result of plasma TG revealed in this study agrees well with the results of Zakia *et al*. (2015), who revealed a significant reduction in plasma TG in a group of rats fed with lemon juice. The plant's polyphenols are the compound probably responsible for the reduction of plasma TG levels, as also stated by Fukuchi *et al*. (2008).

In conclusion, lemon juice consumption was associated with a significant reduction of body weight gain, abdominal and liver fat accumulation, and levels of blood triglycerides in the rats exposed to animal fat. Findings in this study could probably be extrapolated to individuals who are frequent consumers of fats originating from animal products, to include lemon juice in their diet.

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Authors contributions

Dr. Lusekelo M. Mwangengwa, Dr. Frida R. Mgonja, and Dr. James R. Mushi were responsible for the study design and revision, as well as data collection. Dr Lusekelo Mwangengwa drafted the manuscript while Dr. Frida R. Mgonja and Dr. James R Mushi. revised it. All authors read and approved the final manuscript, including authorship.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Macrothink Institute.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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