# The Effect of Lemon Juice on The Concentration of Glucose and Some Hormones in Experimentally Fattened Male New Zeland Rabbits

**Iman Mohammad Said Jallod**
*College of Nursing, University of Telafer, Iraq, imanjallod86@gmail.com*

**Muntaha Mahmoud Al-Kattan**
*Associate professor, Department of Biology, College of Science, Mosul University, Iraq, muntsbio17@uomosul.edu.iq*

## Abstract

The current study was conducted to figure out the effect of experimental fattening of white New Zealand male rabbits and the use of lemon juice on the concentration of glucose and some hormones in the blood serum of these rabbits. 40 white male New Zealand rabbits were used, their ages ranged between 8-10 months, and weights between 1250-1400 g. They were divided into two groups of 20 rabbits/group and placed in separate cages. The first group was fed the standard provender, while the second group was fed a special fattening provender rich in fat for a period of 12 weeks, and after the end of the period, all rabbits were weighed, and each group was divided into two groups with 10 rabbits/group, the control group, the lemon group, the fattening group and the fattening group with lemon: these groups were subjected to standard conditions of provender and water, and the lemon group and the fattening group with lemon were dosed with lemon juice 4 ml / kg for 8 weeks, where the dose was daily. The results showed that treatment with lemon juice led to a significant decrease at the probability level ($p \geq 0.0001$) in the concentration levels of glucose, thyroid stimulating hormone (TSH), thyronine, thyroxine and leptin in both the lemon group and the fattening group with lemon compared to the control group and the fattening group respectively, while there was a significant increase in the insulin hormone and adiponectin in both the lemon group and the fattening group with lemon compared to the control group and the fattening group, respectively.

**Keywords:** *obesity, leptin, adiponectin, insulin, thyronine, thyroxine, thyroid-stimulating hormone, lemon juice.*

## INTRODUCTION

Obesity is a complex multifactorial disease. Since 1980, the prevalence of overweight and obesity has doubled worldwide to nearly a third of the world's population. Obesity rates increased at all ages and both genders, regardless of socioeconomic status and ethnicity, despite the varying rates of obesity prevalence and its geographical distribution (Chooi et al., 2019). According to the World Health Organization, obesity is defined as the abnormal or excessive increase of body fat under the skin or around the organs (Williams et al., 2015) resulting from the consumption of unhealthy food, as the consumption of fast food plays a major role (Lalanza and Snoerenab, 2021), and obesity directly contributes to the development of many chronic diseases such as cardiovascular disease, type 2 diabetes mellitus, hypertension, breathing and sleep disorders (Powell-Wiley et al., 2021). Obesity is also associated with cerebrovascular diseases, which is related to a decrease in the thickness of the cerebral cortex and dementia (Morys et al., 2021). Obesity is a growing health problem in our society, its treatment is a

challenge and many drugs have been used to reduce weight and reduce the risks of associated diseases. However, many weight loss drugs have been withdrawn from the market due to their dangerous side effects, such as aminorex, which causes pulmonary hypertension, and flenfluramine, which causes valvulopathy, and other drugs. (Krentz et al., 2016). Therefore, many natural products and plants have appeared that are used to treat obesity due to their effectiveness and few side effects, such as Lemon Verbena and Hibiscus Flower Extract (Lee et al., 2018), hot peppers have been used as a weight reducer because they contain many compounds such as antioxidants, polyphenols, vitamin C and flavanols, these compounds work to eliminate free radicals caused by obesity that lead to inflammation and metabolic disorders (Azian et al., 2022), the extract of Eucalyptus tereticornis leaves was also used, which is a type of plant used in traditional medicine to treat diabetes, and extracts of eucalyptus leaves have been shown to regulate immune metabolism processes associated with obesity and reduce insulin resistance (Acín et al., 2021) and other plants. Citrus is one of the most important fruit tree crops in the world, and lemon is the third most important type of citrus of the family Rutacecace. Lemon is a health-promoting fruit that is rich in many carotenoids, flavonoids, limonoid, hesperidin, Naringin, flavone citric acid, Terpenes and vitamins such as Vitamin C, E and Thiamine (B1), Riboflavin (B2), Niacin (B3) and minerals such as iron, zinc, potassium, manganese, selenium and copper, as well as fibers such as pectin (Saini et al. 2022). These components gave lemon juice great importance in medicinal uses. Studies have found that lemon juice plays an important role in preventing the formation of kidney stones (Barghouthy and Somani, 2021) Lemon juice can also be used as an antimicrobial for many types of bacteria, such as Escherichia coli; Streptococcus pyogenes, as it inhibits the growth of these types of bacteria (Shuaib et al., 2021) and eating a tablespoon of lemon with 20 g of garlic lowered cholesterol in addition to lowering blood pressure for people suffering from high cholesterol (Aslani et al., 2016) The use of a mixture of lemon juice, onion and garlic to reduce cholesterol level showed a decrease in LDL-c level and total cholesterol level and an increase in HDL-c level (Bassuony et al., 2021). The aim of the study is to investigate the effect of lemon juice on the concentration of glucose, insulin, leptin, adiponectin, thyroid-stimulating hormone, thyronine and thyroxine in experimentally fattened white male New Zealand rabbits.

## Materials and Methods

### Animals under study

In this study, 40 male white New Zealand rabbits were used, their ages ranges between (8-10) months and their weights ranges between (1250-1400) g, obtained from local markets, and after making sure that they are free from diseases, they were placed in metal cages specially made for this purpose, their dimensions are (60 □ 60 □ 50) cm, and under appropriate conditions of temperature ranging between (25-28°c) and a period of illumination of 14 hours per day and good ventilation, they were subjected to an introductory period of one week to acclimatize to the place and the provender before starting the experiment. The standard provender was given to the rabbits using plastic pots in equal quantities and in a constant sequence to all treated rabbits. Water was provided to the rabbits using pots made of plastic and fixed in the cage to prevent water spillage from them.

The plant used in the study

Lemon fruits were used in this study, which were obtained from local markets and after making sure of their classification based on (Cheij, 1984), the fruits were cleaned from the soil, they were squeezed using manual juicer and the resulting juice was filtered using three layers of gauze, and they were squeezed on the same day the treated rabbits were dosed.

Experiment design

The study was conducted in two phases:

The first phase (rabbit fattening phase)

The rabbits were randomly divided into two groups of 20 rabbits/group. After the preliminary period ended, the initial weights of the rabbits were taken, and the first group (the group without fattening) began to be fed with the standard provender only, which is a special provender for rabbits with a protein content of 16.5% and this percentage is approved by the National Research Council (NRC) (1994) [Al-Kattan and Al-Ishlash, 2012], the second group (the fattening group) was fed a special provender rich in fat by mixing 10% fat (2/3 corn oil and 1/ 3 tallow) to the standard provender for 12 weeks [Dharmaraj et al., 2013].

The second phase (rabbits treatment phase)

After the fattening phase ended, the rabbits of each group were weighed, then the rabbit was divided into:

1- Control group (without fattening): This group was treated by giving it a standard provender, and the average weight of rabbits for this group was 1282.3 g.

2- Lemon group: This group was treated by giving it a standard provender with lemon juice at a volume of 4 ml / kg body weight, and the average weight of rabbits for this group was 1291.6 g.

3- Fattening group: This group was treated by giving it a standard provender, and the average weight of rabbits for this group was 2250.3 g.

4- Fattening group with lemon: This group was treated by giving it a standard provender with lemon juice at a volume of 4 ml/kg body weight, and the average weight of rabbits for this group was 2251.1 g.

Note: The rabbits of the control group and the fattening group were dosed with physiological saline solution to equalize the stress of holding rabbits (Al.Kattan and Al-Ishlash, 2012).

Sample collection

Blood samples were obtained from rabbits by drawing blood from the heart directly by heart stab using a 10 ml medical syringe after eight weeks of treatment (slaughter day). The blood was placed in tubes with dry tight covers free of any anticoagulant materials, and left at room temperature for 20 minutes until the blood clotted, and then a centrifugal process was conducted at 3000 rpm for a period of 15 minutes to get the blood serum, and keeping the blood serum by freezing at -20 °c until the blood sugar and hormonal tests are performed.

Estimation of glucose concentration in blood serum

The concentration of glucose in the blood was estimated using the measuring tapes of the Reflotron device manufactured by the German company Roche, based on the enzymatic method mentioned in (Trinder, 1969), by placing 32 microliters of the sample on the test strip, the sample will flow into the reaction area, D-glucose will be oxidized to δ-D-gluconolactone with oxygen and in the

presence of glucose oxidase (GOD) and produce hydrogen peroxide (H2O2), which will oxidize the reagent in the test strip and in the presence of phosphate oxidase (POD) and thus a dye will be formed in proportion to the concentration of glucose in the sample.

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \delta\text{-D-gluconolactone} + H_2O_2$$

$$H_2O_2 + \text{Indicator} \xrightarrow{\text{POD}} \text{Dye} + H_2O$$

These reactions take place at 37 °c, and the dye is directly proportional to the concentration of glucose in the serum, and it is measured at a wavelength of 642 nm, and the glucose concentration appears on the device screen after 125 seconds and in the unit mg/100 ml.

Hormonal tests

The hormones of insulin, leptin, adiponectin, thyroid-stimulating hormone (TSH), T3 thyronine and T4 thyroxine were determined in the blood serum of New Zealand male rabbits using the ready-made Accubind ELISA Microwells assay kit, supplied by the American company Monobind Inc., and using the Semi-Automated ELISA shaker-reader device manufactured by the German company Rayto, this test was based on the principle of Direct Sandwich at wavelength 450 nm and the final results were obtained through the values of the standard curve fixed in the device used (Burtis, 2006).

Statistical analysis

Statistical analysis was carried out using a completely randomized design (C.R.D), one-way analysis of variance, and the differences between groups were determined using Duncan's Multiple Range test for all measurements covered by the study, and the level of statistical discrimination was ($P \leqslant 0.01$), using SAS (2001).

**Results and discussion**

The concentration of glucose in the blood serum

Table (1) shows a significant decrease in the concentration of glucose in the blood serum of white New Zealand male rabbits at the probability level ($P \leqslant 0.0001$) for the lemon group compared to the rest of the treatments. The mean was 3.21 ± 82.77 mg/100 ml compared to the control group, whose mean was ± 107.19 3.26 . While the fattening group showed a significant increase in glucose concentration with a mean of 5.21 ± 153.52 mg/100 ml compared to the rest of the treatments. While the fattening group with lemon showed a significant decrease in glucose concentration compared to the fattening group if its mean was 4.33 ± 102.72 mg/100 ml.

The significant decrease in glucose concentration in all groups treated with lemon juice does not depend on increasing the concentration of the insulin hormone, but through its effect on reducing insulin resistance and a change in glucose metabolism, and this could be related to modifying the action of the insulin hormone and this is consistent with what was stated by (Tejpal et al., 2020), they indicated that mice dosed with lemon juice extract reduced glucose concentration as a result of reducing insulin resistance or outside the effect of the pancreas by inhibiting glucose absorption from the intestine or carbohydrate metabolism or absorption because it contains soluble fiber such as pectin, which works to inhibit glucose absorption in the intestine and inhibiting the activity of the enzyme α-amylase, which is one of the most important enzymes that works to convert carbohydrates into simple sugars and thus reduce the concentration of glucose in the blood (Ohbayashi et al. 2021, Bai et al. 2021) or lemon juice reduces glucose

concentration because it contains compounds such as Hesperidin and Naringin, which are considered flavonoids and improve the regulation of fatty acid and cholesterol metabolism and their effect on the enzymes responsible for the Gluconeogenesis process (Sundaram et al. 2019) or it contains magnesium and phosphorous, which studies have shown its ability to reduce glucose concentration if its deficiency is one of the causes of obesity and type 2 diabetes (Piuri et al. 2021). As for the reason for the significant increase in the concentration of glucose level in the blood serum of New Zealand male rabbits in the fattening group, it may be due to the increase in glucose building from non-carbohydrate sources (gluconeogenesis) (Mizuno 2018) or the increase in the accumulation of white fat as a result of obesity leads to an increase in the secretion of cytokines, as can be adipocytes stimulate the production of types of free radicals, which in turn increases the unregulated production of adipokines and increases the inflammatory state, which leads to an increase in insulin resistance (Marseglia et al. 2015, Ghowsi et al. 2021).

Insulin hormone in blood serum

It is clear from Table (1) that there was no significant difference in the concentration of insulin hormone in the blood serum of white New Zealand male rabbits at the probability level ($P \leqslant 0.0001$) for the lemon group and its mean was $0.16 \pm 6.17$ pg/ml compared to the control group whose mean was $0.06 \pm 6.23$ pg/ml, while the fattening group showed a significant increase in the insulin hormone concentration in the blood serum of white New Zealand male rabbits and its mean was $0.35 \pm 7.25$ pg/ml, while there was a significant decrease in the insulin hormone concentration in the blood serum of white New Zealand male rabbits of the fattening group with lemon, the mean was 0.03±6.66 pg/ml compared to the fattening group.

The results of the current study showed a significant decrease in the concentration of the insulin hormone in the fattening group with lemon compared to the obese group. The reason for this may be due to the fact that lemon juice contains multiple phenols, which have an important role in reducing the concentration of the hormone insulin in the blood, and this was indicated by Fukuchi and his group (2008). Where it was mentioned that feeding mice polyphenols extracted from lemon peels rich in flavonoids with a high-fat diet led to a decrease in the level of the hormone insulin, and this was attributed to the ability of flavonoids to reduce tissue resistance to insulin, thus reducing the level of glucose in the blood and thus reducing the secretion of the insulin hormone. Or, the reason for this is due to the fact that lemon juice contains Naringin, which leads to lowering the level of the insulin hormone, as well as reducing insulin resistance, and this was indicated by (Yang et al., 2022), they observed a significant decrease in the concentration of the hormone insulin and insulin resistance in female mice with polycystic ovary syndrome after being treated with naringin and morin extracts.

As for the reason for the significant increase in the concentration of the insulin hormone in the fattening group compared to the rest of the treatments, it may be attributed to the increase in insulin resistance, as the fatty tissue works to release free fatty acid, reactive oxygen species and pro-inflammatory cytokines, which leads to the occurrence of systemic inflammation that prevents insulin response and increases the level of glucose and thus increases the secretion of insulin (Ahmed et al. 2021). Or the reason may be due to an increase in hepatic glucose

building and its release into the blood, and this leads to an increase in the secretion of the hormone insulin and a rise in its level in the blood (Haedersdal et al. 2018). Or due to an increase in leptin resistance and an increase in leptin secretion in cases of obesity, which increases the secretion of the insulin hormone (Eufert, 2004).

The concentration of leptin in the blood serum

It is evident from Table (1) that there was a significant decrease in the concentration of leptin hormone in the blood serum of white New Zealand male rabbits at a probability level ($P \leqslant 0.0001$) for the lemon group and with a mean of $0.06 \pm 4.39$ pg/ml compared to the control group, whose mean was 0.06±4.58. pg/ml, while the fattening group with lemon showed a significant decrease in the concentration of leptin hormone with a mean of 900.06±4. pg/ml compared to the fattening group whose mean was 0.14 ± 5.85 pg/ml.

The reason for the significant decrease in the concentration of leptin hormone in the blood serum of male New Zealand rabbits in the lemon and fattening with lemon groups, can be explained by the fact that lemon juice contains multiple phenols that reduce leptin resistance and reduce fat mass and this is what (Fukuchi et al., 2008) included, feeding mice with polyphenols extracted from lemon peels with a high-fat diet reduced the concentration of leptin hormone compared to the group that was fed on a high-fat diet only, this was attributed to the ability of multiple phenols to reduce the level of deposited fats, by decreasing leptin resistance and decreasing the level of leptin mRNA. As well as what was indicated by (Boix-Castejón et al., 2018) in a study they conducted for obese people, as the study showed a significant decrease in the concentration of leptin hormone in the blood serum of people treated with multiple phenols extracted from the plant Lemon verbena. (Manuha et al., 2019) also recommended people with obesity to consume lemon juice because lemon juice contains vitamin C, and it showed a percentage of 32.16 mg per 100 ml of lemon juice and its ability to reduce fat mass as well as the level of concentration of the leptin hormone in obese people.

The current study also showed a significant increase in the concentration of leptin hormone in the serum of New Zealand male rabbits in the fattening group, and this result is consistent with what (Tangestani et al., 2021) in their study, as people who eat unhealthy foods rich in fat increases the incidence of obesity as well as an increase in the concentration of the leptin hormone compared to people who eat healthy food and drink fruit juices regularly, and the reason for this is that the hormone leptin is secreted from the white fat tissue accumulated in obese people, as well as increasing the resistance of the leptin hormone, which leads to a decrease in satiety, as the concentration of the leptin hormone is strongly related to the mass of fat (Obradovic et al., 2021).

Adiponectin in the blood serum

Table (1) shows a significant increase in the concentration of the hormone adiponectin in the blood serum of white New Zealand male rabbits in the lemon group, and its mean was 0.34 ± 8.33 mg/L compared to the control group, where its mean was 0.09 ± 6.78 mg/L, while there was a significant decrease in the concentration of adiponectin hormone in the blood serum of New Zealand male rabbits in the fattening group, and its mean was about 0.11 ± 4.45 mg/L compared to the rest of the treatments. It was also noticed that there was an improvement in the concentration of adiponectin hormone in the fattening group

with lemon, and its mean was 6.74 ± 0.25 mg/L, it reached the level of the control group with no significant difference between them.

The results of the current study showed a significant increase in the concentration of the adiponectin hormone in the blood serum of white New Zealand male rabbits in the lemon group and the fattening with lemon group, and this is consistent with what (Kim et al., 2015) stated in the blood serum of overweight women in the Lemon detox program with a low-calorie diet consisting of a mixture of a drink containing lemon juice to a low concentration of the hormone adiponectin, due to the fact that lemon juice contains flavonoids such as Querciten, which act as anti-inflammatory by inhibiting TNF-α (Nair et al., 2006), or that flavonoids increase the effectiveness of the insulin hormone, which affects the gene expression of adiponectin and increases its secretion (Rivera et al., 2008) or as a result of containing a group of B vitamins that increase the secretion of the hormone adiponectin and this is what (Salwa et al., 2012) indicated, as Vitamin B3 stimulates the production of adiponectin hormone, by stimulating receptors in fat cells and activating immune cells such as white blood cells, mononuclear cells and neutrophils and stimulating these receptors leads to a rapid increase in the production of the adiponectin hormone and thus reduces fatty tissue, Vitamin B3 also has an inhibitory effect on TNF-α and interleukin-6 (IL-6), which inhibit adiponectin expression in fat cells (Wanders et al., 2013). (Cassidy et al., 2009) also mentioned that magnesium leads to an increase in the concentration of the adiponectin hormone when it is taken through foods containing magnesium.

As for the reason for a significant decrease in the concentration of the adiponectin hormone in the blood serum of white New Zealand male rabbits in the fattening group compared to the rest of the treatments, the reason for this may be due to the correlation of its ratio with the level of glucose and insulin in the blood (Lara-Castro et al., 2006). Or through its effect on increasing the secretion of TNF-α and IL-6, which are secreted from adipose tissue and increase inflammation in adipose tissue and increase tissue resistance to insulin, thus increasing the release of fatty acids that inhibit the synthesis of the hormone adiponectin and decreasing its concentration in the blood serum (Xu et al., 2021).

**Table No. (1): Effect of lemon juice and boiled cumin seed extract on the concentration of leptin, insulin and adiponectin in the serum of white male New Zealand rabbits.**

| Standards / Groups | blood glucose mg/100 ml | Leptin pg/ml | Insulin pg/ml | Adiponectin |
|---|---|---|---|---|
| control group (Standard provender with plain water) | 3.26±107.19 B | 0.06±4.58 DE | 0.06±6.23 DE | 0.09±6.78 C |
| fattening group (Special provender for fattening with plain water) | 5.21±153.52 A | 0.14±5.85 A | 0.11±7.25 A | 0.11±4.45 E |
| lemon group (lemon juice 4ml/kg body weight) | 3.21±82.77 F | 0.06±4.39 F | 0.16±6.17 E | 0.34±8.33 B |
| Fattening group with lemon (lemon juice 4 ml / kg body weight) | 4.33±102.72 C | 0.06±4.90 C | 0.03±6.66 C | ±6.74 0.25 C |

- Values are expressed as mean (±), standard deviation, and number of rabbits/group = 10
- Diverse numbers with different letters vertically indicate the presence of a significant difference at the level of probability ($P \leqslant 0.0001$).

Serum thyroid-stimulating hormone (TSH) concentration

Table (2) shows a significant increase in the concentration of TSH hormone in the blood serum of white New Zealand male rabbits at the level of probability ($P \leqslant 0.0001$) for the lemon group with a mean of $0.04 \pm 0.89$ compared to the control group, where its mean was ($0.03 \pm 0.73$ ng/ml). While it showed a significant decrease in the TSH concentration in the blood serum of white New Zealand male rabbits for the fattening group, and its mean was 0.05±0.49 ng/ml compared with the rest of the treatments, while the fattening group with lemon showed 0.04±0.70 ng/ml improvement in the level of TSH in the blood serum of white New Zealand male rabbits and reached the level of TSH hormone in the blood serum of white New Zealand male rabbits of the control group with no significant difference between them.

The concentration of thyronine in the blood serum

Table (2) shows a significant increase in the concentration of T3 hormone in the blood serum of white New Zealand male rabbits at the level of probability ($P \leqslant 0.0001$) for the lemon group and with a mean of $0.10 \pm 1.72$ ng/ml compared to the control group, whose mean was 0.03±1.30 ng/ml, while the fattening group showed a significant decrease in the concentration of T3 hormone in the blood serum of white New Zealand male rabbits, with a mean of 0.05±0.76 ng/ml compared to the rest of the treatments. As for the fattening group with lemon, it improved the level of T3 hormone in the blood serum of white New Zealand male rabbits, and its mean was 0.05±1.21 ng/ml with no significant difference between them.

Thyroxine concentration in the blood serum (T4)

While Table (2) shows a significant increase in the concentration of T4 hormone in the blood serum of white New Zealand male rabbits at the level of probability ($P \leqslant 0.0001$) for the lemon group and the mean of the lemon group was $0.30 \pm 13.08$ ng/ml, while the fattening group showed a significant decrease in the concentration of T4 hormone in the blood serum of white New Zealand male rabbits, with a mean of 0.10±8.72 ng/ml compared with the rest of the treatments. As for the fattening group with lemon, there was an increase in the level of T4 hormone to reach the level of the control group with no significant difference between them. Their mean was 0.08±9.33 ng/ml, respectively, while the mean for the control group was 0.06±9.53 ng/ml.

The results of the current study showed a significant increase in the concentration of TSH, T3 and T4 in the serum of New Zealand male rabbits treated with lemon juice compared to the control group and the fattening group. This may be due to the fact that lemon juice contains flavonoids, including rutin, which improve the gene expression of TSH and increase its secretion from the pituitary gland. It also has an effect on the sodium-iodide symporter by which iodide is taken up by the thyroid gland to make its own hormones. It also stimulates the enzyme thyroperoxidase, which is the key hormone for the formation of thyroid hormones, in addition, Rutin has an anti-proliferative effect on thyroid cancer cells

(Gonçalves et al., 2014). Also (Jallod and Al-Kattan 2016) indicated a significant increase in the total capacity of antioxidants in the blood serum of experimentally fattened male rabbits, because lemon juice contains flavonoids, which is one of the multiple phenols and is considered one of the most powerful antioxidants that remove free radicals by reducing Oxidative stress and the production of GSH, thus reducing free radicals, and the resulting increase in thyroid secretion (Upadhya et al., 2004). This is what was indicated by (Pang et al. 2021) that treatment of animals suffering from hypothyroidism with olive oil and the solid residues of olives rich in polyphenols improves the secretion of thyroid gland. Or because it contains selenium, which is necessary for deiodinase activtey, and its deficiency leads to a lack of activity of the latter and thus to a decrease in the activity of the thyroid gland (Pang et al., 2019; Gereben et al., 2008).

The significant decrease in the concentration of TSH, T3 and T4 hormone in the fattening group compared to the rest of the groups was consistent with what was stated by Al-Jedi (2011), as it was shown that the TSH hormone was lower in obese people than in people of normal weight, as well as (Caraccio et al., 2002) pointed out the relationship between obesity and low secretion of thyroid hormones, this may be attributed to the fact that obesity leads to cell hypertrophy as a result of fat accumulation as well as hypoxia and the generation of systemic oxidative stress in fatty tissues such as OH, O, H2O2 etc., although T3 and T4 hormone synthesis is stimulated by thyroid peroxidase and needs ROS, especially H2O2, are an essential component in the early stages of thyroid hormone production during iodine oxidation, and due to thyroid function, they are particularly vulnerable to oxidative damage (Zhou et al., 2021; Thanas et al., 2020), Also, the oxidative stress leads to a decrease in the levels of thyroid hormones from iodothyronin 5- monodeiodination which is a prohormone for T4 hormone, when reduced, it leads to a decrease in the level of T4 hormone and indirectly leads to a decrease in the level of T3 hormone, because T3 hormone consists of transforming T4 hormone by stimulating enzymes by removing one atom of iodine (Singh et al., 2006).

**Table No. (2): Effect of lemon juice and boiled cumin seed extract on the concentration of thyroid stimulating hormone and thyroid hormones in the sera of white male New Zealand rabbits.**

| Standards / Groups | T.S.H ng/ml | $T_3$ ng/ml | $T_4$ ng/ml |
|---|---|---|---|
| control group (Standard provender with plain water) | 0.03±0.73 C | 0.03±1.30 D | 0.06±9.53 C |
| fattening group (Special provender for fattening with plain water) | 0.05±0.49 D | 0.05±0.76 G | 0.10±8.72 F |

| | | | |
|---|---|---|---|
| lemon group (lemon juice 4ml/kg body weight) | 0.04±0.89 A | 0.10±1.72 B | 0.30±13.08 B |
| Fattening group with lemon (lemon juice 4ml/kg body weight) | 0.04±0.70 C | 0.05±1.21 E | 0.08±9.33 D |

- Values are expressed as mean (±), standard deviation, and number of rabbits/group = 10.
- Diverse numbers with different letters vertically indicate the presence of a significant difference at the level of probability ($P \leqslant 0.0001$).

**Reference**


Chooi, Y.C.; Ding , C.; Magkos, F.(2019). The epidemiology of obesity. Metabolism VOL.92: 6-10.

Williams, E.P.; Mesidor ,M.; Winters, K.; Dubbert ,P.M.;Wyatt, S.B., Wyatt, S.B,.(2015). Overweight and Obesity : Prevalence , Consequences , and Causes of a Growing Public Health Problem. Urr. Obes. Rep.4(3) :363–370.

Lalanza,J.F., Snoerenab,E.M.S. (2021).The cafeteria diet: A standardized protocol and its effects on behavior . Neuroscience & Biobehavioral Reviews.122:92-119.

Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association

Powell-Wiley,T.M., Poirier,P., Burke, L.E., Després,J.P. Gordon-Larsen,P., Lavie,C.J., Lear,S.A., Ndumele,C.E., Neeland,I.E., Sanders,P., St-Onge.M.( 2021). Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 143:984–1010.

Morys,F., Dadar, M., Dagher,A.,(2021). Association Between Midlife Obesity and Its Metabolic Consequences, Cerebrovascular Disease, and Cognitive Decline. The Journal of Clinical Endocrinology & Metabolism, Volume 106( 10); 4260–4274.

A. J. Krentz, A.J., Fujioka,K., Hompesch, M. (2016). Evolution of pharmacological obesity treatments: focus on adverse side-effect profiles. Diabetes, Obesity and Metabolism.Vol 18( 6 ):558-570.

Lee ,Y.S., Yang ,W.K., Kim, Y.H., Min ,B. , Caturla ,N., Jones ,J., Park,Y., Lee ,Y. and Kim , S.(2018). Combination of Lemon Verbena and Hibiscus Flower Extract Prevents High-Fat Diet-Induced Obesity through AMP-Activated Protein Kinase Activation. Nutrients , 10(9), 1-18

Azlan, A., Sultana ,S., Huei,C.S., and Rizal Razman,M. Antioxidant, Anti-Obesity, Nutritional and Other Beneficial Effects of Different Chili Pepper.(2022). Journals Molecules. Vol( 27 ):3 p1-11.

Acín, S., Muñoz, D.L.,Guillen, A.,Soscue, D., Castaño, A.,Echeverri, E., Balcazar, N.(2021). Triterpene-enriched fractions from Eucalyptus tereticornis ameliorate metabolic alterations in a mouse model of diet-induced obesity . Journal of Ethnopharmacology. Volume 265:p1-18

Saini , R.K., Ranjit ,A., Sharma ,K., Prasad ,P., Shang , X., Gowda, K.G.M and Keum ,Y. (2022). Bioactive Compounds of Citrus

Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. Antioxidants, 11(239) p 1-27.

Yazeed Barghouthy, Y and Bhaskar K. Somani, B.K. (2021).Role of Citrus Fruit Juices in Prevention of Kidney Stone Disease (KSD): A Narrative Review. Nutrients , 13(11): 1-10.

Shuaib ,M.J., Shailabi ,T.I., Borwis ,E.O., Muhammed,A.S. (2021). Antimicrobial Activity Evaluation of Citrus Lemon Against Streptococcus Pyogenes And Escherichia Coli. IOSR Journal of Pharmacy .Volume 11 (12) :PP. 11-16.

Aslani ,N., Entezari , M.H., Askari, C., Maghsoudi, Z . and Maracy ,M.R. (2016). Effect of Garlic and Lemon juice Mixture on Lipid Profile and son Cardiovascular Risk factors in people 30-60 years old with moderate Hyperlipidaemia : A randomized clinical Trial. International Journal of Preventive Medicine 7(95):1-8.

Bassuony ,N. I., Hafez,M.L., Ibrahim, A.F.M., EISebeay ,A.S. and Omer H.A.A.(2021). Effect of Using Natural Bioactive Mixture Composed of Lemon, Onion and Garlic Juices on Weight and Lipid Profiles for Overweight Rats . European Journal of Applied Sciences (13(1):9-17.

Cheij, R. McDonald Encyclopedia of Medical Plant. (1984). Ltd. McDonald and Co. London, PP:209,309, 313.

Dharmaraj, B.; Srivastava, R.K. and Ghalaut , V. Influence of Liraglutide Alone and in Combination With Glimepiride on Body Weight in Obese-Diabetic Rabbits. J. Pharma. Innov. 2(2013)7: 52-57.

Al.Kattan, M.M. and Al-Ishlash ,H.T. (2012). Effect of cholesterol and Boiled Extract of Red Pepper on the level of some Hormones and Weights of Local Male Rabbits. Rafidain Journal of Science . 23(3):55-67.

Trinder, P.(1969).Determination of in blood using glucose oxidase with an alternative oxygen acceptor . Ann.Clin.Biochem.24-27.

Burtis, C.A., Ashwood,E.R., and Bruns, D.E.(2006) . Tietz Texbook of Clinical Chemistry and Molecular Diagnostics . 4th ed. Elsevier,Saunders USA.

SAS. SAS / STAT Users Guide for Personal Computers. (2001). Release 6:12. SAS Institute Inc. Cary, N. C., USA.

Tejpal , S., Wemyss , A.M., Bastie ,C. C. and Seetharaman ,J.K.(2020). Lemon Extract Reduces Angiotensin Converting Enzyme (ACE) Expression and Activity and Increases Insulin Sensitivity and Lipolysis in Mouse Adipocytes. Journals Nutrients Volume 12 Issue 8 P 1-17.

Ohbayashi, K., Oyama,Y., Yamaguchi,C., Asano,T., Yada,T. and Iwasaki,Y.(2021) .Gastrointestinal Distension by Pectin-Containing Carbonated Solution Suppresses Food Intake and Enhances Glucose Tolerance via GLP-1 Secretion and Vagal Afferent Activation. volume12 . frontiers in Endocrinology. P1-15.

Bai, Y., Atluri ,S., Zhang , Z., Gidley , M.J., Li, E. and Gilbert, R.G.( 2021).Structural reasons for inhibitory effects of pectin on α-amylase enzyme activity and in-vitro digestibility of starch. Food Hydrocolloids . Volume 114 . p1-18.

Sundaram, R., Nandhakumar,E. and Banu , H.H. (2019). Hesperidin, a citrus flavonoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats .Toxicology Mechanisms and Methods . Volume 29, 2019 - Issue 9 p 645 -653.

Piuri, G., Zocchi, M., Porta, M.D., Ficara., V., Manoni, M., Zuccotti, G.V., Pinotti, L., Maier, J.A. and Cazzola, R. (2021). Magnesium in Obesity, Metabolic Syndrome, and Type 2 Diabetes . Nutrients, 13(2), 320; 1-16.

Fukuchi, Y .; Hiramitsu, M .;Okada, M.; Hayashi, S.; Nabeno, Y.; Osawa, T. and Naito, M. (2008).Lemon Polyphenols sSuppress Diet-induced Obesity by Up-Regulation of mRNA Levels of the Enzymes Involved in β-Oxidation in Mouse White Adipose Tissue. J. Clin. Biochem. Nutr., 43:201–209.

Yang, Y., Liu, J., Xu, W.( 2022) .Naringenin and morin reduces insulin resistance and endometrial hyperplasia in the rat model of polycystic ovarian syndrome through enhancement of inflammation and autophagic apoptosis. The Journal of the Polish Biochemical Society and of the Polish Academy of Sciences Vol. 69 No. 1 p 1-23.

Ahmed, B.,Sultana, R., Greenea,M.W.(2021)Adipose tissue and insulin resistance in obese . Biomedicine & Pharmacotherapy . Volume 137 :P1-13.

Hædersdal, S.,Lund , A. , Knop , F.K., TinaVilsbøl ,T. (2018).The Role of Glucagon in the Pathophysiology and Treatment of Type 2 Diabetes .Mayo Clinic Proceedings Volume 93, Issue 2, , Pages 217-239.

Eufert, J. (2004) .Leptin effects on pancreatic beta-cell gene expression and function. Diabetes., 53(1):152-158.

Boix-Castejón, M., Herranz-López,M., Gago , A.,P., Olivares-Vicente , M., Caturla ,N., Enrique Roche ,E.and Micol , V. (2018) .Hibiscus and lemon verbena polyphenols modulate appetite-related biomarkers in overweight subjects: a randomized controlled trial. Food Funct., 2018, 9, 3173-3184

Tangestani,H., Emamat,H., Yekaninejad,S., Keshavarz,S. A., and Mirzaei, K. (2021). Variants in Circadian Rhythm Gene Cry1 Interacts with Healthy Dietary Pattern for Serum Leptin Levels: a Cross-sectional Study. Clin Nutr Res. ; 10(1): 48–58.

Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Swati Arya, S., Stewart, A.J., Gojobori, T.,and Isenovic , E.R.(2021). Leptin and Obesity: Role and Clinical Implication. Frontiers Endocrinology . 12:1-14.

Kim , M. J., Hwang , J.H., JiKo, H., Bock Na, H., Kim, J.H. (2015) . Lemon detox diet reduced body fat, insulin resistance, and serum hs-CRP level without hematological changes in overweight Korean women. Volume 35, Issue 5, May 2015, Pages 409-420.

Nair, M.P.; Mahajan, S.; Reynolds, J.L.; Aalinkeel, R.; Nair, H. and Schwartz, S.A. and Kandaswami, C. (2006).The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor a) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappa beta system. Clin. Vaccine. Immunol., 13(3):319–328.

Rivera, L.; Morón, R.; Sánchez, M.; Zarzuelo, A. and Galisteo, M. (2008).Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. Obesity, 16(9):2081–2087.

Salwa, M.E.; Maha, I.A.M.; Hamed, I.M.;,Salwa, T.T.; Laila, M.H. and Lobna, A.G. (2012).Dietary therapy of obesity: effect on some hormonal and biochemical blood indices. African J. of food, Agricul., Nutr. and Devel., 12 (7):6882-6896.

Wanders, D.; Graff, E.; White, D. (2013). Niacin Increases adiponectin and decreases adipose tissue inflammation in high fat diet-fed mice. Plosone, 8(8):71285-71296.

Cassidy, A.; Skidmore, P.; Rimm, E.; Welch, A.; Fairweather-Tait, S.; Skinner, J.; Burling, K.; Richards, J.; Spector, T. and MacGregor, A. (2009).Plasma adiponectin concentrations are associated with body composition and plant-based dietary factors in female twins. J. Nutr., 139(2):353-358.

Lara-Castro,C., Luo, N., Wallace, P., Klein, R.L. Garvey,W.T.(2006) . Adiponectin multimeric complexes and the metabolic syndrome trait cluster . Diabetes, 55 : pp. 249-259.

Xu , X., Huang , X., Zhang , L., Huang, X., Qin, Z., and Hua, F. (2021) .Adiponectin protects obesity-related glomerulopathy by inhibiting ROS\NF-kB\NLRP3 inflammation pathway. BMC Nephrology . 22(218): 1-17.

Gonçalves, C.; DosSantos, M.; Ginabredo, M.; Fortunato, R.; Carvalho, D. and Ferreria, A. (2014) .Flavonoid rutin increase thyroid iodide uptake in rats. Plosone, 8(9):1-12.

Upadhya, S.; Shanbhgg, K.K.; Suneetha, G.; Balachandra, N.M. and Upadhya ,S. (2004).A study of hypoglycemic and antioxidant activity of Aegle marmelos in alloxan induced diabetic rats. Indian J. Physiol. Pharmacol., 48(4):476-480.

Pang, K.L.; Chin, K.Y. Emerging Anticancer Potentials of Selenium on Osteosarcoma. Int. J. Mol. Sci. 2019, 20, 5318.

Gereben, B.; Zavacki, A.M.; Ribich, S.; Kim, B.W.; Huang, S.A.; Simonides, W.S.; Zeöld, A.; Bianco, A.C. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr. Rev. 2008, 29, 898–938.

Pang , K. Lumintang , J.N., and Chin, K. (2021). Thyroid-Modulating Activities of Olive and Its Polyphenols: A Systematic Review . Nutrients 2021, 13(529):1-14.

Al-Jedi, M.A.R. (2011).Insulin, thyroid stimulating hormone and lipid levels among obese adult males in Gaza Governorate. Master. Biological Sciences. Islamic University-Gaza .

Caraccio, N.; Ferannini, E. and Monzani, F. (2002).Lipoprotein profile in subclinical hypothyroidism:response to levothyroxine replacement, a randomized placebo-controlled study. J. Clin. Endo. and Meta., 87(4):1533-1538.

Zhou, Y., Li, H., and Xia, N.(2021). The Interplay Between Adipose Tissue and Vasculature: Role of Oxidative Stress in Obesity . In cardiovascular medicine . volume 8 p1-14.

Thanas, C.; Ziros, P.G.; Chartoumpekis, D.V.; Renaud, C.O.; Sykiotis, G.P. The Keap1/Nrf2 Signaling Pathway in the Thyroid—2020 Update. Antioxidants 2020, 9, 1082.

Singh, P.; Bobby, Z.; Selvaraj , N. and Vinayagamoorthi , R. (2006).An evaluation of thyroid hormone status and oxidative stress in undialyzed chronic renal failure patients. Indian. J. Physiol. Pharmacol., 50(3):279-284.

Marseglia, L., Manti, S.D., Angelo, G., Nicotera, A, Parisi, E, Di Rosa, G. (2015). Oxidative strees in obesity : a critical component in human diseases . Int.J.Mol Sci. 16(1):378-400.

Ghowsi, M., Qalekhani, F., Mhammad Hosein, F., Fariba, M., Yousofvand , N. and Joshi, T.(2021).Inflammation , oxidative stress, insulin resistance , and hypertension as

mediators for adverse effects of obesity on the brain: A review. BioMedicine . 11(4): 13-22.

Mizuno , T.M.(2018) Fat Mass and Obesity Associated (FTO) Gene and Hepatic Glucose and Lipid Metabolism .Nutrients 10:1-11.