The Use of Green Coffee and Green Tea Extract as Cost-Effective Herbs to Counteract Obesity by Inhibiting PPAR-γ Gene Expression

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Abstract. The use of cost-effective herbs in the health sector is an exciting challenge in this era. Supported by environmentally friendly manufacturing processes, green tea and coffee can benefit health, one of which is metabolic syndrome (MetS). MetS therapy using green tea and green coffee, besides having health benefits, also has great potential in the economic sector because coffee is one of the largest sources of export commodities in Indonesia. Green tea and green coffee products as functional drinks are expected to be beneficial for health, one of which is obesity as a major risk factor in MetS. The purpose of this research is to determine the therapeutic effect of green tea and green coffee on molecular markers of obesity: gene expression of PPAR-γ and intracellular lipid accumulation from 3T3-L1 pre-adipocytes. Based on the results of this study, it was found that there was a significant difference between PPAR-γ and all groups ($p = 0.00$), and lipid accumulation between all groups ($p = 0.00$). There was a positive correlation between PPAR-γ and intracellular lipid accumulation with a p-value $= 0.009$ and an $R = 0.610$. Green tea and green coffee prevent obesity through inhibition of PPARγ gene expression which is characterized by decreased intracellular lipid accumulation in differentiated 3T3-L1 adipocytes.

Keywords: Green Coffee · Green Tea · PPAR-γ · Intracellular Lipid Accumulation

1 Introduction

Metabolic syndrome (SM) is an accumulation of several disorders in the body, which can increase the risk of developing cardiovascular disease, insulin resistance, diabetes mellitus, and vascular and neurological complications. The definition of SM is based on the

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National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria, namely if a person meets at least 3 of 5 agreed criteria, including: abdominal obesity (men's abdominal circumference >102 cm or women >88 cm), high blood triglycerides (>150 mg/dL), low HDL levels (<40 mg/dL), high blood pressure (>130/85 mmHg), and high fasting blood glucose levels (>110 mg/dL) [1]. The average prevalence of SM in the world population is 3.3%, obese children are 11.9%, and adults are 29.2% [2]. While the prevalence of SM in Indonesia is 21.66% [3] where cases in males are higher (26.2%) than in females (21.4%) [4].

Obesity is a major risk factor for SM. This condition occurs due to the accumulation of excess white adipose mass (WAT) which results in an increase in adipocyte cell size and the development of mature adipocytes from undifferentiated precursors [5]. This differentiation process can cause a change in the shape of the pre-adipocyte cells which were shaped like fibroblasts into mature adipocytes that are round and more voluminous. Intensive adipocyte differentiation will lead to the accumulation of mature adipose cells that can be spread in several parts of the body such as the stomach (visceral fat) and the tissue under the skin (subcutaneous fat).

MetS therapy using green tea and green coffee, besides having health benefits, also has great potential in the economic sector because coffee is one of the largest sources of export commodities in Indonesia. Coffee is a commodity that is widely consumed in the world, mainly due to its distinctive organoleptic characteristics and its positive effect on human health due to the bioactive components contained in it. Based on data submitted by [6], it is known that the level of coffee consumption in the world has consistently increased over the last 40 years. Coffee as a source of polyphenols, namely chlorogenic acid, is reported to have many health benefits. Chlorogenic acid is known to be present in unroasted coffee, or green coffee beans. In addition to coffee, tea derived from the Camellia sinensis plant is generally consumed in three different types, namely green, black, and oolong teas. Among the three types of tea that has the most significant effect on human health is the type of green tea [7]. It is estimated that around 2.5 million tonnes of tea leaves are produced annually worldwide, of which 20% of this amount is green tea which is widely consumed in Asia, parts of North Africa, America and Europe [8].

The use of cost-effective herbs in the health sector is an exciting challenge in this era. Supported by environmentally friendly manufacturing processes, green tea and coffee can benefit health, one of which is metabolic syndrome (MetS). Green tea and green coffee products as functional drinks are expected to be beneficial for health, one of which is obesity as a major risk factor in MetS. Research conducted by [9], demonstrated for the first time the effect of CGA on blood glucose levels (fasting blood glucose) in a diabetic mouse model. The diabetic db/db mouse model with homozygous spontaneous mutation of the leptin receptor is a diabetes model that shows the most complete characteristics of type 2 diabetes, such as obesity, elevated blood glucose and uncontrolled insulin. Intraperitoneal CGA injection in this model was able to reduce fasting blood glucose in the db/db mouse model. This is consistent with the report made by [10] in overweight patients. In addition, [9] also found that the effect could persist for 30 min after diabetic animals were administered with 2g/kg intraperitoneal glucose. Meanwhile, the content of EGCG ($C_{22}H_{18}O_{11}$) in green tea is considered a major contributor to various health benefits [11].

Consumption of green tea at optimal doses provides many health benefits, such as preventing cancer [12] and cardiovascular disease [13], regulating blood cholesterol levels [14], increasing fat metabolism thereby increasing the rate of weight loss [15–17]. This research raises the hypothesis that consumption of green coffee as a source of CGA and green tea as a source of EGCG in the long term will be beneficial through regular consumption of both. However, there have not been many studies using these two herbs together and the effective dosage for obesity prevention is not known for certain. So in this study, researchers wanted to know the effect of giving green coffee and green tea in various doses and their combination on the process of cell differentiation. The purpose of this research is to determine the therapeutic effect of green tea and green coffee on molecular markers of obesity: gene expression of PPARγ and intracellular lipid accumulation from 3T3-L1 pre-adipocytes.

An extraction technique that is not only efficient but also has a high level of safety and economic value was used in this investigation. Using a particular water extraction solvent is one strategy that can be applied as the easiest and greenest extraction method. Due to its relatively cheaper costs, water as a solvent can also have positive economic effects. The green coffee and green tea commodities used in this study were produced in powder form due to several factors, such as a higher level of long-term safety for consumption, ease of education and general introduction to society, need for less storage space, reduced production costs, and ease of distribution. It is also hoped that the powder form will extend the product's shelf life and make it more durable. Thus, it is hoped that this research may provide an effective and safe treatment for MetS, thereby lessening the economic burden caused by the disease.

2 Material and Methods

2.1 Green Coffee Preparation and Extraction

Robusta green coffee (*Coffea robusta*) beans were obtained from the Dampit, Malang, Indonesia. To separate the coffee beans from dirt or low-quality coffee beans, perform a sorting process. Roasted using an oven at 180 °C for 3 min (until the first crack). Coffee beans using a grinder to optimize the extraction results. Coffee beans that have been considered and extracted using the infusion method with demineralized drinking water as a solvent with a sample ratio: solvent = 1:15, at a temperature of 90 °C, for 10. The extraction results were filtered using coarse extract paper. Activated carbon for decaffeination of green coffee extract was carried out by adding sugar cane to distilled water as much as 2.5% w/v water, formic acid to 0.5% w/v water, and activated carbon to as much as 25% w/v water. Then the mixture was mixed in a water bath shaker, at a temperature of 80 °C, for 6 h. After that, activated carbon from the solvent and rinsed with water (200% v/w carbon).

Green coffee extract decaffeination can be used with the addition of activated carbon with a ratio of activated carbon: green coffee extract 1:75 (w/v extract). The decaffeination process was reacted using a shaker water bath at 80 °C for 8 h. After the process, we used coarse filter paper to obtain the decaffeinated coffee extract.The decaffeinated coffee extract was added with 5% maltodextrin (w/v extract). Furthermore, drying using

a food dehydrator at a temperature of 60 °C for 5 h. Reduce the size with a dry blender and filter it through an 80 mesh sieve.

2.2 Green Tea Preparation and Extraction

The green tea (*Camelia sinensis*) used is grade 1 quality taken from the top 3 shoots planted in the Ciwidey area of Bandung, Indonesia. A sorting process is carried out to separate dry green tea samples from impurities or low-quality green tea. Dried green tea is ground using a grinder to optimize extraction results. Analysis of dry green tea ingredients included total flavonoids, IC50, and caffeine content.

Green tea was weighed and extracted using the infusion method with demineralized drinking water as a solvent with a sample ratio: solvent = 1:15; at a temperature of 90 °C; for 30 min. After that, it was filtered using coarse filter paper.

Green tea decaffeination was carried out by adapting the blanching method (Liang et al., 2006). Green tea decaffeination was carried out at a temperature of 50^{o} C and within 5 min. The decaffeinated green tea extract was added with 5% maltodextrin (w/v extract). Then drying was done using a Food Dehydrator (T = 60^{o} C, and t = 5 h). It was reduced in size with a dry blender and filtered through an 80 mesh sieve.

2.3 Maintenance of 3T3-L1 Culture Cells

3T3-L1 mouse preadipocytes from ATCC passage 10-15^{th} were cultured in Dulbecco's Modified Eagle's Medium (DMEM high glucose) containing 10% Fetal Bovine Serum (FBS, Gibco) and 1% Penicillin/Streptomycin (P/S, Gibco) in a 5% CO2 incubator at 37 °C using a 60 mm dish culture. Medium is changed every two days. This method is a modification of Aoyagi et al., 2014.

2.4 3T3-L1 Culture Cell Differentiation

To induce cell differentiation, 3T3-L1 preadipocyte cultures obtained two days after confluent were stimulated with 0.5 mM 3-isobutyl-1-methyl-xanthine, 0.25 M Dexamethasone, 2 M Rosiglitazone, and 1 g/mL of insulin (MDI differentiation medium) for one day. Then the cells were kept in a 10% FBS/DMEM medium with 1 g/mL insulin for ten days. Medium is replaced every two days. By this time, more than 90% of the cells will become mature adipocytes with lipid-filled droplets.

2.5 Cell Viability Test

3T3-L1 preadipocyte culture cells were grown as many as 10^4 cells/well on 96 well plates and incubated in a 5% CO2 incubator at 37 °C for 24 h. On the next day, the medium was replaced with green coffee and green tea extracts at concentrations of 0, 20, 40, 80, 160, 320, and 640 g/ml and then incubated for 24 h. Furthermore, the WST -1 based cell proliferation and cytotoxicity assay kit was added from Roche Diagnostics GmbH (Mannheim, Germany) Cat. No. 05015944001 into each well at the end of the treatment period. Then the plate was incubated for 1 h in the dark. After that, the absorbance was measured at 450 nm using a microtiter plate reader (Asys, Cambridge, England).

2.6 Gene Expression Analysis

Total RNA was isolated from cell culture by extraction using guanidium-phenol-chloroform method. The first cDNA strand was synthesized from 1 g of total RNA using 1 unit of Revertra Ace reverse-transcriptase with a random primer. The primers used for cDNA amplification were made in the following sequence (forward and reverse, 5_ to 3_):

PPARγ: AAC TCT GGG AGA TTC TCC TGT TGA dan TGG TAA TTT CTT GTG AAG TGC TCA TA.

B-aktin: TGA GAG GGA AAT CGT GCG TGA CAT dan ACC GCT CAT TGC CGA TAG TGA TGA.

The reverse transcription reaction was performed using the ReverTra Ace-α kit (Toyobo, FSK-101). Furthermore, RNA expression was carried out using Light Cycler PCR96 system (Takara, cat no. TP600) with GoTaq Green Master PCR kit (Promega, cat no, M7822) according to the given protocol. The PCR cycle conditions were as follows: denaturation for 5 min at 95 °C; 35 cycles for 30 s at 95 °C, 30 s annealing process at 52 °C, 55 °C, and 58 °C, followed by an extension for 30 s at 72 °C; and last extension for 10 min at 72 °C. The mRNA levels were quantified using a spectrophotometer at 260 and 280 nm.

2.7 Oil Red O Painting and Quantification

Before painting, a working solution is made from the Oil Red O stock solution. The stock solution was prepared by dissolving 0.5 g of Oil Red O (Sigma) powder into 80 ml of isopropanol (100%) in a water bath at 56 °C overnight. The final volume was adjusted to 100 ml with slow stirring on a magnetic stirrer. Before painting, the stock solution was warmed at 60 °C and filtered with filter paper number 1 (Whatman, UK). The working solution was obtained by dissolving the stock solution in a ratio of 3:2 with de-ionized water, allowed to stand for 10 min at room temperature and filtered (0.22 m, Millipore).

3T3-L1 cells (15x104/well) were grown in 24 well plates containing a differentiation medium for ten days. On the tenth day, cells were washed with phosphate-buffered saline (PBS), added with 10% formalin (pH 7.4) for 30 min, and then washed with 60% isopropanol. After drying, 0.3% Oil Red O solution (Sigma, St. Louis, MO, USA) was added to each well at room temperature for 30 min in the dark. After being separated from the solution, the cells were washed with distilled water three times and dried. Intracellular lipids stained with Oil Red O were extracted with 100% isopropanol and quantified by measuring their optical density at 510 nm. Visual observations were made using a light microscope (Olympus) and photographed using Image ProPlus 6.0 software (Media Cybernetics)33.

2.8 Ethical Clearance

This experimental design has been fulfilled and approved by the Health Research Ethics Committee of Saiful Anwar General Hospital, Malang, Indonesia, by registered number: 400/211/K.3/302/2021.

2.9 Statistical Analysis

Gene expression data were analysed using ImageJ software and expressed as the mean fold change in relative expression. Average fold change and standard deviation (SD) were obtained from three biological replicate samples per condition. Evaluation of statistical data is indicated by the mean ± standard deviation. All data were analyzed by one-way ANOVA, followed by Duncan test to determine the significance of the differences between the treatment groups to test the significance value of $p < 0.05$. Spearman test was used to analyze correlation between PPAR-γ and Intracellular Lipid Accumulation.

3 Results and Discussion

3.1 Gene Expression of PPAR-γ

The expression of the PPAR-γ gene obtained through the gene amplification method by rt-PCR, compared with the expression of β-actin as a housekeeping gene, can be seen in Fig. 1.

The results of One-way ANOVA analysis on PPAR-γ gene expression can be seen in Table 1.

Based on the test results, it can be seen that the significance value of F is 0.000 ($p < 0.05$), so it can be stated that there is a significant difference in PPAR-γ between groups. Further tests were carried out to find out which groups showed differences using Duncan's test.

The results of further tests show that the neg group has the smallest average PPAR-γ expression, which is 0.6247 ± 0.13532, and is not significantly different from several other groups, including; C320, T320, and T160C80. The NEG group was substantially

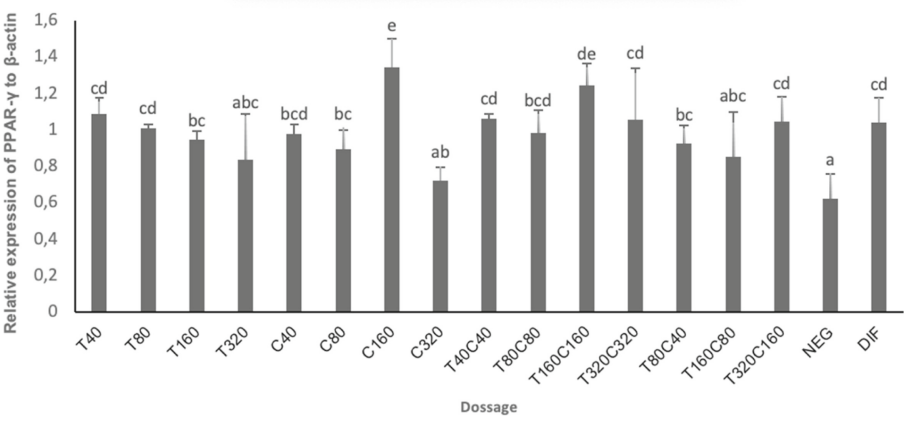

Fig. 1. PPAR-γ gene expression. The gene expression level was normalized based on β-actin level—one-way ANOVA and Duncan posthoc test were used. $P < 0.05$ was considered to indicate a statistically significant difference. Groups that appear in the same homogeneous subset are not significantly different.

Table 1. One-way ANOVA test result based on PPAR-γ gene expression

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	1.423	16	0.089	4.284	0.000*
Within Groups	0.706	34	0.021		
Total	2.129	50			
*Significant for P-value < 0.005					

different from the DIF group. The DIF group was not significantly different from several other groups, including; T320, T160C80, C80, T80C40, T160, C40, TC80, T80, T320C160, T320C320, T40C40, and T40. The group with the highest average PPAR-γ expression was C160, 1.3424 ± 0.15764, and was not significantly different from the T160C160 group.

3.2 Intracellular Lipid Accumulation

The accumulation of Intracellular Lipids obtained through the ORO staining method can be seen in Fig. 2.

The results of the One-way ANOVA analysis on PPAR-γ gene expression can be seen in Table 2.

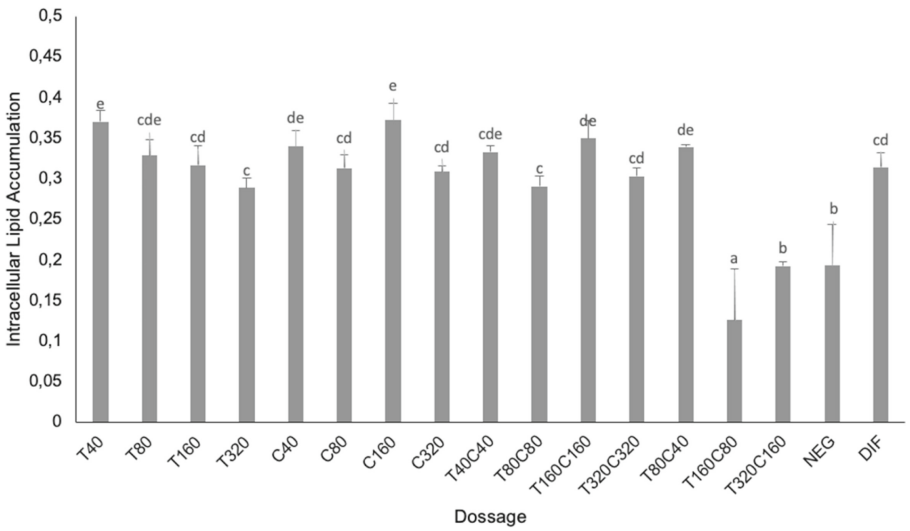

Fig. 2. Intracellular Lipid Accumulation—one-way ANOVA and Duncan posthoc test was used. P < 0.05 was considered to indicate a statistically significant difference. Groups that appear in the same homogeneous subset are not significantly different.

Table 2. One-way ANOVA test result based on Intracellular Lipid Accumulation

Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	0.21	16	0.013	22.548	0.000*
Within Groups	0.02	34	0.001		
Total	0.236	50			
**Significant for P-value < 0.005*					

Based on the test results, it can be seen that the significance value of F is 0.000 (0.000 < 0.05), so it can be stated that there is a significant difference in lipid accumulation between groups. Further tests were carried out to find out which groups showed differences using Duncan's test.

The results of further tests show that the T160C80 group had the smallest average lipid accumulation of 0.126 ± 0.063380 and was different from all other groups. The T320C160 and NEG groups were not significantly different but different from the other groups. The neg group was significantly different from the dif group. The T320 group did not differ from the T80C80, T320C320, C320, C80, DIF, T160, T80, and T40C40 groups but differed from the T80C40, C40, T160C160, T40, and C160 groups. The group with the largest average lipid accumulation was C160, 0.37200 ± 0.020224, but it was not different from the T40, T160C160, C40, T80C40, T40C40, and T80 groups.

3.3 Correlation of PPAR-γ with Intracellular Lipid Accumulation

Based on the table above, it is found that with a test p-value of 0.009, which is less than (0.050), it can be concluded that there is a significant relationship between PPAR-γ and Intracellular Lipid Accumulation. The correlation coefficient shows a positive relationship, which means that when the PPAR-γ variable is high, the Intracellular Lipid Accumulation will also be high, and vice versa. If the PPAR-γ variable is low, the Intracellular Lipid Accumulation is low.

Correlations			
			LIPID
Spearman's rho	PPAR	Correlation Coefficient	.610**
		Sig. (2-tailed)	.009
		N	17
**. Correlation is significant at the 0.01 level (2-tailed).			
*. Correlation is significant at the 0.05 level (2-tailed).			

PPAR-γ is a hormone whose main role is in genes related to carbohydrate and fat metabolism, which causes an increase in insulin sensitivity levels to maintain glucose homeostasis [18, 19]. PPAR-γ activation induces a protein used by cells to reduce inflammation and control free fatty acid delivery, as well as adipogenesis [20]. In this study, it

was found that increasing the concentration of a single dose of tea was proportional to the decrease in PPAR-γ gene expression. Meanwhile, a single dose of coffee at a concentration of 320 had the lowest expression value compared to other doses, and was not significantly different from NEG. It is possible that single-dose coffee is promising for the inhibition of adipocyte differentiation. Meanwhile, the increase in the concentration of the combined dose was not proportional to the decrease in PPAR expression, and only the 2:1 combination was relatively better at inhibiting PPAR-γ than 1:1. Increasing the concentration of a single dose of tea is known to be proportional to the decrease in intracellular lipid accumulation. Meanwhile, the 2:1 combination was relatively better in reducing intracellular lipid accumulation than the single dose or the 1:1 combination. This shows that green tea and green coffee have good potential in preventing obesity.

4 Conclusion

Green tea and green coffee prevent obesity through inhibition of PPARγ gene expression which is characterized by decreased intracellular lipid accumulation in differentiated 3T3-L1 adipocytes. It is hoped that this research may provide an effective and safe treatment for MetS, thereby lessening the economic burden caused by the disease.

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References

1. Ramli, N.S., Areej A. Alkhaldy., Areej A. Alkhaldy. 2021. Review Effects of Caffeinated and Decaffeinated Coffee Consumption on Metabolic Syndrome Parameters: A Systematic Review and Meta-Analysis of Data from Randomised Controlled Trials. Medicina 57(957). https://doi.org/10.3390/medicina57090957.
2. Friend, Amanda., Leone Craig., and Steve Turner. 2013. The Prevalence of Metabolic Syndrome in Children: A Systematic Review of the Literature. Metabolic Syndrome And Related Disorders. Volume 11, Number 2, Mary Ann Liebert, Inc. Pp. 71–80 https://doi.org/10.1089/met.2012.0122.
3. Herningtyas, E. H and Tian Sheng Ng. 2019. Prevalence and distribution of metabolic syndrome and its components among provinces and ethnic groups in Indonesia. BMC Public Health 19(1):377. https://doi.org/10.1186/s12889-019-6711-7.
4. Driyah, S., Oemiati, R., Rustika, & Hartati, N. S. (2019). Prediktor Sindrom Metabolik : Studi Kohor Prospektif Selama Enam Tahun Di Bogor, Indonesia. *Media Litbangkes*, *29*, 215–224. https://doi.org/10.22435/Mpk.V29i3.654.
5. Valli, V., Heilmann, K., Danesi, F., Bordoni, A., Gerhauser, C. 2018. Modulation of Adipocyte Differentiation and Proadipogenic Gene Expression by Sulforaphane, Genistein, and Docosahexaenoic Acid as a First Step to Counteract Obesity. Hindawi Oxidative Medicine and Cellular Longevity Volume 2018, Article ID 1617202, 8 pages https://doi.org/10.1155/2018/1617202.

6. International Coffee Organization. 2012. MONTHLY COFFEE MARKET REPORT. http://www.ico.org/documents/cmr-0712-e.pdf .
7. Cabrera, C., Artacho, R., & Gime, R. (2006). Beneficial Effects Of Green Tea — A Review. *Journal Of The American College Of Nutrition*, *25*(2), 79–99.
8. Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial Effects Of Green Tea: A Literature Review. *Chinese Medicine*, *5*, 1–9. https://doi.org/10.1186/1749-8546-5-13.
9. Ong, Khang Wei., A. Hsu., B.Tan. 2012. Chlorogenic Acid Stimulates Glucose Transport in Skeletal Muscle via AMPK Activation: A Contributor to the Beneficial Effects of Coffee on Diabetes. Plos One.
10. Van Dijk A.E., Olthof M.R., Meeuse J.C., Seebus E., Heine R.J., van Dam R.M. *2009.* Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. *Diabetes Care.* 32(6):1023-5.
11. Chu C., Deng J., Man Y., Qu Y. 2017. Green Tea Extracts Epigallocatechin-3-gallate for Different Treatments. *Biomed. Res. Int.* 2017:5615647.
12. Mukhtar H., Ahmad N. 2000. Tea polyphenols: Prevention of cancer and optimizing health. *Am. J. Clin. Nutr*. 71(Suppl. 6):1698S–1702S.
13. Nagao T., Hase T., Tokimitsu I. 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity.* 15:1473–1483.
14. Moore R.J., Jackson K.G., 2009. Minihane A.M. Green tea (*Camellia sinensis*) catechins and vascular function. *Br. J. Nutr.*102:1790–1802.
15. Zheng X.X., Xu Y.L., Li S.H., Liu X.X., Hui R., Huang X.H. 2011. Green tea intake lowers fasting serum total and LDL cholesterol in adults: A meta-analysis of 14 randomized controlled trials. *Am. J. Clin. Nutr*. 94:601–610.
16. Venables M.C., Hulston C.J., Cox H.R., Jeukendrup A.E. 2008. Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. *Am. J. Clin. Nutr.* 87:778–784.
17. Roberts J.D., Roberts M.G., Tarpey M.D., Weekes J.C., Thomas C.H. 2015. The effect of a decaffeinated green tea extract formula on fat oxidation, body composition and exercise performance. J. Int. Soc. Sports Nutr. 12:1.
18. Hursel R., Viechtbauer W., Westerterp-Plantenga M.S. 2009. The effects of green tea on weight loss and weight maintenance: A meta-analysis. Int. J. Obes. (Lond.). 33:956–961.
19. Mohamed WA, Schaalan MF. Antidiabetic efficacy of lactoferrin in type 2 diabetic pediatrics; controlling impact on PPAR-γ, SIRT-1, and TLR4 downstream signaling pathway. Diabetol Metab Syndr. 2018. https://doi.org/10.1186/s13098-018-0390-x. Return to ref 12 in article.
20. Sahin N, Orhan C, Erten F, Tuzcu M, Defo Deeh PB, Ozercan IH, Juturu V, Kazim S. Effects of allyl isothiocyanate on insulin resistance, oxidative stress status, and transcription factors in high-fat diet/streptozotocin-induced type 2 diabetes mellitus in rats. J Biochem Mol Toxicol. 2019;33:e22328.