ORIGINAL ARTICLE

Symbiotic effect of *lactobacillus acidophilus*, Ginger, Pineapple and Green Coffee in the complex management of obesity in rats

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ABSTRACT

Background: *Obesity is a chronic metabolic disease associated with having excess body fat that could be influenced by many factors. Our study aimed to assess the powerful effect of Lactobacillus acidophilus alone or combined (symbiotic) with Prebiotic such as Ginger, Pineapple and Green Coffee as anti-obesity agents.* ***Methodology****: Using 8 groups (10 rats each) of Sprague-Dawley rats, Group 1 was kept as a negative control, Group 2 positive control, while other groups were orally given Lactobacillus acidophilus, Ginger, Pineapple and Green Coffee individually and in combination with Probiotics, for 45 days till the end of the experiment while the body weight of rats was recorded.Blood samples were collected for biochemical parameters analysis and organs were dissected and homogenized to analyze obesity-related biomarkers,* ***Results:*** *Our results revealed that either individual or mixed administration of this pro and prebiotics decreased the body and organs, specifically those treated with the mixture or probiotic and prebiotic, also serum (HDL), CAT), and (SOD) was decreased (P <0.05), while other biochemical parameters (T.G), (CHOL), (U.A), (Creat), Urea, (GOT),(GPT) and (ALP); (significantly (P<0.05) was decreased when compared with the positive control group, Nevertheless, the histopathological examination showed the reduction of adipose tissue in kidney, liver, and Pancreas showed overestimate reductions in the percentage of body fat.* ***Conclusions:*** *This study showed a promising effect of Lactobacillus acidophilus when it combined with these plants as natural feed additives on obesity.*

INTRODUCTION

Obesity has reached an epidemic rate, millions are diagnosed with obesity, it is a public health burthen worldwide, defined as excessive fat accumulation which is associated with an increase in the adipose tissue mass, which may lead to endocrine malfunction[1]. Some people misconceived the obesity complications, they supposed it's a cosmetics concern, however, many people are not because of food addiction[2], this fatal metabolic disease results from complex factors it could be genetic, behavioral, and environmental it also correlating with economic and an unhealthy lifestyle that increases the risk[3]. Cardiovascular complications and a lot of metabolic syndromes including diabetes mellitus may arise due to obesity because there is a positive association between obesity and insulin resistance and infiltration of adipose tissues[4] it is important to tackle obesity and find out appropriate treatment strategies to achieve optimal weight loss and fat level. This can be executed through surgical, pharmaceutical, or lifestyle interventions[5]. Moreover, the market of anti-obesity medications is hugely associated with many side effects[6], so it is more worthy to think about alternative nutritional therapy which may be the safest and most cost-effective way to control body weight[7].

Lactobacillus acidophilus is one of gut living probiotics, that consists of many different bacterial species, which are involved in the metabolism of nutrients and it also beneficially affect the host animal by improving its intestinal microbial balance [8], they are involved in the treatment of diarrhea, allergy, anti-cancer, as well as playing a vital role in obesity management[9] since it exhibits anti-cholesterolemic properties in mature, antimutagenic and reduction of serum cholesterol[10], besides, improving insulin

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sensitivity it could affect glucose and fat metabolism through gastrointestinal pathways [11]

Green Coffee is one of the most well-known types of coffee, which has characterized by light grain with a distinctive flavor and green color encompass several substances that are important to the human body, most important role of it is the antioxidant activity [12]. Coffee beans consist of a set of complex compounds with an extensive variety of minerals, amino acids, lipids, and other chemical compounds that influence various metabolic pathways processes, for example, trigonelline and caffeine are vital alkaloids has an effective role in the metabolism aiding the digestion of the foods in the stomach [13] as well as a green coffee extract (GCE) has been known as the richest sources of chlorogenic acid which are the ester of caffeic acid and quinic acid. [14] Rustenbeck carried experimental research on mice which displayed the activity of the green coffee on the weight gain, as it has a possible anti-obesity effect by decreasing body fat accumulation by regulating adipogenesis and lipid metabolism-related genes and proteins liver[15].

Ginger is an underground rhizome of plant Zingier Officinale, yellowish-green flowers with purple patterns, it belongs to Zingiberaceae family, that has been used globally in foods as a hot spice or in traditional medicine [16] it is associated with treating a metabolic disorder, particularly it's incredible benefits to human as a natural weight loss agent, due to its pharmacological activity of ginger because of gingerol and shogaol [17].

Pineapple is the most consumed tropical with eight phenolic compounds, including; syringic acid, gallic acid, o-coumaric, acid gentisic acid, syringic acid, vanillin, ferulic acid, ferulic acid sinapic acid, ferulic acid, and sinapic acid[18] in addition to a large amount of bromelain that has a known function in lipolysis[19] as well as high its rich with a high amount of specific dietary fiber, which minimizes the cholesterol level [20]

METHODOLOGY

Bacteria (Probiotics) preparation and activation:

In brief, Lactobacillus acidophilus ATCC 4356) cultures have been cultivated in MRS broth. MRS media (pH 5.5; Acumedia, United States) was prepared by melting 55 g of the medium in one liter of filtered water, mixing it carefully, and autoclaving at 121oC for 15 minutes. Then activate the bacteria by inoculating 1 ml of L. Acidophilus in 50 ml MRS media and incubated at 37°C for 24-48 hours under microaerophilic conditions.

Prebiotics extraction:

The plant materials including rhizomes of *Zingiber officinale* (ginger), green coffee bean, and pineapple were purchased from a fresh plant market. Ginger and green coffee bean were cleaned, cut to fine pieces, and finely granulated in a blender, then extracted with 70% ethanol for 24 hours.The solution was filtered, and lyophilization was performed. The obtained extract was soluble in distilled water. The pineapple fruit was washed with running water to remove sand and dirt from the fruit "and carefully peeled with a kitchen knife to make the peel. Dry and pulverized peels were extracted by cold maceration for 72 hours at room temperature using 70% methanol, then the solution was filtered and lyophilized. The extract obtained was soluble in distilled water.

Micro dilution method:

Micro dilution is the use of thin, disposable plastic plates to test antibacterial activity and MIC. Normal trays contain 96 wells, each containing 0.1 ml in volume. The purpose of this analysis was to determine whether the prebiotic extract is harmful to the probiotic bacteria. We used the McFarland standard to measure bacterial concentration. By obtaining a new culture medium for the test organism as well as for the test organism, we have prepared a test suspension. Micro dilution test was performed by inoculation of 106 CFU [0.5 McFarland St.] of the 100 µl Pineapple, Green Coffee and Ginger extracts separately in each well of the plates by 10 fold dilution. After overnight incubation at 35 C, the spectrophotometer analyzed the evident bacterial growth of the tubes as indicated by turbidity. [21]

Experimental diet:

This research included two forms of diets. The first form was a regular simple diet (SBD). The second diet was a high-fat diet (HFD) prepared by adjusting the SBD by increasing the amount of lipids while lowering the carbohydrate content. The chemical composition of both diets is shown in Table 1.

Table 1: The percentage of chemical constituents of the standard basal diet (SBD) and high-fat diet (HFD)

Constituent	SBD	HFD
Cornstarch (%)	56.07	26.07
Casein (%)	14.00	14.00
Sucrose (%)	10.00	10.00
Corn oil (%)	10.00	10.00
Cellulose (%)	5.00	5.00
Minerals (%)	3.50	3.50
Vitamins (%)	1.00	1.00
Methionine (%)	0.18	0.18
Choline chloride (%)	0.25	0.25
Tert-butyl hydroquinone (%)	0.00	0.00
Lard (%)	0.00	30.00

Animals

Eight adult male Sprague Dawley strains weighing approximately 100-110 g at 7 weeks of age. They were kept under observation for 7 days before the start of the adaptation experiment. During the study era, animals were housed in cages made of stainless steel. The room temperature was held at about 24±2° C and the illumination was a 12-h dark period of 12-h light. Throughout the tests, water was freely given to rats. All the animals were fed for one week on a regular diet.

Experiment design.

Experimental animals will be divided into eight groups (10 rats/group). as shown in Table 2.

Table 2: Summarized experimental design of the study

Weeks num	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Diet type	SBD	HFD						
Green coffee extract (mg/kg diet)	–	–	20	-	-	–	20	–
Ginger extract (mg/kg diet)	-	-	–	200	–	–	200	–
Pineapple extract (mg/kg diet)	–	–	–	–	4	–	4	–
L. acidophilus (CFU)	–	–	–	–	–	2.5×10^8	2.5×10^8	–
Omega3 (mg/kg diet)	–	–	–	–	–	–	–	250
Frequency	Daily							
Duration	For 6 weeks							
Sampling	At the end of the experimental period							

Conditions Experimental groups (N = 80 rats)

Sampling:

At the end of the experiment, the rats fasted for 12 h and were then sacrificed under anesthesia using sodium pentobarbital. Samplings of blood. They were extracted from the vagal vein and then collected in dry test tubes and allowed to stand, then centrifuged at 3000 rpm for 15 minutes to isolate the serum. The transparent serum was put in the deep freezer at −20° C for further biochemical studies. Immediately after scarification, dissection was performed to remove the spleen, kidneys, and liver. In saline physiologic solution.

Biochemical analysis:

Determination plasma biochemical parameters High-density lipoprotein (HDL), triglyceride(T.G), Cholesterol (CHOL), Uric acid (U.A), Creatinine, (Creat), Urea, Glutamic oxaloacetic transaminase (GOT), (MDA) *malondialdehyde*, CAT, (SOD) *superoxide dismutase* (GPT) and Alkaline phosphatase test (ALP) in all groups by using automatic biochemical apparatus

Histological examination:

The liver, kidney, and spleen tissue specimens were fixed in 10 % neutral buffered formalin. Fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol, cleared in xylene, enclosed in paraffin, cut to the 4-6U thickness, and stained with hematoxylin and eosin**.**

Statistical analysis

Results are expressed as mean ± SD (Standard Deviation), Values of $P<0.05$ and $P<0.01$ were considered statistically significant

Results and Discussion

Biological parameters

The Bodyweight gain (BWG), Internal fats, subcutaneous fats, and relative weights of the heart, kidney, and liver of all the experimental rats, after 6 weeks of receiving the appropriate diets, were displayed in Table 3, figure 1 and 2.

Table 3: Bodyweight gain (BWG), feed intake and relative weights of the liver, kidneys, internal fats and subcutaneous fats of all study groups at the end of the tests.

	Groups							
	Control Negative	**Control positive**	**Pineapple**	**Green coffee**	**Ginger**	**Probiotic**	**Mixture**	**Omiga 3**
BWG	$47\pm4_{a}$	$125\pm6_{b}$	$63\pm7_{a,b}$	$117\pm4_{b}$	$110\pm6_{b}$	$110\pm3_{b}$	$73\pm7_{a,b}$	$116\pm3_{b}$
Relative liver weight (g/100 g BW)	$4.06\pm0.4_{a}$	$5.49\pm0.65_{a}$	$3.88\pm0.34_{a}$	$3.9\pm0.5_{a}$	$3.7\pm0.16_{a}$	$2.68\pm0.02_{a}$	$2.8\pm0.13_{a}$	$2.73\pm0.08_{a}$
Relative kidney weight (g/100 g BW)	$0.96\pm0.13_{a}$	$0.767\pm0.14_{a}$	$0.76\pm0.1_{a}$	$0.67\pm0.1_{a}$	$0.67\pm0.06_{a}$	$0.63\pm0.04_{a}$	$0.56\pm0.006_{a}$	$0.65\pm0.017_{a}$
Relative internal fats weight (g/100 g BW)	$1.25\pm0.13_{a,b}$	$2.6\pm0.33_{a,b}$	$1.16\pm0.1_{a,b}$	$1.5\pm0.14_{a,b}$	$1.03\pm0.14_{a,b}$	$1.07\pm0.123_{a,b}$	$0.57\pm0.06_{a}$	$2.03\pm0.08_{b}$
Relative subcutaneous weight (g/100 g BW)	$1.46\pm0.18_{a,b,c}$	$2.2\pm0.3_{a,b,c}$	$1.07\pm0.1_{a,b,c}$	$1.5\pm0.067_{a,c}$	$0.92\pm0.04_{a,b}$	$0.997\pm0.08_{a,b,c}$	$0.68\pm0.07_{b}$	$1.5\pm0.04_{c}$

Data are displayed as mean ± standard error of the mean. Values in the same row and sub table not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost sub table using the Bonferroni correction.

Group 2 and 4 BWG rates were substantially higher than in Group 1. Group 5,6 and 7 rats showed substantial decreases in BWG compared to Groups 1 and 3. Groups 4,5,6 and 7 showed a marked decrease in relative renal weight and a substantial decrease in relative liver weight, relative internal fat weight and relative subcutaneous weight compared to other groups.

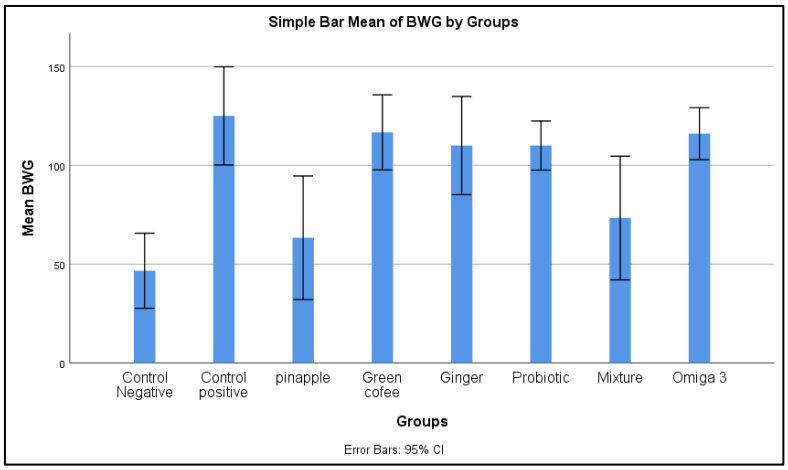

Fig.1: Mean body weight gain (BWG) between all groups

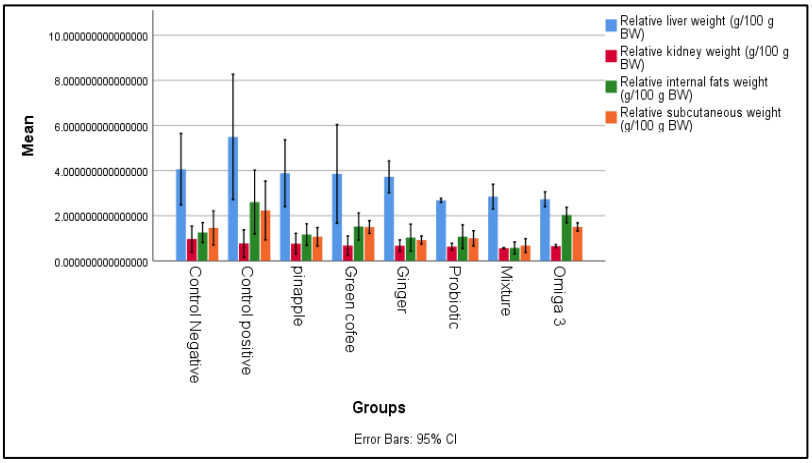

Fig. 2: Simple Bar Mean of Relative liver weight (g/100 g BW), Mean of Relative kidney weight (g/100 g BW), Mean of Relative internal fats weight (g/100 g BW), Mean of Relative subcutaneous weight (g/100 g BW) by groups by INDEX.

Effect on lipid profile in serum

Serum TC, TG, HDL-C, LDL-C, and VLDL-TG levels of all experimental groups were listed in Table 4 and Figure 3. Group 2 and 4 rats showed marked increases in TC, TG, LDL-C, and VLDL-TG serum levels, while HDL-C levels decreased significantly in Groups 3 and 7 compared to Group 2. Compared to Groups 2 and 8, Groups 3,4,5,6, and 7 showed substantial reductions in TC, TG, LDL-C, and VLDL-TG serum levels, although a remarkable increase in HDL-C levels was observed in the control values.

Table 4: Serum levels of total cholesterol (TC), triglycerides (TG), high- and low-density lipoprotein cholesterols (HDL-C & LDL-C), and very-low-density lipoprotein triglyceride (VLDL-TG) of all the studied groups, at the end of the experiments.

	Groups							
	Control Negative	**Control positive**	**Pineapple**	**Green Coffee**	**Ginger**	**Probiotic**	**Mixture**	**Omiga 3**
HDL	$38.0\pm0.5_{a,c}$	$34.2\pm0.4_{b}$	$36.4\pm0.4_{a}$	$38.0\pm0.6_{a,b}$	$37.6\pm0.6_{a}$	$38.9\pm0.2_{a}$	$36.4\pm0.3_{a}$	$39.4\pm0.7_{c,d}$
T.G	$84.6\pm2.3_{a}$	$183.6\pm2.3_{b}$	$134.6\pm1.8_{c}$	$159.4\pm3_{d,e}$	$164.3\pm2.3_{d}$	$154.0\pm1.6_{e,f}$	$140.3\pm0.4_{c,g}$	$146.0\pm1.9_{f,g}$
CHOL	$112\pm1_{a}$	$213\pm1_{b}$	$175\pm2_{c}$	$184\pm1_{d}$	$187\pm2_{d}$	$185\pm2_{d}$	$174\pm1_{c}$	$168\pm3_{c}$
VLDL	$16.92\pm0.46_{a}$	$36.72\pm0.46_{b}$	$26.92\pm0.37_{c}$	$31.89\pm0.6_{d,e}$	$32.86\pm0.46_{d}$	$30.8\pm0.33_{e,f}$	$28.06\pm0.07_{c,g}$	$29.2\pm0.37_{f,g}$
LDL	$59.48\pm1.7_{a}$	$135.98\pm1.3_{b}$	$111.28\pm1.6_{c}$	$113.82\pm1.8_{c}$	$116.54\pm2.17_{c}$	$116.9\pm2.02_{c}$	$109.14\pm0.9_{c,d}$	$101.7\pm2.9_{d}$

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1. Tests are adjusted for all pairwise comparisons within a row of each innermost sub table using the Bonferroni correction.

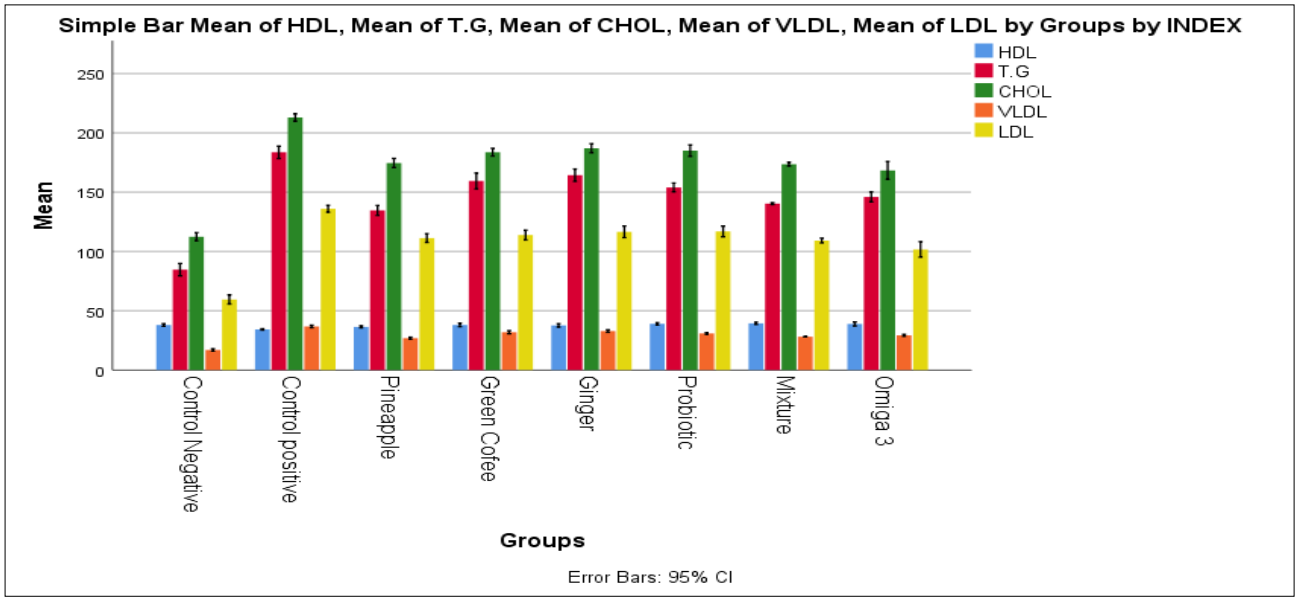


Fig. 3: Mean of HDL, Mean of T.G, Mean of TC, Mean of VLDL, Mean of LDL by groups by INDEX

Effect on activities of aminotransferases, alkaline phosphatase, and creatine kinase, as well as levels of creatinine and urea in serum

Table 5 and figure 4 displayed the serum activities of ALT, AST, and ALP, as well as the serum levels of creatinine and urea of all rats. Rats of groups 2 and 3 exhibited marked elevations in serum activities of ALT, AST, and ALP, in comparison to group 1. The serum activities of ALT, AST, and ALP of group 7 were significantly higher than in group 1, whereas remarkably lower than in groups 3 and 4. Among all the experimental groups, serum activity of creatinine and urea were similar.

Table 5: Serum levels of alanine and aspartate aminotransferases (ASAT and ALAT), alkaline phosphatase (ALP), as well as levels of creatinine and urea of all the experimental groups, at the end of the experiments

	Groups							
	Control Negative	**Control positive**	**Pineapple**	**Green Coffee**	**Ginger**	**Probiotic**	**Mixture**	**Omiga3**
U.A	$3.1\pm0.1_{a}$	$6.1\pm0.1_{b}$	$3.8\pm0_{c,e}$	$5.2\pm0.1_{d}$	$5.2\pm0.1_{d}$	$3.7\pm0.1_{c}$	$4.0\pm0.1_{e}$	$4.7\pm0.1_{f}$
CREAT	$0.60\pm0.01_{a}$	$0.88\pm0.01_{b}$	$0.65\pm0.02_{a,c}$	$0.69\pm0.01_{c,e}$	$0.70\pm0.02_{c,d,e}$	$0.72\pm0.02_{e}$	$0.68\pm0.01_{c,e,f}$	$0.80\pm0.1_{g}$
UREA	$22.0\pm1_{a}$	$33.7\pm0.8_{b}$	$30.6\pm0.6_{b,c,g}$	$29.0\pm0.9_{c,d,g}$	$26.4\pm0.8_{d,f}$	$26.6\pm0.1_{d,e,f}$	$25.2\pm0.2_{f}$	$30.0\pm0.5_{g}$
ALP	$384\pm6_{a}$	$931\pm11_{b}$	$723\pm6_{c}$	$696\pm10_{c}$	$577\pm15_{d}$	$631\pm13_{e}$	$601\pm1_{d,e}$	$515\pm12_{f}$
ALT	$19\pm0_{a}$	$63\pm2_{b}$	$41\pm1_{c}$	$33\pm1_{d}$	$31\pm1_{d}$	$42\pm1_{c}$	$35\pm1_{d}$	$42\pm1_{c}$
AST	$18.7\pm0.5_{a}$	$68.0\pm0.8_{b}$	$48.4\pm0.6_{c}$	$53.0\pm1_{d}$	$42.3\pm0.6_{e,f}$	$44.7\pm0.9_{e}$	$39.0\pm0.7_{f}$	$50.7\pm1.2_{c,d}$

Note: Values in the same row and sub table not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.1
1. Tests are adjusted for all pairwise comparisons within a row of each innermost sub table using the Bonferroni correction.

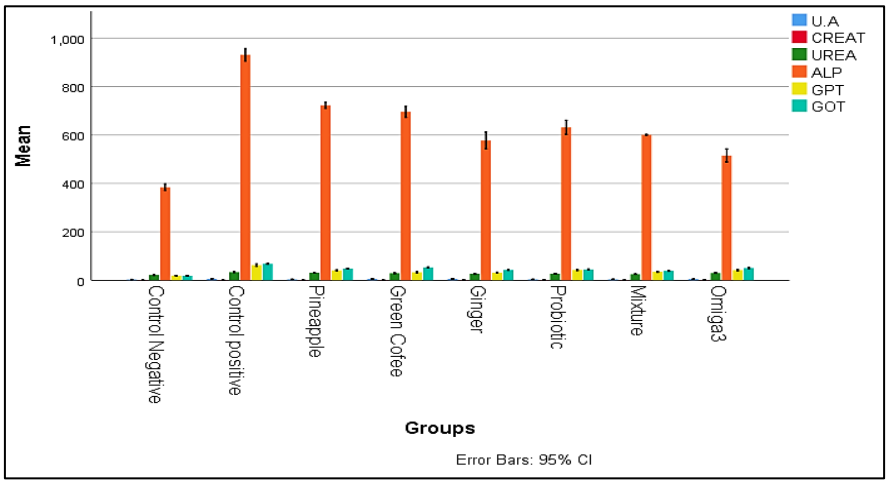


Fig. 4: Mean of U.A, Mean of Creat, Mean of Urea, Mean of GPT, Mean of GOT by groups by INDEX

Effect on serum levels of malondialdehyde and Catalase-peroxidases content and superoxide dismutase activity

The levels of serum MDA, CAT, and SOD activity for all groups were shown in Table 6 and Figure 5. In rats in groups 1,6,7 and 8, substantial increases in serum SOD levels were recorded in combination with a markedly slight increase in CAT and MDA activity compared to groups 2, 3, and 4. Group 2 and 3 rats displayed a marked decrease in serum CAT levels. Besides, CAT and SOD activity levels in Groups 7 and 8 have been restored to normal Group 1 level.

Table 6: The serum levels malondialdehyde (MDA), Superoxide dismutase (SOD), and Catalase-peroxidases (CAT) of all the studied groups, at the end of the experiments

	Groups							
	Control Negative	**Control positive**	**Pineapple**	**Green Coffee**	**Ginger**	**Probiotic**	**Mixture**	**Omiga3**
SOD	$975\pm10_{a}$	$570\pm14_{b}$	$718\pm15_{c}$	$691\pm9_{c}$	$712\pm3_{c}$	$699\pm11_{c}$	$691\pm10_{c}$	$671\pm7_{c}$
CAT	$84\pm3_{a}$	$40\pm1_{b}$	$65\pm2_{c}$	$72\pm3_{c,d,e,f}$	$76\pm3_{a,d}$	$77\pm3_{a,e}$	$65\pm2_{c}$	$80\pm1_{a,f}$
MDA	$174\pm3_{a}$	$87\pm3_{b}$	$15\pm6_{c}$	$149\pm1_{c}$	$142\pm3_{c,d}$	$130\pm1_{d,e}$	$119\pm2_{e,f}$	$110\pm5_{f}$

Note: Values in the same row and subtable not sharing the same subscript are significantly different at p< .05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.1
1. Tests are adjusted for all pairwise comparisons within a row of each innermost sub table using the Bonferroni correction.

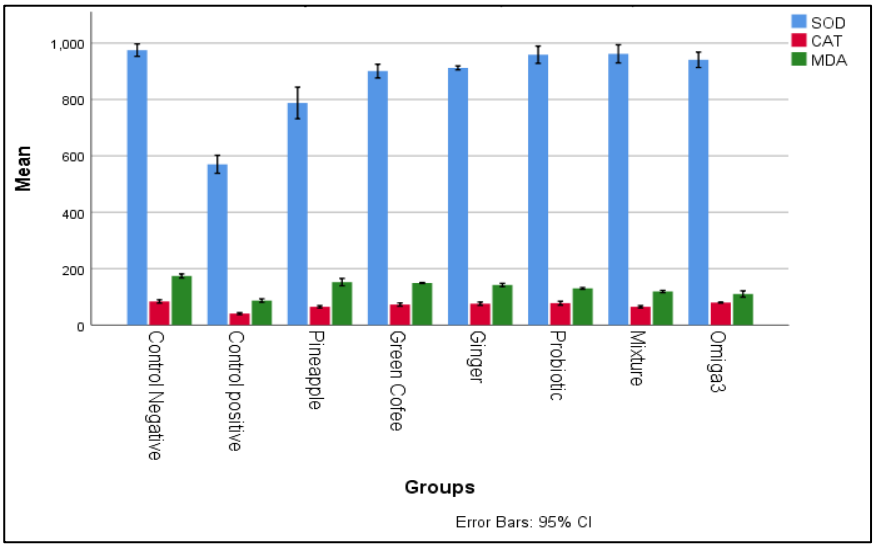


Fig. 5: Simple Bar Mean of SOD, Mean of CAT, Mean of MDA

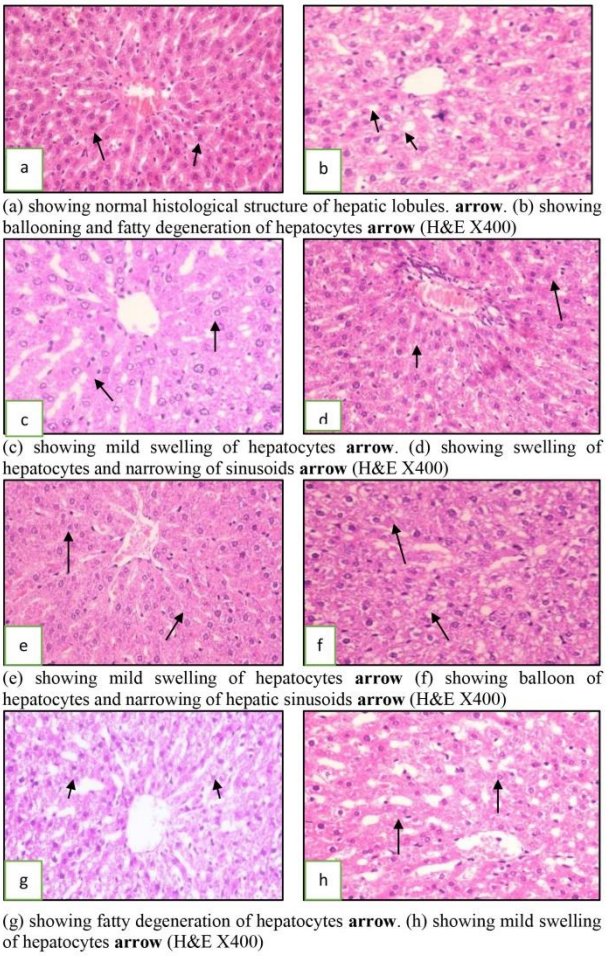
Histological investigations

1. Liver: Table 7 & Figure 6

Light microscopic observation revealed that the control hepatic tissue showed normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells **(grade 0)** fig.(a). The animals of positive control group showed ballooning and fatty degeneration of hepatic cells and narrowing of hepatic sinusoids and hyperplasia of Kupffer cells **(grade III)** fig. (b). On the other side, animals fed on omega showed mild swelling of hepatocytes and granularity of its cytoplasm **(grade I)** fig. (c). Animals (A-B) swelling of hepatocytes and hyperplasia of Kupffer cells **(grade I)** fig. (d&e). Animal group (C) showed ballooning of hepatocytes and narrowing of hepatic sinusoids **(grade II)** fig. (f). Animal **(D)** displayed ballooning of hepatocytes and fatty degeneration with distinct narrowing hepatic sinusoids **(grade III)** fig. (g). The animals **(E)** showed the swelling of hepatocytes and hyperplasia of Kupffer cells **(grade I)** fig. (h).

Table 7: Grading of hepatic lesions:

Group	Grade	Grade Description
-Ve	0	No apparent injury by light microscopy
+Ve	III	Lipid droplets in hepatocytes
Omega	I	Swelling of hepatocytes
Group A	I	Swelling of hepatocytes
Group B	I	Swelling of hepatocytes
Group C	II	ballooning of hepatocytes
Group D	III	Lipid droplets in hepatocytes
Group E	I	Swelling of hepatocytes



(a) showing normal histological structure of hepatic lobules. **arrow**. (b) showing ballooning and fatty degeneration of hepatocytes **arrow** (H&E X400)

(c) showing mild swelling of hepatocytes **arrow**. (d) showing swelling of hepatocytes and narrowing of sinusoids **arrow** (H&E X400)

(e) showing mild swelling of hepatocytes **arrow** (f) showing balloon of hepatocytes and narrowing of hepatic sinusoids **arrow** (H&E X400)

(g) showing fatty degeneration of hepatocytes **arrow**. (h) showing mild swelling of hepatocytes **arrow** (H&E X400)

Fig. 6: Hepatic lesions

Renal lesion scanning is shown in the table 8 and figure 7

Kidney of animal of control group showed normal histological structure characterized by circumscribe glomeruli with normal structure of capillary tufts and Bowman's capsule. The renal tubules of both proximal and distal convoluted tubules showed intact epithelial lining and arrangement **scoring (0)** fig.(a)**.** On the other side, kidney of control **positive and omega groups** showed shrinkage of capillary tufts with widening of Bowman's space of some glomeruli. The renal tubules showed epithelial cell degeneration, and intralumenal albuminus material, with significant necrosis or apoptosis<25% **scoring (2)** fig. (b&c)**.** Animals group (A) showed normal histological structure of renal glomeruli. The renal tubules showed epithelial cell degeneration characterized swelling of epithelial lining and narrowing of tubular lumen, without significant necrosis or apoptosis **scoring (1)** fig. (d)**.** On the other side animal group (B) showed tubular epithelial cell degeneration and tubular epithelial cell necrosis and apoptosis <25%**score (2)** fig. (e)**. Animal groups (C, D, E) revealed** tubular epithelial cell degeneration, without significant necrosis or apoptosis score 1 fig. (f,g,h)

Table 8: Renal lesions scoring

Group	Score	Score Description
Control -Ve	0	Normal histology
Control +Ve	2	Tubular epithelial cell necrosis and apoptosis <25%
Omega	2	Tubular epithelial cell necrosis and apoptosis <25%
Group A	1	Tubular epithelial cell degeneration, without significant necrosis or apoptosis
Group B	2	Tubular epithelial cell necrosis and apoptosis <25%
Group C	1	Tubular epithelial cell degeneration, without significant necrosis or apoptosis
Group D	1	Tubular epithelial cell degeneration, without significant necrosis or apoptosis
Group E	1	Tubular epithelial cell degeneration, without significant necrosis or apoptosis

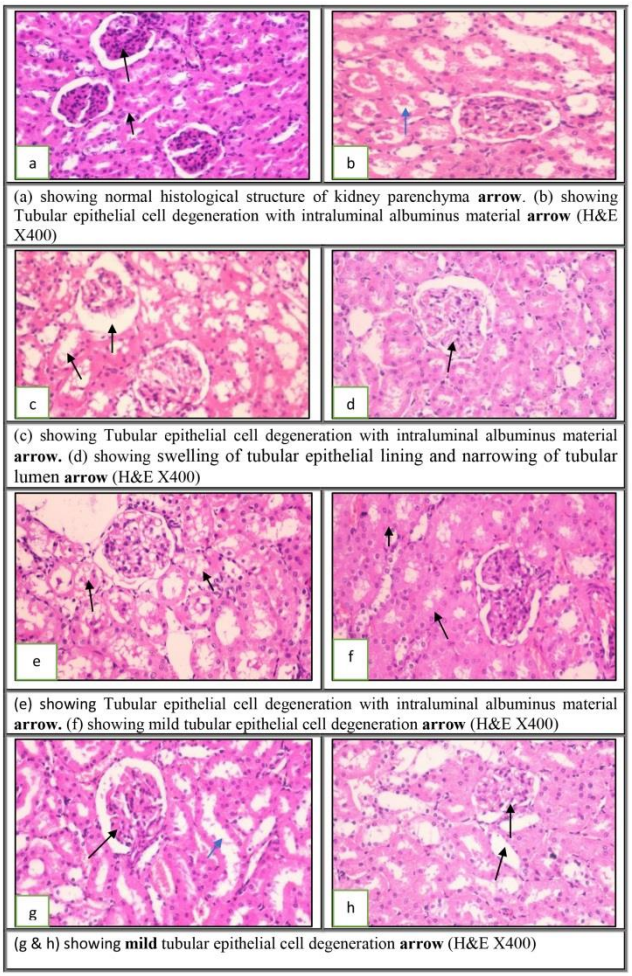


Fig. 7: Renal lesion scanning

Pancreas:

The pancreatic tissues of normal control group showing normal histological structure of both exocrine and endocrine tissues. The islet of Langhan's showed normal arrangement of cellular constituent and, the acinar structure revealed normal proteinous eosinophilic materials fig. (a). Control positive group showed atrophy of pancreatic lobules and vacuolation of acinar epithelial ling fig. (b). Animals groups treated by omega showed vacuolation for few number of acinar epithelial lining in comparison with control positive group fig. (c). Group A-B-C-D revealed normal histological structure of pancreatic parenchyma fig.(d,e,f,g,h). On the other side animals group (E) revealed histopathological picture of animals group treated by omega which characterized by vacuolation of certain number of acinar epithelial lining **fig. 8**.

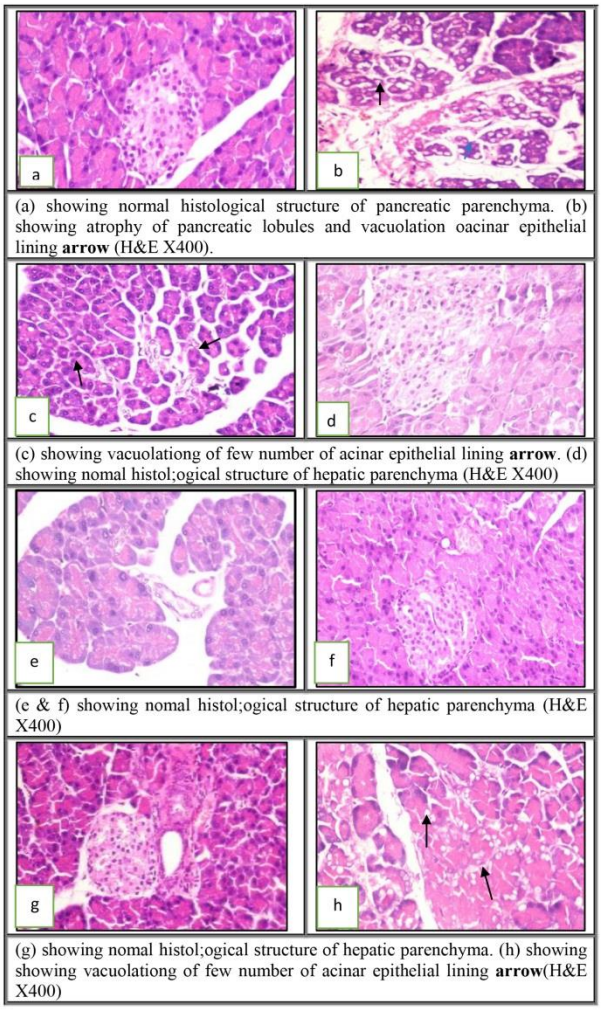


Fig. 8: Pancreatic tissues

DISCUSSION & CONCLUSION

Obesity occurs as a result of the interaction between human actions and unhealthy environmental conditions, genetic history, and epigenetic factors. obesity are highly associated with serious metabolic diseases, moreover, many of most known anti-obesity procedures have negative drawbacks to human health, though alternative natural anti-obesity strategies have proven its efficacy with minimal side effect, Our results demonstrated that both prebiotics and probiotics that reduced the body weight. BWG concentrations for Group 2 and 4 were significantly higher than for Group 1. Groups 5,6 and 7 of the Rats reported major reductions in the BWG compared to Groups 1 and 3. Groups 4,5,6 and 7 showed a marked decline in relative renal weight, relative internal fat weight, and relative subcutaneous weight in contrast to other groups. increase, serum total TG levels, and serum total TC levels in high-fat diet SD rats. Groups 2 and 4 of rats showed substantial increases in serum TC, TG, LDL-C, and VLDL-TG levels, while in Groups 3 and 7, HDL-C levels significantly declined from Group 2. Groups 3,4,5,6 and 7 showed a significant reduction in serum levels TC, TG, LDL-C, and VLDL-TG compared to Groups 2 and 8, but notable increases in HDL-C levels were observed in control values. As well as the creatinine and urea serum levels from rats. Group 2 and 3 rats displayed marked elevations in serum activity of ALT, AST, and ALP compared with Group 1. The ALT, AST, and ALP serum were considerably higher than in Group 1 but slightly lower than in Groups 3 and 4. The serum activity of creatinine and urea was comparable among all experimental groups. Substantial increases in serum SOD levels have been observed in combination with a markedly marginal increase in CAT and MDA activity compared to groups 2, 3, and 4. There was a marked decrease in serum CAT levels in Group 2 and 3 rats. Besides, the levels of CAT and SOD operation in Groups 7 and 8 have been returned to the regular Group 1 level. histopathological examination of lipid accumulation and cellular morphology by H&E staining in the liver, kidney, and pancreas The adipocyte size in the HFD group didn't show an increase compared to the normal diet group, the HFD with mixture groups, showed a marked reduction of adipocyte hypertrophy compared to mice fed the HFD alone, the study showed a promising role of *lactobacillus* in combined with these plants as natural feed additives on obesity GCE mediates the antiobesity effect and it has been reported to have antioxidant activity, pineapple and ginger enhances the antioxidant level of mice with high-fat diet-induced obesity we observed that the administration of *Lactobacillus acidophilus*, a conjugated linoleic acid-producing bacterium, resulted in the lowering of body-weight gain in mice with no change in average energy intake. In another study, the probiotic strain, *Lactobacillus acidophilus*, reduced the body-weight gain in rats fed a high-fat diet with no change in food consumption[22].

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- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat. Rev. Endocrinol. 2012; 8, 228.
2. Glover M A. Employment & (and) Disability Law-Americans with Disabilities Act of 1990-The Weight of Personal Responsibility: Obesity, Causation, and Protected Physical Impairments. UALR L. Rev. 2007; 30, 381.
3. Chan RSM, Woo J. Prevention of overweight and obesity: how effective is the current public health approach. Int. J. Environ. Res. Public Health. 2010; 7, 765–783.
4. Ebrahimzadeh Attari V. et al. A systematic review of the anti-obesity and weight lowering effect of ginger (Zingiber officinale Roscoe) and its mechanisms of action. Phyther. Res. 2018; 32, 577–585.
5. Lavie CJ et al. Reprint of: Healthy Weight and Obesity Prevention: JACC Health Promotion Series. J. Am. Coll. Cardiol. 2018; 72, 3027–3052.
6. Saad F, Yassin A, Doros G, Haider A. Effects of long-term treatment with testosterone on weight and waist size in 411 hypogonadal men with obesity classes I-III: observational data from two registry studies. Int. J. Obes. 2016; 40, 162.
7. Mohamed GA, Ibrahim SRM, Elkhayat ES, El Dine, RS. Natural anti-obesity agents. Bull. Fac. Pharmacy, Cairo Univ. 2014; 52, 269–284.
8. Guarner F, Malagelada J.-R. Gut flora in health and disease. Lancet. 2003; 361, 512–519.
9. Arora T et al. Effect of Lactobacillus acidophilus NCDC 13 supplementation on the progression of obesity in diet-induced obese mice. Br. J. Nutr. 2012; 108, 1382–1389.
10. Shah NP. Functional cultures and health benefits. Int. dairy J. 2007; 17, 1262–1277.
11. Kobyliak N. et al. Probiotics in prevention and treatment of obesity: a critical view. Nutr. Metab. (Lond). 2016; 13, 14.
12. Aguiar J, Estevinho BN, Santos L. Microencapsulation of natural antioxidants for food application–The specific case of coffee antioxidants–A review. Trends Food Sci. Technol. 2016; 58, 21–39.
13. Frost-Meyer NJ, Logomarsino JV. Impact of coffee components on inflammatory markers: A review. J. Funct. Foods. 2012; 4, 819–830.
14. Higdon JV, Frei B. Coffee and health: a review of recent human research. Crit. Rev. Food Sci. Nutr. 2006; 46, 101–123.
15. Mills CE et al. In vitro colonic metabolism of coffee and chlorogenic acid results in selective changes in human faecal microbiota growth. Br. J. Nutr. 2015; 113, 1220–1227.
16. Zhou Y-Q et al. A Review of the Botany, Phytochemical, and Pharmacological Properties of Galangal. in Natural and Artificial Flavoring Agents and Food Dyes. 2018; 351–396.
17. Mahmoud RH, Elnour WA. Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. Eur Rev Med Pharmacol Sci. 2013; 17, 75–83.
18. El-Shazly SA. et al. Physiological and molecular study on the anti-obesity effects of pineapple (Ananas comosus) juice in male Wistar rat. Food Sci. Biotechnol. 2018; 27, 1429–1438.
19. São Paulo Barretto Miranda, Í K et al. The biochemical characterization, stabilization studies and the antiproliferative effect of bromelain against B16F10 murine melanoma cells. Int. J. Food Sci. Nutr. 2017; 68, 442–454.
20. Bhagavathi TP, Bensi PS, Geetha PS. Sensory quality assessment of pineapple–Garcinia cambogia squash, principal component analysis. J. Pharmacogn. Phytochem. 2017; 6, 1332–1359.
21. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis. 2009; 49, 1749–1755.
22. Tanida M et al. High-fat diet-induced obesity is attenuated by probiotic strain Lactobacillus paracasei ST11 (NCC2461) in rats. Obes. Res. Clin. Pract. (2008) doi:10.1016/j.orcp.2008.04.003.