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Assessment the effect of prebiotics, probiotics and synbiotics on Hyperlipidemia

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Abstract

Hypercholesterolemia is a major risk factor for the development of atherosclerosis; a number of pharmacological and non-pharmacological (including dietary) approaches being employed to reduce it. Up until now, the trials to prove the hypocholesterolemia effect of probiotics have not been convincing. There are only few data suggesting that the supplementation of the diet with functional food products containing probiotic bacteria lower LDL-cholesterol (LDL-C) concentration in patients with moderately elevated cholesterol concentration in plasma. The aim of the present study was to evaluate in vitro the anti-atherosclerotic effect of a probiotic (*Lactobacillusrhamnosus, casei, plantarum,* and *reuteri*), prebiotic (Flaxseed & Cinnamon, green coffee, ginger, and green tea extracts) & combination mix using MCF-7cell line. Recording data showed that probiotic compound administration to hyperlipidemia: a significant decrease of the total serum cholesterol & triglycerides, a significant increase of the serum antioxidant potential. In conclusion, our data support the administration of probiotic Lactobacillus bacteria and prebiotic and combination to decrease serum cholesterol, triglycerides, and lipid droplets increase the antioxidant potential in hyperlipidemia subjects.

Key Words: Prebiotics; Probiotics; Symbiotic; Hyperlipidaemia; Activities;

1.Introduction

Hyperlipidemia is defined as a medical condition characterized by an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein along with reduced high-density lipoprotein levels [1,2].Consumption of a high-fat diet (HFD) usually leads to hyperlipidemia, characterized by abnormal lipid levels with the increase of blood total cholesterol (TC) and triglyceride (TG) along with the decrease of high density lipoprotein cholesterol (HDLC). Hyperlipidemia, one of the major risk factors for the development of cardiovascular diseases, such as coronary heart disease, myocardial infarction, cerebral stroke, atherosclerosis and hypertension, is becoming a major health problem in the world. Therefore, effective and safe pharmacological interventions for hyperlipidemia are urgently needed. Recently, functional foods have shown effects on the modulation of lipid levels [3]. Hyperlipidemia results in lipotoxicity and affects several organs. Lipotoxicity is not only an outcome of lipid accumulation in non-adipose tissues but also a result of the hyperlipidemia-associated inflammation and oxidative stress [4]. Elevated levels of LDL cholesterol and reduced amounts of HDL cholesterol in blood have been associated with hyperlipidemia, cardiovascular disease, and the metabolic syndrome. Despite the encouragement of interventions such as life style changes and medication, the prevalence of these diseases continues to increase [5]. Lactose-based prebiotics with probiotics control lipid level in the bloodstream and tissue by (a) suppressing the expressions of lipogenic-genes and enzymes; (b) oxidizing fatty acids in muscle, liver, and adipose

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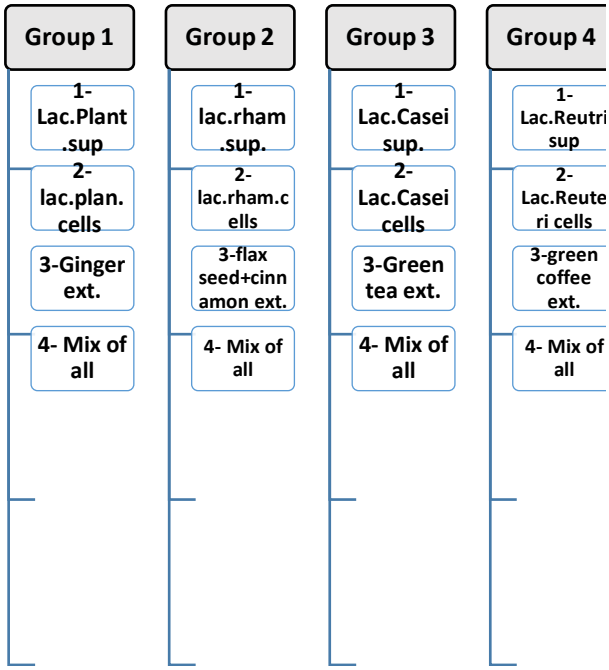
tissue; (c) binding cholesterol with cell membrane of probiotics and subsequent assimilation by probiotics; (d) enzymatic-transformations of bile acids; and (e) converting cholesterol to coprostanol and its defecation. Symbiosis of lactose-based prebiotics with probiotics affect plasma glucose level by (a) increasing the synthesis of gut hormones plasma peptide-YY, glucagon-like peptide-1 and glucagon-like peptide-2 from entero-endocrine L-cells; (b) altering glucose assimilation and metabolism; (c) suppressing systematic inflammation; (d) reducing oxidative stress; and (e) producing amino acids. Clinical investigations show that lactose-based prebiotic galacto-oligosaccharide improves mineral absorption and reduces hyperlipidemia. Another lactose-based prebiotic, lactulose, improves mineral absorption, and reduces hyperlipidemia and hyperglycemia [6]. Administration of probiotics (lactobacilli and bifidobacteria) as live micro-organisms or fermented products achieves proper gut environment. In addition, administration of prebiotics along with probiotics improves the body weight, abdominal fat and intestinal barrier function. Studies are indicating that anti-hyperglycemic and hyperlipidemic effects of probiotics are strain dependent as well as type of animal models. To improve against metabolic disorders, probiotics need to be administered through prebiotics and requires more clinical studies in this area [7]. Manipulation of the gut microbiota through the administration of prebiotics or probiotics may assist in weight loss and reduce plasma glucose and serum lipid levels, decreasing the incidence of cardiovascular diseases and type 2 diabetes mellitus. To the best of our knowledge, short-chain fatty acids (SCFAs), bile salt hydrolase (BSH), metabolic endotoxemia and the endocannabinoid (eCB) system are essential in regulating the initiation and progression of MS through the normalization of adipogenesis and the regulation of insulin secretion, fat accumulation, energy homeostasis, and plasma cholesterol levels. Therefore, the gut microbiota may serve as a potential therapeutic target for metabolic syndrome (MS) [8]. Gut microbiota structure analysis indicated that the anti-obesity effect by Green Tea consumption always accompanied with microbiota structure modification. Preventive consumption of Green Tea by lean mice that never experienced obesity provided persistent protection from High-Fat-Diet HFD-induced obesity, potentially by modifying the gut microbiota [9]. Currently, drug therapy is the principal treatment, but with high relative costs and side effects, it is not considered to be an optimal long-term treatment. Recent studies have demonstrated that different natural products (green tea, green coffee, ginger, cinnamon) and Lactobacilli or Bifidobacteria could exhibit hypolipidemic effects in animal models and in humans [10,11]. This work aims to study the co-administration of probiotics (*(Lactobacillus* rhamnosus, casei, plantarum, and reuteri*), prebiotic (Flaxseed* &Cinnamon, green coffee, ginger, and green tea extracts*) & combination mix* synbiotics effect as anti-hyperlipidemia *using MCF-7 cell line.*

2. Experimental

2.1 Materials, Chemicals, Equipment's, and Samples

Breast cancer cell line MCF7(*ATCC* HTB-22) was supported by *Cell culture department – VACSERA, EGYPT. Green tea, green coffee, ginger, flax and cinnamon mix* obtained from local market in *Giza Egypt*. Probiotic bacteria Standard strains: *Lactobacillus planetarium ATCC 14917; rhamnous 53103ATCC, casei ATCC39539; reuteri ATCC 23272 ATCC were supplied from Grace Company – Egypt.*RPMI medium with L- glutamine *was supported from Biowhittaker – Belgium.* Fetal bovine serum (FBS) *was from GIBCO – USA.* Trypsin 0.25% (w/v) *was from AMRESCO – USA.* Trypan blue dye, Di-methyl sulfoxide (DMSO), Ethanol 70% (v/v), MTT [3-(4, 5-dimethylthiazol-2-yl) 2, 5 diphenyl tetrazolium bromide], Formalin (10%) *were fromSigma-Aldrich – USA.* Normal saline 0.9% solution (w/v) was supplied from *Adwic – Egypt*. Phosphate buffer saline (PBS) was from *Biowhittaker – Belgium.* Total Protein Biuret, carbohydrate, cholesterol, Triglyceride kits were purchased from *Sigma-Aldrich – USA.* The instruments used in this work were: CO_2 incubator, *Jouan – France.* Deep freezer (- 70°C), *RIFCO – USA*. Refrigerator *Ideal – Italy*. Cooling centrifuge, *Jouan GR 412 – France*. Vertical laminar air flow, *Nunclon – USA*. Biological safety cabinet, *Nuair – USA.* Cell culture inverted microscope, *Hund – Germany*. ELISA plate reader, *Dynatech medical products – England*. Plate shaker, *Staurt – England.* PH meter,*Denver – USA*. Vortex, *Thermolyne – USA.* Spectrophotometer, *Biotech Engineering – UK*. Microwave, *JAK – China*. Spin centrifuge, *Firlabo – Belgium*. Multi- and single channel pipette (1-10 µl, 20-200 µl), *Griener – Germany*. Haemocytometer, *New power – Germany*. Quartz cuvettes, *Sigma-Aldrich – USA.* Four groups of samples used in this work were categorized as shown in scheme **1**:



Scheme 1. Extracts Group Sampling

2.2 Preparation of prebiotics extracts

The plant materials were purchased freshfrom a local market, cleaned. The samples were chopped and finely ground in Waring Commercial MX1050XTX 3.5 HP Blender. The ground samples were then extracted in water solution at room temperature for four hours and at 100 ºC for 15 minutes [12]. The water extraction was done only once since prolonged soaking in cold waterwould result in microbial growth. After the extraction was terminated the sampleswere filtered through linen filter cloth. All filtrates were concentrated in a rotary evaporator. Samples were kept in capped dark glass bottles at -20 °C till use.

2.3 Determination of total carbohydrate of prebiotics extracts

Different concentration of glucosesolution starting from 0,1 mg /ml to 0,02 mg /ml were prepared by serial dilution was taken in boiling tubes and the final volumes of each tube was made 1ml by adding distilled water. 1ml of 5% Phenol and 5ml of 96% Sulphuric acid was added one by one in each tubes and shook well so that the Phenol and Sulphuric acid get mixed thoroughly with working standard. After 10 minutes all the tubes were placed in water bath at room tem for 15 minutes. Blank was set with 1ml of distilled water and O.D. of each tube was taken at 490nm with the help of spectrophotometer. Then the whole process following Phenol and Sulphuric acid method was repeated with 0.2ml of different samples and the O.D. of sample solutions were taken [13].

2.4 Preparation of Probiotic (Lactobacillus) culture

Lactobacillus ATCC 3456 was inoculated in 5 mL of MRS broth media (10 g Tryptone + 5 g meat extract + 5 g yeast extract + 10 g NaCl + 1000 ml bi-distilled water) for 24 h at 37°C, 180 rpm in shaking incubator. After one day, the tubes were centrifuged at 8000 rpm for 5 minutes to separate the cells of *Lactobacillus* from MRS growth medium. The culture supernatant was sterilized by filtration through 0.45 µm pore size filters.

2.5 Preparation of Probiotic lactobacillus cell Sonicate

Aliquots of suspension with overnight bacterial culture were sonicated using the intermediate sonication horn (9.5 mm, Sonic 300 Dismembrator Fisher Scientific Co., NJ USA) at 60% intensity, placed in a 15 ml polypropylene tube containing 5-6 ml of cell suspension. Cooling was achieved by putting the polypropylene tube in an ice water bath while sonicating the samples.

2.6 Determination of total protein in *Lactobacillus* supernatant, cell sonicate and mixture of both

A test tube was labeled as blank and tube for each sample to be test. 0.2 ml of 0.85% Sodium Chloride Solution was added to the test tube labeled Blank and 0.2 ml of a test sample solution prepared to the appropriately labeled test tube.To each test tube 2.2 ml of the Biuret Reagent was added. Mix well and allow standing at room temperature for 10 minutes. 0.1 ml of the Folin and Ciocalteau's Phenol Reagent was added to each tube. Mix each tube well immediately after addition. Allow to stand at room temperature for 30 minutes. Transfer the contents of the test tubes to microplate and read absorbance using the Blank as reference. Complete readings within 30 minutes. Determine the protein concentration (mg/ml) of each test sample from the Standard curve**.**

2.7 Maintenance of cell line

Human breast cancer cell line MCF-7 was cultured using RPMI medium containing fetal calf serum (10%), penicillin (100 units/mL), and streptomycin (100

μg/mL), at 37∘C in a humidified atmosphere of 5% CO2 in air. Cell line were maintained in the following way according to previous studies [14], where growth media were discarded from the cell culture flasks and the cell layer was washed gently with sterile PBS. PBS was decanted then the cell monolayer was washed with 5 ml trypsin solution (pre-warmed at 37°C). Trypsin was decanted and the cell culture flasks were incubated with trace trypsin in the incubator at 37°C until the cells detach from the surface. Growth media were added to the detached cells. Cells were re-suspended in growth medium to the desired concentration according to cell count. Cell suspension was cultured in another cell culture flask or in 96 well cell culture plates and incubated at 37°C till confluence.

2.8 Cell Counting

Accurate cell number in the suspension could be calculated by counting the cells using the haemocytometer according to previous literatures [14].Double fold dilution of the original cell suspension was prepared by adding 0.5 ml of undiluted cell suspension to 0.5 ml of 0.4% trypan blue dye. Mixture was mixed well with a fine pipette and immediately aspirated to fill the haemocytometer counting chambers. All viable (unstained) cells in the 8 squares of 2 haemocytometer chambers were counted omitting cells lying on the upper line and left line of each chamber. The volume of each chamber = 0.1 mm^3 (1.0 x 1.0 x 0.1). To perform an accurate cell count, 75% of the cells in the suspension should be viable and the difference between the cell counts in the 2 haemocytometer chambers should be minimal. If cell clumping (aggregation) was observed, the clumps were disaggregated by vigorous aspiration through a pipette. The mean count of the cells in each chamber was calculated. The total number of cells in the suspension was calculated using the following formula: N1 = m x tb x V x 10^4, whereas N1 = number of cells in the cell suspension, m = mean of cell count per 0.1 mm^3. tb = correction of the trypan blue dilution (2 in double fold dilution with trypan blue), V = volume of the original cell suspension in ml, 10^4 = conversion factor for counting chamber volume. N (number of cells per ml) = N1 / V. If new suspension was required to be prepared with new concentration (N2), the new volume (V2) could be calculated as follows: N x V = N2 x V2. The new cell suspension can be prepared by adding growth medium equal to the difference between the new volume (V2) and the original volume (V). Haemocytometer and cover slip were cleaned immediately after use with 70% ethanol.

2.9 Cytotoxicity assay

MTT assay is a sensitive, quantitative and reliable colorimetric method that measures viability of cells. The assay is based on the ability of mitochondrial lactate dehydrogenase enzymes (LDH) in living cells to convert the water soluble substrate 3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyl tetrazolium bromide (MTT) used as 5mg/ml into a dark blue formazan which is water insoluble. A solubilization solution (dimethyl sulfoxide) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring it using spectrophotometer at a wavelength usually between 500 and 600 nm [15].Cytotoxic effect of prebiotics extracts, probiotics and mix of both of them was evaluated using MTT assay where test materials were 2 fold serially diluted on precultured cell lines for 24 hrs. Treatment at 37ºC post decanting growth medium. Treated cell lines were microscopically examined for detection of morphological changes and detached cells. Dead cells were washed-out using phosphate buffer saline, pH 7.2 ±0.2 (PBS-0.05% Tween). Residual live cells were treated with 0.5% MTT stain as 25 µl/ well. Plated were incubated for 3-4 hrs at 37ºC. Developed intra-cytoplasmic MTT formazan crystals were dissolved using 0.05 ml Dimethyl sulphoxide (DMSO) for 30 minutes on plate shaker. Optical densities were read using (Biotek – 8000, USA) ELISA plate reader. Data were reported for three independent experiments [16].Viability percentage was calculated as follows: Cell viability percentage = (OD of treated cells / OD of untreated cells) X 100 [17]. IC_{50} of test extracts were determined using Master –plex-2010 program.

2.10 Morphologic observation using inverted microscope

Precultured ninety-six-well cancer cells plates were treated withtest materials. Morphological changes were observed 24 h post treatment using an inverted phase contrast microscope according to [18].

2.11 Determination of total cholesterol, triglyceride, antioxidant and lipids

Four flasks contain MCF-7 cells were prepared and discard media, then washed by trypsin. Cells were trypsinized and washed by PBS and MCF-7 cells (10^5 cells) were seeded and pre-cultured in RPMI containing 10% (v/v) FCS in 6-well plates in every well 1000µl from media contain MCF-7 cells for 2 days. The plate incubated 48 hrs. in 37c incubator. Discard the old media and then cells were washed twice with PBS. 500µl from RPMI media FCS-free containing 0.1% (w/v) BSA and 500µl from test

samples were added, each group in one plate and make two wells control without treatment in every plate for 1 day. The culture media were subjected to the analysis total cholesterol and TG levels Scraping to adherent cells was done by mechanical way using cell scraper and 100µl PBS. Add scarpered cells into Eppendorf and sonication of cells by Sonicator. Centrifugation and take supernatant to make antioxidant assay.MCF-7 cells were seeded in 6-well plate at density of 1×10^5/ml. After cell adhesion, drug treatment was according to the mentioned above and incubated 24 hours with BSA. Cells were fixed by added 1000 µl formalin (10%) and incubated 5 min and discard formalin. Stained by (Oil-Red-O) solution for 1 hour. Isopropanol was added to determine lipid droplets content in cells. The extracted dye was transferred into a new 6-well plate for determination under 490 nm and the values were presented as the percentage of control.

3. Results and Discussion

Total carbohydrate and total protein were evaluated in order to determine the test samples prebiotics and probiotics concentrations by comparison of OD of the sample (unknown) with standard curve of total protein. The total carbohydrate of 10 % extracted from prebiotic samples (green coffee, green tea, ginger, flax seed and cinnamon) with water extraction show (1.37, 5.99, 5.53 3.49 mg /ml) by comparison of OD of the samples (Figures 1 and 2) with standard curve of total carbohydrate.

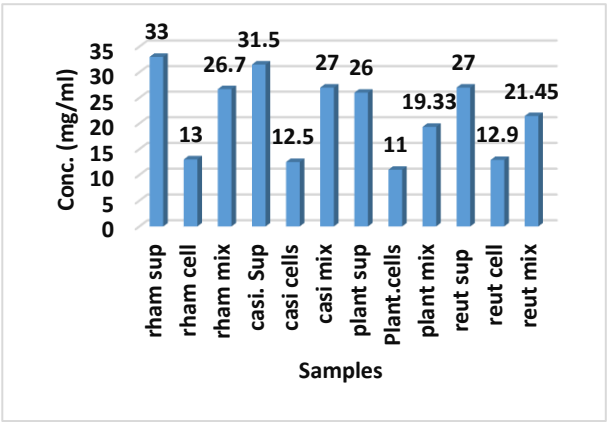


Fig.1. Extracts Total Protein Contents

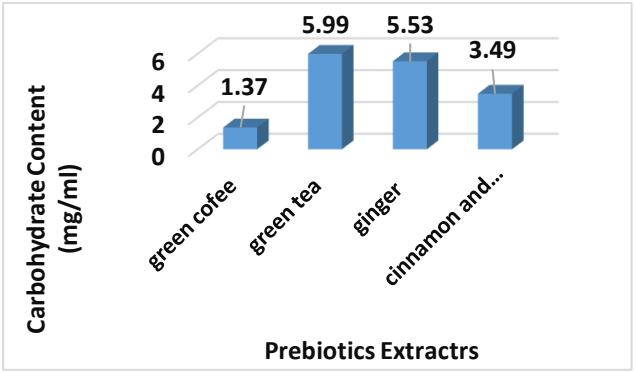
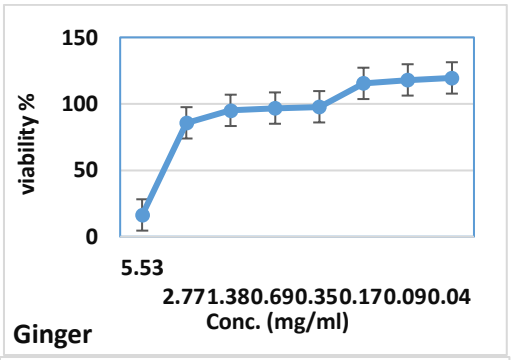
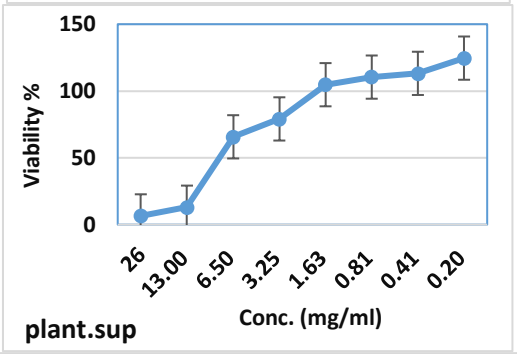
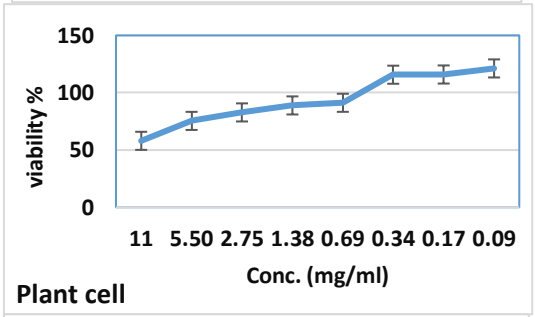


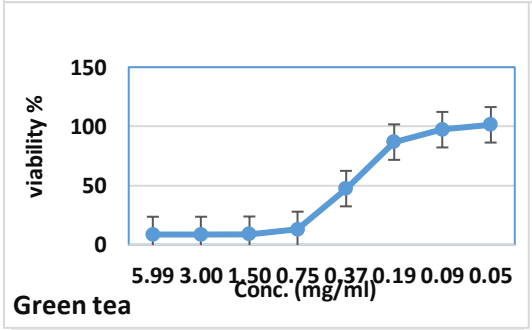
Fig 2. Total carbohydrate Content of PrebioticExtracts

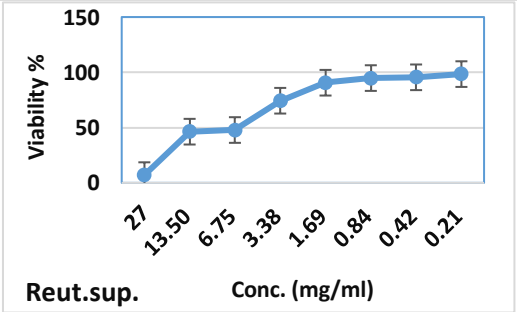
The viability percentages (Figure 3) of treated cells with the different agents were found to be concentration-dependent, where increasing dilutions resulted in the increase in percentage of survived cells in cultures. *Data was processed and IC50 for each sample calculated using Master-plex 2010 software program. The calculated IC50s of utilized samples on MCF-7 as in next figure (Figure 4).* Regarding the cytotoxic effect of test extracts on breast cancer cell line (MCF7) it was found that the IC50 values of probiotic, prebiotic samples and mix of them were: ginger 4.06 mg/ml, *Lactobacillus planetarium* sup is 9.2, *Lactobacillus planetarium* cell NA and mix is 13.5 mg/ml. Green tea was 0.31 mg/ml, *Lactobacillusreutri* sup 6.43 mg/ml, *Lactobacillusreutri* cell 13 mg/ml and mix is 5.35 mg/ml. While flex and cinnamon is 1.19 mg/ml *Lactobacillus rhamnosus* supernatant is 15 mg /ml *Lactobacillus rhamnosus* cell sonicate is NA and combination is NA**.** Finally, green coffee was 0.54 mg/ml, *Lactobacillus casi* supernatant is 11, *Lactobacillus casi* cell NA and mix is 20 mg/ml. Green tea and green coffeehave the most toxic effect then flex and cinnamon then ginger. On the other hand *Lactobacillusreutri* sup was the most toxic from probiotics, *Lactobacillus planetarium* sup and *Lactobacillus casi* supernatant, then *Lactobacillus rhamnosus* supernatant. Concurrently, potential synergetic effect for *Lactobacillusreutri* mix which reduced the IC50 value.

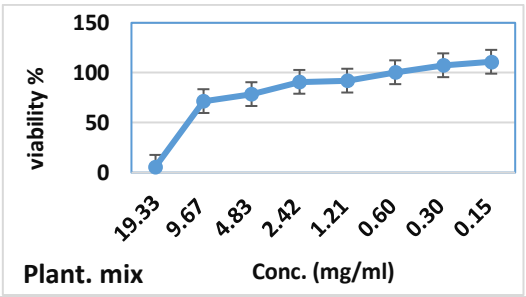

viability %
150
100
50
0
5.53
2.771.380.690.350.170.090.04
Conc. (mg/ml)
Ginger

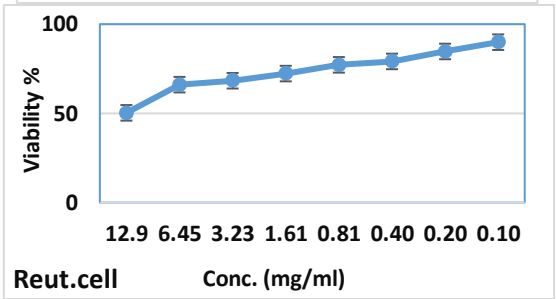

Viability %
150
100
50
0
26
13.00
6.50
3.25
1.63
0.81
0.41
0.20
Conc. (mg/ml)
plant.sup

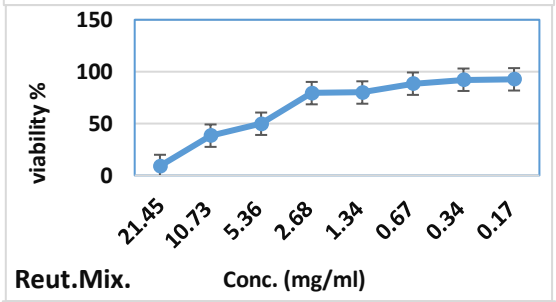

viability %
150
100
50
0
11 5.50 2.75 1.38 0.69 0.34 0.17 0.09
Conc. (mg/ml)
Plant cell

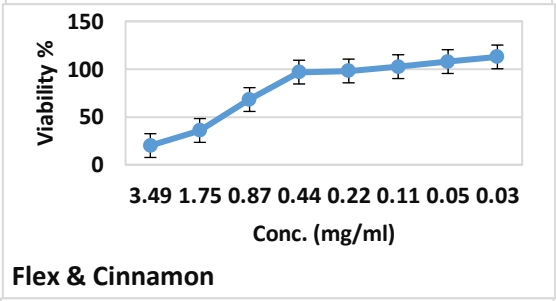

viability %
150
100
50
0
5.99 3.00 1.50 0.75 0.37 0.19 0.09 0.05
Conc. (mg/ml)
Green tea

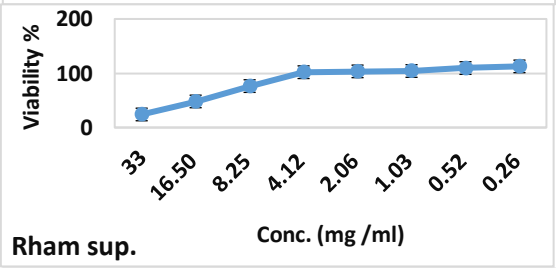

Viability %
150
100
50
0
27
13.50
6.75
3.38
1.69
0.84
0.42
0.21
Conc. (mg/ml)
Reut.sup.

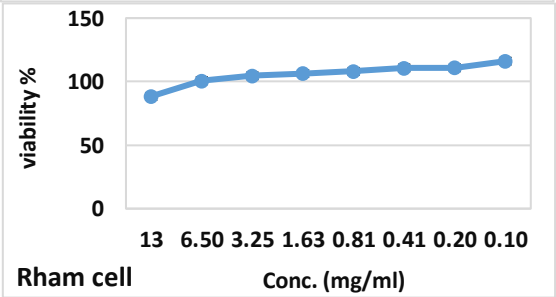

viability %
150
100
50
0
19.33
9.67
4.83
2.42
1.21
0.60
0.30
0.15
Plant. mix
Conc. (mg/ml)


Viability %
100
50
0
12.9 6.45 3.23 1.61 0.81 0.40 0.20 0.10
Reut.cell
Conc. (mg/ml)


viability %
150
100
50
0
21.45
10.73
5.36
2.68
1.34
0.67
0.34
0.17
Reut.Mix.
Conc. (mg/ml)


Viability %
150
100
50
0
3.49 1.75 0.87 0.44 0.22 0.11 0.05 0.03
Conc. (mg/ml)
Flex & Cinnamon


Viability %
200
100
0
33
16.50
8.25
4.12
2.06
1.03
0.52
0.26
Conc. (mg /ml)
Rham sup.


viability %
150
100
50
0
13 6.50 3.25 1.63 0.81 0.41 0.20 0.10
Conc. (mg/ml)
Rham cell

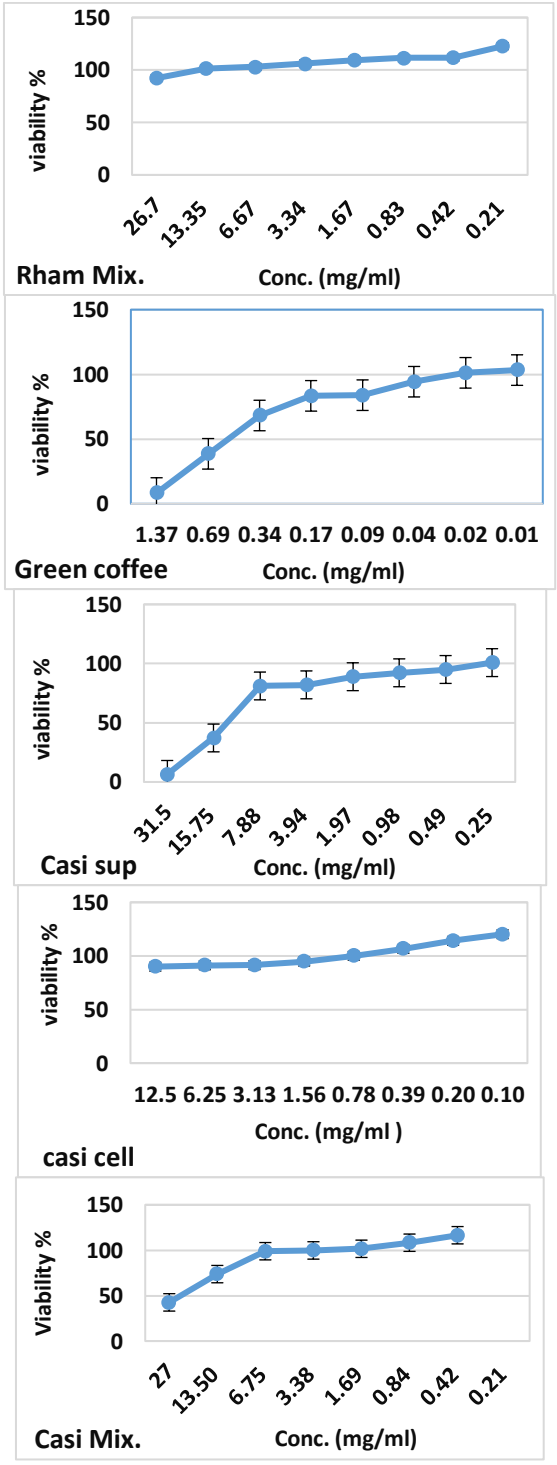


Fig 3. Viability % of Extracts

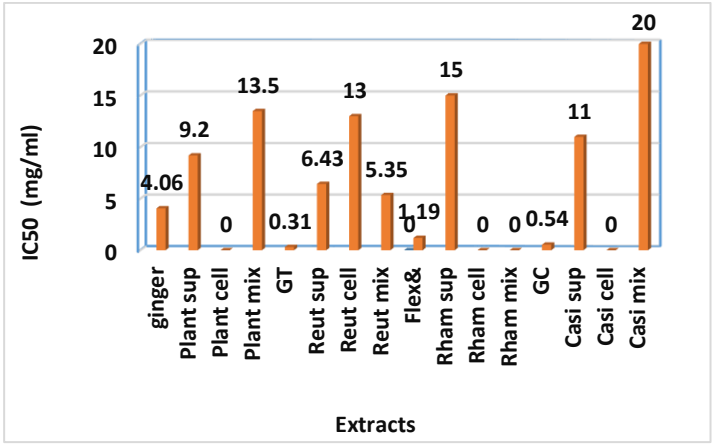


Fig. 4. IC_{50} (mg/ml) of Extracts

Regarding the determination oftotal cholesterol (Figure 5), our results showed reduction percentage level after treatment with probiotic (*Lactobacillus planetarium), prebiotic (*ginger) and combination the most effective is combination that make reduction 66% and the lowest sample is *Lactobacillus. planetarium* supernatant 36%.in group 1, while Reduction percentage level after treatment with probiotic (*Lactobacillus. reuteri),* prebiotic *(*green tea) and combination and the most effective is combination with reduction 65% and the lowest sample is *Lactobacillus reuteri* cell sonicate 21%in group 2. Meanwhile in group 3 reduction percentage level after treatment with probiotic (*Lactobacillus. rhamnosus), prebiotic (*Cinnamon and flax seed) and combination, the most effective is combination that make reduction 71% and the lowest sample is *Lactobacillus. rhamnosus* cell sonicate 33%. Also, reduction percentage level after treatment with probiotic (*Lactobacillus. casi),* prebiotic *(*green coffee) and combination showed that the most effective is combination that make reduction 62% and the lowest sample is *Lactobacillus. casi* cell sonicate 45%.in group 4. Obviously the most effective combination was in group 3 flex seed and cinnamon with *Lactobacillus rhamnosus* in agreement of the previous results[6].

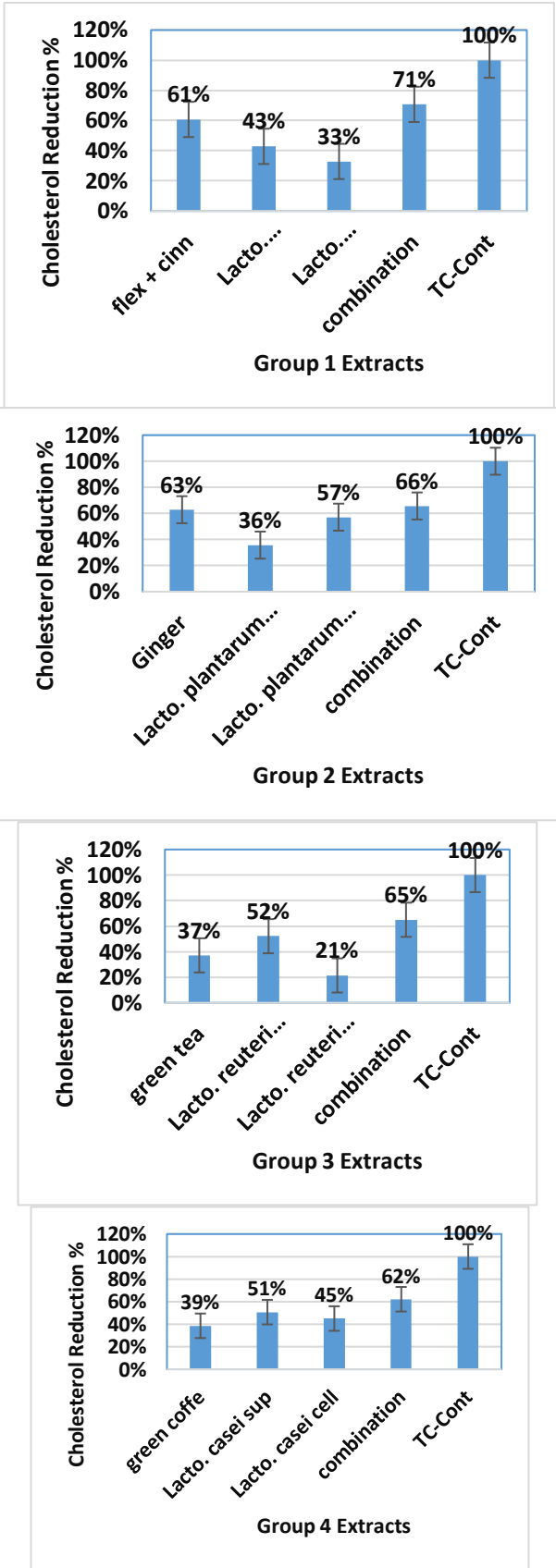


Fig. 5. Cholesterol Reduction % of Extracts

Lipid was determined using Oil-Red-O test, the data are presented as proportional lipid reduction (%) by comparing the treated group with the untreated group.

It was noticed that using prebiotics and probiotics mixture showed a significant increment in the reduction percentage in lipid level in MCF7 cell line after treatment compared with their values in control treated cells and in sole application of each test drugs ($P<0.05$).

This is in agreement with the previous studies[19]. Prebiotics hashepatic lipid lowering properties. Other nondigestible/fermentable nutrients, which also modulate intestinal flora activity, exhibit cholesterol or triglyceride lowering effects**.**

Also a recent research [6] mentioned that Lactose-based prebiotics with probiotics control lipid level in the bloodstream and tissue by (a) suppressing the expressions of lipogenic-genes and enzymes; (b) oxidizing fatty acids in muscle, liver, and adipose tissue; (c) binding cholesterol with cell membrane of probiotics and subsequent assimilation by probiotics; (d) enzymatic-transformations of bile acids; and (e) converting cholesterol to coprostanol and its defecation.

Table 1. Triglyceride in cell culture

G 1	R %	G 2	R %	G 3	R %	G 4	R %
ginger	65	Flex &cinn	62	Green coffee	51	Green tea	66
Plant.sup.	57	Rham sup	51	Casi sup	47	Reut.sup	42
Plant. cell	35	Rham cell	31	Casi cell	27	Reut cell	25
Plant. Mix	66	Rham mix	65	Casi mix	61	Reut mix	67
Total Triglyceride control	49.7 =100%		100		100		100

G: equals Group
R: equals Reduction

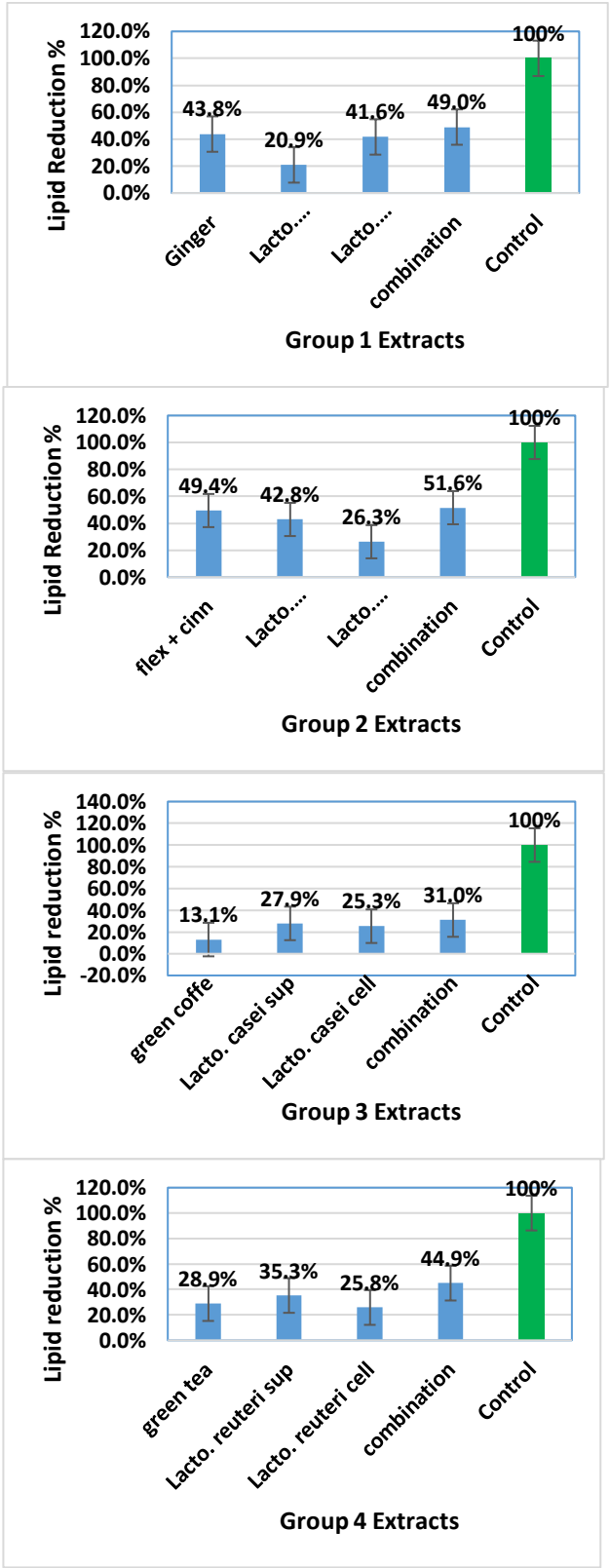


Fig. 6. Lipid reduction percentage of MCF7 cell line after treatment with extracts

According to cholesterol reduction result weevaluated triglyceride in cell culture (Table 1) treated with test groups samples, results showed significant reduction percentage level after treatment with that means potential effect in reducing triglycerides.as in (Figure 6). The results showed reduction percentage in lipid level in MCF7 cell line after treatment with prebiotic, probiotic and mix and the most effective sample was found combination of *lactobacillus planetarium* supernatant + cell sonicate with ginger 49%. This graph shows reduction percentage in lipid level in MCF7 cell line after treatment with prebiotic, probiotic and mix and the most effective sample was found combination of *lactobacillus rhamonsous* supernatant + cell sonicate with Flex & cinnamon 51.6%. This graph shows reduction percentage in lipid level in MCF7 cell line after treatment with prebiotic, probiotic and mix and the most effective sample was found combination of *lactobacillus Casi* supernatant + cell sonicate with green coffee 31%. This graph shows reduction percentage in lipid level in MCF7 cell line after treatment with prebiotic, probiotic and mix and the most effective sample was found combination of *lactobacillus reuteri* supernatant + cell sonicate with green tea 44.9%.

We studied the antioxidant effect of our four group's agents, which contain prebiotic, probiotic and mix. In our study the measuring of antioxidant concentrations made regarding to the cytotoxic effect of the test samples. The results indicated that the combination form of ginger and bacteria possessed significant elevated antioxidant levels 150% compared with ginger alone 98%. Also in second group the highest antioxidant level was combination of flaxseed and cinnamon with *lactobacillusrham* bacteria (146%) followed by flex& cinn 110%, *lactobacillusrham* supernatant 86% and last was *lactobacillusrham* cell sonicate78%. In group 3 the highest antioxidant was green tea and *lactobacilluscasei* bacteria combination (160%) followed by *lactobacilluscasei* Supernatant 100% , green tea 80% and last was *lactobacilluscasei* cell sonicate50% .finally group 4 results showed the highest antioxidant was combination of green coffee and *lactobacillusreuteri* bacteria (154%) followed by *lactobacillusreuteri* supernatant 138% , *lactobacillusreuteri* cell sonicate 122% and last was green coffee78% .From results it was noticed that the drugs showed oxidative stress (in percentage%), in which ROS was significantly elevated in sole and in combined tested samples, treated cells compared with its level inuntreated cell control. Additionally, the combined samples showed a significant synergetic activity of samples toxicity than that in each sample sole form.

Our results (Figure 8) showing Increase in antioxidant level in MCF7 cell line after treatment with probiotic (*lactobacillus plantarum*supernatant and cell sonicate), Prebiotic (ginger) and combination, and the most effective sample that give the highest antioxidant is combination (150%) followed by ginger 98%, cell sonicate58% and last is supernatant 24% .

This graph showed that Increase in antioxidant level in MCF7 cell line after treatment with probiotic (*lactobacillusreuteri* supernatant and cell sonicate), prebiotic (green tea) and combination, and the most effective sample that give the highest antioxidant is combination (160%) followed by Supernatant 100%, green tea 80% and last is cell sonicate50%.

Our results (Figure 8) showing Increase in Antioxidant level in MCF7 cell line after treatment with probiotic (*lactobacillus rhamnosus*supernatant and cell sonicate), prebiotic (flex& cinn) and combination, and the most effective sample that give the highest antioxidant is combination (146%) followed by flex& cinn 110%, supernatant 86% and last is cell sonicate78%.

Our results (Figure 8) showing Increase in Antioxidant level in MCF7 cell line after treatment with probiotic *(lactobacillus casei*supernatant and cell sonicate), Prebiotic (green coffee) and combination, and the most effective sample that give the highest antioxidant is combination (154%) followed by supernatant 138%, cell sonicate 122% and last is cell green coffee78%.

It was stated those probiotic bacteria such as *L. plantarum* CAI6 and *L. plantarum* SC4 may protect against cardiovascular disease by the regulation of lipid metabolism and induced antioxidative defense in hyperlipidemic mice [20]. Also [21] reported anti-hyperglycemic and anti-hyperlipidemic activities of probiotic strain in diabetic rats. Further, [22], also reported the lipid lowering and antioxidant effect of aqueous suspension of L. acidophilus (109 CFU/ml) on high fat diet fed rats. They found a significant decrease in plasma cholesterol, triglycerides, and LDL as well as an increase in HDL levels.

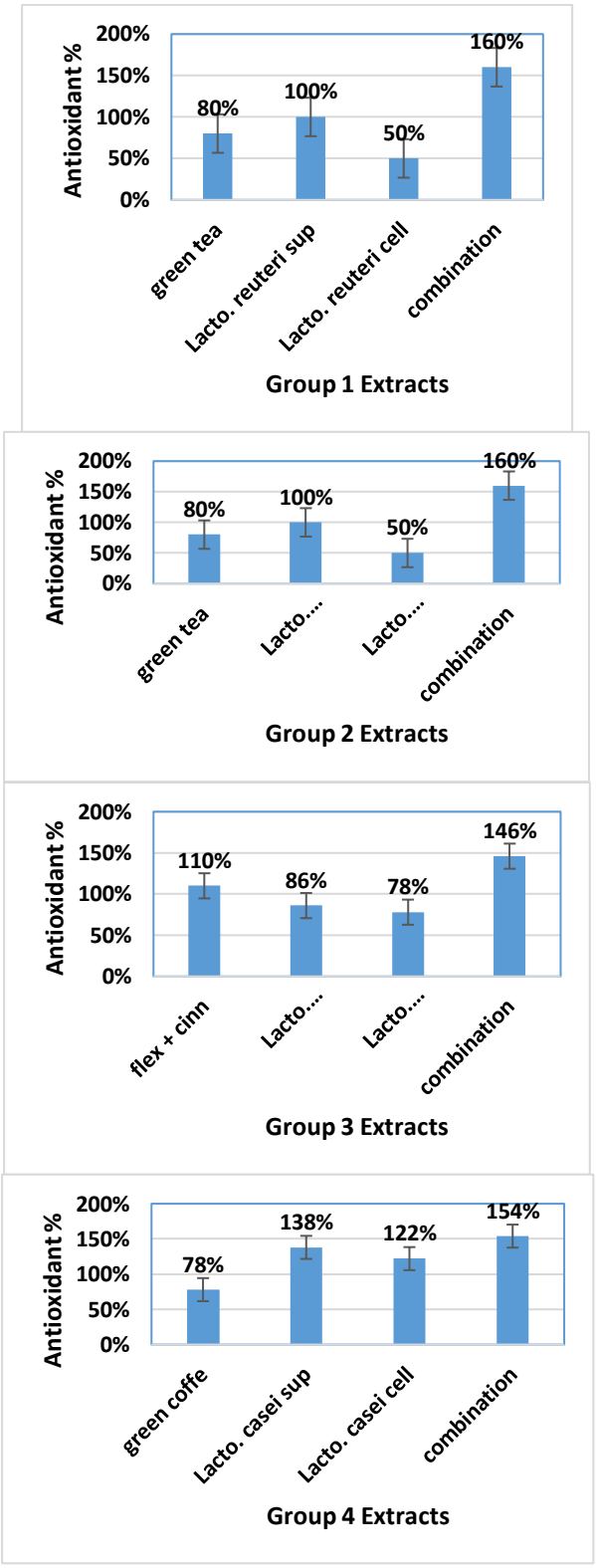


Fig. 7. Antioxidant percentage after treatment with extracts

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4.Conclusions

Finally, it appears that the potential of probiotics. *Lactobacillus* strains, used in this study may have hypocholesterolemic properties. It could be concluded that a diet rich in prebiotics, probiotics and synbiotics might confer numerous health benefits to the host possibly due to positive gut microbiota modulation. However, further studies should provide reliable mechanistic, safety and clinical evidence before recommending prebiotics, probiotics and synbiotics to individuals. The combination of probiotics and prebiotics significantly reduces the serum cholesterol and lipid levels and that can be used as an alternative remedy for hypercholesterolemic problems without any side effects to the consumers.

5.Conflicts of interest

There are no conflicts to declare.

6.Acknowledgments

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7. References

1. Reynolds, J.C., Laura, G., Usen, I. C., Cheng, H., Jeyabalan, S., Palayan, M. Antihyperlipidemic activity of Sapindus emarginatus in Triton WR-1339 induced albino rat. Research Journal of Pharmacy and Technology, 2(2), 319-323. (2009).
2. Mishra, S., Verma, P. "Flaxseed-bioactive compounds and health significance." IOSR Journal of Humanities and Social Science, 17(3), 46-50. (2013).
3. Li, C., Li, Y., Ma, Y., Wang, D., Zheng, Y., Wang, X." Effect of black and white sesame on lowering blood lipids of rats with hyperlipidaemia induced by high-fat diet." Grain & Oil Science and Technology, 3(2), 57-63. (2020).
4. Manzoni, A. G., Passos, D. F., Leitemperger, J. W., Storck, T. R., Doleski, P. H., Jantsch, M. H., Leal, D. B.." Hyperlipidaemia-induced lipotoxicity and immune activation in rats are prevented by curcumin and rutin". International immunopharmacology, 81, 106217. (2020).
5. Iqbal, U.H., Westfall, S., Farahdel, L., Prakash, S." A novel probiotic blend for potential use in the metabolic syndrome: Design and in-vitro characterizations." international Journal of Probiotics and Prebiotics,13 (1), pp. 25-36. (2018).
6. Nath, A., Molnár, M.A., Csighy, A., Kőszegi, K., Galambos, I., Huszár, K.P., Koris, A., Vatai, G." Biological activities of lactose-based prebiotics and symbiosis with probiotics on controlling osteoporosis, blood-lipid and glucose levels". Medicina (Lithuania), 54 (6), art. no. 98, (2018).
7. Pothuraju, R., Sharma, R.K." Interplay of gut microbiota, probiotics in obesity:" A review.Endocrine, Metabolic and Immune Disorders - Drug Targets, 18 (3), pp. 212-220. (2018).
8. He, M., Shi, B. "Gut microbiota as a potential target of metabolic syndrome": The role of probiotics and prebiotics Cell and Bioscience, 7 (1), art. no. 54. (2017).
9. Zhu, J., Cai, R., Tan, Y., Wu, X., Wen, Q., Liu, Z., Yang, H." Preventive consumption of green tea modifies the gut microbiota and provides persistent protection from high-fat diet-induced obesity." Journal of Functional Foods, 64, 103621. (2020).
10. Xiao, J. Z., Kondo, S., Takahashi, N., Miyaji, K., Oshida, K., Hiramatsu, A., Hosono, A. "Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers." Journal of dairy science, 86(7), 2452-2461. (2003).
11. Jones, M.L., Martoni, C.J., Parent, M. and Prakash, S. "Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active Lactobacillus reuteri NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults." Brazilian Journal of Nutrition. 107: 1505- 1513. (2012).
12. Pranakhon,Ratree,PatchareewanPannangpetch, and Chantana Aromdee. "Antihyperglycemic activity of agarwood leaf extracts in STZ-induced diabetic rats and glucose uptake enhancement activity in rat adipocytes." Songklanakarin Journal of Science & Technology 33.4 (2011).
13. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., and Smith, F. "Colorimetric method for determination of sugars and related substances." Analytical chemistry, 28(3), 350-356.) (1956).

14. James, A. M., and John, K. D. "Basic cell culture technique and the maintenance of cell lines". Basic cell culture. 99-138. (1998).
15. Cory, A. H., Owen, T. C., Barltrop, J. A., Cory,J.G.."Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture." Cancer communications.3(7): 207-212. (1991).
16. Berridge, M. V., Tan, M. S., Herst, P. M. "Tetrazolium dyes as tools in cell biology. New insights into their cellular reduction". Biotechnology annual review.11: 127-152. (2005).
17. Chen, X., Ping, L., Liu, J., Kangsen, X. "Apoptosis of human hepatocellular carcinoma cell (HepG2) induced by cardiotoxin III through S-phase arrest." Experimental and Toxicologic Pathology. 61 (4): 307-315. (2009).
18. Zhang, J., Li, Y., Liu, T., He, W., Chen, Y., Chen, X., Li, X., Zhou, W., Yi, J., Ren, Z. "Antitumor effect of matrine in human hepatoma G2 cells by inducing apoptosis and autophagy." World J Gastroenterol. 16 (34): 4281-4290 (2010).
19. Delzenne, N.M., Williams, C.M. "Prebiotics and lipid metabolism". Current Opinion in Lipidology, 13 (1), pp. 61-67. (2002).
20. Wang, L.X., Liu, K., Gao, D.W. and Hao, J.K. "Protective effects of two Lactobacillus plantarum strains in hyperlipidemic mice." World Journal of Gastroenterology. 19: 3150-3156. (2013).
21. Alsayadi, M., Al Jawfi, Y., Belarbi, M., Soualem-Mami, Z., Merzouk, H., Sari, D.C., Sabri, F. and Ghalim, M. "Evaluation of anti-hyperglycemic and anti-hyperlipidemic activities of water kefir as probiotic on streptozotocin-induced diabetic wistar rats." Journal of Diabetes Mellitus. 4: 85-95. (2014).
22. Shrivastava, A., Chaturvedi, U., Singh, S. V., Saxena, J. K., & Bhatia, G. "Lipid lowering and antioxidant effect of miglitol in triton treated hyperlipidemic and high fat diet induced obese rats". Lipids, 48(6), 597-607. (2013).