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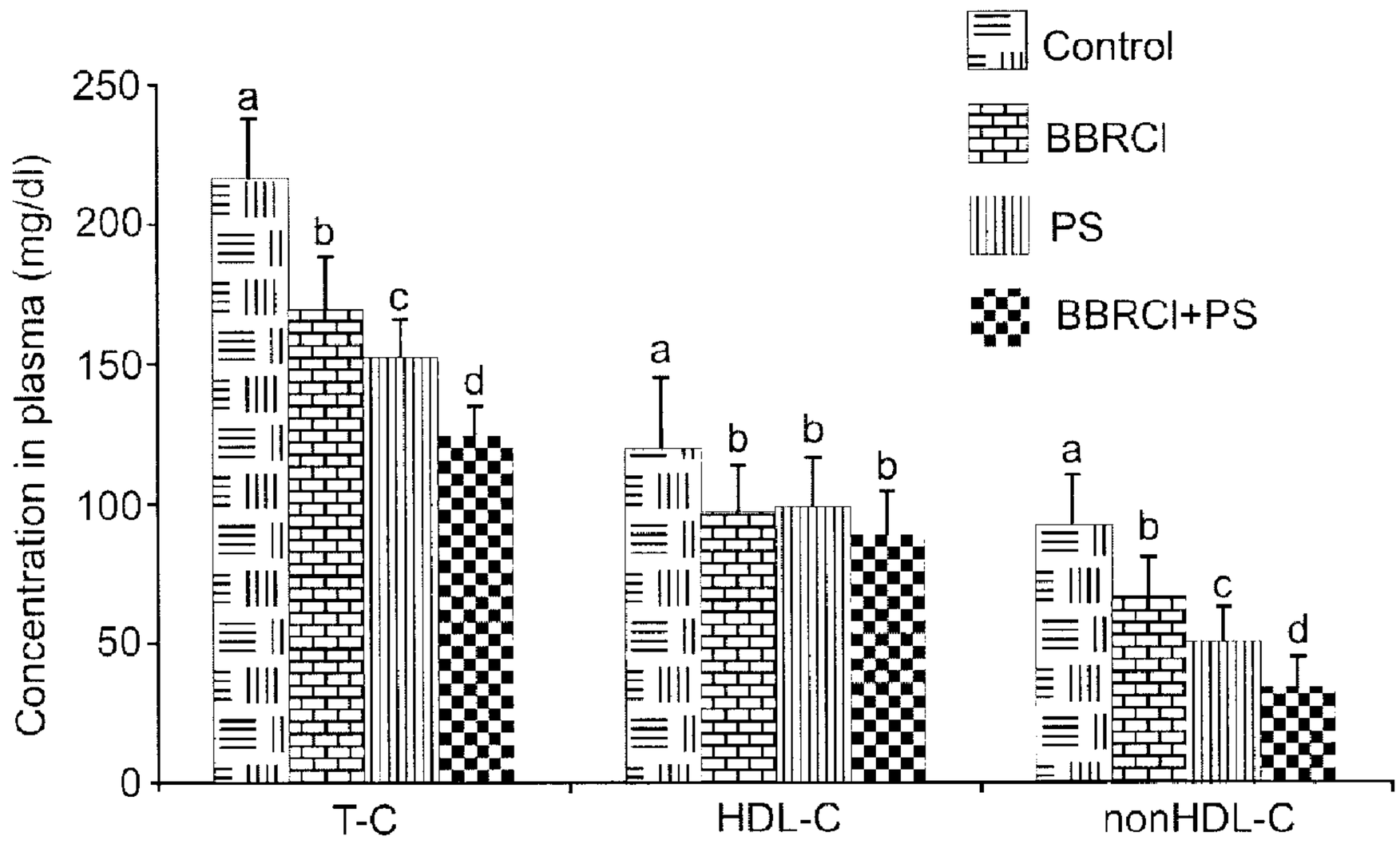


(57) Abrégé/Abstract:
A blood lipid lowering agent that functions through a same mechanism as berberine (e.g. berberine, one or more pharmacologically acceptable salts of berberine or a mixture thereof) and a blood lipid lowering agent that functions through a different mechanism than berberine (e.g. phytosterols, phytostanols, esters thereof or mixtures thereof) act synergistically to improve blood lipid profiles, for example, lowering total cholesterol, LDL-C or nonHDL-C, and triglyceride, and increasing the ratio of HDL-C to nonHDL-C. The two may be used in combination to treat or reduce the chance of contracting cardiovascular disease, hyperlipidemia, atherosclerosis, coronary heart disease, angina, cerebrovascular disease, stroke, overweight or obesity, diabetes, insulin resistance, hyperglycemia, hypertension, arrhythmia, diseases of the central nervous system, diseases of the peripheral nervous system and/or inflammation. The blood lipid lowering agent that functions through a same mechanism as berberine, with or without the blood lipid lowering agent that functions through a different mechanism than berberine, may also be used to control weight.

ABSTRACT

A blood lipid lowering agent that functions through a same mechanism as berberine (e.g. berberine, one or more pharmacologically acceptable salts of berberine or a mixture thereof) and a blood lipid lowering agent that functions through a different mechanism than berberine (e.g. phytosterols, phytostanols, esters thereof or mixtures thereof) act synergistically to improve blood lipid profiles, for example, lowering total cholesterol, LDL-C or nonHDL-C, and triglyceride, and increasing the ratio of HDL-C to nonHDL-C. The two may be used in combination to treat or reduce the chance of contracting cardiovascular disease, hyperlipidemia, atherosclerosis, coronary heart disease, angina, cerebrovascular disease, stroke, overweight or obesity, diabetes, insulin resistance, hyperglycemia, hypertension, arrhythmia, diseases of the central nervous system, diseases of the peripheral nervous system and/or inflammation. The blood lipid lowering agent that functions through a same mechanism as berberine, with or without the blood lipid lowering agent that functions through a different mechanism than berberine, may also be used to control weight.

COMBINATIONS OF BOTANICAL EXTRACTS FOR PROMOTING CARDIOVASCULAR HEALTH

Field of the Invention

The present invention relates to medicinal compositions, particularly to combinations of botanical extracts for promoting cardiovascular health.

Background of the Invention

Cardiovascular disease (CVD), including coronary heart disease, atherosclerosis, stroke, myocardial infarction, sudden death syndrome, is the number one cause of death in most developed countries all over the world. Also, in developing countries, the prevalence of CVD is on the increase and appears to be linked to people adopting a more Westernized (North American) diet (high fat) and lifestyle (sedentary). Elevated circulating cholesterol levels, in particular low-density-lipoprotein cholesterol (LDL-cholesterol) levels, have been well established as one of the major risk factors for the development and progression of CVD. A high level of circulating triglycerides is also a critical risk factor in the increased incidence of CVD. Accordingly, reducing total cholesterol and/or triglyceride levels is advised to high risk patients to reduce cardiovascular-related risk factors that are known and demonstrated to be associated with a higher incidence of morbidity and mortality. Subjects with obesity, diabetes and hyperlipidemia are three major subgroups of the population that are adversely affected by high cholesterol and triglyceride levels.

To date, it is known that plant sterols/stanols and their various analogues can reduce circulating blood cholesterol concentration by inhibiting dietary and biliary cholesterol absorption in the intestine. Red Yeast Rice supplements lower blood cholesterol through inhibiting the activity of the rate-limiting enzyme, HMG-CoA reductase that essentially governs cholesterol biosynthesis in mammals. Berberine was most recently reported to be able to lower blood cholesterol through enhancing cholesterol clearance by increasing LDL-receptor mediated cholesterol clearance. These three types of bioactive compounds (berberine, plant sterols/stanols, and Red Yeast Rice) work through distinct mechanisms.

Presently available products have been demonstrated to work via different mechanisms and achieve the expected results to certain degree; however, the efficacy of presently available products is limited and/or is accompanied by side effects. Statin drugs reduce blood cholesterol through suppressing the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, but this class of compounds has little or no effect on lowering triglycerides. The major drawback of statin or statin-like compounds is that the synthesis of an important mitochondrial enzyme called Q10 is inhibited, depending on HMG-CoA reductase to be intact and functional. Blockage of HMG-CoA reductase by statins causes reduced coenzyme Q10 levels and this is thought to underlie the cause of a number of statin-associated muscle-related myopathies reported, such as muscle soreness, muscle weakness, muscle tenderness, intense muscle pain, peripheral neuropathy and muscle protein breakdown called rhabdomylosis and may underlie other side effects that are dependent on the presence of normal physiological levels of coenzyme Q10. In particular, rhabdomylosis can be both a serious and a life threatening side effect clearly associated with the use of statin drugs where the muscle breakdown causes major organ damage to both the liver and kidney that has resulted in many reported deaths. The all-cause discontinuation rate of statin use was about 10% and discontinuation because of adverse events was about 4%. Plant sterols and their different analogues inhibit cholesterol absorption and thus reduce cholesterol concentration in the plasma. However, when blood cholesterol concentration is reduced through the inhibition of cholesterol absorption, cholesterol synthesis increases simultaneously as a compensation mechanism to counteract the reduced absorption of dietary and biliary cholesterol. Plant sterols and their different analogues have not previously been shown to have any significant effect in reducing serum triglyceride levels.

A recent discovery of a botanical bioactive alkaloid compound berberine, contained in Chinese Huanglian, goldenseal, or goldthread, lowers cholesterol levels through increasing LDL-receptor mediated cholesterol clearance.

Summary of the Invention

It has now been found that a blood lipid lowering agent that functions through a same mechanism as berberine and a blood lipid lowering agent that functions through a different mechanism than berberine act synergistically to improve blood lipid profiles. Such improvements may be manifested, for example, in lowering blood lipids such as total cholesterol (T-C), low-density-lipoprotein cholesterol (LDL-C) or non-high-density-lipoprotein cholesterol (nonHDL-C), and/or triglycerides (TG), and/or in increasing the ratio of high-density-lipoprotein cholesterol (HDL-C) to LDL-C or nonHDL-C. Blood lipid

modulations (e.g. the lowering effects) of the two administered in combination are more than the sum of the two administered separately.

Thus, in one aspect of the invention, there is provided a blood lipid level lowering composition comprising a synergistic mixture of a first blood lipid lowering agent comprising berberine, one or more pharmacologically acceptable salts thereof or a mixture thereof; and a second blood lipid lowering agent comprising a plant sterol, plant stanol, ester of a plant sterol, ester of a plant stanol or a mixture thereof, for lowering blood lipid levels in a blood stream of a mammal.

In a second aspect of the invention, there is provided use of a blood lipid level lowering synergistic combination of a first blood lipid lowering agent comprising berberine, one or more pharmacologically acceptable salts thereof or a mixture thereof; and a second blood lipid lowering agent comprising a plant sterol, plant stanol, ester of a plant sterol, ester of a plant stanol or a mixture thereof for lowering blood lipid levels in a blood stream of a mammal.

In a third aspect of the invention, there is provided a dosage form comprising the blood lipid level lowering composition of the invention.

In a fourth aspect of the invention, there is provided a commercial package the blood lipid level lowering composition of the invention together with instructions for use in lowering blood lipid levels in a mammal.

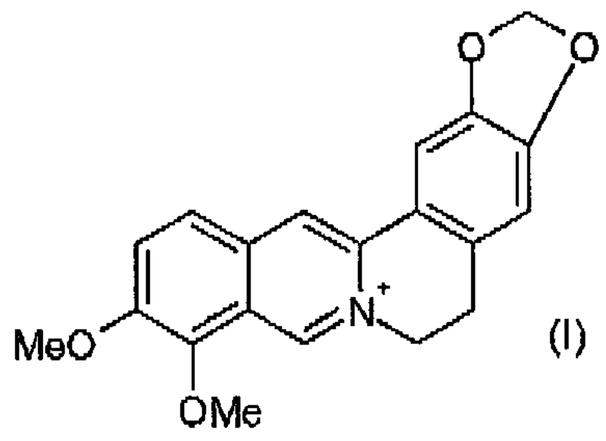
In a fifth aspect of the invention, there is provided a food or beverage comprising the blood lipid level lowering composition of the invention.

In a sixth aspect of the invention, there is provided a use of a blood lipid lowering agent that functions through a same mechanism as berberine for controlling weight of a mammal.

The blood lipid lowering agent that functions through a same mechanism as berberine (hereinafter referred to as "BBR") may be from a naturally occurring source or from a synthetic or semi-synthetic source. BBR in a naturally occurring source may be used "as is", for example, plant material containing the BBR may be used directly. BBR from any source may be subject to one or more isolation or concentration steps (e.g. extraction, crystallization, filtration) to provide a purer and/or more concentrated form of

the BBR. Preferably, BBR is used as a crude extract from a natural source, as a concentrated extract from a natural source, as a partially purified extract from a natural source, or in a substantially pure form from a natural, synthetic or semi-synthetic source. BBR may be available commercially from a number of suppliers.

BBR may comprise, for example, berberine, one or more berberine derivatives or analogs, one or more pharmacologically acceptable salts thereof, or a mixture thereof. Berberine is an isoquinoline alkaloid of formula (I):



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Berberine, berberine derivatives or analogs, salts thereof or mixtures thereof may be found in a variety plants, for example *Coptis chinensis* rhizomes (*huanglian, coptis, goldthread*), goldenseal, goldthread, *Phellodendron amurense* bark, *Berberis sargentiana, Berberis thunbergii, Berberis vulgaris (Barberry), Berberis aquifolium (Oregon grape), Hydrastis Canadensis (goldenseal), and Berberis aristata (tree turmeric).* A recent paper demonstrated that a berberine mixture from goldenseal is more effective than pure berberine chloride alone.

Berberine, berberine derivatives or analogs, and salts thereof may be prepared synthetically or semi-synthetically by a variety of chemical and/or enzymatic methods known in the art, for example, as described in United States patent publication 2006/0223838 published October 5, 2006.

In one embodiment, the BBR comprises berberine, one or more pharmacologically acceptable salts of berberine or a mixture thereof. Preferred pharmacologically acceptable salts of berberine include, for example, acid addition salts, e.g. chlorides, sulfates, carbonates, phosphates, citrates and acetates. Acid addition salts may be produced by reacting berberine with an appropriate acid.

The blood lipid lowering agent that functions through a different mechanism than berberine (hereinafter referred to as "BLLA") may be, for example, a plant sterol

(phytosterol), a plant stanol (phytostanol), a statin, an isoflavone, a natural product containing one or more of the above and/or other blood lipid lowering agents, a derivative thereof or a mixture thereof.

In particular, a plant sterol, a plant stanol, an ester of a plant sterol, an ester of a plant stanol or a mixture thereof provide surprisingly synergistic effects when used in conjunction with BBR. Plant sterols comprise alcoholic derivatives of cyclopentanoperhydrophenanthrenes. Plant stanols are saturated forms of the sterols. Some representative examples of plant sterols are beta-sitosterol, campesterol and stigmasterol. Some representative examples of plant stanols are sitostanol and campestanol. Particularly preferred esters of plant sterols or plant stanols are esters with unsaturated fatty acids, for example omega-3 fatty acids (e.g. docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA)), and esters with any other acids that are suitable for consumption and/or that benefit health (e.g. ascorbic acid). Plant sterols and plant stanols are found in legumes, fruits, vegetables, trees and other plants as well as in fungi and other microorganisms. Plant material containing plant sterols and/or stanols may be used directly, or the plant sterols and/or stanols may be extracted from the plant material and used in a more purified and/or concentrated form. Preferably, plant sterols and plant stanols are extracted from plant or other phytosterol/phytostanol-containing materials. Some examples of plant materials include, for example, soybean oil, tall (pine tree) oil, wood pulp, leaves, nuts, vegetable oils, corn and rice. Plant sterols and plant stanols are available commercially from a number of suppliers.

Some natural products contain a variety of BLLAs. For example, Red Yeast Rice contains several different naturally-occurring statins, including lovastatin, which are naturally produced during the fermentation process involved with producing Red Yeast Rice. However, the active lipid lowering components in Red Yeast Rice are not solely statins since the amount of statins obtained from Red Yeast Rice consumption is much lower than the dose of statin drug. The lipid lowering effect of Red Yeast Rice is more likely a result of the combination of lovastatin with other different statins and isoflavones found in Red Yeast Rice extracts. In addition, Red Yeast Rice is a fermented rice product that has been used in Chinese cuisine and as a medicinal food to promote blood circulation for centuries, without significant side effects that occurs after statin drug supplementation.

Blood lipid levels may include, for example, triglyceride (TG) levels, total cholesterol (T-C) levels and non-high-density-lipoprotein cholesterol (nonHDL-C) levels

(e.g. very low-density lipoprotein cholesterol (VLDL-C) levels, intermediate-density lipoprotein cholesterol (IDL-C) levels and/or low-density-lipoprotein cholesterol (LDL-C) levels). Compositions, uses and methods of the present invention may lower one or more of these levels in the blood. The lowering of blood lipid levels may be measured in whole blood, blood plasma and/or blood serum.

BBR and BLLA are used in amounts effective to provide a daily dose of the combination of ingredients that lowers blood lipid levels. For example, the daily dose of each ingredient may in some cases be 5 mg or more per kg of body weight of a subject. In other cases, doses of each ingredient of 10 mg or more per kg of body weight may be appropriate. In yet other cases, doses of each ingredient of 50 mg or more per kg of body weight may be appropriate. For an 80 kg subject, a dose of each ingredient of 10 mg or more per kg of body weight is about 0.8 grams or more of each ingredient per day. Daily dosages can be given all at once in a single dose or can be given incrementally in several smaller dosages. Thus, the composition of the present invention can be formulated such that the recommended daily dose is achieved by the administration of a single dose or by the administration of several smaller doses.

BBR and BLLA may be used for any suitable length of time to reduce blood lipid levels in the subject. Preferably, BBR and BLLA are used for at least two weeks. Longer periods of usage can provide greater reduction in blood lipid levels and are envisaged as continued use of all known lipid control therapeutic approaches is required to effectively control or maintain lower lipid levels in the long term. For example, discontinuation of statin therapy results in blood lipid profiles returning to pre-intervention levels.

BBR and BLLA may be formulated in a dosage form. Dosage forms include powders, tablets, capsules, softgels, solutions, suspensions, emulsions and other forms that are readily appreciated by one skilled in the art. The compositions may be administered orally, parenterally, intravenously or by any other convenient method. Oral administration is preferred.

BBR and BLLA may be administered simultaneously or within a short period of time of one another. Preferably, BBR and BLLA are administered simultaneously. If they are administered within a short period of time of one another, the period time should not be so long that the synergistic effect of BBR and BLLA is not realized.

BBR and BLLA may be formulated together with other pharmacologically acceptable ingredients typically used in the nutraceutical and/or pharmaceutical compositions, for example antiadherents, binders (e.g. starches, sugars, cellulose, hydroxypropyl cellulose, ethyl cellulose, lactose, xylitol, sorbitol and maltitol), coatings (e.g. cellulose, synthetic polymers, corn protein zein and other polysaccharides), disintegrants (e.g. starch, cellulose, cross-linked polyvinyl pyrrolidone, sodium starch glycolate and sodium carboxymethyl cellulose), fillers/diluents (e.g. water, plant cellulose, dibasic calcium phosphate, vegetable fats and oils, lactose, sucrose, glucose, mannitol, sorbitol and calcium carbonate), flavors and colors, glidants, lubricants (e.g. talc, silica, vegetable stearin, magnesium stearate and stearic acid), preservatives (e.g. vitamin A, vitamin E, vitamin C, selenium, cysteine, methionine, citric acid, sodium citrate, methyl paraben and propyl paraben), antioxidants, sorbents, sweeteners, and mixtures thereof.

BBR and BLLA may also be admixed with a food or beverage and taken orally in such a manner. Fortified foods and beverages may be made by adding BBR and BLLA during the manufacturing of the food or beverage. Alternatively, the consumer may add BBR and BLLA to the food or beverage near the time of consumption. Each ingredient may be added to the food or beverage together with the other ingredients or separately from the other ingredients. Examples of foods and beverages are, but not limited to, cereals, snack bars, dairy products, fruit juices, powdered food and dry powder beverage mixes.

BBR and BLLA may be packaged together in a commercial package together with instructions for their use. Such packages are known to one skilled in the art and include, for example, bottles, jars, blister packs, boxes, etc.

BBR and BLLA may be used to treat or reduce the chance of contracting or the progression of a number of diseases or conditions in a subject. Diseases or conditions include, for example, cardiovascular disease, hyperlipidemia, atherosclerosis, coronary heart disease, angina, cerebrovascular disease, stroke, overweight or obesity, diabetes, insulin resistance, hyperglycemia, hypertension, arrhythmia, diseases of the central nervous system, diseases of the peripheral nervous system and inflammation. BBR and BLLA are particularly effective at preventing cardiovascular diseases, for example, coronary heart disease, atherosclerosis, stroke, arrhythmia, myocardial infarction and sudden death syndrome. BBR and BLLA may also be used to control weight in a subject.

Subjects are humans and animals with blood circulatory systems, particularly mammals, for example, humans, dogs, cats, horses and rodents (e.g. hamsters, mice and rats).

Without being held to any particular mechanism of action, synergy between BBR and BLLA is thought to arise by affecting two or more blood lipid control mechanisms to achieve a blood lipid-lowering efficacy greater than the additive effect. BBR and BLLA also provide an anti-inflammatory effect that provides additional health benefits and protection, especially to the cardiovascular system. In addition to the synergistic effect, there is a reduction in side effect profile compared to targeting a single mechanism. For example, BBR and BLLA may simultaneously affect three independent pathways to synergistically lower serum cholesterol levels, lower triglycerides and reduce or down-regulate chronic inflammatory mechanism.

Circulating cholesterol concentration is a function of input from absorption (dietary and biliary cholesterol) and de novo synthesis relative to clearance through hepatic and non-hepatic removal mechanisms as well as cholesterol elimination through excretion. There are three major pathways involved in controlling cholesterol homeostasis in the human body. Many of the available natural products that lower body cholesterol target a single distinct pathway and either show limited efficacy or require a high dose level to control cholesterol. Currently there is a high consumer demand for a novel product that can significantly reduce cholesterol levels at a reasonable daily dose and without causing significant side effects. Embodiments of the present invention can meet this demand.

Further features of the invention will be described or will become apparent in the course of the following detailed description.

Brief Description of the Drawings

In order that the invention may be more clearly understood, embodiments thereof will now be described in detail by way of example, with reference to the accompanying drawings, in which:

Fig. 1 is a graph depicting effect of berberine chloride and plant stanols on circulating cholesterol levels in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (for each lipid parameter, values with different superscripts (a, b, c or d) are significantly different);

Fig. 2 is a graph depicting effect of berberine chloride and plant stanols on plasma triglyceride concentration in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (values with different superscripts (a or b) are significantly different);

Fig. 3 is a graph depicting percent changes of plasma cholesterol and triglyceride concentration in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols;

Fig. 4 is a graph depicting effect of berberine chloride and plant stanols on liver cholesterol concentration in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (values with different superscripts (a, b or c) are significantly different);

Fig. 5 is a graph depicting effect of berberine chloride and plant stanols on the weekly body weight in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks;

Fig. 6 is a graph depicting effect of berberine chloride and plant stanols on average daily feed intake in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks;

Fig. 7a is a graph depicting effect of berberine chloride and plant stanols on intestinal ABCG5 mRNA expression in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (values with different superscripts (a or b) are significantly different);

Fig. 7b is a graph depicting effect of berberine chloride and plant stanols on intestinal ABCG8 mRNA expression in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (values with different superscripts (a or b) are significantly different);

Fig. 8a is a graph depicting effect of berberine chloride and plant stanols on liver HMG-CoA reductase mRNA expression in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks (values with different superscripts (a or b) are significantly different);

Fig. 8b is a graph depicting effect of berberine chloride and plant stanols on liver CYP7A1 mRNA expression in hamsters fed an atherogenic control diet and the

atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks; and,

Fig. 8c is a graph depicting effect of berberine chloride and plant stanols on liver CYP27A1 mRNA expression in hamsters fed an atherogenic control diet and the atherogenic control diet supplemented with berberine chloride and/or plant stanols for 4 weeks.

Description of Preferred Embodiments

An objective of the following examples was to show that a combination of BBR and BLLA results in a synergistic improvement in blood lipid profile than each intervention on its own, toward positive health benefits to the cardiovascular system, cerebrovascular vasculature, liver, and body weight.

Materials:

Table 1 lists materials used in the following examples.

Table 1

Material	Source
Berberine chloride (BBRCl)	Sigma-Aldrich Co., purity > 98%
Plant stanols (PS)	Forbes-Medi Tech Inc., purity >92%*
Casein	MP Biomedicals
Corn starch	MP Biomedicals
Sucrose	MP Biomedicals
Cholesterol	MP Biomedicals
Male Golden Syrian Hamsters	Charles River Co.
Male Sprague-Dawley rats	Charles River Co.

* Plant stanols (PS) consist of 10% campestanols and 82% sitostanols, with overall 92% of plant stanols.

Example 1:

This example provides data for the combination of berberine chloride (BBRCl) with plant stanols (PS) to improve blood lipid profiles toward positive health benefits to the cardiovascular system, cerebrovascular system, vasculature, liver, and body weight.

A controlled, four week animal study was performed to compare and contrast the effects of three interventional strategies on blood cholesterol and triglyceride levels.

Four randomized groups of 15 male Golden Syrian hamsters were fed isocaloric diets as follows:

Control	a semi-synthetic casein-corn starch-sucrose diet that contained 0.15% (w/w) cholesterol and 5% (w/w) fat
BBRCI	control diet containing 100 mg/kg·d of BBRCI
PS	control diet containing 1% (w/w) PS
BBRCI+PS	control diet containing 100 mg/kg·d BBRCI and 1% (w/w) PS

BBRCI and PS were introduced into the diet by blending. The primary endpoint at the end of the study was to determine the effects of each intervention on circulating blood lipids including total cholesterol (T-C), high-density-lipoprotein cholesterol (HDL-C), nonHDL-C (which is indicative of LDL-C levels) and triglyceride (TG) profiles. All lipids were measured by the standard enzymatic method.

The hamster has been established as a good model for studying human cholesterol metabolism and was used to examine the effects of BBRCI and plant stanols each alone and in combination on circulating blood lipid levels. As well, tissues were collected to determine the associated biochemical and molecular mechanisms that are activated, induced or augmented by the experimental interventions tested.

Male Golden Syrian Hamsters were purchased from Charles River Co. housed individually in cages for two weeks prior to the commencement of the study. During this adjustment period, animals were fed with regular rodent chow diet with free access to both food and water. A total of 60 hamsters were weighed and randomly assigned to one of four groups of 15 animals each. Animals are subjected to a temperature-controlled environment with a 12:12h light/dark cycle. Each group was fed with one of the four isocaloric experimental diets for a 4 week period. A preliminary study demonstrated that the addition of BBRCI to the chow does not induce a taste aversion effect as a food switch from the control diet to one containing 100 mg/kg BBRCI did not change the food intake. During the course of the experiment, animals were weighed weekly and food consumption was determined on a daily basis over the course of the experiment.

At the conclusion of the study all animals were anaesthetized with isoflurane and killed by decapitation. Blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Red blood cells and plasma were separated and stored at -80°C until further analysis. Plasma was analyzed for total cholesterol, HDL-C and TG concentrations, for which results are reported below. NonHDL-C was calculated by subtracting HDL-C from total cholesterol.

Figs. 1-3 and Table 2 represent the effects of BBRCI and PS alone or in combination on blood cholesterol and triglyceride levels. Values are means ± SD, n=15. Data were analyzed by one-way ANOVA followed by Tukey test if significance was detected. A significant difference was indicated by a $p < 0.05$.

The results obtained strongly support that BBRCI and the combination of BBRCI with plant stanols (for this example) induces a beneficial effect on blood lipid profiles (see Figs. 1-3 and Table 2). The results showed a novel and very important finding that dietary supplementation of BBRCI and plant stanols acted synergistically to lower circulating TG levels (see Fig. 2), whereas neither BBRCI (100 mg/kg·d) alone nor plant stanols (1% (w/w)) alone showed any significant reduction of TG levels (BBRCI reduced TG by only 4% and PS reduced TG by 11%). When BBRCI and PS were combined together, plasma TG levels in this group of 15 animals decreased by an average of 36%. Moreover, both materials showed significant effects in lowering T-C and nonHDL-C levels (see Fig. 1).

Table 2

	T-C	HDL-C	nonHDL-C	HDL-C/nonHDL-C	TG
Concentration (mg/dl)					
Control	217±21[a]	119±26[a]	92±17[a]	1.35±0.46[b]	339±137[a]
BBRCI	169±20[b]	100±17[b]	66±14[b]	1.60±0.58[b]	326±111[a]
PS	152±14[c]	99±18[b]	51±21[c]	2.21±1.34[ab]	297±58[a]
BBRCI+PS	125±11[d]	89±15[b]	34±11[d]	3.10±1.82[a]	215±68[b]
% difference from Control					
Control	0	0	0	0	0
BBRCI	-21.9	-16.2	-28.2	19	-3.9
PS	-29.8	-17.2	-45.0	64	-10.6
BBRCI+PS	-42.5	-25.4	-63.2	130	-36.6

In terms of converting the data to a more conventional reporting format where the % change of blood total and sub-fraction of cholesterol levels relative to control is calculated and presented, it was found that BBRCI reduced T-C by 22% and nonHDL-C by 28% while plant stanols reduced T-C and nonHDL-C by 30% and 45%, respectively. The combination of both materials lowered T-C by 42% and nonHDL-C by 63% (see Fig. 3). BBRCI and PS tended to improve the ratio of HDL-C to nonHDL-C. When BBRCI and

PS were supplemented simultaneously in the diet, the ratio of HDL-C to nonHDL-C was improved significantly through a synergistic action mode.

Histopathological testing and examination of gross and microscopic examination of thin sections of the various organs were conducted by a certified pathologist. There were no significant changes in the appearance or weight of brain, lung, heart, spleen and kidney. However, the livers of control animals were observably yellowish and heavier than those of animals treated with BBRCl or plant stanols, or the combination of either.

Table 3 summarizes the effect of BBRCl and plant stanols on the percent tissue weight relative to body weight. For each tissue weight to body weight parameter in Table 3, values with different superscripts (a or b) are significantly different. The results suggest beneficial effects to the liver and liver function as BBRCl and PS alone and especially BBRCl and PS in combination markedly reduced the appearance of fatty liver, and significantly lowered organ weight compared to the livers of the control animals.

Table 3

	Brain	Heart	Lung	Liver	Spleen	Right kidney
Control	0.81±0.05	0.38±0.04	0.52±0.08	5.0±0.04[a]	0.12±0.02	0.34±0.02
BBRCl	0.83±0.06	0.36±0.03	0.54±0.06	4.4±0.4[b]	0.11±0.01	0.32±0.03
PS	0.79±0.06	0.37±0.04	0.51±0.07	4.6±0.4[a]	0.12±0.01	0.33±0.02
BBRCl+PS	0.81±0.07	0.36±0.03	0.54±0.06	3.9±0.3[b]	0.11±0.02	0.33±0.02

Fig. 4 shows the effect of BBRCl and PS alone and in combination the cholesterol content in the liver. Dietary supplementation of BBRCl and PS either alone or combined together dramatically reduced cholesterol concentration in the liver of hamsters. This observation, together with lower liver weight, implies that either BBRCl or PS or combination of either can be used to treat fatty liver.

During the course of the experiment, data were obtained with regard to food consumption and bodyweight so that the dose of BBRCl was maintained on a mg/kg basis as the animals grew over the 4 weeks experimental period. Data showed that the body weight and food intake were not immediately affected by the switch from the control diet to any of the three test diets used (BBRCl alone, PS alone, or BBRCl/PS combination, see Figs. 5-6). Analysis of the data showed an unexpected trend toward reduced body weights after two weeks of feeding with BBRCl alone and after 3 weeks of

feeding with BBRCI+PS combination (see Fig. 5). These results indicate that BBRCI and the combination of BBRCI and PS may be useful in weight control in addition to the beneficial effects of lipid lowering. This may have important implications on weight control, healthy weight management strategies, and body composition (increase lean body mass) in humans and animals.

Similarly, during the early segments of the experiment the daily food intake was not affected by BBRCI until after 3 weeks (see Fig. 6). Thereafter, significant differences in food intake was observed from days 22-25 but this effect disappeared during days 25-28 as a small surgical intervention was performed on each animal on day 25 on the neck area of the animal, which reduced food consumption in each treatment. Surgical intervention was performed to permit intravenous injection of a stable isotope cholesterol tracer.

The experimental results described above show that a powerful synergistic effect of BBRCI and PS on TG reduction exists. Dramatic reductions in serum T-C and nonHDL-C occurred when BBRCI and PS were combined at the levels used in these experiments.

Experiments were also conducted to determine the effect on the expression of genes associated with cholesterol in the liver and intestine. Total RNA was extracted and mRNA was converted to cDNA. The mRNA expression was measured by real-time PCR with four repeats and calculated as relative expression in reference to internal control of a housekeeping gene for each sample.

Table 4 and Figs. 7a-7b summarize the effect of BBCI and PS alone or in combination on intestinal ABCG5 (Fig. 7a) and ABCG8 (Fig. 7b) mRNA expression. BBRCI and PS alone did not affect ABCG5 and ABCG8 mRNA expression. However, when they were administered simultaneously, the mRNA expression of both genes was significantly reduced in a synergistic mode. The function of ABCG5 and ABCG8 in the intestine is to transport cholesterol out of enterocytes and back to the intestine for elimination via the feces. Recent observations have demonstrated that the mRNA expression of ABCG5 and ABCG8 is closely and positively associated with blood cholesterol levels. When a strong action occurs on cholesterol reduction, the expressions of these two genes are down-regulated. The observation of our study (Example 1) has provided a strong support to the synergistic action of BBRCI and PS on cholesterol reduction.

Table 4

	ABCG5	ABCG8
Control	2.36±0.72[a]	1.30±0.25[a]
BBRCI	2.37±0.70[a]	1.33±0.45[a]
PS	1.97±0.72[ab]	0.99±0.32[ab]
BBRCI+PS	1.55±0.29[b]	0.69±0.17[b]

Table 5 and Fig. 8a summarize the effect of BBRCI and PS alone or in combination on liver HMG-CoA reductase. BBRCI and PS alone did not affect HMG-CoA reductase mRNA expression. However, when they were combined, the mRNA expression of HMG-CoA reductase was increased by 7-fold. HMG-CoA reductase is a rate-limiting enzyme in cholesterol biosynthesis. A large body of evidence has indicated that cholesterol synthesis is altered reciprocally with cholesterol absorption. When a strong action occurs on cholesterol reduction through inhibiting cholesterol absorption, cholesterol biosynthesis is increased as compensatory response to the cholesterol loss due to the reduced absorption. The powerful synergistic action of BBRCI and PS on HMG-CoA reductase mRNA expression implies that the combination of these materials may act synergistically to reduce blood cholesterol, possibly by inhibiting cholesterol absorption.

Table 5, Fig. 8b and Fig. 8c summarize the effect of BBRCI and PS on the mRNA expression of CYP7A1 and CYP27A1 in the liver. BBRCI and PS alone tended to increase mRNA expression of both genes. CYP7A1 and CYP27A1 are two rate-limiting enzymes controlling bile acid synthesis, which is one mechanism through which the body removes cholesterol by converting it into bile acids. When BBRCI and PS were combined, a synergistic action appeared to happen. This result implies that BBRCI and PS reduce blood cholesterol by affecting cholesterol catabolism in the liver through a synergistic action mode.

Table 5

	HMG-CoA reductase	CYP7A1	CYP27A1
Control	1.01±0.19[b]	1.03±0.44	1.06±0.27
BBRCI	0.98±0.10[b]	1.30±0.66	1.27±0.31
PS	1.34±0.48[b]	1.32±0.67	1.40±0.37
BBRCI+PS	7.52±2.60[a]	1.83±1.05	1.60±0.75

There is a balance between synthesis and clearance of cholesterol in the body. Despite efficient clearance of cholesterol, the body will compensate to try to maintain a certain baseline cholesterol level. In Example 1, the strong action of the combination of BBRCI and PS may have reduced cholesterol levels to a near minimum or base level. In such a case, no observable synergistic effect is expected even though a synergistic action was implied by the gene expressions. For this reason, a synergistic action on cholesterol reduction was not detected in Example 1 as a maximum or near maximum reduction in blood lipids was achieved. Accordingly, if a sufficiently higher blood cholesterol level is achieved by increasing dietary cholesterol intake and/or if sufficiently less BBRCI and/or PS are used in the diet, a synergistic action of cholesterol reduction should be observable. Examples 2 and 3 below describe such studies.

Example 2:

In this example, a controlled, five week animal study was performed. A total of 48 male Golden Syrian hamsters were randomized into 4 groups of 12 and fed isocaloric diets as follows. Hamster husbandry, living and feeding conditions were similar to that used for Example 1.

Control	a semi-synthetic casein-corn starch-sucrose diet that contained 0.25% (w/w) cholesterol
BBRCI	control diet containing 100 mg/kg·d of BBRCI
PS	control diet containing 1% (w/w) PS
BBRCI+PS	control diet containing 100 mg/kg·d BBRCI and 1% (w/w) PS

One objective of this example was to determine the effects of each intervention on circulating blood lipids including total cholesterol (T-C), high-density-lipoprotein cholesterol (HDL-C), nonHDL-C (which is indicative of LDL-C levels) and triglyceride (TG) profiles when hamsters were fed a diet containing a higher concentration of cholesterol, for example, 0.25% by weight in the diet.

Table 6 summarizes the effect on blood lipid levels (values are means ± SD, n=12). Data were analyzed by one-way ANOVA followed by Tukey test if significance was detected. A significant difference was indicated by a $p < 0.05$. Values with different superscripts (a, b or c) within a specific lipid group are significantly different.

Results of this study indicate that BBRCI and PS synergistically decrease blood TG levels. BBRCI and PS did not show a synergistic action on cholesterol reduction. However, as discussed in Example 1, the reason for this may still be that the

very strong cholesterol lowering action of combined BBRCI and PS was maximized under the experimental conductions (i.e. baseline levels of cholesterol were reached). Animals in Example 2 were heavier and supplemented with a higher level of dietary cholesterol in a longer feeding period than those in Example 1. As can be seen in Example 3 below, a synergistic effect on cholesterol lowering is observed when the level of cholesterol is sufficiently high and the amount of PS supplemented in the diet is reduced by 50%. The ratio of HDL-C to nonHDL-C tended to increase by BBRCI or PS alone. The combination of BBRCI and PS significantly increased the ratio of HDL-C to nonHDL-C.

Table 6

	T-C	HDL-C	nonHDL-C	HDL-C/ nonHDL-C	TG
Concentration (mg/dl)					
Control	287.2±39.3^{a}	91.5±20.1^{ab}	195.2±46.1^{a}	0.51±0.20^{b}	550.3±224.9^{a}
BBRCI	264.7±49.8^{a}	93.1±27.1^{ab}	173.5±66.5^{a}	0.66±0.41^{ab}	549.1±225.3^{a}
PS	180.2±28.2^{bc}	77.2±13.7^{b}	103.4±27.2^{b}	0.84±0.44^{ab}	369.6±137.3^{ab}
BBRCI+PS	160.9±23.8^{c}	76.3±15.7^{b}	84.6±25.3^{b}	1.00±0.49^{a}	310.3±127.3^{b}
% difference from Control					
Control	0	0	0	0	0
BBRCI	-7.8	1.7	-11.1	29	-0.2
PS	-37.3	-15.6	-47.0	65	-32.8
BBRCI+PS	-44.0	-15.6-16.6	-56.7	96	-43.6

Table 7 summarizes the effect on tissue weight to body weight ratio (values are means ± SD). Data were analyzed by one-way ANOVA followed by Tukey test when a significant treatment effect was detected. A significant difference was indicated by a $p < 0.05$. Values with different superscripts (a, b or c) for each tissue weight to body weight parameter within a tissue group are significantly different. The average liver weight was statistically lower in the PS group compared to the Control group but was not significantly lower when comparing the BBRCI group with Control. The combination of BBRCI and PS (BBRCI+PS), however, reduced the average liver weight in a strong, statistically significant, and synergistic manner compared to liver weights in the BBRCI and PS groups. The BBRCI+PS induced effect on the average liver weight was also significantly lower than in Control.

Table 7

	Heart	Liver	Right kidney
Control (n=12)	0.37±0.05	5.03±0.29[a]	0.35±0.04
BBRCI (n=12)	0.37±0.03	4.67±0.38[ab]	0.35±0.03
PS (n=12)	0.37±0.03	4.56±0.37[b]	0.36±0.03
BBRCI+PS (n=12)	0.35±0.04	3.94±0.33[c]	0.35±0.03

Example 3:

In this example, a controlled, five week animal study was performed. Four randomized groups of 12 or 6 male Golden Syrian hamsters were fed isocaloric diets as follows. The dosage of PS was reduced from 1% to 0.5% (w/w) in the diet. Hamster husbandry, living and feeding conditions were similar to that in Example 1.

Control	a semi-synthetic casein-corn starch-sucrose diet that contained 0.25% (w/w) cholesterol
BBRCI	control diet containing 100 mg/kg·d of BBRCI
PS	control diet containing 0.5% (w/w) PS
BBRCI+PS	control diet containing 100 mg/kg·d BBRCI and 0.5% (w/w) PS

One objective of this example was to determine the effects of each intervention on circulating blood lipids including total cholesterol (T-C), high-density-lipoprotein cholesterol (HDL-C), nonHDL-C (which is indicative of LDL-C levels) and triglyceride (TG) profiles.

Table 8 summarizes the effect on blood lipid levels (values are means ± SD, n=12). Data were analyzed by one-way ANOVA followed by Tukey test if significance was detected. A significant difference was indicated by a $p < 0.05$. Values with different superscripts (a, b or c) within a specific lipid group are significantly different. When BBRCI and PS were given to hamsters simultaneously, significant synergistic actions were observed on T-C, nonHDL-C and TG levels. A synergistic action of BBRCI and PS was also observed on the ratio of HDL-C and nonHDL-C. It has been demonstrated by the results of this study that BBRCI and PS act synergistically to reduce blood total cholesterol, nonHDL-C and triglyceride levels.

Table 8

	T-C	HDL-C	nonHDL-C	HDL-C/ nonHDL-C	TG
Concentration (mg/dl)					
Control	287.2±39.3[a]	91.5±20.1	195.2±46.1[a]	0.51±0.20[b]	550.3±224.9[a]
BBRCI	264.7±49.8[a]	93.1±27.1	173.5±66.5[ab]	0.66±0.41[b]	549.1±225.3[a]
PS	212.1±20.3[b]	108.2±19.1	111.2±19.0[bc]	1.02±0.37[ab]	341.4±121.7[ab]
BBRCI+PS	164.3±17.5[b]	98.5±12.7	65.9±12.3[c]	1.56±0.47[a]	278.2±89.9[b]
% difference from Control					
Control	0	0	0	0	0
BBRCI	-7.8	1.7	-11.1	29	-0.2
PS	-26.2	18.3	-43.0	100	-37.9
BBRCI+PS	-42.8	7.7	-66.2	206	-49.5

Example 4:

In this example, a controlled, six week animal study was performed in a different animal model. Six randomized groups of 10 male Sprague-Dawley rats were fed isocaloric diets as follows. The PS was introduced into the diet by blending and BBRCI was introduced by gavage feeding twice a day.

Control	a semi-synthetic casein-corn starch-sucrose diet that contained 2% (w/w) cholesterol and 28% (w/w) fat
BBRCI-1	control diet containing 100 mg/kg·d of BBRCI
PS	control diet containing 1% (w/w) PS
BBRCI-1+PS	control diet containing 100 mg/kg·d BBRCI and 1% (w/w) PS
BBRCI-2	control diet containing 200 mg/kg·d of BBRCI
BBRCI-2+PS	control diet containing 200 mg/kg·d BBRCI and 1% (w/w) PS

One objective of this example was to determine the effects of each intervention on plasma cholesterol levels in a different animal model.

Table 9 summarizes the effect on blood total cholesterol levels (values are means ± SD, n=10). A significant synergistic total cholesterol reduction was seen for the combination of either of two doses of BBRCI and PS. Results of this study demonstrate in a different animal model that BBRCI and PS synergistically reduce blood total cholesterol levels.

Table 9

	T-C (mg/dl)
Control	109.4±22.8
BBRCI-1	102.1±30.6
PS	90.0±29.0
BBRCI-1+PS	67.2±14.8
BBRCI-2	109.1±26.7
BBRCI-2+PS	83.1±18.1
	% difference from Control
Control	0
BBRCI-1	-6.7
PS	-17.8
BBRCI-1+PS	-38.6
BBRCI-2	-0.3
BBRCI-2+PS	-24.0

Other advantages that are inherent to the structure are obvious to one skilled in the art. The embodiments are described herein illustratively and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments will be evident to a person of ordinary skill and are intended by the inventor to be encompassed by the following claims.

Claims:

1. Use of a blood lipid level lowering synergistic combination of:

(a) a first blood lipid lowering agent comprising berberine, one or more pharmacologically acceptable salts thereof or a mixture thereof; and,

(b) a second blood lipid lowering agent comprising a plant sterol, plant stanol, ester of a plant sterol, ester of a plant stanol or a mixture thereof,

for lowering blood lipid levels in a blood stream of a mammal.

2. The use according to claim 1, wherein the first blood lipid lowering agent comprises berberine.

3. The use according to claim 1 or 2, wherein the second blood lipid lowering agent comprises beta-sitosterol, campesterol, stigmasterol, sitostanol, campestanol, an ester comprising a plant sterol and an unsaturated fatty acid, an ester comprising a plant stanol and an unsaturated fatty acid, or a mixture thereof.

4. The use according to claim 3, wherein the ester comprising a plant sterol and an unsaturated fatty acid, and the ester comprising a plant stanol and an unsaturated fatty acid comprise an omega-3 fatty acid.

5. The use according to claim 4, wherein the omega-3 fatty acid comprises docosahexaenoic acid, docosapentaenoic acid, eicosapentaenoic acid or alpha-linolenic acid.

6. The use according to any one of claims 1 to 5 further comprising the use of Red Yeast Rice.

7. The use according to any one of claims 1 to 6, wherein the blood lipid levels that are lowered comprise triglyceride levels.

8. The use according to any one of claims 1 to 6, wherein the blood lipid levels that are lowered comprise low-density-lipoprotein cholesterol or non-high-density-lipoprotein cholesterol levels.

9. The use according to any one of claims 1 to 6, wherein the blood lipid levels that are lowered comprise total cholesterol levels.

10. The use according to any one of claims 1 to 9, wherein the lowering of blood lipid levels treats or reduces chance of contracting cardiovascular disease, hyperlipidemia, atherosclerosis, coronary heart disease, angina, cerebrovascular disease, stroke, overweight or obesity, diabetes, insulin resistance, hyperglycemia, hypertension, arrhythmia, diseases of the central nervous system, diseases of the peripheral nervous system, fatty liver or inflammation.

11. The use according to any one of claims 1 to 9, wherein the lowering of blood lipid levels treats or reduces chance of contracting coronary heart disease, atherosclerosis, stroke, arrhythmia, myocardial infarction or sudden death syndrome.

12. The use according to any one of claims 1 to 11, wherein the mammal is a human, dog, cat or horse.

13. The use according to claim 12, wherein the mammal is a human.

14. A blood lipid level lowering composition comprising a synergistic mixture of:

(a) a first blood lipid lowering agent comprising berberine, one or more pharmacologically acceptable salts thereof or a mixture thereof; and,

(b) a second blood lipid lowering agent comprising a plant sterol, plant stanol, ester of a plant sterol, ester of a plant stanol or a mixture thereof,

for lowering blood lipid levels in a blood stream of a mammal.

15. The composition according to claim 14, wherein the first blood lipid lowering agent comprises berberine.

16. The composition according to claim 14 or 15, wherein the second blood lipid lowering agent comprises beta-sitosterol, campesterol, stigmasterol, sitostanol, campestanol, an ester of a plant sterol comprising an unsaturated fatty acid, an ester of a plant stanol comprising an unsaturated fatty acid, or a mixture thereof.

17. The composition according to claim 16, wherein the ester of a plant sterol comprising an unsaturated fatty acid and the ester of a plant stanol comprising an unsaturated fatty acid, comprise an omega-3 fatty acid.

18. The composition according to claim 17, wherein the omega-3 fatty acid comprises docosahexaenoic acid, docosapentaenoic acid, eicosapentaenoic acid or alpha-linolenic acid.

19. The composition according to any one of claims 14 to 18 further comprising Red Yeast Rice.

20. The composition according to any one of claims 14 to 19 further comprising an antiadherent, a binder, a coating, a disintegrant, a filler or diluent, a flavor, a color, a glidant, a lubricant, a preservative, a sorbent, a sweetener, an antioxidant or a mixture thereof.

21. A dosage form comprising a composition as defined in any one of claims 14 to 20.

22. The dosage form according to claim 21, which is a powder, a tablet, a capsule, a softgel, a solution, a suspension or an emulsion.

23. The dosage form according to claim 21, which is an oral dosage form.

24. A food or beverage comprising a composition as defined in any one of claims 14 to 20.

25. The food or beverage according to claim 24, which is a cereal, a snack bar, a dairy product, a fruit juice, a powdered food or a dry powder beverage mix.

26. A commercial package comprising a composition as defined in any one of claims 14 to 20 together with instructions for use in lowering blood lipid levels in a mammal.

27. A commercial package comprising a dosage form as defined in any one of claims 21 to 23 together with instructions for use in lowering blood lipid levels in a mammal.

28. A commercial package comprising a food or beverage as defined in claim 24 or 25 together with instructions for use in lowering blood lipid levels in a mammal.

29. The commercial package according to any one of claims 26 to 28, which is a bottle, jar, blister pack or box.

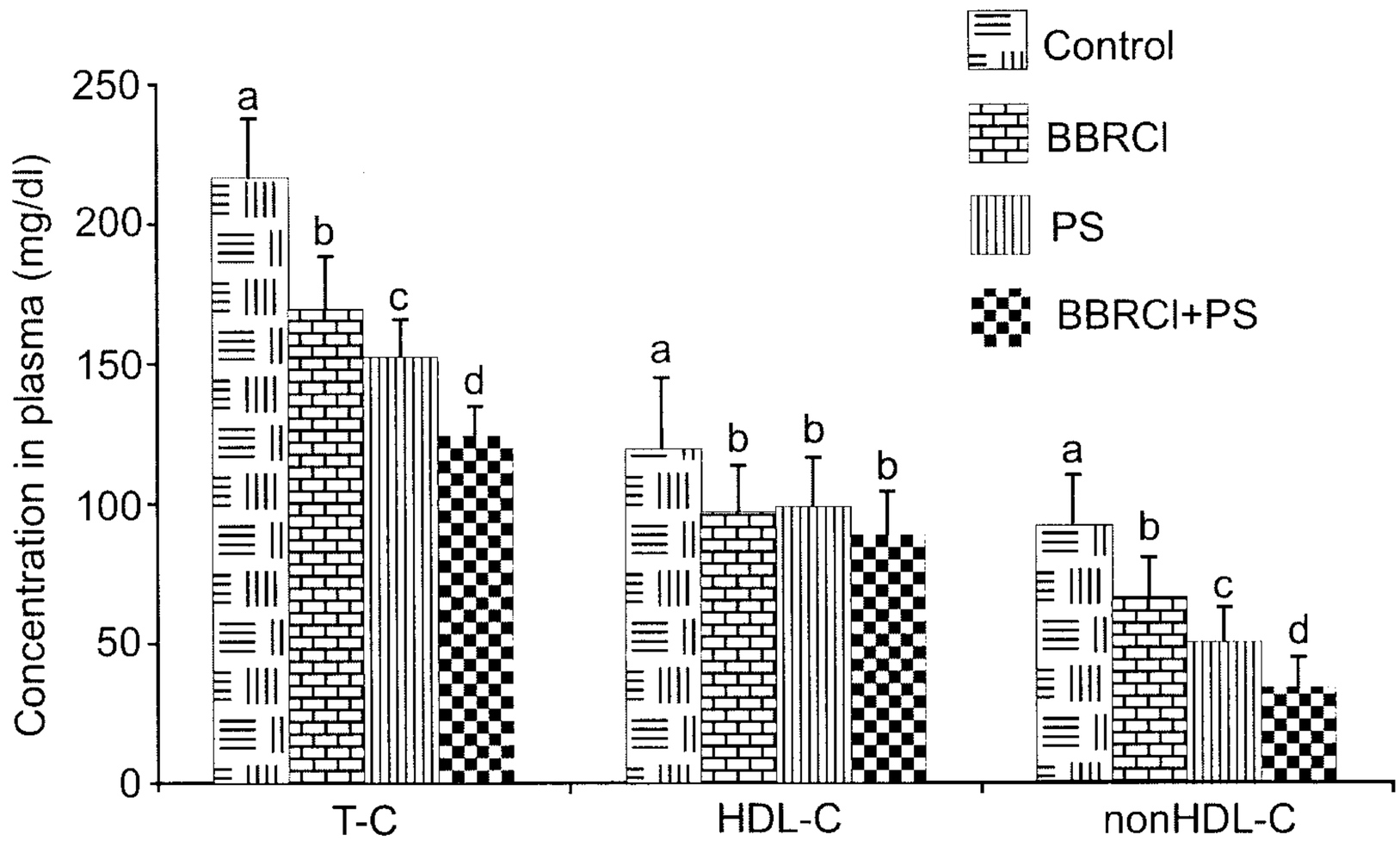

Concentration in plasma (mg/dl)
250
200
150
100
50
0
a
b
c
d
a
b
b
b
a
b
c
d
T-C
HDL-C
nonHDL-C
Control
BBRCl
PS
BBRCl+PS

FIG. 1

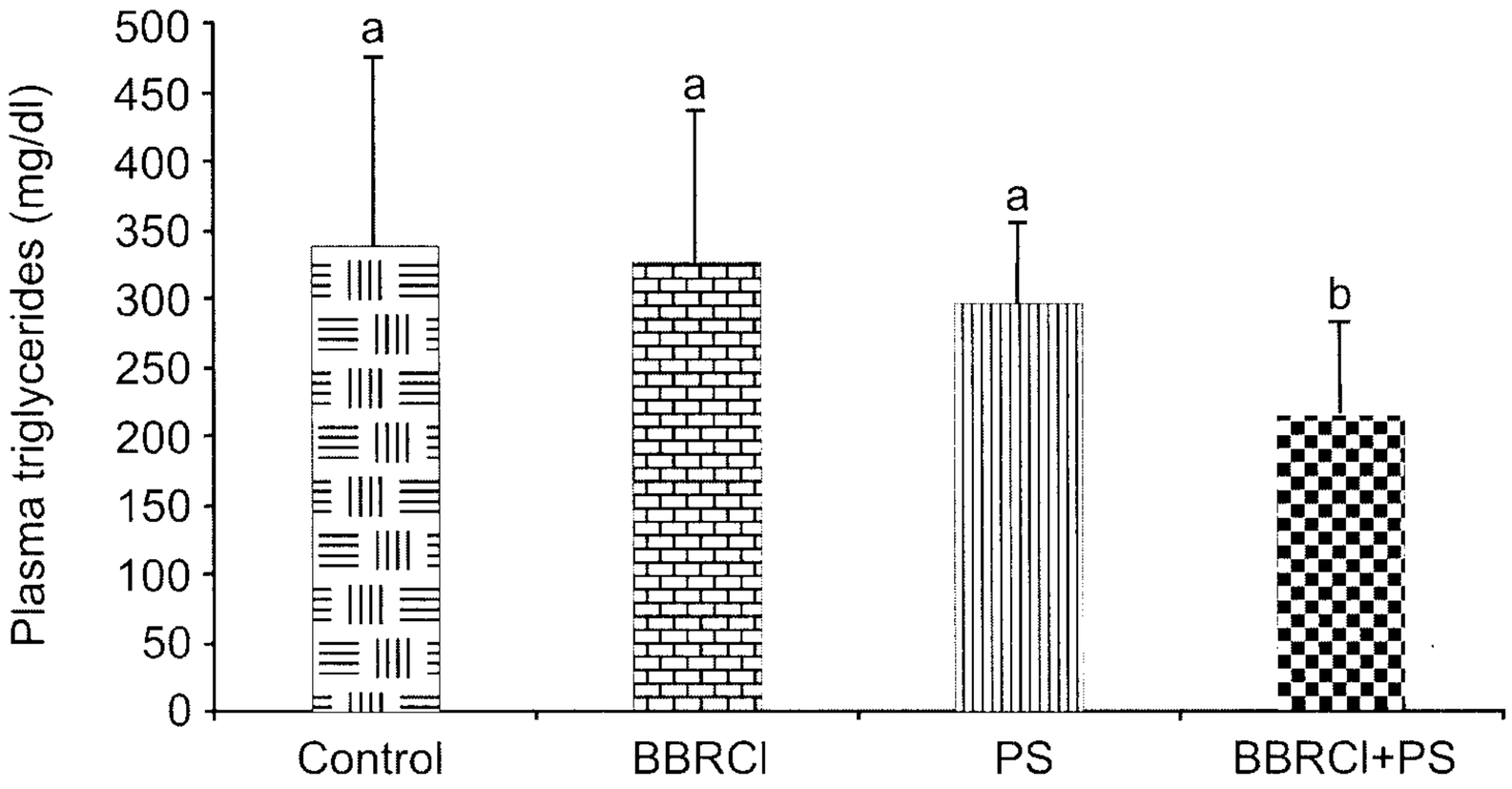

Plasma triglycerides (mg/dl)
500
450
400
350
300
250
200
150
100
50
0
a
a
a
b
Control
BBRCl
PS
BBRCl+PS
Dietary Treatment

FIG. 2

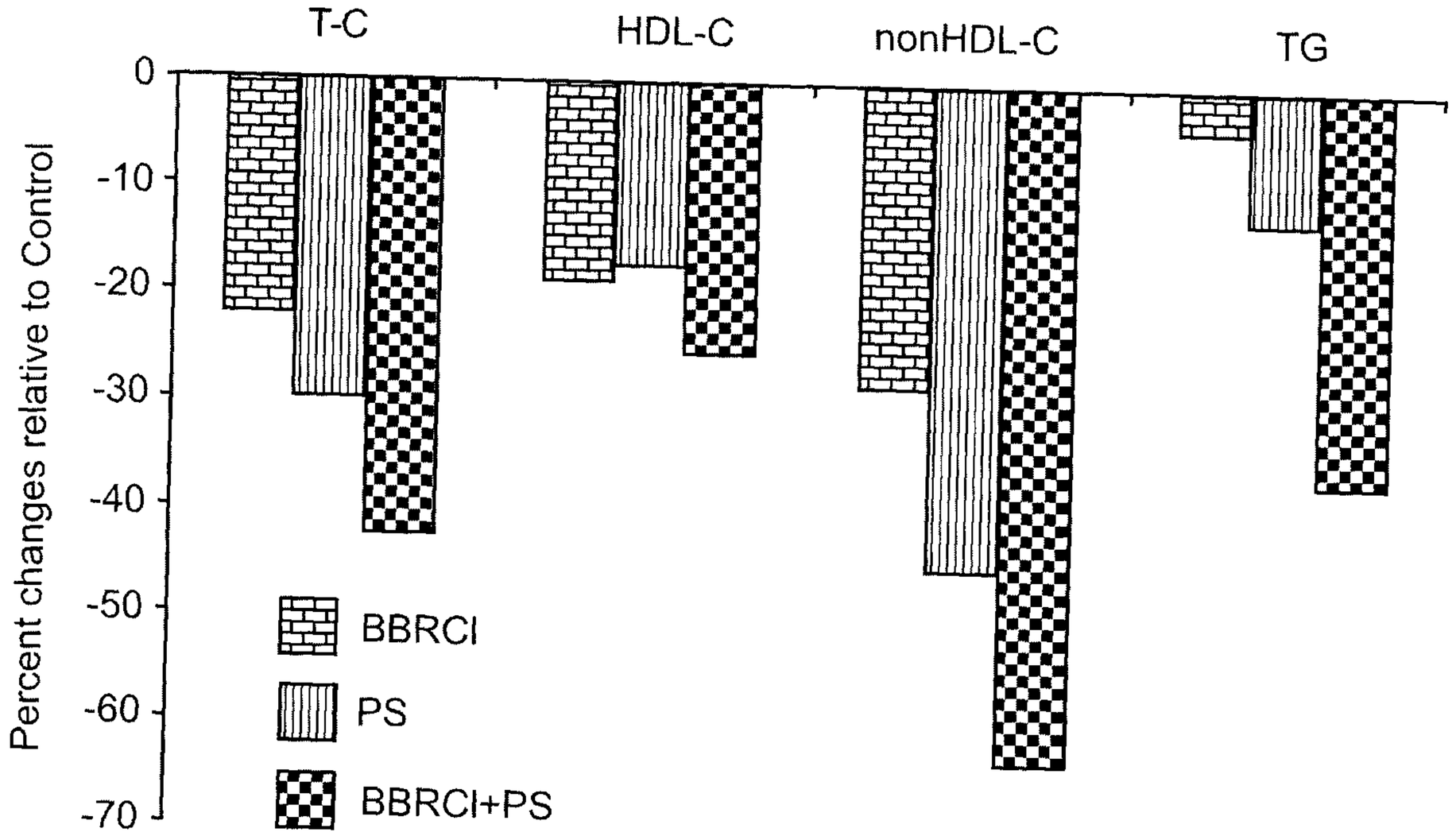

T-C
HDL-C
nonHDL-C
TG
0
-10
-20
-30
-40
-50
-60
-70
Percent changes relative to Control
BBRCI
PS
BBRCI+PS

FIG. 3

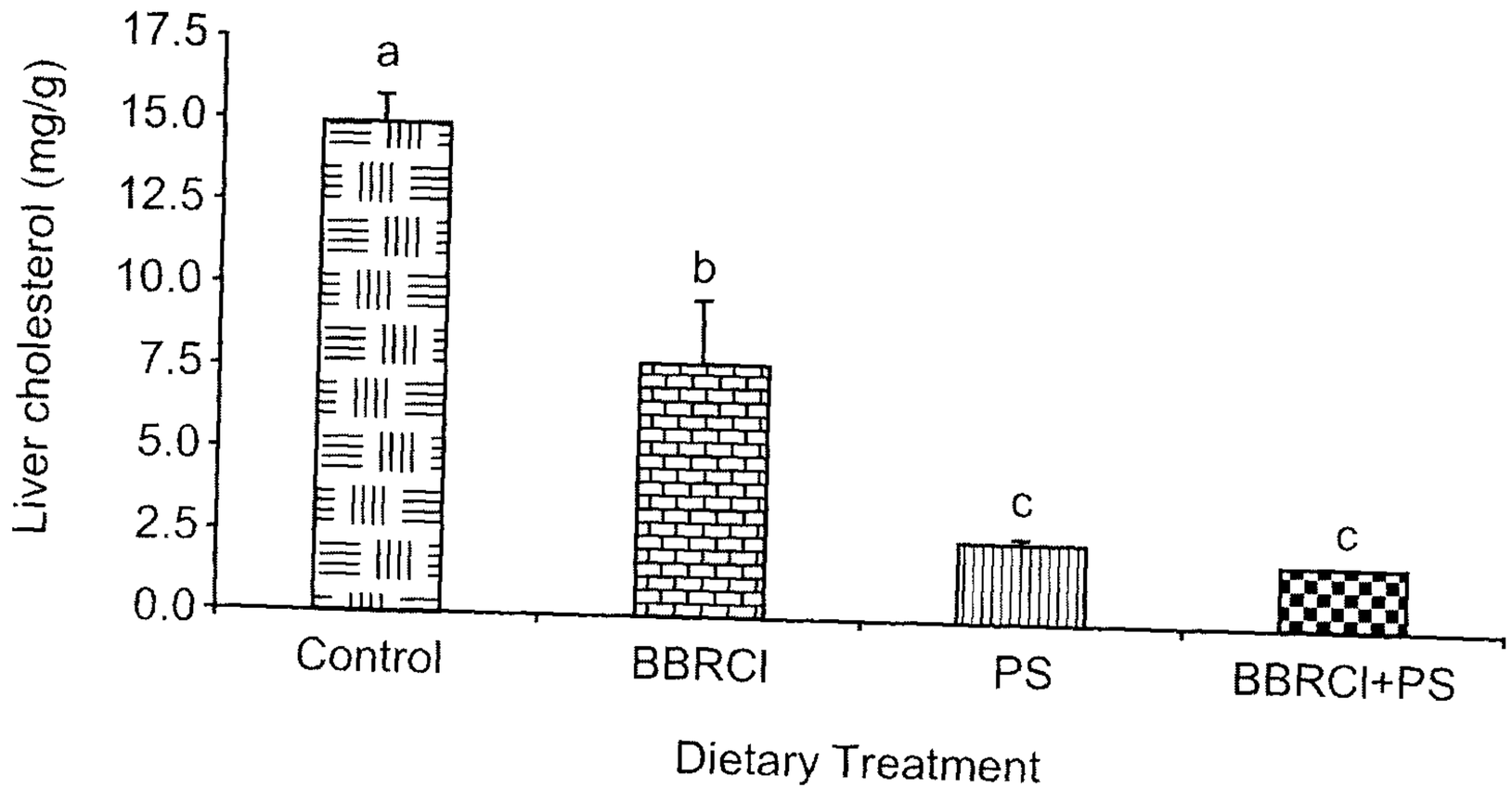

17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
Liver cholesterol (mg/g)
a
b
c
c
Control
BBRCI
PS
BBRCI+PS
Dietary Treatment

FIG. 4

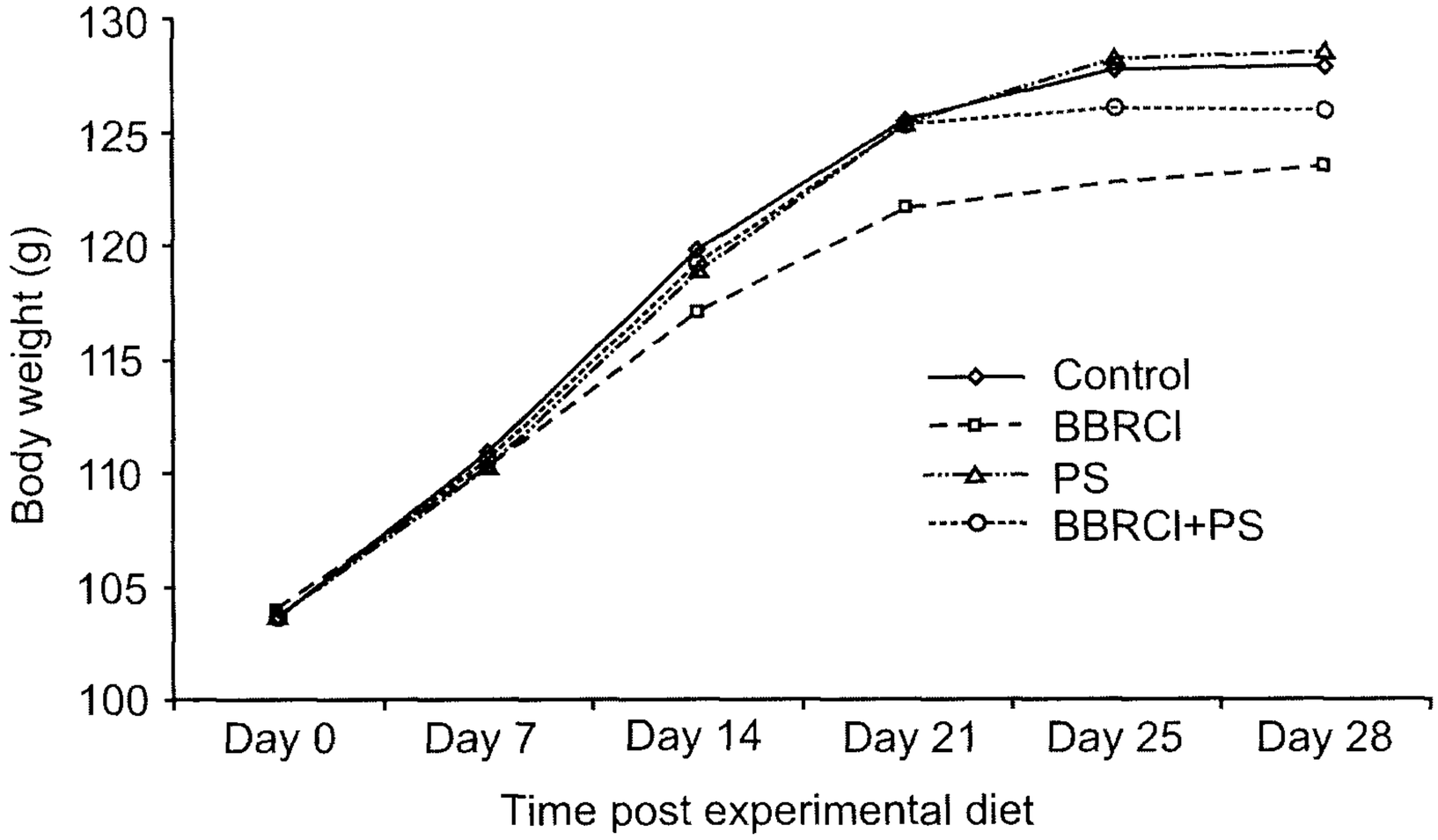

130
125
120
115
110
105
100
Body weight (g)
Day 0
Day 7
Day 14
Day 21
Day 25
Day 28
Time post experimental diet
Control
BBRCI
PS
BBRCI+PS

FIG. 5

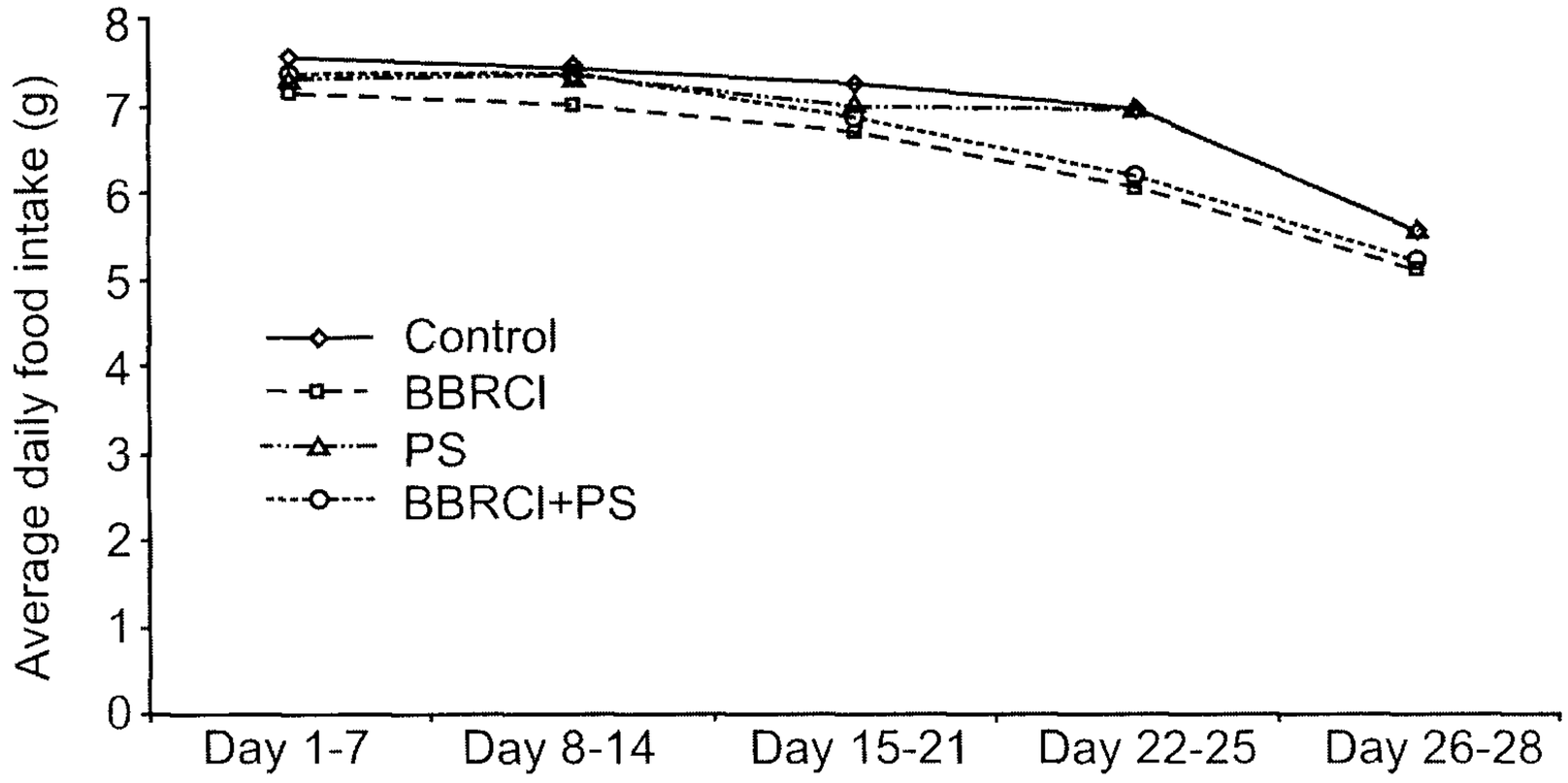

8
7
6
5
4
3
2
1
0
Average daily food intake (g)
Day 1-7
Day 8-14
Day 15-21
Day 22-25
Day 26-28
Control
BBRCI
PS
BBRCI+PS

FIG. 6

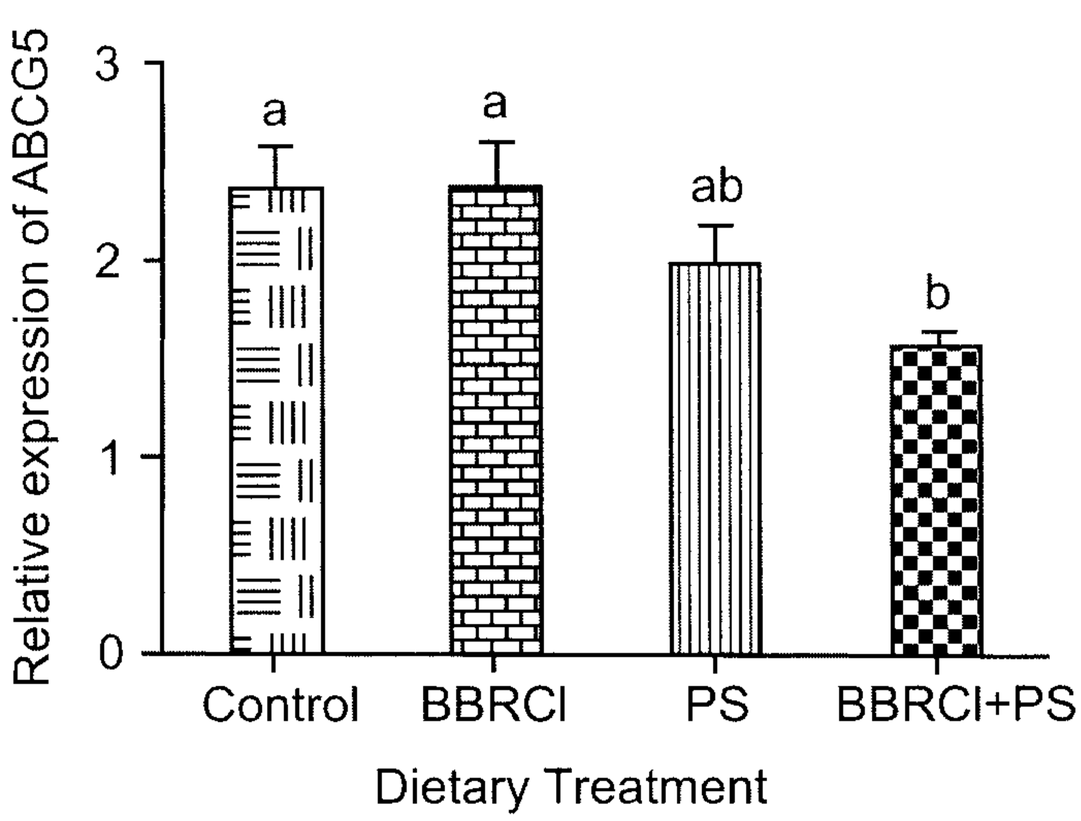

Relative expression of ABCG5
3
2
1
0
a
a
ab
b
Control
BBRCI
PS
BBRCI+PS
Dietary Treatment

FIG. 7a

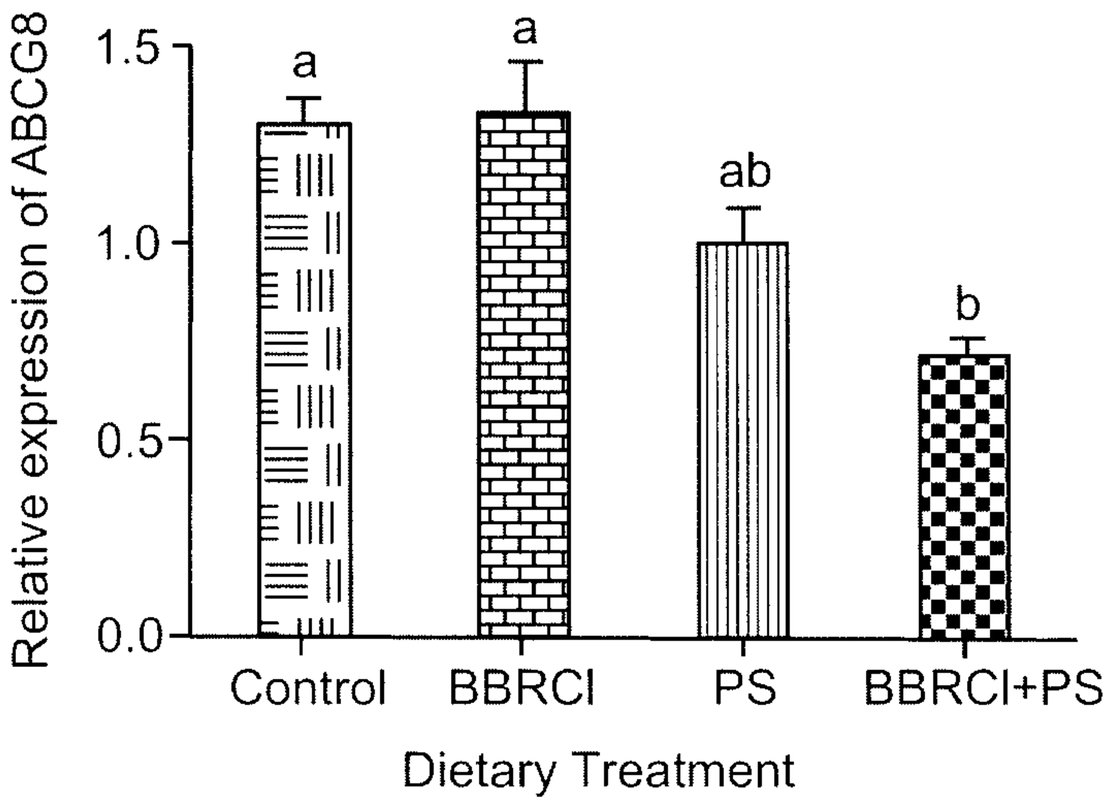

Relative expression of ABCG8
1.5
1.0
0.5
0.0
a
a
ab
b
Control
BBRCI
PS
BBRCI+PS
Dietary Treatment

FIG. 7b

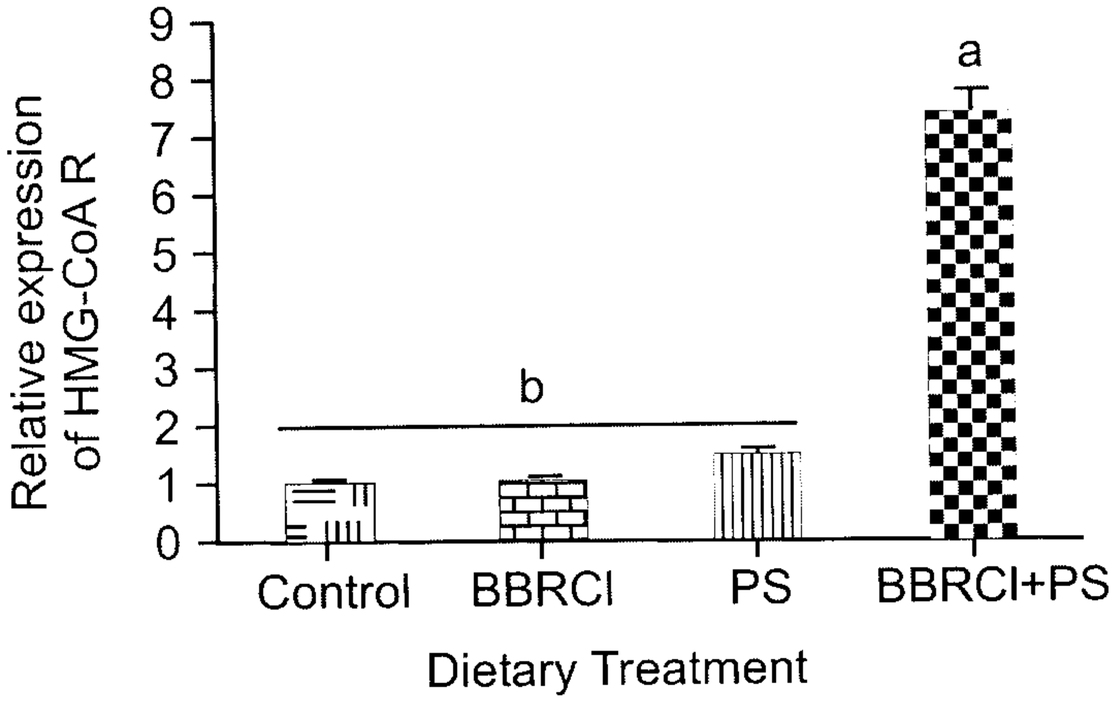


FIG. 8a

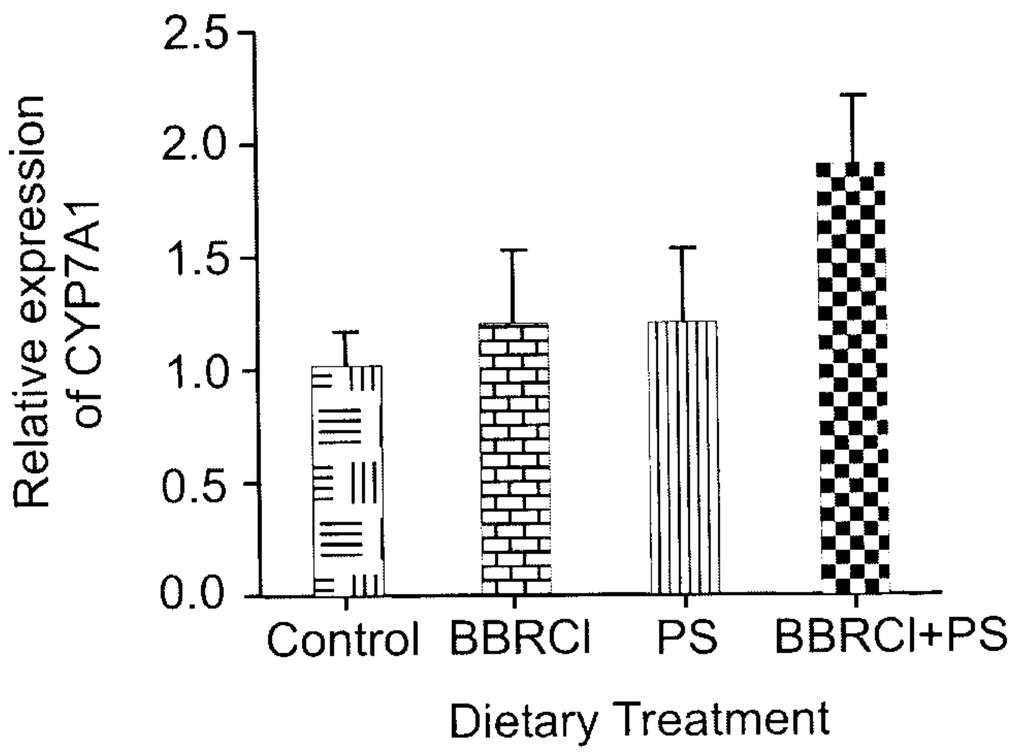


FIG. 8b

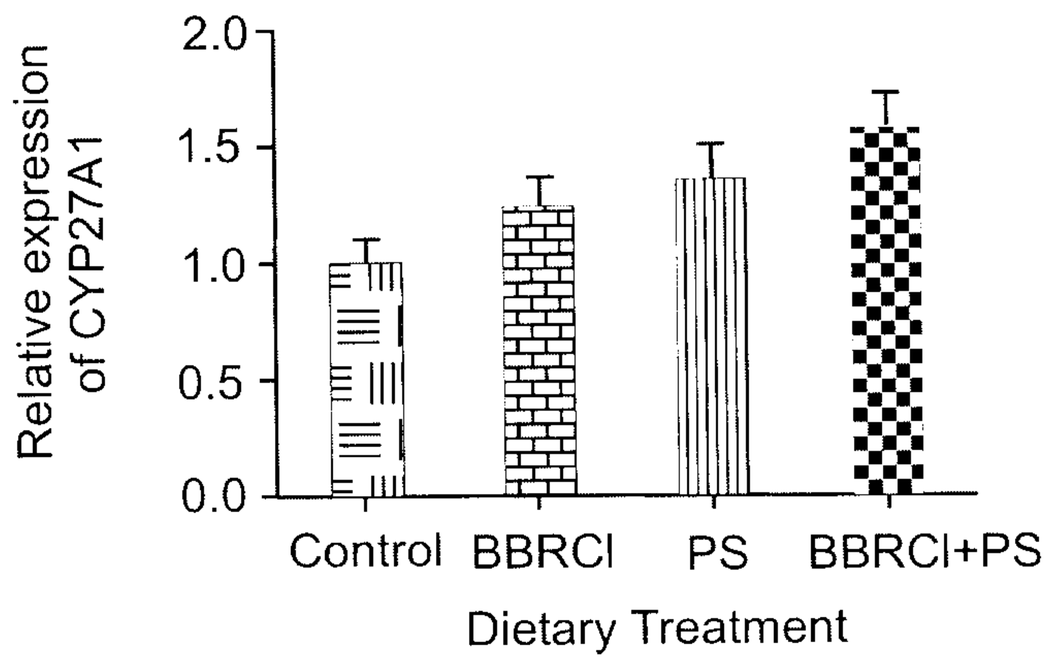
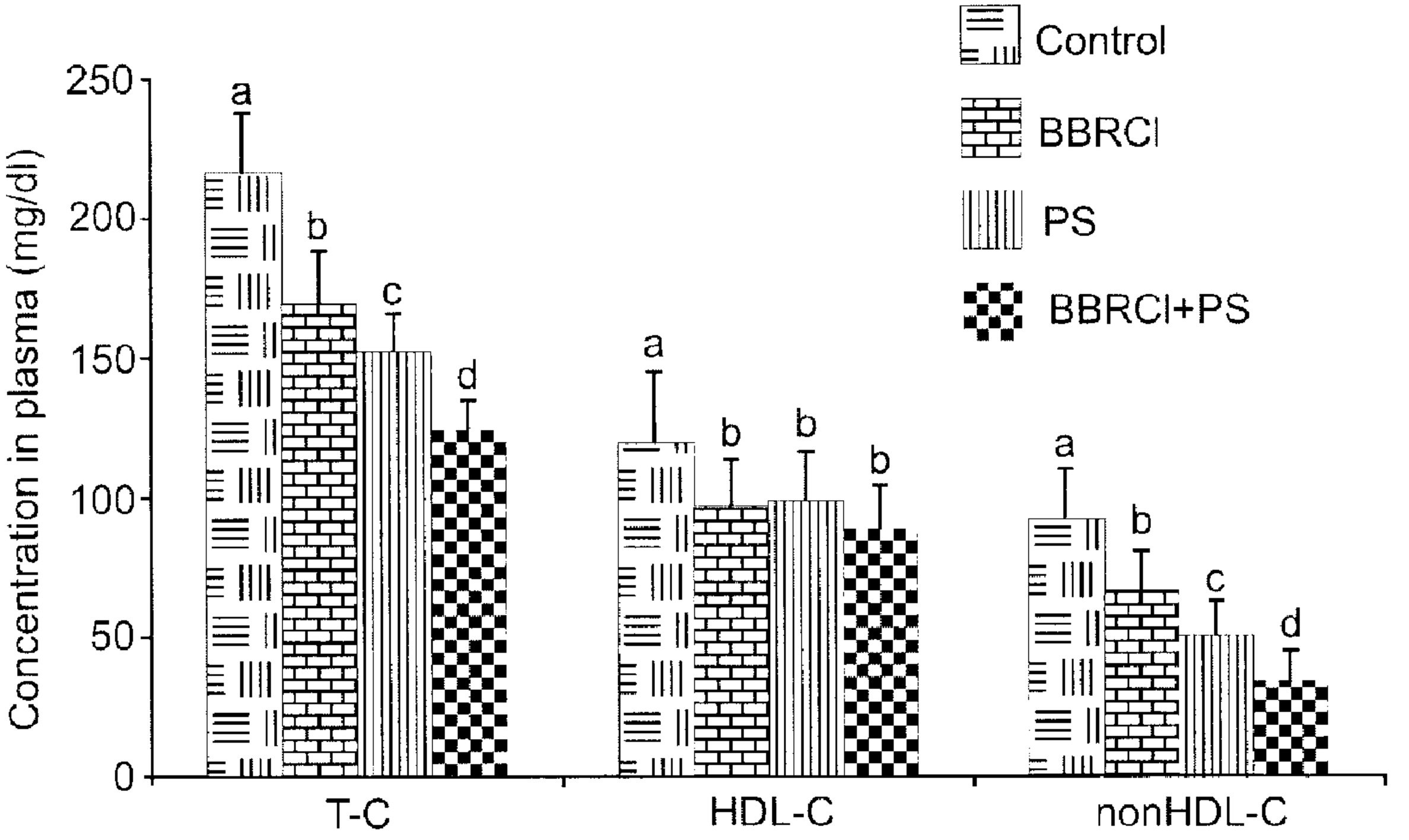


FIG. 8c


Control
BBRCI
PS
BBRCI+PS
Concentration in plasma (mg/dl)
250
200
150
100
50
0
a
b
c
d
a
b
b
b
a
b
c
d
T-C
HDL-C
nonHDL-C