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Lipomodulatory and anti-oxidative stress effects of a polyherbal formulation based on garlic and avocado seed extracts on high fat high sucrose diet fed rats

Boris K.G. Azantsa [a,*], Ntentie F. Raissa [a,b], Mbong A. Mary-Ann [a], Mafongang Amelie [a], Kamtchoum Alexine [a], Momo Cliffbrown [a], Chimou N. Lauriane [a], Fonkoua Martin [a], Edoun E. Ferdinand [a,c], Ngondi J. Laure [a], Julius E. Oben [a]

[a] Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, Faculty of Sciences, University of Yaounde 1, PO Box: 812, Yaounde, Cameroon
[b] Department of Life and Earth Science, Higher Teachers' Training College, PO Box: 55, Maroua, Cameroon
[c] Centre for Food and Nutrition Research, Institute of Medical Research and Medicinal Plant Studies, MINRESI, P.O Box 13033, Yaounde, Cameroon

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ABSTRACT

Objective: To determine antioxidant potentials of *Allium sativum and Persea americana* seeds extracts and three formulation-based extracts *in vitro*, and to evaluate the effects of the best formulation on oxidative stress and dyslipidemia on rats fed with high fat and high sucrose diet (HFHSD).

Methods: Aqueous extracts of *Allium sativum*, *Persia. americana* and three formulations were mixed at various portions (A. s/P. a; w/w): F (1:1), F (3: 1), and F(1:3). They were then tested for their antioxidant potentials *in vitro* using FRAP, DPPH and NO radicals to identify the best formulation. Four hundred (400) mg/kg b.w. of formulation F(1:1) were administered once daily for 21 days to rats previously fed with HFHSD for 8 weeks. Standard diet, vitamin E, and Atorvastatin were used as controls. After 21 days, body weight, blood glucose, lipid markers, activities of transaminases and markers of the antioxidant systems were assessed.

Results: The Formulation F(1:1) showed the best *in vitro* activity with IC_{50} values of 6.5 and 2.23 mg/mL respectively for FRAP and DPPH- radical scavenging capacity. HFHSD caused a depletion of antioxidants associated with an increase of pro-oxidants and all the lipid markers except HDL-c Treatment with F(1:1) significantly increased TAC, SOD, and catalase activities, while MDA, protein carbonyls, and NO levels decreased ($p < 0.05$). Formulation F(1:1) decreased triglycerides (119.88 ± 4.25 mg/dL) and LDL-c (3.78 ± 0.66 mg/dL) levels and significantly increased the HDL-c level: (108.07 ± 6.29 mg/mL). Furthermore, Formulation F(1:1) significantly caused weight loss (2.31%), reduced blood glucose levels (27.38%) and ALT activity.

Conclusion: The formulation F(1:1) could be a good candidate for the prevention and treatment of oxidative stress, dyslipidemia and features of metabolic syndrome.

1. Introduction

Nutrition transition is associated with changes in lifestyles and eating habits. It is a global issue mainly among populations living in low and middle-income countries [1]. Nutrition transition is responsible for the increased prevalence of cardio-metabolic diseases and metabolic syndrome worldwide [1,2]. Metabolic syndrome is defined as a constellation of conditions, including hypertension, abdominal obesity, raised lipid levels, and increased blood glucose levels [3]. It results from energy imbalance due to excessive dietary intake couple with lack of physical activity [3]. For approximately two decades, the eating habits of African have been undergoing changes marked by increased

List of abbreviations: A. s, Allium sativum extracts; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DM, Dry matter; DPPH, 2,2diphenyl -1-picrylhydrazyl; EAA, equivalent ascorbic acid; F, Formulation; FRAP, Ferric Reducing Antioxidant Power; HDL-c:, High density lipoprotein; HFHSD, high fat high sucrose diet;; IC_{50}, inhibitory concentration 50; LDL:, Low Density Lipoprotein; MDA, Malondialdehyde;; NO, Nitric oxide;; P a., Persea americana extracts; RONS, reactive oxygen and nitrogen species; RSC, Radical scavenging capacity; SD, standard diet;; SOD, Superoxide Dismutase; TAC, Total Antioxidant capacity; TC, Total Cholesterol; Vit C, Vitamin C.; vit E, vitamin E.

* Corresponding author.
E-mail address: borisazantsa@yahoo.fr (B.K.G. Azantsa).

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consumption of fast and street foods, which are rich in fats and sucrose, because of the westernization of their lifestyle [4]. Such changes are associated with the development of non-communicable diseases such as abdominal obesity, insulin resistance, and oxidative stress [5]. Consumption of high fat and high sucrose diet (HFHSD) leads to increase of excess energy supply that induces oxidative activity, alongside an insufficient antioxidant defence [6,7] involving an overproduction of reactive oxygen species (ROS) in mitochondria and mitochondrial dysfunction associated with metabolic syndrome. The excessive production of ROS damages proteins, lipids, and nucleic acids, altering physiological and biochemical functions [8].

Several drugs such as metformin, glibenclamide, statins, and inhibitors of angiotensin have been used to treat conditions associated with metabolic syndrome [3,9]. However, their use is associated not only with numerous side and adverse effects but also to huge costs. Fortunately, bioactive compounds from both edible and non-edible plants tested on animal models of metabolic syndrome have been proven to be an alternative [10]. Edible parts of plants, including fruits, vegetables, teas, and oils have been intensively used. Interest for non-edible plants as whole or as parts such as bark, stems and roots has considerably increased. This also includes parts considered as wastes, which possess bioactive compounds of therapeutic importance [11]. Avocado seeds, for instance, are reported to contain secondary metabolites, such as alkaloids, triterpenoids, tannins, flavonoids, and saponins [10,11].

Avocado or *Persea americana* belongs to the family of Laureaceae. It grows in tropical and sub-tropical regions. Almost all its parts are used in traditional medicine. The fruits are highly consumed because of their nutritional values. Fruits contain rutin, quercetin, gallic, ellagic, vanillic acids, and phytosterols of known medicinal importance [12]. Avocado oils have been proven to decrease oxidative stress and mitochondrial dysfunction in the pathogenesis of metabolic disorders [12,13]. Edible plants like garlic have been used to manage the metabolic syndrome. Garlic or *Allium sativum* belongs to the family of Alliaceae. It has been used since 1500 BC for its magical power by ancient Egyptians and has become a widely consumed spice in central Africa and around the globe. Garlic is very rich in sulfur compounds, including allicin and aliin, enzymes like allinase, and seventeen amino acids, minerals, and trace elements [14]. Extracts of garlic and avocado seeds have both the capacity to decrease cholesterol, glucose levels, body weight, cardio-protective, hypotensive, and anti-atherogenic properties [12,14]. Despite the diversity of their bioactive compounds, little data is available on the use of a formulation based on the two plant extracts. The research on the formulation reveals that *Allium sativum* potentiates the action of *Persea americana* in reducing postprandial hyperglycemia [15]. However, the effects of such a formulation on body weight, oxidative stress, and dyslipidemia have never been evaluated. This study was then carried out to: (i) determine *in vitro,* the radical scavenging capacity (DPPH-rsc, NO -rsc) and the ferric antioxidant reducing power (FRAP) of aqueous extracts of *Allium sativum (A.s) and Persea americana* seeds *(P.a)* and three formulation-based extracts; (ii) to evaluate the effects of the best formulation on oxidative stress *in vivo*, as well as its modulatory effects on dyslipidemia, hyperglycemia and body weight of rats fed with high fat and high sucrose diet.

2. Methods

2.1. Chemicals and reagents

Reagents were purchased from Sigma Aldrich (Germany Inc. Co. Ltd). Vitamins C and E, ethanol 95° and atorvastatin were purchased in a local pharmacy.

2.2. Plant sample collection and extracts' preparation

Allium sativum was bought at Mokolo market (Yaounde). Fruits *of Persea americana* were harvested at Mendong quarter in the locality of Yaounde- Cameroon. They were identified at the National Herbarium under the voucher numbers: No. 44810HNC and No 31940 HNC for *Allium sativum* and *Persea americana* respectively. Bulbs of *Allium sativum* and seeds of *Persea americana* were removed, chopped into small pieces, and air-dried until constant weight. Each dried part was then blended into powder. One hundred grams (100 g) of powder was macerated in 500 ml of distilled water for 24 h. A rotavapor was used to remove water and the recovered extract was dried in an oven at 50 °C for 4 h. The powder obtained was stored in polyethylene bags to avoid rehydration.

2.3. Preparation of formulation-based extracts

Aqueous extracts of *Allium sativum* (A.s) and *Persea americana* seeds (P. a) were prepared. Upon this, they were then mixed in various proportions of *Allium sativum* and *Persea americana* respectively (weigh/weight) as follows:

i the first formulation: F(1:1) made up of 200 mg of *A. s.* + 200 mg of *P. a.*;
ii the second formulation: F(1:3), made up of 100 mg of *A. s.* + 300 mg of *P. a.* and
iii the third formulation: F (3:1) made up of 300 mg of *A. s.* + 100 mg of *P. a.*

Each extract and each formulation was then dissolved in 1 ml distilled water and used for *in vitro* assays.

2.4. In vitro assessment of antioxidant potential and selection of the best formulation

Each extract and each formulation was tested for Ferric Reducing Antioxidant Power (FRAP) and (2,2) -diphenyl -picrylhydrazyl (DPPH) radical scavenging capacity (RSC).

2.5. Determination of DPPH radical scavenging capacity (DPPH –RSC)

DPPH-RSC was evaluated as described by Katalinié et *al.* [16]. Fifty (50) µL of extracts or formulations at the concentrations of 20, 25, 30, 35, and 40 mg/mL were added to 1.95 ml of alcoholic DPPH freshly prepared. The mixture was incubated in darkness for 30 min and the optical density was read at 515 nm. The control consisted of a mixture of vitamin C (50 µg/mL) and DPPH reagent. DPPH –RSC was calculated as follows:

$$\text{DPPH} - \text{RSC}(\%) = \frac{[\text{OD}(0\text{min}) - \text{OD sample } (30\text{min})]}{\text{OD Control}(0\text{ min})} \times 100$$

OD: optical density

Determination of Ferric Reducing Antioxidant Power (FRAP)

The capacity of an antioxidant to reduce Fe^{3+} into Fe^{2+} was evaluated as described by Jayaprakash et al. [17]. Results were expressed as percentage (%) reduction as follows:

$$\text{Reduction}(\%) = \frac{\text{OD FeCl3} - \text{OD control}}{\text{OD control}} \times 100$$

OD: optical density.

2.7. In vivo assessment of the best formulation

2.7.1. Animals and ethical considerations

Twenty-five (25) albino Wistar male rats weighing 190–200 g and aged ten (10) weeks were obtained from the animal house of the Department of Biochemistry of the University of Yaounde 1 in

Cameroon. They were handled with good care during the experiment accoding to ARRIVE guidelines. The rats were acclimated for two weeks in cages of 5 each. They were fed with a standard diet (SD) in 12 h day -light cycle; under ambient temperature (25 °C). They received water *ad-libitum*. After acclimation, the rats were randomly allocated to two groups, Group-SD (05 rats) and group-HFHSD (20 rats), fed during 8 weeks with standard diet (SD), high fat and high sucrose diet (HFHSD) respectively. Different diets were formulated (Supplementary Table 1) from modified protocols of Ble-Castillo et al. [18]: the standard diet or Normal Diet (SD) and the high-fat high sucrose diet (HFHSD). The HFHSD contained carbohydrates (57.66%) and lipids (37.46%) for an energy intake of 414.3 kcal. In addition, HFHSD groups of rats were supplemented with 5% fructose added in their water. Food consumption was measured daily, while weight (Fig. 1A) and fasting blood glucose (Fig. 1C) were monitored weekly. The study protocol was approved by the ethical review board of the University of Yaounde1.

2.7.2. Experimental design

After feeding the animals for 8 weeks, those with weight increase above 32% and persistently high hyperglycemia (Fatsting Blood Gluose >126 mg/dL) were selected in the HFHSD group and randomly assigned to four groups of five animals each. All the groups were treated as follows:

Group 1 (Negative control; SD): rats under Standard Diet (SD) + distilled Water; n = 5.

Group 2 (Positive control, HFHSD): rats under HFHSD + distilled water; n = 5.

Group 3: rats under HFHSD + 400 mg/kg b.w of the formulation F (1:1, w/w). The formulation contained (200 mg P. a.). + 200 mg A.s); n = 5.

Group 4: rats under HFHSD +10 mg/kg b.w of Atorvastatin; n = 5.

Group 5: rats under HFHSD +10 mg/kg b.w of Vitamin E; n = 5.

Formulation F(1:1) was prepared and administered once daily through oral route for 21 days in a volume of distilled water of 5 mL/kg b w. Vitamin E was dissolved in 5 mL/kg b. *w* of vehicle. Food intake, body weight, and blood glucose level were monitored. Atorvastatin and vitamin E were used as reference drugs for quality control of results.

2.7.3. Sacrifice and sample preparation

At the end of the experiment, all animals were sacrificed by gentle cervical dislocation. Blood was collected from the neck in EDTA tubes to prepare plasma and hemolysates. Organs including liver, heart, brain, and pancreas were isolated and chopped to prepare homogenates.

2.8. Assessment of markers of oxidative stress in vivo

1. Total antioxidant capacity (TAC)

The total antioxidant capacity was determined by the method described by Prieto et al. [19]. It was based on the reduction of molybdenum Mo (VI) into molybdenum Mo (V) by an extract to form a green complex of phosphate Mo(V) at acidic pH, which absorbs at 695 nm. Results are expressed as milligram equivalent ascorbic acid/g of dry matter (mg EAA/g DM).

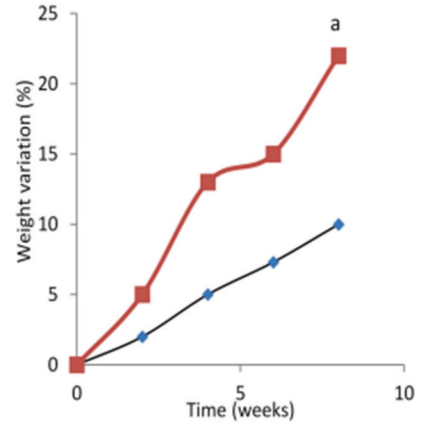

Fig. 1A: Weight variation during induction

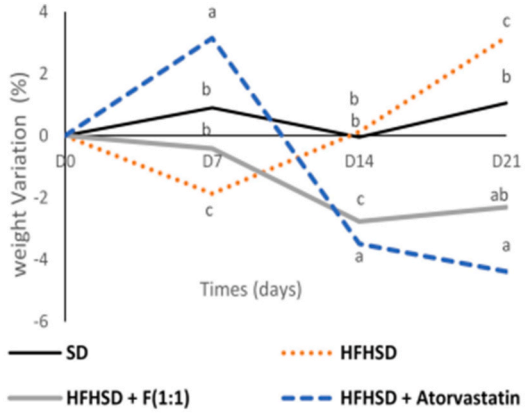

Fig 1B: Weight variation during treatment

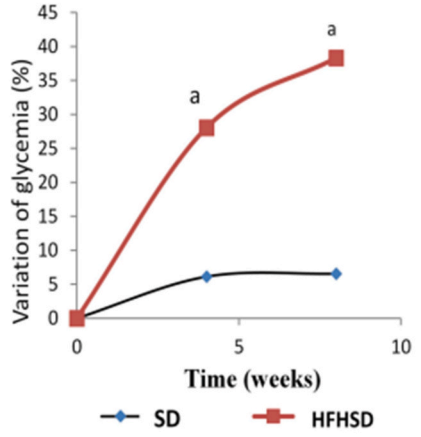

Fig. 1C: Glycemic variation during induction

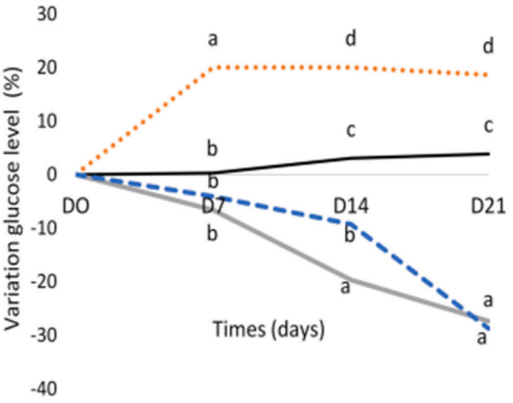

Fig. 1D: Glycemic variation during treatment

Fig. 1. Variation of weight and fasting blood glucose during induction (A, C) and treatment (B, D)
Results are expressed as % variation; HFHSD: High fat, high sucrose Diet; SD: Standard diet; Different letters indicate a significant difference between groups at each time; F: Formulation;
F
(1:1): 200
mg of *Allium sativum* + 200 mg of *Persea americana* seeds (1:1; w/w).

2. *Assessment of the* superoxide dismutase (SOD) activity

Determination of superoxide dismutase (SOD) activity was done based on sample's ability to inhibit adrenaline oxidation into adrenochrome. Adrenochrome develops a colour that absorbs at 480 nm as described by Misra and Fridovish [20]. Briefly, 0.2 mL of sample was added to 2.5 ml of sodium carbonate. The reaction started by adding 0.3 mL of adrenaline freshly prepared in a buffer. Absorbance was read at 480 nm at 30 and 120 s. Standards contained 2.5 ml buffer, 0.3 mL adrenaline and 0.2 ml distilled H_2O. A unit SOD was defined as the quantity of SOD needed to cause 50% inhibition of adrenaline's oxidation into adrenochrome in 1 min following the formula:

$$\Delta \mathrm{DOmin} = \frac{OD\ (t = 120s) - DO\ (t = 30s)}{2}$$

Inhibition was calculated as follows:

$$\mathrm{Inhibition}(\%) = \frac{\Delta \mathrm{OD\ blank} - \Delta \mathrm{OD\ sample}}{\Delta \mathrm{OD\ blank}} \times 100$$

$$\mathrm{SOD\ unit/mg\ protein} = \frac{\mathrm{SOD\ unit/m}}{\mathrm{Protein\ mg/ml}} \times \mathrm{dilution,\ with\ 50\%\ inhibition} = 1\ \mathrm{unit}$$

3. Assessment of catalase activity

Catalase activity was evaluated according to a protocol developed by Singh [21] based on the fact that catalase hydrolysis hydrogen peroxide into water and oxygen.

4 ***Assessment of lipid peroxidation*: *Malondialdehyde (MDA)***

Malondialdehyde reacts with thiobarbituric acid (TBA) to form chromophores which absorb at 532 nm as described by Yagi [22]. The concentration of MDA was determined using a molecular extinction coefficient (ε = 1.53 105 M − 1 cm-1) expressed as:

Concentration of malondialdehyde (MDA) in (μmol/L) = OD/ε L

5. Assessment of p rotein carbonyls

In presence of dinitrophenyl hydrazine, carbonyl (-COO) groups of proteins form dinitrophenyl hydrazone yellowish orange colour which absorbs at 370 nm according to the protocol defined by Levine et al. [23] Results were expressed using Beer Lambert formula: **OD = εLC**;

Molecular Extinction coefficient ε = 22000 M^{-1}cm; L = 1 cm

6 ***Assessment of cardiac nitric oxide (NO•) radical level***

It is based on the di-azotation reaction as described by Griess [24]. Results were expressed according to the Beer-Lambert formula: **NO (μM) = OD/εL:**

L = 1 cm, Molecular Extinction coefficient ε = 39500 M^{-1} cm^{-1}

7. Evaluation of plasma and homogenates total proteins

Total proteins were determined based on a method described by Lowry et *al.* [25] and results were expressed as follows:

$$\mathrm{protein\ Concentration\ (g/L)} = \frac{\mathrm{DO\ (sample)}}{\mathrm{DO\ (standard)}} \times \mathrm{standard\ concentration\ (g/l)}$$

Albumin concentration = 4.24 g/dl

2.9. Assessment of lipid markers

Plasma Triglycerides and Cholesterol levels were determined using commercial assay kits (CHRONOLAB™).

1. Measurement of triglycerides' concentration

Triglycerides level were evaluated as described by Fossati and Principe [26], with a combination of enzymes, involving lipoprotein lipase, glycerol kinase (G.K), and peroxidase (POD). Colour intensity was proportional to the quantity of triglycerides in the sample.

2. Assessment of total cholesterol level

The total cholesterol (TC) level was determined using the protocol developed by Roeschlau et *al.* [27]. Briefly, 10 μL of sample or standard were mixed with 1000 μL of reagent followed by homogenization and incubation for 10 min at 25 °C. Absorbance was read at 505 nm against a blank containing reagent only.

3. Measurement of HDL-cholesterol level

HDL-cholesterol was analysed using a protocol described by NCLS [28]. The reaction is based on the precipitations of LDL-c, VLDL, and chylomicrons by the use of surfactant. The quantification of HDL-c followed by a procedure similar to that of total cholesterol.

4. Determination of LDL-cholesterol concentration

LDL-cholesterol level was obtained from a formula developed by De Cordova et al. [29] as follows:

LDL-cholesterol = ¾ (TC – HDL-c)

2.10. Treatment biosafety: determination of transaminase activities

Liver integrity and function were examined via transaminase activities: alanine aminotransferase (ALT) and aspartate aminotransferase (AST), according to protocols developed by Reitman et Frankel [30].

2.11. Data processing and statistical analysis

The results were expressed as Mean ± Standard Error or as percentages. They were analysed using statistical packages for social sciences 17.0 (*Chicago Illinois Inc.*) Variations were compared between groups using Chi-square test and one-way analysis of variance (ANOVA) followed by post hoc Tukey. Regression analysis conducted on *Graphpad prism 5.0* permitted the determination of inhibition concentration 50 (IC_{50}). The results were considered significant for $p < 0.05$.

3. Results

3.1. Antioxidant potentials of extracts and selection of the most active formulation

DPPH radical scavenging capacity increased with increasing concentrations of extracts and formulations. *Persea americana* extracts showed the highest DPPH RSC compared to F(3:1) (Supplementary Fig. 1B). As concerns the Iron reducing power (FRAP) of extracts and formulations, it was observed that at lower and higher concentrations, the percentage reduction of Fe^{3+} into Fe^{2+} of extracts remained relatively low for the formulation F(1:3). At concentrations greater than 7.5 mg/mL, FRAP was higher in the other extracts and vitamin C used as reference (Supplementary Fig. 1B). Regression analysis from DPPH and

FRAP data permitted the determination of the inhibitory concentration 50 (IC_{50}) of extracts and formulations. The formulation F(1:1) showed the lowest IC_{50} for both DPPH free radicals and ferric reducing antioxidant power compared to other formulations and was then selected as the best or the most active (Table 1).

3.2. Induction of parameters of metabolic syndrome

Feeding rats with HFHS during 8 weeks increased body weight ($p < 0.05$) up to 23% compared to 10% in rats under a standard diet ($p < 0.05$). The increase in body weight (Fig. 1A) was accompanied by an excessive increase in blood glucose level 38.33% against 6.57% in the group under standard diet. During the first four weeks, the increase was proportional to time, and from week 4 to week 8 the rise in glycaemia reduced in HFHS fed rats but remained constant in standard diet-fed rats (Fig. 1C). As regards food consumption of rats, it was observed that from week 1, rats under standard diet and HFHS consumed more food than those in the groups treated with F (1:1) and atorvastatin while in the group receiving formulation F(1:1), food consumption level remained unchanged all through the experimental period (Supplementary Fig. 1).

3.3. Effect of formulation F (1:1) on weight's variation

Rats treated with the formulation F (1:1) showed a significant and constant reduction of their weight all through the treatment period (up to 2.31% at the end) compared to the group receiving atorvastatin, which rather exhibited an increase of weight in the first week followed by a decrease in the next two weeks (reduction of 4.39%) (Fig. 1B).

3.4. Hypoglycemic effects of the formulation F(1:1)

The high-fat high sucrose diet caused a significant increase of blood glucose level from week 1 to the end of the induction period (final increase of 18.6% versus 3.86% for the SD group at week 8) (Fig. 1C). On the other hand, treatment with the formulation F(1:1) gradually and consistently reduced blood glucose level (reduction of 27.38%) during the treatment period (21 days). Such reduction was also observed with the group receiving atorvastatin ($p < 0.05$) (Fig. 1D).

3.5. Modulatory effects of the formulation F(1:1) on oxidative stress

Formulation F(1:1) reduced Malondialdehyde (MDA) levels and increased total antioxidant capacity. The HFHS diet caused an increase ($p < 0.05$) of MDA levels in the liver and plasma compared to the standard diet. Administration of the formulation once daily for 21 days reduced MDA levels in all the organs (Table 2). In addition, the results revealed a depletion of TAC in the heart and pancreas of rats fed with the HFHS diet compared to those fed with the Standard Diet. Treatment with F(1:1) increased total antioxidant capacity (TAC) in those organs though their levels remained lower than in the SD group.

Table 1
Inhibitory Concentration 50 (IC_{50}) of extracts and formulations.

	Extracts		Formulations			Reference
	A. s only	*P. a* only	F (1:3)	F (3:1)	F (1:1)	Vit C
DPPH (mg/mL)	29.81	17.79	30.61	28.88	2.23	0.49
FRAP (mg/mL)	6.17	18.53	9.84	86.27	6.50	2.83

A. s: Allium sativum; P. a.: Persea americana; F: formulation; IC_{50}: inhibitory concentration 50; F (1:3): represents 1 portion (weight) of *A. sativum* and 3 portions (weight) of *P. americana*; Vit C: Vitamin C. FRAP: Ferric Reducing Antioxidant Power; DPPH: 2,2 diphenyl -1-picrylhydrazyl. Assays were done in triplicates.

Table 2
Effect of the formulation on malondialdehyde and Total antioxidant capacity.

	Malondialdehyde (MDA) in µmol/L			Total Antioxidant Capacity (TAC) in (mg EAA/g DM)	
	Liver	Plasma	Pancreas	Heart	Pancreas
SD	7.84 ± 0.09¶	4.34 ± 0.04†	3.79 ± 0.13†	4.38 ± 0.01¶	4.09 ± 0.04¶
HFHSD	8.97 ± 0.06§	5.17 ± 0.12¶	3.94 ± 0.09†	3.40 ± 0.02*	1.88 ± 0.11*
HFHSD + F (1:1)	6.95 ± 0.08†	3.48 ± 0.48*	2.42 ± 0.41*	3.75 ± 0.03†	2.65 ± 0.05†
HFHSD + Vit E	1.41 ± 0.12*	4.46 ± 0.02¶†	4.03 ± 0.06†	3.68 ± 0.02†	2.75 ± 0.01†

Results are expressed as mean ± standard error; EAA: equivalent ascorbic acid; DM: matter.
SD: standard diet; HFHSD: high fat high sucrose diet; vit E: vitamin E; values with different.
Symbols *, †, ¶, § in the same columns are significant ($p < 0.05$). F (1:1): represents 1 portion.
(weight) of *A. sativum* and 1 portion (weight) of *P. americana.*

3.6. Formulation F(1:1) reduced protein carbonyls and cardiac nitric oxide

Feeding rats with HFHS induced an increased level of protein carbonyls in the brain and liver as well as the NO radical in the heart compared to animals fed with the standard diet. Treatment of rats with F (1:1) at 400 mg/kg b. w. reduced nitric oxide level compared to the group under HFHSD. However, in the liver of treated rats, carbonyls level was lower compared to the control group HFHSD + Vitamin E (Table 3).

3.7. Formulation F(1:1) improves enzymatic antioxidant systems: SOD and catalase activities

In rats fed with HFHSD for 8 weeks, a depletion of SOD was noted in the brain, liver and hemolysate as compared to those fed with standard diet. Administration of the formulation F(1:1) at 400 mg/kg b. w, once daily during 21 days increased catalase activity in the erythrocytes (Table 4).

3.8. Modulatory effects of formulation on markers of lipid metabolism and liver integrity

In HFHSD fed rats, alteration of the lipid markers was noted compared to the group fed with the Standard Diet (SD). Total cholesterol, triglycerides, LDL-c cholesterol significantly increased, while HDL-c decreased in the group fed with High fat high sucrose diet (HFHSD) compared to the group fed with Standard Diet. Administration of the formulation F(1:1) for 21 days, significantly improved the profile compared to the positive control (group receiving only HFHSD) and the group receiving atorvastatin (Fig. 2A). Formulation F(1:1) decreased

Table 3
Effect of the formulation on carbonyls and nitric oxide.

	Protein carbonyls (µmol/g proteins)		Nitric oxide (µmol/L)
	Brain	Liver	Heart
SD	1.59 ± 0.04¶	0.19 ± 0.02*	25.787 ± 0.44¶
HFHSD	2.08 ± 0.09§	1.72 ± 0.22†	30.58 ± 0.07§
HFHSD + F (1:1)	1.23 ± 0.06†	0.38 ± 0.08*	16.83 ± 0.20†
HFHSD + Vit E	0.95 ± 0.05*	1.10 ± 0.04†	10.94 ± 0.19*

Results are expressed as mean ± standard error; SD: standard diet; HFHSD: high fat high sucrose diet; vit E: vitamin E; values with different symbols *, †, ¶, § in the same columns are significant ($p < 0.05$). F (1:1): represents 1 portion (weight) of *A. sativum* and 1 portion (weight) of *P. americana*.

Table 4
Effect of the formulation F(1:1) on Superoxide dismutase (SOD) and catalase.

	Superoxide dismutase (unit/mg protein)				Catalase (*μmol*/min/mg)
	Heart	Brain	Liver	Hemolysate	Hemolysate
SD	2.18 ± 0.03†¶	1.88 ± 0.03†	1.97 ± 0.07†	2.17 ± 0.08†	19.0 ± 0.3*
HFHSD	1.99 ± 0.02†	1.55 ± 0.04*	1.49 ± 0.02*	0.88 ± 0.07*	19.0 ± 0.3*
HFHSD + F(1:1)	2.37 ± 0.10¶	1.98 ± 0.03†	2.04 ± 0.01†	2.16 ± 0.07†	20.0 ± 0.1†
HFHSD + Vit E	1.68 ± 0.04*	1.97 ± 0.05†	2.34 ± 0.07¶	2.48 ± 0.04¶	19.0 ± 0.2*

Results are expressed as mean ± standard error; SD: standard diet; HFHSD: high fat high sucrose diet; vit E: vitamin E; values with different symbols *, †, ¶, § in the same columns are significant ($p < 0.05$). F (1:1): represents 1 portion (weight) of *A. sativum* and 1 portion (weight) *P. americana*.

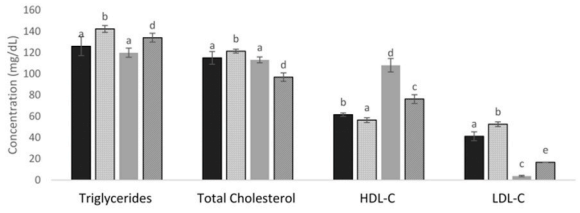

Fig. 2A: Effects of F(1 :1) on lipid markers

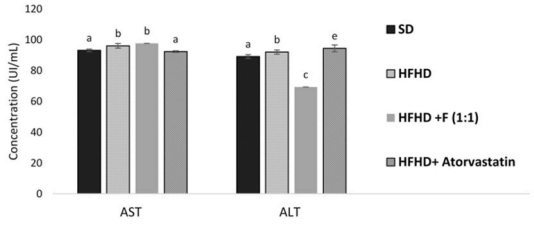

Fig. 2B: Effects of F(1 :1) on transaminases activities

Fig. 2. Effects of F(1:1) on lipid markers (A) and transaminase activities (B) Results are expressed as mean ± standard error; HFHSD: High fat, high sucrose Diet; SD: Standard diet; AST Aspartate aminotransferase; ALT: Alanine Aminotransferase; Different letters indicate a significant difference between groups; F: Formulation; F(1:1): 200 mg of *Allium sativum* + 200 mg of *Persea americana* seeds (1:1; w/w); n = 5 rats/groups.

triglycerides (119.88 ± 4.25 mg/dL) and LDL-c (3.78 ± 0.66 mg/dL) levels and significantly increased the HDL-c level: (108.07 ± 6.29 mg/mL). Furthermore, it was observed that HFHSD caused a significant increase in enzyme activity of transaminases (AST, ALT) when compared to the negative control (SD). The treatment with F(1:1) slightly increased the activity of aspartate aminotransferase (97.72 ± 2.32 UI/mL) compared to the positive control (95.07 ± 1.20 UI/mL). However, Formulation (1:1) protected the liver through a decreased activity of alanine aminotransferase compared to the group fed with HFHSD and atorvastatin (Fig. 2B).

4. Discussion

The prevalence of metabolic syndrome is increasing in Cameroon and worldwide. Metabolic syndrome is associated with oxidative stress, obesity, hyperglycemia and dyslipidemia [31]. Phytochemicals are capable to prevent free radicals which damage proteins lipids, DNA and impaired cellular functions. Three formulations prepared from mixed portions (weight/weight) of *Allium sativum* and *Persea americana* [F (1:3), F(3:1). F(1:1)] scavenged or inhibited reactive oxygen species and reduced Fe^{3+} to Fe^{2+}. Among the three formulations, F(1:1, w/w) made up of equal portions of each plant extract (200 mg of *Allium .sativum* and 200 mg of *Persea americana*) showed the lowest IC_{50}. F(1:1), hence the most active. This suggests that F(1:1) probably combines the efficacy of each plant through the diversity of their bioactive components to scavenge free radicals. Previous studies also reported the antioxidant potentials of the two plants [15,32]. However, the lowest IC_{50} obtained with F(1:1) was higher compared to those reported with ethanolic extracts of *Persea americana* seeds solely [32] (Table 1). This justifies why formulation F(1:1) was tested *in vivo* on rats fed with high fat high sucrose diet as established models of metabolic.

4.1. Induction of oxidative stress and metabolic disorders

During the induction of the metabolic syndrome state, an excessive increase in body weight and fatness was observed (Fig. 1A) alongside with dyslipidemia, involving disturbances of triglycerides, total cholesterol, HDL and LDL cholesterol levels (Fig. 2A). In addition, feeding animals with HFHSD for 8 weeks caused a depletion of antioxidants coupled with an increase of pro-oxidant markers (Tables 2 and 4). These results corroborate several other studies reported on animal models of metabolic syndrome induced with diets [5,18,33]. In fact, HFHSD brought perturbations of metabolic pathways, inducing insulin resistance and liberation of free fatty acids that generate visceral fat accumulation due to deregulation of hydroxy-methyl glutaryl-CoA reductase [5,6]. Insulin resistance resulting from hyperglycemia is accompanied with generations of ROS, which attacked unsaturated fats causing lipid peroxidation, membrane fragility and reduction in the of level HDL-cholesterol [18,34]. During induction of metabolic syndrome, weight increases (Fig. 1A) because of the high energy intake and reduced energy expenditure that causes fat accumulation in white tissue. The increase in body weight and fat has been proven to lead to protein degradation and atrophy of skeletal muscles, impairing peripheral glucose transport and therefore hyperglycemia as observed in Fig. 1C [35]. In addition, research has shown that hypercholesterolemia degenerates cardiomyocytes, starting earlier in the myocardium, degeneration of muscle fibres, cell death and leukocytic infiltration (Fig. 2A) [6,35]. Mitochondrial damages release reactive oxygen and nitrogen species (RONS) in the cardiovascular system as confirmed by cardiac NO radical levels (Table 4). RONS could oxidise LDL and cause cell death through alterations of apoptotic markers like Bcl-2 and caspase-3 [35].

4.2. Formulation F(1:1) improved oxidative stress status, reduced dyslipidemia and metabolic disorders

Persea americana seeds considered as waste can be functional food ingredients and source of bioactive compounds against several diseases [32]. This is also explained by the fact that during the 21 days treatment, the formulation F(1:1) at 400 mg/kg b. w, (200 mg A s + 200 mg P. a) successfully reduced MDA in the liver, plasma and pancreas (Table 2), protein carbonyls in the liver and brain, and nitric oxide in the heart (Table 3). Therefore, reduced lipid peroxidation obviously protects cell membranes from ROS damages through free radical-scavenging mechanisms as demonstrated *in vitro* (Supplementary Fig. 1, Table 1). Similar findings were reported with leaves' extracts of *Persea americana* [10,32].

Several studies demonstrated that seeds of avocado and garlic are great sources of antioxidants, and are capable of scavenging free radicals or preventing their generation [15,36]. *Persea americana* seeds and *Allium sativum* extracts possess anti-hemolytic and anti-lipid peroxidation effects which contribute to improve oxidative status of animals [37]. In fact, flavonoids from both plants induce suppression of RONS generation, inhibit enzymes or chelate trace elements involved in free radical formation [13]. A portion of garlic (50%) in the formulation can promote weight loss by downregulating CCAAT/enhancer-binding protein (C/EBP) α and β and peroxisome proliferator-activated receptor (PPAR)γ leading to a decrease in fatty acid synthase and lipid accumulation in 3T3-L1 adipocytes [38] and also through the upregulation of UCP-1 and the enhancement of energy expenditure [39]. The joint

action of avocado seeds and garlic aqueous extracts (1:1 w/w) likely reduces body weight (−2.31%) and glycaemia (−27%) compared to atorvastatin (Fig. 1) through mechanisms such as inhibition of digestives enzymes, reduction of intestinal bioavailability of glucose, reduction of glucose absorption, and increase glucose uptake by muscles via enhanced insulin action as demonstrated by Azantsa et al. [15,37].

Formulation F(1; 1) also modulated lipid profile by correcting dyslipidemia established on rats displaying features of metabolic syndrome induced by a high-calorie diet. Indeed, the lowering of total cholesterol, triglycerides, LDL-c levels and increase HDL-c levels induced by the formulation F (1:1) may be due to their richness in phenolic compounds such as phytosterols (found in avocado) in particular the beta-sitosterols which are known to induce a decrease in plasma lipoprotein and cholesterol levels by decreasing cholesterol solubility and absorption across the intestinal barrier [6,40,41]. Bioactive compounds of the formulation such as allicin can also increase the activation of AMPK to suppress cholesterol synthesis [42].

4.3. F(1:1) protected and restored the liver of rats damaged by HFHS diet

During the 8 weeks' induction of metabolic syndrome features (Fig. 2B), the hepatic function was altered through the NADPH oxidase pathway, resulting in decrease glycogen levels and increase in transaminase ALT and AST activities [43] as well as hepatic MDA levels. The formulation F(1:1) was able to protect and restore the liver as confirmed by reductions of transaminase activities (Fig. 2B) and MDA levels (Table 2). This may be attributed to the beneficial action of the diallyl sulfur, an antioxidant found in garlic and other flavonoids of the mixture [44].

5. Conclusions

The results for this research evince that the use of avocado seeds combined to garlic extract F(1:1, w/w), significantly caused weight loss (−2.31%), reduced blood glucose levels (−27.38%), and protected the liver integrity through reduced ALT activity. Also, Formulation F(1:1) reduced pro-oxidant levels and improved enzymatic and non-enzymatic antioxidant systems. This, therefore, suggests that a polyherbal formulation based on the plant extracts mixed in the formulation F (1:1) at 400 mg/kg b w could be a good candidate for the management and prevention of oxidative stress and features of metabolic syndrome, including dyslipidemia, obesity, and diabetes.

CRediT authorship contribution statement

Boris K.G. Azantsa: Conceptualization, Investigation, Formal analysis, Project administration, and, Writing – original draft, Writing – review & editing, Funding acquisition. **Ntentie F. Raissa:** Software, Formal analysis, Validation, Writing – original draft. **Mbong A. Mary-Ann:** Software, Investigation, Visualization, Writing – review & editing. **Mafongang Amelie:** Methodology, Data curation, Resources. **Kamtchoum Alexine:** Methodology, Resources. **Momo Cliffbrown:** Investigation, Data curation, Resources. **Chimou N. Lauriane:** Investigation, Visualization, Resources. **Fonkoua Martin:** Methodology, Data curation. **Edoun E. Ferdinand:** Methodology, Data curation, Investigation. **Ngondi J. Laure:** Supervision, Writing – review & editing. **Julius E. Oben:** Supervision.

Declaration of competing interest

Authors declare that there is no conflict of interest relevant to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2022.100195.

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