Haematological indices, *in-vivo* antioxidant and hypolipidemic activities of infused beverages from *Red sorghum Bran-Roselle Calyx-Avocado* leaf flour blends.

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Abstract

Hyperlipidemia is a heterogeneous disorder with multiple etiologies resulting in major healthcare problem such as risk of heart disease, stroke and micro-vascular complications such as blindness, renal failure and peripheral neuropathy. The research was aimed at evaluating the haematological indices, *in-vivo* antioxidants and anti-hyperlipidemic potentials of infused beverages from Red Sorghum Bran-Roselle calyx-avocado leaf flour blends in diabetic mellitus induced rats. The flour samples were prepared in varied proportion according to central composite design and the samples with highest DPPH, overall acceptability and other chemical composition was picked as experimental sample. Hypolipidemic activity was evaluated by measuring various biochemical parameters like total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and high density lipoprotein. Haematological indices are PVC, Hb, WBC and RBC, of rats fed on samples were in the range of 25-41%, 6.85-11.20g/100ml, 166.35-272.81 (x50wbc/cubic mm) and 224.34-366.80 (x103rbc/cubic mm) respectively. The results showed that serum total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein levels of the infused sample was statistically higher the commercial herbal control. The observed hypolipidemic effect can be associated with high antioxidant activities of the Red Sorghum Bran-Roselle Calyx-Avocado leaf infused beverage sample, particularly R50Z40A10. The present study therefore supports the consumption of the infused beverage for the obese and those watching weights.

Keywords: Beverage, Hypolipidemia, Haematology, *In-vivo* antioxidants.

Introduction

Hyperglycaemia is associated with disturbance in carbohydrate, protein and lipid metabolism as a result of absolute or relative deficiency in insulin secretion or action as well as increased cellular resistance to that hormone with dysfunction in organ systems. The increasing incidence of the disease worldwide may be due to sedentary life style, unhealthy diet, obesity and other predisposing risk factors [1,2]. Atherosclerosis and microcirculatory disorder [3], retinopathy, nephropathy, and foot ulceration are results from diabetes mellitus complications [4]. It is projected by 2040 to become of the world's main disablers and killers, as the number of people with obesity multiplies worldwide [5]. The World Health Organization has also recommended the evaluation of the effective use of plants, because the use of modern drugs is not safe [6,7] with 70-80% of people worldwide use herb for management of mild to moderate illnesses [8-10]. The synthetic hypolipidemic agents used in clinical practices have serious side effects like haematological effects, coma, disturbances of liver and kidney [11-13]. Many medicinal plants used in ethnomedical practices in Nigeria are known or little known to scientific world.

Sorghum grain (*Sorghum bicolor (L.) Moench*) is used by various food industries, including milling, starch production, brewing and distilling, resulting in numerous by-products [14]. *Red sorghum bran* (sorghum offal, sorghum milling waste, sorghum mill feed) is a mixture of grain pericap (bran) and of variable amounts of grain fragments (endosperm, germ). Red sorghum bran has been used as ingredient in preparation of foods such as bread, biscuits and snacks to improve their antioxidant properties [15]. Red sorghum bran components could be a potential source of nutrients and phytochemicals for industrial applications. It can also be used in cereal-based products to enhance their phytochemical profile because nowadays consumers demand healthy food [16].

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Hibiscus sabdariffa calyx extract has been reported to show therapeutic promise in decreasing and preventing the development of atherosclerosis and possible related cardiovascular pathologies linked with diabetes. The authors suggest that this activity might be linked to polyphenolic compounds and dihydrobenzoic acids, like protocatechuic acids, but further identification of the active compounds is warranted [17]. A similar effect was reported by Huang et al., 2009 with the extract suppressing the high-glucose-induced migration in a vascular smooth muscle cell model. The sour tea was able to significantly affect the blood lipid profile by increasing highdensity lipoprotein-cholesterol, decreasing total cholesterol, low density lipoproteincholesterol, triglycerides and Apo-B100, with no effect on apolipoprotein-A1 and lipoprotein a [18].

Avocado (*Persea americana*) is originated in the state of Puebla, Mexico [19]. The leaf has been greatly appreciated in recent times and researchers have found scientific support for its use in folk medicine [20]. The parts of this plant have various applications in ethnomedicine, ranging from treatment of diabetes, hypertension, hypercholesterolemia, diarrhea, dysentery, toothache, anemia, intestinal parasites to the area of skin treatment and beautification [21]. The leaves of P. Americana are reported to possess anti-inflammatory, analgesic, antimicrobial, antiviral, antihypertensive, antihypoglycemic, antiulcer, anticonvulsant, larvicidal antihepatotoxic, vasorelaxant, toxicological activities [22,23]

The infused beverage from the flour blends may turn out to be the most unique for health support involving cholesterol regulation and may play an important role in the development of neutraceuticals [24-26]. The main objective of this study was to assess the haematological indices, *in vivo* antioxidant activities and hypolipidemic potentials of infused beverage from *Red Sorghum Bran-Roselle Calyx-Avocado* Leaf flour blends.

Materials and Methods

Sources of food materials and wistar albino rats

Main materials are *Red Sorghum* (*Sorghum bicolor*) bran, *Roselle calyx* and Avocado Leaf. *Red Sorghum* (*Sorghum bicolor*) bran, *Roselle calyx* were obtained from Oja-Oba market in Akure while Avocado's leaf and three weeks old Albino wistar strain weanling rats (male and female) were purchased from Federal College of Agriculture and Research Farm in Akure, Nigeria. The plant materials were authenticated by Crop, Soil and Pest Management Department, Federal University of Technology, Akure. All chemicals were of analytical grade and were obtained from Sigma-Aldrich, London, United Kingdom.

Preparation of red sorghum bran

Red Sorghum bran flour was processed using method described by Famurewa et al. [27]. About 500g of mature Red Sorghum Bran was purchased from Oja-Oba market in Akure. The bran was sorted to remove damaged or discoloured ones and winnowed to remove dirt. The red bran was dried in a locally fabricated hot air dryer at 60oC for 4hrs to a constant weight. The dried bran was milled into flour using Merlex Excella dry mill (Marlex Appliances PVT, Daman). The dry extract was then cooled and stored in an airtight polyethylene bag at room temperature (~27oC) until required for use.

Preparation of avocado leaf flour

Avocado leaf flour was processed using method described by Owolabi et al. [20]. About 500g of mature young Avocado young leaves were stripped off the stems after harvesting. The leaves were sorted to remove damaged or discoloured leaves. Leaves will then be rinsed in clean water to remove dirt. The leaves will be dried in a locally fabricated hot air dryer at 60oC (Plus11 Sanyo Gallenkamp PLC) to a constant weight. The dried leaves will then be milled into fine powder using Merlex Excella dry mill (Marlex Appliances PVT, Daman), cooled and kept at room temperature (~27oC) in an airtight polyethylene bag until further use.

Preparation of roselle calyx flour

Roselle calyx flour was flour was processed using method described by Ogiehor et al. About 500g of mature Roselle calyx was purchased from Oja-Oba market in Akure. The leaf will be sorted to remove damaged or discoloured ones. The leaf was winnowed to remove dirt and dried in a locally fabricated hot air dryer at 60oC for 4hrs to a constant weight. The dried leaf was milled into flour using Merlex Excella dry mill (Marlex Appliances PVT, Daman). The dry extract was then cooled and stored in an airtight polyethylene bag at room temperature (~27oC) until required for use.

Preparation of red sorghum Bran-Roselle calyx-avocado leaf infused beverage

Blends of the flours were produced using the central composite design of Design Expert using variables red sorghum bran flour (50-62.5%), Roselle calyx flour (25-45%) and Avocado leaf flour (5-17.5%) which targeted antioxidant activity and overall acceptability of the infused beverage. The flour samples were blended to obtain the following food sample combinations, that is, R100Z0A0 (RSBF=100%; ZCF=0%, ALF=0%); R0Z100A0 (RSBF=0%; ZCF=100%, ALF=0%); R0Z0A100 (RSBF=0%; ZCF=0%, ALF=100%); R50Z40A10 (RSBF=50%; ZCF=40%, ALF=10%); R50Z42.5A7.5 (RSBF=50%; ZCF=42.5%, ALF=7.5%); R50Z45A5 (RSBF=50%; ZCF=45%, ALF=5%); Herbal tea (H100) was used as commercial control sample. The modified method was adopted with some modifications [27]. Red Sorghum bran, Roselle Calyx and Avocado Leaf flour were mixed in varied proportions as described above. The flour blends were packed separately into infused bags. The bags were infused in hot water (90^{o}C) for 5mins. The extract was cooled and stored at refrigerated temperature (-0^{o}C) until required for use.

Hypolipidemic assay

Hypolipidemic activity of the extract was evaluation after 4 weeks of extract administration the rats were anaesthetized with chloroform vapour and the blood was collected through cardiac puncture into sample bottles devoid of anticoagulant. The samples were centrifuged at 1000 rpm for 15 mins to obtain the sera. Serum total cholesterol, triglyceride and

High Density Lipoprotein (HDL) levels were measured by enzymatic colorimetric methods using Randox diagnostic kits. The concentrations of Low Density Lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated from the formula of [26].

Experimentanl induction of diabetics and High Fat Diet (HFD)

Experimental type-2 diabetes mellitus was induced by subjecting rats to High Fat Diet (HFD) (or hyperlipidemic diet) which contained 20% fat, 45% carbohydrate, 22% protein, for 4 weeks [28]. The intra-peritoneal injection of single low dose of STZ (35 mg/kg, i.p.) was carried out on the fourth week on overnight fasted rats. The STZ was prepared by dissolving appropriate amount in freshly prepared 5 mmol/L citrate buffer, pH 4.5. After STZ injection, the rats had free access to glucose solution (5%) for 24 h to avoid and/or attenuate subsequent inevitable hyper-insulinemia and hypoglycemic shock. Forty-eight hours post-STZ injection, animals were fasted overnight and a drop of blood samples were analyzed for glucose levels (mg/dL) by using strips on glucometer (ACCU-CHEK ACTIVE, Roche, Germany). Animals with glucose levels above 250 mg/dL was considered as diabetic and used in the therapeutic study.

Evaluation of oxidative stress indices (in-vivo antioxidant potentials) of infused beverage

Glutathione Peroxidase (GPx) activity was evaluated according to the method of [29]. Glutathione Transferase Activity [30], Reduced Glutathione (GSH) Concentration [31], Superoxide Dismutase (SOD) activity [32] and catalase activity [33].

Haematological determination

The Automated Haematologic Analyzer (Sysmex, KX-21, Systmex Corporation, Kobe, Japan) was used to analyze the haematological parameters, that is, red blood cells (RBC), Pack Cell Volume (PCV), haemoglobin concentration (Hbc), white blood cells (WBC), neutrophil (NEU) and lymphocytes (LYM) using methods described by Dacie and Lewis [34]. Mean corpuscular haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentrated (MCHC) and Mean Corpuscular Volume (MCV) were calculated from values obtained from RBC, PCV and Haemoglobin (Hbc) content [34].

Statistical analysis

Data are presented as mean ± SEM and were analyzed statistically using one-way ANOVA followed by Tukeykramer multiple comparison test and values with $P<0.05$ were considered significant.

Results and Discussion

Criteria for choice of best outfit samples for biochemical assay

The three flour blends were ranked according to their chemical composition to know the choice of best sample for biochemical assay (Table 1). Ranking percentage of the enriched samples was in the order of: R50Z40A10 (82.20%) > R50Z42.5A7.5 (66.67%) > R50Z45A5 (51.10%). Sample R50Z40A10 had the highest % chemical score and so was picked for the biochemical assay.

In-vivo antioxidant potentials of red sorghum bran-roselle calyx-avocado leaf infused beverages on type-2-diabetic mellitus (T2DM) induced rats

Plants contain active components that are known to act as antioxidants [35]. It is known that only about 5% of phenolic intake in the diet can be absorbed and the rest will pass to the large intestine for microflora fermentation [36]. Given the low phenolic absorption in the human body, it should be health-beneficial to ingest food supplements with higher level of phenolics because health effects of phenolics are dependent on intake and their bioavailability [37]. It has been suggested that a high intake of fruits and vegetables, the main sources of antioxidant such as phenols in the diet, could decrease the potential stress caused by ROS *via* a number of mechanisms, including the protection of target molecules (lipids, proteins and nucleic acids) from oxidative damage, suppressing the inflammatory response and modulating vascular homeostasis

Table 1. *Ranking selection of the best outfit samples for biochemical assay.*

Parameters	R50Z45A5	R50Z42.5A7.5	R50Z40A10
Sensory	3	2	1
Phenolic Profile	1	2	3
Diffusion Rate	3	2	1
Amino Acid	1	2	3
Na/K	1	2	3
DPPH	3	2	1
Fe-Che	1	2	3
ABTS	1	2	3
FRAP	1	2	3
OH	1	2	3
Anthocy	1	2	3
Carotenoid	1	2	3
Flavonoid	3	2	1
Phenol	1	2	3
Saponin	1	2	3
Total	23	30	37
%	51.1	66.67	82.2

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[38]. A large body of literature is devoted to vitamins effects. However, the healthy properties of foods lacking in vitamins as wine, mainly phenolics compounds the scope of interest. Phenols have been considered powerful antioxidants *in vitro* and have been proved to be more potent antioxidants than vitamins E and C and carotenoids [38-40]. The effects of fruit and vegetable intake and risk of cardiovascular and neurodegenerative diseases, cancer, diabetes or osteoporosis have partially been ascribed to phenols [41]. The in- vivo antioxidant activities of the beverages (G-Tea, AV, RSB, Zobo, and F-Blend) were revealed in Table 2. The antioxidant indices investigated were the activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione transferase, as well as the concentration of reduced glutathione. Antioxidant biomarkers (CAT, SOD, GPx, GST, GSH) those present in the tissues that can inhibit free radical production by minimizing reactive oxygen species (ROS) and/or scavenging radical, chelating proxidant metals and breaking chain reactions [42-48].

The antioxidant enzymes; catalase and Superoxide Dismutase (SOD) represent the primary intracellular antioxidant defense mechanism against oxidative stress. The activities of catalase decreased significantly due to the induction of diabetes mellitus ($p<0.05$), all the beverages administered caused significant increased activity of catalase at all the doses compared to the positive control ($p<0.05$). The formulated beverages competed significantly with the G-Tea for all the amounts (2.5, 5.0, 7.5, and 10.0) administered, F-blend caused increase in the activity of catalase for the doses compared to AV, RSB, and Zobo. F-blend beverage demonstrated effects against the progression of free radical activity in diabetic rats (Table 2). The high antioxidant properties of beverages could be attributed to it high amount of flavonoids and phenols as phenols have been known to possess high concentrations of isoquercitrin and quercetin which are known as potential chemo preventive agents and are essential for counteracting oxidative stress; possess potent free radicals scavenging and antioxidant properties.

Superoxide Dismutase (SOD) is one of the principal antioxidant enzymes which is responsible for the elimination of superoxide radicals. In this present study, induction of type 2 diabetes mellitus using high fat diet and single-low dose of STZ resulted in a significant decrease in the activity of SOD expresses in the untreated diabetic rats ($p<0.05$) as compared to the groups treated with varying doses of G-tea, AV, zobo, RSB and F-blend and the negative control (non-diabetic) group (Table 2). However, this reduced enzyme activity might lead to increase in hydrogen peroxide (H202) that can also produce hydroxyl radicals, which could then result in lipid peroxidation (Malondialdehyde). There was a significant ($p<0.05$) increase in the SOD activity in the rats as the dose increased from 2.5 to 10 of all the samples. The group treated with 10 mg/kg bwt of the G-tea had highest activity of SOD which was not significantly different from the SOD activity in the F-blend (10 mg/kg) treated group. ($p<0.05$).

Glutathione is a small tri-peptide protein synthesized in the liver. It is a potent antioxidant with high redox potential and

Table 2. *In-vivo antioxidant activities of Red Sorghum bran-Roselle Calyx-Avocado leaf Infused beverages (mg/kg bwt) on (T2DM) induced rats.*

Samples	Conc.	CAT	SOD	GPx	R-GSH	GSH-T
Controls	NC	8.02 ± 0.63^{a}	18.39 ± 0.77^{a}	131.43 ± 0.85^{a}	6.33 ± 0.04^{a}	1.24 ± 0.01^{a}
	PC	1.28 ± 0.10^{f}	3.57 ± 0.41^{i}	23.42 ± 1.85^{i}	1.12 ± 0.09^{e}	0.22 ± 0.02^{h}
	GC	4.64 ± 0.44^{d}	10.54 ± 0.05^{d}	73.7 ± 1.33^{e}	3.55 ± 0.06^{c}	0.70 ± 0.01^{cd}
H100	2.5	5.34 ± 0.24^{c}	12.58 ± 0.43^{c}	87.8 ± 1.84^{d}	4.23 ± 0.06^{b}	0.83 ± 0.02^{c}
	5	5.75 ± 0.11^{c}	13.54 ± 0.44^{b}	94.80 ± 1.31^{e}	4.56 ± 0.06^{b}	0.89 ± 0.01b^{c}
	7.5	5.81 ± 0.16b^{c}	13.69 ± 0.19^{b}	95.33 ± 1.21^{c}	4.58 ± 0.06^{b}	0.89 ± 0.01b^{c}
	10	6.24 ± 0.13^{b}	13.97 ± 0.28^{b}	104.87 ± 1.95^{b}	5.05 ± 0.09^{b}	0.99 ± 0.02^{ab}
R0Z0A100	2.5	3.86 ± 0.31^{de}	8.44 ± 0.49^{f}	52.46 ± 0.60^{g}	2.52 ± 0.04^{d}	0.49 ± 0.01^{ef}
	5	3.89 ± 0.26^{de}	8.85 ± 0.61^{f}	63.65 ± 1.73^{f}	3.06 ± 0.09^{c}	0.60 ± 0.02^{d}
	7.5	4.66 ± 0.15^{d}	10.59 ± 0.35^{d}	72.68 ± 1.36^{e}	3.50 ± 0.07^{c}	0.68 ± 0.01^{cd}
	10	5.76 ± 0.21^{c}	12.75 ± 0.75^{c}	85.65 ± 1.76^{d}	4.13 ± 0.09^{b}	0.81 ± 0.02^{c}
R100Z0A0	2.5	3.00 ± 0.44^{e}	5.48 ± 0.17^{h}	38.01 ± 1.22^{h}	1.83 ± 0.06^{e}	0.36 ± 0.01^{g}
	5	4.15 ± 0.11^{d}	9.43 ± 0.24^{e}	65.38 ± 1.67^{f}	3.15 ± 0.08^{c}	0.63 ± 0.02^{d}
	7.5	5.24 ± 0.12^{c}	11.91 ± 0.28^{c}	83.18 ± 0.07^{d}	4.01 ± 0.05^{b}	0.79 ± 0.01^{c}
	10	6.09 ± 0.07^{b}	13.85 ± 0.16^{b}	95.97 ± 1.07^{c}	4.62 ± 0.05^{b}	0.91 ± 0.01^{b}
R0Z100A0	2.5	3.69 ± 0.38^{de}	7.70 ± 0.30^{fg}	53.39 ± 2.05^{g}	2.57 ± 0.09^{d}	0.51 ± 0.02^{e}
	5	4.57 ± 0.33^{d}	9.39 ± 0.29^{e}	66.50 ± 1.02^{f}	3.13 ± 0.06^{c}	0.61 ± 0.02^{d}
	7.5	4.57 ± 0.22^{d}	9.70 ± 0.14^{e}	73.57 ± 2.34^{e}	3.55 ± 0.11^{c}	0.69 ± 0.02^{cd}
	10	4.71 ± 0.09^{d}	10.71 ± 0.22^{d}	84.20 ± 1.53^{d}	4.06 ± 0.07^{b}	0.79 ± 0.01^{c}
R50Z40A10	2.5	3.56 ± 0.32^{e}	8.08 ± 0.73^{f}	52.65 ± 1.23^{g}	2.54 ± 0.06^{d}	0.50 ± 0.01^{ef}
	5	4.11 ± 0.22^{d}	9.33 ± 0.50^{e}	65.65 ± 2.20^{f}	3.16 ± 0.11^{c}	0.62 ± 0.02^{d}
	7.5	5.56 ± 0.16^{c}	13.41 ± 0.19^{b}	92.91 ± 1.33^{c}	4.48 ± 0.06^{b}	0.88 ± 0.01^{bc}
	10	6.14 ± 0.23^{b}	13.93 ± 0.51^{b}	97.55 ± 2.36^{c}	4.70 ± 0.11^{b}	0.92 ± 0.02^{b}

Values with different alphabet along the same column are significantly different at $P<0.05$. NC: Negative Control; PC: Positive Control (HFD/STZ (35mg/kg BWT)-induction); GC: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg BWT Glybenclamide; H100: (Commercial herbal tea flour); R100Z0A0: (100% Red sorghum bran + 0% zobo calyx+0% Avocado leaf), R0Z0A100: (0% Red sorghum bran + 0% zobo calyx + 100% Avocado leaf) flour, R0Z100A0: (0% Red sorghum bran + 100% zobo calyx +0% Avocado leaf)flour, R50Z45A5 (50% Red sorghum bran + 40% zobo calyx + 10% Avocado leaf)flour; CAT: Catalase Activity (mmol H2O2/mg of protein); SOD: Superoxide dismutase activity (U/mg protein); GPx: Glutathione peroxidase activity (µmoles GSH consumed/min/mg protein); R-GSH: Reduced glutathione concentration (mmol/g protein); GSH-T: Glutathione transferase activity (µmole CDNB conjugated/min/mg protein).

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also served as a co-factor for antioxidant enzymes. Glutathione conjugation serves as protective mechanism whereby potentially toxic metabolites are "mopped up" as cellular redox status (Chance et al.). The activities of glutathione transferase (GST) and Glutathione Peroxidase (GPx) is a function of the availability of reduced glutathione (GSH). The diabetic state of the rats were characterized by reduced activities of GST and GPx, as well as the concentration of GSH. F-blend treated diabetic rats had increased levels of GPx, GST and GSH, compare to the positive control group and the other experimental groups except G-tea. The antioxidant potentials of these samples, were significantly higher than glibenclamide which is a standard drug used in the treatment of diabetes mellitus. These functional samples prepared as beverages will not only contribute nutritionally but will mitigate the oxidative stress caused by hyperglycemia and excessive glycolytic processes which result into excessive generation of free radicals which the endogenous system could not overwhelm. This study agreed with the reports of Bouabid, who reported the antioxidant properties of aqueous and organic extracts of *atractylisgummifera* and Abiola on antioxidant activity of pomegranate seeds.

Effects of red sorghum bran-roselle calyx-avocado leaf infused beverages on the lipid profile of T2DM induced rats

Natural products have attracted increasing attention for the management of dyslipidemias and associated cardio-metabolic diseases. The results (Table 3) of this anti-diabetic effects of these beverages especially F-blend suggest that treatment of subjects with T2DM with these functional beverage (F-blend) causes reduction of serum total cholesterol, insignificantly lower than the total cholesterol concentration in the group treated with G-tea ($p<0.05$), but significantly lower cholesterol concentration compared to the AV, RSB and zobo ($p<0.05$); the Low Density Lipoprotein (LDL) level in the G-tea treated group was lower than the concentration in F-blend group, however all the experimented beverages demonstrated LDL-reducing potential significantly, compared to the LDL concentration in the positive control group. The triglyceride concentration in the F-blend treated group was least among the treatment groups; while there was an increase in High Density Lipoprotein (HDL) concentration when compared with positive control group, although the concentration of HDL in the F-blend treated group was lower compared to the G-tea treated group, the concentration was higher compared to AV, RSB and zobo et al. the doses. There was a significant decrease in serum levels of TCHOL, LDL and TG in the groups but this, at least in part, can be explained by better control of the subjects and most probably their better adherence to the therapeutic nutritional (diet) pattern. The finding that the functional beverages can elevate low HDL-C serum levels in the rats with T2DM is also important because the problem with increasing HDL-C is much more complex than lowering LDL-C. Although HDL-C is, according to the guidelines, not recommended as a target for treatment, it is

Table 3. *Effect of Infused beverage samples (mg/kg b.wt) on the blood lipid profile of T2DM rats.*

Samples	Conc.	TCHOL (mg/dL)	HDL (mg/dL)	TAG (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	CRI	AC
Controls	NC	92.14 ± 2.21f	47.4 ± 3.64a	35.93 ± 4.33j	36.75 ± 0.50g	7.98 ± 1.41jf	1.94	0.73
	PC	258.99 ± 9.86a	8.44 ± 0.65g	195.76 ± 9.46a	207.04 ± 9.74a	43.5 ± 7.65a	30.68	29.22
	GC	117.12 ± 5.84d	26.58 ± 2.04d	112.46 ± 6.54e	65.55 ± 2.44de	24.99 ± 6.93bc	4.46	3.27
H100	2.5	116.37 ± 5.76d	31.66 ± 3.43bc	133.53 ± 6.50d	55.03 ± 2.42e	29.67 ± 6.88bc	3.67	2.33
	5	109.25 ± 6.56e	34.19 ± 4.63b	108.48 ± 3.96f	50.95 ± 1.48ef	24.1 ± 4.19bc	3.19	2.16
	7.5	108.9 ± 5.97e	34.26 ± 2.63b	107.05 ± 3.60f	50.84 ± 1.34ef	23.79 ± 3.82bc	3.17	2.33
	10	99.54 ± 7.95f	37.82 ± 2.90b	70.39 ± 4.80h	46.07 ± 1.79ef	15.64 ± 5.08h	2.63	1.43
R0Z0A100	2.5	142.23 ± 9.74c	18.92 ± 1.45e	140.55 ± 5.88c	92.07 ± 2.19c	31.24 ± 6.22bc	7.51	6.66
	5	123.05 ± 9.16d	22.96 ± 1.76d	108.79 ± 5.57f	75.91 ± 4.31d	24.16 ± 3.25c	5.35	4.82
	7.5	116.21 ± 7.52d	26.21 ± 2.01d	105.86 ± 6.96f	66.47 ± 2.59de	23.52 ± 2.36c	4.43	3.94
	10	109.22 ± 6.81e	30.89 ± 2.37bc	30.89 ± 2.37g	56.41 ± 2.43e	21.92 ± 3.91c	3.53	2.66
R100Z0A0	2.5	178.7 ± 6.82b	13.71 ± 1.05ef	170.28 ± 6.23b	127.15 ± 3.29b	37.84 ± 3.34b	13.03	12.16
	5	129.35 ± 7.63d	23.58 ± 1.81d	143.37 ± 7.64c	73.91 ± 3.97d	31.88 ± 3.27bc	5.48	4.52
	7.5	111.36 ± 6.84e	30.34 ± 2.30bc	104.79 ± 4.13f	58.07 ± 1.54e	23.28 ± 4.37bc	3.67	2.11
	10	104.09 ± 5.22ef	34.61 ± 2.66b	86.16 ± 3.15gh	50.33 ± 1.18ef	19.14 ± 3.34c	3	2.17
R0Z100A0	2.5	136.89 ± 8.58c	19.25 ± 1.48e	121.96 ± 3.66de	90.54 ± 4.33c	27.09 ± 2.31bc	7.11	6.28
	5	123.39 ± 9.81d	23.45 ± 2.80d	114.95 ± 4.16e	74.34 ± 4.91d	25.54 ± 3.94bc	5.59	4.96
	7.5	112.9 ± 9.13e	26.53 ± 2.04d	93.86 ± 3.55g	65.7 ± 4.31de	20.66 ± 2.23c	4.38	3.26
	10	103.84 ± 9.68ef	30.36 ± 2.33bc	72.22 ± 5.84h	57.38 ± 2.18e	16.05 ± 1.19d	3.29	2.42
R50Z40A10	2.5	135.42 ± 9.67c	18.99 ± 3.46e	109.43 ± 3.87f	91.76 ± 4.43c	24.31 ± 2.57bc	7.22	6.13
	5	117.81 ± 6.35d	23.68 ± 3.82d	92.29 ± 3.49g	73.62 ± 5.03d	20.51 ± 4.29c	4.97	3.84
	7.5	101.57 ± 6.84ef	33.51 ± 4.57b	72.31 ± 4.13h	51.99 ± 1.54ef	16.06 ± 4.37d	3.03	2.36
	10	96.54 ± 6.93f	35.18 ± 3.70b	53.22 ± 6.60i	49.53 ± 2.46ef	11.82 ± 6.99e	2.44	1.74
*Standards		<200.00	<50.00	<150.00	<150.00	<100.00		

Means (± SEM) with different alphabetical superscripts in the same column are significantly, NC: Negative Control; PC: Positive Control (HFD/STZ (35mg/kg BWT)-induction); GC: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg BWT Glybenclamide; H100: (Commercial herbal tea flour); R100Z0A0: (100% Red sorghum bran + 0% zobo calyx +0% Avocado leaf), R0Z0A100: (0% Red sorghum bran + 0% zobo calyx +100% Avocado leaf) flour, R0Z100A0: (0% Red sorghum bran + 100% zobo calyx +0% Avocado leaf)flour, R50Z45A5 (50% Red sorghum bran + 40% zobo calyx +10% Avocado leaf)flour; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TRI: Triglyceride; VLDL: Very low-density lipoprotein; TCHOL: Total cholesterol. *American medical association (2018)

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well known that low HDL-C is a risk factor for CVD. Low HDL-C can be increased by lifestyle changes such as reducing intake of dietary Trans fats, increasing habitual physical activity, reducing excessive body weight, reducing dietary carbohydrates and replacing them with unsaturated fat and consuming very modest quantities of therapeutic substances. However, when drugs are concerned, it is well known that statins increase HDL-C only very modestly and the influence of these drugs is not completely understood while the efficacy of fibrates to increase HDL-C may be attenuated in people with T2DM. A number of clinical studies have yielded disappointing results about the therapeutic benefit of elevating low serum HDL-C with different new drugs. The most important reason is probably that it is not the quantity of HDL-C that mattersbut HDL dysfunctionality, particularly in some conditions like T2DM contributes to elevated risk of CVD. The results of this study indicating an increase of HDL-C caused by the functional beverages fit well with the idea that protective properties of these individual beverage might influence HDL functionality when combined to form a more functional beverage.

Effects of red sorghum bran-roselle calyx-avocado leaf infused beverages on hematological parameters of Type-2-Diabetic Mellitus (T2DM) induced rats

Parameters obtained from hematologic counts can provide insight into changes that occur in hematological indices such as white blood cells (WBC), red blood cells (RBC), and the other parameters. Analysis of these parameters could contribute in the following-up of the development of degenerative complications in DM (Advia systems, 2017). Table 4 revealed the effects of G-tea, F-blend, AV, RSb and zobo samples treatment on hematological parameters in type 2 diabetic rats (Table 4). The induction of HFD/STZ significantly ($p<0.05$) decreased the percentages of total red blood cell count (222.7 ± 3.017 rbc/cubic mm), white blood cell count (166.3 ± 2.945 wbc/cubic mm), blood hemoglobin (6.8 ± 0.081 g/100 mL), lymphocyte (10.0 ± 1.469%), neutrophil (9.3 ± 1.869%) and packed cell volume (25 ± 0.662 %). G-tea had significant increase in the PCV of animals treated with the doses compared to the positive control group ($p<0.05$). The 10 mg/kg dose of F-blend had the highest effects on the PCV. Comparatively, these values were within the recommended values for PVC (37.6-50.6), Hb (11.5-16.1), WBC (6.6-12.6) and RBC (6.76-9.75) [5]. This observation indicates that the beverage samples in this study are of high nutritional quality and contain appreciate amount of essential nutrients to support production of blood components in rats, and besides, the beverage samples were free from toxic chemicals and microorganisms, which may result in heamolytic of the blood. Hence, the infused samples may be suitable for the prevention of anaemia [13]. Comparatively, the haematogical indices obtained in this study were higher than that of diabetic- induced rats treated with Glibenclamide (a synthetic antidiabetic agent).

Some phytochemicals such as anthocyanins, are well known to boost the red blood cell levels which is a key index in the determining the PCV levels of the blood. All the treatment samples significantly increased the PCV levels in a dose-

Table 4. *Effects of Red Sorghum bran-Roselle Calyx-Avocado leaf infused beverage samples on the hematological indices of T2DM rats.*

Samples	Conc.	PCV (%)	HBC (g/100 mL)	WBC x 50 wbc/cubic mm	RBC x 103 rbc/cubic mm	Lymphocyte (%)	Neutrophil (%)
Controls	NC	42 ± 0.38	11.50 ± 0.82	279.47 ± 2.37	376.63 ± 3.21	26.9 ± 0.62	49.2 ± 5.82
	PC	25 ± 0.66	6.80 ± 0.08	166.35 ± 2.95	222.70 ± 3.02	10 ± 1.47	9.3 ± 1.87
	GC	29 ± 0.28	7.90 ± 0.27	192.97 ± 4.97	258.73 ± 2.23	18.5 ± 2.63	34.0 ± 0.83
H100	2.5	36 ± 0.37	9.80 ± 0.11	239.54 ± 3.41	320.95 ± 3.07	23 ± 1.39	42.2 ± 2.39
	5	38 ± 0.62	10.40 ± 0.18	252.85 ± 3.15	340.60 ± 3.06	24.3 ± 1.46	44.5 ± 2.86
	7.5	39 ± 0.84	10.60 ± 0.16	259.87 ± 3.20	347.15 ± 3.12	25.4 ± 2.87	45.8 ± 0.27
	10	39 ± 0.35	11.00 ± 0.12	267.51 ± 3.06	360.25 ± 4.16	25.7 ± 3.03	47.1 ± 1.03
R0Z0A100	2.5	28 ± 0.81	8.23 ± 0.17	206.27 ± 3.92	269.53 ± 2.72	19.8 ± 1.71	36.3 ± 5.31
	5	28 ± 0.72	8.49 ± 0.18	206.74 ± 4.13	278.05 ± 3.18	19.9 ± 0.52	36.4 ± 4.12
	7.5	29 ± 0.37	8.96 ± 0.10	212.97 ± 2.97	293.44 ± 4.30	20.5 ± 0.23	37.5 ± 3.43
	10	30 ± 0.44	9.22 ± 0.15	219.62 ± 5.01	301.96 ± 3.20	21.1 ± 4.75	38.7 ± 1.55
R100Z0A0	2.5	26 ± 0.72	7.10 ± 0.29	173.00 ± 3.20	232.53 ± 4.83	16.6 ± 2.60	30.4 ± 3.00
	5	26 ± 0.67	7.40 ± 0.39	183.45 ± 3.09	242.35 ± 2.08	17.6 ± 2.62	32.3 ± 4.22
	7.5	20 ± 0.82	8.20 ± 0.18	199.62 ± 3.19	268.55 ± 3.07	19.2 ± 2.15	35.1 ± 1.95
	10	39 ± 0.78	10.68 ± 0.39	259.51 ± 3.11	349.77 ± 3.16	24.9 ± 1.99	45.7 ± 4.99
R0Z100A0	2.5	28 ± 0.75	7.60 ± 0.83	186.31 ± 3.09	248.90 ± 2.88	17.9 ± 1.65	32.8 ± 4.45
	5	28 ± 0.54	7.72 ± 0.63	189.55 ± 2.94	252.83 ± 1.63	18.2 ± 1.89	33.4 ± 0.89
	7.5	29 ± 0.67	7.94 ± 0.33	192.97 ± 2.34	260.04 ± 4.88	18.5 ± 2.20	34 ± 1.85
	10	35 ± 0.53	9.59 ± 0.18	232.89 ± 3.10	314.07 ± 3.13	22.4 ± 1.86	41.0 ± 4.26
R50Z40A10	2.5	25 ± 0.43	6.85 ± 0.67	166.35 ± 4.10	224.34 ± 3.38	16.0 ± 1.47	29.3 ± 1.87
	5	27 ± 0.27	7.39 ± 0.12	179.66 ± 2.36	242.02 ± 3.23	17.2 ± 2.55	31.6 ± 2.35
	7.5	37 ± 0.18	10.18 ± 0.63	246.20 ± 2.10	333.40 ± 2.30	23.7 ± 0.81	43.4 ± 0.40
	10	41 ± 0.20	11.20 ± 0.17	272.81 ± 3.10	366.80 ± 3.96	26.2 ± 1.60	48.0 ± 1.40

Means (± SEM) with different alphabetical superscripts in the same column are significantly, NC: Negative Control; PC: Positive Control (HFD/STZ (35mg/kg BWT)-induction); GC: HFD/STZ (35mg/kg BWT)-induction and 100 mg/kg BWT Glybenclamide; H100: (Commercial herbal tea flour); R100Z0A0: (100% Red sorghum bran + 0% zobo calyx +0% Avocado leaf), R0Z0A100: (0% Red sorghum bran + 0% zobo calyx +100% Avocado leaf) flour, R0Z100A0: (0% Red sorghum bran + 100% zobo calyx +0% Avocado leaf)flour, R50Z45A5 (50% Red sorghum bran + 40% zobo calyx +10% Avocado leaf)flour; ESR: Total erythrocyte count; RBC; Total red blood cell count; WBC: Total white blood cell count; HB: Blood hemoglobin; PCV: Packed cell volume

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dependent manner compared to the level of PCV in the positive control group. Haematological examination of blood serum is a common and important method used for the assessment of new compounds, such as foods and drugs, and for the detection of some changes in health status of animals and their vital organs, and consequently their fitness. Hematological and biochemical parameters can be used to determine the extent of deleterious effect of foreign compounds (toxic or microorganisms) in the blood constituents of an animal [13]. White blood cells are well established biomarker of inflammation in cardiovascular disease as well as in T2DM and its complications. They could be activated by advanced glycation end products, angiotensin II, and oxidative stress in T2DM, induced by hyperglycemia. Recent studies have shown that the number of red blood cells is decreased; its lifespan is reduced in T2DM due to elevated blood glucose. This study has further established that decreased Hb levels are associated with increased risk of T2DM microangiopathy progression, and has further established this functional beverage, as potential against hematologic complications that could result from diabetes mellitus. Possible mechanisms combine reduced erythrocytes survival, hypoxemia and decreased erythropoiesis. Activated leukocytes at the sites of damaged endothelium release vasoactive substances such as leucotrien, serotonin, thromboxane A2 that lead to vasospasm and favorably influence the development of thrombus, the inhibition of this activation by the administration of this functional beverage could be on the long run responsible for mitigating dissiminated thrombosis. Additionally, platelets express numerous adhesion molecules and ligands that facilitate interactions among platelets, leukocytes, and endothelium, by direct modulation of the activity of leukocytes (neutrophils, lymphocytes) with phagocytosis and oxidative stress; and endothelium with adhesion molecule and chemokine expression.

Conclusion

This study indicated that Red Sorghum bran-Roselle Calyx-Avocado leaf infused beverage has potentials to improve hyperlipidemia. The observed effects can be associated with the levels of in-vitro and *in-vivo* antioxidant present in the extract. The present study also supported the traditional claim in the infused sample R50Z40A10 can serve as hypolipidemic herbal drink. Hence, the beverage can be recommended in the management of atherosclerosis and cardiovascular diseases.

Conflict of Interest

All authors do not have any possible conflicts of interest.

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