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Antioxidant and Toxicological Evaluation of *Vigna subterranea* Seed (Bambara Nut) Fractions on High-Fat Diets-Induced Oxidative Stress in Male Wistar Rats

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ABSTRACT

High-fat diets (HFD) produce free radicals that trigger oxidative stress, increasing the risk of several health challenges. Medicinal plants have shown good antioxidant properties that ameliorate oxidative stress related diseases. This study examined how the hexane (HF) and aqueous (AF) fractions of *Vigna subterranea* (VS) reduce oxidative stress caused by HFD in Wistar rats. A total of 49 adult male Wistar rats were placed into seven groups of seven each. Group 1 (normal control) received standard feed and water, while groups 2–7 were fed HFD for 12 weeks. Afterwards, treatments were given daily for 4 weeks as: Group 2 (HFD + water), Group 3 (HFD + 100 mg/kg orlistat), Groups 4 and 5 (HFD + 200/400 mg/kg HF), and Groups 6 and 7 (HFD + 200/400 mg/kg AF). Various biochemical, haematological, oxidative stress markers, and tissue analyses (liver and kidney) were conducted using standard techniques. Results showed that both HF and AF significantly ($p<0.05$) reduced food intake, body weight, cholesterol levels, AST, ALT, creatinine, urea, and malondialdehyde (MDA), while increasing SOD, CAT, GSH and HDL. No liver or kidney inflammation was observed. These findings suggest that *Vigna subterranea* extracts may help reduce high serum cholesterol, MDA, and tissue damage without adverse effects, supporting needs for additional studies.

Keywords: *Vigna subterranea*, Antioxidants, Toxicology, Metabolic syndrome, High-fat diet, Oxidative stress.

Introduction

Antioxidants are substances that act on free radicals to slow down their oxidation and neutralise or prevent their effects from damaging the biological system.[1] Free radicals exist in the form of reactive oxygen, nitrogen and sulphur species (RONS) such as hydrogen peroxide (H_2O_2), nitric oxide (NO) and superoxide radical anion (O_2 •−).[2] They are generated by oxidative processes from foods, drugs, smokes/gases, chemical agents and biological materials ingested, injected, or inhaled from the environment.[3] RONS activate the body's antioxidant defence systems, both enzymatic and non-enzymatic, using mechanisms such as hydrogen atom transfer (HAT), single electron transfer (SET), and transition metals.[4] These processes inactivate and eliminate RONS,[4] and are considered essential functions of RONS to strike a balanced oxidant and antioxidant level.[5]

However, an imbalance in favour of oxidant levels may overwhelm the antioxidant defence mechanisms, leading to oxidative stress and subsequently oxidative burst and attracting inflammatory cells in vital areas or sites, tissues, organs and the entire system.[6] This may cause acute and chronic health conditions affecting the kidneys,[7] liver,[8] gastrointestinal tract,[9] and other endothelial cells.[10] This may pose risk of cardiovascular diseases, hypertension, diabetes, reproductive abnormalities, respiratory disorders and promote certain kinds of cancer.[1]

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Oxidative stress may also induce DNA damage and inhibit DNA repair mechanisms, leading to some disease conditions.[11] DNA damage can lead to distortion (down or upregulation) of certain transcriptional and posttranslational factors, thereby enhancing alteration of key gene expression and functions.[12]

High-fat diets (HFD) are the chief cause of metabolic syndrome, characterised by increased body weight, high serum lipid concentration, total cholesterol, triglycerides, and low-density lipoproteins.[13] The increase in production, accumulation and deposition of triglycerides in body cells, tissues, and organs may further lead to oxidative stress, subsequently initiating hypercholesterolemia, infiltration of inflammatory cells, platelets activation, abnormal blood pressure, obesity and cardiovascular diseases.[14] Studies have shown that plant metabolites from food and nutritional supplements may be an excellent source for exogenous antioxidants, strengthening the antioxidant defence system.[15] However, toxicity of plant compounds may cause acute and chronic organ damage, which are always limiting factors for utilisation, especially where there is insufficient scientific knowledge.[16]

Vigna subterranea (VS), also referred to as Bambara groundnut, belongs to one of many legume crops in sub-Saharan Africa (SSA). The crop is of the *Fabaceae* and *Faboidea* family and sub-family respectively.[17] It is grown in Africa and is called by different names Okpa by Igbo in South-Eastern Nigeria, Gurujia by Hausa-Fulani in Northern speaking and known as earth pea, while others call it jugo bean, although it is also known as nyimo beans, or ditloo by the people of Southern Africa.[18] The plant is cultivated mainly as a source of protein in food for its seeds are highly nutritious.[19] *Vigna subterranea* has been utilised in folk medicine due to its health benefits and therapeutic properties.[20] It's been reported to be used to treat bacterial infections such as those that cause typhoid fever, abdominal pains and watery stool, sexually transmitted infections, menstrual irregularities, morning illness, and obesity.[19]

In vitro enzyme studies with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) have shown high RONS scavenging, reduction and chelating potentials.[17,20,21] However, *in vivo* antioxidant capacity and their toxicological properties are still under

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investigation. This study is intended to explore the antioxidant and toxicological effects of *Vigna subterranea* fractions in high-fat diet-induced male Wistar rats. We hypothesised that *Vigna subterranea* fractions would exhibit significant antioxidant activity with minimal toxicological effects *in vivo*. *Vigna subterranea* is available and cheap in Africa, identifying its antioxidant property will increase its acceptability and its therapeutic utilisation in disease management, especially those associated with oxidative stress. This study will provide added knowledge on the therapeutic properties and validate the traditional claims on the usage.

Materials and Methods

Plant and Identification

Vigna subterranea seeds were collected from local farmers in Auta-Balefi, Karu Local Government of Nasarawa State, Nigeria located at approximately 8°56′48.8″ N latitude and 7°40′44.0″ E longitude. The plant seed was identified and authenticated in the Department of Botany, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, with specimen voucher number NIPRD/H/7349 issued by Mr. Akeen Lateef Adeyanju in May 2023.

Plant Extraction

The *Vigna subterranea* seeds were washed with clean tap water to remove debris and stones. The seeds were air-dried for two weeks under shed and ground into fine powder using the local grinding mill. The powder was packaged in 2 kg capacity polystyrene zip lock and stored at 20°C-25°C in dry place prior to extraction procedures.

Preparation of hexane and aqueous fractions

Exactly 500 g of *Vigna subterranea* powder was macerated in 1000 ml of absolute ethanol with intermittent shaking at 3-6 hours interval at room temperature for 3 days. The mixture was first sieved using a muslin cloth (spore size 0.22 mm). The filtrate was again filtered using Whatman No. 1 filter paper. The resultant filtrates were concentrated at reduced pressure and temperature of 60°C in a Biobase rotary evaporator (model name RE-501, product no: 22125159 by Biobase Bioyu CO., LTD) fitted with a chiller and a water circulating vacuum pump (Model: SHZ-D(III), Batch No: 22125059). The slurry extracts collected were stored at 2-8ºC prior to fractionations.

Fractionation was carried out using solvent/solvent partitioning, adopting the method described by Hostettmann[22] with slight modification. About 250 g of the methanol extract was suspended in 300 ml of distilled water, then 300 ml of hexane solvent was added, and the mixture was stirred vigorously, releasing accumulated pressure. The mixture was then left to settle for 15 minutes in a separating funnel, forming two separate layers of solvent hexane (top) and water (bottom) which were collected in two different beakers as hexane and aqueous fractions. This procedure was repeated on the aqueous fraction with chloroform, ethyl acetate (3×300ml) separately, and the last fraction was collected as the aqueous fraction. A slurry fractions of hexane, chloroform, ethyl acetate and aqueous obtained after subjecting to rotary evaporator under reduced pressure as described earlier. The different yields were stored at 4ºC prior to analysis.

Acute oral toxicity evaluation

The acute toxicity of hexane (HF) and aqueous (AF) fractions of *V. subterranea* was evaluated following OECD (2002) guidelines. Male Wistar rats (130 ± 11g) were selected at random, labelled then placed in 4 respective groups of 7 rats each. The rats were given 300 and 2000 mg/kg body weight,[23] of HF and AF of VS respectively as a single oral dose after overnight fasting with the aid of distilled water as solvent vehicle. The rats were observed from the first hour after administration to day 7 for abnormal behaviours and signs reflecting toxicity.

GC-MS analysis of HF and AF of Vigna subterranea

The compounds in the hexane and aqueous fractions of *Vigna subterranea* seeds were determined using Shimadzu GC-MS-QP2010 ultra.[24] The compounds were analysed at column oven temperature of 60.0ºC, at 250°C injection temperature and split mode ratio of 10:1 at a pressure of 100 kpa. The column flow was set at 0.8 mL/min, total flow of 13:9 mL/min and linear velocity of 23.1 cm/sec over 5.0 ml/min purge flow. The equilibrium time was maintained at 3 min, with 230ºC ion source temperature under interface temperature of 250 °C at 4.50 min. The ACQ scan was programmed at 28 min at 35-500 m/z at ionization of 70 ev for the system (EI) for the electronic ionization system. The peaks were identified by validating using reference standard NIST11 (National Institute of Standards and Technology) library and literature.

Experimental animals and diet

In this study, 49 male adult Wistar rats of 120 to 150g weight were procured from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The rats were randomly grouped in seven separate cages of seven (n=7) at the Bingham University animal house. The rats were allowed to adapt at ambient temperature, providing access to a 12:12 h light/dark cycle, water and food for 2 weeks prior to study commencement. All ethical procedure was approved by Bingham University Teaching Hospital Health Research and Ethics Committee (BHUTH HREC), Jos, Plateau State, Nigeria, with number NHREC/21/05/2005/01398. Animal grouping was done, thus, group 1 fed on the normal pelleted diet (NPD), while groups 2-7 had HFD *ad libitum* for 12 weeks. The high-fat diet contained 50% fat, 27% carbohydrates, 16% protein, and 7% fibre as described by Anyanwu et al.[25] Thereafter, rats in groups 1 (normal control) and 2 (HFD control) received distilled water, while group 3 received 100 mg/kg body orlistat as the standard drug. However, rats in groups 4 to 5 and 6 to 7 were administered 200 and 400 mg/kg hexane and aqueous fractions, respectively, using normal saline as the vehicle for fraction administration. All interventions were orally introduced daily for 28 days.

Blood collection and processing

After 4 weeks of treatment, all rats were anaesthetised by exposing to inhale 5 ml of 3% Isoflurane applied on absorbent cotton wool placed in the chamber, blood sample was collected by cardiac puncture. Blood was transferred into plain and EDTA tubes to target serum and whole blood respectively. The blood in plain tubes were centrifuged at 3000 rpm at 4ºC in a refrigerated centrifuge for 15 min. The serum was stored in 2ml capacity cryovials at -20ºC prior to analysis. Haematological analysis was performed on the whole blood collected in EDTA tubes.

Biochemical analysis

The following biochemical tests were determined: total cholesterol (Trinder,1969), Triglycerides (Tietz, 1990), high density lipoprotein cholesterol (Tietz, 1976), while very low-density lipoproteins (VLDL) and low-density lipoprotein cholesterol (LDL) were calculated using the (triglyceride/5) and Friedewald equation respectively. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total protein, Albumin, Urea and creatinine were assayed using the manufacturer's protocols (ELITEC assay kits, Netherlands) and analyzed with Selectra Pros S spectrophotometer (Vital Scientific, Netherlands).

Haematology analysis

Full blood count was performed on EDTA collected samples using XN-350, analyser (Sysmex XN-Series technology) for RBCs, Hb, HCT, WBC, lymphocytes % and PLT count and recorded adhering to the equipment operation manual and laboratory standard operating procedure.

Enzymatic antioxidant assays

The antioxidant activities were determined using Elabscience for total superoxide dismutase (T-SOD), glutathione (GSH), and catalase (CAT) activities were performed following the assay kit instructions provided by the manufacturer. In this study, measurement of antioxidant activities was done by determining the serum TSOD, GSH and CAT

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activities measured with spectrophotometer at wavelength of (405 nm) while MDA levels were calculated by the difference between wavelength absorbance at 532 nm, 450 nm and 600 nm described by the manufacturer.

Histopathology

The organs were rinsed in several changes of distilled water and stored in 10% neutral buffered formalin for histological analysis. The liver and kidney were sliced into small pieces of ≤ 4 mm thick and placed into labelled bottle containing fixative for a period of 24h. The fixed tissues were examined at X100 objectives of the microscope after staining with haematoxylin and eosin, observing standard laboratory practice.

Statistical analysis

The experimental data were determined using GraphPad Prism 5 and SPSS version. 23 choosing one-way ANOVA, followed by Duncan's multiple range test at $P<0.05$. The results were shown in Mean ± S.E.M. in tabular format, demonstrating significant differences in parameters among the groups.

Results and Discussion

Antioxidants exert important roles in eliminating RONS, preventing cellular and tissue deformations that will disturb metabolic functionality.[6] Studies have shown that high-fat diets may elevate serum lipid profiles which can also increase free radicals generation by lipid peroxidation, leading to both acute and chronic diseases.[26,27] Plant compounds have exhibited tremendous free radical scavenging potential in preventing oxidative stress and its associated risk.[28]

There were no adverse effects or deaths recorded in groups administered with hexane and aqueous fractions of *V. Subterranea* up to 2000 mg/kg dose. These observations may be attributed to low or no acute toxicity observed for the hexane and aqueous fractions of *V. subterranea*, which justifies the dose used in this study. The HF showed the presence of 5 compounds with 9,12-Octadecadienoic acid (Z,Z)- (67.19%) and Octadecanoic acid, ethylester (18.06%) as the most abundant compounds in the fraction (Table 1). The aqueous fraction showed the presence of 17 compounds with 4-O-Methylmannose (11.37%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (11.22%) and 9,12-Octadecadienoic acid (Z,Z)- (11.15%) as most abundant compounds (Table 2). The compounds in HF of VS were Docosanoic acid, ethyl ester, Octadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- and Octadecanoic acid, ethyl ester. The AF of VS contained various bioactive compounds such as 5-Hydroxymethylfurfural, Maltol, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2,3,4-Trihydroxy-6-methylcyclohexanone, 4-O-Methylmannose.

Table 1: GC-MS of compounds found in hexane fraction of *V. subterranea*

Peak #	R. Time	Compounds	Area%
1	19.120	Docosanoicacid, ethylester	8.23
2	21.932	Octadecanoicacid, ethylester	18.06
3	24.102	9,12-Octadecadienoicacid (Z,Z)-	67.19
4	24.236	9,12,15-Octadecatrienoicacid (Z,Z,Z)-	2.24
5	24.370	Octadecanoicacid, ethylester	4.27

Table 2: GC-MS of compounds found in aqueous fraction of *V. subterranea*

Peak#	R. Time	Compounds	Area%
1	8.984	Dihydroxyacetone	6.28
2	10.578	Diglycerol	5.77
3	11.709	Maltol	6.06
4	12.811	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	4.58
5	12.931	dl-Glyceraldehydedimer	2.77
6	13.975	5-Hydroxymethylfurfural	3.28
7	16.841	1,3-Propanediol,2-(hydroxymethyl)-2-nitro-	11.22
8	18.975	Ethyl.alpha.-d-glucopyranoside	3.38
9	19.140	Ethyl.alpha.-d-glucopyranoside	12.60
10	19.426	4-*O*-Methylmannose	11.37
11	19.455	2,2-Dimethyl-5-(2-methyl-[1,3] dioxolan-2-yl)	4.31
12	19.545	Methyl4,8-dimethylnonanoate	5.88
13	19.565	Cyclohexanone,2,3,4-trihydroxy-6-methyl-,[	3.23
14	21.925	Hexadecanoicacid,ethylester	4.41
15	24.094	9,12-Octadecadienoicacid(Z,Z)-	11.15
16	24.354	Octadecanoicacid,ethylester	1.23
17	24.465	Cyclohexanol,4-(1-methylethyl)-	2.48

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Docosanoic acid, ethyl ester, Octadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z)- in HF of *Vigna subterranea* and Hexadecanoicacid, ethylester in the AF of *Vigna subterranea* were also part of the compounds identified in study by Abdulmumin et al.[29] While Maltol, 9,12-Octadecadienoic acid (Z,Z)-, and Octadecanoic acid were said to be present in the GC-HRT of fermented bambara nuts by Adebiyi et al.[30] Generally, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- in HF of *Vigna subterranea* are predominantly polyunsaturated fatty acid (PUFA) while AF are predominantly esters (Methyl 4,8-dimethylnonanoate, Hexadecanoic acid, Docosanoic acid, ethyl ester, Octadecanoic acid, ethyl ester).[30] These compounds have been investigated and reported to have antioxidant properties.[31] Maltol, 9,12-Octadecadienoic acid (Z,Z)-, Octadecanoic acid, ethyl ester, Cyclohexanol, 4-(1-methylethyl) were reported for their anti-inflammatory potentials.[32] Other groups in AF are pyranones (4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6, maltol), carbohydrate derivatives (dl-Glyceraldehyde dimer, Dihydroxyacetone, Ethyl α-D-glucopyranoside, 4-O-Methylmannose), Cyclohexanones (Cyclohexanone,2,3,4-trihydroxy-6-methyl-, Cyclohexanol,4-(1-methylethyl)-), Furan derivatives; 5-Hydroxymethylfurfural, Nitro Alcohols; 1,3-Propanediol,2-(hydroxymethyl)-2-nitro-, Dioxolanes; 2,2-Dimethyl-5-(2-methyl-[1,3]dioxolan-2-yl). These compounds have shown positive effects in reducing free radicals and protecting the body against inflammation due to oxidative burst.[33] Hence, these fractions are rich in compounds known to play tremendous roles in enhancing free radicals neutralisation and elimination. This suggests that *V. substerranea* may help in reducing effects of oxidative stress and inflammation.

Tables 3, 4 and 5 illustrated the effects of HFD on food intake, body and organ weights, respectively. There were significant decrease ($p < 0.05$) in food intake, body weight and organ weights of HF and AF groups of VS when compared to HFD control at week 4. The decrease in body weights in treatment groups was more prominent in 200 and 400 mg/kg AF exerting dose-dependent fall in body weights. But liver, kidney and fat weights decreased in dose-dependent levels in both 200 and 400 mg/kg of HF and AF of VS, respectively, notably in AF, causing peak decrease in liver, kidney and fat weights.

Table 3: Effect of hexane and aqueous fractions of *Vigna subterranea* on food intake of HFD male Wistar rats

	Food intake (g)					
		Week 0	Wk1	Wk 2	Wk 3	Wk 4
Groups	Dosage mg/kg					
NPD control		144.75 ± 1.31^{a}	144.00 ± 0.91^{a}	143.75 ± 1.44^{a}	144.50 ± 0.96^{ab}	145.50 ± 0.87^{a}
HFD control		146.75 ± 0.75^{a}	144.75 ± 0.63^{a}	146.00 ± 1.68^{a}	147.00 ± 0.71^{a}	146.75 ± 0.48^{a}
HFD+ Orlistat	100	145.00 ± 0.41^{a}	140.75 ± 0.48^{c}	135.25 ± 0.48^{b}	139.00 ± 4.34^{b}	139.75 ± 6.01^{a}
HFD+ Hexane fraction	200	144.75 ± 2.21^{a}	141.50 ± 0.29^{ab}	123.75 ± 1.89^{c}	111.25 ± 3.71^{c}	96.00 ± 1.78^{b}
HFD+ Hexane fraction	400	145.50 ± 0.87^{a}	143.00 ± 0.41^{ab}	124.50 ± 1.55^{c}	114.25 ± 2.46^{c}	94.25 ± 0.63^{b}
HFD+ Aqueous fraction	200	145.50 ± 1.55^{a}	143.00 ± 0.82^{ab}	125.00 ± 2.42^{c}	115.00 ± 1.41^{c}	95.00 ± 1.22^{b}
HFD+ Aqueous fraction	400	145.00 ± 1.08^{a}	143.50 ± 0.65^{ab}	125.00 ± 1.15^{c}	116.50 ± 1.50^{c}	97.50 ± 1.19^{b}

The reported values are in Mean ± S.E.M. Mean of ($p < 0.05$) are significant for those in the same column having different letter(s). Wk (Week)

Table 4: Effect of Hexane and aqueous fractions of *Vigna subterranea* on body weights of HFD male Wistar rats

	Weight (g)					
		Wk 0	Wk 1	Wk 2	Wk 3	Wk 4
Groups	Dosage mg/kg					
NPD control		202.67 ± 3.71^{a}	224.67 ± 2.03^{b}	231.67 ± 0.88^{b}	236.33 ± 1.20^{b}	240.67 ± 2.19^{b}
HFD control		204.67 ± 1.45^{a}	249.00 ± 2.52^{a}	257.67 ± 6.44^{a}	260.67 ± 4.91^{a}	266.33 ± 0.88^{a}
HFD+ Orlistat	100	207.00 ± 1.53^{a}	195.00 ± 2.65^{c}	193.67 ± 2.33^{c}	185.00 ± 2.65^{c}	170.33 ± 0.88^{c}
HFD+ Hexane fraction	200	206.33 ± 1.86^{a}	179.67 ± 2.85^{de}	170.00 ± 1.15^{e}	163.67 ± 1.76^{e}	157.33 ± 0.67^{d}
HFD+ Hexane fraction	400	206.00 ± 2.08^{a}	173.33 ± 1.20^{e}	158.33 ± 0.33^{f}	150.67 ± 0.88^{f}	143.33 ± 2.85^{e}
HFD+ Aqueous fraction	200	204.00 ± 2.08^{a}	193.33 ± 2.19^{c}	184.00 ± 1.15^{d}	176.00 ± 1.53^{d}	136.33 ± 2.19^{c}
HFD+ Aqueous fraction	400	205.33 ± 0.33^{a}	185.00 ± 1.00^{d}	179.33 ± 2.03^{d}	166.00 ± 0.58^{e}	156.00 ±1.155^{d}

The reported values are in Mean ± S.E.M. Mean of ($p < 0.05$) are significant for those in the same column having different letter(s). Wk (Week)

Table 5: Effect of hexane and aqueous fractions of *Vigna subterranea* organ weights of HFD male Wistar rats

Groups	Dosage mg/kg	Liver (g)	Kidney (g)	Fat tissues (g)
NPD control		7.10 ± 0.46^{ab}	1.96 ± 0.17^{a}	1.57 ± 0.03^{bc}
HFD control		9.03 ± 0.66^{a}	1.91 ± 0.05^{a}	2.98 ± 0.59^{a}
HFD+ Orlistat	100	7.33 ± 0.45^{ab}	1.54 ± 0.03^{bc}	2.00 ± 0.10^{bc}
HFD+ Hexane fraction	200	6.56 ± 0.81^{b}	1.71 ± 0.05^{ab}	1.97 ± 0.32^{bc}
HFD+ Hexane fraction	400	6.27 ± 0.87^{b}	1.33 ± 0.09^{c}	1.39 ± 0.06^{c}
HFD+ Aqueous fraction	200	5.83 ± 0.36^{b}	1.57 ± 0.04^{bc}	2.33 ± 0.12^{ab}
HFD+ Aqueous fraction	400	5.77± 0.59^{b}	1.40 ± 0.10^{c}	1.50 ± 0.20^{bc}

The reported values are in Mean ± S.E.M. Mean of ($p < 0.05$) are significant for those in the same column having different letter(s).

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HFD can increase body weight, inducing metabolic syndrome and free radicals due to increased accumulation of triglycerides in the adipose tissues,[13] in obese individuals.[33] Weight reduction is key to preventing oxidative processes and free radical generation to prevent cell damage.[1,37] Reduction in body weights in this study was achieved in rats fed with both HF and AF of VS.[26] Although, weight reduction may be achieved through different mechanisms such as satiety, gastrointestinal inhibition and thermogenesis by plant compounds. However, the mechanism by which weight loss was achieved in this study is under investigation. The reduction in body and organ weights is synonymous with decrease in food intake recorded.[34] These effects may be connected to the role of docosanoic acid, octadecanoic acid, 9,12-octadecadienoic acid and diglycerol in lipid metabolism,[37] which were present in the HF and AF of VS (Tables 1 and 2) and require further investigation. Therefore, the compounds in fractions of VS may decrease food intake, fat tissue and organ weights, hence translating to possible reduction in oxidative stress.

The TCHO (total cholesterol), TRIG (triglycerides), VLDL-C (very low-density lipoproteins- calculated) and LDL-C (low density lipoproteins-calculated) were significantly reduced ($p < 0.05$) in HF and AF treatment groups in dose dependent levels when compared to HFD control groups. However, 400 mg/kg of AF had the lowest effect on TCHO, TRIG, VLDL-C and LDL-C values in all the treatment fractions of VS (*Vigna subterranea)* (Table 6). Studies have reported that HFD increases total cholesterol, triglycerides, VLDLs, LDL and decrease HDL.[34] The HFD-fed control groups when compared to NPD control groups had significant increase in TCHO, TRIG, VLDL-C and LDL-C. But HF and AF of VS treatment groups revealed reduced TCHO, TRIG, VLDL-C and LDL-C with an increase in HDL-C when compared with HFD control Wistar rats. *Vigna substerranea* was observed to reduce lipid profile in normal Wistar rats fed with standard diets.[35] Similarly, VS fractions reduce the lipid profile in Streptozotocin induced diabetic rats.[36] Decrease in lipid profile may also be one of the reasons there was decrease in body and organ fats observed earlier in this study. Reduction in lipid profile in plant fractions treatment groups could suggest these fractions possess anti-hyperlipidemic or hypolipidemic properties. Hence, this may also suggest that VS could be beneficial in reversing the hyperlipidemia and lipid peroxidation processes that could produce free radicals in HFD metabolic syndrome.

Table 6: Effect of hexane and aqueous fractions of *Vigna subterranea* on fasting lipid profile of HFD male wistar rats

Groups	Dosage mg/kg	TCHO (mmol/L)	TRIG (mmol/L)	HDL (mmol/L)	VLDLC (mmol/L)	LDL-C (mmol/L)
NPD control		1.59 ± 0.03^{b}	0.79 ± 0.05^{c}	0.31 ± 0.02^{c}	0.40 ± 0.02^{c}	1.12 ± 0.03^{b}
HFD control		2.07 ± 0.06^{a}	0.95 ± 0.01^{b}	0.13 ± 0.01^{c}	0.19 ± 0.00^{d}	1.75 ±0.07^{a}
HFD+ Orlistat	100	1.14 ± 0.02^{cd}	1.00 ± 0.07^{b}	0.28 ± 0.03^{c}	0.20 ± 0.01^{d}	0.63 ± 0.01^{c}
HFD+ Hexane fraction	200	1.17 ± 0.00^{c}	1.12 ± 0.05^{a}	0.83 ± 0.03^{a}	0.56 ± 0.01^{a}	0.12 ± 0.01^{e}
HFD+ Hexane fraction	400	1.07 ± 0.00^{d}	0.97 ± 0.00^{b}	0.68 ± 0.02^{ab}	0.48 ± 0.00^{b}	0.20 ± 0.03^{e}
HFD+ Aqueous fraction	200	1.16 ± 0.02^{bc}	0.97 ± 0.00^{b}	0.55 ± 0.16^{c}	0.49 ± 0.00^{b}	0.42 ± 0.15^{d}
HFD+ Aqueous fraction	400	1.08 ± 0.01^{d}	0.81 ± 0.04^{c}	1.68 ± 0.02^{bc}	0.40 ± 0.01^{c}	0.24 ± 0.02^{de}

The reported values are in Mean ± S.E.M. Mean of ($p < 0.05$) are significant for those in the same column having different letter(s). Total cholesterol (TCHO), Triglycerides (TRIG), calculated very low-density lipoproteins (VLDL-C) and calculated low-density lipoproteins (LDL-C).

Serum concentrations of ALT, AST, creatinine and urea were significantly decreased ($p < 0.05$) in HF and AF treatment groups of VS in dose-dependent levels when compared to HFD control (Table 7). The 400 mg/kg of HF and AF of VS were observed to be more effective. Although there was no significant changes in albumin concentration irrespective of the dose used for the HF or AF of *V. subterranea*. Oxidative stress promotes the progression of many diseases,[1,37] organ injury in liver [38] and kidneys.[7] Increased serum liver AST and ALT enzymes, creatinine and urea levels are indications of liver and kidney alterations.[39] This was observed in HFD control groups but HF and AF treatment groups of VS significantly decreased ($p<0.05$) serum AST, ALT, creatinine and urea levels (Table 7). In similar studies, AST and ALT were reported to remain unaltered in Wistar rats treated with VS.[40,41] This is suggestive that the fractions of *V. subterranea* possessed compounds that might have ameliorated acute oxidative alterations in liver and kidney of Wistar rats [41] with no harm or injuries to the liver and the kidney.[40]

Table 7: Effect of hexane and aqueous fractions of *Vigna subterranea* on Kidney and Liver function of HFD male wistar rats

Groups	Dosage (mg/kg)	Creatinine (µmol/L)	Urea (mmol/L)	Albumin (g/L)	ALT (U/L)	AST (U/L)
NPD control		66.80 ± 7.30^{b}	7.08 ± 0.87^{b}	30.17 ± 1.27^{a}	68.33 ± 10.52^{b}	159.67 ± 3.84^{b}
HFD control		95.33 ± 3.89^{a}	9.31 ± 0.56^{a}	28.10 ± 3.60^{a}	108.67± 14.11^{a}	196.00 ± 8.96^{a}
HFD+ Orlistat	100	65.33 ± 6.92^{b}	5.17 ± 0.27^{c}	30.77 ±1.32^{a}	109.33 ± 7.26^{a}	163.00 ± 6.92^{b}
HFD+ Hexane fraction	200	58.50 ± 2.03^{c}	5.50 ± 0.12^{d}	28.50 ± 0.28^{a}	67.50 ± 4.33^{b}	151.00 ± 8.66^{bc}
HFD+ Hexane fraction	400	51.33 ± 0.33^{c}	5.10 ± 0.70^{c}	28.00 ± 0.57^{a}	70.33 ± 9.53^{b}	149.33 ± 8.67^{bc}
HFD+ Aqueous fraction	200	51.67 ± 1.45^{c}	5.50 ± 0.17^{d}	28.00 ±1.53^{a}	70.67 ± 4.48^{b}	157.66±10.33^{bc}
HFD+ Aqueous fraction	400	51.03 ± 0.30^{c}	5.30 ± 0.70^{d}	28.00 ± 0.58^{a}	70.60 ± 9.53^{b}	149.33 ± 8.67^{bc}

The reported values are in Mean ± S.E.M. Mean of ($p < 0.05$) are significant for those in the same column having different letter(s). Alanine amino transferase (ALT), Aspartate amino transferase (AST).

Treatment with n-hexane and aqueous fractions of VS increased but not significantly ($p < 0.05$) RBC counts in 200 HF and 200/400 mg/kg AF treatment groups of VS. However, 400 mg/kg HF of VS were observed to be significantly increased in RBCs count, HGB concentration and HCT level in 200 HF and 200/400 mg/kg AF treatment groups of VS, maintaining a higher value in 400 mg/kg HF of VS when compared to

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the HFD control groups. The WBCs values were significantly reduced ($p < 0.05$) in HF and AF of VS treatment groups. This corresponds to significant decrease ($p < 0.05$) in (LYMPER) lymphocytes percentage and PLT count in dose-dependent levels in HF and AF treatment groups of VS when compared to HFD control groups (Table 8). Metabolic Syndrome may impact negative changes in some haematological parameters such as RBCs, WBC and platelets in animal models due to oxidative burst.[42] The liver and kidneys play crucial roles in haemopoiesis. Plant compounds are also sources of iron that may help maintain the required blood cells and volume in cases of anaemia. Defects in liver and kidney may cause iron deficiency and decrease blood cells production significantly and loss of blood, especially in the red blood cell indices (RBCs counts, Hb concentration and HCT) levels as observed in HFD control group.[42] However, the n-hexane and aqueous fraction treatment of VS exhibited a significant increase in RBCs counts, Hb concentration levels and HCT compared with HFD control group. The compounds in HF and AF may be enriched with compounds with haemopoietic effects.[40]

Table 8: Effect of hexane and aqueous fractions of *Vigna subterranea* on some selected haematological parameters in HFD male Wistar

Groups	Dosage (mg/kg)	RBC (10^6/uL)	HGB (g/dl)	HCT (l/l)	WBC (10^9/l)	LYMPH (%)	PLT (10^3/uL)
NPD control		7.29 ± 0.24^{c}	11.44 ± 0.62^{c}	30.15 ± 0.16^{b}	11.09 ± 1.01^{b}	76.05 ± 1.81^{b}	538.40 ± 19.87^{d}
HFD control		8.47 ± 0.15^{b}	10.72 ± 0.36^{c}	45.66 ± 0.40^{c}	15.68 ± 0.92^{a}	86.17 ± 1.74^{a}	854.67 ± 21.40^{a}
HFD + Orlistat	100	8.83 ± 0.42^{b}	15.28 ± 0.35^{b}	49.83 ± 0.23^{b}	8.73 ± 0.20^{c}	78.00 ± 1.43^{ab}	727.67 ± 3.53^{b}
HFD + Hexane fraction	200	8.82 ± 0.13^{b}	15.20 ± 0.06^{b}	51.60 ± 0.69^{ab}	9.15 ± 0.40^{bc}	69.15 ± 1.82^{b}	665.67 ± 4.48^{c}
HFD + Hexane fraction	400	10.20 ± 0.21^{a}	16.57 ± 0.41^{b}	52.37 ± 0.62^{a}	9.86 ± 0.52^{bc}	74.33 ± 3.85^{b}	665.43 ± 20.91^{c}
HFD + Aqueous fraction	200	8.67 ± 0.32^{b}	14.37 ± 0.55^{b}	50.00 ± 0.71^{b}	10.02 ± 0.52^{bc}	71.30 ± 0.85^{b}	650.33 ± 22.45^{c}
HFD + Aqueous fraction	400	9.09 ± 0.14^{b}	14.90 ± 0.06^{b}	50.77 ± 0.78^{ab}	8.29 ± 0.42^{c}	74.33 ± 6.16^{b}	627.67 ± 3.53^{c}

Values are reported as Mean ± S.E.M. Mean considered significantly ($p < 0.05$) are those in the same column having different letter(s). Red Blood Cell (RBC), haemoglobin (HGB), white blood count (WBC), lymphocyte (LYMPH), platelet (PLT).

High WBC, especially lymphocytes, may suggest the ability of the animals to respond to biological agents and stress.[40] This was evident in the HFD control groups, which had an increase in WBC and lymphocytes in this study. However, the HF and AF of VS showed significant decrease ($p<0.05$) in WBC and lymphocytes when compared to HFD control groups. Oxidative burst or raptures are damages that occur in cells and tissues or organs due to the effects of high free radicals generated during lipid peroxidation in HFD.[3] Neutrophils and other phagocytes are recruited to the sites of injury to initiate healing mechanisms.[43] Therefore, the increase in WBCs and lymphocytes in the HFD group observed in this study may be in response to oxidative damage in tissues and organs as an attempt to engage in tissue repair mechanisms.[37] However, treatment with HF and AF suggests ameliorative effects of fractions of *V. subterranea* eliciting oxidative stress responses.[40]

Oxidative insults may also stimulate platelet activity.[44] Increased platelet count as observed in HFD control groups in this study. The implication is that an increase in platelet count increases the risk of platelet aggregation, posing high risk of development and progression of atherosclerosis which are likely to increase cardiovascular events in HFD-induced oxidative stress due to increased cholesterol, fat deposition and body weight.[44,45] The significant decrease in platelet counts in HF and AF treatment of VS may be a pointer that the fraction contains compounds that inhibit platelet aggregation, thereby suppressing the platelet mechanism PCSK9 (Proprotein Convertase Subtilisin Kexin 9) which could lead to cardiovascular issues.

There were significant increase ($p < 0.05$) in CAT, TSOD and GSH activity in HF and AF of VS in dose dependent fashion compared to HFD control group (Figure. 1). MDA levels were observed to be significantly deceased in a dose dependent manner in HF and AF of VS treatment groups when compared to HFD control ($p < 0.05$). High-fat diets increase total cholesterol and triglycerides, which in turn increase free fatty acid β oxidation in the mitochondria (lipid peroxidation),[37] generating increased free radicals with detrimental consequences.[13] This is evident by increased MDA level and decreased TSOD, CAT, GSH activities makers of oxidative stress, indicating high free radical production and impacting oxidative stress, hence overwhelming the antioxidant defence mechanisms at the same time, favouring the oxidant levels.[46] In this study, increased MDA and decreased TSOD, CAT, and GSH concentrations were observed in HFD control groups. However, there was a reversal in HF and AF of *V. subterranea* treatment groups with significant decreased in MDA levels and increased TSOD, CAT, GSH (Figure.1). Megwas et al, had also highlighted decreased MDA levels and increased SOD, CAT, GSH activities in alcoholic induced oxidative stress in Wistar rats after treatment with *V. subterranea*.[27] Therefore, HF and AF of *V. substerranea* may increase SOD, CAT, GSH activities and decrease MDA level hence strengthening the endogenous antioxidant defence system and enhancing elimination of free radicals and preventing oxidative processes.

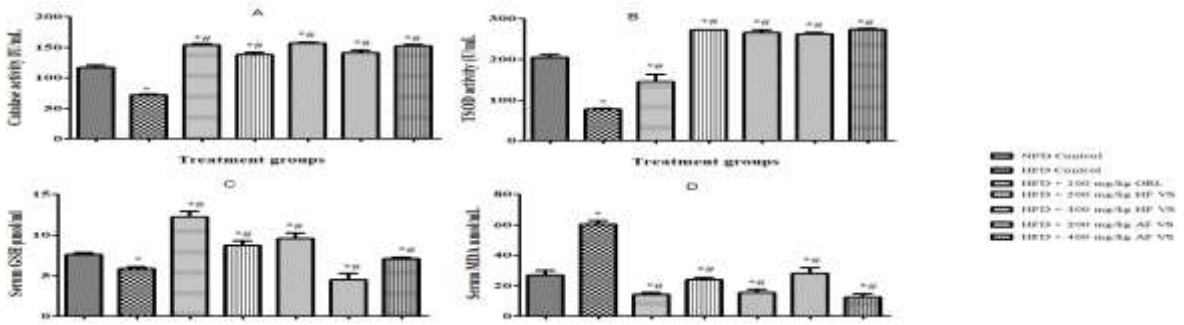

Figure 1: Effects of hexane and aqueous fractions on oxidative stress markers

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Bars with * and /or # are reported as significantly different ($p<0.05$) from NPD and HFD controls respectively. NPD=Normal pelleted diets, HFD=High Fats diets, ORL=Orlistat, HF=hexane fraction, AF=Aqueous fraction. Total superoxide dismutase (TSOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA).

Figure. 2 showed the liver tissues X100 magnification. The HFD control showed the sinusoids had a mild intrusion of inflammatory cells (slim arrow). The 200 and 400 mg/kg HF and AF of VS showed that the central venules (white arrow), hepatocytes shape (blue arrow) and sinusoids were normal and were not harboured by inflammatory cells (slender arrow). Figure. 3 revealed kidney tissues stained X100 magnification. The HF and AF of VS treatment groups have normal renal, cuboidal epithelial arrangement on tubular basement membrane (blue arrow). The interstitial spaces, however, in 200 mg/kg HF of VS showed mildly congested vessel (slim arrow). But the 400 mg/kg HF VS treatment group did not show infiltration by inflammatory cells (slender arrow). The 200 and 400 mg/kg AF VS treatment groups also have normal renal tubules, cuboidal epithelial and tubular basement membrane (blue arrow) arrangements when compared with HFD control groups.

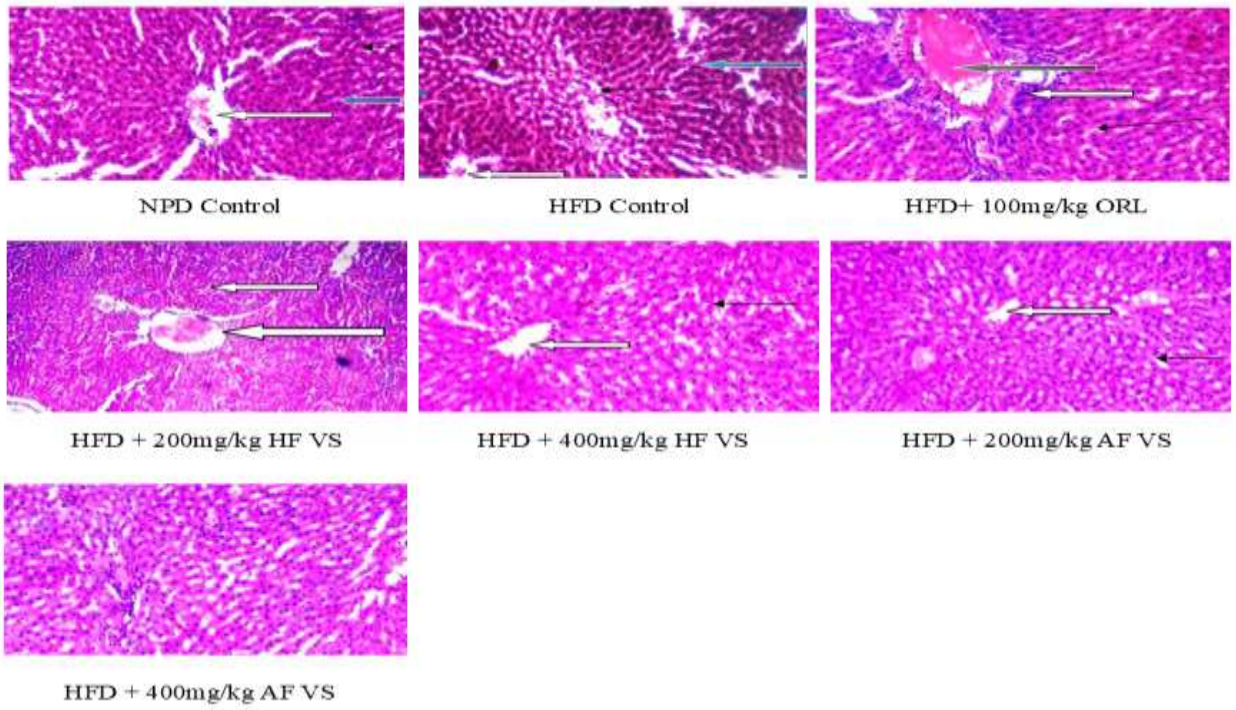

Figure 2: The liver photomicrograph section stained by Haematoxylin and Eosin showing effects of n-Hexane and aqueous fractions (X100). NPD= Normal pelleted diets, HFD=High Fats diets, ORL=Orlistat, HF= hexane fraction, AF=Aqueous fraction.

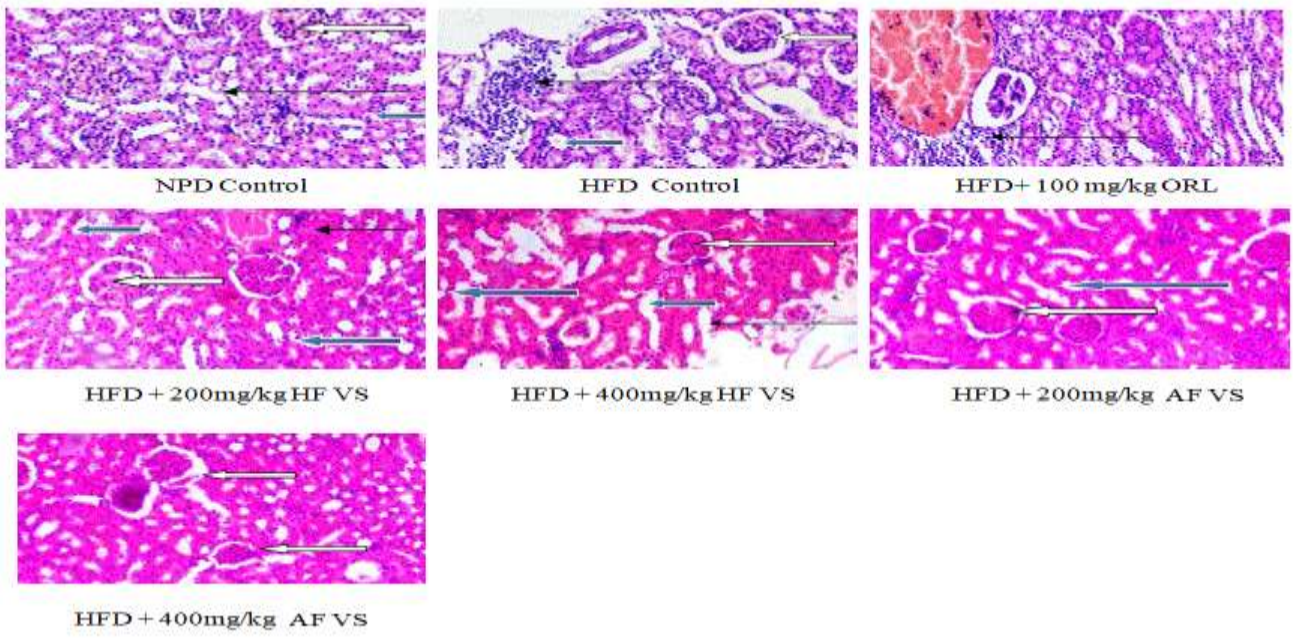

Figure 3: The kidney photomicrograph section stained by Haematoxylin and Eosin showing effects of hexane and aqueous fractions (X100). NPD= Normal pelleted diets, HFD=High Fats diets, ORL=Orlistat, HF= hexane fraction, AF=Aqueous fraction.

The liver and kidneys are two vital organs responsible for detoxification [48] and excretion [49] of waste products. While intact and undisturbed organ morphology is a key requisite for optimum liver and kidney functions, some plant compounds may have toxic effects on these organs, thereby limiting their functions.[44] Maintaining a healthy organ function can be achieved by antioxidant functions and the non-toxicity of plant compounds. The liver section showed normal sinusoids' appearance with no infiltration of inflammatory cells caused by HF and AF of VS in 200 and 400 mg/kg. The kidneys of the rats treated with HF and AF of *V. substerranea* had normal architecture with no infiltration of inflammatory cells. Inflammatory cells infiltration could on one hand, signify a response to tissue damage due to oxidative injuries[50] in the liver and kidneys[7] and on the other hand, reflecting toxicity damage or effects of the plant fractions.[51] The infiltration of inflammatory cells observed in the HFD control group revealed oxidative stress since there was no plant fraction administration in this group. The absence of inflammatory cells in the liver and kidney of the HF and AF treatment group of VS may also have been due to the antioxidant effects of the plant fractions on the organs on one hand, and on the other hand, the less or no toxicity effects of the fraction on the other hand. This effect was also established by Dos Santos Lacerda et al,[39] who observed restoration of cell sloughing and dilation in the tubules of the kidney in HFD-induced rats, but on treatment with grape juice, there was no glomerular distortion, irregularity in tubular size. The normal liver and kidney structure observed further agreed with the decrease in AST, ALT, creatinine and urea levels, suggesting hepatoprotective and renoprotective effects of VS in obesity, diabetes, hypertension and other kidney-related risk conditions.

Conclusion

This study illustrated promising antioxidant properties of n-hexane and aqueous fractions of VS by enhancing increase activities of superoxide dismutase and catalase, glutathione levels, and reducing malondialdehyde levels in high-fat diet Wistar rats, particularly in the aqueous fraction. These findings highlight the antioxidant ability of VS fractions to ameliorate oxidative stress induced by high-fat diet, with a notable protective effect on the liver and kidney. This is suggestive that n-hexane and aqueous fractions of VS may have therapeutic potential in oxidative stress and related conditions. Future studies on understanding the specific antioxidant mechanisms at the molecular level and long-term effects is highly encouraged to enhance drug advancement.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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