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Phytochemical Screening, Antioxidant Activity and Lipid Profile Effects of *Citrus reticulata* Fruit Peel, *Zingiber officinale* Rhizome and *Sesamum indicum* Seed Extracts

Samar Saadeldin Abdelmotalab Omer, Ikram Mohamed Eltayeb Elsiddig, Amna El Hassan Hamad Mohammed, Saad Mohamed Hussein Ayoub

***Abstract*—**Many herbal medicinal products are considered potential anti-hypercholesterolemic agents with encouraging safety profiles, however only a limited amount of clinical research exists to support their efficacy. The present study was designed to compare the antihypercholesterolemic and antioxidant activities of the crude ethanolic extracts of *Citrus reticulata* fruit peel, *Zingiber officinale* rhizome and *Sesamum indicum* seeds.

Forty-five rats were used throughout the experiment which are extended for four weeks. These were divided into nine groups, five rats per each group as follows; group 1 was the normal control group (rats only fed standard normal rat diet), group 2 was the hypercholesterolemic control group (rats fed only hypercholesterolemic diet which contained 1% cholesterol plus 10% saturated animal fat added to the normal rat diet), groups 3 and 4 were fed hypercholesterolemic diet in addition to *Citrus reticulata* ethanolic extract at doses of (250mg/kg (group 3) and 500mg/kg (group 4)) administered daily via oral route, groups 5 and 6 were given hypercholesterolemic diet in addition to *Zingiber officinale* ethanolic extract at doses of (250mg/kg (group 5) and 500mg/kg (group 6)) daily through oral route, groups 7 and 8 fed on hypercholesterolemic diet in addition to *Sesamum indicum* ethanolic extract at doses of (250mg/kg (group 7) and 500mg/kg (group 8)) daily orally; and group 9 rats were given hypercholesterolemic diet in addition to atorvastatin (0.18mg/kg) daily via oral route as a standard reference antihypercholesterolemic drug. Blood samples from all groups were drawn from the retro-orbital venous plexus four weeks following treatment after overnight fasting and the lipid profile (total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) and triglyceride levels) were measured and the risk ratio (TC/HDL-C) was assessed. The antioxidant activity of the three plants extracts was determined using DPPH free-radical antioxidant assay. Results of *in vivo* and *in vitro* antihypercholesterolemic and antioxidant assay respectively, revealed that the three extracts possess comparable antioxidant and antihypercholesterolemic activities.

***Keywords*—**Antihypercholesterolemic effects, Antioxidant activity, HDL, LDL, TC, TGs, *Citrus reticulata, Sesamum indicum, Zingiber officinale.*

Samar Omer is with the University of Medical Sciences and Technology, Khartoum, Sudan (Phone: +249912731913; e-mail: sammorano@live.com).

Dr. Ikram Mohamed Eltayeb Elsiddig and Prof. Dr. Saad Mohamed Hussein Ayoub are with the department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology.

Dr. Amna El Hassan Hamad Mohammed is in the department of Pharmacology, Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

I. Introduction

DYSLIPIDEMIA is a lipid profile abnormality encompassing a variety of disorders related to the elevation of total cholesterol, LDL, TGs, or conversely, lower levels of HDL. It may present as a single disorder i.e. affecting only one lipoprotein parameter, or a combination of lipoprotein abnormalities, such as elevated TGs and low HDL levels. Elevated non-HDL serum lipids and lipoproteins are amongst the most critical risk factors for atherosclerotic diseases, particularly coronary heart disease (CHD) [1]. Proper recognition and management of dyslipidemia can reduce cardiovascular and total mortality rates [2]. Current lipid lowering drugs include statins (pravastatin, rosuvastatin, atorvastatin and simvastatin are the most commonly used), fibrates (e.g. fenofibrate, clofibrate and gemfibrozil), niacin (vitamin B_3), bile acid sequestrants (cholestyramine, colestipol), however LDL-aphaeresis and liver transplantation are used in severe cases refractory to other interventions.

Clinically, statins are the most widely prescribed drugs for hypercholesterolemia [3]. Statins effectively lower the plasma concentration of low-density lipoprotein cholesterol (LDL-C) and reduce mortality and morbidity rates due to CHD [4]. However, many patients under statin treatment alone do not achieve the desired LDL-C goal suggested by the recent guidelines of the National Cholesterol Education Program's Adult Treatment Panel III (ATP III). In addition, intensive statin therapy has been associated with increased risk of discontinuation, hepatotoxicity, and myalgia [5].

Citrus reticulata is commonly known as mandarin fruit. Mandarin oranges are native to the tropical and sub-tropical regions of Asia where they exist in many different varieties. The oil of the fruit has been found to possess a variety of valuable properties including Antioxidant, Anti-inflammatory, Antifungal, Anxiolytic properties and cardioprotective effects [6]-[10]. All these precious activities emphasize the importance of evaluating the activity of the fruit rinds grown locally in the northern region of Sudan.

Z. Officinale is an herbaceous perennial comprising a rhizome, fibrous roots and aerial shoots. The constituents of ginger are numerous and vary depending on the place or geographical location of cultivation and whether the rhizomes are extracted in the fresh (fleshy) or dry (powdered) state [11].

The pungent odor of ginger is mainly due to its volatile oil [12]. Over 50 components of the oil have been characterized and the pungency is mainly due to gingerol, an oily liquid consisting of homologous phenols formed in the plant from phenylalanine, malonate and hexonate [13]. Ginger has proven to be very useful to Asian and Arabic cultures. It has also been greatly used for centuries for its medicinal properties. In various cultures, ginger has been used to treat common ailments such as headaches, nausea, vomiting, indigestion, flu, diarrhea, arthritis, colic, and even painful menstruation [14]. Many researches have demonstrated the powerful biological activities of *Z. Officinale* including Anti-inflammatory, Anti-microbial and Anti-thrombotic effects in addition to a great sum many other useful activities [15]-[20]. *Sesamum indicum* is a member of *Pedaliaceae* family, an annual shrub with white, bell-shaped flowers. Sesame is mainly grown in tropical, subtropical, and southern temperate areas of the world, particularly in India, China, South America, and Africa. It has an utmost economic importance, and it is grown primarily by farmers in developing countries. Today, India and China are the world's largest producers of sesame, followed by Burma, Sudan, Mexico, Nigeria, Venezuela, Turkey, Uganda, and Ethiopia. Sesame seeds contain essential fatty acids (EFAs) such as linoleic acid and high levels of lignans that consist of sesamin, sesaminol, sesamol and sesamolin [21]. Several studies have reported the health-promoting properties of sesame seeds. Sesame oil (SO) contains unique class of compounds known as lignans. Lignans comprise sesamin, sesamolin, and sesamol [21]. They have multiple physiological functions, such as decreasing arachidonic acid levels, increasing antioxidative ability, γ-tocopherol bioavailability, and exhibiting anti-inflammatory effects [22]. Some sesame lignans (e.g., sesamin) are converted to the mammalian lignans, which may exert weak estrogenic and antiestrogenic activities [23]. Sesame oil is a mild laxative, emollient, and demulcent [24]. Sesamin present in sesame oil has been found to protect the liver from damage due to oxidative stress [25]. These properties are of vital importance and thus prompt further investigation of sesame seeds grown locally for their enormous biological activities.

II. Materials and Methods

A. Plant Material Collection and Preparation

Citrus reticulata fruits, *Zingiber officinale* rhizomes, and *Sesamum indicum* seeds were purchased from the Local Market. *Citrus reticulata* fruit rinds were carefully peeled, *Zingiber officinale* fresh rhizomes were cut into smaller pieces, the plant material was shade-dried and ground into fine powder for extraction. *Sesamum indicum* seeds were ground into fine powder in preparation for extraction. All Plant materials obtained were authenticated at the Medicinal and Aromatic Plants Research Institute (MAPRI) and a voucher specimen was deposited at the herbarium.

B. Phytochemical Screening

1. Preparation of Extract

The phytochemical constituents (secondary metabolites) of the extracts were detected using standard procedures as described in [36], [37]. The extract was prepared by boiling 20 grams of powdered plant material with 250 ml of 70% ethanol; the macerate was then filtered and used for phytochemical screening.

2. Detection of Flavonoids

Two ml of the extract were mixed with diluted NaOH to produce yellow coloration. Disappearance of the color upon addition of dil. HCl indicates the presence of flavonoids.

3. Detection of Tannins

Two ml of the extract were stirred with about ten ml of distilled water then filtered. Few drops of 10% ferric chloride solution were added to two ml of the filtrate. Occurrence of a blue, black, green, and blue – green indicates the presence of tannins.

4. Detection of Saponins

Two ml of the extract were boiled with five ml distilled water, filtered; about three ml of distilled water were further added to the filtrate and shaken vigorously for about five minutes. Frothing which persists for about thirty minutes indicates the presence of saponins.

5. Detection of Cardiac Glycosides (*Keller Killiani Test)*

Two ml of extract were evaporated to dryness, the residue was dissolved in two ml of (3.5%) ferric chloride in glacial acetic acid, transferred to clean dry test tube, and two ml of conc. H_2SO_4 poured carefully on the wall of the test tube. Development of reddish brown ring at the junction between the two layers indicates the presence of cardiac glycosides.

6. Detection of Sterols

Two ml of the extract were evaporated to dryness, the residue was then dissolved in two ml of chloroform and transferred to clean dry test tube, two ml of acetic anhydride were added followed by addition of conc. H_2SO_4 carefully to the wall of the tube. Color development from violet to blue or green indicates presence of a steroidal moiety.

7. Detection of Alkaloids

Two ml of the extract were acidified with 1% HCl, few drops of Mayer's reagent were added, appearance of turbidity indicates the presence of alkaloids.

8. Detection of Terpenoids (Salkowski Test)

Two ml of the extract were mixed with two ml of chloroform. Three ml of conc. H_2SO_4 were added carefully to form a layer, formation of a reddish brown color at the interface Indicates the presence of terpenoids.

9. Detection of Carbohydrates

The alcoholic extract was dissolved in five ml distilled water and filtered. The filtrate was treated with two drops of alcoholic α-naphthol. Formation of a violet ring at the junction

of the two layers indicates the presence of carbohydrates.

10. Detection of Reducing Sugars

Two ml of the extract were heated with equal volumes of Fehling solutions A and B. Appearance of a precipitate indicates the presence of reducing sugars.

11. Detection of Compound reducing sugars

Two ml of the extract were hydrolyzed by boiling with five ml diluted HCl; the resulting solution was neutralized with NaOH solution. A few drops of Fehling solution were added, then heated on a water bath for two minutes. Appearance of a reddish-brown precipitate indicates the presence of compound reducing sugars.

C. Preparation of Large Scale Plant Extract

The plant material obtained consisted of *Zingiber officinale* rhizomes, *Sesamum indicum* seeds and *Citrus reticulata* fruit peels. The powdered plant material was weighed (100g) and extracted with 96% ethanol using Soxhlet apparatus for 24 hrs at 70^0C. The extracts were obtained and the solvent residue removed using rotatory evaporator where the concentrated extracts were air dried in order to remove any traces of solvent remaining.

D. Assay of Hypolipidemic Activity

1. Materials

- Cholesterol Powder (Central Drug House Ltd., India).
- Atorvastatin (Troikaa pharmaceuticals Ltd., India).
- Standard rat chow (Vitamin and mineral premix, minced meat, oil, starch) obtained from Eilaf veterinary supplies Ltd., Sudan. Saturated Animal Fat.
- Weighing balance, Stainless steel gastric tubes, Syringes, Cotton pads, Centrifuge, Blood collection tubes, plain containers for serum collection, Capillary tubes, Pasteur pipette.

2. Experimental Animals

Male and female Albino rats of weight ranging from 100-270 grams purchased from the animal house of National Research Center; the rats were kept under standard conditions of temperature and pressure, 12hrs light/dark and were given access to water *ad libitum*.

3. Procedure

Forty-five adult albino rats of both sexes with weight ranging from 100-270g purchased from the animal house of the National Research Center were used throughout the experiment. The animals were housed in standard metal cages at 12-hr light and dark cycles, given access to food and water *ad libitum*. The rats were divided into nine groups, five rats per each group as follows; first group was the normal control group (normal rats fed on standard diet). The second group was the hypercholesterolemic control group (rats fed on hypercholesterolemic diet consisting of 1% cholesterol C27H46O + 10% saturated animal fat added to the standard rat diet. The third group received hypercholesterolemic diet + (250 mg/kg) of 96% crude ethanolic *Citrus reticulata* fruit peel extract. The fourth group received hypercholesterolemic diet + (500 mg/kg) of 96% crude ethanolic *Citrus reticulata* fruit peel extract. The fifth group received hypercholesterolemic diet + (250 mg/kg) of 96% crude ethanolic Zingiber *officinale* rhizome extract. The sixth group received hypercholesterolemic diet + (500 mg/kg) of 96% crude ethanolic Zingiber *officinale* rhizome extract. The seventh group received hypercholesterolemic diet + (250 mg/kg) of 96% crude ethanolic *Sesamum indicum* seed extract. The eighth group received hypercholesterolemic diet + (500 mg/kg) of 96% crude ethanolic *Sesamum indicum* seed extract. The ninth group received hypercholesterolemic diet + Atorvastatin (0.18mg/kg) daily orally via stainless steel gastric tubes throughout the four weeks experimental period, all extracts were administered orally via stainless steel gastric tubes for the four weeks experimental period [26].

The extracts and standard drug were dissolved in distilled water prior to administration, where each rat received the recommended dosage based on determined weight throughout the experimental period of four weeks. Blood samples were obtained after four weeks of the treatment period from the retro-orbital venous plexus of all rat groups after 12hr fasting [26]. The blood was allowed to flow into a clean, dry centrifuge tube and left to stand for 30 minutes before centrifugation to avoid hemolysis. Samples were centrifuged for 15 minutes at 2,500 rpm. The clear supernatant serum was collected and placed into a clean dry test tube using Pasteur pipette to perform the following biochemical tests (Lipid profile: TC (total cholesterol), TGs (triglycerides), HDL-C (high density lipoprotein-cholesterol) and LDL-C (low density lipoprotein-cholesterol).

E. DPPH Radical Scavenging Antioxidant Activity Assay

The DPPH radical scavenging activity was determined according to the method of [27] with some modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37^0 C. The concentration of DPPH was kept as (300 micromoles). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured at 517 nm using multi-plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

F. Statistical Analysis

Values were expressed as Mean ± SD (standard deviation). Results were analyzed using one-way analysis of variance (ANOVA) to compare the different groups and determine the significance (P- value) of data.

III. Results

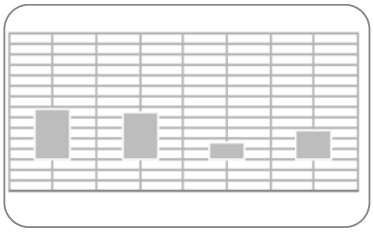

Fig. 1 The mean values of Lipid profile (TC, TGs, HDL and LDL) of *Zingiber officinale* 500mg/kg dose

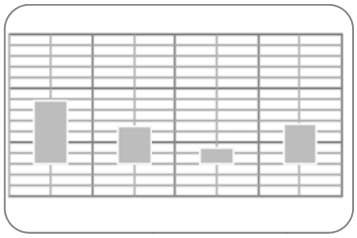

Fig. 2 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of *Zingiber officinale* 250mg/kg dose

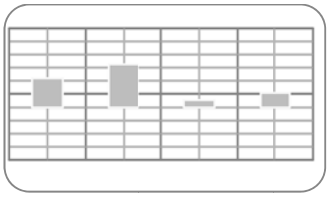

Fig. 3 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of Atorvastatin 0.18 mg/kg dose

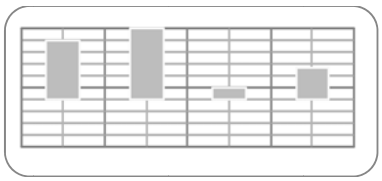

Fig. 4 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of Hypercholesterolemic control group

TABLE I
Results of Phytochemical Screening

Screening Test	*Z. officinale*	*S. indicum*	*C. reticulata*
Alkaloids	+Ve	+Ve	-Ve
Saponins	+Ve	+Ve	+Ve
Tannins	+Ve	+Ve	+Ve
Molish's Test for carbohydrates	+Ve	+Ve	+Ve
Reducing Sugars	+Ve	-Ve	+Ve
Compound reducing Sugars	+Ve	-Ve	+Ve
Terpenoids	+Ve	+Ve	+Ve
Flavonoids	+Ve	+Ve	+Ve
Sterols	+Ve	+Ve	+Ve

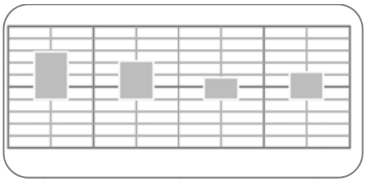

Fig. 5 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of *Citrus reticulata* 250mg/kg dose

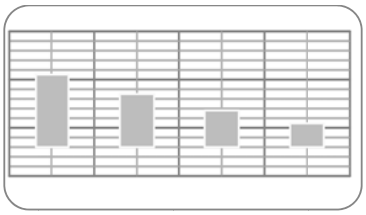

Fig. 6 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of *Citrus reticulata* 500mg/kg dose

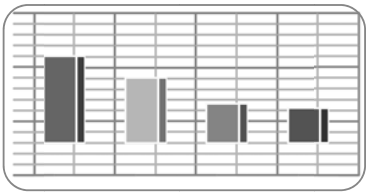

Fig. 7 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of *Sesamum indicum* 250mg/kg dose

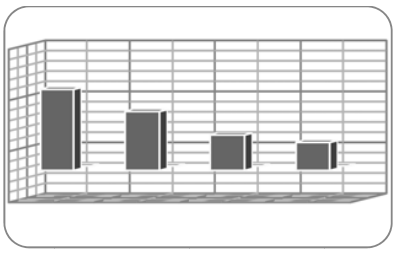

Fig. 8 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of *Sesamum indicum* 500mg/kg dose

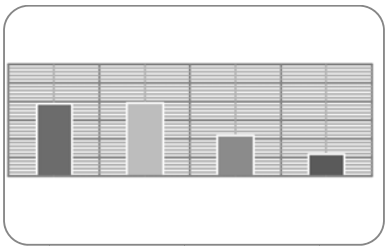

Fig. 9 The mean values of Lipid profile parameters (TC, TGs, HDL and LDL) of Normal control group

TABLE II
RESULTS OF ASSAY OF EFFECTS ON LIPID PROFILE PARAMETERS

Group	TC Mean ± SD	TGs Mean ± SD	HDL Mean ± SD	LDL Mean ± SD
Normal Control Group	77.6667 ± 4.62	78.5000 ± 6.35	43.5000 ± 1.29	23.5000 ± 11.58
Hypercholesterolemic Control Group	99.3333 ±14.434	131.3333 ± 34.31	19.6667 ± 4.51	53.3333 ± 12.66
Zingiber officinale (250mg/kg)	58.5000 ± 3	34.7500 ± 16.7	14.7500 ± 7.1	36.8000 ± 7.04
Zingiber officinale (500mg/kg)	48.0000 ± 8.60	44.6667 ± 6.35	16.0000 ± 1.29	27.6667 ± 11.58
Citrus reticulata (250mg/kg)	78.0000 ± 11.14	62.7500 ± 19.17	35.2500 ± 4.78	44.3333 ± 9.29
Citrus reticulata (500mg/kg)	75.2000 ± 10.03	55.0000 ± 10.09	38.0000 ± 7.62	24.6000 ± 10.31
Sesamum indicum (250mg/kg)	80.8000 ± 10.03	60.8000 ± 10.09	36.2000 ± 7.62	32.2000 ± 10.31
Sesamum indicum (500mg/kg)	79.5000 ± 7.33	57.8000 ± 17.02	34.8000 ± 11.37	27.5000 ± 14.66
Atorvastatin (0.18mg/kg)	43.8000 ± 7.05	65.0000 ±18.32	11.4000 ± 1.52	21.4500 ± 6.68

TABLE III
RESULTS OF ANTIOXIDANT ASSAY REPRESENTED AS % RSA (RADICAL SCAVENGING ACTIVITY)

Plant Sample	% RSA ± SD (DPPH)
Sesamum indicum	24 ± 0.08
Citrus reticulata	46 ± 0.06
Zingiber officinale	72 ± 0.05
PG (Propyl-gallate)	90 ± 0.01

IV. STATISTICAL ANALYSIS

TABLE IV
COMPARISON OF *ZINGIBER OFFICINALE* TO HYPERCHOLESTEROLEMIC CONTROL GROUP

Comparison to Hypercholesterolemic Control Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Zingiber officinale (250mg/kg)	0.002**	0.004**	0.346	0.076
Zingiber officinale (500mg/kg)	0.002**	0.012*	0.518	0.049*

* Significant at P < 0.05 for group versus Hypercholesterolemic control group.
** Highly significant at P < 0.01 for group versus Hypercholesterolemic control group.

TABLE V
COMPARISON OF *CITRUS RETICULATA* TO HYPERCHOLESTEROLEMIC CONTROL GROUP

Comparison to Hypercholesterolemic Control Group	**TC**	**TGs**	**HDL**	**LDL**
Citrus reticulata (250mg/kg)	0.113	0.019*	0.007**	0.337
Citrus reticulata (500mg/kg)	0.03*	0.007**	0.01*	0.012**

* Significant at P < 0.05 for group versus Hypercholesterolemic control group.
** Highly significant at P < 0.01 for group versus Hypercholesterolemic control group.

TABLE VI
COMPARISON OF *SESAMUM INDICUM* TO HYPERCHOLESTEROLEMIC CONTROL GROUP

Comparison to Hypercholesterolemic Control Group	**TC**	**TGs**	**HDL**	**LDL**
Sesamum indicum (250mg/kg)	0.037*	0.004**	0.001**	0.02*
Sesamum indicum (500mg/kg)	0.06	0.006**	0.075	0.059

* Significant at P < 0.05 for group versus normal control groups.
** Highly significant at P < 0.01 for group versus normal control group.

TABLE VII
COMPARISON OF *ZINGIBER OFFICINALE* TO NORMAL CONTROL GROUP

Comparison to Normal Control Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Zingiber officinale (250mg/kg)	0.001**	0.003**	0.000**	0.097**
Zingiber officinale (500mg/kg)	0.003**	0.000**	0.001**	0.635

* Significant at P < 0.05 for group versus normal control groups
** Highly significant at P < 0.01 for group versus normal control group

TABLE VIII
COMPARISON OF *CITRUS RETICULATA* TO NORMAL CONTROL GROUP

Comparison to Normal Control Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Citrus reticulata (250mg/kg)	0.964	0.170	0.016*	0.05*
Citrus reticulata (500mg/kg)	0.709	0.008**	0.202	0.884

* Significant at P < 0.05 for group versus normal control groups
** Highly significant at P < 0.01 for group versus normal control group

TABLE IX
COMPARISON OF *SESAMUM INDICUM* TO NORMAL CONTROL GROUP

Comparison to Normal Control Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Sesamum indicum (250mg/kg)	0.453	0.028*	0.006**	0.201
Sesamum indicum (500mg/kg)	0.000**	0.538	0.002**	0.481

* Significant at P < 0.05 for group versus normal control groups
** Highly significant at P < 0.01 for group versus normal control group

TABLE X
COMPARISON OF *ZINGIBER OFFICINALE* TO ATORVASTATIN (STANDARD DRUG) CONTROL GROUP

Comparison to Standard Drug (Atorvastatin) Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Zingiber officinale (250mg/kg)	0.006**	0.038*	0.331	0.02*
Zingiber officinale (500mg/kg)	0.446	0.115	0.248	0.352

* Significant at P < 0.05 for group versus Atorvastatin group
** Highly significant at P < 0.01 for group versus Atorvastatin group

TABLE XI
COMPARISON OF *CITRUS RETICULATA* TO ATORVASTATIN (STANDARD DRUG) CONTROL GROUP

Comparison to Standard Drug (Atorvastatin) Group	**TC Sig.**	**TGs Sig.**	**HDL Sig.**	**LDL Sig.**
Citrus reticulata (250mg/kg)	0.002**	0.863	0.000**	0.012*
Citrus reticulata (500mg/kg)	0.000**	0.364	0.000**	0.616

* Significant at P < 0.05 for group versus Atorvastatin group
** Highly significant at P < 0.01 for group versus Atorvastatin group

TABLE XII
COMPARISON OF *SESAMUM INDICUM* TO ATORVASTATIN (STANDARD DRUG) CONTROL GROUP

Comparison to Standard Drug (Atorvastatin) Group	**TC**	**TGs**	**HDL**	**LDL**
Sesamum indicum (250mg/kg)	0.000**	0.675	0.000**	0.05*
Sesamum indicum (500mg/kg)	0.722	0.05*	0.117	0.683

* Significance at P < 0.05 for group versus Atorvastatin group
** Highly significant at P < 0.01 for group versus Atorvastatin group

IV. DISCUSSION

The present study intended evaluation and comparison of

extract of *Sesamum indicum* seeds, *Citrus reticulata* fruit peels and *Zingiber officinale* rhizomes in terms of their antioxidant and hypolipidemic activities as an attempt to predict their cardioprotective properties, since dyslipidemia and oxidative stress are considered as major factors contributing to the pathogenic development of cardiovascular atherosclerotic disease.

Preliminary Phytochemical screening of the plant material was carried out, Screening results for *Zingiber officinale* revealed the presence of alkaloids, tannins, saponins, carbohydrates, sterols, reducing sugars, compound reducing sugars, terpenoids and flavonoids.

Results obtained for *Citrus reticulata* peels revealed the presence of saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, compound reducing sugars, terpenoids, flavonoids and sterols, however alkaloids were not detected.

Sesamum indicum screening results revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, carbohydrates, terpenoids, flavonoids, sterols and absence of reducing sugars and compound reducing sugars. However, these plant extracts shared the presence of tannins, terpenoids, saponins, cardiac glycosides, carbohydrates and sterols. These secondary metabolites have been implicated in many biological activities, for example tannins are considered as major biological antioxidants, while flavonoids have been implicated in the protection against CAD (Coronary Artery Disease) through inhibition of LDL oxidation and reduction of plasma LDL levels, the latter is the main lipoprotein involved in atherogenesis.

Assay of antioxidant activity using DPPH radical scavenging technique revealed modest antioxidant activity for *S. indicum*, a moderately higher activity for *C. reticulata* while the highest antioxidant activity obtained belonged to *Z. officinale,* these results revealed the antioxidant properties of the three plants extracts as has been reported in literature. The antioxidant activity of the extracts is partly attributed to their flavonoid content as flavonoids are considered as major biological antioxidants [28], the essential oils occurring in both *Zingiber officinale* and *Citrus reticulata* contain terpenoids which exhibit antioxidant properties [29], *Citrus* peels contain ascorbic acid which possess powerful antioxidant activity. Sesame seeds contain lignans e.g. sesamol in addition to Gamma-tocopherol, are both powerful biological antioxidants [30].

Study of the hypolipidemic activity of these plants resulted in multiple findings. A dose of 250 mg/kg and 500 mg/kg of *Z. officinale* given daily for a period of four weeks resulted in 41.11% and 51.7% reduction in serum TC (Total Cholesterol) respectively in comparison to the hypercholesterolemic control group. LDL cholesterol that plays a major role in atherogenesis was reduced by 30.996% and 59.8% for 250mg/kg and 500mg/kg respectively. The 500mg/kg and 250mg/kg doses of *Zingiber officinale* resulted in 31% and 48.12% elevation in serum HDL levels respectively when compared to hypercholesterolemic control group suggesting that elevation in serum HDL levels is not correlated to dose increment i.e. the elevation in serum HDL is not dose dependant. A significant reduction in serum TGs of 66% and 73.54% was obtained for the 250mg/kg and 500mg/kg doses of *Zingiber officinale* respectively in comparison to hypercholesterolemic control group. These results reveal that the crude 96% ethanolic extract of ginger administered in doses of 250mg/kg and 500mg/kg for four weeks induced a decrease in TC, LDL and TGs with an increase in serum HDL in comparison to the hypercholesterolemic control group. CVS (cardiovascular) risk ratio was calculated as the ratio between TC and HDL-C.

An improved CVS risk ratio with risk reduction of 21.4% and 40% was attained with the 250mg/kg and 500mg/kg *Zingiber officinale* doses respectively in comparison to the hypercholesterolemic control group, these findings demonstrate the cardioprotective effects of this extract.

The Atorvastatin control group exhibited 56%, 50.5% and 60% reduction in serum TC, TGs and LDL levels respectively, while the CVS risk ratio was reduced by 24%; thus, the 250mg/kg dose of *Zingiber officinale* resulted in 78.34% reduction in TC, 130.95% reduction in TGs and 89.2% reduction in LDL in comparison to the atorvastatin treated group while the 500mg/kg dose of *Zingiber officinale* resulted in 92.32%, 145.62%, 99.67% reduction in serum TC, TGs and LDL respectively compared to the atorvastatin treated group. Statistical analysis by one-way ANOVA revealed a significant reduction in serum TC and TGs obtained from the 250mg/kg dose of *Zingiber officinale* and a significant reduction in serum TC, TGs and LDL levels was obtained with the 500mg/kg dose in comparison to the hypercholesterolemic control group, however no significant elevation in serum HDL was observed. A highly significant reduction of serum TC and TGs was obtained with 250mg/kg and 500mg/kg of *Zingiber officinale*, however no significant reduction in serum LDL was observed in comparison to the normal control group receiving standard rat diet. These results can be attributed to the disruption of cholesterol absorption from the diet by oleoresins present in ginger extract [31], this in addition to the pharmacological action of ginger in elevation of 7 α-hydroxylase which is the rate limiting enzyme in conversion of cholesterol into bile acids leading to the excretion of cholesterol from the body [32], moreover polyphenolic flavonoids present in ginger protect against coronary artery disease by reducing plasma cholesterol levels and inhibiting LDL oxidation [33]. The major antioxidant principles of ginger namely shogaols and gingerols inhibit lipid peroxidation, which plays a vital role in attenuation of atherosclerotic disease. The lipid lowering effect of ginger can also be attributed to the presence of lignans that have been reported to reduce serum cholesterol and glucose concentrations in hypercholesterolemic subjects. According to the present study, increasing the dose of ginger extract resulted in a greater reduction in serum Total cholesterol, however, the dose dependant effect was most evident in reduction of serum LDL levels.

Administration of *Citrus reticulata* fruit peel extract at 250mg/kg and 500mg/kg doses orally for four weeks resulted

in significant changes in lipid profile parameters in comparison to the hypercholesterolemic control group. The 250mg/kg dose resulted in 21.5%, 52.22% and 16.9% reduction in serum TC, TGs and LDL respectively, however serum HDL level was increased by 46.1%, all in comparison to the hypercholesterolemic control group. The 500mg/kg dose of *Citrus reticulata* fruit peel extract resulted in 24%, 58.12% and 53.97% reduction in serum TC, TGs and LDL levels respectively in comparison to the hypocholesterolemic control group, while serum HDL level increased by 48.24% when compared to the hypocholesterolemic control group. Apparently, the effect on serum TC, TGs, and HDL levels is slightly dose-dependant, however the decrease in serum LDL appears to be highly dose-dependent. In contrast to *Zingiber officinale*, *Citrus reticulata* fruit peel extract resulted in a significant elevation of serum HDL level in comparison to the hypercholesterolemic control group. Increase in serum HDL is associated with an increased reduction in risk ratio; the latter was reduced by 56.18% and 60.8% for the 250mg/kg and 500mg/kg doses respectively in comparison to the hypercholesterolemic control.

Citrus reticulata 250mg/kg dose resulted in 38.4%, 103.41% and 28.17% reduction in serum TC, TGs and LDL respectively, when compared to the atorvastatin treated group, the 500mg/kg dose resulted in 42.86%, 115.09% and 89.95% reduction in TC, TGs and LDL respectively in comparison to the atorvastatin standard control group. The 500mg/kg dose of *Citrus reticulata* fruit peel extract produced a highly significant reduction in serum TC, TGs and LDL levels, and a significant elevation in serum HDL level in comparison to the hypercholesterolemic control group, however a significant elevation in serum HDL was obtained with the 250mg/kg dose in comparison to the hypercholesterolemic control group. When compared to the normal control group, the higher dose of 500mg/kg resulted in a significant reduction in serum triglycerides. These effects demonstrate the effectiveness of the *Citrus reticulata* peel extract on lipid profile parameters that might be attributed to the presence of polymethoxylated flavones that occur in *Citrus reticulata* fruit peels and have been reported to significantly reduce serum LDL levels with a reduction in serum TC and TG levels and a significant elevation in serum HDL levels.

Assay of the biological effects of *Sesamum indicum* seed extract on lipid profile revealed a statistically significant reduction in serum TGs with a moderate reduction in serum LDL, TC accompanied by a significant elevation of serum HDL levels in comparison to the hypercholesterolemic control. Administration of 250mg/kg dose of *Sesamum indicum* seed extracts orally for four weeks resulted in a significant reduction in serum TGs of 53.7% and moderate reduction in serum TC and LDL of 18.65% and 39.62% respectively when compared to the hypercholesterolemic control. The higher dose of 500mg/kg of extract yielded more or less similar results suggesting that the effects might not be dose dependent. Results obtained with the higher dose revealed a significant reduction in serum TGs level of 55.99% and a more or less similar reduction to the 250mg/kg dose in serum TC of 19.96% and a reduction in serum LDL of 48.43% in comparison to the hypercholesterolemic control. The higher and lower doses of *Sesamum indicum* resulted in 43.5% and 45.7% elevation in serum HDL levels respectively, hence the effect of sesame seed extract was not dose dependant.

In comparison to the atorvastatin treated group, the 250mg/kg and 500mg/kg doses of *Sesamum indicum* seed extracts resulted in 66.03% and 80.72% reduction in serum LDL levels respectively, reduction in serum TGs levels was 106.34% and 110.87% for the 250mg/kg and 500mg/kg doses respectively in comparison to the atorvastatin control group, TC was reduced by 33.3% and 35.64% for the 250mg/kg and 500mg/kg doses respectively when compared to the atorvastatin treated group.

The CVS risk ratio obtained for the *Sesamum indicum* treated groups on comparison to the hypercholesterolemic control group revealed 55.84% and 54.9% reduction in CVS risk for the 250mg/kg and 500mg/kg doses respectively. The effect of the crude ethanolic extract of sesame seeds on lipid profile parameters following four weeks of administration can be partly attributed to their sterol content that have been reported in previous studies to reduce the absorption of dietary cholesterol which in turn lowers serum cholesterol levels [34], in addition to this, the lignan content of sesame seeds contributes greatly to its influence on lipid profile parameters [35].

V. Conclusion

In conclusion, the results were obtained in this study reveal that daily administration of 250mg/kg and 500mg/kg doses of *Zingiber officinale* rhizome, *Citrus reticulata* fruit peel and *Sesamum indicum* seed extracts to albino rats for four weeks produces a reduction in serum LDL, TC and TGs levels. Moreover, the elevation in serum HDL levels in comparison to the hypercholesterolemic control; these findings demonstrate their cardioprotective effects and their potential as therapeutic antihypercholesterolemic agents with enormous safety profiles and high nutritional value. This as well is in addition to their antioxidant properties, where combination of these two biological activities namely; antioxidant and lipid lowering effects constitute a prophylactic approach against atherosclerotic disease. These results inspire future attempts to develop new formulations and provide guidance towards a better understanding of the exact mechanisms underlying these biological properties, which is of great importance in pharmaceutical drug development and novel drug discovery.

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