

EVALUATION OF THE EFFECTS OF AQUEOUS EXTRACT OF SOME COMMON TRADITIONAL SPICES (CTS) ON HYPERCHOLESTEROL-INDUCED OXIDATIVE STRESS IN WISTAR- ALBINO RATS.

Obiefule Jennifer Chidera*, Prof Oze Gabriel, Peter Akwukwaegbu, Dr Uche Mercylyn Ezinne, Asiegbu Fitzgerald

Department is Medical Biochemistry and my Faculty is Basic Medical Sciences.

***Corresponding Author: Obiefule Jennifer Chidera**

Department is Medical Biochemistry and my Faculty is Basic Medical Sciences.

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ABSTRACT

Evaluation of the effects of aqueous extract of some common traditional spices (CTS) on hypercholesterol-induced oxidative stress in Wistar-albino rats was done using standard analytical methods, involving GC-MS, Randox Laboratory Biochemical kits and spectrophotometer. The acute toxicity studies were carried out. Thirty-five Wistar albino rats weighing 100-150 g were purchased and housed in seven plastic cages of five rats per group. The rats were fed with normal rat chow. The group fed with chow and normal saline constituted the group 1 (normal control), group 2 was administered 300 mg/kg body weight CTS extract only, while group 3 (Negative control) was induced oxidative stress using 30mg/kg body weight cholesterol in coconut oil. Group 4 was the standard and treated with 3mg/kg body weight atorvastatin. Groups 5,6, and 7 were treated with 100, 200 and 400mg/kg body weight CTS after stress induction respectively. All the treatments lasted for 21 days. Phytochemical analysis of the CTS extract revealed 11 bioactive compounds. For the negative control, there was a significant ($p < 0.05$) increase in the serum levels of the biomarkers of the liver, kidney, haematology and lipid profile parameters such as total proteins, AST, ALT, ALP, urea, creatinine, K⁺, Cl⁻, and total cholesterol. TAG, LDL-c, VLDL-c, MDA, LDH, CPK, TWBC and PLTs, when compared with the saline control groups. There was a significant decrease ($p < 0.05$) in GSH, GPx, SOD, catalase and Hb levels of the hypercholesterolemic rats. However, the administration of CTS extract restored the serum levels of these parameters to their approximate saline control concentrations. The decrease in the biomarkers were statistically significant ($p < 0.05$). There was no significant ($p > 0.05$) increase in the serum Na⁺ and HCO³⁻ concentrations for the studied groups. Histopathological examinations of the liver, heart and kidney cells showed normal histology in the control rats, and cellular distortions of the liver and kidney sections in the hypercholesterolemic rats. But the test groups which received the CTS extracts showed normal histology of the hepatocytes and renal cells. The study indicates that the CTS leaf extracts clearly ameliorated the oxidative damage caused by the hypercholesterol in the liver, kidney and the cardiovascular tissues of the rats. This could justify their use in local settings to treat stress related ailments.

INTRODUCTION

Oxidative stress has been reported to increase under hypercholesterolemic conditions (Al-Rejaie *et al.*, 2013; Lee *et al.*, 2017). In addition, oxidative stress has been suggested to be the mechanism through which hypercholesterolemia induces tissue damage (Bin-Jumah, 2018). Dyslipidemia/hypercholesterolemia leads to increased accumulation of lipids in the liver and cardiovascular system, hence reducing its ability to mobilize lipids (Lee *et al.*, 2017; Bin-Jumah, 2018). Alteration in cholesterol and triglycerides metabolism as

a result of hypercholesterolemia has been shown to exacerbate oxidative stress biomarkers and promotes production of reactive oxygen species (ROS), thus increasing lipid peroxidation (Abuhashish *et al.*, 2013; Otunola *et al.*, 2014). Increased production of free radicals and decreased enzymatic and non-enzymatic antioxidant activities are the main features of oxidative stress (Anila & Vijayalakshmi, 2003). The accumulation of cholesterol in the endothelial cells of the liver, heart, kidney, lymph and blood vessels provokes the production of reactive oxygen species (ROS) and reduces

antioxidant defenses (Vijayalakshmi, 2003 & Forstermann, 2008; Anila). This can lead to redox imbalance, oxidative stress, and metabolic alterations (Seifried *et al.*, 2007; Bin-Jumah, 2018).

Therefore, agents that combine lipid-lowering and antioxidant potentials can ameliorate the negative impacts of cholesterol on the cardiovascular, hepatic, renal and other somatic cells.

Several researches suggest that limiting ROS may be a more successful strategy for preventing cardiovascular illnesses than diets rich in fat and cholesterol (Otunola *et al.*, 2014; Reddy & Katan, 2004). There is growing evidence that ROS play a major role in oxidative damage induction to biomolecules (Hazra, Biswas and Mandal, 2008). A number of degenerative diseases, including aging, cancer, and cardiovascular disorders, have been linked to the oxidative destruction of cell membranes, DNA, and proteins caused by reactive oxygen species (ROS) (Fang *et al.*, 2009).

A number of researches have demonstrated that some of our local traditional spices and plant extracts have significant antioxidant properties. Some of these spices and plant extracts include *Piper guineense*(oziza), *Xylopiia aethiopia*(uda), *Monodora myristica*(ehuru), *Ocimum gratissimum*(nchanwu) and *Gongronema latifolium*(utazi).

Natural compounds such as minerals, vitamins, flavonoids, alkaloids, terpenoids and sesquiterpenes have been found in common traditional spices (Srinivasan, 2005). These compounds provide spices their potent antioxidant effects, which aid in the body's defense against free radicals, attenuate lipid peroxidation, ameliorate DNA mutation and reduce the incidence of healthy cells turning malignant (Shobana and Naidu, 2013; Otunola *et al.*, 2014).

METHODOLOGY

Study area

- The study was carried out in the Animal House of the College of Medicine, Imo State University, Owerri. The university is located in Owerri city, one of the largest cities in Imo State within the rain forest region.

Grouping of rats (n=5): The experimental animals were divided into seven groups, containing five animals each:

GROUPS	NUMBER OF RATS	TREATMENT
1	5	Control (Normal Saline)
2	5	CTS Extract only (300mg/kg)
3	5	Stress-induced rats only (hypercholesterolemic rats, 30mg/kg cholesterol in coconut oil) (Innih and Oimage, 2022)
4	5	Stress-Induced rats + 3mg/kg Atorvastatin (Standard)
5	5	100mg/kg CTS + Stressor
6	5	200mg/kg CTS + Stressor
7	5	400mg/kg CTS + Stressor

Sample collection

- The resource plants (*Occimum gratissimum* leaf, *Gongronema latifolium* leaf, *Piper guineense* seed, *Xylopiia aethiopia* seed, and *Monodora myristica* seed) parts were obtained from the market in Orji, Owerri North Local Government Area of Imo State. The procured plant samples were identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri.

- Ethical Approach:** All animal experiments were conducted according to ethical guidelines of World Health Organization of Good Laboratory Practice Regulations of 1998 for the care and use of laboratory animals; and were approved by the Research and Ethical Committee of the College of Medicine, Imo State University Owerri.

Sample preparation

The leaves of the plants and other components of the plants were washed, sorted, macerated and oven-dried at 40-50°C to steady weight. The dried leaves were milled into fine powder, packed into airtight plastic bottles and stored in a refrigerator for use. Two hundred grams of each ground component of the sample were mixed to constitute the cocktail, making up 1000g(1kg). From the uniformly mixed powdered samples, 200g of the spices was extracted with 1 L of distilled water and concentrated using rotatory evaporator. The concentrate was constituted into appropriate doses and orally administered to the rats, twice daily for 21 days (Shanmugam *et al.*, 2010; Abduljawad *et al.*, 2013; Ahmadi *et al.*, 2013).

Experimental Design

Housing of Animals: A total of thirty five (35) healthy Wistar albino rats (100 - 150g) were purchased from the Animal House of the College of Medicine, Imo State University for this study. Animals were individually housed under temperature of 25-28±2.0 °C in cages with 12hours light dark cycle. They were given access to standard rat chow and water *ad libitum*.

Acclimatization: The animals were acclimatized to the facility for two weeks before experimentation.

Induction of Oxidative Stress: Hypercholesterolemia was induced in the rats by administering 30mg/kg of cholesterol powder (dissolved in coconut oil) on the rats (Innih and Oimage, 2022). Cholesterol powder was purchased from Kentin Chemical Store, Douglas Road Owerri, Imo State.

Collection of blood and organ samples: After feeding for twenty-one days, the rats were fasted overnight (12 hours), weighed and anaesthetized by exposure to chloroform. The rats were sacrificed painlessly and blood was collected through cardiac puncture into plain and ethylenediaminetetra-acetic acid (EDTA) sample bottles for biochemical and hematological analyses respectively. The anti-coagulated blood in heparin sample bottles was centrifuged at 1000 rpm for 10

minutes to obtain serum that was used for the analysis of liver, renal, cardiac, lipid profile and antioxidants parameters. Organs (liver and kidney) were harvested by dissecting the rats in longitudinal section from the chest region and preserved in 10% formalin and then sent to Department of Anatomy, Imo State University Owerri for histopathological examination.

DATA ANALYSIS

Statistical Package for Biological and Social Sciences (SPSS) Inc. 27.0 Software program was used. Mean values (M) ± SD were calculated and one-way analysis of variance (ANOVA) was performed for multiple comparison. The probabilities were processed at 95% confidence limit.

RESULTS

Table 1: Phytochemical Composition of CTS Extract.

PEAK	RT	COMPOUND NAME	BIOACTIVE ACTIVITIES	%COMPOSITION
1	7.081	10-Undecen-4-one, 2,2,6,6-tetramethyl-	Anti-microbial and ant-inflammatory activity	2.61
2	7.691	2,6-Octadienal, 3,7-dimethyl-, (E)	Anti-inflammatory and anti-microbial property	4.30
3	16.993	Hexadecanoic acid, methyl ester	Antioxidant activity	16.40
4	17.641	n-Hexadecanoic acid	Antioxidant properties and hypocholesterol activity\	15.53
5	18.808	cis-13-Octadecenoic acid, methyl ester	Anti-inflammatory activity	28.44
6	18.974	Fumaric acid, pent-4-en-2-yl tridecyl ester	Analgesic, anti-inflammatory and antioxidant property	0.81
7	19.050	Heptadecanoic acid, 16-methyl-, methyl ester	Anti-oxidant, anti-cancer activity	6.03
8	19.448	cis-Vaccenic acid	Anti-bacterial and hypolipidemic activity	17.19
9	19.647	Oleic Acid	Anti-cancer, anti-inflammatory and anti-bacterial activity	6.04
10	19.699	12-Methyl-E,E-2,13-octadecadien-1-ol	Anti-bacterial, antioxidant, analgesic and antiseptic activity	2.10
11	19.799	1H-Indene, 2-butyl-5-hexyloctahydr o-	Anti-bacterial, antioxidant, anti-inflammatory properties and promote wound healing	0.58

Data obtained using Gas Chromatographic Mass Spectrometry machine (capillary column: Rxi-Sms, 30 m × 0.25 mm × 0.25 µm; carrier gas: helium; purity level: 99.99%; flow rate: 1.2 ml/min).

ACUTE TOXICITY: Acute toxicity study were carried out using graded doses up to 10,000 mg/kg of the extract as shown in Tables 2A, 2B and 2C. Results of the acute toxicity studies produced no mortality in the rats. The treated rats also did not show signs of severe toxicity such as tremor, convulsions, writhing reflexes and agitations, but remained active and physically stable throughout the 24 hour period and a further 7 days of observation.

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where; D_0 is the highest dose that gave no mortality

D_{100} is the lowest dose that produced mortality

Table 2A: Stage 1 Acute Toxicity (LD₅₀) Evaluation of the Extract in Wistar Albino Rats.

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	100	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	1000	0/3	0.00	No mortality observed, instead animals remained active and physically stable

Table 2B: Stage 2: Acute Toxicity (LD₅₀) Evaluation of the Extract in Wistar Albino Rats.

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	1600	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	2900	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
3	5000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within one hour of administration.

Table 2C: Stage 3: Acute Toxicity (LD₅₀) Evaluation of the Extract in Wistar Albino Rats.

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	6600	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	7900	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within 2 hours of administration..
3	10000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within 24 hours of administration.

LD₅₀ > 10,000 mg/kg body weight

$$LD_{50} = LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where:

D₀: Highest dose that gave no mortality.

D₁₀₀: Lowest dose that produced mortality

$$LD_{50} = \sqrt{10000 \times 6600}$$

$$LD_{50} = 8,124.04 \text{ mg/kg body weight}$$

Table 3: Effect of CTS Extract on Liver Function Parameters of Oxidative Stress-Induced Wistar Albino Rats.

Treatment groups	AST (u/l)	ALT (u/l)	ALP (u/l)	Total protein (g/l)	Total bilirubin (μmol/l)	Albumin (g/l)	Globulin (g/dl)
Group I	41.33±2.52 ^a	30.67±1.15 ^a	73.67±6.11 ^a	5.77±0.26 ^a	0.52±0.03 ^a	3.27±0.07 ^a	2.50±0.19 ^a
Group II	36.67±2.89 ^a	28.00±2.00 ^a	74.33±4.04 ^a	6.05±0.25 ^a	0.46±0.03 ^a	3.44±0.36 ^a	2.61±0.11 ^a
Group III	55.33±3.51 ^b	43.33±2.89 ^b	81.67±2.52 ^b	6.33±0.24 ^b	0.61±0.06 ^b	4.10±0.03 ^b	2.23±0.24 ^b
Group IV	49.67±1.53 ^c	42.67±1.15 ^b	76.00±1.73 ^{ab}	6.25±0.13 ^{ab}	0.52±0.04 ^{ac}	3.75±0.14 ^{bc}	2.49±0.04 ^{ac}
Group V	41.00±3.60 ^{ac}	42.67±3.06 ^b	76.33±2.52 ^{ab}	5.87±0.07 ^{ac}	0.50±0.03 ^{ac}	3.36±0.07 ^{ac}	2.51±0.05 ^{ac}
Group VI	41.00±2.65 ^{ac}	41.67±2.08 ^b	76.67±1.53 ^{ab}	5.89±0.24 ^{ac}	0.50±0.03 ^{ac}	3.30±0.15 ^c	2.59±0.21 ^{ac}
Group VII	39.67±4.04 ^{ac}	39.33±1.15 ^c	74.33±4.04 ^{ac}	5.99±0.15 ^{ab}	0.49±0.04 ^{ac}	3.37±0.18 ^{ac}	2.62±0.03 ^{ac}

Values are (M±S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different (p<0.05) down the column when compared to groups I and III.

Note: AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, ALP- Alkalinephosphatase, u/L- units per litre, g/l- grams per liter, mmol/L- micromoles per litre, g/dl- grams per deciliter.

Table 3 showed the effect of CTS extract on liver function parameters.

Results showed that there was a significant increase (p<0.05) in all the liver function parameters of animals in group III when compared with group I. There was a significant decrease (p<0.05) in the globulin concentration of animals in group III when compared with group I. There was a significant decrease (p<0.05) in the plasma concentration of AST, total bilirubin and albumi of animals in groups V, VI and VII when

compared with group III. There was a significant decrease ($p < 0.05$) in the ALT and ALP activity of animals in group VII when compared with group III. There was a significant increase ($p < 0.05$) in the globulin plasma concentration of animals in groups V, VI and VII

when compared with group III. There was a significant difference ($p < 0.05$) in the AST, total bilirubin, albumin and globulin concentration of animals in group IV when compared with group III.

Table 4: Effect of CTS Extract on Kidney Function Parameters of Oxidative Stress-Induced Wistar Albino Rats.

Treatment groups	Urea (mg/dl)	Creatinine(mg/dl)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)	HCO ₃ ⁻ (mmol/L)
Group I	20.22±0.69 ^a	0.84±0.05 ^a	131.03±0.71 ^a	4.49±0.06 ^a	87.23±1.70 ^a	19.63±0.15 ^a
Group II	20.08±0.19 ^a	0.80±0.03 ^a	130.97±1.46 ^a	4.45±0.10 ^a	88.40±2.62 ^a	19.70±0.20 ^a
Group III	24.27±0.97 ^b	1.05±0.08 ^b	130.57±0.76 ^a	4.30±0.08 ^b	84.23±1.22 ^b	20.10±0.26 ^a
Group IV	22.17±0.45 ^a	0.90±0.06 ^a	131.07±1.03 ^a	4.45±0.06 ^a	87.30±0.66 ^a	19.93±0.15 ^a
Group V	20.94±0.31 ^a	0.86±0.03 ^a	131.73±0.80 ^a	4.51±0.03 ^a	87.47±0.81 ^a	19.97±0.15 ^a
Group VI	20.81±0.60 ^a	0.87±0.03 ^a	131.80±0.62 ^a	4.50±0.04 ^a	88.70±0.89 ^a	19.70±0.36 ^a
Group VII	20.19±0.20 ^a	0.88±0.04 ^a	131.27±1.42 ^a	4.51±0.04 ^a	88.57±0.80 ^a	19.57±0.78 ^a

Values are (M±S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different ($p < 0.05$) down the column when compared to groups I and III.

Note: Na⁺ - sodium ion, K⁺ - potassium ion, Cl⁻ - chloride ion, HCO₃⁻ - carbonate ion, mg/dL- milligrams per deciliter, mEq/L- milliequivalents per litre, mmol/L- millimoles per litre.

Table 4 showed the effect of CTS extract on kidney function parameters of oxidative stress-induced rats. Results showed that there was a significant increase ($p < 0.05$) in the urea and creatinine concentrations of rats in group III when compared with group I. There was a significant decrease ($p < 0.05$) in the K⁺ and Cl⁻ concentrations of rats in group III when compared with group I. There was a significant decrease ($p < 0.05$) in the

urea and creatinine concentrations of rats in groups V, VI and VII when compared with group III. There was a significant increase ($p < 0.05$) in the K⁺ and Cl⁻ concentrations of rats in groups V, VI and VII when compared with group III. There was a significant difference ($p < 0.05$) in the urea, creatinine, K⁺ and Cl⁻ concentrations of rats in group IV when compared with group III. There was no significant difference ($p < 0.05$) in all the kidney function parameters of rats in group II when compared with group I. There was no significant difference ($p < 0.05$) in all the comparison of Na⁺ and HCO₃⁻ carried out.

Table 5: Effect of CTS Extract on Lipid Profile Parameters of Oxidative Stress-Induced Wistar Albino Rats.

Treatment groups	Total Cholesterol (mg/dl)	TAG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Group I	114.71±4.49 ^a	76.60±3.45 ^a	63.80±1.63 ^a	66.24±3.08 ^a	15.32±0.69 ^a
Group II	107.90±2.04 ^c	74.52±3.74 ^a	64.73±2.28 ^a	58.08±3.55 ^c	14.90±0.75 ^a
Group III	125.04±5.87 ^b	95.12±3.58 ^b	61.36±2.48 ^a	82.70±7.53 ^b	19.03±0.71 ^b
Group IV	102.74±2.05 ^c	76.49±5.46 ^a	64.11±0.93 ^a	53.93±3.66 ^c	15.30±1.09 ^a
Group V	107.49±2.63 ^c	76.72±2.29 ^a	62.89±0.70 ^a	59.94±3.47 ^a	15.35±0.46 ^a
Group VI	107.09±2.59 ^c	76.86±1.11 ^a	63.65±0.31 ^a	58.82±2.70 ^c	15.37±0.22 ^a
Group VII	105.25±0.68 ^c	72.82±0.61 ^a	66.03±3.61 ^a	53.78±3.62 ^c	14.56±0.12 ^a

Values are (M±S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different ($p < 0.05$) down the column when compared to groups I and III.

Note: TAG- triglyceride, HDL-c – high density lipoprotein cholesterol, LDL-c - low density lipoprotein cholesterol, VLDL-c – very low density lipoprotein cholesterol, mg/dL- milligrams per deciliter

Table 5 showed the effect of CTS extract on lipid profile parameters of oxidative stress-induced rats. Results showed that there was a significant decrease ($p < 0.05$) in the total cholesterol and LDL-c of rats in group II when compared with group I. There was a significant increase ($p < 0.05$) in all the lipid profile parameters of rats in group III when compared with group I except in HDL-c.

There was a significant decrease ($p < 0.05$) in the total cholesterol, TAG, LDL-c and VLDL-c of rats in groups V, VI and VII when compared with group III. There was a significant decrease ($p < 0.05$) in the total cholesterol, TAG, LDL-c and VLDL-c of rats in group IV when compared with group III. There was no significant difference ($p < 0.05$) in all the kidney function parameters of rats in group II when compared with group I. There was no significant difference ($p < 0.05$) in HDL-c for all the comparison carried out except in group III. There was a significant increase ($p < 0.05$) in the MDA of rats in group III when compared with group I.

Table 6: Effect of CTS Extract on Serum Antioxidant Parameters of Oxidative Stress-Induced Wistar Albino Rrats

Treatment groups	GSH (mg/dl)	GPx (μ /l)	SOD (μ /l)	Catalase (μ /l)	MDA (mmol/L)
Group I	8.40 \pm 0.39 ^a	34.60 \pm 1.76 ^a	41.43 \pm 1.68 ^a	18.63 \pm 0.78 ^a	0.32 \pm 0.04 ^a
Group II	8.99 \pm 0.34 ^b	40.60 \pm 1.08 ^b	42.57 \pm 1.66 ^a	21.70 \pm 1.57 ^b	0.28 \pm 0.02 ^a
Group III	7.65 \pm 0.40 ^b	31.30 \pm 0.56 ^b	38.03 \pm 1.04 ^b	18.03 \pm 0.85 ^a	0.71 \pm 0.02 ^b
Group IV	7.82 \pm 0.16 ^b	33.03 \pm 0.76 ^b	39.57 \pm 1.46 ^b	20.67 \pm 1.03 ^c	0.51 \pm 0.04 ^c
Group V	7.75 \pm 0.12 ^b	33.90 \pm 1.25 ^a	40.93 \pm 0.93 ^a	20.23 \pm 0.40 ^{bc}	0.54 \pm 0.03 ^c
Group VI	8.64 \pm 0.35 ^a	33.97 \pm 0.93 ^a	42.67 \pm 1.55 ^a	21.20 \pm 0.66 ^c	0.52 \pm 0.01 ^c
Group VII	8.85 \pm 0.08 ^a	36.20 \pm 0.79 ^a	43.33 \pm 1.19 ^a	21.73 \pm 1.17 ^c	0.52 \pm 0.03 ^c

Values are (M \pm S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different (p<0.05) down the column when compared to groups I and III.

Note: GSH- Glutathione superoxide, GPx- glutathione peroxidase, SOD- superoxide dismutase, MDA- malondialdehyde, mg/dL- milligrams per deciliter, μ /L- micromoles per litre, mmol/L- millimoles per litre.

Table 6 showed the effect of CTS extract on serum antioxidant parameters of oxidative stress-induced rats. Results showed that there was a significant increase (p<0.05) in the GSH, GPx and catalase activity of rats in group II when compared with group I. There was a significant decrease (p<0.05) in the GSH, GPx and SOD of rats in group III when compared with group I. There

was a significant increase (p<0.05) in the GPx, SOD and catalase activity of rats in groups V, VI and VII when compared with group III. There was a significant decrease (p<0.05) in the MDA activity of rats in groups V, VI and VII when compared with group III. There was a significant increase (p<0.05) in the GSH activity of rats in groups VI and VII when compared with group III. There was a significant difference (p<0.05) in the catalase and MDA of rats in group IV when compared with group III. There was no significant difference (p<0.05) in the GSH, GPx and SOD of rats in group IV when compared with group III.

Table 7: Effect of CTS Extract on Cardiac Function Parameters of Oxidative Stress-Induced Wistar Albino Rrats

Treatment groups	LDH (μ /L)	CPK (μ /L)
Group I	199.10 \pm 5.09 ^a	243.07 \pm 10.68 ^a
Group II	170.30 \pm 60.35 ^a	241.97 \pm 10.04 ^a
Group III	343.97 \pm 54.53 ^b	437.23 \pm 11.28 ^b
Group IV	264.67 \pm 5.88 ^c	371.23 \pm 40.39 ^c
Group V	300.10 \pm 5.17 ^b	348.47 \pm 24.88 ^c
Group VI	286.53 \pm 6.96 ^c	311.97 \pm 11.63 ^c
Group VII	270.40 \pm 6.71 ^c	302.30 \pm 15.75 ^c

Values are (M \pm S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different (p<0.05) down the column when compared to groups I and III.

Table 7 showed the effect of CTS extract on cardiac function parameters. Results revealed that there was a significant increase (p<0.05) in the LDH activity of rats in group III when compared with group I. There was a significant decrease (p<0.05) in the LDH activity of rats in groups VI and VII when compared with group III.

There was a significant decrease (p<0.05) in the CPK activity of rats in groups V, VI and VII when compared with group III. There was a significant decrease (p<0.05) in the LDH and CPK activity of rats in group IV when compared with group III.

Table 8: Effect of CTS Extract on Hematological Parameters of Oxidative Stress-induced Rrats.

Treatment groups	RBC ($\times 10^6/\mu$ L)	PCV (%)	Hb (g/dl)	TWBC ($\times 10^3/\text{mm}^3$)	PLT ($\times 10^3/\text{mm}^3$)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group I	7.03 \pm 0.12 ^a	43.33 \pm 1.15 ^a	16.43 \pm 0.40 ^a	8.56 \pm 0.41 ^a	253.67 \pm 4.16 ^a	61.66 \pm 0.62 ^a	23.39 \pm 0.22 ^a	37.92 \pm 0.37 ^a
Group II	7.73 \pm 0.18 ^b	48.00 \pm 1.00 ^b	17.57 \pm 0.51 ^b	9.13 \pm 0.15 ^b	253.33 \pm 5.51 ^a	62.10 \pm 0.20 ^a	22.72 \pm 0.15 ^b	36.59 \pm 0.37 ^b
Group III	6.73 \pm 0.31 ^a	41.33 \pm 1.53 ^a	15.60 \pm 0.46 ^b	9.65 \pm 0.26 ^b	269.00 \pm 8.00 ^b	61.47 \pm 0.74 ^a	23.20 \pm 0.40 ^a	37.75 \pm 0.54 ^a
Group IV	6.93 \pm 0.27 ^a	42.33 \pm 2.08 ^a	16.20 \pm 0.36 ^{ab}	9.14 \pm 0.16 ^c	258.67 \pm 5.03 ^{ab}	61.04 \pm 0.65 ^a	23.38 \pm 0.43 ^a	38.15 \pm 0.80 ^a
Group V	6.90 \pm 0.16 ^a	42.33 \pm 0.58 ^a	16.23 \pm 0.21 ^{ab}	9.15 \pm 0.10 ^c	258.00 \pm 7.55 ^{ab}	61.36 \pm 0.75 ^a	23.53 \pm 0.42 ^a	38.35 \pm 0.61 ^a
Group VI	7.18 \pm 0.12 ^{ac}	44.67 \pm 1.15 ^{ac}	16.87 \pm 0.35 ^{ac}	9.16 \pm 0.18 ^c	251.33 \pm 4.73 ^a	62.17 \pm 0.62 ^a	23.51 \pm 0.15 ^a	37.77 \pm 0.56 ^a
Group VII	7.31 \pm 0.04 ^{ac}	46.33 \pm 0.58 ^c	17.27 \pm 0.21 ^c	9.24 \pm 0.12 ^c	250.00 \pm 10.58 ^a	63.38 \pm 0.50 ^b	23.62 \pm 0.20 ^a	37.26 \pm 0.11 ^a

Values are (M \pm S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different (p<0.05) down the column when compared to groups I and III.

Note: RBC- red blood cell count, PCV- packed cell volume, Hb- haemoglobin, TWBC- total white blood cell count, PLT – platelet, MCV – mean cell volume, MCH – mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, fl- femtoliters, pg- pictograms.

Table 8 showed the effect of CTS extract on hematological parameters of oxidative stress-induced rats. Results showed that there was a significant increase ($p < 0.05$) in the RBC, PCV, Hb and TWBC of rats in group II when compared with group I. There was a

significant decrease ($p < 0.05$) in the MCH and MCHC of rats in group II when compared with group I. There was a significant difference ($p < 0.05$) in the Hb, TWBC, and PLT of rats in group III when compared with group I. Furthermore, there was a significant increase ($p < 0.05$) in the RBC, PCV and Hb of rats in groups VI and VII when compared with group III. There was a significant decrease ($p < 0.05$) in the PLT of rats in groups VI and VII when compared with group III. Also, there was a significant decrease ($p < 0.05$) in the TWBC of rats in groups V, VI and VII when compared with group III. There was a significant decrease ($p < 0.05$) in the TWBC.

Table 9: Effect of CTS Extract on White Blood Cell Differentials of Oxidative Stress-Induced Wistar Albino Rats.

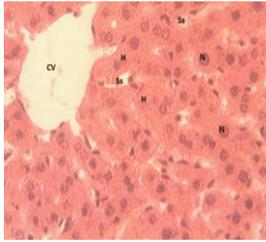
Groups	Monocytes (%)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)
Group I	3.67±0.58 ^a	38.67±1.15 ^a	55.67±1.53 ^a	2.00±0.00 ^a	0.00±0.00 ^a
Group II	3.33±0.58 ^a	41.00±1.00 ^a	53.33±2.08 ^a	2.33±0.58 ^a	0.00±0.00 ^a
Group III	3.00±0.00 ^a	37.00±2.65 ^a	57.33±2.89 ^a	2.67±0.58 ^a	0.00±0.00 ^a
Group IV	3.67±0.58 ^a	39.33±0.58 ^a	54.67±0.58 ^a	2.33±0.58 ^a	0.00±0.00 ^a
Group V	3.33±0.58 ^a	40.00±1.00 ^b	54.33±1.15 ^b	2.33±0.58 ^a	0.00±0.00 ^a
Group VI	3.67±0.58 ^a	39.33±1.15 ^b	54.00±1.00 ^b	3.00±0.00 ^b	0.00±0.00 ^a
Group VII	3.33±0.58 ^a	40.33±1.53 ^b	53.67±1.53 ^b	2.67±0.58 ^a	0.00±0.00 ^a

Values are (M±S.D) of five determinations (n=5). Values bearing different superscript letters (a, b, c) are significantly different ($p < 0.05$) down the column when compared to groups I and III.

Table 9 showed the effect of CTS extract on white blood cell differentials of oxidative stress-induced rats. Results showed that there was no significant difference ($p < 0.05$) in all the white blood cell differentials of rats in group III when compared with group I. There was a significant

decrease ($p < 0.05$) in the percentage of neutrophils of rats in groups V, VI and VII when compared with group III. There was a significant increase ($p < 0.05$) in the percentage of lymphocytes of rats in groups V, VI and VII when compared with group III.

HISTOPATHOLOGY OF THE LIVER



H&E staining; magnification X400
Plate 1.1: Photomicrograph of liver section of a normal control rat. The central vein is clear, the hepatocytes radiates hexagonally, the hepatic plates sinusoid and the nuclei are seen intact and open faced respectively which implies histological normal liver section.

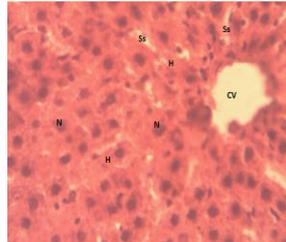
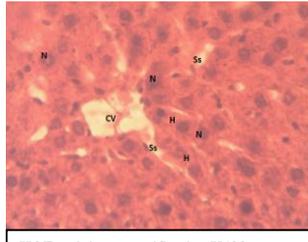
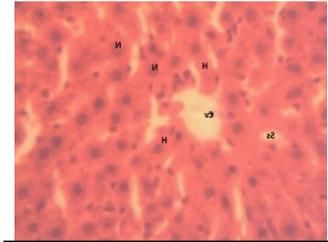


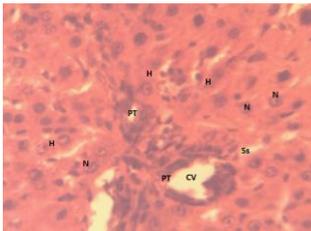
Plate 1.2: Photomicrograph of liver section of a Wistar rat fed with CTS. It showed that the hepatocytes radiates hexagonally, the central vein (CV) is clear, the sinusoid intact but the nuclei were affected which implies histological normal liver section.



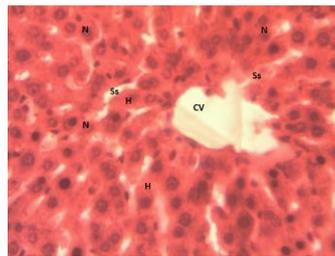
H&E staining; magnification X400
Plate 1.3: Photomicrograph of liver section of hypercholesterolemic rat (oxidative stress-induced) showed a compacted or dilated central vein (CV) with fatty deposits which also influenced part of the sinusoid, the nuclei and partly darkened and partly opened faced which implies histologically distorted liver section.



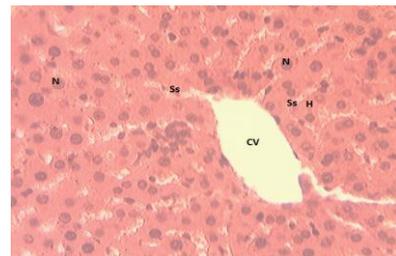
H&E staining; magnification X400
Plate 1.4: Photomicrograph of liver section of an oxidative stress-induced rat treated with atorvastatin drug showed a regenerating liver as the fatty deposits found in group III is clearing drastically and the nuclei are seen with open faced majority the hepatocytes are also radiating hexagonally which implies histologically normal liver section.



H&E staining; magnification X400
Plate 1.5: Photomicrograph of liver section of an oxidative stress-induced rat treated with 100 mg/kg of CTS indicated that the fatty deposits are not found here, the hepatocytes radiates hexagonally and the nuclei are healthy which implies histologically normal liver section.

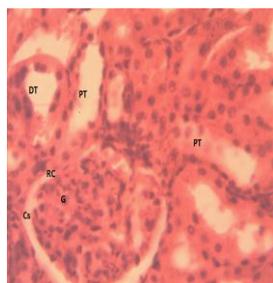


H&E staining; magnification X400
Plate 1.6: Photomicrograph of liver section of an oxidative stress-induced rat treated with 200 mg/kg of CTS showed normal liver with clear central vein, hexagonal radiation of the hepatocytes open faced nuclei and healthy sinusoids which implies histologically normal liver section.

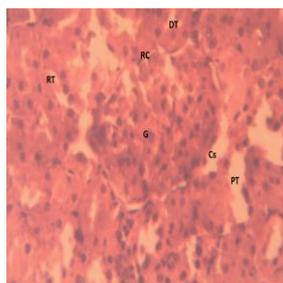


H&E staining; magnification X400
Plate 1.7: Photomicrograph of liver section of oxidative stress-induced rat treated with 400 mg/kg of CTS showed normal liver with clear central vein, hexagonal radiation of the hepatocytes open faced nuclei and healthy sinusoids which implies histologically normal liver section.

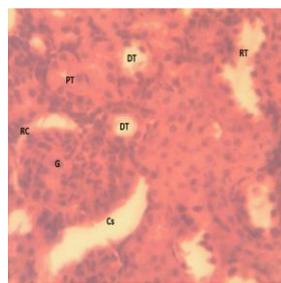
HISTOPATHOLOGY OF THE KIDNEY



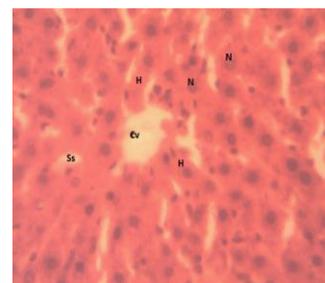
H&E staining; magnification X400
Plate 1.8: Photomicrograph of kidney section of a normal control rat showed normal kidney structure as the glomerulus, renal tubules and nucleus are seen in a normal kidney architecture which implies histological normal kidney section.



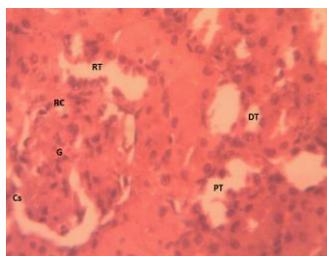
H&E staining; magnification X400
Plate 1.9: Photomicrograph of kidney section of a Wistar rat fed with CTS which looks normal but with slight fatty deposits in the distal and proximal convoluted tubules with wider capsular space (CP) which implies histological normal kidney section.



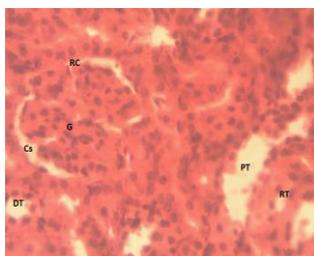
H&E staining; magnification X400
Plate 1.10: Photomicrograph of kidney section of hypercholesterolemic rat (oxidative stress-induced) showed an influenced kidney structure which could be caused by an intake or condition in which the rat was subjected to, though much adverse effect were not found but the disease seems not to be okay with the rat or that the rat was trying to adapt. The glomerulus and tuft-glomerular apparatus are intact but the proximal tubules are slightly touched and eroded which implies that the kidney is affected.



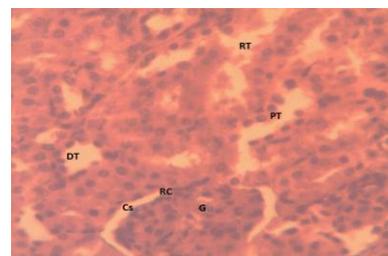
H&E staining; magnification X400
Plate 1.11: Photomicrograph of kidney section of an oxidative stress-induced rat treated with atovastatin drug showed a kidney structure undergoing a healing process. The glomeruli and the tubules are seen regenerating which implies histologically normal kidney section.



H&E staining; magnification X400
Plate 1.12: Photomicrograph of kidney section of an oxidative stress-induced rat treated with 100 mg/kg of CTS showed a relatively normal kidney. The distal and proximal tubules are free from fatty acid deposits and the capsular space is seen normally (regeneration) which implies histologically normal kidney.



H&E staining; magnification X400
Plate 1.13: Photomicrograph of kidney section of an oxidative stress-induced rat treated with 200 mg/kg of CTS showed a restored kidney structure. The glomeruli, capsular space, distal and proximal tubules are seen to be intact and healthy which implies histologically normal kidney section.



H&E staining; magnification X400
Plate 1.14: Photomicrograph of kidney section of an oxidative stress-induced rat treated with 400 mg/kg of CTS showed a normal kidney structure as every feature looks normal despite the fat cells noticed on the proximal tubule which implies histologically normal kidney section.

DISCUSSIONS

Oxidative stress has been implicated in the development and progression of a number of diseases such as atherosclerosis with several studies focusing on lowering high cholesterol content in the body by antioxidants as a possible way of addressing the condition (Wang *et al.*, 2012; AlAlmri *et al.*, 2020). As much as the nutritional importance of the Common Traditional Spices (CTS) used in this study have been understood in the past decades, the need to further analyze their phytochemical content became imperative in view of their potential therapeutic and pharmacological benefits. The results of the GC-MS screening of the phytochemical contents revealed substantial bioactive compounds which include cis-13-octadecanoic acid methyl ester, 2,6- octadienal 3,7- dimethyl, hexadecanoic acid methyl ester, n-hexadecanoic acid and heptadecanoic acid 16-methyl-methyl ester which have been reported to confer anticancer, antioxidant, hepatoprotective, antimicrobial, anti-inflammatory and cholesterol lowering effects

(Ganesan *et al.*, 2018; Ralte *et al.*, 2022; Johra *et al.*, 2023).

The acute toxicity of the CTS indicate that the extract was not toxic to the rats as they did not show signs of toxicity such as tremor, convulsions, writhing reflexes and agitations, but remained active and physically stable throughout the 24 hour period; and a further 7 days of observation at doses as high as 10,000mg/kg.

Elevation of serum liver enzymes marked by increase in the levels specific biomarkers (AST, ALT and ALP) shows damage to the liver particularly with increase in ALT revealing the extent of liver damage (Jain *et al.*, 2008; Sijabat *et al.*, 2023). The results of the effect of CTS extract on hypercholesterol-induced rats showed that high cholesterol caused significant increase ($p < 0.05$) in the AST, ALT, ALP, total protein, total bilirubin, albumin and globulin concentrations. However the administration of CTS extract particularly at 400 m/kg

restored the concentrations of the biomarkers to normalcy (see Figures 4.1 to 4.7). Compared with the standard atorvastatin drug, findings on the effect of CTS on liver function parameters of oxidative stress induced rats due to hypercholesterol is similar to the studies of Al-Obeidyeen *et al.* (2023) and Bin-Jumah (2018) who independently reported modulatory potentials of extracts of *Bassia muricata* and *Monolluma quadrangular* on AST, ALT and ALP activity of hypercholesterolemic rats. However, the results disagreed with the study of Taheri *et al.* (2012) who reported that extract of *Berberis vulgaris* had no significant effect on AST and ALP activity of hypercholesterolemic rats at the respective dosages administered. The possible reason for this variation could be the dosage administered and its tendency to suppress the action of acetylcholinesterase at cholinergic junctions of the central nervous system which inhibit oxidative stress that in turn reduce the serum levels of these biomarker (Friday, *et al.*, 2015). More so, the high proteolytic activity which enhanced metabolic function could be responsible for the significant increase in the total protein concentration in the hypercholesterolemic group (Abu-Backer *et al.*, 2010). Albumin supports glomerular filtration function of the kidney (Iwu *et al.*, 2020). The findings on the effect on liver function parameters is indicative of the modulatory potential of CTS extract on the liver, and the tendency to ameliorate hepato-biliary disorders.

The development of kidney disease in the body can partly be attributed to high cholesterol and high lipid content as it triggers the onset of metabolic disorders, obesity in its actual sense and obesity-related glomerular malfunction (Faran *et al.*, 2019). Hypercholesterolemia is a significant pointer for chronic renal disorder which can be exhibited as high levels of urea and creatinine, as well as electrolyte imbalance in the body (Faran *et al.*, 2019; Albrahim and Robert, 2022). These conditions are associated with higher oxidative stress and death of kidney cells (Albrahim, 2022).

In line with earlier studies (Faran *et al.*, 2019; Albrahim, 2022), the present study showed that administering 30mg/kg cholesterol in coconut oil significantly increased ($p < 0.05$) the concentrations of urea, creatinine, potassium ion and chloride ion in the body which showed that the kidney function has been compromised (Table 4.3). The possible explanations for this could be that hypercholesterolemia may have caused necrosis of renal cells; thereby distorting glomerular filtration, altered electrolyte balance and renal tubular reabsorption process. These physiologic phenomena could cause suppression of renal clearance, cellular seepage and consequent elevated plasma concentrations of the renal function biomarkers observed in the hypercholesterolemic group. Administering CTS extract at all concentrations restored the standard levels of urea, creatinine and potassium and chloride ions which might be attributed to ameliorative tendency and antioxidant potentials of these CTS as a result of presence of

bioactive compounds that inhibit metabolic free radicals production under hypercholesterolemic conditions (Albrahim, 2022). The positive effect of CTS extract on chloride ion tend to suggest a diuretic potential (Crook, 2006). Findings on the ameliorative potential of CTS on hypercholesterolemia-induced rats is similar to the study by Othman *et al.* (2022) who reported renoprotective action of lycopene against urea and creatinine levels in rats.

The administration of cholesterol in coconut oil had similar effect on lipid profile parameters as it had on the renal parameters. Hypercholesterolemia significantly increased total cholesterol, TAG, LDL-c and VLDL-c in rats. CTS extract restored them to normalcy although not better than atorvastatin standard drug (Table 4.4). A possible explanation for the protective effect of CTS extracts on the lipid parameters of hypercholesterolemic rats could be due to the presence of phytochemicals with the tendency to arrest lipid peroxidation (Ikewuchi and Ikewuchi, 2010; Ikewuchi *et al.*, 2011). Increased levels of total cholesterol in the body can cause atherosclerosis (Ademuyiwa *et al.*, 2005). Low concentration of Low Density Lipoprotein Cholesterol triggers cardiovascular disease (Ademuyiwa *et al.*, 2005; Rang *et al.*, 2005) while high TAG is a pointer for compromised heart (Dobiášová, 2004; McBride, 2007) which might eventually trigger some coronary heart diseases such as hypertension (Lopes *et al.*, 1997; Zicha *et al.*, 1999). Results on the effect of CTS extract on total cholesterol, TAG, LDL-c and VLDL-c is in line with the study of Bin-Jumah (2018) and Albrahim (2022).

Glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase are antioxidant enzymes that synergistically neutralize plasma levels of O_2 and H_2O_2 in the body by suppressing oxygen radicals and mopping up organic peroxides formed as a result of exposure to oxidative stress (Okoduwa *et al.*, 2017; Ojo *et al.*, 2019). The results obtained from this study indicate that hypercholesterolemia significantly decreased GSH, GPx, catalase and SOD but significantly increased MDA levels (Table 4.5). However, the administration of CTS extract had a positive effect on these antioxidant enzymes as it restored their levels to normalcy even better than the standard Atorvastatin. The perceived modulatory effects of CTS is indicative of the scavenging potential of the extracts of these spices (Ojo *et al.*, 2019). Data from this study agreed with the findings of Pal *et al.* (2023) who reported significant increase in MDA levels and significant decrease in GSH, SOD and catalase activity of acute restraint stressed rats which were restored to normalcy by nitric oxide precursor L-arginine, ascorbic acid and L-NAME at the concentrations administered. Furthermore, the results of the antioxidant parameters are similar to the study by Al-Sowayan and Almarzougi (2024) who reported significant reduction in MDA levels and significant increase in superoxide dismutase and glutathione

peroxidase levels by vitamin E in the brain of albino rats induced with oxidative stress. The current findings are equally in agreement with the work of Otunola *et al.* (2014) who earlier reported the potential of selected spices (*Allium ativum*, *Zingiber officinale* and *Capsicum fructensces*) administered singly and in combination to positively modulate antioxidant enzymes in the liver, heart and kidney. These results further provide evidence of the possible therapeutic and antioxidant potential of the extracts of these common traditional spices.

Furthermore, hypercholesterolemia significantly ($p < 0.05$) increased the plasma levels of lactate dehydrogenase and creatine kinase; however the administration of CTS extract significantly ($P < 0.05$) decreased their levels better than atorvastatin drug in the case of creatinine kinase (Figures 4.8 and 4.9). Results from this study are in agreement with the studies by Bin-Jumah (2018) and Johra *et al.* (2023) who independently reported cardiac enhancing potential of *Monolluma quadrangular* and mushroom extract on oxidative stressed rats.

The administration of high cholesterol significantly reduced haemoglobin but increased total white blood cell count and platelet. Treatment with CTS extract had positive haemopoetic effect on these hematological parameters (Table 4.6). It was reported that white blood cell helps in body defense, but excess WBC can trigger coronary artery diseases (Takeda *et al.*, 2003), while damage to TWBC and lymphocytes compromise the immune system. Hypercholesterolemia had no significant ($p > 0.05$) impact on the red blood cell count and packed cell volume, and white blood cell differentials (Table 4.7). The CTS extract enhanced up to 85% protection of the red blood cells from lysis as can be seen in Table 4.8, against atorvastatin with CTS.

Histopathological examination of the liver and kidney organs revealed histological distortion in the liver and kidney of hypercholesterolemic rats but the administration of CTS extract at all concentrations restored the liver and kidney to normalcy (Plate 4.1-4.14). This further supports the hepato and reno-protective potentials of CTS extract on oxidative stress rats as a result of hypercholesterolemia.

CONCLUSION

Conclusively, this study demonstrated the protective potentials of CTS on cardiac, hepatocellular and renal cells. The effects on lipid profile and antioxidant parameters revealed the ameliorative effects of the CTS on the oxidative-stress induced rats. This study has revealed that GC-MS screening of the phytochemical contents of CTS extract revealed substantial bioactive compounds which include cis-13-octadecanoic acid methyl ester, 2,6- octadienal 3,7- dimethyl, hexadecanoic acid methyl ester, n-hexadecanoic acid and heptadecanoic acid 16-methyl-methyl ester which are of therapeutic importance. Furthermore, administering

cholesterol on rats triggered hypercholesterolemia by causing significant increase on all the liver function parameters, urea, creatinine, K^+ , Cl^- , total cholesterol, TAG, LDL-c, VLDL-c, MDA, LDH, CPK, TWBC and PLT induced a significant decrease in GSH, GPx, SOD and Hb levels which showed that the various organs for which these biomarkers are specific to have been compromised. Treatment with the above mentioned CTS (*Occimum gratissimum*, *Gongronema latifolium*, *Piper guineense*, *Xylopia aethiopica*, and *Monodora myristica cocktail*) extract restored normalcy in the levels of these biomarkers even more than the standard atorvastatin drug in some cases such as the lipid profile parameters. Treatment with CTS extract gave positive results at all concentrations administered, although 200 mg/kg and 400 mg/kg were the most effective. The outcome validates the use of these CTS in traditional medicine and in facilitating the immune response and positive modulating effect on oxidative stress induced rats.

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