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# PROTECTIVE EFFECTS OF *Xylopia aethiopica* FRUIT ETHANOL EXTRACT ON CADMIUM-INDUCED INFLAMMATION AND DYSLIPIDEMIA IN MALE ALBINO RATS

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## ABSTRACT

The wide use of cadmium (Cd) in industry causes great environmental health problems to humans and animals. The aim of this study was to investigate the protective effects of *Xylopia aethiopica* fruit ethanol extract (XAFEE) on cadmium-induced inflammation and dyslipidemia in male albino rats. Thirty albino rats weighing 120 – 180 g were randomly selected into six groups (n = 5). A: control rats (administered distilled water only), B: Cd alone group (10 mg/ kg bw), C: Cd + 150 mg/kgbw XAFEE, D: Cd + 300 mg/kgbw XAFEE, E: 150 mg/kgbw XAFEE and F: 300 mg /kgbw XAFEE group. After 2-week acclimatization and 21 days of the experiment, blood sample was collected via cardiac puncture. Changes in tumor necrosis factor (TNF- α ), interleukin 10 (IL-10), total cholesterol (TC), triacylglycerol (TAG), phospholipids and free fatty acids (FFAs) concentrations in serum were determined. The results of the present study indicated that Cd exposure remarkably increased ($p < 0.05$) the TC, TAG, phospholipids, FFAs and TNF- α concentrations, and significantly decreased IL-10 concentration ($p < 0.05$) compared with control. These findings suggest that inflammatory changes and alterations in lipid metabolism might be one of the mechanisms underlying the subtle effects of Cd-induced inflammation and dyslipidemia. XAFEE expressed protective role against the toxic influence of Cd on affected parameters. The results raised the possibility of *Xylopia aethiopica* fruit being considered as a condiment in soup, local drinks, supplements or herbs preparations in areas where people have chances to Cd exposure, occupationally or environmentally.

**Key words**: Medicinal plant, spices, condiment, anti-dyslipidemic effect and Cd toxicity.

## 1. Introduction

Cadmium, Cd is one of the most toxic heavy metals present in the environment, and causes serious environmental and occupational hazards to humans[1,2], with wide range of organ toxicity and long elimination half-life (10-35 years). Environmental contamination by Cd results from its industrial use and its presence in agricultural fertilizers. Non-occupational exposure to Cd in humans predominantly results from smoking, air pollution, and consumption of Cd-contaminated sea foods and water [3]. Cd is implicated in the pathogenesis of several diseases, including cardiovascular disease (CVD) which may ensue from metabolic disorders such as diabetes and dyslipidemia amongst others [4]. The available conventional drugs used as anti-dyslipidemic agents are expensive and have adverse effects on health [5].

*Xylopia aethiopica* or Ethiopian pepper belongs to the family “Annonaceae” and is among the species that thrive in the evergreen rain forests of tropical and subtropical Africa [6]. It has its English name as

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Negro pepper or grains of Selim. In Nigeria, Yoruba call it 'Eeru', Igbo call it 'Uda' and Hausa call it 'Chimba' [6]. This plant possesses great nutritional and medicinal values in African traditional medicine for several centuries owing to its wide array of therapeutic indications in the treatment of cough, bronchitis, malaria among other disease conditions [7].

**Figure. 1: Snapshot of *Xylopia aethiopica* fruit**

In both human and animal studies, it has been shown that Cd perturbs cholesterol, triacylglycerol (TAG) and lipoproteins metabolism [7,8,9].Oxidative stress and inflammatory changes have been proposed as one of the numerous mechanisms in the pathogenesis of Cd toxicity, e.g. dyslipidemia, diabetes mellitus and cancer amongst others [10].

Nevertheless, several studies have investigated how various medicinal plants and spices containing natural anti-oxidants and anti-dyslipidemic potentials exert their effects, which subsequently prevent the damaging consequences of inflammation and dyslipidemia [11,12]. Thus, the present study was designed to evaluate the anti-inflammatory and anti-dyslipidemic effects of *Xylopia aethiopica* fruit ethanol extract (XAFEE) against cadmium-induced inflammation and dyslipidemia in male albino rats.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Cadmium chloride and chemicals used for this study was bought from Bridge Biotech. Ilorin, Kwara state. Kits were purchased from Elabscience$^{R}$ Biotechnology Co. LTD, USA. Other chemicals were of analytical grade.

### 2.2 Collection and identification of plant material (fruit)

Dried fruit of *Xylopia aethiopica* were purchased from a local herb store, Osiele, Abeokuta, Ogun State, Nigeria. Its botanical identification and authentication (FUNAAB H-0061) was done by a Botanist in the Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, Ogun State**,** Nigeria. The fruit were washed with clean tap water and allowed to dry again.

### 2.3. Extraction of plant material (fruit)

The dried fruit of *Xylopia aethiopica* were pulverized in a clean dry mortar. Six hundred grams (600 g) of the pulverized sample was cold macerated in 6.0 L of absolute ethanol (1:10 w/v) over 48 h

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periods. The extract was filtered using clean Whatmann No.1 filter paper, pore size of 100 (195 mm by 195 mm). The filtrate was concentrated using rotatory evaporator, and placed on water bath to allow evaporation of the solvent.

2.4. Determination of median lethality dose, $LD_{50}$

The $LD_{50}$ of the XAFEE was tested on sixteen (16) albino rats using the modified method and calculation proposed by Lorke [13]. Different doses on single administration of XAFEE were administered orally to the rats in four groups (n = 4). Each group received 1000 mg/kg b.w, 2000 mg/kg b.w, 3000 mg/kg b.w and 4000 mg/kg b.w respectively. The rats were monitored and examined for 24 hrs for any sign of toxicity and mortality after the XAFEE administrations. Maximum dose with 0 % and minimum dose with 100 % mortality were recorded, and used to determine the **$LD_{50}$** of the XAFEE as follows:

$LD_{50} = \sqrt{a \times b}$

Where a = Maximum dose with 0 % mortality b = Minimum dose with 100 % mortality.

Ten (10 %) of the $LD_{50}$ was used for therapeutic doses selection.

2.5. Experimental rats

The approval of the departmental animal ethical committee (FUNAAB- BCH) was taken prior the experiment with ethical number: FUNAAB- BCH- APF0441, dated 20th November, 2019. All the protocols and the experiments were conducted in strict compliance according to the guidelines approved by the committee.

Thirty (30) male albino rats weighing 120–180 g were purchased from College of Veterinary Medicine, FUNAAB, and randomly distributed into six (6) groups (n = 5). The rats were fed *ad libitum*, kept on a 12 h light–dark cycle periods and acclimatized for two weeks prior the experiment, which lasted for a period of 21 days.

**Table 1**: Experimental design

| Group (n =6) | Treatment |
|---|---|
| A (Control) | Distilled water only |
| B | Cd as $CdCl_2$ in distilled water (10 mg/kg b.w/day) |
| C | Cd as $CdCl_2$ in distilled water (10 mg/kg b.w/day) + XAFEE ( 150 mg/kg b.w/day) |
| D | Cd as $CdCl_2$ in distilled water (10 mg/kg b.w/day) + XAFEE (300 mg/kg b.w/day) |
| E | XAFEE (150 mg/kg b.w/day) |
| F | XAFEE (300 mg/kg b.w/day) |

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2.6. Sacrifice

The oral route was selected because it depicts more reasonably how the general human population is exposed to cadmium. After 21 days of the experiment, the rats were fasted overnight and sacrificed under light diethylether anaesthesia.

2.7. Serum biochemical estimation

Blood sample was collected via cardiac puncture into plain tubes. Thereafter, centrifuged at 3000 rpm for 10 mins to obtain serum for the biochemical analyses.

2.8. Determination of inflammatory biomarkers

TNF-α and IL-10 concentrations were determined using Sandwich - enzyme-linked immunosorbent assay (ELISA) method.

2.9. Determination of lipid profile biomarkers

2.9.1. Determination of total cholesterol concentration

Total cholesterol concentration was determined spectrophotometrically according to the method described by Allain *et al.* [14]. The method involves the use of three enzymes; cholesterol esterase, cholesterol oxidase and peroxidase. In the presence of the enzymes, the mixture of N-ethyl-N-propyl-M-anisidine (ADPS) and 4-aminoantippyrine (4-AA) are condensed by hydrogen peroxide to form quinoneimine dye, which is proportional to the concentration of cholesterol in the sample.

2.9.2. Determination of triacylglycerol concentration

Triacylglycerol concentration was determined spectrophotometrically according to the method described by Buccolo and David [15]. This method is based on the enzymatic hydrolysis of triglyceride to glycerol and free fatty acids by Lipoprotein lipase. The glycerol was phosphorylated by adenosine triphosphate in the presence of glycerokinase to form glycerol-3-phosphate and adenosine diphosphate.

2.9.3. Determination of phospholipids concentration

Phospholipids concentration was determined spectrophotometrically according to the method described by Stewart [16]. Phospholipids are hydrolyzed by phospholipase D and the liberated choline is subsequently oxidized by choline oxidase to betaine with the simultaneous production of hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide couples oxidatively with 4-aminophenazone and dichlorophenol to form a quinonemine dye.

2.9.4. Determination of free fatty acids concentration

Concentration of free fatty acids was determined according to the method described by Brunk and Swanson, [17].

2.10. Data Analysis

All values were expressed as the mean ± standard error of mean (SEM). The data were analyzed using one-way analysis of variance (ANOVA) and significant means were separated by post hoc Duncan's multiple range test at $p < 0.05$.

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## 3. Results

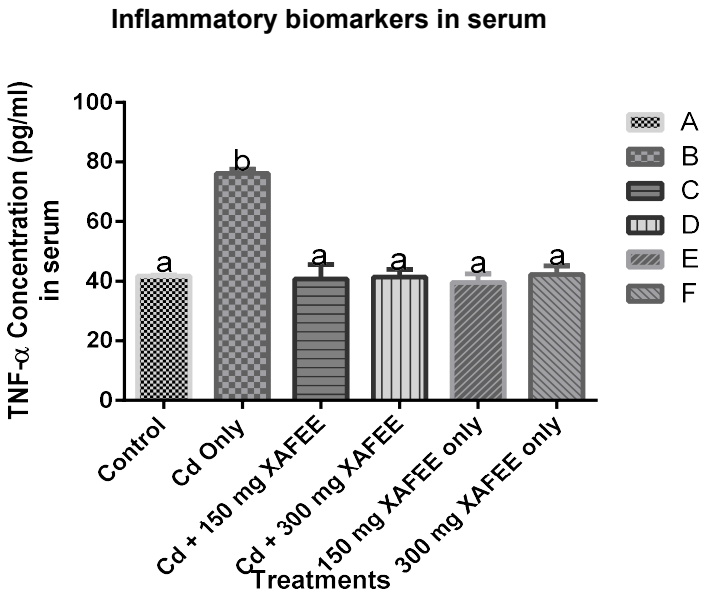


Figure 2: Effects of Cd and XAFEE on TNF-α concentration

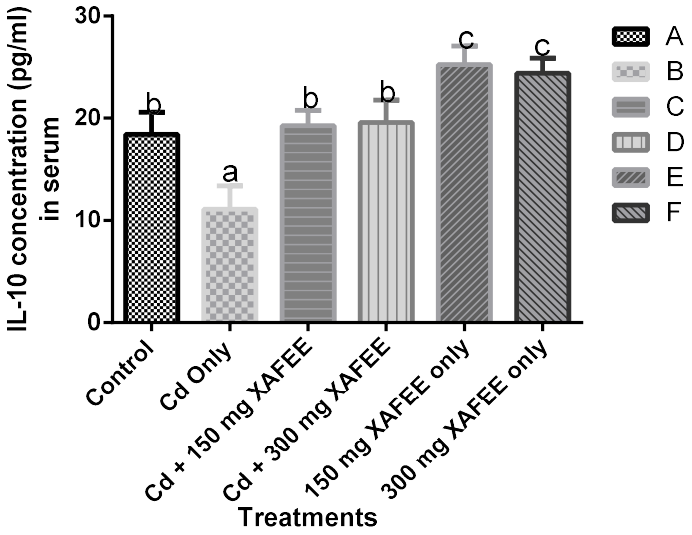


Figure 3: Effects of Cd and XAFEE on IL- 10 concentration

Figures 2 showed the effects of Cd and XAFEE on inflammatory biomarkers in serum. The group exposed to Cd only showed a significant increase ($p < 0.05$) in TNF-α concentration compared with control. In figure 3, a remarkable decrease in IL-10 concentration was observed compared to control. Treatment with XAFEE remarkably reversed the concentrations of TNF- α and IL-10 towards control level, in both figures respectively.

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**Lipids profile assay in serum**

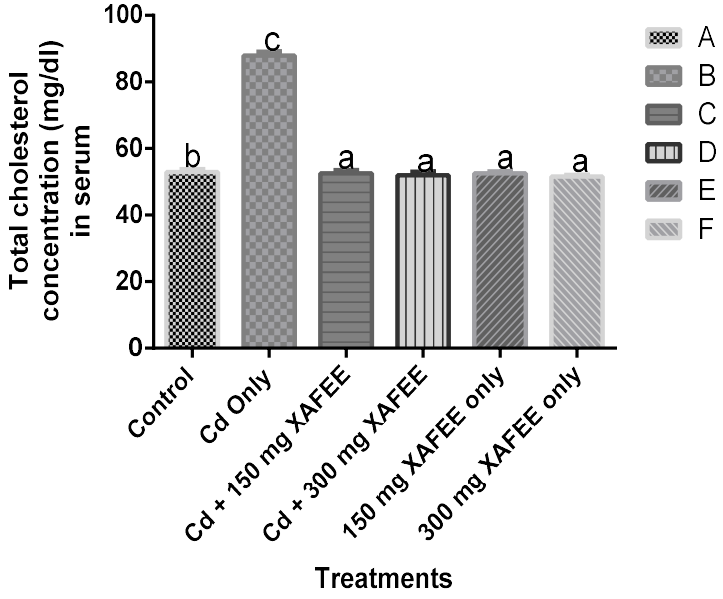


Figure 4: Effects of Cd and XAFEE on total cholesterol concentration

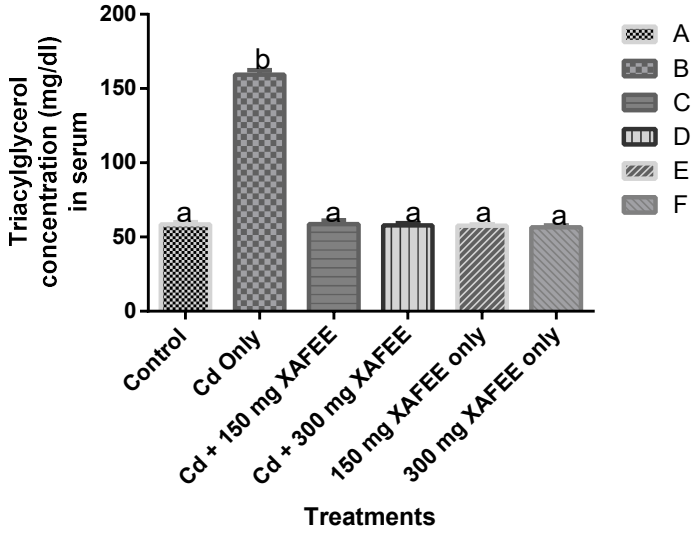


Figure 5: Effects of Cd and XAFEE on triacylglycerol concentration

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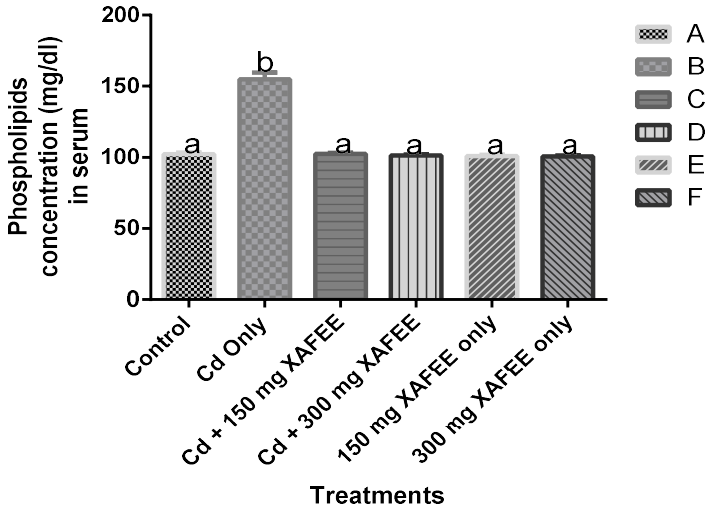


Figure 6: Effects of Cd and XAFEE on phospholipids concentration

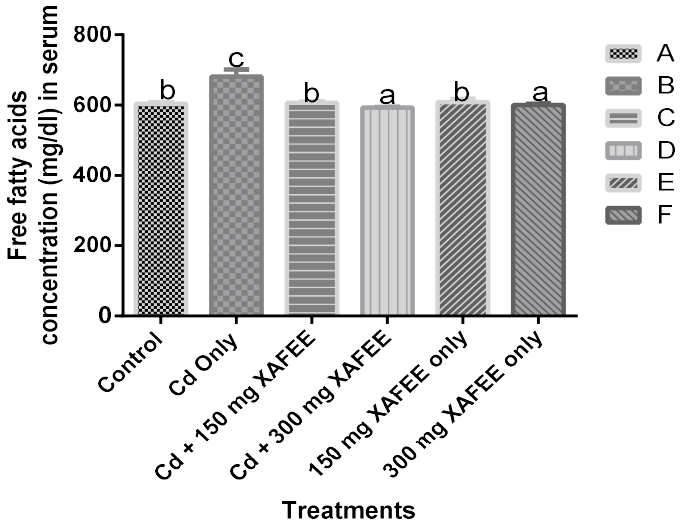


Figure 7: Effects of Cd and XAFEE on free fatty acids concentration

Figure 4-7 showed the effects of Cd and XAFEE on lipid profile in serum. The group exposed to Cd only showed a remarkable increase in the concentrations of total cholesterol, triacylglycerol, phospholipids and free fatty acids compared with the control rats. Treatment with XAFEE normalized this perturbation towards the control level.

## 4. Discussion

Cadmium (Cd) is a toxic metal that is widely used in different industries. Exposure to Cd causes damage to various organs including liver, kidneys, lungs, bones, testes and placenta depending on the dose, route of administration and duration of exposure [18]. It promotes an early oxidative stress and

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afterward contributes to the development of serious pathological conditions because of its long retention in some tissues [19]. The present results have clearly shown the ability of Cd to induce inflammatory changes and dyslipidemia.

The remarkable increase in TNF- α (Figure 2) and decrease in IL-10 (Figure 3) concentrations is a reflection of pathological event, precisely, inflammation. Inflammation is a series of defense mechanism in response to harmful effects, exposure to toxicant, injury and infection, associated with the release of pro-inflammatory cytokine, alongside with swollenness, redness, hardness, heat and pain in tissues. However, Cd promotes an early oxidative stress and afterward contributes to the development of inflammation and serious pathological conditions like dyslipidemia and other metabolic disorders, because of its long retention in tissues [20].

The ability of XAFEE to reverse the concentrations of TNF- α and IL-10 towards control level might be due to the anti-inflammatory property of the *Xylopia aethiopica* fruit [21].

Changes in the distribution of lipids after Cd exposure is associated with a change in the turn-over of lipids in medium of high oxidative stress which is known to modify the lipid properties of membranes. Increased concentration of phospholipids in serum may lead to the formation of numerous multi-lamella inclusion bodies in cell, causing a loss of cell functions which compromise the cell integrity, thereby promoting cellular dysfunction [22]. The observations in Figure 4-7 established the fact that Cd causes alteration in lipid metabolism following production of free radicals and oxidative damage caused by Cd, which plays an important role in the metabolic reactions of the animals. These findings correlate with the studies reported on the contribution of oxidative stress to the modification of lipids which is associated with oxygen free radical production, resulting in oxidative degradation of lipids [23]. In the current study, an increases in TC, TAG, phospholipids and FFAs were observed in rats exposed to Cd alone as compared with the control group. Other studies have demonstrated similar increases after administration of Cd to rats [24]. In the study, *Xylopia aethiopica* fruit ethanol extract exerted anti-inflammatory and antidyslipidemic effects.This might be due to the presence of the phytochemical constituents of the XAFEE, as shown in the supplementary file (file 1), some of which have been reported to possess anti-oxidative, anti-inflammatory and ant-dyslipidemic properties [25], enabling it to normalize the inflammatory changes and the lipids levels [26, 27].

## 5. Conclusion

The results raised the possibility of *Xylopia aethiopica* fruit as a good treatment agent for inflammation and dyslipidemia. However, further study could be done on the specific components of the extract that are responsible for the anti-inflammatory potential, and the mechanism at which the fruit exerts its anti-dyslipidemic effect.

## Aknowlegement

We appreciate all participants, including the laboratory technologists who jointly worked with us towards the success of this wor but couldn't be listed in the authors list.

## Authors contribution

All authors contributed equally towards this research.

## Conflict of interest

All authors declared no conflict of interest.

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