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Effect of Lime Juice Extract of Cashew Bark on Lipid Biomarkers in Indomethacin-Induced Gastric Ulcerated Wistar Rats

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ABSTRACT

This study investigated the effect of lime juice extract of cashew bark (LJECB) on lipid profile in indomethacin-induced gastric ulcer in Wistar rats. Freshly pulverised *Anacardium occidentale* stem bark (1 kg) was soaked in freshly prepared *Citrus aurantifolia* fruit juice (3 L) for 48 hours inside a refrigerator (4 ^{o}C). The filtered solution was concentrated *in vacuo* using rotary evaporator to produce the crude extract labeled as LJECB. Forty-two (42) female Wistar rats (100-150 g) were randomized into seven groups (n = 6) and treated as follow. Group 1: normal control (Distilled water 1 ml/100g body weight); Group 2: 30 mg/kg indomethacin Only (ulcerated group); Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg LJECB; Group 5: 30 mg/kg indomethacin + 800 mg/kg LJECB. Group 6: 400 mg/kg LJECB; Group 7: 800 mg/kg LJECB. Prior to ulcer induction, the animals were fasted overnight and allowed free access to water *ad libitum*. Then indomethacin (30 mg/kg, 1 ml) was orally administered to the rats in Group 2 - 5. Treatment with LJECB or antacid commenced 4 hours post-induction for 14 days. After the last dose, the animals were fasted overnight and sacrificed under mild inhalation of diethyl ether. Serum samples were prepared and used for lipid biomarker analyses (total cholesterol, triglycerides, HDL-c, LDL-c, and VLDL-c). Castelli risk index-1 and 2 were calculated from an established equation to determine the cardiovascular risk of the extract. Results showed that the untreated ulcerated group had a significant reduction in total cholesterol levels compared to the normal control group. Treatment with a high dose of LJECB further caused a significant decrease in the total cholesterol levels. Also ulcerated group had a significant reduction in triglyceride levels compared to the normal control group. Treatment with 400 mg/kg dose of LJECB further lowered the triglyceride levels in ulcerated rats, however, higher dose of LJECB (800 mg/kg) increased the triglyceride levels. Untreated ulcerated rats had a significant increase in HDL compared to the normal control group. Administration of LJECB (400 mg/kg) had no significant ($p<0.0001$) effect on HDL levels in the ulcerated group. However, 800 mg/kg of LJECB administered to a normal control group was associated with an increase in HDL. Moreover, the LDL was significantly low across all the groups. The normal control group had a higher Castelli risk index-1 (CRI-1), while the ulcer group and LJECB-treated groups had a relatively lower CRI-1 value. The study concludes that administration of LJECB to *Wistar* rats was associated with a reduction in total cholesterol and LDL levels, elevated HDL levels, and low Castelli risk index, in both ulcerated and non-ulcerated rats.

Keywords: Lime fruit juice, cashew, lipid biomarkers, Castelli risk index,

INTRODUCTION

Lipids including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), serves as an important indicator of cardiovascular health and overall lipid metabolism [1]. Disturbances in lipid profile have been strongly associated with an increased risk of developing cardiovascular diseases, including atherosclerosis and coronary artery disease [2, 3].

Cashew bark (*Anacardium occidentale*) has been recognized as a potential source of bioactive compounds that exhibit significant health benefits [4]. Cashew bark contains beneficial phytochemicals, including phenolics [5], flavonoids, and tannins [6] which possess antioxidant [7] and anti-inflammatory properties [8]. These bioactive compounds have shown promising effects on lipid metabolism [9,10] suggesting their potential roles in managing lipid disorders.

Similarly, *Citrus aurantifolia* (lime; family: Rutaceae), also known as the miracle fruit for its delightful aroma and taste, is a globally cultivated plant [11]. Lime fruit juice is a globally consumed cuisine due to the multi-medicinal purposes such as antibacterial, anti-inflammatory, anticancer, antidiabetic, anti-hypertensive, antioxidant properties [12-13].

Ulceration is a prevalent health condition that affects the gastrointestinal tract [14], leading to disturbances in vital metabolic processes, including lipid metabolism [15]. Ulcerated rats serve as a suitable model for studying the impact of ulcer conditions on lipid metabolism [14]. Studying the effects of lime juice extract of cashew bark on lipid profiles of both normal and ulcerated rats can provide an information on potential therapeutic applications of the extract in mitigating lipid disturbances caused by ulceration.

Hyperlipidemia is a medical condition characterized by an elevation in lipid profile or lipoproteins in the blood [16]. It is also called hypercholesterolemia or hyperlipoproteinemia. Elevated low-density lipoprotein cholesterol (LDL-c) is considered as the best indicator of atherosclerosis risk [17]. However, studies have shown that dyslipidemia can also be characterized by elevated total cholesterol (TC), triglycerides (TG), or low levels of high-density lipoprotein cholesterol (HDL-c) [18-19].

Human body is characterized as a complex machine for maintaining a constant homeostatic balance in various tissues and organs [20]. Dysregulation in lipid metabolism can adversely impact the normal physiology and homeostasis thereby leading to a disease condition [21]. Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides [22]. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides are fuel molecules which are oxidized or stored in the fat cells

depending on the cellular need. TG is synthesized by the liver from dietary intake or intestinal absorption [22].

Previous studies have primarily focused on finding substances that can reduce blood lipid levels, often motivated by the link between lipid dysregulation and various diseases. However, the connection between lipid levels and gastric ulcers is less well-understood. This study aimed to investigate if there is a relationship between lipid profiles and the development of gastric ulcers in rats exposed to indomethacin. Indomethacin is a commonly used nonsteroidal anti-inflammatory drug used to induce gastric ulcers in Wistar rat models [14]. It's well-established mechanism of action of inhibiting cyclooxygenases 1 and 2 causes a reduction in the production of prostaglandins, whose function is to maintain a healthy gastric mucosal wall. The disruption of such protective mucosal layer leads to an increased susceptibility to ulcer formation, making it a reliable and reproducible model for studying gastric ulceration and the efficacy of potential therapeutic interventions.

Ulceration and cardiovascular disease (CVD) may share a complex (indirect) relationship rooted in common risk factors and underlying pathophysiological mechanisms. Both conditions occur in individuals with underlying health issues such as diabetes, hypertension, and peripheral arterial disease. Chronic inflammation is largely implicated in ulceration and in the development and progression of atherosclerosis which is the primary cause of CVD. Presence of ulceration may serve as a potential marker for increased cardiovascular risk [18].

In this study however, we aim to investigate the potential therapeutic effects of lime juice extract from cashew bark on indomethacin-induced gastric ulcers in rats. This would assist in a better understanding of the mechanisms underlying the development and progression of gastric ulcers and its association to cardiovascular risk, as well as exploring the potential of this natural remedy as a complementary or alternative therapy.

MATERIALS AND METHODS

Ethical Consideration

An ethical approval (AUERC/2024/66IR/01) was collected from Adeleke University Research Ethics Committee.

Experimental Animals

Healthy forty-two (42) female Wistar rats (150 – 200 g) were purchased from Adegoke Animal Breeding Laboratory, Ogbomoso, Oyo State, Nigeria. The animals were acclimatized for seven (7) days at room temperature (25±2 ^{o}C) and humidity (40-60%) in the Animal Facility Unit, Department of Biochemistry, Adeleke University, Ede, Nigeria. The facility is equipped with a ventilation system that ensured optimal air flow to prevent the accumulation of harmful gases. During this time, they had unrestricted access to food and water.

Collection of Plant Samples

Fresh *Anacardium occidentale* stem bark was collected at Adeleke University Campus, Ede, Osun State. *Citrus aurantifolia* fruits were purchased at Owode local market in Ede of Osun State, Nigeria. *Anacardium occidentale* (Voucher

Number: IFE-18169) and *Citrus aurantifolia* fruits (Voucher Number: IFE-18170) were identified and authenticated by the Ife Herbarium at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of *Citrus aurantifolia* Juice Extract of *A. occidentale*

Preparation of *C. aurantifolia* Juice for Extraction

The *C. aurantifolia* fruit juice was prepared as formerly described [24].

Extraction of *A. occidentale* Stem Bark

The extraction of *A. occidentale* stem bark (1 kg) was carried out as previously reported [24]. The crude extract was labeled as the lime juice extract of cashew bark (LJECB).

Experimental Design

Indomethacin is a commonly used nonsteroidal anti-inflammatory drug used to induce gastric ulcers in Wistar rat models [14]. The forty-two (42) *Wistar* rats were randomised into seven groups (n = 6) and treated as follow. Group 1: Normal control (Distilled water 1 ml/100 g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract;

Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg LJECB Only; Group 7: 800 mg/kg LJECB Only. Group 6 and 7 receiving only the extract were included to address important questions. First, to establish a baseline for the effects of the extract in healthy rats, without the confounding factor of indomethacin-induced ulceration. Second, to assess the safety of the extract and identify any potential adverse effects. We believe that the inclusion of these groups was essential for a comprehensive evaluation of the extract's effects and for ensuring the validity of our findings. On the other hand, the selected doses (400 & 800 mg/kg) were based on the obtained LD_{50} - 4303 mg/kg of the crude extract LJECB.

Induction of gastric ulcer

Prior to gastric ulcer induction, the experimental animals were fasted overnight but were given access to water *ad libitum*. Then, gastric ulcer was induced by oral administration of 1 ml of indomethacin (30 mg/kg) in Group 2 to Group 5 of the experimental design as earlier reported [24]. The animals were further fasted for 4 h after induction, to allow for ulcer manifestation (see Plate 1).

Treatment with Extract or Drug

The extract LJECB (400 or 800 mg/kg; 1 ml) or Gecrol antacid (5 ml/kg; 1 ml) was administered orally to the animals in Group 3 to Group 5 after 4 hours induction. The treatment was continued for 14 days between 9 and 10 a.m. During this period, the animals were given free access to food and water.

After administering the last dose of the extract/drug on Day 14, the animals were fasted overnight until the 15th day when they were sacrificed.

Animal Sacrifice, Dissection, and Tissue (blood, stomach) Sample Collection

The animals were anaesthetized under mild inhalation of diethyl ether and sacrificed via cardiac puncture. The blood samples were collected into plain sample vials for serum preparation. The stomach tissues were separately excised and rinsed in isosaline to remove gastric suspension. The stomach was examined for macroscopical mucosal lesions.

Preparation of the Blood Serum

The serum sample was prepared as formerly reported [24]. The serum samples were used for lipid biochemical analyses.

Lipid Biochemical Assays

Cholesterol Assay

The total cholesterol level of the serum samples was determined using the Randox kit method as reported by Akinlusi et al. [25]. Both serum sample and standard (0.01 ml) were separately pipetted in duplicate. Then, 1 ml of Reagent 1 was added to each of the samples. The reagent blank was prepared by adding 0.01 ml of distilled water and 1 ml of Reagent 1. The composition of Reagent 1 includes PIPES buffer (80 mmol/l, pH 6.8), 4-aminoatipyrine (0.25 mmol/l), phenol (6 mmol/l), peroxidase (0.5 U/ml), cholesterol esterase (0.15 U/ml), and cholesterol oxidase (0.10 mmol/l). The reaction mixture was incubated at 37 ^{o}C for 10 minutes. The absorbance was read at 546 nm within 1 hr against the reagent blank.

Triglycerides Assay

The concentration of triglycerides in the serum samples was determined using the Randox kit method [26-27]. The serum and standard sample (0.01 ml) were pipetted in duplicate, followed by addition of 1 ml of Reagent 1. The constituents of Reagent 1 were PIPES buffer (40 mmol/l, pH 7.6), 4-chloro-phenol (5.5 mmol/l), magnesium ions (17.5 mmol/l), 4-aminophenazone 0.5 (mmol/l), ATP (1.0 mmol/l), lipase (150 U/ml), glycerol kinase (0.4 U/ml), glycerol-3-phosphate oxidase (1.5 U/ml), and peroxidase (0.5 U/ml). The reaction mixture was incubated at 37 ^{o}C for 10 minutes and the absorbance was read at 546 nm within 1 hr against the reagent blank containing 0.01 ml of distilled water in place of the sample.

High Density Lipoprotein (HDL) Assay

The concentration of HDL-c was also determined [26-27]. The serum sample or standard (0.2 ml) was precipitated using 0.5 ml of precipitating reagent (phosphotungstic acid, 0.55 mM and manganese chloride, 25 mM). The suspension was mixed, allowed to settle, and centrifuged for 10 min at 400 rpm. The supernatant was collected and used for estimation of HDL-c. The reaction mixture contained 0.1 ml supernatant/standard in duplicate and 1 ml Reagent 1. The reagent blank contained distilled water (0.1 ml) and 1 ml of Reagent 1 {PIPES buffer (80 mmol/l, pH 6.8), 4-aminoantipyrine (0.25 mmol/l), phenol (6 mmol/l), peroxidase (0.5 U/ml), cholesterol esterase (0.15 U/ml), and cholesterol oxidase (0.10 mmol/l). The reaction mixture was incubated at 37 ^{o}C for 10 minutes. The absorbance was read against the reagent blank at 500 nm within 1 hr of incubation.

Estimation of Very Low-Density Lipoprotein (VLDL) Cholesterol

The concentration of LDL in the serum samples was estimated from the Friedelwald *et al.* [28] equation as: VLDL = Triglycerides/5 (mg/dl).

Parameters for Assessing Cardiovascular Risk

Atherogenic Indices

Atherogenic Coefficient (AC) was calculated as described [29].

AC = (TC – HDL-c)/ HDL-c; Where non-HDLc = TC – HDL-c.

Atherogenic Index of Plasma

Atherogenic index of plasma (AIP) was calculated as described [28].

AIP = Log (TG/ HDL-c)

Non-HDL-C

Non-HDL-c was calculated described [28]. Non-HDL-c = TC –HDL-c

Data Analysis

Statistical analysis was performed using GraphPad Prism 5.0. Differences between experimental groups and normal control was determined by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Values were considered statistically significant at $p<0.0001$. Data were expressed as Mean ± standard error of mean SEM (n = 5).

RESULTS AND DISCUSSION

Effect of LJECB on Total Cholesterol Level in Ulcerated and Normal Rats

The effect of LJECB on serum total cholesterol level in ulcerated and normal Wistar rats is shown in Figure 1 (See Plate 1). The normal control group had a cholesterol level of 189.00±0.04 mg/dl. The negative control group (indomethacin 30 mg/kg, only) showed a significantly lower cholesterol level (20.58± 0.07 mg/dl) compared with the normal control (189.00±0.04 mg/dl). This shows that indomethacin had a cholesterol-lowering property. Treatment of gastric ulceration with commercial antacid (Gecrol) elevated the cholesterol level (169±0.00 mg/dl) compared with the negative control (20.58± 0.07 mg/dl). Suggesting that Gecrol antacid promoted cholesterol synthesis in gastric ulceration. Treatment with 400 mg/kg of LJECB showed a nonsignificant difference with Gecrol positive control. However, treatment with 800 mg/kg of LJECB significantly increased the cholesterol levels to 127.00±0.05 mg/dl compared with the negative control (20.58±0.07 mg/dl). Compared with the negative control group (20.58± 0.07 mg/dl), treatment of the ulcerated rats with 400 and 800 mg/kg of LJECB caused significant elevation in the cholesterol levels. For healthy Wistar rats administered with 400 and 800 mg/kg of LJECB as extract control, the cholesterol level was significantly ($p<0.0001$) reduced to 55±0.02 and 92±0.00 mg/dl.

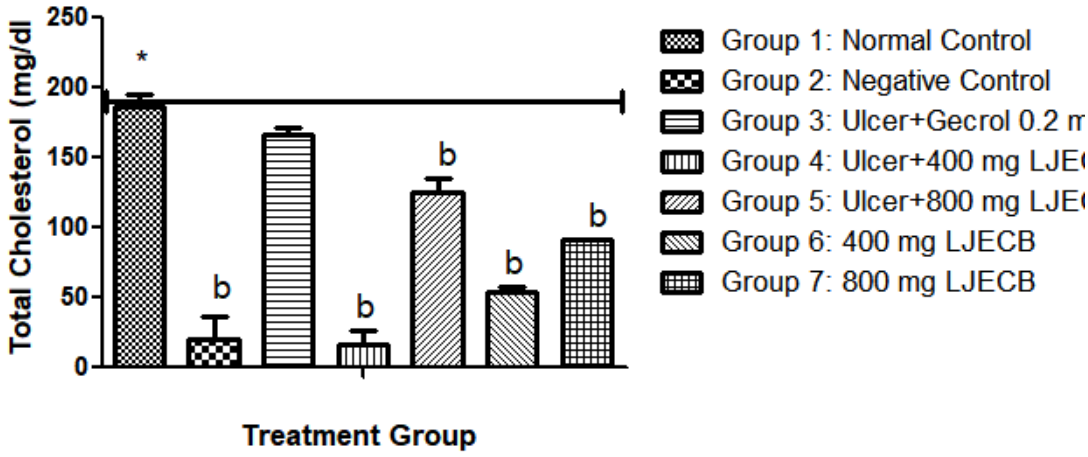

Figure 1: Effect of the lime juice extract of cashew bark on total cholesterol level of ulcer-induced and control groups of Wistar rats. Values expressed as Mean ± SEM (n = 6). Group 1: Control (Distilled water 1 ml/100g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract; Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg extract; Group 7: 800 mg/kg extract. (b): Values significantly ($p<0.000$) lower than normal control.

Effect of Lime Juice Extract of Cashew Bark (LJECB) on Triglyceride Concentration in Ulcerated and Normal Rats

The effect of LJECB on serum triglyceride level in ulcerated and normal Wistar rats is shown in Figure 2 (See Plate 1). Compared with the normal control group (1360± 0.00 mg/dl), the negative control group (indomethacin 30 mg/kg, only) had a significantly lower ($p<0.0001$) (953.60±0.01 mg/dl) triglyceride level. This suggests that indomethacin may have lowered the triglyceride level and could be employed in lowering the blood lipid levels. Treatment of the gastric ulcer with Gecrol antacid significantly increased the cholesterol level (1521.6± 0.06 mg/dl) compared with the negative control (953.6± 0.01 mg/dl). Treatment of the gastric ulcer with 400 mg/kg of LJECB caused a significant reduction (436.8± 0.03 mg/dl) in triglyceride level compared with the negative control (953.6± 0.01 mg/dl). However, a higher dose (800 mg/kg) of LJECB in animals with gastric ulceration caused a significant increase (1654.8± 0.18 mg/dl) in triglyceride levels. In extract control groups administered with 400 and 800 mg/kg of LJECB, the triglyceride level was significantly reduced to 780.8± 0.20 and 712± 0.09 mg/dl, respectively compared with the negative control (953.6± 0.01 mg/dl) and normal control (1360± 0.00 mg/dl) groups. These findings indicate that both doses of LJECB are more effective at lowering the blood triglyceride levels than the Gecrol ($p<0.0001$).

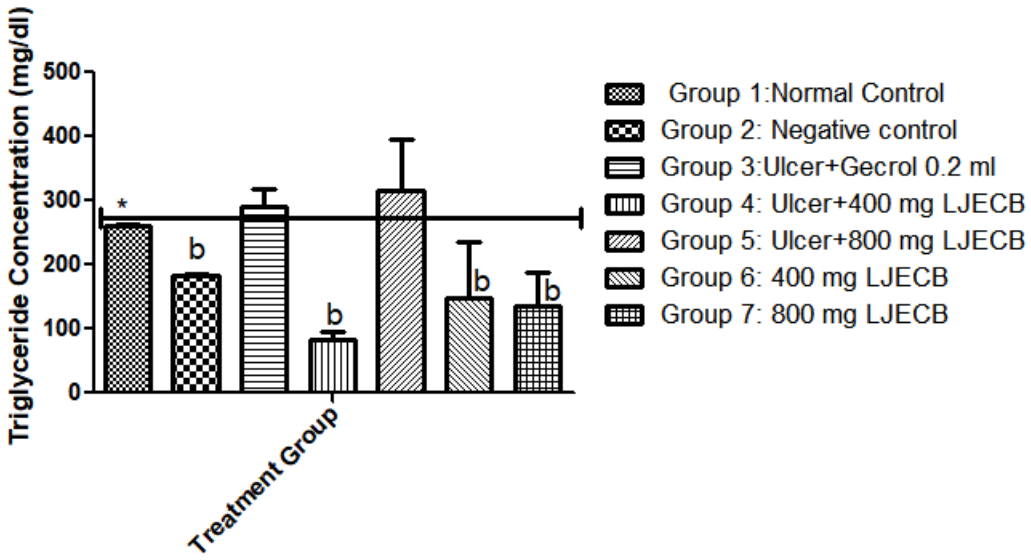

Figure 2: Effect of the lime juice extract of cashew bark on the triglyceride values of ulcer-induced and control groups of Wistar rats.

Group 1: Control (Distilled water 1 ml/100g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract; Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg extract; Group 7: 800 mg/kg extract. (b): Values significantly lower than normal control. (a): Values significantly higher ($p<0.0001$) than normal control.

Effect of Lime Juice Extract of Cashew Bark on HDL-Cholesterol Level

The effect of LJECB on serum HDL level in ulcerated and normal Wistar rats is shown in Figure 3 (See Plate 1). Results showed that the HDL level of normal control (79.18±0.06 mg/dl) was significantly lower than the negative control (309.26±0.01 mg/dl), indicating that indomethacin enhanced the biosynthesis of high-density lipoprotein in the untreated negative group. Treatment with Gecrol significantly lowered the HDL level to 18.36±0.00 mg/dl. Suggesting that Gecrol antacid had a negative impact on HDL levels in ulcerated rats. Treatment with 400 and 800 mg/kg of LJECB was found to raise the HDL to 20.08±0.00 and 22.37±0.00 mg/dl, respectively compared with the positive control (18.36±0.00 mg/dl). However, administration of 400 and 800 mg/kg of LJECB to healthy Wistar rats caused a significant increase in HDL levels to 289.75±0.04 and 232.95±0.00 mg/dl, respectively compared with the normal control (79.18±0.06 mg/dl).

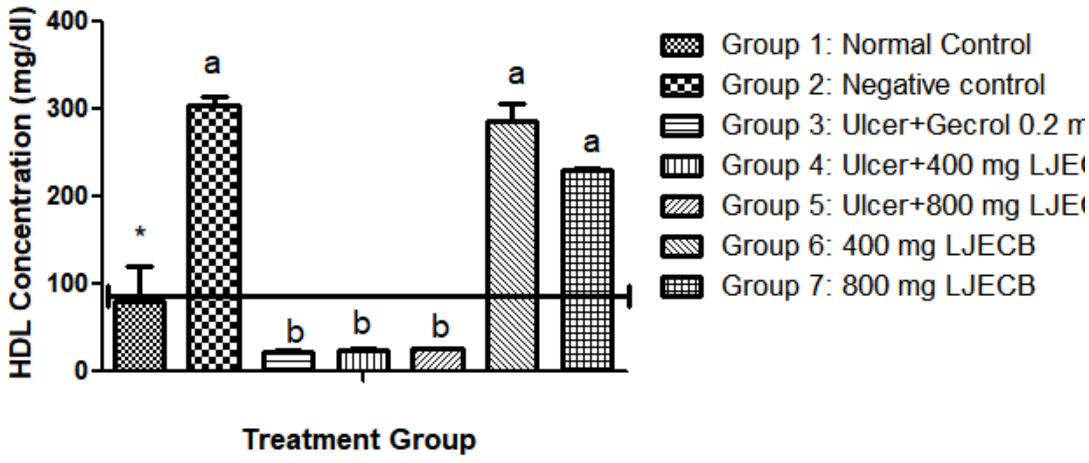

Figure 3: Effect of the lime juice extract of cashew bark on the HDL-cholesterol level of ulcer-induced and control groups of Wistar rats.

Group 1: Control (Distilled water 1 ml/100 g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract; Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg extract; Group 7: 800 mg/kg extract. (b): Values significantly lower ($p < 0.0001$) than normal control (Group 1). (a): Values significantly higher ($p<0.0001$) than normal control (Group 1).

Effect of LJECB on Serum Very Low-Density Lipoprotein (VLDL) Ulcerated and Normal Wistar Rats

The effect of LJECB on serum VLDL level in ulcerated and normal Wistar rats is shown in Figure 4 (See Plate 1). Results showed that the VLDL level in normal control (272.4±0.03 mg/dl) was significantly higher than the negative group (190.72±0.00 mg/dl). Implying that indomethacin significantly lowered the blood VLDL level. Treatment with Gecrol antacid showed a higher VLDL (304.32±0.01 mg/dl) compared with the negative control (190.72±0.00 mg/dl). Treatment with 400 mg/kg of LJECB significantly lowered the VLDL to

87.36±0.00 mg/dl compared with the normal control. However, treatment with 800 mg/kg of LJECB significantly raised the VLDL to 330.88±0.01 mg/dl.

Administration of 400 and 800 mg/kg of LJECB to normal rats significantly lowered the VLDL to 156.16±0.05 and 142.44±0.00 mg/dl, respectively compared with the normal control (272.4±0.03 mg/dl).

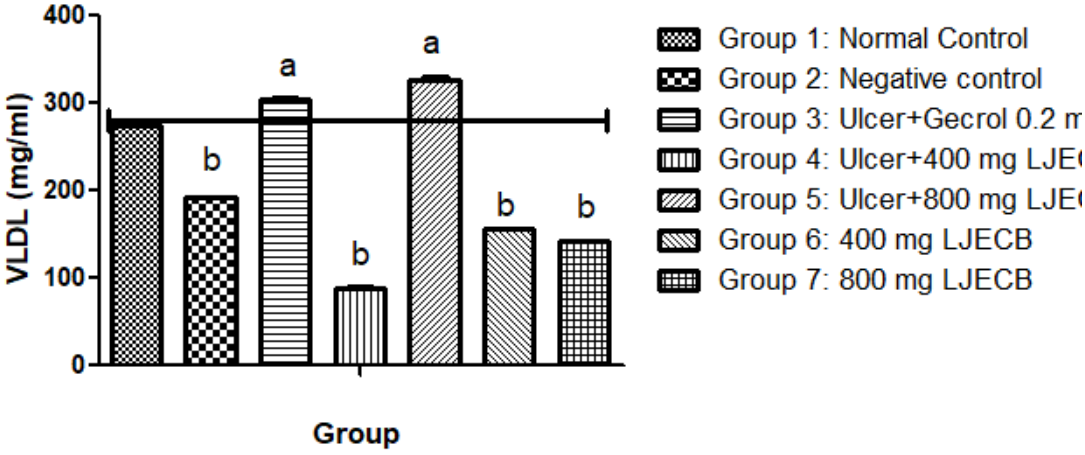

Figure 4: Effect of lime juice extract of cashew bark on VLDL-cholesterol in ulcer-induced Wistar rats. Group 1: Control (Distilled water 1 ml/100g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract; Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg extract; Group 7: 800 mg/kg extract. (b): Values significantly lower than normal control (Group 1). (a): Values significantly higher than normal control (Group 1). Significantly lower ($p<0.0001$) than negative control (Group 2). (#): Significantly higher than negative control (Group 2).

CARDIAC PARAMETERS

Atherogenic Coefficient (AC)

The results of AC are presented in Figure 5. The atherogenic coefficient was generally low for the normal control, negative control, and the groups administered with 400 and 800 mg/kg of LJECB, suggesting a low risk of developing atherosclerosis. However, treatment with Gecrol antacid and 800 mg/kg of LJECB resulted in higher AC, which may suggest an increased risk of atherosclerosis.

Atherogenic Index of Serum

The results indicate that the normal group had a higher atherogenic index compared to the negative control group (Figure 5), suggesting a potentially increased risk of atherosclerosis in the normal control group. Treatment with Gecrol and 800 mg/kg of LJECB showed a higher atherogenic index. Treatment of the gastric ulceration with 400 mg/kg of LJECB was associated with a significant reduction in the atherogenic index comparable with the extract control group that received the same dose of the extract.

Castelli Risk Index-1 (CRI-I)

The results showed that the normal control group had a higher CRI-I compared with the negative control group. The normal control groups administered with 400 and 800 mg/kg of LJECB, showed a relatively lower CRI-1 value, suggesting a potentially reduced risk. However, treatment with Gecrol leads to a significantly higher Castelli risk index-1, indicating an increased risk of cardiovascular disease.

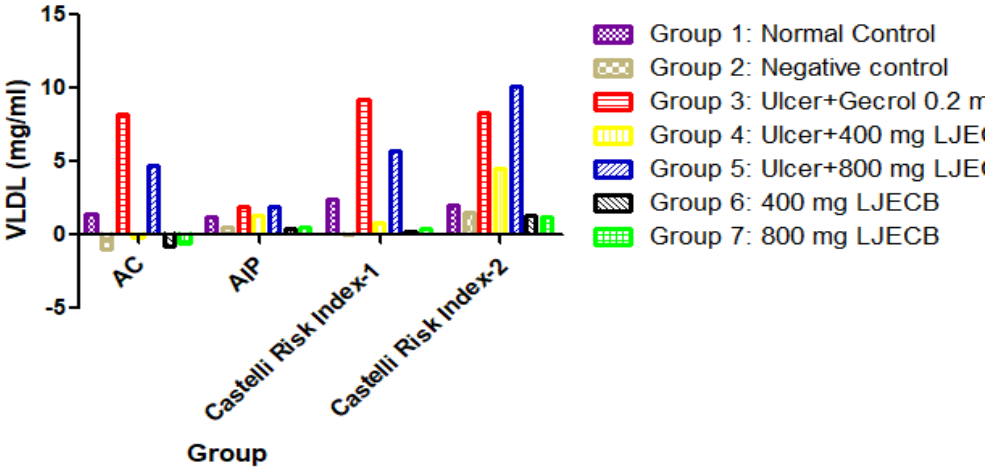

Figure 5: Effect of the lime juice extract of cashew bark on the cardiac parameters on ulcer-induced and control groups of Wistar rats.

Group 1: Control (Distilled water 1 ml/100g body weight); Group 2: 30 mg/kg indomethacin Only; Group 3: 30 mg/kg indomethacin + 0.2 ml/kg Antacid; Group 4: 30 mg/kg indomethacin + 400 mg/kg extract; Group 5: 30 mg/kg indomethacin + 800 mg/kg extract. Group 6: 400 mg/kg extract; Group 7: 800 mg/kg extract. Group 3 treated with Gecrol had an increased risk of developing cardiovascular disease.

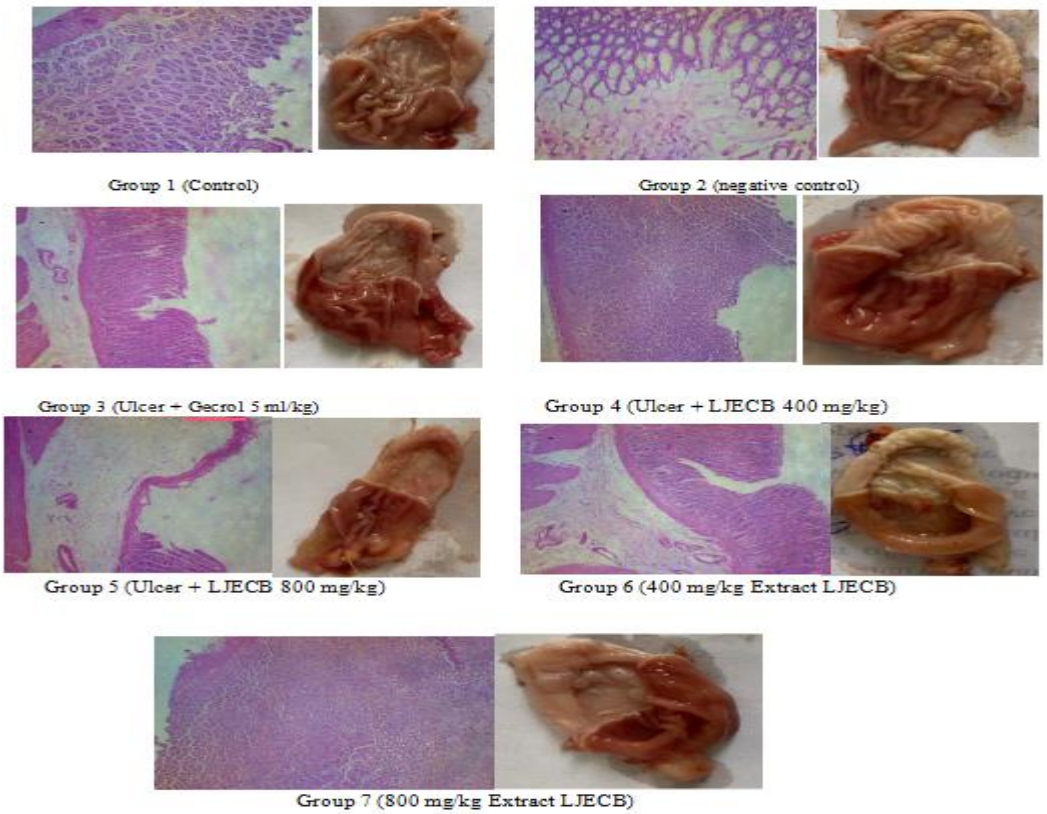

Plate 1: Photomicrographs of stomach tissues excised from ulcerative Wistar rats administered with lime juice extract of cashew bark.

DISCUSSION

Gastric ulcers induced by non-steroidal anti-inflammatory drugs (NSAIDs) consumption is a major public health concern [14]. The NSAIDs are effective in the treatment of inflammatory conditions through the inhibition of cyclooxygenases (COXs). Unfortunately, the same mechanism of action of NSAIDs on COX is responsible for the ulcerogenic potential of NSAIDs in the stomach. Indomethacin elicits its ulcerogenic effect via the inhibition of PGE_2 synthesis by COX. Hence, indomethacin is employed in experimental protocols to induce gastric ulcer [30, 23]. PGE_2 plays a protective role in the gastric mucosa by stimulating the gastric epithelial cells to synthesize mucus to enhance mucosal resistance to gastric insult [30]. PGE_2 also promotes blood flow in the gastric tissues [31].

Many of the anti-ulcer therapies, including antacids and proton pump inhibitor drugs, produce adverse effects in the patients [30-31]. Gastric ulcers are often associated with increased production of gastric acid which damages the gastric mucosal walls [28]. Alterations in lipid levels have been implicated in ulcer development [14].

In this study however, the group induced with 30 mg/kg of indomethacin (negative control) showed a significantly lower cholesterol level (20.58± 0.07 mg/dl) than the normal control group (189.00±0.04 mg/dl). Indicating that indomethacin possibly had a cholesterol-lowering property as well as ulcerogenic property through the inhibition of PGE_2 (a lipid from eicosanoid family) [30]. Indomethacin is well-known for its ulcerogenic properties due to PGE2 inhibition. However, its cholesterol-lowering effect appears to be intriguing. We therefore suggest a potential influence of indomethacin in the production or activity of anti-inflammatory lipid mediators such as lipoxins, resolvins, or protectins. Further investigation into the specific mechanisms underlying these interactions is warranted.

Over the years, studies on dyslipidemia have been geared towards finding agents capable of lowering the blood lipid levels and promoting cardiovascular health [32]. The prevalent form of lipid disorder in the human body is hypercholesterolemia [33]; with low-density lipoprotein (LDL) cholesterol as the predominant carrier of cholesterol particles which are deposited in blood vessel walls [34], leading to the buildup of plaque in arteries [34]. High LDL cholesterol increases the risk of heart disease. HDL (High-Density Lipoprotein) helps remove excess cholesterol from the arteries [32]. This study has shown that, indomethacin had anti-lipidemic property by lowering the blood cholesterol levels.

A similar study that evaluated the lipid profile levels in patients with *H. pylori* (ulcer causative agent) and normal healthy individuals was compared [35]. Their result showed a significant increase in the serum levels of total cholesterol, triglycerides, and LDL cholesterol in patients with *H. pylori* [35]. Suggesting that pathogen-associated mechanism induced lipid synthesis. The amount of cholesterol in cancer cells was also found to be higher than in normal cells, suggesting the pro-

cancer activity of cholesterol [36]. Patients with peptic ulcer disease were found to have lower cholesterol levels than healthy individuals. These findings show that the impact of ulcer on lipid profile is dependent on specific etiology. The *H. pylori* pathogen was found to raise the serum lipid levels [37-38], while in this study, indomethacin was associated with decreased cholesterol levels.

Furthermore, treatment of the indomethacin-induced gastric ulcer with commercial Gecrol antacid, was associated with increased cholesterol levels. Sperber *et al*. [39] reported a reduction in both LDL and HDL levels in hypercholesterolemic individuals treated with an aluminum hydroxide-containing antacid for two months. The effect of antacids on cholesterol levels may vary depending on the specific antacid used and their mechanisms involved [38]. It is possible that the antacid treatment may have influenced cholesterol metabolism indirectly through effects on gastric acid secretion. Further investigation is needed to understand the precise mechanisms and implications of antacids on cholesterol levels. Treatment of the gastric ulcer in this study with 400 and 800 mg/kg of LJECB resulted in a significant decrease in cholesterol levels. This effect on cholesterol level may have been elicited by influencing cholesterol metabolism around the gastric mucosa.

Furthermore, the negative control group (indomethacin-induced only) had a significant decrease in triglyceride levels compared with the normal control (group 1). Treatment with 400 mg/kg of LJECB was associated with a significant reduction in serum triglyceride levels compared with the negative control. However, treatment with 800 mg/kg of LJECB or Gecrol antacid was associated with a significant increase in serum triglyceride levels. This indicates that LJECB had a dose-dependent function on triglyceride metabolism in ulcerated rats. Conversely, normal healthy rats administered with 400 and 800 mg/kg of LJECB showed a significant reduction in triglyceride levels compared with the normal control group. This shows that 400 mg/kg of LJECB had a triglyceride-lowering property in both ulcerative and non-ulcerative conditions. Oral administration of indomethacin (1 mg/kg) for 3 days was found to significantly raised the serum cholesterol and triglycerides levels [39]. However, a higher dose (10 mg/kg) of indomethacin for the same 3 days produced a significant increase in non-esterified fatty acids and triglycerides with an overall reduction in total lipid levels [39-41].

Also, the HDL concentration in the negative control group (indomethacin only) was significantly higher than the normal control group. Our finding in this study corroborated Dhawan *et al*. [42] who had reported an increase in serum HDL in monkeys, separately fed with stock diet and atherogenic diet and then treated with 2.5 mg/kg of indomethacin on alternate days for 6 months. Raised blood levels of HDL (often referred to as 'good cholesterol') are associated with lower risk of cardiovascular disease [43]. HDL helps in the removal of excess LDL cholesterol from the arteries [43] thereby, preventing the accumulation of toxic hydroperoxides (LOOH) [44]. High-density lipoprotein (HDL) plays a critical role in protecting the protein and

lipid components of LDL from free radical damage. This prevents the formation of both initial and more complex oxidation products associated with cardiovascular disease [45]. Therefore, this study has found a positive correlation between indomethacin consumption (at 30 mg/kg) and a raised blood level of HDL. Morakinyo *et al*. [46] reported a significant reduction in HDL level in the normal rat control group when compared with the negative control group induced with isoproterenol (85 mg/kg). Dhawan *et al*. [42] reported an increase in serum HDL in indomethacin-treated monkeys.

The HDL cholesterol level was found to decrease significantly upon treatment of the ulcer group with Gecrol. This suggests that Gecrol antacid had a HDL-lowering property, which might increase cardiovascular risk. There is dearth of scientific literature on the HDL-lowering effect of Gecrol. Therefore, based on the findings of this study, prolonged use of Gecrol antacid in the control of ulcer flares may be associated with serious dyslipidemia and increased cardiovascular risk. Both 400 and 800 mg/kg of LJECB raised the HDL levels in healthy rats, but significantly lowered the blood HDL level in ulcer groups.

The VLDL levels in normal control was significantly higher than the negative group, which suggests that indomethacin lowered the blood VLDL level. The gecrol antacid elevated the blood VLDL compared with the negative. Treatment with 400 mg/kg of LJECB significantly lowered the VLDL, while treatment with 800 mg/kg of LJECB significantly raised the VLDL. However, administration of 400 and 800 mg/kg of LJECB to normal rats significantly lowered the VLDL compared with the normal control. VLDL carries more triglycerides synthesised by the liver and intestinal tissues to the adipose and muscle tissues [45-46]. In these tissues, the triglycerides are hydrolysed to fatty acids by lipases for ATP production. In contrast, LDL carries more cholesterol.

The Castelli risk index-1 CRI-1 is the ratio of total cholesterol to HDL [47-48]. CRI is an indicator for assessing cardiovascular risk. High CRI-1 value suggests an increased risk of cardiovascular disease, while a low value indicates a potentially reduced risk. The ulcer group and LJECB-treated groups were found to have a relatively lower CRI-1 values, which suggests a low risk of cardiovascular disease. This observation implies that the indomethacin-induced ulcer condition and/or subsequent treatment with LJECB impacted lipid profile differently (reduction in TC, TG, and increase in HDL level) so that on the overall, no risk of cardiovascular complications was observed. The observed effect (i.e., cardiovascular risk lowering effect) of the extract could be due to the presence of certain phyto-constituents which were able to interact synergistically to lower the atherosclerotic plaques along the arterial vessels. However, Gecrol raised the CRI-1 value, indicating a possible risk of cardiovascular disease. Hence, caution should be applied when using such antacid for control of ulcer flares.

Conclusion

The study concludes that oral administration of 30 mg/kg indomethacin induced gastric

ulceration in Wistar rats. Additionally, the gastric ulceration was associated with lower levels of total cholesterol (TC) and triglycerides (TG), higher levels of high-density lipoprotein (HDL), and improved cardiovascular health as indicated by reduced coronary risk index (CRI-1) scores. Treatment with LJECB caused a significant ($p<0.0001$) decrease in TC and TC levels, an increase in HDL level, and a reduced cardiovascular risk as compared with the normal control or positive (Gecrol) control groups. The study observed that the lipid parameters were significantly reduced in the ulcer groups treated with the extract (LJECB) when compared with the normal control groups that were only administered with the plant extract.

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Conflict of Interest

The authors declare no conflict of interest or personal gain.

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