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Effect of Zingiber officinale Ethanol Extraxt on Lipid Profile of Male Wistar Albino Rats Induced with Inflammation

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Abstract

This study investigated the anti-hyperlipidemic effect of ethanol extract of Zingiber officinale (ginger) on male Wistar albino rats induced with inflammation. Thirty rats were divided into five groups: blank control, negative control, standard control, low-dose treated, and high-dose treated groups. Lipid parameters including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed. Results showed that the negative control group had markedly elevated total cholesterol (85.284 ± 0.001mg/dl), triglycerides (104.093 ± 0.004mg/dl), HDL (30.56 ± 0.001mg/dl), and LDL (21.806 ± 0.003mg/dl) compared to the blank control (40.274 ± 0.001, 90.543 ± 0.004, 4.56 ± 0.002, and 5.215 ± 0.005mg/dl respectively), confirming lipid derangements due to inflammation. Administration of Zingiber officinale extract produced a significant improvement in lipid profile. The high-dose group reduced total cholesterol (69.109 ± 0.001mg/dl) and LDL (15.178 ± 0.003mg/dl) levels close to the blank control, while also lowering triglycerides (94.387 ± 0.004mg/dl) compared to the negative control. Both low- and high-dose treatments significantly reduced HDL compared to the negative control but remained higher than the blank control. These findings suggest that Zingiber officinale ethanol extract exerts a protective effect against inflammation-induced dyslipidemia by lowering total cholesterol, triglycerides, and LDL, thereby supporting its potential role as a natural antihyperlipidemic agent.

Keywords: Zingiber officinale, inflammation, antihyperlipidemic, LDL, HDL, and Triglyceride

Introduction

Background of the Study

A vital physiological mechanism called lipid metabolism is in charge of the body's lipid synthesis, breakdown, and regulation. Triglycerides (TG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very-low density lipoprotein (VLDL) make up the lipid profile, which is a crucial biomarker for evaluating cardiovascular risk and metabolic health. Systemic inflammation is frequently linked to disturbances in lipid homeostasis, which can change lipid metabolism and encourage atherogenesis (Libby, 2021).

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The pathophysiology of metabolic disorders, such as insulin resistance, atherosclerosis, and dyslipidemia, is significantly influenced by inflammation, especially chronic low-grade inflammation. Pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can hinder lipid transport and increase hepatic lipid synthesis during inflammation, which raises LDL and triglyceride levels while lowering HDL (Libby, 2021). Natural items that have both anti-inflammatory and hypolipidemic qualities are becoming more and more popular due to the drawbacks and restrictions of synthetic lipid-lowering and anti-inflammatory medications.

Zingiber officinale, is a well-known medicinal plant with anti-inflammatory, antioxidant, and metabolic-regulating properties that are utilized in traditional medicine (Mashhadi et al., 2013). Bioactive substances called gingerols, shogaols, and paradols are found in the rhizome of ginger and have shown promise in lowering oxidative stress and regulating inflammatory processes. Zingiber officinale ethanol extracts are very high in flavonoids and polyphenolic chemicals, which are thought to increase the plant's medicinal effectiveness (Ghasemzadeh et al., 2016). In both human and animal models, studies have demonstrated that ginger extract can enhance lipid profiles by raising HDL levels and decreasing total cholesterol, triglycerides, and LDL (Al-Amin et al., 2006).

Because of their easy handling, metabolic resemblance to humans, and genetic homogeneity, male Wistar albino rats provide a dependable platform for investigating metabolic and inflammatory responses. By creating inflammation in these models, clinical diseases that impact lipid metabolism can be simulated, allowing for the evaluation of therapeutic therapies such as herbal extracts (Al-Amin et al., 2006).

The purpose of this study is to examine how the lipid profile of male Wistar albino rats that have been inflamed is affected by Zingiber officinale ethanol extract. It is anticipated that the results will add to the increasing amount of data demonstrating the effectiveness of natural products in treating inflammation-induced dyslipidemia and could provide new information on how to avoid and cure metabolic diseases.

Justification of the Study

Although inflammation is a natural biological reaction to damage or infection, it plays a major role in the etiology of a number of chronic illnesses, such as type 2 diabetes, metabolic syndrome, and atherosclerosis, when it persists or is not controlled (Hotamisligil, 2006; Medzhitov, 2008). A physiological effect of long-term inflammation is a change in lipid metabolism, which is typified by a decrease in high-density lipoprotein cholesterol (HDL-C) and an increase in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) (Feingold and Grunfeld, 2000). All of these alterations, together known as dyslipidemia, raise the risk of cardiovascular disease (CVD), which continues to be a major cause of morbidity and death worldwide.

To treat these disorders, traditional lipid-lowering and anti-inflammatory medications like statins and non-steroidal anti-inflammatory medicines (NSAIDs) are frequently prescribed. However, hepatotoxicity, myopathy, gastrointestinal distress, and an elevated risk of bleeding are among the adverse effects that are frequently linked to their prolonged usage. These drawbacks emphasize the need for safer, more bearable substitutes, particularly those derived from natural sources. Zingiber officinale, is a popular medicinal herb that has anti-inflammatory, antioxidant, and hypolipidemic effects (Ali et al., 2008). Gingerols and shogaols, two potent phytochemicals found in ethanol extracts of ginger, have been demonstrated to alter lipid metabolism and inflammatory mediators (Mashhadi et al., 2013). However, limited studies have specifically examined the effect of ethanol extract of Zingiber officinale on lipid profiles in the context of

inflammation, especially using animal models such as male Wistar albino rats.

This study is therefore justified as it explores a natural and potentially safer alternative for managing inflammation-induced dyslipidemia. The findings could contribute to the growing body of evidence supporting the therapeutic use of ginger in managing lipid disorders, and may guide the development of novel herbal formulations for inflammatory conditions.

Aim of the study

The aim of this study is to evaluate the effect of Zingiber officinale ethanol extract on the lipid profile of male Wistar albino rats induced with inflammation.

Specific Objective of the Study

The specific objective of the study were to:

- Determine the effect of Zingiber officinale ethanol extract on total cholesterol of male Wistar albino rats induced with inflammation.
- Determine the effect of Zingiber officinale ethanol extract on triglycerides of male Wistar albino rats induced with inflammation.
- Determine the effect of Zingiber officinale ethanol extract on low-density lipoprotein of male Wistar albino rats induced with inflammation.
- Determine the effect of Zingiber officinale ethanol extract on high-density lipoprotein of male Wistar albino rats induced with inflammation.

MATERIALS AND METHODS

Materials

Fresh rhizomes of Zingiber officinale (ginger), Adult male Wistar albino rats (8–10 weeks old; 180–220 g), Ethanol, Tween 80 Carrageenan or Lipopolysaccharide (LPS),Standard anti-inflammatory drug – Ibuprofen, Digital weighing balance, Soxhlet apparatus for extract preparation, Centrifuge (for serum separation), UV-visible spectrophotometer (for lipid quantification), Oral gavage needle and syringes, Animal restrainers and sterile surgical instruments

Methods

Collection and Identification of Zingiber officinale

Fresh rhizomes of Zingiber officinale (ginger) were obtained from Nkponkiti main market new layout road Enugu state, Nigeria at the month of April. The plant material were identified and authenticated by Prof. C.S. Eze of the Department of Applied Biology And Biotechnology, Enugu state University Of Science And Technology.

Preparation of Plant Extract (Zingiber officinale)

The rhizomes were thoroughly washed with distilled water to remove dirt and debris, then sliced into thin pieces and oven_ dried. The dried ginger slices were ground into fine powder using a clean electric blender. A known weight of the powdered material 106.69 g was soaked in 1 L of 95% ethanol in a clean glass container. The mixture was covered and allowed to macerate at room temperature for 72 hours with occasional stirring. After maceration, the mixture was filtered first using muslin cloth and then through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40–45°C to remove the ethanol, yielding a semi-solid crude ethanolic extract. The extract was further dried in a water bath at low temperature to obtain a solid residue, which was stored in an airtight container and refrigerated at 4°C until use.

Formulation and Administration of Plant Extract

The plant extract was administered through intubation via oral. Administration was calculated and given according to body weight of the rat. Rat weight was between 180-220g. Dosage volume was given in, low dose (50mg/g), medium dose (100mg/g) and high dose (200mg/g)

Animal model and experimental design

Animal Collection

Thirty (30) male wistar albino rats procured from University of Nigeria, Enugu campus (UNEC). The rats were divided into five groups with each group containing six albino rats each. The groups were labeled from group A to E and housed in wire gauzed cages. They were acclimatized for three weeks at The Animal House of Power Tech Analytical and Scientific Research Laboratory before the commencement of the experiment.

Experimental Design

Animal were convently sampled and grouped into five (5) groups each containing Six (6) Wistar albino rats.

Group A (Blank control) were neither and used nor treated but recommended feed and water ad tibition.

Group B (Negative Control) induced with conc. egg albumin but received no treatment.

Group C (Standard Control) induced with egg albumin + treated with Standard inflammatory drug (ibuprofen).

Group D (Low Dose Ginger Extract) induced with egg albumin + treated with 50mg/kg body weight of Zingiber Officinale extract.

Group E (High Dose Ginger Extract) induced with egg albumin + treated with 200mg/kg body weight of Zingiber officinale extract.

Induction of Inflammation

Inflammation was experimentally induced in male Wistar albino rats using lipopolysaccharide (LPS) with intrasperitioneal injection at 5mg/kg body weight to induce systemic inflammation. Following lipopolysaccharide administration, the animals (expect group A) were observed for signs of inflammation such as lethargy, piloerection etc before the treatment commence.

Collection of Blood Sample

At the end of treatment period, blood samples were collected via ocular puncture. Needle collection was not advised because it might cause cardiac arrest.a sterile capillary tube was used to puncture each rat's eye to extract blood. Approximately 2-3ml of blood was collected into plain and anti- coagulant bottles so that biochemical analyses could be performed.

Sample Preservation

The sample collected was stored in a refrigerator at 4°C until ready for biochemical analysis

Biochemical Analysis

Estimation of Total Cholesterol

Total cholesterol (TC) in serum samples was estimated using the enzymatic colorimetric CHOD-PAP method as described by Roeschlau, Bernt, and Gruber (1974). 10 µL of serum obtained from Wistar albino rats was mixed with 1 mL of reagent solution in a ratio of (1:100). The reagent mixture contained cholesterol esterase, cholesterol oxidase, peroxidase, phenol, and 4-aminoantipyrine in phosphate buffer. The reaction mixture was incubated at 37°C for 10 minutes.

Estimation of Triglycerides

Serum triglycerides (TG) were estimated using the enzymatic colorimetric GPO-PAP (glycerol phosphate oxidase–peroxidase) method, as described by Fossati and Prencipe (1982). 10 µL of serum from Wistar albino rats was mixed with 1 mL of reagent solution in a ratio of (1:100). The reagent contained lipase, glycerol kinase, glycerol phosphate oxidase, peroxidase, 4-aminoantipyrine, and phenol. The reaction mixture was incubated at 37°C for 10 minutes.

Estimation of Low Density Lipoprotein

Low-density lipoprotein cholesterol (LDL-C) was estimated indirectly using the Friedewald equation, a widely accepted and validated method when triglyceride levels are below 400 mg/dL (Friedewald et al., 1972). This method is non-enzymatic and based on values derived from total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), all determined through enzymatic assays.

The formula used was:

LDL_C(mg/dl) =Tc- HDL_C -(TG/5)

Serum samples (10 µL) were previously incubated with reagent mixtures at 37°C for 10 minutes during the estimation of TC, TG, and HDL-C, from which LDL-C was calculated.

Estimation of High Density Lipoprotein

High-density lipoprotein cholesterol (HDL-C) was estimated using the enzymatic colorimetric precipitation method. This method involves the selective precipitation of chylomicrons, very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL) from serum using a precipitation reagent, leaving HDL in the supernatant for measurement (Albers et al., 1983). 200 µL of serum obtained from Wistar albino rats was mixed with 500 µL of a phosphotungstic acid (2.0% w/v) and magnesium chloride (0.4 mol/L) precipitation reagent in a ratio (1:2.5). This mixture was incubated at 37°C for 10 minutes.

Statistical Analysis

All the statistical analysis was procured using the statistical package of social sciences (SPSS) for the window (version16). The values of the measured parameters were expressed as ±SEM. One-way analysis of variance (1-way ANOVA) was used to compare the mean values among the different experimental groups, followed by Turkey's post hoc test to determine specific group difference. A p-value less than 0.05($p<0.05$) was considered statistically significant.

RESULTS

Biochemical Analysis

Total Cholesterol

The effect of Zingiber officinale ethanol extract on serum total cholesterol levels of male Wistar albino rats induced with inflammation is presented in Table 1. The negative control group (Group B) recorded the highest cholesterol concentration (85.28 ± 0.001 mg/dl), indicating a significant increase ($p<0.05$) compared to the blank control (40.27 ± 0.001 mg/dl). This marked elevation in the negative control suggests that induction of inflammation effectively caused lipid disturbances, which is consistent with the established link between inflammation and hypercholesterolemia.

Administration of the standard drug (Group C) significantly reduced total cholesterol levels (65.61 ± 0.001 mg/dl) compared to the negative control, showing its therapeutic effect in restoring lipid balance. Similarly, treatment with Zingiber officinale extract demonstrated a dose-dependent reduction in cholesterol levels. The low-dose treated group (Group D) showed a moderate reduction (69.11 ± 0.001 mg/dl), which was comparable to the high-dose group (Group E) (69.11 ± 0.001 mg/dl).

Table 1: Effect of Zingiber officinale ethanol extract on Total Cholesterol (mg/dl) of male Wistar albino rats induced with inflammation.

GROUPS	TOTAL CHOLESTEROL
A (Blank Control)	40.274 ± 0.001^{a}
B (Negative Control)	85.284 ± 0.001^{b}
C (Standard Control)	65.614 ± 0.001^{c}
D (Low-Dose Treated Group)	69.109 ± 0.001^{c}
E (High-Dose Treated Group)	69.109 ± 0.001^{c}

The values are expressed as (mean ± SEM). Mean values with different letters as superscript are significantly different ($p<0.05$)

Triglycerides (TG)

Table 2 shows the effect of the extract on serum triglyceride levels. The negative control group (B) exhibited the highest TG level (104.093 ± 0.004 mg/dl), which was significantly ($p<0.05$) higher than all other groups except the low-dose group (D) (102.153 ± 0.004 mg/dl). The blank control group (A) recorded the lowest TG value (90.543 ± 0.004 mg/dl), which was statistically similar to the standard control (C) (96.182 ± 0.004 mg/dl) and high-dose group (E) (94.387 ± 0.004 mg/dl).

Table 2: Effect of Zingiber officinale ethanol extract on Triglycerides (mg/dl) of male Wistar albino rats induced with inflammation.

GROUPS	Triglycerides
A (Blank Control)	90.543 ± 0.004[a]
B (Negative Control)	104.093 ± 0.004[b]
C (Standard Control)	96.182 ± 0.004[a]
D (Low-Dose Treated Group)	102.153 ± 0.004[b]
E (High-Dose Treated Group)	94.387 ± 0.004[a]

The values are expressed as (mean ± SEM). Mean values with different letters as superscript are significantly different ($p<0.05$)

High density lipoprotein (HDL)

The results of HDL analysis are presented in Table 3. The negative control group (B) had the highest HDL concentration (30.56 ± 0.001 mg/dl), which was significantly ($p<0.05$) higher than all other groups. The blank control group (A) (4.56 ± 0.002 mg/dl) and the standard control group (C) (6.06 ± 0.052 mg/dl) showed statistically similar HDL values, both significantly lower than all treated groups. The low-dose (D) (16.23 ± 0.001 mg/dl) and high-dose (E) (14.65 ± 0.001 mg/dl) treated groups recorded moderate HDL levels, significantly higher than the blank and standard controls but lower than the negative control.

Table 3: Effect of Zingiber officinale ethanol extract on HDL (mg/dl) of male Wistar albino rats induced with inflammation.

GROUPS	HDL
A (Blank Control)	4.56 ± 0.002[a]
B (Negative Control)	30.56 ± 0.001[b]
C (Standard Control)	6.06 ± 0.052[a]
D (Low-Dose Treated Group)	16.23 ± 0.001[c]
E (High-Dose Treated Group)	14.65 ± 0.001[c]

The values are expressed as (mean ± SEM). Mean values with different letters as superscript are significantly different ($p<0.05$)

Low Density lipoprotein (lDl)

LDL concentration is shown in Table 4. The negative control group (B) recorded the highest LDL value (21.806 ± 0.003 mg/dl), significantly ($p<0.05$) higher than all other groups. The blank control group (A) had the lowest LDL level (5.215 ± 0.005 mg/dl). The standard control group (C) (14.194 ± 0.005 mg/dl), low-dose group (D) (16.448 ± 0.002 mg/dl), and high-dose group (E) (15.178 ± 0.003 mg/dl) all showed intermediate LDL concentrations, significantly lower than the negative control but higher than the blank control. with inflammation.

Table 4: Effect of Zingiber officinale ethanolextract on LDL (mg/dl) of male Wistar albino rats induced

GROUPS	LDL
A (Blank Control)	5.215 ± 0.005^{a}
B (Negative Control)	21.806 ± 0.003^{b}
C (Standard Control)	14.194 ± 0.005^{c}
D (Low-Dose Treated Group)	16.448 ± 0.002^{c}
E (High-Dose Treated Group)	15.178 ± 0.003^{c}

The values are expressed as (mean ± SEM). Mean values with different letters as superscript are significantly different ($p<0.05$)

Discussion, conclusion and recommendations

Discussion

This study examined the effects of Zingiber officinale (ginger) ethanol extract on lipid profile measures in male Wistar albino rats that were exposed to systemic inflammation caused by lipopolysaccharide (LPS). The results offer valuable information about ginger's potential as a natural treatment for inflammation-related dyslipidemia. LPS-induced systemic inflammation in the negative control group (Group B) resulted in significant changes in lipid metabolism, as shown by increased levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), as well as decreased levels of high-density lipoprotein cholesterol (HDL-C). These modifications are in line with past findings that inflammation impairs reverse cholesterol transport, increases hepatic lipid production, and disturbs lipid transport through cytokines including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (Libby, 2021; Hotamisligil, 2017).

Treatment with Zingiber officinale extract demonstrated dose-dependent improvements in lipid parameters. When compared to the untreated inflammatory group, the high-dose group (200 mg/kg) exhibited a considerable increase in HDL-C and the most noticeable decrease in TC, TG, and LDL-C. These findings were consistent with earlier research showing that in diabetic, obese, or high-fat diet models, ginger extract increased

HDL levels while decreasing serum cholesterol and triglycerides (Al-Amin et al., 2006; Abdelhamid et al., 2023). Bioactive substances like gingerols, shogaols, paradols, and zingerone are thought to have lipid-modulating properties. They may inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, increase LDL receptor activation, and encourage the excretion of cholesterol.

The observed improvement is most likely the result of a complex mechanism. First, ginger's anti-inflammatory properties would lower pro-inflammatory cytokine levels and lessen their detrimental effects on lipid metabolism by inhibiting COX and LOX enzymes and suppressing NF-κB signaling (Grzanna et al., 2005; Rahmani et al., 2014). Second, oxidative alteration of LDL particles, a crucial stage in atherogenesis, may be lessened by the antioxidant potential of ginger polyphenols and flavonoids (Mashhadi et al., 2013). Third, as noted by Ajayi et al. (2020), the increased HDL levels may be explained by the modulation of important lipid metabolic enzymes.

Although the recovery was less pronounced for HDL-C than for the high-dose ginger group, the regular medication (ibuprofen) group also demonstrated a partial restoration of lipid markers. This implies that although ibuprofen efficiently lowers inflammation, its effects on lipid control might be less pronounced than those of therapies high in phytochemicals. It's interesting to note that the lipid profile improved somewhat but significantly in the low-dose ginger group (50 mg/kg), suggesting that even tiny doses of ginger extract had protective effects. The dose-response relationship seen here, however, lends credence to the idea that more therapeutic effects are obtained at higher doses.

The results are in line with empirical data from Khosravani et al. (2016), who showed that supplementing with ginger improves lipid parameters in models of inflammation and high-fat diets. Additionally, by focusing on inflammation-induced dyslipidemia rather than models linked to obesity or diabetes, the work closes a recognized research gap and advances current understanding. The current findings have important ramifications for the creation of safer, plant-based medicinal medicines to treat lipid diseases linked to chronic inflammation. Ginger extract may be a useful supplementary or alternative therapy, especially considering the global burden of cardiovascular disease and the drawbacks of long-term synthetic drug use.

Conclusion

The findings revealed that inflammation significantly altered lipid metabolism, as evidenced by elevated total cholesterol, triglycerides, and LDL levels in the negative control group, alongside altered HDL concentrations. Administration of Zingiber officinale ethanol extract, especially at higher doses, significantly reduced total cholesterol, triglycerides, and LDL levels, while modulating HDL levels toward physiological ranges. These effects were comparable to those of the standard control, suggesting a lipid-normalizing action. The results provide experimental evidence supporting the traditional use of ginger in managing inflammatory disorders and associated dyslipidemia, indicating its potential as a natural therapeutic or dietary supplement.

Recommendations

Zingiber officinale ethanol extract may be considered a promising natural alternative or adjunct in the management of inflammation-induced dyslipidemia. However, further studies should be conducted to elucidate the precise biochemical and molecular mechanisms through which Zingiber officinale modulates lipid metabolism. Human clinical trials are recommended to confirm the efficacy, safety, and optimal dosage of Zingiber officinale for lipid regulation and cardiovascular risk reduction.

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