



Effects of Citrus Limon Juice on Serum Bilirubin, High Density Lipoprotein and Low Density Lipoprotein in Adult Male Wistar Rats under Variable Models of Stress

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Authors' contributions

This work was carried out in collaboration among all authors. Author AKK designed the study and wrote the protocol. Authors DTME and EOL managed the animals and the field study. Authors AKK, EOL and OJC conducted laboratory analysis and collected all data. Authors DTME and EOL performed the statistical analysis and author AKK wrote the first draft of the manuscript. Authors AKK and DTME did the literature search and completed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: There has been sufficient awareness on atherosclerosis but this has not reduced the number of people with it. Atherosclerosis is promoted by low density lipoprotein (LDL) without sufficient removal of fats and cholesterol by high density lipoprotein (HDL). Stress is linked with hyperlipidemia, lowering HDL and increasing LDL levels. Citrus Limon is known for its rich minerals and flavonoid content which have been employed widely. This study is aimed at assessing the hypolipidemic effects of citrus Limon on Bilirubin, HDL and LDL levels.

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Place and Duration of Study: Department of physiology, Delta State University Abraka.
Methods: Experimental animals were divided into six groups. Group I were control group rats fed with feed *ad libitum*, Group II were negative control rats (stressed but not treated), Group III-V were rats stressed and treated with 0.5 ml/kg, 1 ml/kg and 2 ml/kg of Citrus Limon juice respectively, Group VI treated with 0.2 ml/kg of Vitamin C. The animals were sacrificed by cervical dislocation after 14 days and blood samples were collected for biochemical analysis. Data were analyzed using SPSS and expressed as Means±Standard Error of Mean with significance at $p<0.05$.
Results: Results showed that stress significantly ($p<0.05$) increased bilirubin and LDL and reduced HDL levels. Result also showed that Citrus limon administration significantly reduced serum bilirubin and LDL but increased HDL levels in experimental animals after 14 days.
Conclusion: This study concludes that stress raises serum bilirubin and LDL levels and reduces HDL levels thus a major risk factor towards development of atherosclerosis. Administration of Citrus limon juice however reduces serum bilirubin and LDL while increasing HDL levels.

Keywords: Stress; citrus limon; bilirubin; high density lipoprotein (HDL); low density lipoprotein (LDL); vitamin C; atherosclerosis.

1. INTRODUCTION

Stress is a threat to homeostasis [1] which affects different body systems [2] and causes several diseases in both humans and in experimental animals [3]. Many studies have reported the effect of stress on lipid metabolism [4]. Stress increases low-density lipoprotein (LDL) and decreased high density lipoprotein (HDL) level [5,6]. LDLs cause atherosclerosis [7, 8], but HDL reduces the risk of cardiovascular diseases [9-12]. Stress also increases bilirubin level [13].

LDLs are referred to as “bad Cholesterol” because they transport cholesterol into arterial walls, attract macrophages and thus drive atherosclerosis [7,8]. HDL are referred to as “good cholesterol” because they are involved in reverse cholesterol transport, thus reducing the risk of coronary artery diseases [9].

Bilirubin, a catabolic product of heme is often used to assess liver and blood disorders. Increase in formation or retention of bilirubin (hyperbilirubinemia) by the body may result in a disease condition called jaundice (icterus), which is characterized by the presence of yellowish pigmentation in the skin and sclera of the eyes [14,15].

Medicinal plants are gifts of nature and have been associated with a 10-40% reduction in the risk of cardiovascular diseases [16]. Lemon is a medicinal plant of the family *Rutaceae* and third most important of the citrus family following orange and mandarin, and it is used in different ways due to its numerous medicinal abilities [17]. The health benefits of lemon are due to its

various nourishing elements such as proteins, carbohydrates, vitamin C, vitamin B and phosphorous [18].

Citrus Lemon fruit juice possesses alkaloids, flavonoids, steroids, terpenoids, saponins, cardiac glycosides, and reducing sugars which has been shown to have broad spectrum of biological activities [19-23] and has significantly decreased LDL level [24], but raises HDL level in animal models [25] and decreased bilirubin levels in carbon tetrachloride (CCl_4)-intoxicated rats [26].

Some other plant extracts have also been found to possess antihyperlipidemic effects. Administration of 200 mg/kg/day of *moringa oleifera* fruit extracts significantly reduced serum blood cholesterol and LDL levels in rabbits fed with hypercholesterolaemic diet for 120 days [27]. *Moringa oleifera* leaf powder was also found to significantly lower blood total cholesterol, glycated haemoglobin, fasting blood glucose and low density lipoproteins in type 2 diabetes subjects [28]. Maruthappan and Sakthi, [29] reported that the aqueous extract of *Phyllanthus reticulatus* (250 mg and 500 mg/kg) produced significant reduction in triglyceride, VLDL-cholesterol, total cholesterol (TC), LDL-cholesterol and oxidative stress (protein carbonyl) and increased HDL-cholesterol in atherogenic diet-induced hypercholesterolemic rats.

Mahmoud et al. [30] also reported that administration of a hydroalcoholic extract of *Ferulago angulata* can reduce serum levels of total cholesterol, triglycerides, and LDL. Furthermore graded doses of zingiber officinale

showed potent antilipidemic effects after 2 and 4 weeks of treatment of hypercholesteromic rats [31].

The nutritional values of Citrus limon are enormous and has been implicated to have wide range of medicinal value. The tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, which has both culinary and cleaning uses. It's a common source of food ingredient in our diet. It has been shown to have antioxidant and antinociceptive effects [32]. This protective effects may be associated with the presence of bioactive components such vitamin C, beta-carotene, limonoids, folic acids and flavonoids. High intake of citrus limon is also known to reduce the risk of degenerative diseases [33]. Citrus limon showed the presence of phenols, reducing sugars, steroids, terpenes and tannins [34] and has strong antimicrobial action [35]. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [36].

Citrus limon tree is a small evergreen tree native to Asia that produces citrus limon fruit. It is a perennial tree of the citrus family- Rutaceae. It grows up to 3m. Lemon fruit is oval with a broad, low, apical nipple and 8 to 10 segments. The juice of the lemon is about 5% to 6% citric acid, which gives lemon a sour taste. The outer rind or peel is yellow when ripe and rather thick in some varieties, is prominently glandular-dotted. This study was undertaken to analyse the effects of citrus limon fruit juice in rats under variable models of stress. It will specifically determine effects of citrus limon juice on plasma bilirubin, high density lipoproteins (HDL) and low density lipoproteins (LDL) in stressed animals so as to evaluate whether the administration of citrus limon juice could be beneficial in the management of stress and hyperlipidemia.

2. MATERIALS AND METHODS

2.1 Animal House and Experimental Animals

The animals were kept for a two weeks period for acclimatization in a standard animal house of the faculty and were maintained in standard iron cages under standard laboratory and environmental conditions of light. They were fed with standard rat chow and water *ad libitum*. The animals were handled in compliance with the

guidelines approved by the institution's Ethics Committee.

The rats used in this study were adult male Wistar rats weighing between 150-200 g. The animals were bred and procured from the Laboratory Animal Centre of the Faculty of Basic Medical Sciences, University of Nigeria Nsukka, Nigeria.

2.2 Preparation of Plant

Fresh *Citrus limon* fruits was obtained in Abraka, Ethiope East Local Government Area of Delta State, Nigeria. The fresh *Citrus limon* fruits were washed with tap water, peeled and the juice squeezed out with manual juicer. The juice obtained was filtered with Whatmann filter paper.

2.3 Preparation of Drug

One hundred milligram (100 mg) of vitamin C tablets was used. Each tablet (100 mg) was dissolved in 10ml of distilled water. The mixture was centrifuged to obtain clear Vitamin C solution, which was then administered orally (0.2 ml/kg) for fourteen days.

2.4 Animal Grouping and Administration of Juice

Forty two (42) adult male wistar rats were divided into 6 groups (n=7). Administration of Citrus limon juice was done once daily (only in the morning) using orogastric cannula for a period of fourteen days. 0.5 ml/kg, 1ml/kg and 2ml/kg was administered to the rats.

- Group 1: Control
- Group 2: Negative control (stressed and untreated)
- Group 3: Stressed rats + low dose (0.5ml/kg)
- Group 4: Stressed rats + medium dose (1ml/kg)
- Group 5: Stressed rats + high dose (2ml/kg)
- Group 6: Stressed rats + 0.2ml/kg of Vitamin C

2.5 Stress Models

- i. Movement restriction (immobilization) model of stress was performed on the experimental rats. The rats were placed in plastic tubes (inner diameter, 5.7cm; length, 20.3 cm) that completely restrict lateral movement of the rats and markedly reduced their front-to-back movement. One end of the tube remained open to allow breathing. Once inside the tubes, the plastic tubes containing the rats were

placed in a horizontal orientation. The animals were exposed daily to three hours (4 hrs) of stress in the morning (between 9:00 am and 1:00 pm).

- ii. Water restriction stress was done by restricting the animals from water for 12 hours after which Citrus limon juice was administered.
- iii. Feed restriction stress was also done by restricting the animals from feed for 12 hours after which Citrus limon juice was administered.

2.6 Analysis of Sample

Total bilirubin, HDL and LDL was determined using the Prietest Auto analyzer, Easy Lab, Version 2.1 (Labtech Product, UK).

2.7 Sacrificing of Animals and Collection of Sample

Blood samples were collected on day 7 and 14 and the animals were sacrificed by cervical dislocation. Blood was collected by cardiac puncture using 2 ml syringes into plane containers and centrifuged to obtain serum for bilirubin, HDL and LDL analysis.

2.8 Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis were performed by one way analysis of

variance (ANOVA) using the SPSS software (version 21). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS

This result shows representation of the effects of Citrus limon juice on serum total bilirubin, HDL and LDL levels.

3.1 Effects of Citrus lemon Juice on Total Bilirubin Level of Stress Induced Wistar Rats

This study shows the effect of Citrus limon fruit juice on serum bilirubin. Stress causes significant ($p=.05$) increase in serum bilirubin level at day 7 and day 14 respectively. Citrus lemon juice reduced the serum bilirubin level in a dose dependent manner. 0.2ml/kg of Vitamin C caused similar effect to the rats in negative control by decreasing the serum bilirubin level. At day 14, Citrus lemon juice and Vitamin C also caused a significant ($p=.05$) decrease in serum bilirubin level when compared with negative control.

3.2 Effects of Citrus Limon Juice on High Density Lipoprotein (HDL) Level of Stress Induced Wistar Rats

This study shows the effect of Citrus limon juice on serum HDL level. Stress causes a significant ($p=.05$) decrease in serum HDL level at day 7

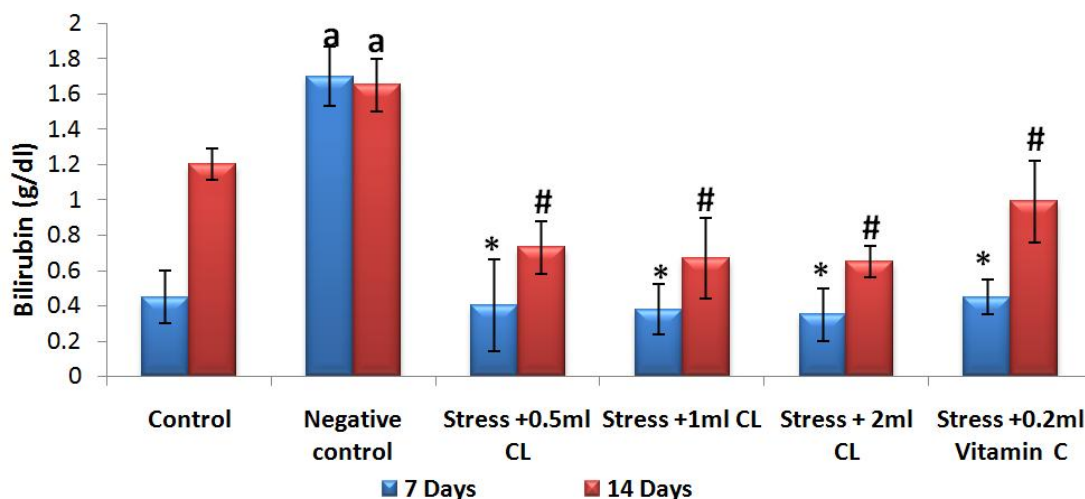


Fig 3.1. Effects of Citrus limon juice on total bilirubin level of stress induced Wistar rats

a $p=.05$ when compared to control at day 7 and 14
 * $p=.05$ when compared to negative control at day 7
 # $p=.05$ when compared to negative control at day 14

and 14 days respectively. At day 7, Citrus limon juice does not significantly increase HDL in experimental animals when compared with negative control rats. However, at day 14, Citrus limon juice (2 ml/kg) significantly ($p=.05$) increased HDL level when compared with negative control rats. At day 7 and 14 respectively, administration of 0.2 ml/kg of Vitamin C does not cause any significant change in the HDL level when compared with negative control rats.

3.3 Effects of Citrus Limon Juice on Low Density Lipoprotein (LDL) Level of Stress Induced Wistar Rats

This study shows the effect of Citrus limon on serum LDL level. Stress causes a significant ($p=.05$) increase in serum LDL level at day 7 and 14 respectively. At day 7, Citrus limon (1 ml/kg and 2 ml/kg) administration significantly ($p=.05$) decreased the serum LDL level in stressed rats. Also, administration of vitamin C has no significant effect in reducing serum LDL level at day 7. However, at day 14 of stress, Citrus limon (2 ml/kg) causes significant ($p=.05$) reduction in serum LDL level when compared with negative control. Vitamin C administration at day 14, also does not reduce the LDL level. Hence, Citrus limon (1 ml/kg, 2 ml/kg) shows a more potent effect in reducing serum LDL level than 0.2 ml/kg of vitamin C.

4. DISCUSSION

Plants are one of the most important sources of active substances with therapeutic potential to cure a variety of diseases in humans. The evaluation of pharmacological effects can be used as a strategy for discovering new drugs of plant origin [37]. In Nigeria, herbalists use Citrus lemon juice in the treatment of diarrhea, dysentery, typhoid fever, wound infections, urinary tract infection and arthritis [38]. The present work was designed to study the ameliorative effect of C. limon juice extract against stress effects on lipid profile parameters.

At the end of the experiment, there was significant increase in plasma bilirubin as a result of stress. However, upon administration of Citrus limon juice, there was a significant ($p<0.05$) decrease in serum bilirubin level in a dose dependent manner. This observation may be ascribed to the presence of certain phytochemicals of the Citrus limon juice, which was previously reported to contain polyphenols, terpenes and flavonoids [22,39]. This observation is also in agreement with the study reported by Shefalee [26]. Since Citrus limon juice has been reported to contain great deal of vitamin C an antioxidant, the consequent significant reduction in bilirubin following Citrus limon juice may be ascribed to the antioxidant

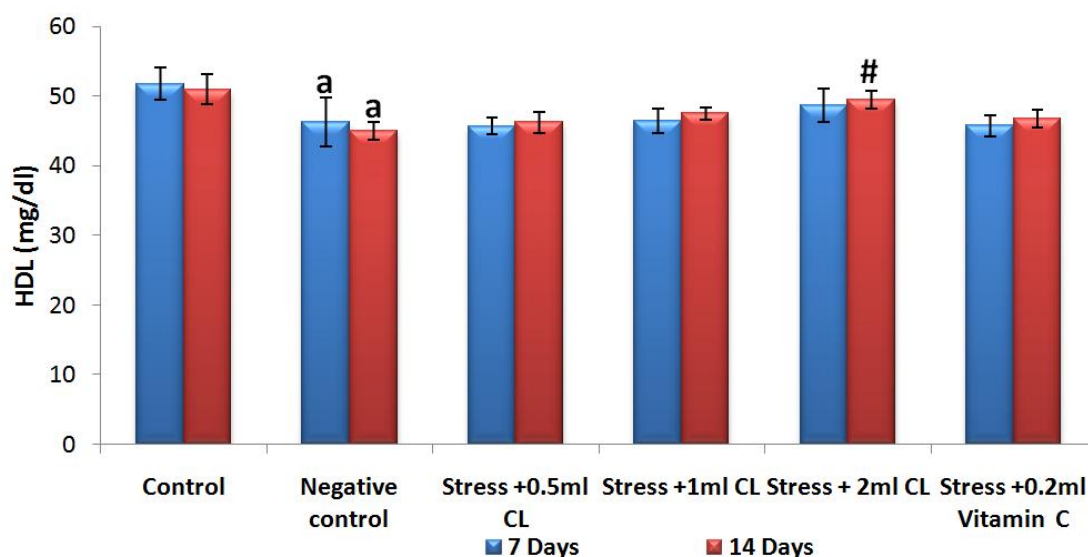


Fig. 3.2. Effects of Citrus limon juice on HDL of stress induced wistar rats

a $p=.05$ when compared to control at day 7 and 14

$p=.05$ when compared to negative control at day 14

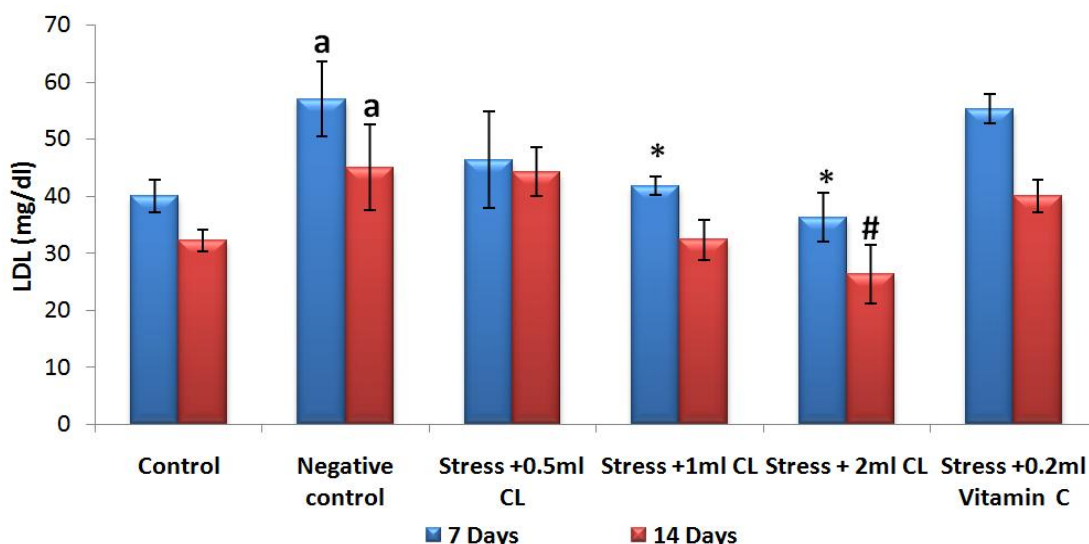


Fig. 3.3. Effects of Citrus limon juice on LDL of stress induced Wistar rats. a p=.05 when compared to control

* p=.05 when compared to negative control at day 7

p=.05 when compared to negative control at day 14.

effect exerted by this fruit due to the presence of this compound [40].

High density lipoprotein (HDL) is known for removing and transporting unused and excess cholesterol from the tissues back to the liver (reverse cholesterol transport) for catabolism and excretion; this therefore connotes that HDL is beneficial to health and hence commonly called “good cholesterol”. In the present study, stress decreases HDL level significantly, a result that corroborates previous studies [6]. HDL level in the present study was increased significantly in the experimental animals administered with Citrus limon juice (2 ml/kg) for 14 days. This shows that Citrus limon juice have HDL-boosting effect. This result is also consistent with the results reported by Kamal [25] that citrus peels powders raised HDL cholesterol level but reduces LDL level. Administration of vitamin C for 7 and 14 days respectively does not show similar effect as Citrus limon. However, Itoh [41] shows that vitamin C increases HDL level, a result contrary to findings in this study. The differences observed in this result may be due to the low dosage (0.2 ml/kg) used in this study.

Low density lipoproteins (LDLs) are also known as “bad cholesterol” as they transport cholesterol from the liver to tissues [9] and as such an important predictor of the likelihood of atherosclerotic conditions [12]. This study reports that stress increases serum LDL level at 7 and 14 days respectively. This result is in agreement

with one study by Neves [6] who also affirmed that LDL level increases during stress. Citrus limon juice (1 ml/kg, 2 ml/kg) significantly decreased the LDL level at 7 days. Vitamin C administration for 7 days has no significantly effect in reducing the level of LDL in serum when compared with stressed rats. However, administration of Citrus limon juice (2 ml/kg) for 14 days reduces LDL level. This result is consistent with those obtained by Olukanni [24] and Gorinstein [42] that citrus juice possesses hypolipidemic effect and capable of reducing serum LDL level. Also, Monforte [43] reported similar trend using citrus flavonoids.

The reduction in LDL levels may be due to its antioxidant effect as it has been suggested that antioxidant (vitamin C) administration in animal model ameliorates endothelial function of coronary and peripheral vessels [44]. At 14 day of 0.2ml/kg vitamin C administration, serum LDL level was not significantly reduced in stressed rats, a result contrary to that reported by Nerissa [45]. This action could be dose-dependent causing an inability to reduce LDL level raised by stress as observed in this study.

5. CONCLUSION

This study concludes that stress raises serum bilirubin and LDL levels and reduces HDL levels. Citrus limon juice administration however provided ameliorating effect by reducing bilirubin

and LDL while increasing HDL level. The similarity in some actions of Citrus limon and vitamin C shows that the activities of Citrus limon may be via antioxidant mechanism pathway. Hence Citrus limon can be beneficial in the management of stress or stress related disorders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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