

## **Original Research Article**

### **Effects of citrus limon juice on serum Bilirubin, High Density Lipoprotein and Low Density Lipoprotein on adult male wistar rats under variable models of stress**

#### **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 effects of citrus Limon on Bilirubin, HDL and LDL levels.

**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 0.5ml/kg, 1ml/kg and 2ml/kg of Citrus Limon juice respectively, Group VI treated with 0.2ml/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.

Key-words: Stress, *Citrus limon*, Bilirubin, High density lipoprotein (HDL), Low density lipoprotein (LDL), vitamin C, atherosclerosis.

## 1.0 Introduction

Stress is a threat to homeostasis<sup>[1]</sup> which affects different body systems<sup>[2]</sup> and causes several diseases in both humans and experimental animals.<sup>[3]</sup> Many studies have reported the effect of stress on lipid metabolism.<sup>[4]</sup> Stress increases low-density lipoprotein (LDL) and decreased high density lipoprotein (HDL) level<sup>[5, 6]</sup> LDLs cause atherosclerosis<sup>[7, 8]</sup>, but HDL reduces the risk of cardiovascular diseases.<sup>[9-12]</sup> Stress also increases bilirubin level.<sup>[13]</sup> 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<sup>[14-18]</sup> and has significantly decreased LDL level<sup>[19]</sup>, but raises HDL level in animal models<sup>[20]</sup> and decreased bilirubin levels in carbon tetrachloride (CCl<sub>4</sub>)-intoxicated rats.<sup>[21]</sup>

## 2.0 Materials and Methods

### 2.1 Experimental Animals

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

## 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 (100mg) of vitamin C tablets was used. Each tablet (100mg) was dissolved in 10ml of distilled water. The mixture was centrifuged to obtain clear Vitamin C solution, which was then administered orally (0.2ml/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. 0.5ml/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

## Stress models

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 (4hrs) of stress in the morning (between 9:00am and 1:00pm).

Water restriction stress was done by restricting the animals from water for 12hours after which Citrus limon juice was administered.

Feed restriction stress was also done by restricting the animals from feed for 12hours 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 2ml 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  $\pm$  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.

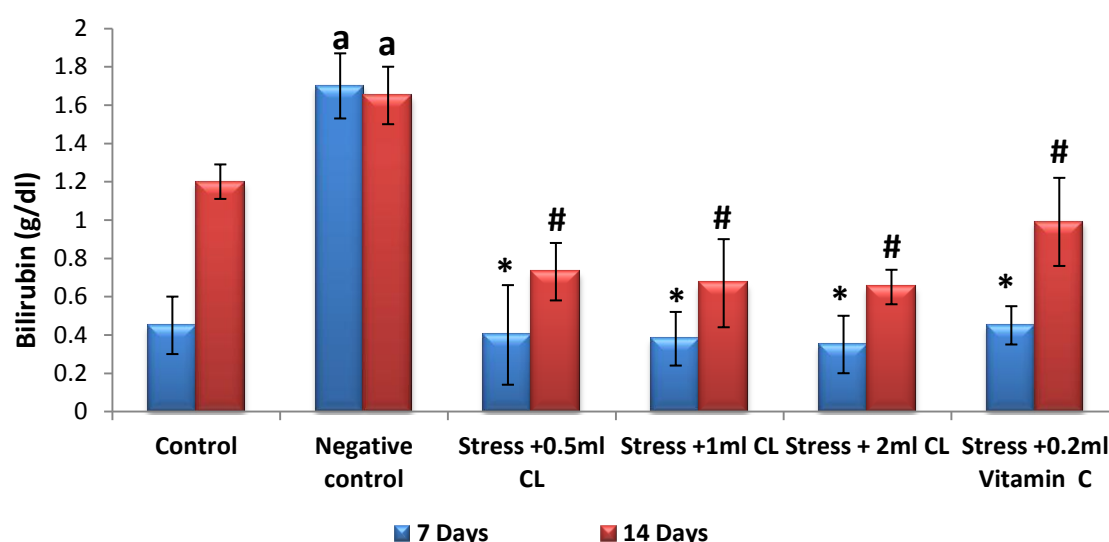
## **3.0 Results**

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.**

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

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

110 <sup>a</sup>  $p=.05$  when compared to control at day 7 and 14

111 <sup>\*</sup>  $p=.05$  when compared to negative control at day 7

112 <sup>#</sup>  $p=.05$  when compared to negative control at day 14

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

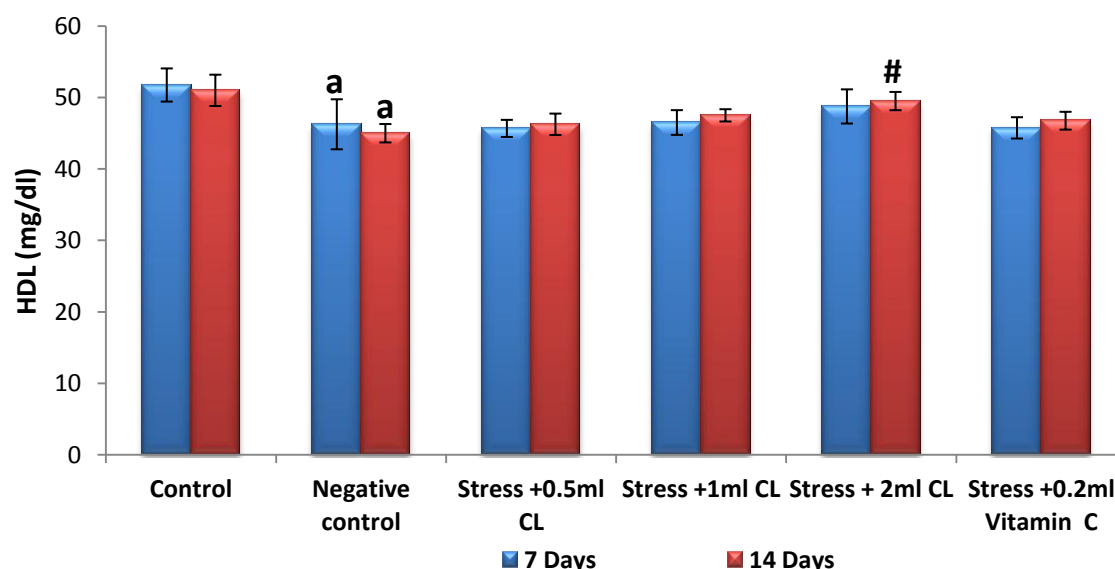


Fig. 3.2. Effects of Citrus limon juice on HDL of stress induced Wistar rats.

<sup>a</sup>  $p=.05$  when compared to control at day 7 and 14

<sup>#</sup>  $p=.05$  when compared to negative control at day 14

**Fig 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 (1ml/kg and 2ml/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 (2ml/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 (1ml/kg, 2ml/kg) shows a more potent effect in reducing serum LDL level than 0.2ml/kg of vitamin C.

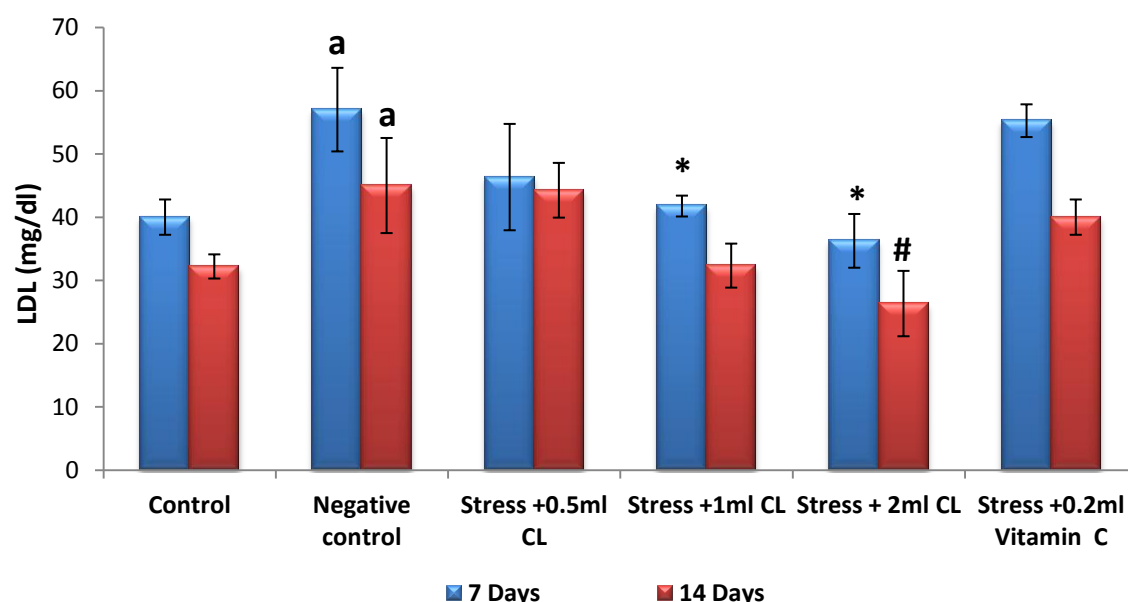


Fig. 3.3. Effects of Citrus limon juice on LDL of stress induced Wistar rats. <sup>a</sup>  $p=.05$  when compared to control

<sup>\*</sup>  $p=.05$  when compared to negative control at day 7

<sup>#</sup>  $p=.05$  when compared to negative control at day 14.

#### 4.0 Discussion

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.<sup>[17, 22]</sup> This observation is also in agreement with the study reported by Shefalee.<sup>[21]</sup> 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 effect exerted by this fruit due to the presence of this compound.<sup>[23]</sup>

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<sup>6</sup>. HDL level in the present study was increased significantly in the experimental animals administered with Citrus limon juice (2ml/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<sup>[20]</sup> 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<sup>[24]</sup> 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 (0.2ml/kg) used in this study.

Low density lipoproteins (LDLs) are also known as “bad cholesterol” as they transport cholesterol from the liver to tissues<sup>[9]</sup> and as such an important predictor of the likelihood of atherosclerotic conditions.<sup>[12]</sup> 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 <sup>[6]</sup> who also affirmed that LDL level increases during stress. Citrus limon juice (1ml/kg, 2ml/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 (2ml/kg) for 14 days reduces LDL level. This result is consistent with those obtained by Olukanni <sup>[19]</sup> and Gorinstein <sup>[25]</sup> that citrus juice possesses hypolipidemic effect and capable of reducing serum LDL level. Also, Monforte<sup>26</sup> 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.<sup>[27]</sup> 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.<sup>[28]</sup> This action could be dose-dependent causing an inability to reduce LDL level raised by stress as observed in this study.

## **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.**

There was no conflict of interest.

200     **References**

2011. Goldstein DS. Stress as a scientific idea: A homeostatic theory of stress and distress.  
202     Homeostasis. 1995;4: 177-215
2032. McEwen BS. Physiological and neurobiology of stress and adaptation: Central role of the  
204     brain. *Physiol Rev.*2007;87(3):873-904
2053. Rozanski A, Blumenthal JA, Davidson KW, Saab PG, Kubzansky L. The epidemiology,  
206     pathophysiology, and management of psychosocial risk factors in cardiac practice: the  
207     emerging field of behavioral cardiology. *Brain BehImm.* 2005; 20(2):113-9.
2084. Black PH. Stress and the inflammatory response: a review of neurogenic inflammation.  
209     *Brain, behavior, and immunity.* 2002;16(6):622-653
2105. Stoney CM. Cholesterol and Lipoproteins, In: *Encyclopedia of Stress*, George Fink (editor).  
211     San Diego, CA – USA. 2007; 478-483
2126. Neves VJ, Moura MJCS, Tamascia ML, Ferreira R, Silva NS, Costa R, Montemor PL,  
213     Narvaes EAO, Bernardes CF, Novaes PD, Marcondes FK. Proatherosclerotic effects of  
214     chronic stress in male rats: Altered phenylephrine sensitivity and nitric oxide synthase  
215     activity of aorta and circulating lipids. *Stress:The international journal on the biology of*  
216     *stress.* 2009;12(4):320-327
2177. Berliner JA, Navab M, Fogelman AM, Frank JS. Atherosclerosis: basic mechanisms  
218     oxidation, inflammation, and genetics. *Circulation.*1995; 91 (9): 2488-2494.
2198. Holvoet P, Harris TB, Tracy RP. Association of high coronary heart disease risk status with  
220     circulation oxidizing LDL in the well elderly; findings from the health, aging and body  
221     composition study. *Atherosclerosis Thrombosis Vascular Biology.*2003;23:1444-1448
2229. Colpo A. LDL cholesterol; Bad science or bad cholesterol. *Journal American Physicians and*  
223     *Surgeons.*2005;10(3):83-89

22410. Assmann G, Schulte H, Von Eckardstein A, Huang Y. High density lipoprotein as a predictor  
225 of CHD risk; the PROCAM experience and pathophysiological implication for reverse  
226 cholesterol transport. *Atherosclerosis*. 1996;124:11-20
22711. Sharret AR, BallantyneCM, Candy SA. Coronary heart disease prediction from lipoprotein,  
228 cholesterol levels, triglycerides, lipoprotein (a), apolipoprotein A-1 and B, and HDL desity  
229 sub-fractions; the atherosclerosis risk in communities.104:1108-1113
23012. Curb JD, Abbot RD, Rodriquez BI. A prospective study of HDL-C and cholesteryl ester  
231 transfer protein gene mutation and the risk of coronary heart disease in the elderly. *Journal of*  
232 *Lipid Reserve*. 2004;45:948-953
23313. Dohi K, Satoh K, Ohtaki H, Shioda S, Miyake Y, Shindo M, Aruga T. Elevated plasma level  
234 of bilirubin in patients with neurotrauma reflects its pathophysiological role in free radical  
235 scavenging. *In vivo*. 2005; 19:855-860
23614. Ehigbai I. Oikeh, Ehimwenma S. Omoregie, Faith E. Oviasogie, and Kelly Oriakhi.  
237 Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates  
238 *Food Science and Nutrition*. 2016; 4(1): 103–109.
23915. Giuseppe Gattuso, Davide Barreca, Claudia Gargiulli, Ugo Leuzzi and Corrado Caristi.  
240 Flavonoid Composition of Citrus Juices. *Molecules*. 2007; 12: 1641-1673
24116. Abdur Rauf, Ghias Uddin and Jawad Ali Phytochemical analysis and radical scavenging  
242 profile of juices of Citrus sinensis, Citrus anrantifolia and Citrus limonum. *Organic and*  
243 *Medicinal Chemistry Letters*. 2014; 4:1-5

24517. Al-snafi AE. Nutritional value and pharmacological importance of citrus species grown in Iraq.

246 ISOR Journal of Pharmacy. 2016; 6(8):77-108

24718. Kawaii YSI, Eriko K, Kazunori O, Masamichi Y, Meisaku K, Chihiro I, Hiroshi F. Quantitative

248 study of flavonoids in leaves of citrus plant. Journal of Agricultural Food Chemicals.

249 2000;48:3865-3871

25019. Olukanni OD, Akande OT, Alagbe YO, Adeyemi SO, Olukanni AT, Daramola GG. Lemon juice

251 elevated level of reduced glutathione and improved lipid profile in Wistar rats. American-

252 Eurasian Journal of Agricultural and Environmental Science. 2013;13(9):1246-1251

25320. Kamal M, Youssef E, Hanan MK, Youssef E, Rasha M, Mousa A. Evaluation of

254 Antihyperlipidemic Activity of Citrus Peels Powders Fortified Biscuits in Albino Induced

255 Hyperlipidemia. Food and Public Health. 2014; 4(1): 1-9

25621. Shefalee K, Bhavsar, PJ, Mamta BS, Santani DD. Investigation into Hepatoprotective Activity

257 of Citrus limon. Pharmaceutical Biology. 2007; 45(4): 303–311

25822. Muhammad NO, Soji-Omoniwa O, Usman LA, Omoniwa BP. Antihyperglycemic activity of

259 leaf essential oil of Citrus sinensis o alloxan-induced diabetic rats. Annual Review and Research

260 in Biology. 2013; 3(4): 825-834

26123. Tounsi WMS, Wannes A, Ouerghemmi I. Juice components and antioxidant capacity of four

262 Tinsian Citrus varieties. Journal of Science of Food and Agriculture. 2011; 91(1):142—151

26324. Itoh R, Yamada K, Oka J, Suyama Y, Murakami K. Serum ascorbic acid and HDL cholesterol in

264 a healthy elderly Japanese population. International Journal of Vitamin and Nutrition Resources.

265 1990;60(4):360-365

26625. Gorinstein S, Leontowicz H, Leontowicz M, Krzeminski R, Gralak M, Martin-Belloso O,  
267 Delgado-Licon E, Haruenkit R, Trakhtenberg S, Katrich E, Park YS, Jung ST. Comparison of the  
268 contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and  
269 his new hybrid. *LebensmWiss Technol.* 2004; 37: 337-343.

27026. Monforte MT, Trovato A, Kirjavainen S, Forestieri AM, Galati EM. Biological effects of  
271 hesperidin, a Citrus flavonoid. (Note II): hypolipidemic activity on experimental  
272 hypercholesterolemia in rat. *IlFarmaco.* 1995; 50: 595-599.

27327. Grover-Páez F, Zavalza-Gómez AB. Endothelial dysfunction and cardiovascular risk factors.  
274 *Diabetes Res. Clin. Pr.* 2009; 84: 1-10.

27528. Nerissa MS, Sunga MD, Pascual A. Effects of ascorbic acid on dyslipidemia (A study among  
276 Philipine hear Center employee). *Philipines Heart Center Journal.* 2012; 16(2):

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