The Cholesterol Lowering Effects of *Eurycoma longifolia* Jack (Tongkat Ali) Root Extract in Male Rats

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Abstract

**Background:** To investigate the effect of *Eurycoma longifolia* Jack root extract on serum lipids in rats. **Methods:** Twenty-six mature male albino Wistar rats were used in this study. A group of 18 rats were fed a high fat and high cholesterol diet for 4 weeks, after which their lipid profile was compared to the control group, who were kept on a normal diet. The rats were then further divided into three groups, the C group that continued to feed on a high fat and cholesterol diet only, and group A and group B who continued on a high fat diet with the addition of 5 mg/kg and 10 mg/kg of *Eurycoma longifolia* Jack root extract respectively for 4 weeks. After the 4 week period, the rat’s lipid profiles were analysed again. **Results:** Group A and B showed significant total cholesterol reduction when compared to the C group, 140 ± 7.23, 139.63 ± 7.95, 192.14 ± 8.96 mg/dL respectively (*p* < 0.001). The total cholesterol/HDL ratio in group A was 5 however there was a sharp increase in group B to a high-risk level of 9.2 indicating a significant drop in HDL levels. The LDL level increased significantly in both group A and B compared to the C group. **Conclusions:** *Eurycoma longifolia* Jack root extract is effective in lowering total cholesterol, however the dose needs to be adjusted to prevent an excessive decrease in HDL levels.

Keywords: wistar rats, cholesterol, *Eurycoma longifolia* Jack, HDL, LDL

Introduction

Over the past three decades there has been an increasing trend to use natural products rather than conventional treatments as remedies for many diseases.¹ A study conducted in the USA showed that a high percentage of well-educated people prefer to use alternative medicines.²

A very common tropical herbal plant in Southeast Asian countries is *Eurycoma longifolia* Jack (E.L), which is also known as Tongkat Ali. This tree is found in the forests of Burma, China, Thailand, Malaysia, Sumatra, Borneo, and the Philippines.³ The plant’s roots, stems, and bark are used as folk medicines for the treatment of many conditions including fevers, sexual dysfunction, dysentery, and fatigue. Traditionally the water decoction of *E. longifolia* root is consumed orally and research has shown a wide range of biological activities including antimalarial, cytotoxic, antiulcer, and antipyretic properties. These properties may be attributed to the various quassinoids, squalene derivatives, biphenylneolignans, tirucallane-type triterpenes, canthine-6-one, and 1-carboline alkaloids found in the plant. However, the main use of this plant is as a sexual enhancer with studies proving that it can raise the serum testosterone levels leading to improved strength and power during sexual activities and an increase in male virility.⁹

Recent studies have also shown that the E.L root has antibacterial and antifungal properties against human pathogenic bacteria and fungi.¹⁰,¹¹ Additionally, antioxidant and anti-inflammatory properties were also detected in the root extract.¹²

Lipids serve several important physiologic functions within the human body, such as stabilising the cell membrane and facilitating membrane transport. They are also important for the biosynthesis of hormones and the production of bile acids for the absorption of fat in mammals. Lipids are insoluble in water and must be packaged into lipoproteins to circulate within the plasma. The core of the lipoprotein, containing cholesterol ester and triglycerides (TG), is nonpolar and hydrophobic, and the outer layer of the lipoprotein particle (containing free cholesterol, phospholipid, and specific apolipoproteins) is polarised, permitting the lipoprotein particles to be...
transported in the circulation. Each lipoprotein class (chylomicrons, very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL)) varies in size, density, and lipid composition within the core of the particle.15

LDL particles carry the majority of the cholesterol in the blood, supplying cholesterol to the cells. LDL receptors in peripheral cells or the liver bind with LDLs and clear it from the blood and the peripheral cells utilise LDL cholesterol. LDL is an atherogenic lipoprotein particle, and it is established that higher levels of LDLs are associated with increased cardiovascular disease risk.16

HDL particles are synthesised and catabolised in the liver and intestines. Nascent HDL obtains free cholesterol from peripheral tissues. It is well established that increased HDL levels are associated with a decreased risk of coronary heart disease, whereas reduced HDL levels increase risk. The cardioprotective role of HDLs is to facilitate the transfer of cholesterol from atherogenic lipoproteins and peripheral tissues to the liver.15

Hyperlipidaemia involves abnormally elevated levels of any or all lipids and lipoproteins in the blood.17 Lipid and lipoprotein abnormalities are common in the general population and are regarded as a modifiable risk factor for cardiovascular disease due to their influence on atherosclerosis.17

Previous studies addressed the effect of testosterone supplementation on the serum lipid levels of men and found that the administration of testosterone improved lipid metabolism by decreasing total cholesterol and low-density lipoprotein cholesterol.18,19 Researchers have also found that testosterone administration slows the process of atherosclerosis and leads to attenuation of fatty streaks.20

Because the consumption of Eurycoma longifolia Jack root leads to a rise in serum testosterone levels, we hypothesised that the root extract would indirectly lead to a decrease in serum lipid levels.

Methods

The water soluble, freeze-dried extract was purchased from the Ljack Sdn Bhd Company in Malaysia; the product was supported by a certificate indicating it was pure and of a high concentration.

Twenty six mature male albino Wistar rats were used in this study. The sample size was calculated according to the resource equation method.21 They were housed in standard cages and fed on standard pellets for 7 days before commencing the experiment. Serum cholesterol, HDL, LDL, and testosterone were analysed using commercial kits manufactured by Abnova, with kit numbers KA0829, KA1671, and KA0309 respectively. They were purchased from Axon scientific Sdn Bhd Malaysia.

Eight rats were randomly chosen to represent the control group C; they did not receive any dietary changes or E.L root extract throughout the experiment. At the start of the experiment fasting serum cholesterol, LDLs, and HDLs were recorded for the control group. The rest of the 18 rats were maintained exclusively on a high fat and high cholesterol diet for 4 weeks. At the end of this period we analysed the fasting cholesterol, HDLs, and LDLs for the high fat fed rats.

The 18 high fat fed rats were randomly split into three groups; the control group that continued to feed on a high fat diet only (C), and group A and B both of which continued to be fed on a high fat diet with the addition of E.L root extract. Group A received 5 mg/kg, and group B received 10 mg/kg of E.L extract twice daily that was dissolved in 10 mL of distilled water and manually fed to the rats using a mouth gavage. The treatment continued for 4 weeks at the end of which, the fasting serum lipid and testosterone analysis were repeated.

Statistical analysis. Statistical analysis of the data was carried out using Statistical Package for Social Sciences (SPSS) version 20.0. One-way analysis of variance and the Tukey post hoc test for average comparison were completed. Mean values ± standard deviation were calculated. Values of \( p < 0.05 \) were considered statistically significant.

Ethical approval. This research was approved by the Kulliyyah of Dentistry Research Committee and the Research Management Centre, of the International Islamic University of Malaysia, Kuantan Campus, 2100 Kuantan, Pahang, Malaysia.

Results

The results of this study showed that there was a statistically significant increase in fasting levels of Cholesterol, HDLs, and LDLs in the rats fed on high cholesterol diets when compared to the control group \( (p < 0.001) \). This implies that the high cholesterol diet caused an elevation of blood lipids. The cholesterol/HDL ratio was maintained at the same level (Table 1).

After administration of the E.L extract the level of blood lipids found in group A showed a statistically significant reduction in total cholesterol levels when compared to the C group, 140 ± 7.23, 192.14 ± 8.96 mg/dL respectively. The total cholesterol of group A dropped down to nearly the same level as the control group, 136.6 ± 3.77 mg/dL. The levels of LDLs significantly increased to 74.81 ± 2.21 mg/dL \( (p < 0.001) \). However, the HDL levels dropped
The effects of Eurycoma longifolia Jack root extract on cholesterol levels were investigated in this study. The total cholesterol levels were significantly reduced in group B when compared with the C\textsubscript{f} group (p < 0.001). Due to the significant decrease in HDLs, the total cholesterol/HDL ratio increased greatly to 9.2 in group B (Table 3).

When comparing the results of group A and B, we can see that the decrease in cholesterol seen in group B is not significant when compared to group A (p = 0.729). However, the decrease in HDLs and the increase in LDLs in group B are statistically different than group A (p < 0.001) (Table 4).

Testosterone levels were slightly increased in group A when compared to the C\textsubscript{f} group, however the increase was not significant. In group B, there was a sharp rise in testosterone levels to 1.75 ± 0.10 when compared to group A (p = 0.729). In comparing the testosterone levels between group A and B, there was a statistically significant increase in testosterone in group B (p < 0.001) (Table 2, 3, and 4).

**Discussion**

Modern lifestyle and the increased consumption of a high fat diet and processed foods has led to an increase in the serum lipids across different age groups in the population. This has increased the need for medical intervention using drugs or invasive techniques to prevent or treat coronary heart disease, a common consequence of high blood lipids.

Raised total cholesterol is a significant risk factor for coronary heart disease, and the risk is significantly higher in men than women. The currently available lipid lowering agents are not free from side effects, some of which are quite serious.

The current trend in research is to look for natural resources with medical applications that are safe for human use as well as being environmentally friendly.

Many recent studies have documented the inverse relationship between testosterone and cardiovascular related mortality, stating that low serum testosterone is associated with increased risk of cardiovascular disease and mortality, and that increasing endogenous testosterone can lead to higher survival rates.

Eurycoma longifolia Jack root extract is a well-known natural product that has been documented as safe for human use and can be used to increase testosterone levels in males.

In this study, we fed sample rats a high cholesterol diet, which lead to an actual rise in cholesterol levels when...
compared to the rats fed a normal level. We then used two different concentrations of E.L root extract administered twice daily.

In a previously conducted study using the same doses of E.L root extract, the 10 mg/kg dose resulted in very sharp rise in testosterone levels when compared to the 5 mg/kg dose.29

In both treatment groups there was a significant lowering of cholesterol levels in comparison with the C group. However, when comparing the cholesterol levels of group A and group B, there was no statistically significant difference (p = 0.729), indicating that an increased E.L dose does not improve lipid lowering effects (Table 4).

The effect of E.L extract on HDLs and LDLs appears to be reciprocal as E.L caused a decrease in HDLs and an increase in LDLs, an effect that is considered unfavourable. However, studies have shown that the most important predictor of cardiovascular risk is the total cholesterol/HDL ratio and not high levels of LDLs alone. It has been reported that subjects with low LDL cholesterol levels and high total cholesterol/HDL ratios (>5) had a 2.5 times higher incidence of CHD than those with similar LDL cholesterol levels and low total cholesterol/HDL cholesterol ratios.30

Looking at the effect of E.L extract on the cholesterol/HDL ratio, we found that in group A the ratio was equal to 5, which means that it is still within the safe range, however for group B the ratio had increased well beyond safe levels. The rise in the ratio and the consequent sharp drop in HDL levels seen in group B can be explained by the effect of sharply increasing testosterone levels in this group. Previous studies have shown the effect of testosterone on lowering HDL cholesterol, a phenomenon observed in athletes using androgenic-anabolic steroids.31 This effect has also been achieved consistently with the administration of supraphysiologic doses of oral androgens in young men, however it is far less consistent when testosterone therapy is used at physiologic doses to restore eugonadal serum levels, particularly in older men.32,33

As such, we can expect that treatment with 5 mg/kg of E.L extract should maintain testosterone levels close to the normal physiological range to help reduce total cholesterol levels, whilst also maintaining a safe cholesterol/HDL ratio. While the higher dose of 10 mg/kg led to a significantly higher level of testosterone leading to a sharp rise in the cholesterol/HDL ratio and no increased benefit of cholesterol lowering ability.

**Conclusions**

E.L root extract is beneficial in lowering total cholesterol level, however the dose should be regulated to prevent unnecessary lowering of HDL levels. This extract may be useful to lower total cholesterol as well as helping to return testosterone to normal physiological levels in elderly males with hypercholesterolaemia.

**Conflict of Interest Statement**

The authors declare no conflict of interest in conducting this research.

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**References**

1. Ji H-F, Li X-J, Zhang H-Y. Natural products and drug discovery. *EMBO Rep.* 2009;10:194-200.
2. Frass M, Strassl RP, Friedl H, Mullner M, Kundi M, Kaye AD. Use and acceptance of complementary and alternative medicine among the general population and medical personnel: a systematic review. The Ochsner Journal. 2012;12:45-56.
3. Bhat R, Karim AA, Tongkat Ali (*Eurycoma longifolia* Jack): a review on its ethnomedical and pharmacological importance. *Fitoterapia* 2010;81:669-79.
4. Low BS, Teh CH, Yuen KH, Chan KL. Physico-chemical effects of the major quassinoids in a standardized *Eurycoma longifolia* extract (Fr 2) on the bioavailability and pharmacokinetic properties, and their implications for oral antimarialar activity. *Natural Product Commun.* 2011;6:337-41.
5. Wong PF, Cheong WF, Shu MH, Teh CH, Chan KL, Abu Bakar S. Eurycomanone suppresses expression of lung cancer cell tumor markers, prohibitin, annexin 1 and endoplasmic reticulum protein 28. *Phytomedicine.* 2012;19:138-44.
6. Park S, Nhim NX, Van Kiem P, Van Minh C, Tai BH, Kim N, et al. Five new quassinoids and cytotoxic constituents from the roots of *Eurycoma longifolia*. *Bioorg Med Chem Lett.* 2014;24:3835-40.
7. Tada H, Yasuda F, Otani K, Doteuchi M, Ishihara Y, Shiro M. New antiucler quassinoids from *Eurycoma longifolia*. *Eur J Med Chem.* 1992;26:345-9.
8. Chan KL, Lee SP, Yuen KH. Antipyretic activity of quassinoids from *Eurycoma longifolia* jack. Proceedings of the 11th Chemical Seminar on Natural Products, UNIMAS, Sarawak, Malaysia; 1995. p.197-204.
9. Tambi MI1, Imran MK, Henkel RR. Standardised water-soluble extract of *Eurycoma longifolia*, Tongkatatali, as testosterone booster for men with late-onset hypogonadism. *Andrologia.* 2012;44 Supplement s1:226-30.
10. Faisal GG, Zakaria SM, Najmuldeen GF, Al-Ani IM. Andrological activity of *Eurycoma longifolia* jack (Tongkat Ali) Root Extract. *J Int Dent Med Res.* 2016;9:70-4
11. Faisal GG, Zakaria SM, Najmuldeen GF. In vitro antibacterial activity of *Eurycoma longifolia* jack (Tongkatatali) root extract. *Int Med J Malaysia.* 2015; 14:77-81.
12. Varghese CP, Ambrose C, Jin SC, Lim YJ, Keisaban T. Antioxidant and anti-inflammatory activity of *Eurycoma longifolia* Jack, a traditional medicinal plant in Malaysia. *Int J Pharm Sci Nanotechnol*. 2013;1:875-8.

13. Hall JE. Guyton and Hall textbook of medical physiology. 13th ed. Philadelphia: Saunders; 2015.

14. Chapman MJ, Ginsberg HG, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J*. 2011;32:1345-61.

15. Rader DJ. *Guyton and Hall textbook of medical physiology*. 13th ed. Philadelphia: Saunders; 2015.

16. Chapman MJ, Ginsberg HN, Amarenco P, Andreotti F, Borén J, Catapano AL, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J*. 2011;32:1345-61.

17. Rader DJ, Hovingh GK, HDL and cardiovascular disease. *The Lancet*. 2014;384:618-25.

18. Dorlands Medical Dictionary for Health Consumers. For Dorland’s Medical Dictionary: Iatrogenic. 2007. hay.dorland.com/Iatrogenic.

19. Kuwabara M, Kondo F, Hamada T, Takahashi JI, Takenaka N, Furuno T. Impact of Statins Therapy for Ischemic Heart Disease Patients with Low-Density Lipoprotein Cholesterol Levels Less Than 100 mg/dL. *Acta Cardiologica Sinica*. 2016;32:565.

20. Surampudi PN, Wang C, Swerdloff R. Hypogonadism in the aging male diagnosis, potential benefits, and risks of testosterone replacement therapy. *Int J Androl*. 2012;14;2012.

21. Traish AM, Haider A, Doros G, Saad F. Long-term testosterone therapy in hypogonadal men ameliorates elements of the metabolic syndrome: An observational, long-term registry study. *Int J Clin Prac*. 2014;68:314-29.

22. Nettleship JE, Jones TH, Channer KS, Jones RD. Physiological Testosterone Replacement Therapy Attenuates Fatty Streak Formation and Improves High-Density Lipoprotein Cholesterol in the Tfm Mouse. *Circulation*. 2007;116:2427-434.

23. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR J*. 2002;43:244-58.

24. Li Y, Wang DD, Ley SH, Howard AG, He Y, Lu Y, Danaei G, Hu FB. Potential impact of time trend of lifestyle factors on cardiovascular disease burden in China. *J Am Coll Cardiol*. 2016;68:818-33.

25. Peters SA, Singhateh Y, Mackay D, Huxley RR, Woodward M. Total cholesterol as a risk factor for coronary heart disease and stroke in women compared with men: A systematic review and meta-analysis. *Atherosclerosis*. 2016;248:123-31.