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In-Vivo Anti-Hyperlipidemic Activity and Preliminary Phytochemical Screening of *Canephora robusta*

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Abstract

The main aim of this study is to determine the anti-hyerlipidemic and anti-obesity activity of *Canephora robusta* in hyperlipidemia induced rats. Prepared coffee bean extract (GCE) was procured from the market which is unroasted and contains more quantity of caffeine and chlorogenic acid when compared to roasted coffee. Male albino Wister rats are fed with high fat diet (HFD) for weeks to induce hyperlipidemia in rats, which are divided into 4 groups with 4 animals in each group. Test GCBE was given in doses of 200 mg/kg and 400 mg/kg to III and IV groups which are fed with HFD for 30 days. Then blood samples were collected through retro-orbital sinus by capillaries and serum is separated for analysis. The result obtained from lipid profile which includes total cholesterol, triglycerides, very low density lipoproteins, and low density lipoproteins shows the decreased level when compared to the hyperlipidemic control. This shows the significant reduction of total body weight ($p < 0.05$) when given with dose of 200 mg/kg and 400 mg/kg. The present study suggests that GCBE has anti-obesity and anti-hyperlipidemic activity, where 400 mg/kg is more effective to reduce the total body weight and lipid levels when compared to 200 mg/kg. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

Keywords

Anti-Hyerlipidemic, Anti-Obesity, Lipoproteins Hyperlipidemia,

Canephora robusta

1. Introduction

Nowadays obesity is the most prevailing conditions in world wide. Obesity is a disorder involving in excessive accumulation of body fat. It increases risk of diseases and health problems such as heart disease, diabetes, high blood pressure [1]. According to WHO overweight and obesity are defined as the abnormal fat accumulation that causes the risk to health [2]. Therefore, lifestyle changes such as exercise and eating healthy diet are the first line against high fat [3]. Hyperlipidemia is elevated lipid levels in blood. This is also leading to increased risk of heart diseases [4]. Coffee is a drink prepared from roasted and unroasted coffee beans, which is widely taken beverages all over the world [5]. *Coffee canefora* is better known as Coffee robusta which is classified as variety of *C. canefora* [6] *Coffee robusta* [unroasted green coffee beans] of the Rubiaceae family is rich in chlorogenic acid and caffeine called as green coffee beans. The roasting process of coffee bean reduces amount chlorogenic acid, which is thought to have health benefits [7]. People take green coffee for obesity, diabetes, high blood pressure, Alzheimer's disease and bacterial infections [8]. Green coffee bean is promising as an energy supplement which has chlorogenic acid active ingredient which is well known to reduce the cholesterol and triglyceride levels in serum [9]. It is found to have presence chemical constituents like green coffee which includes flavonoids, glycosides, alkaloids, tannins and phenols which are detected and proved by using standard methods [10]. Coffee bean extract appears to effectively suppress body fat and serum triglyceride levels through at least in part the decrease in fatty acid synthesis and the acceleration of fatty acid oxidation, showing that it may be a novel functional food material for suppressing fat deposition [11]. Antioxidant and radical scavenger activities of coffee beans can improve wound healing to control overexposure of oxidative stress in the wound bed. The coffee beans press cake has shown a lower commercial value; however it might gain value as thought of as a valuable biomass source of bioactive compounds interesting for the human health usage. Indeed, the residual coffee biomasses studied are able to improve the regeneration of damaged skin tissue, allowing new product development in the cosmetic and pharmaceutical industries [12].

2. Materials and Methods

2.1. Plant Material

Coffee bean powder of *Canephora robusta* was procured from the market.

2.2. Plant Extract

2.2.1. Preparation of Aqueous Extract [12]

The bean powder of *Canephora robusta* is soaked in water for 1 hour in a

beaker. Then the powder is filtered by using filter paper. Then obtain extract is evaporated in water bath.

2.2.2. Preparation of Alcoholic Extract [13]

The bean powder of *Canephora robusta* is extracted with ethanol by the soxhlation method by using soxhlet apparatus. The extract was evaporated on a water bath until the solvent has been evaporated and the extract was completely dry and weighed and pure extract was obtained.

2.3. Phytochemical Screening [14]

All the chemicals used in the study were analytic graded. Chemical tests were carried out with alcohol and aqueous extract by adapting standard procedure.

2.4. Experimental Animals

Mature male albino Wistar rats of weight 150 - 200 gm were used for the experiment. Animals selected were free from diseases. Animals were housed in a temperature of 22˚C ± 1˚C. At 12:12 hours light and dark cycle was maintained. All the animals were allowed to free access of water and feed off the normal pellet diet. The composition of atherogenic diet is used during the study. The experimental procedure and protocol used in the study were viewed by the Institutional Animal Ethical Committee (IAEC) No: (VCP/cology/10/11/2018) and were in accordance with the guidelines of the CPCSEA (VCP/cology/10/11/2018).

2.5. Induction of Obesity

In order to induce obesity and hyperlipidemia method reported by Bopanna *et al.* was followed by feeding the male Wistar albino male rats (150 - 200 g) with high fat diet for 30 days. The composition of Atherogenic Diet was followed according to Yash Prashar *et al.* [15], the composition of the diet is included in Table 1. Acute toxicity studies were conducted in Wistar albino male rats as per OECD guidelines 423 [16]. The alcoholic extract of *Canephora robusta* was found

Table 1. Atherogenic diet used during the study [15].

S. No.	Composition	Normal Diet (%)	Atherogenic Diet (%)
1	Protein (Milk Powder)	12	10
2	Carbohydrates (Wheat Powder)	71	61
3	Sugar	05	05
4	Fat (Butter)	05	16
5	Salt	04	04
6	Vitamin	01	02
7	Fibres	02	01
8	Cholesterol	-	01
	Total	100 gm	100 gm

the safe dose at 4000 mg/kg.

2.6. Experimental Design

Sixteen mature male albino Wistar rats were randomly grouped into 4 groups of 4 animals each.

Group 1: Normal control;

Group 2: Atherogenic/hyperlipidemic control;

Group 3: Standard control;

Group 4: Ethanolic extract of 200 mg/kg and 400 mg/kg.

Group I rats are fed with Normal Diet and remaining three groups are fed with the high fat diet to induce obesity. Other 3rd and 4th groups are given with standard and test along with high fat diet. The standard used in this study is atorvastatin at a dose of 10 mg/kg for 1 week and group 4 is given with GCBE at doses of 200 mg/kg and 400 mg/kg through oral route using cannula for 1 week. The end of the experiment body weight of rats was recorded.

2.7. Blood Sampling Analysis

Blood was withdrawn by using heparinised capillaries from the retro-orbital sinus in overnight fasted animals and the obtained blood is centrifuged at 3000 rpm for 15 - 20 mins to obtain serum which was used to estimate the concentration of biochemical parameters TC, HDL, LDL, VLDL using the semi-auto analyser and relevant lipid profile kits.

2.8. Statistical Analysis

Appropriate statistical methods were used to analyze the data to fulfil the objectives. Graph pad prism Results were expressed in mean ± SD. Probability values of $P < 0.05$ were considered to be statistically significant.

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical screening is very important for determining biological compounds. The results showed that phenols, flavonoids, alkaloids, glycosides and tannins. This Phytochemical showed different biological activities which play an important role in the protection against chronic diseases.

3.2. Anti-Hyperlipidemic Activities

Body weight of rats was significantly increased when they were fed with high fat diet (HFD) for 4 weeks when compared with normal control. By inducing GCBE the body weight reduced significantly ($P < 0.05$), when compared to normal and hyperlipidemic control and the results were expressed in **Table 2** and **Figure 1**.

By administering the high fat diet to group 4 animals for 4 weeks continuously, the lipid levels get increased significantly. The dose of green coffee bean extract of 200 mg/kg and 400 mg/kg given orally and the serum lipid levels reduced

Table 2. Effect of *Canephora robusta* on body weight and lipid profile.

Groups	Weight	Total cholesterol	Triglycerides	Low density lipoprotein	High density lipoprotein	Very low density lipoproteins	Atherogenic index
Normal Rats	182.5 ± 23.6	23.6 ± 67	142.9 ± 9.1	26.7 ± 38	33.1 ± 4.06	26.5 ± 2.07	0.71
Hyperlipidemic Rats	222.5 ± 18.9	52.2 ± 3	235.9 ± 1.6	172.05 ± 3.1	27.1 ± 3.9	52.2 ± 2.0	1.92
Standard (Atorvastatin)	165 ± 19.1	45.7 ± 2.2	276.6 ± 10.7	180.7 ± 4.1	40.8 ± 1.7	46.7 ± 2.6	1.12
Ethanolic extract 200 mg/kg	165 ± 5.7	35.2 ± 4.8	146.3 ± 3.8	163.5 ± 0.8	33.7 ± 2.4	35.6 ± 2.7	0.91
Ethanolic extract 400 mg/kg	162 ± 19.1	35.4 ± 3.5	186.1 ± 4.7	65.5 ± 1.4	38.8 ± 15	35.3 ± 2.7	1.04

Values are expressed as Mean ± SD using ANOVA.

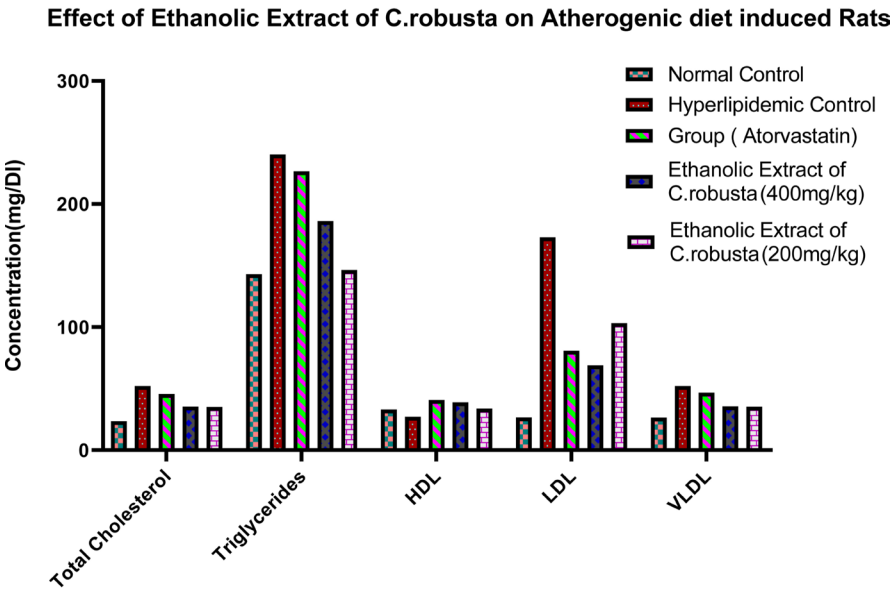

Figure 1. Effect of *C. robusta* on atherogenic diet induced rats.

significantly by ($P < 0.05$). The HDL levels get decreased by inducing the obesity by HFD for 4 weeks. So, this green coffee bean given at 200 mg/kg and 400 mg/kg shows the significant increase in the HDL in the body shown in **Figure 1** and the atherogenic index of the treated group was reduced when compared with Hyperlipidemic group in **Figure 2**. So, the following results have shown the effect of the drug which leads to decrease the serum lipid levels and body weight of obese rats. Moreover, the previous studies on *Canephora robusta* on anti-hyperlipidemic activity with methanolic extract at doses of 100 and 200 mg/kg also shown the significant decrease of body weight, lipid levels like LDL, VLDL, TC, Triglycerides and increase in the HDL levels [17]. Bong keun *et al.* confirmed that the active compound 3-CQA from GCBE, reduces body fat accumulation by the regulation of adipogenesis and lipogenesis in obesity. Thus, those findings demonstrate that GCBE represents a potential activity that prevents the development of obesity, hyperlipidemia and its complications in diseases [18]. Our present study shows the significant decrease of weight and lipid levels were better than previous studies because of high dose and more effectiveness.

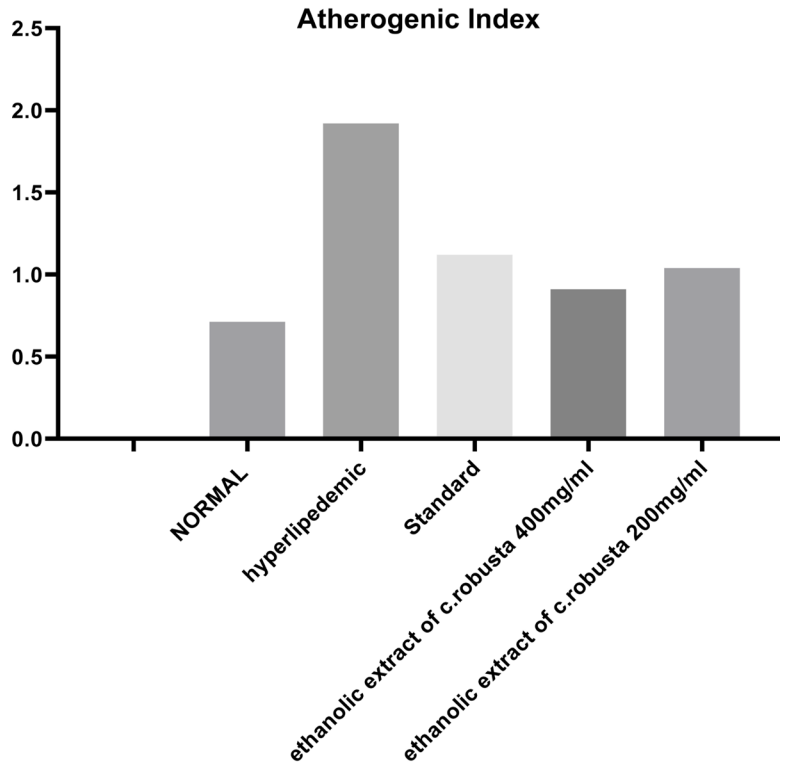

Figure 2. Effect of ethanolic extract of *C. robusta* on atherogenic diet index of rats.

4. Conclusion

The main aim of study is to show the significant anti-obesity and anti-hyperlipidemic activity of the plant. This was conducted to determine that *Canephora robusta* can act as anti-hyperlipidemic agent which was proven by many researchers. They were determined that their plant was reduced body weight in previous studies. Here, study was performed to determine effective dose and the amount of a dose which is safe to reduce body weight.

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Authors' Contributions

All the authors have contributed equally.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Wanderley, A.B. (2017) Functional Benefits of Green Coffee in Metabolic Syndrome Prevention: A Review Study. *Journal of Chemical and Pharmaceutical Research*, **9**,

5-12.

[2] WHO (2015) Obesity and Overweight Fact Sheet.

[3] Uptodate.com (2005) R Rosenson-UpToDate. Up to Date, Wellesley.

[4] Levy, R.I. and Fredrigkson, D.S. (1968) Diagnosis and Management of Hyperlipoproteinemia. *The American Journal of Cardiology*, **22**, 576-583.

[5] (2019) What Is Green Coffee Bean Uses and Benefits. https://vitagene.com

[6] Dagoon, J. (2005) Agriculture & Fishery Technology IV. Rex Bookstore, Inc., Manila, 58.

[7] Kathleen Davis, F.N.P. (2015) Article, 295385. Medical News Today.

[8] O'Keefe Osborn, C. (2017) What We Should Know about Hyperlipidemia.

[9] Diabetis.co UK (2013) Green Coffee Bean Extract Has Anti-Diabetic Benefits.

[10] Tanaka, K., Nishizono, S., Tamaru, S. and Kondo, M. (2009) Anti-Obesity and Hypotriglyceridemic Properties of Coffee Bean Extract in Sprague Dawley Rats. *Food Science and Technology Research*, **15**, 147-152. https://doi.org/10.3136/fstr.15.147

[11] Dong, W. (2015) Characterization of Fatty Acid, Amino Acid and Volatile Compound Compositions and Bioactive Components of Seven Coffee (*Coffea robusta*) Cultivars Grown in Hainan Province China. *Molecules*, **20**, 16687-16708. https://doi.org/10.3390/molecules200916687

[12] Affonso, R.C.L., Voytena, A.P.L. and Maraschin, M. (2016) Phytochemical Composition, Antioxidant Activity, and the Effect of the Aqueous Extract of Coffee (*Coffea arabica* L.) Bean Residual Press Cake on the Skin Wound Healing. *Oxidative Medicine and Cellular Longevity*, **2016**, Article ID: 1923754. https://doi.org/10.1155/2016/1923754

[13] McNaugh, A.D. and Wilkinson, A. (1997) Compendium of Chemical Terminology. 2nd Edition, Blackwell Scientific Publications, Oxford.

[14] Khandelwal, K.R. (2008) Practical Pharmacognosy. Nirali Prakashan Publications.

[15] Prashar, Y., Gandhare, B. and Mendhe, B. (2010) Anti-Hyperlipidemic Activity of Bauhinia Variegate Root Extracts. *International Journal of Biomedical and Advance Research*, **1**, 96-102. https://doi.org/10.7439/ijbar.v1i3.7

[16] OECD Guideline for Testing of Chemicals, Acute Oral Toxicity—Acute Toxic Class Method.

[17] Blum, J., Lemaire, B. and Lafay, S. (2007) Effect of a Green Decaffeinated Coffee Extract on Glycaemia: A Pilot Prospective Study. *Nutrafoods*, **6**, 13-17.

[18] Choi, B.-K., Park, S.-B. and Lee, B.-R. (2016) Green Coffee Bean Extract Improves Obesity by Decreasing Body Fat in High Fat Diet Induced Obese Mice. *Asian Pacific Journal of Tropical Medicine*, **9**, 635-643. https://doi.org/10.1016/j.apjtm.2016.05.017