OPENING MINDS:
RESEARCH FOR SUSTAINABLE
DEVELOPMENT

Effect of Crude Methanolic Extracts of *Emblica officinalis* on Cholesterol Induced Wistar Albino Rats (*Mus norvegicus albinus*)

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1 INTRODUCTION

Hypercholesterolemia, elevated serum cholesterol levels in the body, is a serious health condition affecting a large population of the world today (Yadav *et al.*, 2014). Medications used at present to reduce body cholesterol levels are associated with unwanted side effects. Therefore, there is a growing interest in search of hypocholesterolemic plant metabolites of herbal origin (Maruthappan and Sakthi, 2010). The plant family Euphorbiaceae is a systematically complex family but consists of plants with various medicinal properties (Asha *et al*., 2006, Chauhan *et al*., 2010 and Maruthappan and Sakthi, 2010). *Emblica officinalis* is one of the species of this family that exhibits various therapeutic properties. In Ayurveda, a class of drugs derived from *E. officinalis* is believed to promote health and longevity by improving defense against diseases (Deshmukh *et al*., 2010 and Fatima *et al.,* 2014). Studies of *E. officinalis* on anti-ulcer activity, anti-cancer activity, anti-inflammatory activity, hypoglycemic activity, blood cholesterol reduction etc. have been reported by Jain *et al*., 2015). Few studies of *E. officinalis* on hyperlipidemia are available using an Ayurvedic powder at the concentration of 540 mg/kg (Santoshkumar *et al*., 2012) and fresh fruit juice (Mathur *et al*., 1996). Compared to the water extract of plant materials, methanolic extract results in the highest extraction yield with maximum presence of phytoconstituents (Azwanida, 2015). Studies on hypocholesterolemic activity of *E. officinalis* using the crude methanolic extract of fruits are lacking and its dose-dependent response and effective dose has not been established. Hence the present study was carried out to explore the potential of the use of crude methanolic extract as an alternative to fresh *E. officinalis* fruit juice in a rat model and to determine its effective dose.

2 METHODOLOGY

2.1 Plant collection and Extraction

Fresh fruits of *Emblica officinalis* were collected from Gampaha district. Seeds were removed and the fruits dried in the shade for three weeks. Dried fruits were powdered mechanically and samples were

subjected to soxhlet extraction with 80% methanol at 64°C for 6-8hr. Extracts were evaporated at 40°C using a rotary evaporator. Evaporated samples were dried in vacuum oven until attaining constant weight.

2.2 Experimental animals

Wistar albino rats were used in this study as they have similarities with humans in terms of physiology, anatomy, nutrition, pathology, and metabolism and according to published literature, they are the most common animal model used in cholesterol studies.

Only male rats were used as they are less affected by reproductive hormonal changes. Ethical approval for the study was obtained from the Ethical review committee, Institute of Biology, Sri Lanka. Male Wistar albino rats (*Mus norvegicus albinus*) weighing 180-200 g were purchased for this study from the Medical Research Institute, Colombo 08. They were kept under standard animal house conditions (photoperiod: approx. 12h natural light per day, temperature: 28-30°C, RH: 55%-60%) and given water and standard diet *ad libitum* throughout the experimental period**.** Rats were acclimatized for 7 days.

2.3 Experimental design

Rats were divided into groups (n=06/group) of six animals each. Except the rats of negative control group (NCG), hypercholesterolemia was induced in rats in treatment groups and the positive control group (PCG), by feeding a mixture of cow ghee, butter and egg yolk (1:1:2 by weight) orally once a day throughout the experimental period in addition to the standard diet given *ad libitum*.When induced total cholesterol levels were significantly higher ($p \leq 0.05$) than normal total cholesterol level in blood (75± 10 mg/dL reported by Samaranayaka, 2005) treatments started. Crude methanolic extract of *E. officinalis (EO*-CME*)* was given orally once a day to rats of treatment groups at the dosage of 400 mg/kg (*EO*-CME400), 800mg/kg (*EO*-CME800) and 1200mg/kg (*EO*-CME1200). Rats in the NCG and ECG were given distilled water as the vehicle.

2.4 Evaluation of blood parameters

Animals were anesthetized and held in a rat holder to collect blood samples from tail vein. Samples were centrifuged within one hour after collection, at 3500 rpm for 30 minutes to obtain serum. Total cholesterol (TC), Triglyceride (TG), HDL Cholesterol (HDL-C) and LDL Cholesterol (LDL-C) were measured using standard kits (Biolabo reagents-Maizy, France). Blood parameters were evaluated at the beginning, 14th, 28thand 42nd days of the experiment.

2.5 Statistical analysis

Descriptive statistics such as means and standard deviations were calculated. The significance of difference between the controls and treated groups were determined using one-way analysis of variance (ANOVA) using SPSS Ver. 20. The acceptable level of significance was $p < 0.05$.

3 RESULT AND DISCUSSION

Increasing EO-CME concentrations resulted in dose dependent negative responses ($p < 0.05$) with total cholesterol, triglyceride and LDL-C along with a dose dependent positive response ($p < 0.05$) with HDL-C. When compared with cholesterolemic untreated group (PCG), the levels of total cholesterol, triglyceride and LDL-C were lowered significantly and HDL-C the response increased significantly ($p < 0.05$) by all three doses ($p < 0.05$) of the crude methanolic extract of E .officinalis. These trends increased with time.

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There was a significant decrease ($p < 0.05$) in total cholesterol in rats for all three doses of EO-CME throughout the experiment (Figure 1a). By the 42nd day of the experiment EO-CME1200 treated group reached the normal total cholesterol level of the group NCG, making the total cholesterol levels insignificant ($p \geq 0.05$) in these two groups.

Although the TGL levels were insignificant ($p \geq 0.05$) at 14th day of the experiment between EO-CME-400mg/kg treated group and PCG, all three doses of EO-CME treated groups indicated significant decrease ($p \leq 0.05$) throughout the experiment. At 42nd day of the experiment, TGL level of rats in EO-CME-1200mg/kg treated group became insignificant ($p \geq 0.05$) with NCG (Figure 1b)

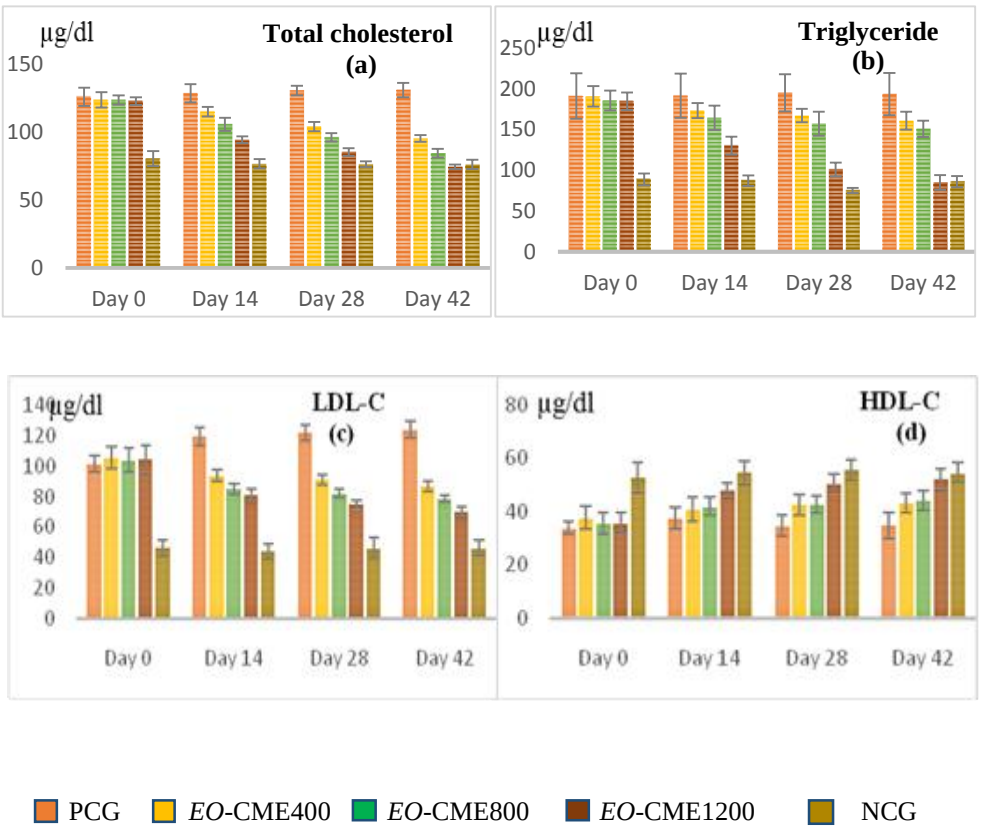

Figure 1: Mean variation of Total Cholesterol (a), Triglyceride (b), LDL-C (c), HDL-C (d) in *EO*-CME treated groups and control groups.

4 CONCLUSIONS AND RECOMMENDATIONS

The crude methanolic extract of E. *officinalis* was able to reduce levels of total cholesterol and triglyceride and increase of HDL-C in rats up to normal level within 42 days. The most effective dose was found to be 1200mg/kg body weight.

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REFERENCES

Asha, V.V., Sheeba, M.S., Suresh V. and Wills, P.J. (2007). Hepatoprotection of Phyllanthus maderaspatensis against experimentally induced liver injury in rats.Fitoterapia, 78, 134–141.

Azwanida, N. N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants, 4(3), 1-6.

Chauhan, A., Sharma, P. K., Srivastava, P., Kumar, N. and Dudhe, R. (2010). Plants having potential antidiabetic activity: A Review. Der Pharmacia Lettre, 2(3), 369-387.

Deshmukh C. D., Veeresh B. and Pawar A.T. (2010). Protective effect of Emblica Officinalis fruit extract on acetic acid induced colitis in rats.Journal of Herbal Medicine and Toxicology, 4 (2), 25-29.

Fatima, N., Pingali, U. and Pilli, R. (2014). Evaluation of Phyllanthus emblica extract on cold pressor induced cardiovascular changes in healthy human subjects. Pharmocognocy Research,6 (1), 29–35.

Jain, R., Pandey, R., Mahant, R.N. and Rathore, D.S. (2015). A review of medicinal importance of Emblica officinalis. International journal of pharmaceutical sciences and research, 6(1), 72-84.

Maruthappan, V. and Sakthi S., K. (2010). Effects of Phyllanthus reticulatus on lipid profile and oxidative stress in hypercholesterolemic albino rats. Indian J Pharmacology, 42(6), 388–391.

Mathur, R., Sharma, A., Dixit, V. P. and Varma, M. (1996). Hypolipidaemic effect of fruit juice of Emblica officinalis in cholesterol-fed rabbits. Journal of Ethnophamacology, 50, 61-68.

Samaranayaka, M. D. P. (2000). Effects of garlic (Allium sativam) on chemically induced hepatocarcinogenesis in normal and hypocholesterolemic wistar rats. (M.phill thesis). University of Sri Jayawardanapura, Sri Lanka.

Santoshkumar1, J., Manjunath S. and Sakhare, P. M. (2012). A study of Anti-hyperlipidamia, Hypolipidamic and Anti-atherogenic activity activity of Emblica offcinalis (Amla) in high fed albino rats. International Journal of Medical Research and Health Sciences, 2(1), 70-72.

Yadav, V., Upadhyay, V. and Ravinder, D.D. (2014). Importance of herbs in the treatment of hyperlipidaemia. Scholars Academic Journal of Pharmacy, 3(3), 306-312.

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