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LIPID LOWERING ACTIVITY OF *Aegle marmelos* AND *Ocimum basilicum* EXTRACT IN HYPERLIPIDEMIA INDUCED RATS

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ABSTRACT

Hyperlipidemia causes the development of atherosclerosis and is a risk factor for cardiovascular diseases and myocardial infarction, which is common cause of mortality and morbidity.. The aim of this study is to compare the effect of Aegle marmelos (A.marmelos) and Ocimum basilicum (O.basilicum) aqueous extract on hyperlipidemia induced albino rats. This investigation had been performed on 36 male Wistar albino rats which were arbitrarily classified into 6 groups (n=6). Group I: negative control group was given basal diet. Group II: positive control was fed with 2% high fat diet (HFD). Group III: the rats were treated with 250 mg/kg A.marmelos aqueous extract. Group IV: the rats were treated with 800 mg/kg O.basilicum. Group V: the rats were administrated with combination of 250 mg/kg A.marmelos and 800 mg/kg O.basilicum. Group VI: the rats in this group were treated with standard drug atorvastatin. All the groups excluding negative control were induced hyperlipidemia by feeding them 2% HFD throughout the experiment and received the treatment for 2 weeks. The blood samples were obtained through cardiac puncture to analyze serum total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) level by using Enzymatic Colorimetric Test Kits. The results were statistically analyzed by using one-way ANOVA followed by Dunnet's Multiple Comparison Test. High fat feeding caused significant ($p<0.05$) increases in serum levels of total cholesterol, triglycerides and LDL-C and decreases HDL-C as compared with the rats fed basal diet. Administration of the individual aqueous extract of A.marmelos and O.basilicum to HFD fed rats significantly ($p<0.05$) prevented the increases in serum total cholesterol, triglycerides and LDL-C and decrease of HDL-C. Besides, the results were more significant in rats treated with combination extract of A.marmelos and O.basilicum. The standard drug atorvastatin showed slightly better effect than the treatment of the both extracts. In conclusion, the combination treatment aqueous extraction of A.marmelos and O.basilicum produces more significant effect than individual treatment of the extracts. Aqueous extract of A.marmelos and O.basilicum can serve as an alternative source in the

management of hyperlipidemia causing diseases.

Key words: Hyperlipidemia, Cholesterol, *Aegle Marmelos*, Myocardial infarction, *Ocimum basilicum*

INTRODUCTION

Hyperlipidemia is the elevation levels of triglycerides or cholesterol which occur as a consequence of several inter-related factors that may be lifestyle, genetic, metabolic or other conditions that influence plasma lipoprotein metabolism (Vijaya et al., 2008). The increase of deadth rates in cardiovascular disease which is caused by myocardial infraction happened due to hyperlipidemia. An elevation in the amount of liver and kidney produced enzymes indicates fatty liver development as well as kidney injuries which causes dangerous increase in blood lipids.The excessive assembly of body lipids can cause disruption in blood circulation, cell growth and functionality. (Rashmi et al., 2011). Lipid lowering therapy is the primary and secondary prevention of cardiovascular disease in addition to the management of other risk factors such as smoking, diabetes and obesity (Vijaya et al., 2008). *Aegle marmelos*, also known as Bael in english, is an aromatic tree indigenous to India. In folk medicine, its leaves are used as astringent, digestive, laxative and febrifuge. Its leaves also were reported as exhibiting blood glucose lowering effect in normal, streptozotocin and alloxan diabetic rats (Rajadurai and Prince., 2005). In addition, the leaves of *Aegle marmelos* were also reported to reduce the serum cholesterol in alloxan diabetic rats and possess antihyperlipidemic effect in rats with isoproterenol induced myocardial infarction (Vijaya et al., 2008). Ocimum basilicum is a plant belonging to Lamiacea family locally known as sweet basil and frequently utilized due to its culinary as well as therapeutics quality in treating hyperlipidemia and cardiovascular disease prevention.Sweet basil also reported has the properties of anti-inflammatory and antioxidant (Hicham et al., 2009). The aim of present study is to compare the effect of *Aegle marmelos* and *Ocimum basilicum* aqueous extract on hyperlipidemia induced in male albino rats.

MATERIAL AND METHODS

PLANT MATERIALS AND EXTRACTION

Aegle marmelos and *Ocimum basilicum* leaves were collected from the available sources. The leaves were washed with water and dried in shade for 3-5 days. The powdered *Aegle marmelos* and *Ocimum basilicum* up to 100g were mixed with distilled water and extracted with boiling water for 48 hours. The resulting extracts were filtered and concentrated separately in rota-vapour reduced pressure. The resulted extracts were collected and stored separately at the temperature of -4ºC.

PREPARATION OF ANIMALS

36 eight-week-old male albino rats weighing around 180-200g were purchased from Management and Science University, Shah Alam, Malaysia. They were housed in the institutional experiment animal laboratory. Before being used in the experiment, the rats were being adapted for 7 days in order to get used to the environment. The rats were kept in cages in the room maintained at 25-29ºC and 55-65% relative humidity with a 12-hour light-dark cycle and were supplied with a standard pellet diet and water *ad libitum*. All the animal procedures were carried out in strict compliance with the institutional animal ethical committee regulations.

PREPARATION OF HIGH FAT DIET

Commercially available rats pellet was purchased from Management and Science University, Shah

Alam, Malaysia and used as the normal diet. The high fat diet was formulated by adding 2kg cheese powder into 10kg of powdered normal diet and made into pellet again.

EXPERIMENTAL DESIGN

All rats were randomly divided into six groups with each group containing 6 rats. Group 1 served as a negative control group in which the rats were being fed with standard pellet diet and water *ad libitum* throughout the experiment. The rats in group 2, 3, 4, 5 and 6 were supplied with high fat diet throughout the experiment to induce hyperlipidemia. After 2 weeks induction of hyperlipidemia, group 2 hyperlipidemic rats were force fed orally with distilled water. On the other hand, group 3 hyperlipidemic rats were treated with *Aegle marmelos* extract dose of 250 mg/kg body weight and group 4 hyperlipidemic rats were administered *Ocimum basilicum* extract dose of 800 mg/kg body weight. While, group 5 hyperlipidemic rats were treated with the combination of *Aegle marmelos* extract dose of 250 mg/kg body weight and *Ocimum basilicum* extract dose of 800mg/kg body weight. And, group 6 rats were treated with 10 mg/kg standard drug of atorvastatin. The treatment was given orally to the rats and this procedure was carried out daily for 2 weeks. All the five groups were kept on the same high fat diet throughout drug treatment. On the last day of treatment, after 1 hour of dosing, the serum total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol were estimated.

BIOCHEMICAL ANALYSIS

At the end of treatment, all non-fasted animals are anaesthetized by diethyether solution and blood samples were collected via cardiac puncture using 23G needles and 3-ml syringes. Blood samples obtained were used to collect serum. And, serum was used to estimate serum total cholesterol, triglycerides and high-density lipoprotein by using Enzymatic Colorimetric Test Kits. Low-density lipoprotein cholesterol was calculated using the Friedewald formula:

LDL-C = TC – [HDL-C + TG/5]

STATISTICAL ANALYSIS

The experimental results were expressed as mean ± SEM and statistically analyzed by using one way analysis of variance (ANOVA) SPSS software version 20 followed by Dunnet's Multiple Comparison Test. Values were considered significant at $p<0.05$.

RESULT AND DISCUSSION

EFFECT OF *Aegle marmelos* AND *Ocimum basilicum* ON SERUM TOTAL CHOLESTEROL LEVEL

The level of serum total cholesterol of normal and hyperlipidemia rats were measured during this study. As shown in Figure 1, there is no significant differences ($P>0.05$) in the level of serum total cholesterol of basal diet group throughout the study. Positive control group with only hyperlipidemic rats is significantly higher than the negative control group which the basal diet group. The treatment with individual and combination extract of *Aegle marmelos* and *Ocimum basilicum* revealed a significant lowering of serum total cholesterol when compared to the control group of high fat diet. The *Aegle marmelos* treatment group had slightly better effect than the treatment group of *Ocimum basilicum* The combination treatment of *Aegle marmelos* and *Ocimum basilicum* group is more significantly reduced in total cholesterol compared to the individual treatment group of *Aegle marmelos* and *Ocimum basilicum*. The lipid lowering effect of the standard hypolipidemic drug, atorvastatin given to high fat diet fed rats was significantly higher

than that exhibited by both extracts. The standard drug treated group is significantly better than the individual extract treatment groups but slightly better than the group of combination treatment of the both extracts.

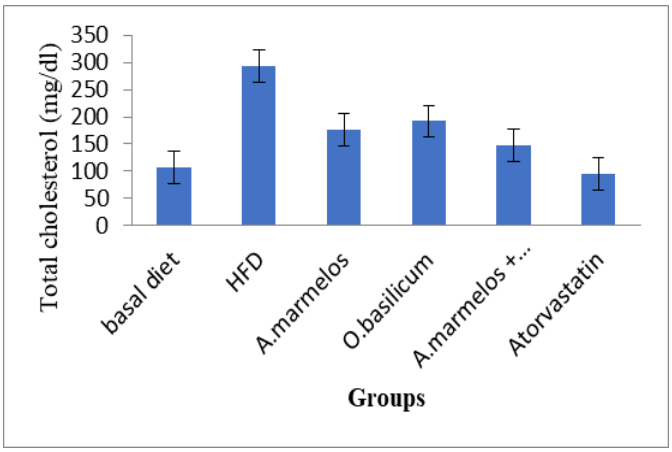

Figure 4.1 The serum total cholesterol levels in control and experimental rats treated with *Aegle marmelos* and *Ocimum basilicum* extract. All values are expressed mean ± standard deviation.

EFFECT OF *Aegle marmelos* AND *Ocimum basilicum* ON SERUM TRIGLYCERIDE LEVEL

Triglyceride level was significantly reduced as compared with high fat diet control group. Decrease in triglyceride level were observed due to treatment of *Aegle marmelos* and *Ocimum basilicum* and values are expressed in Figure 4.2 ($P<0.05$). The triglyceride level for the high fat diet group which serves as positive control group is significantly higher compared to the basal diet control group. There is a significant decrease of triglyceride level in the group treated with *Aegle marmelos* only, group treated with *Ocimum basilicum* only, the group of combination treatment of *Aegle marmelos* and *Ocimum basilicum* extract. The result of triglyceride level shows more significant decrease in the treatment group of the combination of *Aegle marmelos* and *Ocimum basilicum* compared to individual treatment of the extract. The *Aegle marmelos* treatment group had slightly better effect than the treatment group of *Ocimum basilicum.* The atorvastatin treated group shows slightly better result than the group of combination treatment of *Aegle marmelos* and *Ocimum basilicum* extract and significantly better result compared to the groups of individual treatment of the *Aegle marmelos* and *Ocimum basilicum* extract.

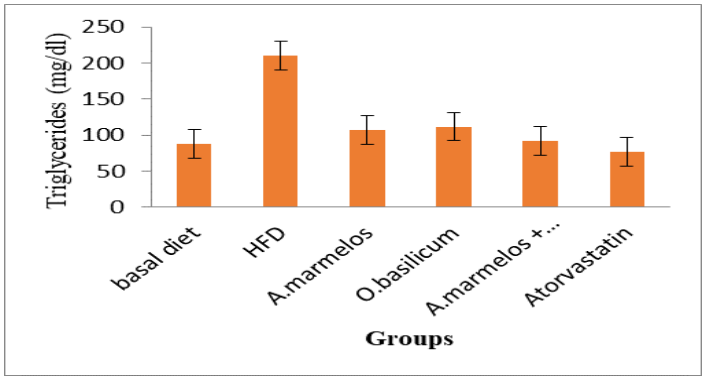

Figure 4.2 The serum triglyceride levels in control and experimental rats treated with

Aegle marmelos and *Ocimum basilicum* extract. All values are expressed mean ± standard deviation.

EFFECT OF *Aegle marmelos* AND *Ocimum basilicum* ON SERUM

LOW- DENSITY LIPOPROTEIN CHOLESTEROL

Differences in the serum LDL-C levels of all groups were observed at the end of treatment (P<0.05) as shown in Figure 4.3. Serum LDL-C level were significantly increase in high fat diet group when compared with basal diet group. Oral administration of individual and combination aqueous extract of *Aegle marmelos* and *Ocimum basilicum* had significantly reduced the serum LDL-C levels. The level of LDL-C in the treatment group of the combination of *Aegle marmelos* and *Ocimum basilicum* is more significantly reduced compared to individual treatment of the extract. The *Aegle marmelos* treatment group had slightly better effect in reducing LDL-C than the treatment group of *Ocimum basilicum*. The atorvastatin treated group shows a significantly better result when compared to the groups of individual treatment of the *Aegle marmelos* and *Ocimum basilicum* extract. The atorvastatin treated group also revealed a slightly better result than the group of combination treatment of *Aegle marmelos* and *Ocimum basilicum* extract.

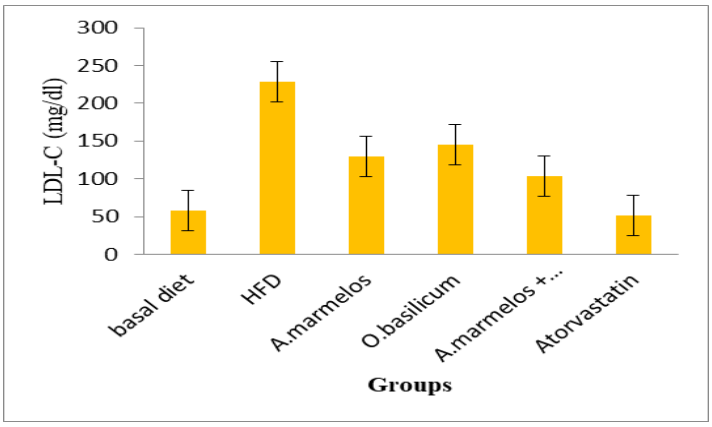

Figure 4.3 The serum LDL-C levels in control and experimental rats treated with *Aegle marmelos* and *Ocimum basilicum* extract. All values are expressed mean ± standard deviation.

EFFECT OF *Aegle marmelos* AND *Ocimum basilicum* ON SERUM HIGH- DENSITY LIPOPROTEIN CHOLESTEROL

The effect of aqueous extract of *Aegle marmelos* and *Ocimum basilicum* on serum HDL-C levels is shown in Figure 4.4. The high fat diet shows a significant reduced in serum HDL-C when compared to basal diet group (P<0.05). In hyperlipidemia rats, significant increase in HDL-C level was observed after the oral administration of individual and combination aqueous extract of *Aegle marmelos* and *Ocimum basilicum*. The *Aegle marmelos* treatment group had slightly better effect in increasing HDL-C than the treatment group of *Ocimum basilicum*. The level of HDL-C in the treatment group of the combination of *Aegle marmelos* and *Ocimum basilicum* is more significantly increase compared to individual treatment of the extract. The standard drug treated group yield significantly better result than the individual extract of *Aegle marmelos* and *Ocimum basilicum* treatment groups. The standard drug treated group also shows a slightly better result than the group of combination treatment of *Aegle marmelos* and *Ocimum basilicum* extract.

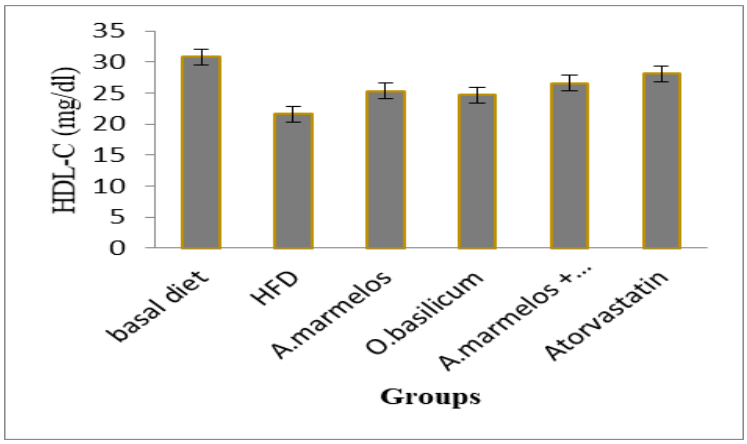

Figure 4.4 The serum HDL-C levels in control and experimental rats treated with *Aegle marmelos* and *Ocimum basilicum* extract. All values are expressed mean ± standard deviation.

DISCUSSION

Researchers have proven and reported atherosclerosis and cardiovascular impairments caused by increased concentration of overall amount of cholesterol as well as low-density lipoprotein(LDL-C) in human (Dominiczak, 1998). Oxidative modification of LDL-C appears to be the significant role in initiation and progression of atherogenic changes in aorta. The agent of serum cholesterol lowering and scavenge or inhibit free radicals formation have gained wide therapeutic value (Jain et al., 2007).

Modern lipid lowering agents like simvastatin and atorvastatin are widely used by the patients. These agents are expensive and cause a number of adverse effects. The most important adverse effects of the agents are liver and muscle toxicity. Besides, the modern lipid lowering agent also causes hypothyroidism, renal insufficiency, hepatic dysfunction, advanced age and serious infections (Khyati., 2010).

Based on various existed researches, it is shown that high fat diet induces elevation in LDL-C, cholesterol and triglyceride.According to Durrington(1995), hyperlipidemia are caused by ingestion of foods that contain high amount of lipids compared to total calories in 4:1 ratio or more than 10% saturated fat. (Defronzo et., 1992).

In the present study, the high fat diet rat pellet supplemented with additional 2% cholesterol in the normal diet contrasted with normal rat pellet which did not include any additional. Serum total cholesterol, triglyceride and LDL-C were higher and HDL-C was lower in HFD rats group than in the normal diet rats group. Administration of either *Aegle marmelos* or *Ocimum basilicum* aqueous extracts to HFD induced rats significantly reversed the hyperlipidemic effect. But the lipid lowering effect is not to the same extent to which the standard hypolipidemic drug, atorvastatin. Lipid lowering drugs in the market fall into various categories which including those that inhibit HMG CoA reductase, sequester bile acids, reduce intestinal fat absorption and activate lipoprotein lipase. The active compound present in the *Aegle marmelos* and *Ocimum basilicum* extracts may have acted in one or more of these ways.

There are several limitations that cannot be avoided present during the study. The mechanisms

underlying these associations are still to be explored. Histological study was not performed to determine the effectiveness of the extracts in the liver. This is due to the lack of time to conduct the study. Other than that, the leaves of *Aegle marmelos* and *Ocimum basilicum* are not identified and authenticated by Forest Research Institute Malaysia (FRIM). Due to the lack of instruments and chemicals, the biochemical analysis to determine total cholesterol, triglycerides, LDL-C and HDL-C cannot be performed as soon as the serum withdrawn.

Hyperlipidemia has been well documented to increase production of reactive oxygen species (ROS) by polymorphonuclear leukocytes and monocytes (Wilson and Gelb., 2002). According to Flora (2007), these ROS cause damage to subcellular structures including membrane components, DNA and certain proteins. The damage contributes to the etiology of diseases associated with hyperlipidemia such as cardiovascular diseases and type 2 diabetes. Since the *Aegle marmelos* and *Ocimum basilicum* have the antioxidant properties, future study can be done to evaluate the antioxidant activity.

This study showed that aqueous extract of *Aegle marmelos* and *Ocimum basilicum* reduces the level of serum total cholesterol, triglyceride and LDL-C and at the same time increases serum HDL-C level in male albino rats. Aqueous extract of *Aegle marmelos* and *Ocimum basilicum* contains active components which act on the serum lipid profile in the rats. Further studies are required to gain more insight into the possible mechanism of action.

CONCLUSION

In a nutshell, there is lipid lowering properties in the aqueous extract of *Aegle marmelos* and *Ocimum basilicum* and the effect exhibited is almost the same when the extracts are given individually. In contrast, combination treatment aqueous extraction of *Aegle marmelos* and *Ocimum basilicum* produces more significant better effect than individual treatment of the extracts. Thus, aqueous extract of *Aegle marmelos* and *Ocimum basilicum* can serve as an alternative source in the management of hyperlipidemia causing diseases.

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