

**Hypolipidemic potential of the ethanolic extract of
Caesalpinia Pulcherrima Linn. (Kabalyero) flowers in hyperlipidemic
Sprague-Dawley rats**

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Abstract - Heart diseases substantially top the major causes of mortality in the Philippines. Hyperlipidemia is considered the greatest risk factor of a coronary heart disease. In recent years, various herbal medicines have been studied and are proven to have beneficial effects against it. Pharmaceutical active ingredients like sterols, glycosides and flavonoids are claimed to reduce hyperlipidemia. In previous studies, *Caesalpinia pulcherrima* phytochemically showed the presence of the said contents. The present study was undertaken to investigate the effects of *Caesalpinia pulcherrima* ethanolic flower extract in high-fat diet induced hyperlipidemic rats for 37 days to evaluate its antihyperlipidemic activity. Atorvastatin (10 mg/kg) was used as a standard drug. Both 200 mg/kg and 400 mg/kg proved a constant lipid lowering activity on fasted hyperlipidemic rats. Moreover, 200 mg/kg is most efficient in decreasing triglyceride and low density lipoprotein cholesterol and enhancing high density lipoprotein cholesterol; while 400 mg/kg is most exceptional in lowering total cholesterol and very low density lipoprotein cholesterol. Consequently, both 200 and 400 mg/kg *Kabalyero* flower extract is more effectual and exhibited the same therapeutic outcome with atorvastatin. For that reason, the findings obtained in the present study demonstrates that the extract of *Caesalpinia pulcherrima* (*Kabalyero*) possess a promising hypolipidemic activity in hyperlipidemia thus preventing its occurrence.

Keywords - antihyperlipidemic, hypolipidemic, *Caesalpinia pulcherrima*, flavonoids

INTRODUCTION

Hyperlipidemia (characteristic high level of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) along with low level in high-density lipoprotein (HDL) cholesterol) contributes to the prediction of coronary heart disease (CAD) (Ghule et al., 2009). Hyperlipidemia associated diabetic and liver disorders are considered to cause thrombotic, atherosclerotic cardiovascular disease (Adeyemi et al., 2009) related to ischemic heart disease such as hypercholesterolemia and hypertriglyceridemia (Saravanakumar et al., 2010). The epidemiologic data shows that almost 12 million people die of cardiovascular diseases each year all over the world (Chen and Li, 2007). Therefore, it is very important to pay consideration to early stage prevention and control of hyperlipidemia in a comprehensive way (Chen and Li, 2007). The reduction of the risks of the disease would be attained through the consumption of flavonoids and their glycosides. (Chen and Li, 2007). This could be possible through the use

of plant materials and extracts which are characterized by minimal adverse effects and multiple targets in preventing and curing hyperlipidemia (Feng et al., 2010).

Caesalpinia pulcherrima belonging to the family Caesalpinaceae/Leguminosae (Pawar et al., 2009a; Das et al., 2010; Chakraborty et al., 2009; Sudhakar et al. 2006) is a leguminous, perennial, large shrub or small tree that is widely distributed in the tropics (Zhao et al., 2004; Srinivas et al., 2003). A variety of extracts of *Kabalyero* contains phenolic acids and flavonoids, each of which has their own beneficial actions on human health (Pawar et al., 2009a). The ethanol extract of its leaves has reported to demonstrate anticonvulsant effects due to the presence of flavonoids, glycosides and tannins (Kumar et al., 2009). Isolated flavonoids, isovoucaperol, and sitosterol of this plant is observed to have anti-inflammatory, neuropharmacologic, and analgesic activities (Pawar et al., 2009a; Rao et al., 2005; Bose et al., 2011; Patel et al., 2010; Chakraborty et al., 2009). Its antiviral ability (Chiang et al., 2003) can also be detected through the said components together with cassane diterpenoids, pulcherrins, and neocaesalpins (Pranithanchai et al., 2009). Furthermore, its leaf extracts which is indicative of hydrocyanic acid, tannins, benzoic acid and flavonoids can be used as a potential antimicrobial and antipyretic agent (Chakraborty and Kaushik, 2009; Pawar et al., 2009).

Aqueous and methanol extracts of *C. pulcherrima* has significant antibacterial activity (Parekh et al., (2005); Parekh et al., (2006). In addition, this plant can be utilized with purgative, stimulant, cytotoxic, and antioxidant potential using extracts from its flower and other aerial parts (Rao et al., 2007; Pawar et al. 2009b). The aerial contents also possess a high percentage of peristaltic inhibition which helps treat gastrointestinal disorders (Calzada et al., 2010). Its seeds served as a good source for the minerals involved in bone formation like iron (Yusuf et al., 2007). It has also been reported that its flower extracts contained an especially high concentration of gallic acid (Samee and Vorarat, 2007). Moreover, birds-of-paradise has also been used as anti-diabetic in Nigeria (Olabanji et al., 2008). Another study includes the evaluation of mucilage of *C. pulcherrima* as binder for tablets (Selvi et al., 2010).

Since flavonoids from different plants are proven to have hypolipidemic effect (Feng et al, 2010; Chen and Li, 2007; Koshy et al, 2001; Bansal et al., 2011), and studies have shown that *Caesalpinia pulcherrima* plant contains high flavonoid contents (Srinivas et al., 2003; Maheswara et al., 2006; Rao et al., 2008), an attempt has been made to investigate the hypolipidemic potential of ethanolic extracts of *Caesalpinia pulcherrima* L. plant in high-fat diet-induced hyperlipidemic rats by: (a) estimating serum lipid profile; (b) estimating biochemical parameters and (c) determining the weight gain by experimental animals.

MATERIALS AND METHODS

Reagents and Materials

Eight kilograms of Kabalyero flowers will be collected from Rosario, Batangas. Vouchers of the plant specimen will be submitted to the Herbarium of University of Santo Tomas, Espana Manila for authentication. Lipid profile test kits will be purchased from Sigma-Aldrich, MO, USA.

Preparation of the Extract

Flowers of *Caesalpinia pulcherrima* will be dried in shade, powdered and stored in an air tight container at room temperature. Dried flower powder will then be extracted with ethanol (95%) using soxhlation method. The extract will be concentrated to dryness using rotary evaporator and preserved in a refrigerator. Aliquot portions of the ethanolic extract of *Caesalpinia pulcherrima* flowers (CPEE) will be weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day of our experiments. Doses of the extract will be prepared according to body weight of the animals (Kumar et al., 2009).

Determination of Phytochemicals

The qualitative determination of phytochemicals will be confirmed by the Industrial Technology Development Institute of the Department of Science and Technology, Taguig City, Metro Manila using qualitative chemical determination.

Test Animals

Male Sprague-Dawley Rats, weighing 150-200 g, will be obtained from Bio Philippines Manila. These will be housed in cages under controlled temperature ($22 \pm 3^\circ\text{C}$) and a 12-h light/12-h dark cycle will be maintained. The animals will be allowed to acclimatize to the environment for 7 days and will be supplied with a standard pellet diet and water will then be given ad libitum. The animals will be fasted for 12 hours with free access to water before lipid profile determination. (Feng et al., 2010). All procedures that will require the use of laboratory animals will be secured from the Bureau of Animal Industry (BAI) for approval.

Induction of Hyperlipidemia

A total of 24 rats will be fed with basic diet for 1 week in the experimental environment. Once they had adapted to the environment, the animals will be divided into two groups (normal and high-fat diet induced). Four rats will be selected randomly as the normal control group (NC); these will be fed with basic diet (Feng et al., 2010). Other animals will be fasted for 12 h prior to the induction of high-fat diet as presented in Table 1. Ten grams of diet will be administered orally to each animal every day. Development of hyperlipidemia will be confirmed by measuring lipid profile 5 days after the administration of high-fat diet. Rats with cholesterol level of above 94.06 ± 8.66 mg/dl will be considered to be

hyperlipidemic and will be used for the studies (Ghule et al., 2009). If the lipid profile is not yet elevated, additional five days of feeding will be conducted. Then, another lipid profile test will be done until the hyperlipidemic level has been achieved. In addition, all animals will be given the said diet during the course of the experiment.

Table 1
Compositions (%) of the experimental diets (Feng et al., 2010).

| Ingredients | Basic diet (%) | High-fat diet (%) |
|--------------|----------------|-------------------|
| Corn meal | 30 | 26.3 |
| Soybean meal | 20 | 17.5 |
| Wheat bran | 25 | 21.9 |
| Wheat flour | 16 | 14.0 |
| Fish meal | 5 | 4.4 |
| Bone meal | 2 | 1.8 |
| Yeast powder | 1 | 0.9 |
| NaCl | 1 | 1.2 |
| Cholesterol | 0 | 2 |
| Lard | 0 | 10 |
| Total | 100 g | 100 g |

Experimental Procedure

The rats will be randomized into six groups comprising of four animals in each groups as given below. NSS, Atorvastatin and CPEE will be administered orally once daily for four weeks.

- i. Normal control group (three rats) 0.90 % NSS
- ii. Model control group (three rats) 0.90 % NSS
- iii. Positive control group (three rats) 1 mg/kg B.W. atorvastatin
- iv. Low-dosage group (three rats) 100 mg/kg B.W. CPEE
- v. Medium-dosage group (three rats) 200 mg/kg B.W. CPEE
- vi. High-dosage group (four rats) 400 mg/kg B.W. CPEE

Biochemical Analysis

Every 5 days during the experimental period, animals will be weighed (Pengzan et al., 2003) and blood samples will be collected from the tail vein of each rat (Adeyemi et al., 2009) and centrifuged at 3500 rpm for 15 minutes to obtain serum (Feng et al, 2010). The levels of serum total triglycerides (TGL), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-c) will be determined, enzymatically, using a commercial kit (Elitech, France) in a semi-automated spectrophotometer (Statfax, Awareness Technology, USA). Low-density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c) will be calculated using the Friedewald equation.

(Saravanakumar, et al., 2010; Adeyemi et al., 2009). These parameters will be done to monitor the lipid profile of each laboratory animal.

Statistical Analysis

Each result will be expressed as means \pm Standard Error. The grouped data was to be evaluated statistically using one-way analysis of variance (ANOVA) and t-test for independent variables. $P < 0.05$ will be considered significant (Feng et al, 2010)

RESULTS

Determination of Phytochemicals

Preliminary phytochemical screening revealed that CPEE showed the presence of flavonoids, sterols, saponins, glycosides and tannins.

Antihyperlipidemic Effects

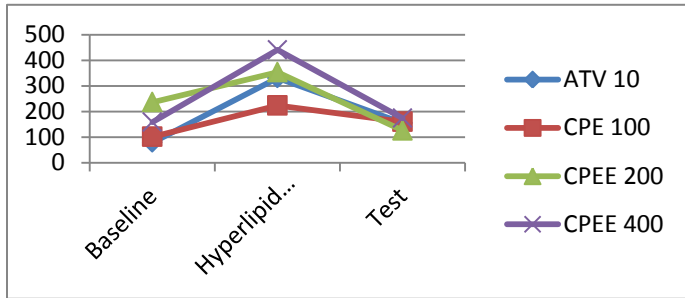
Hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases (Chen & Li., 2007). Several studies revealed that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases. Therefore, prime consideration in the therapy for hyperlipidemia and arteriosclerosis is to attenuate the elevated serum/plasma levels of lipids (Ghule et al., 2009). Currently, available hypolipidemic drugs have been associated with number of side effects (Savaranakumar et al., 2010).

The flower of *Caesalpinia pulcherrima* showed the presence of lupeol, B-sitosterol, myricetin and flavonoids (Pawar et al., 2003) that can inhibit the various stages thought to be involved in the initiation of atherosclerosis (Koshy et al., 2001).

This study aims to determine the effects of *Kabalyero* flowers in the lipid profile (cholesterol, TAG, HDL, LDL and VLDL) of hyperlipidemic Sprague-Dawley rats. The experiment has an interval of seven weeks and blood samples and weight of rats were determined pre-treatment and through the course of the experiment.

To evaluate the anti-hyperlipidemic effect of *Caesalpinia pulcherrima* ethanolic extract, rats were fed with high-fat diet the week after the rats had acclimatized to the environment. When rats showed marked hyperlipidemia, these were then divided into control and experimental groups, each containing three rats, except for the high dosage experimental group which contains four rats.

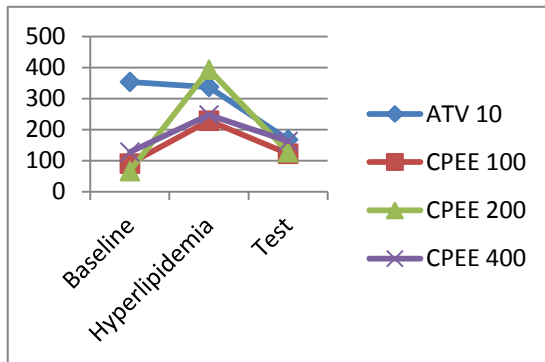
Figure 1
Average Cholesterol levels



The level of total cholesterol increased after the inducement of high-fat diet due to the high cholesterol content of the said diet (Feng, et al. 2010). After treatment with atorvastatin and different doses of the extract (100, 200, 400), cholesterol level produce a total reduction of 178.89, 63, 227.512 and 266.23 mg/dl respectively. These results showed that *Caesalpinia pulcherrima* at a dose of 400 mg/kg B.W had the best hypocholesterolemic potential, followed by 200 mg/kg B.W CPEE, atorvastatin and 100 mg/kg B.W. CPEE.

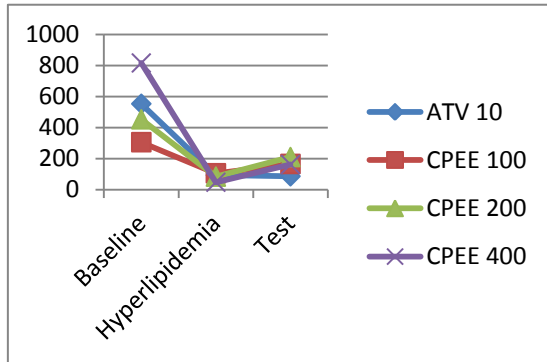
Figure 1

Average Triglyceride levels



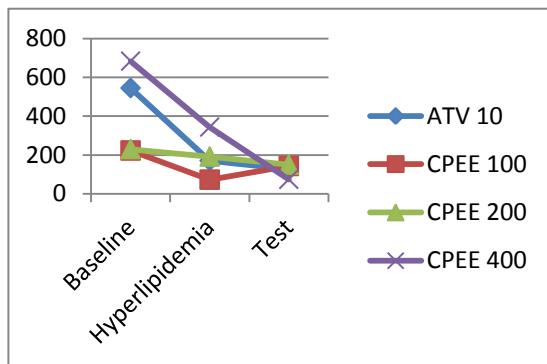
Triglyceride levels had increased after the rats were fed with high-fat diet, except for the third group. Following the treatment of rats with atorvastatin and extract in varying concentrations, triglyceride levels had a decrease of 169.8 mg/dl for atorvastatin, 106.36 mg/dl for low-dosage CPEE, 265.78 mg/dl for medium-dosage CPEE, and 84.958 mg/dl for high-dosage CPEE. Thus, the medium-dosage of *Kabalyero* flower extract is the best in lowering this particular component of lipid. Atorvastatin go after MCPEE, then LCPEE. HCPEE had the least effect on triglyceride levels of the experimental rats.

Figure 1
Average HDL levels



A demonstrable drop in HDL values was observed in all treatment groups, from atorvastatin through *Caesalpinia pulcherrima* ethanolic extract in dosages of 100, 200 and 400 mg/kg B.W, which proves that hyperlipidemia was evident (Ghule et al., 2009) on the rats. The levels suppressed from 93.47 to 86.23 mg/dl after treatment with atorvastatin. On the other hand, an additional of 60.106 mg/dl, 125.84 mg/dl and 114.36 mg/dl were detected after the administration of 100, 200 and 400 mg/kg B.W of CPEE in that order. As a result, 200 mg/kg B.W extract of *Kabalyero* turned out to have the greatest effect on increasing the level of good cholesterol.

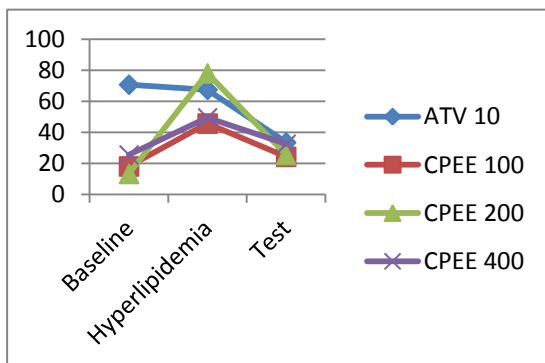
Figure 1
Average LDL levels



All the treatment groups exhibited decreased values after the high-fat diet was given to the rats. The highest dosage of the extract had a large amount of decrease in LDL levels which is 269.34 mg/dl. Atorvastatin and medium

dosage treatment comes next which lowered as follows: 169.59 to 126.36 and 191.33 to 149.494. Low dosage has the least activity on high-fat diet induced hyperlipidemia.

Figure 1
Average VLDL levels



Levels of VLDL had markedly increase when rats were administered with high-fat diet. As with the TAG and HDL levels, 200 mg/kg B.W Caesalpinia pulcherrima ethanolic extract had the most excellent activity in diminishing the VLDL component of the lipid profile. It is followed by atorvastatin, low-dosage CPEE and lastly high-dosage CPEE.

Analysis of the results revealed that Caesalpinia pulcherrima has a potential hypolipidemic activity. Furthermore, at a dose of 200 mg/kg B.W is most outstanding in creating hypolipidemic activity on triglyceride, HDL and VLDL while 400 mg/kg is best in lowering total cholesterol and LDL levels in hyperlipidemic rats stimulated by high-fat diet.

CONCLUSION

The results of the study indicates that the powder of Kabalyero flower is beneficial in exerting antihyperlipidemic effect in serum lipid profiles of hyperlipidemic Sprague Dawley rats in terms of lowering the TC, TAG, LDL and VLDL simultaneously with increasing HDL levels.

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