Assessment and Evaluation of the Leaf Extract of *Begonia barbata* to the Reduction LDL-Cholesterol in Carbamazepine Induced Obese Rats

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**ABSTRACT**

Scientific endeavor has made it possible to discover and synthesize lipid lowering drugs but, in most of the cases, their beneficial effects are overshadowed by their adverse effects. Hence, research interest on screening of medicinal plants has intensified in recent years with a view of discovering potential antioxidants, lipid and glucose lowering phytochemicals. Four month feeding of carbamazepine (both 5 mg/kg and 20 mg/kg body weight) with normal diet increased the body mass of rats. Low density lipoprotein (LDL) cholesterol level was increased based on the oral execution of carbamazepine. But high density lipoprotein (HDL) cholesterol level and weight of liver increased slightly and the level of triacylglycerol (TG) and total cholesterol (TC) level remain unchanged. Nonetheless, the *Begonia barbata* feeding with normal diet reduced the carbamazepine induced obesity at both high and low doses. The level of LDL cholesterol and liver weight was significantly decreased due to the oral execution of *B. barbata* together with normal diet and carbamazepine, where HDL level was changed but not significantly.

**Keywords:** Assessment, *Begonia barbata*, Leaf extract, LDL-Cholesterol, Obese Rats, HDL, and Carbamazepine.

**INTRODUCTION:**

Obesity is most commonly caused by a combination of excessive food intake, lack of physical activity and genetic susceptibility (Yazdi et al., 2015). Obesity progresses towards metabolic syndrome which is defined by a constellation of interconnected physiological, biochemical and clinical factors including dyslipidemia, hypertension, and diabetes, pro inflammatory and pro thrombotic state. These conditions are directly linked to higher level of LDL cholesterol, lower level of HDL cholesterol, oxidative stress and elevated blood glucose. On average, obese person have higher energy expenditure than their normal counterparts due to the energy needed to maintain a raised body mass (Kushner, 2007). Obesity results from an imbalance of food intake, basal metabolism, and energy expenditure. At an individual level, multiple endogenous or environmental causes could lead to obesity (Flier, 2014). However, in most cases, a coalescence of excess energy intake and availability of energy-dense meals is thought to be the main contributor to obesity (Wisse and Kim, 2007).

In the past 20–30 years, there have been many studies characterizing the responses of animals exposed to high-fat diets (Zhang, 2010). In the mice, the A/J mouse and C57BL/KsJ mouse are relatively resistant to high-fat diet when compared to C57BL/6J mouse (Rossmeis, 2005). The B6 mouse is a distinctly better
model mimicking human metabolic insanity that are showed in obesity because when fed ad libitum with a high-fat foods, these mice progress obesity, hyperinsulinemia, hyper-glycemia, and hyper-tension, but when fed ad libitum to chow diet, they remain lean without metabolic abnormalities (Collins and Martin, 2004). The high-fat diets effects on blood glucose level are more contrary and based on the type of nutritive regimen. Hyperglycemia usually develops within 4 weeks of a high-fat diet (Sato, 2010).

Inhibition of Akt and mTOR pathway by rapamycin has effects in longevity (Cox and Mattison, 2009; Firoz et al., 2016), adipocyte differentiation, and obesity (Chang, 2009). Recent studies have shown that S6K1-deficient mice and Akt1 knockout mice exhibit are prevented from diet-induced obesity through model of murine high-fat diet induced obesity described below (Um, 2004). While a specific amount of fat is required for normal physiological functioning (Powers and Howley, 2001), obesity and overweight are correlated with numerous health problems, viz heart diseases, diabetes, stock, hyper-lipidemia, osteo-porosis, gout, cancer and osteoarthritis (Corbin et al., 2002; Akinpelu and Akinola, 2009; Gbiri et al., 2010). It is used in schizophrenia along with other medications and as a second-line agent in bipolar disorder (The American Society of Health-System Pharmacists, 2015). Carbamazepine shows to functioning as well as phenytoin and valproate (Nolan et al., 2017). There is few argument concerning the teratogenic effects of carbamazepine, but maximum of scientists believe that malformations associated with maternal usage of can carbamazepine be frequently cleaved into key malformations viz craniofacial defects, heart defects, and neural tube defects and minor anomalies such as growth retardation, developmental delay, and hyperplasia of the nails or distal phalanges (Jallon and Picard, 2001).

This interaction has been demonstrated through hypothalamic-mediated mechanisms in rat models with epilepsy (St-Pierre et al., 2009), amygdala-mediated mechanisms in kindled rats (Hum et al., 2009), and hippocampus and fornix-mediated mechanisms in rats (Davidson et al., 2009; Sharif et al., 2019) and humans (Metzler-Baddeley et al., 2013). One of those drugs, vigabatrin, is normally better tolerated than the older compounds, but has recently been shown to be more frequently associated with weight gain than carbamazepine (Chadwick, 2003). However, they are not recommended for usage due to the toxicity associated with them (Patel et al., 2013). Plants synthesize several antioxidants to them against damage caused by active, reactive oxygen species (ROS) (Rad and Sen, 2013; Rad and Mohsenzadeh, 2014).

These compounds include chlorophyll derivatives, alkaloids, essential oils, phytosterols, phenolics and polyphenolics (Rad and Alfatemi, 2001). Some of the antioxidants that have been isolated from plants include curcumin, eugenol, flavonoids, coumarins, carotenoids, tannins, gallic acid, limonene, terpenoids, Β-sitosterolete (Sha-rifi et al., 2014; Gupta and Sharma, 2006). Tubers of this plant are found to be rich in starch, mucilage, sugar, phosphate, chloride and a glucoside-loroglossin (Pant and Rinchen, 2011; Sarkar et al., 2015). The gum resin comprising of mainly Β-boswellic acids along with 11-keto-Β-boswellic acids and their ace-tates has been focused to have anti-bacterial activity (Raja and Ali, 2011). The gum resin has been reported to have a definite role in the treatment of rheumatoid arthritis and boswellic acid has been manifested as most potent inhibitor of 5-lipoxygenase, a key enzyme involved in inflammation (Siddiquedi et al., 2011). Anti-bacterial activity against gram-positive bacteria like S. aureus, B. subtilis and all gram-negative bacteria have been demonstrated in a recent study (Singh and Khajuria 2007; Das 2012; Mosaib et al., 2020).

Numerous clinical studies performed in hyperlipidemic subjects have manifested a beneficial effect of RYR extract supplements (Bogsrud and Ose, 2010) resulting in a decrease in plasma total cholesterol (TC), LDL-C and triacylglycerols (TG). In some cases, an increase in high-density-lipoprotein cholesterol (HDL-C) was also demonstrated (Liu and Zhang, 2006). Depending on the Monascusstrains use and the conditions of fermentation, they may hold in polyketides called monacolins (Heber and Lembertas, 2001). A data review and a meta-analysis also revealed a more favorable action of policosanols on serum lipids compared with phyto-sterols and stanols, and an equivalent effect to statins (Chen and Wesley, 2005; Gouni-Berthold and Bert-hold, 2002). Oxidized LDL-cholesterol
It has been shown that men and women supplemented with daily doses exceeding 100 IU of vitamin E for over 2 years showed a significant reduction in heart attacks (Bowen and Borthakur, 2004).

In the recent meta-analysis that indicated a TG-lowering effect of plant stanols (Naumann et al., 2008; Rony et al., 2019), significant interaction was observed between baseline TG concentrations and plant stanol intake, resulting in larger TG reductions (shown in mmol/L) with more baseline TG concentrations. As HDL-C metabolism is closely related to that of TG via the action of the cholesterol-ester transfer protein (CETP) (Chapman and Le, 2010) the effect of PS-enriched food consumption on HDL-C concentrations was also evaluated. In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), treatment with lovastatin resulted in a 6% mean increase in HDL-cholesterol (Gotto and Bocuzzi, 2000). Healthy diet and physical exercise have beneficial effects on the improvement of the serum lipid profile, with reduction of total cholesterol (TC), TG, and LDL-C with increase of HDL-C (Chapman, 2004; Kelly, 2010; Uddin et al., 2016). The mixed controlling role of these two food models has been defined as “Mediterr Asian diet” (Nicklas and O’Neil, 2014; Pallauf and Giller, 2013). In two recent works (Laing et al., 2000) manifested that the quantum growth of photosystem II (PSII) and the oxidation state of PSII (qP) decrease as PPFD increases, while electron transport ratio and non-photo-chemical quenching enlarge. The aim of this work was to examine the ability of carbamazepine to induce obesity and related dyslipidemia in female Wister rats. And subsequently to evaluate ability of B. barbata leaf extract to reduce carbamazepine induced weight gain and dyslipidemia (Shahen et al., 2019; Habib et al., 2019; Talukder et al., 2020).

MATERIALS AND METHODS:

Animal - The whole study protocol to carry out several experiments related to this project will be approved by Ethical Committee of Dept. of Pharmaceutical Sciences, North South University (NSU), Bangladesh, for animal care and experimentation. For investigating the effect of each leaf extract, 30 days old rats (for each group) having similar body weight will be obtained from animal production unit of Dept. of Pharmaceutical Sciences, North South University, and will be housed at room temperature of 22±3°C, humidity of 55%, in 12 h dark/light cycles with standard laboratory chow diet and drinking water ad libitum (Khatun et al., 2016). The animal’s usage in this experiment was Wistar rat (Rattus norvegicus) approved by Dept. of Pharmaceutical Sciences, NSU. There were 25 female normal Wistar rats used & age was 30 days. The animals housed were under controlled environment room 22–25°C and humidity (50%) and a 12/12 h dark/light cycle.

Experiment design - Wistar rats were kept in animal house under room temperature and divided in 5 groups. Each group contains 5 rats. Different type of food is given to the different group and observed their body weight, liver weight and cholesterol level.

Group-1: Normal diet

Group-2: Normal diet + Carbamazepine low dose (5 mg/kg) orally everyday

Group-3: Normal diet + Carbamazepine high dose (20 mg/kg) orally everyday

Group-4: Normal diet + Carbamazepine high dose (5 mg/kg) + B. barbata (10 mg/kg) orally everyday

Group-5: Normal diet + Carbamazepine high dose (20 mg/kg) + B. barbata (10 mg/kg) orally everyday

Blood sample collection - Rats were anesthetized with ketamine (100 mg/kg body weight, 0.1 ml) before sacrificing. Then blood was collected from aorta and heart. Then the blood was transferred to the Eppendorf tube and the blood was centrifuged at 10,000 rpm keeping temperature at 4°C for 10 minutes to separate the blood cells as pellet. Finally serum was collected carefully by micropipette. The liver collected carefully by opening the abdominal cavity and wet weight was measured by electronic balance.

Measurement of body weight of rats - The body weight was taken daily when rats were 4 weeks old and was continued up to 20 weeks and this body weight was taken by electronic balance.

Measurement of HDL cholesterol value of rats

HDL Cholesterol LS test - Phosphotungstic acid and magnesium ions fastidious precipitating all lipoproteins without the HDL fraction – cholesterol.
present in the supernatant can be determined by the same method used for total cholesterol. The reactions are as follows:

1) ApoB containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO4 --> soluble non-reactive complexes with apoB-containing lipoproteins
2) HDL-cholesterol esters PEG-cholesterol esterase > HDL-unesterified cholesterol + fatty acid
3) Unesterified chol + O2 PEG-cholesterol oxidase > cholestenone + H2O2
4) H2O2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'- succinyl ethylene diamine+ H2O + H+ Peroxidase > quinoneimine dye + H2O

Reagents
R1 Precipitating reagent - Phosphotungstic acid Magnesium chloride 0.02 mol/L1 mol/L2

Determination of HDL-Cholesterol

|                         | Blank (ml) | Standard (ml) | Sample (ml) |
|-------------------------|------------|---------------|-------------|
| Distilled water         | 0.05       | ........       | ........     |
| Standard (R2)           |            | 0.05          | ........     |
| Supernatant             | ........    | ........       | 0.05        |
| Working reagent         | 1.00       | 1.00          | 1.00        |

Mix, incubate at 37ºC for 10 min. Read absorbance of sample (A Sample) and standard (A Standard) against the blank at 490 nm.

HDL – Cholesterol in sample (mg / dl)

Measurement of LDL cholesterol level - Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is determined from calculated values of sum cholesterol, triglycerides and HDL cholesterol

According to the relationship

[LDL-chol] = [total chol] - [HDL-chol] - [TG]/5

Where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/Dl

Reagents
R1 Precipitating reagent - HEPARIN0.68g/l

SODIUM CITRATE 0.064mol/l
STABILIZERS 2%
R2 Standard cholesterol 50 mg / dL (1.29 mmol/L)

Procedure
Each cholesterol standard and sample should be evaluated in duplicate or triplicate. A newly prepared standard curve should be utilized each time the assay is performed. Assay Protocol

a) Add 50 µL of the diluted cholesterol standards or the diluted HDL fraction samples to the 96-well microtiter plate.
b) Add 50 µL of the pursued Cholesterol Reaction to each well and mix the well contents thoroughly.
c) Cover the plate wells to save the reaction from heat. Incubate the plate for 45 minutes at 37ºC.
d) IMMEDIATELY count the plate with a fluorescence microplate counter equipped for exci-
tation in the 530-570 nm order and for emission in the 590-600 nm order.

e) Determine the concentration of cholesterol within samples by differentiating the sample RFU to the cholesterol standard bow.

| Blank (ml) | Standard (ml) | Sample (ml) |
|------------|---------------|-------------|
| Distilled water | ***** | ***** |
| Standard (R2) | 100 ul | ***** |
| Supernatant | ***** | 100 ul |
| Working reagent | 1000 ul | 1000 ul |

Mix, incubate at 37°C for 10 min. Read absorbance of sample (A Sample) and standard (A Standard) against the blank at 490 nm.

**Quality Control -** For accuracy and reproducibility control: - Assayed Multi – Sera, Normal and Elevated.

**Measurement of TG value of rats**

**Triglycerides** - Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which TG is hydrolyzed to yield glycerol.

Glycerol is then oxidized utilizing glycerol oxidase and H₂O₂ one of the reaction goods, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

1. Lipase
2. Triglycerides + 3H₂
   
   O \rightarrow glycerol + fatty acids

3. Glycerokinase
   
   Glycerol + ATP \rightarrow glycerol-3-phosphate + ADP

4. Glycerophosphate oxidase
   
   Glycerol-3-phosphate + O₂ \rightarrow dihydroxyacetone phosphate + H₂O₂

5. Peroxidase
   
   H₂O₂ + 4-aminophenazone + 4-chlorophenol \rightarrow 4-(p-benzoquinone-monoimino) phenazone + 2H₂O + HCl

**Reagent**

R1

Precipitating reagent - p-chlorophenole 2 mol/L; lipoprotein lipase 150000u/l

| Blank (ml) | Standard (ml) | Sample (ml) |
|------------|---------------|-------------|
| Distilled water | ***** | ***** |
| Standard (R2) | 10 ul | ***** |
| Supernatant | ***** | 10 ul |
| Working reagent | 1000 ul | 1000 ul |

Mix, incubate at 37°C for 10 min. Read absorbance of sample (A Sample) and standard (A Standard) against the blank at 490 nm.

**Quality Control -** For accuracy and reproducibility control: - Assayed Multi – Sera, Normal and Elevated.

**Measurement of TC level of rats**

**Total Cholesterol** - Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H₂O₂ is counted quantitatively in a peroxidase catalyzed reaction that yields a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction chronology is as follows:

1. Cholesteryl ester hydrolyase
2. Cholesteryl ester + - H₂O \rightarrow cholesterol + fatty acid
3. Cholesterol oxidase
4. Cholesterol + O₂ \rightarrow cholest-4-en-3-one + H₂O₂
5. Peroxidase
   
   2H₂O₂ + 4-aminophenazone + phenol \rightarrow 4-(p-benzoquinonemonoimino)-phenazone + 4 H₂O

**Samples:** Serum, heperanised plasma

**Procedure**

1. Add 50 µL of the triglyceride samples to the 96-well microtiter plate.
2. Add 50 µL of the prepared triglyceride Reaction to each well and mix the well contents thoroughly.
3. Cover the plate wells to save the reaction from heat. Incubate the plate for 45 minutes at 37°C.
4. IMMEDIATELY count the plate with a fluorescence microplate count equipped for excitation in the 530-570 nm order and for emission in the 590-600 nm order.
5. Examine the concentration of cholesterol within the samples by differentiating the sample RFU to the cholesterol standard bow.
Reagent concentration

R1
Precipitating reagent - Pipes buffer, pH 6.990 mmol/L; Phenol 26 mmol/L; Cholesterol oxidase 200 u/l; Cholesterol esterase 300 u/l; Peroxidase 1250 u/l; 4-aminoantipyrine, 4 mmol/l

R2
Standard cholesterol 200 mg/dL (5.17 mmol/L)

Samples: Serum, plasma collected on heparin

Procedure
Each cholesterol standard and sample should be examined in duplicate or triplicate. A newly prepared standard bow should be utilized each time the assay is performed. Assay Protocol –

a) Add 50 µL of the diluted cholesterol standards or the diluted HDL snippet samples to the 96-well microtiter plate.

b) Add 50 µL of the prepared Cholesterol Reaction to each well, and mix the well contents thoroughly.

c) Cover the plate wells to save the reaction from heat. Incubate the plate for 45 minutes at 37ºC.

d) IMMEDIATELY count the plate with a fluorescence microplate count equipped for excitation in the 530-570 nm order and for emission in the 590-600 nm order.

e) Dertermine the concentration of cholesterol within samples by differentiating the sample RFU to the cholesterol standard bow.

Blank (ml) Standard (ml) Sample (ml)
Distilled water ---- 10ul ----
Standard (R2) ------- 10ul ----
Supernatant ----- ---- 10ul
Working reagent 1000ul 1000ul 1000ul

Mix, incubate at 37ºC for 10 min. Read absorbance of sample (A Sample) and standard (A Standard) against the blank at 490 nm, (490 nm).

Measurement of liver weight of rats - After sacrificing the rat liver weight was taken and liver weight was taken by electronic balance.

RESULTS AND DISCUSSION:
Carbamazepine (CRZ) able to increase weight - Simultaneously B. barbata leaf extract was given to other two groups of rats which were also fed with low dose (ND+CRZLD+BB) and high dose (ND+CRZLD+BB) of carbamazepine daily with normal diet. Feeding of carbamazepine and B. barbata started when rats were 4 weeks old and was continued up to 20 weeks.

Fig 1: Feeding of rats with normal diet (ND), carbamazepine in low dose (ND+CRZLD) and carbamazepine in high dose (ND+CRZLD) with normal diet. Body weights were measured every week by electronic balance and initial (A); and final (B) body weights of different groups of rats are represented in the bar diagram.
**B. barbata able to increase HDL**

In group 1 we can see HDL value is normal due to intake of normal food. Small amount of CBZ (5mg/kg) decrease the HDL value shown in group 2. And large amount of CRZ (20 mg/kg) decrease more HDL value shown in group 3. In case of small amount of BB (5mg/kg) HDL value is increasing that is shown in group 4. Finally large amount of bb (20 mg/kg) increase the HDL value most that is shown in group 5.

![HDL Concentration](image)

Fig 2: Feeding of rats with normal diet (ND), carbamazepine in low dose (ND+CRZLD) and carbamazepine in high dose (ND+CRZLD) with normal diet.

Simultaneously *B. barbata* leaf extract was given to other two groups of rats which were also fed with low dose (ND+CRZLD+BB) and high dose (ND+CRZLD+BB) of carbamazepine daily with normal diet (Fig 2). Feeding of carbamazepine and *B. barbata* started when rats were 4 weeks old and was continued up to 20 weeks. HDL cholesterol was measured of different groups of rats are represented in the bar diagram (Alam et al., 2015).

**B. barbata able to decrease LDL**

In this graph we can see different amount of CRZ (5mg/kg and 20 mg/kg) increase the LDL value as followed by group 2 and 3. And different amount of BB (5mg/kg and 20 mg/kg) is decreasing the value of LDL that is shown in group 4 and 5.

Simultaneously *B. barbata* leaf extract was given to other two groups of rats which were also fed with low dose (ND+CRZLD+BB) and high dose (ND+CRZLD+BB) of carbamazepine daily with normal diet. Feeding of carbamazepine and *B. barbata* started when rats were 4 weeks old and was continued up to 20 weeks. LDL cholesterol was measured of different groups of rats are represented in the bar diagram (Fig 3).

![LDL Concentration](image)

Fig 3: Feeding of rats with normal diet (ND), carbamazepine in low dose (ND+CRZLD) and carbamazepine in high dose (ND+CRZLD) with normal diet.

**B. barbata able to decrease Triglyceride level**

In this graph we can see different amount of CRZ (5mg/kg and 20 mg/kg) increase the TG value as followed by group 2 and 3. And different amount of BB (5mg/kg and 20 mg/kg) is decreasing the value of TG that is shown in group 4 and 5.

Simultaneously *B. barbata* leaf extract was given to other two groups of rats which were also fed with low dose (ND+CRZLD+BB) and high dose (ND+CRZLD+BB) of carbamazepine daily with normal diet. Feeding of carbamazepine and *B. barbata* started when rats were 4 weeks old and was continued up to 20 weeks. TG cholesterol was measured of different groups of rats are represented in the bar diagram (Fig 4).

![TG Concentration](image)

Fig 4: Feeding of rats with normal diet (ND), carbamazepine in low dose (ND+CRZLD) and carbamazepine in high dose (ND+CRZLD) with normal diet.
**B. barbata able to reduce Total cholesterol**

In this graph we can see different amount of CRZ (5mg/kg and 20 mg/kg) increase the TC value as followed by group 2 and 3. And different amount of BB (5mg/kg and 20 mg/kg) is decreasing the value of TC that is shown in group 4 and 5.

**Simultaneously** B. barbata leaf extract was given to other two groups of rats which were also fed with low dose (ND+CRZLD+BB) and high dose (ND+CRZLD+BB) of carbamazepine daily with normal diet. Feeding of carbamazepine and B. barbata started when rats were 4 weeks old and was continued up to 20 weeks. Liver weight was measured of different groups of rats are represented in the bar diagram (Fig 6).

**CONCLUSION:**

Carbamazepine (CRZ) intake is increasing obesity and weight gain of rats. This is the side effect of this drug. Carbamazepine increased appetite deposition of fat in body. On the other hand apply the leaf extract of B. barbata is decreasing that weight of rates. This plant extract can reduce cholesterol. In this study we observed B. barbata can reduce TG, LDL, TC and can increase the LDL value which are good for our health and this plant extract can be play a great role to control weight.

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**CONFLICTS OF INTEREST:**

The author’s declared there are no potential conflicts of the interest.

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