# Anti-hyperlipidemic Activity of Methanolic Extract of *Impatiens Balsamina L.* in Hypercholestrolemic Induced Sprague Dawley Rats

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Keywords : Anti-cholesterol, Anti-hyperlipidemia, *Impatiens Balsamina L*., Simvastatin.

Abstract : Background & Aim: The occurrence of hyperlipidemia, is currently increasing at a remarkable rate throughout the world. Hyperlipidemia graded as one of the greatest risk factors that contributes to the prevalence and severity of life threatening coronary heart diseases. Medicinal plants and their products are safer than their synthetic counterparts, including those involved in the anti-hyperlipidemic drugs statins. *Impatiens Balsamina (IB)* is used medicinally for various ailments. No study was carried out on the anti-cholesterol activity of *IB*. Objective: To study anti-hyperlipidemic activity of methanolic extracts of *IB* (*MEIB*) in hypercholesterolemia induced Sprague Dawley rats. Materials & Method: *MEIB* leaves were prepared using maceration method. Toxicity study was carried out using OECD guidelines. Hypercholesterolemia in rats was induced by using 6% of lard oil, 2% of cheese and egg yolk. Two different doses 200 and 400mg/kg of *MEIB* were used to study for anti-hyperlipidemic activity. Histopathological study was carried out in rats. Results: No mortality was observed even up to 2g/kg. Only 400mg/kg of *MEIB* statistically decreased in total cholesterol ($P<0.05$), LDL-cholesterol ($P<0.05$) and an increase in HDL-cholesterol ($P<0.05$) as compared to the positive control. Histopathology study revealed that 400mg/kg *MEIB* leaves administered group have mild steatosis and no inflammation as compared to control group. Conclusion: *MEIB* could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia.



## 1 INTRODUCTION

The occurrence of hyperlipidemia, is currently increasing at a remarkable rate throughout the world and there is a close connection between the hyperlipidemia and cardiovascular diseases (CVD) which has been well documented (Suanarunsawat et al 2010). Hyperlipidemia represents a spectrum of metabolic disorders that can be found in many humans today. The manifestation of the hyperlipidemia is defined as an abnormal increase in one or more of the serum lipids profile such as either total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) (Bencze et al., 2012). Hyperlipidemia need to be given special attention since it has been one of the greatest risk factors that contributes to the prevalence and severity of coronary heart diseases (Sudha et al 2011). Statins reducing the risk of coronary events, has been shown in large scale studies of both primary and secondary intervention to reduce coronary artery disease. These drugs will correct the modified blood lipid profile by inhibiting the biosynthesis of cholesterol and also by improving the clearance of triglycerides rich lipoproteins (Girija et al 2011). The use of synthetic hypolipidemic drugs may lead a person to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and also abnormal liver function (Subramaniam et al 2011).

Hence, there is a need to find other essential materials from natural source that can give less toxicity or side effects with better safety and efficacy on long term usage. The cost of the therapy also should be taken into consideration. Natural products that come from a plant are used for centuries in order to cure various ailments (Desu, 2013). Besides that, in many cases, medicinal plants and its active ingredients have shown the ability to cure or improve diseases.



Fattepur, S., Nilugal, K., Balan, T., Bacayo, M., Choon, W., Razik, M., Asmani, F. and Yusuf, E.
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DOI: 10.5220/0009842200002406
In *Proceedings of BROMO Conference (BROMO 2018) - Symposium on Natural Product and Biodiversity*, page 1
ISBN: 978-989-758-347-6

Various research studies have been conducted from many natural sources having anti-hyperlipidemic effects from the dietary fiber, plant sterols, herbal extracts, and some yeast extracts (Yoon et al., 2008). Unlike conventional medicines or treatments, herbal treatments are less expensive, more effective in certain chronic conditions, reduced occurrence of adverse effects as well as widespread availability or in other words, it is easily obtained. Lot of pharmacological work have been carried out on the *Impatiens Balsamina L.* extracts. The leaves were selected to investigate the anti hyperlipidemic activity of methanolic extract of *Impatiens Balsamina* (*MEIB*). However there is lack of scientific report, on the anti hyperlipidemic activity of *MEIB*.

# 2 METHODOLOGY

## 2.1 Preparation of Plant Materials

The fresh leaves of *Impatiens Balsamina* L. were obtained from Temerloh, Pahang, Malaysia and were authenticated by Forest Research Institute Malaysia. Fresh plant materials were washed, shade dried for about 7 days and subjected for coarse powder. Then, these dry leaves were powdered using a mechanical grinder. The coarse powder was subjected for methanolic extraction using soxhlet apparatus and extracts were concentrated using rotary evaporator under reduced pressure. The final MEIB was stored in umber colored bottle and kept refrigerator at 4, until use for the pharmacological and phytochemical screening. (Abdelwahab et al 2011)

## 2.2 Animals Maintenance

Healthy male Sprague Dawley rats weight ranging between (150 to 175gm) were selected for the study. The animals were brought form the local vender and are kept in animals for 1 week to acclimatized to the laboratory condition. During the study, they were kept at temperature (25 ±1) $^{O}$C, with relative humidity (50 ± 15) % in 12 h light - dark cycles. They were maintained in standard diet and water ad-libitum. Institutional animal ethics clearance (IAEC) was obtained from management and science university, Malaysia before the study.

## 2.3 Preparation of High Cholesterol Diet

Eggs and high cholesterol cheese were procured from local market at Klang Valley, Malaysia. The lard oil was prepared by rendering method where mutton fats was bought from Masai, Johor Market and chopped to fine cubes. These fat cubes were placed in a large stockpot and heated slowly, delicately over medium heat. The fat was stirred at regular interval for 30min. The high cholesterol diet was prepared by mixing 60ml of melted lard oil, with 2 egg yolk and 20ml of melted cheese. These ingredients were mixed at mild heat water bath to prevent solidification of oil and cheese at room temperature. This high cholesterol diet was fed to the SD rats for 4 weeks according to their body weight by using gavage needle. Each rat was weighed and received 20ml/kg of this semi-solid high cholesterol diet twice daily (Morning and evening) according to table 3 in order to induce cholesterol (Balasubramanian et al 2008).

## 2.4 Toxicity Study

Toxicity study was carried out using the OECD guidelines. Single administration of *MEIB* extracts of 500, 1000 and 2000 mg/kg of the extract was given by intra-gastric intubation by gavage needle to SD rats (n=3) respectively. The rats were observed for mortality and toxicity signs for 14 days. Animals were observed individually at least once during the first 30 min, periodically during the first 4 h, and daily thereafter, for a total of 14 days.

## 2.5 Experimental design

The rats were randomly divided into five groups (n=8). Tail marker was used for identification and all the animals were housed in cage according to their group at ambient temperature. The feeding and drug administration schedule for the five groups was presented in Table 1.

Table 1: The feeding and drug administration schedule for the 5 groups of rats.

| Group Schedule | |
|---|---|
| Group 1 | Normal control rats were fed with the normal diet for 4 weeks. |
| Group 2 | High cholesterol rats fed with high cholesterol diet for 4 weeks. |
| Group 3 | Rats fed with high cholesterol diet for 4 weeks. During the last 2 weeks, daily administration of 200 mg/kg of *MEIB* extracts by intra-gastric intubation. |

| | |
|---|---|
| Group 4 | Rats fed with high cholesterol diet for 4 weeks. During the last 2 weeks, daily administration of 400 mg/kg of *MEIB* extracts by intra-gastric intubation. |
| Group 5 | Rats fed with high cholesterol diet for 4 weeks. During the last 2 weeks, the reference drug Simvastatin at dose of 10 mg/kg was administered by intra-gastric intubation. |

## 2.6 Collection of Blood and Biochemical Analysis

Blood was collected by retro-orbital sinus puncture which is a good choice when less quantity aseptic sample is needed on 1st, 14th and 31st day of the test under mild ether anesthesia Isoflurane 300µL by using drop jar method where the cotton with anesthesia will be placed inside the jar with the SD rats.

These blood samples were tested using the blood cholesterol testing kit in order to check Total cholesterol, LDL-Cholesterol and HDL-Cholesterol for each rat. Diagnostic kits to screen for cholesterol and LDL-Cholesterol was procured from Mercury Pharmacy, Temerloh, Malaysia (Dhulasavant et al 2010).

## 2.7 Histopathological Assessment

The liver sections of the SD rats were fixed in 10% formaldehyde, dehydrated in gradual ethanol (50% to 100%), cleared in xylene and embedded in paraffin. Sections (4 to 5 µm thick) were prepared and stained with hematoxylin and eosin (HE) dye and observed under a microscope. (Arsad et al 2014)

## 2.8 Statistical Analysis

The results were evaluated for statistical significant difference by one-way ANOVA followed by post hoc Dunnett's test using SPSS software version 24. Significant difference was accepted at the level of $P < 0.05$.

# 3 RESULTS

## 3.1 Toxicity Study

There were no toxicity symptoms nor mortality were observed in all the SD rats fed with 500mg/kg, 1 and 2g/kg of the *MEIB* extracts

## 3.2 Biochemical Analysis

### 3.2.1 Effect on Body Weight

Table 2: Average body weight of experimental rats (n=6 per group) are expressed as mean ± S.E.M

| Group | Average Body Weight of Experimental Rats (g) | | |
|---|---|---|---|
| | Week 0 | Week 2 | Week 4 |
| I | 124.83 ± 3.21 | 155.46 ± 3.42 | 173.72 ± 2.98 |
| II | 119.04 ± 2.49 | 185.33 ± 2.98 | 210.75 ± 1.59 |
| III | 129.61 ± 1.08 | 189.84 ± 1.93 | 190.66 ± 2.53 |
| IV | 131.43 ± 3.13 | 183.36 ± 2.95 | 187.39 ± 1.88 |
| V | 128.32 ± 1.84 | 181.68 ± 3.31 | 185.78 ± 1.42 |

### 3.2.2 Effect On Total Cholesterol (TC)

Table 3: Anti-hyperlipidemic effect of *MEIB* extracts 200mg/kg and 400mg/kg in hyperlipidemia induced rats. The values of Total Cholesterol (TC) are expressed as mean ± S.E.M of six rats per group, [a] $P < 0.05$; [b] $P > 0.05$ compared with positive control.

| Groups | Treatment | Total Cholesterol (TC), mmol/L | | |
|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 |
| I | Control with normal diet | 106.88 ± 2.75 | 109.76 ± 6.01 | 111.47 ± 4.71 |
| II | Control with high cholesterol diet | 105.75 ± 4.97 | 139.49 ± 5.53 | 143.51 ± 4.55 |
| III | High Cholesterol diet treated with 200mg/kg of *MEIB* extracts | 103.61 ± 3.28 | 137.09 ± 5.53 | 142.27 ± 2.98[b] |
| IV | High Cholesterol diet treated with 400mg/kg of *MEIB* extracts | 110.47 ± 2.49 | 135.52 ± 9.60 | 133.12 ± 8.44[a] |
| V | High cholesterol diet treated with Simvastatin 10mg/kg | 106.02 ± 4.77 | 140.80 ± 5.72 | 107.44 ± 8.09[a] |

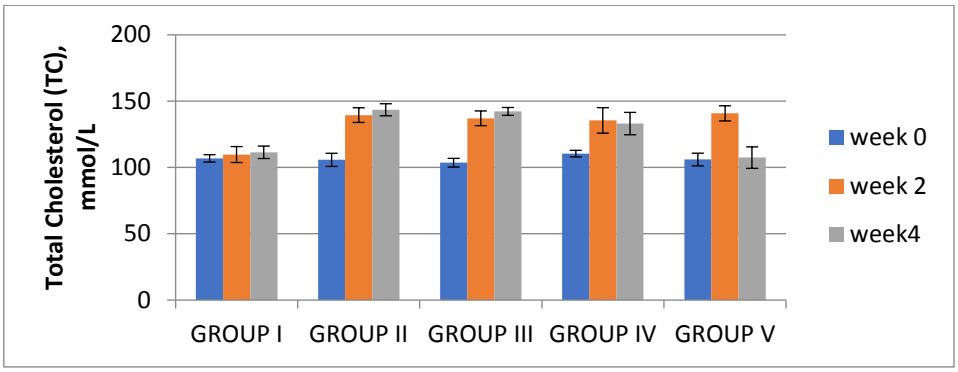



Figure 1: Graph shows the effect of *MEIB* extracts on Total Cholesterol (TC) of the hypercholesterolemia rats according to different time.

### 3.2.3 Effect on Low-Density Lipoprotein (LDL)

Table 4: Anti-hyperlipidemic effect of *MEIB* extracts 200mg/kg and 400mg/kg in hyperlipidemia induced rats. The values of Low-Density Lipoprotein (LDL) are expressed as mean ± S.E.M of six rats per group, [a] $P < 0.05$ and [b] $P > 0.05$ compared with positive control.

| Groups | Treatment | Low-Density-Lipoprotein Cholesterol (LDL) mmol/L | | |
|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 |
| I | Control with normal diet | 79.51 ± 1.65 | 82.52 ± 2.39 | 82.70 ± 1.51 |
| II | Control with high cholesterol diet | 80.88 ± 4.72 | 106.95 ± 4.48 | 112.91 ± 4.37 |
| III | High Cholesterol diet treated with 200mg/kg of *MEIB* extracts | 75.36 ± 3.43 | 101.72 ± 5.21 | 103.50 ± 6.03[b] |
| IV | High Cholesterol diet treated with 400mg/kg of *MEIB* extracts | 82.28 ± 3.02 | 105.98 ± 7.33 | 100.71 ± 6.20[a] |
| V | High cholesterol diet treated with Simvastatin 10mg/kg | 79.80 ± 3.97 | 101.94 ± 6.81 | 79.37 ± 11.55[a] |

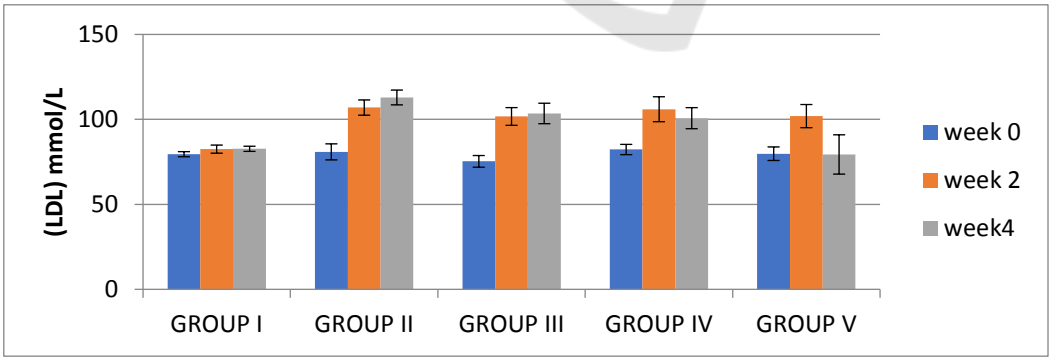



Figure 2: Graph shows the effect of *MEIB* extracts on Low Density Lipoprotein Cholesterol (LDL-C) of the hypercholesterolemia rats according to different time.

### 3.2.4 Effect on High-Density Lipoprotein (HDL)

Table 5: Anti-hyperlipidemic effect of *MEIB* extracts 200mg/kg and 400mg/kg in hyperlipidemia induced rats. The values of High-Density Lipoprotein (HDL) are expressed as mean ± S.E.M of six rats per group, [a] $P < 0.05$; and [b] $P > 0.05$ compared with positive control.

| Groups | Treatment | High-Density-Lipoprotein Cholesterol (HDL) mmol/L | | |
|---|---|---|---|---|
| | | Week 0 | Week 2 | Week 4 |
| I | Control with normal diet | 12.26 ± 0.97 | 12.10 ± 0.99 | 12.27 ± 0.87 |
| II | Control with high cholesterol diet | 12.65 ± 1.21 | 11.20 ± 0.72 | 12.65 ± 1.04 |
| III | High Cholesterol diet treated with 200mg/kg of *MEIB* extracts | 13.41 ± 0.82 | 12.64 ± 1.19 | 13.91 ± 1.16[b] |
| IV | High Cholesterol diet treated with 400mg/kg of *MEIB* extracts | 12.30 ± 1.12 | 10.72 ± 1.79 | 14.50 ± 1.17[a] |
| V | High cholesterol diet treated with Simvastatin 10mg/kg | 12.85 ± 1.30 | 11.71 ± 1.57 | 15.73 ± 0.69[a] |

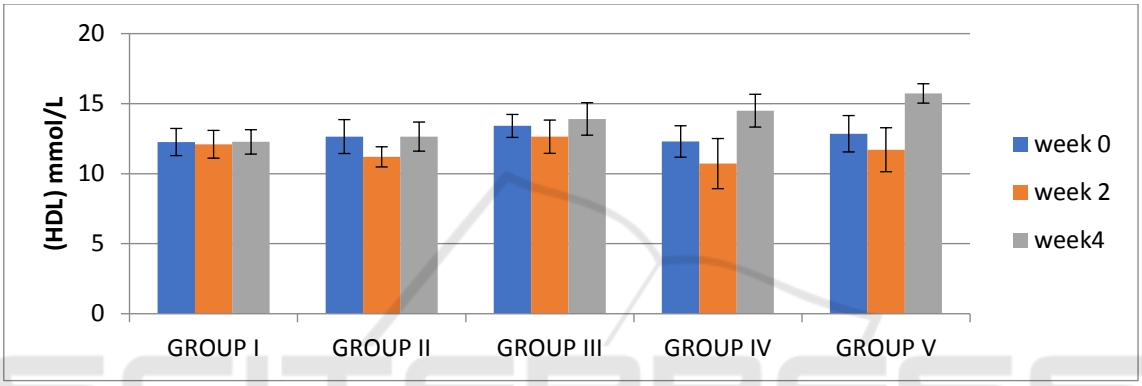
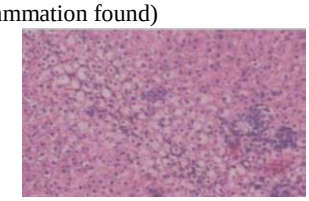
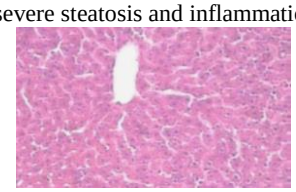
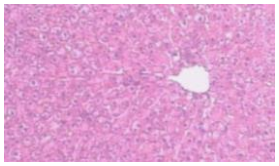
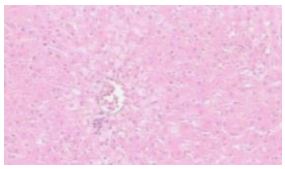



Figure 3: Graph shows the effect of *MEIB* extracts. leaves on High Density Lipoprotein Cholesterol (HDL-C) of the hypercholesterolemia rats according to different time.

Table 6: Anti-hyperlipidemic effect of *MEIB* extracts 200mg/kg and 400mg/kg in hyperlipidemia induced rats on 4$^{th}$ week (28$^{th}$ day). The increase (+) or decrease (-) value of Total Cholesterol, Low-Density Lipoprotein and High-Density Lipoprotein (HDL) are expressed as percentage.

| Groups | Treatment | Percentage increase (+) or decrease (-) of lipid values at week 4 (28$^{th}$ Day), mmol/L (%) | | |
|---|---|---|---|---|
| | | Total Cholesterol | Low Density Lipoprotein Cholesterol | High Density Lipoprotein Cholesterol |
| I | Control with normal diet | 111.47 ± 4.71 | 82.70 ± 1.51 | 12.27 ± 0.87 |
| II | Control with high cholesterol diet | 143.51 ± 4.55 | 112.91 ± 4.37 | 12.65 ± 1.04 |
| III | High Cholesterol diet treated with 200mg/kg of *MEIB* extracts | 142.27 ± 2.98 (-0.86%) | 103.50 ± 6.03 (-8.32%) | 13.91 ± 1.16[c] (+9.96%) |
| IV | High Cholesterol diet treated with 400mg/kg of *MEIB* extracts | 133.12 ± 8.44 (-7.24%) | 100.71 ± 6.20 (-10.80%) | 14.50 ± 1.17[a] (+14.62) |
| V | High cholesterol diet treated with Simvastatin 10mg/kg | 107.44 ± 8.09 (-25.13%) | 79.37 ± 11.55 (-29.70) | 15.73 ± 0.69[b] (+24.35) |

## 3.3 Histopathological Result

| | |
|---|---|
| High cholesterol diet rat (severe steatosis, and inflammation found)  | Hypercholestrolemia rats treated with 200mg/kg of *MEIB* extracts (severe steatosis and inflammation found),  |
| Hypercholestrolemia rats treated with 400mg/kg of *MEIB* extracts (mild steatosis and no inflammation found),  | Hypercholestrolemia rats treated with 10mg/kg of Simvastatin (very mild steatosis and no inflammation found).  |

## 4 DISCUSSION

Natural products that come from a plant are used for centuries in order to cure various ailments. Plants have been the found to be companions to man and formed the basis for various drugs synthesis since they are less toxic than synthetic drugs (Srujana et al 2012). Screening of medicinal plants presents basic path for the discovery of new drugs. From very long ancient times medicinal plants are considered to be important source for drug discovery having with potential therapeutic effect and safer entity. Plant species that have been traditionally used as an anti-cholesterol folk medicine should be seen as strategy in research for new anti-cholesterol drugs with better therapeutic effect and less side effects. Many drugs used for the treatment are found to be associated with side effects when used. These may leads to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and also abnormal liver function (Katzung et al 2012) . Hence there is an increase in demand to produce anti hypolipidemic activity from the natural source.

According to the toxicity test result, *MEIB* extracts produced non-toxic symptoms or mortality up to dose level of 2000mg/kg body weight orally in rats and hence the drug was considered safe for further pharmacological screening. Phytochemical analysis of the *MEIB* extracts revealed the presence of alkaloid, flavonoid, terpenoid and tannins.

In the present study, the anti-cholesterol activity of the test extract was measured by inducing hypercholesterolemia in rats model. *MEIB* extracts was used to evaluate the anti-cholesterol in this hypercholesterolemia induced rats. The doses of the test extract used for this study are 200mg/kg and 400mg/kg body weight respectively. The result obtained from this study has been showed in lipid profile of rats where 200mg/kg of *MEIB* extracts shown to have non-significant anti-cholesterol effect ($P>0.05$), whereas 400mg/kg of *MEIB* extracts has significant anti-cholesterol effect ($P<0.05$) when compared to the positive control. The results are statistically analyzed using One Way ANOVA.

From previous studies, it showed that flavonoids have the ability to reduce LDL-C and increase HDL-C in hypercholesterolemia induced rats. The antihyperlipidemic activity may also be attributed to some of its active principles. The hypolipidemic activity of natural products can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism (Borradaile et al 2003). So in this study, Flavonoids presence in BPR extract might be the reason for reducing TC, LDL-C and increasing HDL-C in 400mg/kg treated rats. Further experiments are required to prove the exact mechanism and active ingredients involved in the hypolipidemic activity.

## 5 CONCLUSION

The findings of the study revealed that *MEIB* extracts are having anti-cholesterol properties with the dose of 400mg/kg, and the anti-cholesterol effect is not significant with 200mg/kg. This was proven statistically with that $P> 0.05$.

Therefore, *MEIB* extracts could be a potential herbal medicine as adjuvant with existing therapy for the treatment of hyperlipidemia. Further studies

to isolate, identify, and characterize the active principle(s) present in the extract have to be done.

## ACKNOWLEDGEMENT

The author is thankful to Professor Tan Sri Dato' Wira Dr Mohd Shukri Ab Yajid, President, Management & Science University, Malaysia, for providing financial help and providing facilities to complete the research.

## CONFLICT OF INTERESTS

Authors shows no conflict of interests.

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