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Original Research Article

**Hypolipidemic activity of *Madhuca longifolia* in triton induced hyperlipidemic rats**

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Dhamangaon Road, Yavatmal, Maharashtra 445001, India**\*Article History:****Received:** 13/05/2017**Revised:** 04/07/2017**Accepted:** 04/07/2017**DOI:** <https://doi.org/10.7439/ijbar.v8i7.4157>**Abstract****Objective:** In the present study, an aqueous extract from *Madhuca longifolia* bark was evaluated for its hypocholesterolaemic and hypotriglyceridaemic activities using Triton WR-1339 induced hyperlipidemic rats as experimental model.**Material and Method:** Hyperlipidemia was induced by a single injection of Triton WR 1339 (400 mg/kg i.p.) in sprague dawley rats. Aqueous extract of *Madhuca longifolia* bark (ML) (250, 500 and 750 mg/kg/day) was administered to hyperlipidemic rats for one week. Harvested serum was analyzed for lipid profile such as cholesterol, triglyceride, and lipoproteins. Oxidative stress parameters like Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione reductase (GRh) and activity of lipolytic enzyme such as lecithin-cholesterol-acyltransferase (LCAT) & post-heparin lipolytic activity (PHLA) were estimated in the liver tissues of hyperlipidemic rats.**Results:** Result of the study suggested that treatment with ML 750mg/kg/day significantly ( $p < 0.01$ ) lowered the level of serum cholesterol, triglyceride phospholipids and increased in lecithin-cholesterol-acyltransferase activity & post-heparin lipolytic activity compared to Triton-treated rats. In addition, ML 750mg/kg/day significantly ( $p < 0.01$ ) reduced oxidative stress and normalizes the activities of SOD, CAT, GPx and GRh compared to Triton-treated rats.**Conclusion:** The current study provides strong evidence that intragastric administration of ML 750mg/kg/day has a beneficial effect in treating dyslipidemia with decrease in oxidative stress.**Keywords:** *Madhuca longifolia*; triton WR 1339; dyslipidemia; oxidative stress, antioxidant activity.**1. Introduction**

Hypercholesterolemia and hypertriglyceridemia are major risk factors either, alone or together. They accelerate the development of coronary artery disease and the progression of atherosclerosis [1]. High levels of low-density lipoprotein (LDL) accumulate in the extracellular sub endothelial space of arteries and are highly atherogenic and toxic to vascular cells thereby leading to atherosclerosis, hypertension, obesity, diabetes, functional depression in some organs, etc [1]. In hyperlipidemic conditions enzymatic as well as non-enzymatic antioxidative defense systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx),

ascorbic acid, and reduced glutathione (GSH) are altered leading to reactive oxygen species (ROS) mediated damage[2]. Lipid lowering drugs like fibrates, statins and bile acid sequestrants are used to treat hyperlipidemia and are known to possess some side effects [3]. Therefore, there is an urgent need to have drugs with lipid lowering and antioxidant activities with no side effects and natural products are the best claimed option. World ethnobotanical information reports a number of herbal medicines from plants and vegetables that can be used to control hyperlipidemia and related complications in patients [4]. *Madhuca longifolia* (Sapotaceae, common name: Mahua) a

common plant in Asia, has been widely used for external application in treating skin diseases, rheumatism, headache, chronic constipation, piles, hemorrhoids and sometimes used as an emetic and galactagogue[5]. Different parts of *Madhuca longifolia* have different therapeutic properties. Mahua flower are used as a food as well as used as an exchanger in tribal and rural areas. Mahua seeds are rich in edible fats so they have economic importance. Mahua fruits are used as vegetable and widely consumed by the tribes of western Odisha [6]. *Madhuca longifolia* contains several active constituents such as saponins, triterpenoids, steroids, saponins, flavonoids and glycosides that are known to have scavenging activities against free radicals and to prevent the cardiovascular disease[6]. Therefore, present study is designed to investigate the lipid lowering activity of MLB in hyperlipidemic rats and also to study its effect on free radical scavenging activity.

## 2. Material and method

*Madhuca longifolia* Bark was collected from local region of Yavatmal district, Maharashtra, India. The bark was dried under shade thoroughly and powdered. About 1000 g of the dry bark powder was extracted thrice with water. The combined extracts were concentrated in rotary evaporator at reduced pressure to obtain about 133.50 g (13.35%, w/w) and stored at 4°C until further use. During the study, the residual extract was suspended in distilled water and orally administered to the animals [7].

Hyperlipidemia was induced in the experimental rats by a single intravenous (IV) injection of triton WR 1339 (300 mg/kg b.w.) and after 48 h rats depicted elevated levels of cholesterol and triglyceride in serum [8].

Male albino rats of Wistar strain, 140–170 g of body weight were selected under hygienic conditions and kept at standard environmental conditions (temperature: 24 ± 1 °C, light/dark cycle: 12/12 h) in central animal facility of P. Wadhvani College of Pharmacy, Yavatmal. All procedures complied with the standards for the care and use of animal subjects as stated in the guidelines laid by Institutional Animal Ethical Committee (IAEC). The rats were fed with standard pellet diet and water *ad libitum*. Rats randomly selected were divided into 6 Groups, comprising of six rats each. After induction of hyperlipidemia (48 h), the MLB or Atorvastatin was administered to hyperlipidemic rats for 14 days. Blood samples were collected during three different time intervals from both control and experimental rats. On the 14th day, rats were sacrificed by decapitation and the liver was removed from

each rat for further analysis. The treatment schedule was followed: Group-I: Control rats. Group-II: Triton 1339 treated Hyperlipidemic rats. Group-III: Hyperlipidemic rats administered with MLB (250 mg/kg b.w./day) Group-IV: Hyperlipidemic rats administered with MLB (500 mg/kg b.w./day). Group-V: Hyperlipidemic rats administered with MLB (750 mg/kg b.w./day). Group-VI: Hyperlipidemic rats administered with Atorvastatin (10 mg/kg b.w./day).

The liver samples (100 mg/ml) were homogenized in 50 mM phosphate buffer (pH 7.4) and the homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant obtained was used for biochemical analysis.

Total cholesterol (TC), triglyceride, and HDL in serum and tissues were determined using enzymatic kits (Ambica Diagnostics, India) following the manufacturer's instructions. The atherogenic index (AI) was calculated as  $AI = (TC - HDL)/HDL$  and the LDL was calculated by Friedewald's formula [9]. The antioxidant enzyme activities namely SOD [10], Catalase[11], GPx[12] and antioxidants like reduced GSH[13] were measured.

Statistical analysis was performed using analysis of variance ANOVA and a Least Significant Difference (LSD) post hoc test was used to compare individual means. The results were expressed as the mean ± SD of six values in each group, and a statistical probability of  $P < 0.05$ ,  $P < 0.001$  was considered to be significant.

## 3. Results

The effect of MLB on serum lipid profile in control and experimental rats after 14 days treatment is shown in Table 1. The acute injection of Triton WR 1339 caused a significant increase ( $P < 0.001$ ) in lipid levels when compared with Group I rats. Group V showed a significant ( $P < 0.001$ ) reduction in cholesterol, triglyceride, LDL and VLDL levels, whereas significant increase ( $P < 0.001$ ) was observed in HDL after 14 days treatment of MLB when compared to Group II. The AI was also significantly ( $P < 0.001$ ) decreased in Group V after 14 days treatment. It was observed that dose dependent reduction of lipid profile and maximum antihyperlipidemic effect ( $P < 0.001$ ) was in Group V (750 mg/kg/b.w. of MLB administered) when compared to Group II. Atorvastatin significantly reduced the lipid parameters; MLB however, was more effective. MLB restored LCAT and PHLA activity in liver tissue and it was comparable to standard drug Atorvastatin and is depicted in Table 2. There was no significant difference in both serum and liver lipid profiles, in Group I rats.

**Table 1: Effect of MLB on level of serum lipids and lipoproteins in hyperlipidemic rats after 14 days**

| Group No. | Values are expressed as mg/dl |                 |                |               |                          |
|-----------|-------------------------------|-----------------|----------------|---------------|--------------------------|
|           | Total Cholesterol             | Triglyceride    | LDL            | VLDL          | HDL                      |
| I         | 83.94±9.61                    | 190.09±16.25    | 13.15±2.3      | 28.61±4.56    | 39.85±3.83               |
| II        | 246.51±16.55***               | 293.91±15.8***  | 78.88±10.56*** | 65.44±3.68*** | 18.99±4.07***            |
| III       | 214.91±14.72*                 | 175.32±12.15*** | 25.16±4.37***  | 43.06±5.55*** | 20.59±3.15 <sup>ns</sup> |
| IV        | 191.80±15.06***               | 170.85±18.99*** | 20.89±4.73***  | 38.09±3.35*** | 30.11±4.58*              |
| V         | 179.61±20.91***               | 144.03±10.15*** | 16.4±3.56***   | 30.80±2.25*** | 36.15±3.56***            |
| VI        | 164.45±11.50***               | 134.77±18.88*** | 15.38±4.65***  | 30.95±2.61*** | 38.68±4.25***            |

All values are expressed as mean±SD and n=6 for all groups. Triton treated group was compared with control; MLB 250mg/kg, MLB 500mg/kg, MLB 750mg/kg and Atorvastatin were compared with triton treated rats. \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

**Table 2: Effect of MLB on antidyslipidemic and antiatherogenic potential in hyperlipidemic rats after 14 days**

| Group No. | LCAT<br>(n mole Cholesterol<br>released/h/L) | PHLA<br>(n mole free fatty acid<br>released/h/L) | Atherogenic<br>Index | HDL/LDL<br>Ratio |
|-----------|--|--|----------------------|------------------|
| I         | 65.89±4.35                                   | 22.82±0.56                                       | 0.72±0.12            | 3.63±0.23        |
| II        | 25.89±3.56***                                | 16.54±1.24***                                    | 19.07±2.01**         | 0.07±0.001**     |
| III       | 29.58±2.89 <sup>ns</sup>                     | 12.58±1.87***                                    | 0.57±0.02**          | 2.58±0.04**      |
| IV        | 39.38±2.56***                                | 14.52±1.45**                                     | 0.36±0.04**          | 3.67±0.06**      |
| V         | 42.59±3.34***                                | 18.85±1.94**                                     | 0.38±0.03**          | 5.07±0.09**      |
| VI        | 47.78±2.92***                                | 20.45±1.25***                                    | 0.20±0.01**          | 2.19±0.07**      |

All values are expressed as mean±SD and n=6 for all groups. Triton treated group was compared with control; MLB 250mg/kg, MLB 500mg/kg, MLB 750mg/kg and Atorvastatin were compared with triton treated rats. \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

Significant reduction ( $P < 0.001$ ) in the antioxidant enzyme activities were observed in hyperlipidemic rats and depicted in Table 3. When MLB was administered to hyperlipidemic rats, there was a significant increase in SOD, GPx in serum. Antioxidant enzymes in liver homogenate showed a similar trend. The level of SOD was increased in liver homogenate. GPx and CAT were

increased in liver tissue. Atorvastatin similarly increased enzymatic status in the serum and liver samples of hyperlipidemic rats. Collectively, results suggest that administration of MLB significantly decreases the both serum and tissues lipids accompanied with antioxidant activity.

**Table 3: Effect of MLB on antioxidant potential in hyperlipidemic rats after 14 days**

| Group No. | Values are expressed as Units/mg protein |               |              |                |
|-----------|--|---------------|--------------|----------------|
|           | SOD                                      | CAT           | GPx          | GRh            |
| I         | 14.89±1.25                               | 77.58±6.89    | 12.25±0.45   | 0.91±0.014     |
| II        | 8.14±0.89**                              | 21.3±1.25***  | 7.52±0.36*** | 0.58±0.0075*** |
| III       | 9.56±0.62 <sup>ns</sup>                  | 45.58±3.36*** | 8.83±0.82*   | 0.65±0.026***  |
| IV        | 11.54±0.88***                            | 56.85±4.85*** | 9.2±0.67***  | 0.72±0.029***  |
| V         | 12.54±0.87***                            | 69.7±4.56***  | 9.7±0.62***  | 0.81±0.017***  |
| VI        | 13.36±1.12***                            | 66.27±5.67*** | 9.83±0.58*** | 0.84±0.04***   |

All values are expressed as mean±SD and n=6 for all groups. Triton treated group was compared with control; MLB 250mg/kg, MLB 500mg/kg, MLB 750mg/kg and Atorvastatin were compared with triton treated rats. \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

#### 4. Discussion

The current work is a preliminary one to assess antihyperlipidemic and antioxidant activity of MLB in Triton WR1339 induced hyperlipidemic rats. India and most other developing countries are endowed with vast resources of medicinal and aromatic plants. Currently, there has been a considerable interest in finding new drugs from these plant materials to replace synthetic drugs due to its adverse effects [14] and there is an urgent need for the development

of hypolipidemic drugs from natural resources that are safe. Many natural products recently have been screened for lipid lowering and antioxidant activities. In the present investigation, *Madhuca longifolia* bark was studied for its antihyperlipidemic activity. Traditionally it is a well-known medicinal plant with diverse biological activities, pharmacological functions including antidiabetic and antioxidant activities [15,16]. The bark has been reported to contain good amount of several active constituents [17].

Hyperlipidemia was noted along with increase in oxidative stress hence we measured the oxidative parameters in all groups [16].

The effect of MLB on serum and liver lipids were analysed in control and experimental rats and we have observed significant antihyperlipidemic activity. Evidenced reports showed that MLB possess significant amount of active components [18] and may be responsible for the antihyperlipidemic activity.  $\alpha$ -Terpeneol, an antioxidant reduces blood cholesterol either by interrupting with the recirculation of bile acids or reduces the absorption of cholesterol. Flavonoids have diverse biological activities [19] and decrease LDL, increase HDL and may also hasten the removal of cholesterol from peripheral tissues to liver for catabolism and excretion [20]. Sesquiterene alcohol,  $\alpha$ -terpeneol and  $3\beta$ -monocaprylic ester of eythrodinol Anthracene derivatives and dimeric proanthocyanidins are polyphenols reported to be present in *Madhuca longifolia* bark [18]. They possess antiatherogenic effect and decrease LDL/HDL ratio and LDL oxidation [21]. Moreover, the polysaccharides present in MLB may also exert beneficial effects. Our results suggest that MLB possesses antihyperlipidemic activity and may have a major role in maintaining cholesterol homeostasis. Increased HDL, decreases plasma TC and prevents atherosclerosis development [22]. The decreased LDL concentration may also be responsible for the reduction of TC in hyperlipidemic rats treated with MLB. The de-novo biosynthesis of cholesterol occurred mainly in liver and search for new drugs to inhibit cholesterol biosynthesis has long been pursued as a means to treat hyperlipidemia. We observed significant reduction of hepatic cholesterol and the possibility exists that MLB may diminish the endogenous cholesterol biosynthesis by inhibiting the hepatic HMG-CoA reductase [23] or by up regulating the hepatic LDL receptor which is involved in cholesterol clearance reported that TG is independently related to coronary heart diseases and most of the current anti-hyperlipidemic drugs do not decrease TG levels. Interestingly in our present study treatment of MLB significantly decreased serum TG level and is an important property of MLB, because it has antihyper-cholesterolemic, antihyper-triglyceridemic activity and increases antioxidant activity during hyperlipidemic rats treated with MLB. Hyperlipidemia causes oxidative stress and reduces the antioxidant defense system, thereby elevating the lipid peroxides [24]. Free radical scavenging enzymes such as SOD, CAT, and GPx are the first line of cellular defense against oxidative injury and are involved in the disposal of superoxide anions, hydrogen peroxide, etc and pronounced alterations were observed during hyperlipidemic conditions in rats.

Aqueous extract of *Madhuca longifolia* produces high NADP<sup>+</sup> which results in down regulation of lipogenesis and lowers the oxidative stress. Reduction of lipid peroxidation was associated with antiatherogenicity and naturally occurring dietary antioxidants have antiatherogenic effect [25], scavenge free radicals and superoxide anions thereby inhibiting lipid per-oxidation and also showing antihyperlipidemic effects [24]. We observed that administration of MLB suppresses lipid peroxidation in hyperlipidemic rats by increasing the level of antioxidants. Treatment with MLB significantly increases the activities of antioxidative enzymes in hyperlipidemic rats. Apart from alteration in enzymatic antioxidants we observed a significant ( $P < 0.001$ ) reduction of serum GSH (reduced form). Increase in free radicals during hyperlipidemia was reverted back to near normal values with MLB treatment. During hyperlipidemic condition depletion of GSH occurred due to enhanced oxidation/consumption by electrophilic compounds which are due to increased formation of ROS. In addition to antihyperlipidemic effect of MLB, increased antioxidant enzyme activities prevent oxidative damage. There was no difference between control and MLB administered to control rats, which suggest that extract has no adverse side effects.

## 5. Conclusions

It is concluded from the data that MLB possess potent antihyperlipidemic and antioxidant activity and these beneficial activities may contribute to its cardio protective and anti-atherosclerotic role without any known adverse effect. Pronounced activity was observed at dose of 750 mg/kg b.w. of MLB for 2 weeks. In addition, MLB is better in lowering the lipids and improving the antioxidant effects than Atorvastatin and the later is known to have side effects whereas MLB is free from side effects.

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