EVALUATION OF ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF PTEROSPERMUM ACERIFOLIUM (L.)WILLD.

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Summary

The present study investigates the antihyperlipidimic and antioxidant property of methonalic extract of flowers of *Pterospermum acerifolium (L.) Wild* (250 mg/ kg body weight, 500 mg/ kg body weight). The extract was evaluated for antihyperlipidimic activity in high fat diet induced hyperlipidemic model in wistar albino rats by measuring the biochemical parameters [Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C), High density lipoprotein cholesterol (HDL-C) and Triglycerides (TGs)], Marker enzymes [Alanine transaminase (ALT) & Alkaline phosphatase (ALP)], Cardiac risk indicator [Atherogenic ratio] and Body weight. The antioxidant status of the extract was evaluated by calculating the level of Superoxide dismutase (SOD), Catalase and Malondialdehyde (MDA). The extract exhibited the significant reduction in total cholesterol,
triglycerides, Low density lipoprotein cholesterol, Very low density lipoprotein cholesterol and increase in the level of High density lipoprotein cholesterol when compared to atorvastatin. Artherogenic index and LDL-C: HDL-C ratio was also reduced to significant extent. In the case of in vivo antioxidant evaluation, the extract exhibited the significant reduction in the levels of Malondialdehyde, and significantly increase the levels of Superoxidedismutatase and Catalase. Thus the antihyperlipidemic action of the extract may be due to their antioxidant property.

Key Words: *Pterospermum acerifolium*, Antihyperlipidimic activity, Antioxidant activity.

**Introduction**

Hyperlipidemia is a metabolic disorder specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) and Triglycerides (TGs) with a concominant decrease in the concentrations of High density lipoprotein cholesterol (HDL-C) in the blood circulation [1]. Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases [2]. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as Coronary Heart Disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease [3]. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease [4]. Reduction in serum cholesterol levels reduces the risk for CHD [5]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease [6].
Oxidative stress occurs when there is an imbalance between free radical production and antioxidant defenses, resulting in deregulation of cellular function [7]. Increased oxidative stress has been suggested in the pathogenesis and progression of many diseases like hyperlipidemia and diabetes mellitus and their associated complications such as atherosclerosis, myocardial infarction, neuropathy, nephropathy, retinopathy, micro and macro vascular damage and poor wound healing [8]. Antioxidant substances and enzymes in the body are not wholly effective in preventing oxidative damage especially in conditions like hyperlipidemia and diabetes mellitus where free radicals are produced in excess. Currently available hypolipidemic drugs have been associated with a number of side effects [9].

The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [4]. An herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system [10]. Medicinal plants are being found to play a major role in antihyperlipidemic activity [4]. Thus, there is a considerable interest on development of lipid-lowering drugs from natural products in the recent years. Since, plants contain substantial amount of antioxidants such as, carotenoids, ascorbic acid, flavonoids and other polyphenols, tannins, terpenoids and α-tocopherol, they are believed to exert their antihyperlipidemic and antidiabetic effect, at least in part, through their anti-oxidant property [11].

Pterospermum acerifolium (L) Willd (Family: Sterculiaceae) is found in the sub-himalayan tract and outer valleys from Yamuna eastwards to West Bengal, and in Assam and Manipur, up to an altitude of 1200 meters. It is also found in Ramnagar hills of Bihar and in western ghats of Konkan and North Kanara; it is also common in Andamans. Also found in Chittagong and Khasi hills. It is an ever green large tree up to 24 meters in height and 2.5 in girth with smooth bark, greyish brown in colour. Leaves are variable in size and shape, 25-30 cm x 15-30 cm, entire or variously lobed, oblong cordate and sometimes peltate; flowers are large, 12-15 cm in diameter, axillary, solitary or in pairs, white and fragrant [12, 13].
According to Ayurveda, *Pterospermum acerifolium* flowers are used as tonic, laxative, anthelmintic, removes “kapha”, inflammation, abdominal pain, ascites, cures ulcers, leprosy, urinary discharges and tumours [13-15]. In the Konkan the flowers and bark are charred and mixed with Kamala and are applied in suppurating small pox [13, 15]. The leaves are used as haemostatic and antimicrobial agent [13-15]. Antihyperglycemic activity has been found in the leaves of this plant in type 2 diabetic model rats. *Pterospermum acerifolium* is commonly used herb in ayurvedic anticancer treatment. The flowers are mixed with sugar to be applied locally [16]. The leaves of *Pterospermum acerifolium* have been found to possess antidiabetic activity. Hyperlipidemia/Dyslipidemia accompanies Diabetes mellitus. Thus the present objective is to determine whether the flowers of *Pterospermum acerifolium* have hypolipidemic activity as because hyperlipidemia also precipitates in presence of various metabolic and cardiovascular disorders. The present study was carried out to understand the antihyperlipidimic and antioxidant property of methonalic extract of *Pterospermum acerifolium (L)* Willd.

Materials and Methods

Plant Collection and Extraction: The flowers of *Pterospermum acerifolium* were collected from BIT, Mesra, Ranchi in the month of december 2010. Previously the plant had been identified and authenticated by Dr. Narasima, Botanical Survey of India, Howrah, West Bengal, and the voucher specimens were retained in Department of Pharmaceutical Sciences, B.I.T. Mesra, Ranchi, for future reference. The flowers of *Pterospermum acerifolium* were dried in the shade of about 30°C and crushed into a coarse powder. The dried and powdered plant material (flowers) was subjected to successive hot extraction in a soxhlet continuous extraction apparatus with methanol as a solvent. The average time period for extraction was 48 hours. The extract was filtered and concentrated using a Rotary evaporator (Buchi US). The yield was found to be 8.74%w/w.

Animals: Thirty male Wistar Albino rats (100g to 250g body weight) were used in this study. Animals were procured from Institutional Animal House (Reg no. BIT/PH/IAEC/31/2010/15-9-10) of Birla Institute of Technology, Mesra. All animals were kept in
polyacrylic cages and maintained under standard housing conditions (room temperature 24 – 27°C and humidity 60 – 65 % with 12: 12 light: dark cycles). Food was provided in the form of dry pellets and water ad libitum. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

**Preliminary Phytochemical screening:** Preliminary tests were carried out for the presence or absence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Sterols, Terpenes and Tannins in the extract [17].

**Preparation of the dose:** The two doses of the methanolic extract (250 mg/ kg body weight, 500 mg/ kg body weight) and one dose of the standard drug Atorvastatin (10mg/ kg body weight) were suspended in 5% (w/v) Tween 80 and given at a dose of 0.1 ml/ 10 g body weight.

**Composition of Hyperlipidemic diet [18]:**
- Rodent chow: 83 %
- Coconut oil: 15%
- Cholesterol: 2%

**Protocol for Induction of Hyperlipidemia and Treatment:** The experimental animals were divided into 5 groups with 6 animals in each group.

- **Group 1 - Normal diet control** [Treated with Tween 80 (5% w/v) orally]
- **Group 2 - Hyperlipidemic control** [Treated with Tween 80 (5 % w/v) orally]
- **Group 3 - Hyperlipidemic treated with Standard drug** Atorvastatin (10mg/kg body weight [19], orally)
- **Group 4 - Hyperlipidemic treated with methanolic extract of flowers of Pterospermum acerifolium (L) Willd.** (250 mg/kg body weight, orally)
Group 5 - Hyperlipidemic treated with methanolic extract of flowers of *Pterospermum acerifolium* (L) Willd. (500 mg/kg body weight, orally)

Both normal and hyperlipidemic diets were administered for a period of 60 days [20]. Treatment was administered once daily throughout the diet period (60 days). The animals were weighed at the beginning and at the end of the diet period.

**Blood sample collection and analysis:** On the 61st day, blood was collected by retro-orbital puncture technique under mild ether anesthesia after 8 hours fasting and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and refrigerated until biochemical estimations were carried out.

**Assesments of Biochemical parameters and Marker enzymes:** The main biochemical parameters recommended by the National Cholesterol Education Program (NCEP) guidelines (2002) for lipid screening ie. Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C), High density lipoprotein cholesterol (HDL-C) and Triglycerides (TGs) were evaluated from the serum [2] using diagnostic kits which were procured from Span Diagnostics Ltd. (Surat). The composition of reagents, principles, procedures and formulae of all the above mentioned assays have been taken from the instruction manuals of the kits provided by Span Diagnostics Ltd. (Surat). Clinical analyzer-Systronics 635 was used for spectrophotometric measurements.

The Cardiac risk ratio recommended by NCEP guidelines (2002) was estimated by calculating “Atherogenic Index” [21], LDL-C and HDL-C ratio. Biological parameters like body weights was determined just before the animals were sacrificed.

Marker enzymes Alanine transaminase (ALT) & Alkaline phosphatise (ALP) activities were also measured using diagnostic kits which were procured from Span Diagnostics Ltd. (Surat).
In vivo anti-oxidant activity: [22, 23]

**Preparation of liver homogenate:** The liver was separated by sacrificing the rats of all groups (Group 1 to Group 5) after 30 min observation period. Liver tissues were homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer pH 7.4 followed by centrifugation (5000 rpm for 10 minutes at 4°C) and the supernatant was used to prepare aliquots of homogenates which were used to carry out SOD (Superoxide dismutase), Catalase and MDA (Malondialdehyde) assays.

**Superoxide dismutase assay:** An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activities in blood plasma. Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent prepared as described by Onwurah, to a mixture of epinephrine (3 x10^{-3} M), Na_{2}CO_{3} (10^{-3}M), EDTA (10^{-4}M), and 1.0ml of deionised water at a final volume of 6 ml. The auto-oxidation was read in a spectrophotometer at 480 nm every 30 sec for 5 min. The experiment was repeated with 1.0 ml of the liver homogenate collected from different groups of rats (Group1 to Group 5). A graph of absorbance against time was plotted for each, and the initial rate of auto-oxidation calculated. One unit of SOD activity was defined as the concentration of the enzyme (mg protein/ml) in the plasma that caused 50% reduction in the auto-oxidation of epinephrine. Superoxide dismutase activity was subsequently calculated for each sample.

**Catalase assay:** The enzyme catalase (CAT) catalyses the conversion of Hydrogen Peroxide (H_{2}O_{2}) into water. The CAT activity was measured using the method of Chance and Maehly with some modification. The CAT reaction solution 3 ml contained; 100 mM phosphate buffer (pH 7.0), 30mM H_{2}O_{2} and 0.1ml of enzyme extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were noted after every 30 Seconds. One unit CAT activity was defined as the amount of enzyme required to decompose 1 micromole H_{2}O_{2}/min calculated by using molar extinction coefficient 0.0394/mM/min for H_{2}O_{2}. One unit of CAT activity was defined as an absorbance change of 0.01 unit/min.
Malondialdehyde (MDA)-Lipid peroxidation assay: Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard according to the method of Wills. One ml of supernatant was added to 1.5 ml of 20% TCA (Trichloroacetic acid) solution, and allowed to stand for 15 min at room temperature. The tube containing this mixture was centrifuged and supernatant was separated. To this 2 ml of the supernatant was added to 1.5 ml of 0.67% of TBA (Thiobarbituric acid) solution and this mixture was heated in a boiling water bath for 15 min. After cooling at room temperature, a pink coloured chromophore was formed. The absorbance of the chromophore was measured at 532 nm. The concentration of MDA or lipid peroxide was expressed as nanomole of MDA/mg liver tissue. As 99% TBARS are Malondialdehyde (MDA), So TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is $1.56 \times 10^5 \text{M}^{-1}\text{cm}^{-1}$.

Statistical analysis: Results were expressed as Mean ± SEM (Standard error of Mean) of six rats. Data was analyzed by one-way ANOVA followed by Dunnett’s multiple comparison tests against Normal control and Hyperlipidemic control. The $P$ values < 0.05 were considered to be statistically significant. The statistical analysis was done using the trial version of Graph Pad INSTAT 3.10 software.

Results

Phytochemical Screening: The results showed that the methanolic extract of the flowers yield the highest number of positive tests for phytoconstituents like carbohydrates, flavanoids, reducing sugars, glycosides, saponins and terpenes.

Anti-Hyperlipidemic Activity

Biochemical Parameters: In diet induced hyperlipidemic model, treatment with standard drug Atorvastatin exhibited the greatest antihyperlipidemic activity (Table 1).

Treatment with the MEFPA (Methanolic extract of flowers of *P. acerifolium*) also showed significant antihyperlipidemic activity, especially at the higher dose of 500 mg/kg body weight. Administration of both the standard drug Atorvastatin and the higher
dose (500 mg/kg body weight) of MEFPA led to significant decrease in serum Total Cholesterol (TC), LDL-C, VLDL-C and Triglycerides (TGs) while leading to a significant increase in HDL-C. The lower dose (250 mg/kg body weight) of the flower extract did not lead to any significant increase in HDL-C but serum levels of TC, LDL-C, VLDL-C and Triglycerides showed significant decrease which was less than that showed by the higher dose of the extract. Thus the extract exhibits dose-dependent relationship.

Table 1: Effect of Methanolic extract of Flowers of *P. acerifolium* (MEFPA) on Serum Lipid profile of Hyperlipidemic rats after 60 days of treatment

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Normal Control (HC)</th>
<th>Hyperlipidemic Control (HC)</th>
<th>Hyperlipidemic Atorvastatin (10mg/kg b.w)</th>
<th>Hyperlipidemic MEFPA-250mg/kg body weight</th>
<th>Hyperlipidemic MEFPA-500mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>75.57 ± 0.66</td>
<td>174.24 ± 2.83a</td>
<td>92.55 ± 3.90b</td>
<td>139.59 ± 1.87b</td>
<td>120.75 ± 2.39b</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>38.81 ± 1.83</td>
<td>22.49 ± 0.76a</td>
<td>35.50 ± 2.03b</td>
<td>25.58 ± 1.54c</td>
<td>30.72 ± 1.40b</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>21.86 ± 1.68</td>
<td>118.30 ± 2.85a</td>
<td>40.69 ± 1.99b</td>
<td>85.22 ± 1.97b</td>
<td>66.06 ± 2.96b</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>14.90 ± 0.14</td>
<td>33.45 ± 0.51a</td>
<td>16.36 ± 0.54b</td>
<td>28.20 ± 0.81b</td>
<td>23.97 ± 0.78b</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>74.53 ± 0.70</td>
<td>167.24 ± 2.57b</td>
<td>81.78 ± 2.69b</td>
<td>144.00 ± 4.06b</td>
<td>119.87 ± 3.88b</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (Standard error of mean), n = 6. *Represents values statistically significant w.r.t. Normal control group (p< 0.05). †Represents values statistically significant w.r.t. Hyperlipidemic control group (p<0.05). ‡Represents values statistically insignificant w.r.t. Hyperlipidemic control group (p>0.05).
The Cardiac risk indicator—“Atherogenic Index” (AI) showed the highest value for the hyperlipidemic diet control group; the value for the normal control group was significantly lower. The treatment groups showed significantly lower values of Atherogenic index compared to the hyperlipidemic control group; among the treatment groups, Atorvastatin treatment resulted in the lowest values of AI; The higher dose of the extract showed greater decline in AI than the lower dose (Figure 1).

**Figure 1: Effect of Methanolic extract of Flowers of P. acerifolium (MEFPA) on Atherogenic Index after 60 days of treatment**
Administration of hyperlipidemic diet produced significant weight gain in the Hyperlipidemic control group with respect to the normal control group while the groups treated with the standard drug Atorvastatin and the two doses of the MEFPA showed significantly less weight gain compared to the hyperlipidemic control group (Table 2). This can be attributed to the antihyperlipidemic activity of the extracts and the standard drug Atorvastatin. These results also exhibited the dose-response relationship of the extract as the higher dose of the extract produced a decline in weight gain greater than the decline produced by the lower dose. The highest decline in weight gain was exhibited by the group treated with the standard drug Atorvastatin.

Table 2: Effect of Methanolic extract of Flowers of *P. acerifolium* (MEFPA) on Percentage weight gain after 60 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight (b. wt.) in grams</th>
<th>Gain in b. wt. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Normal control</td>
<td>134.67 ± 5.73</td>
<td>155.29 ± 6.30</td>
</tr>
<tr>
<td>Hyperlipidemic (HD) control</td>
<td>156.24 ± 8.31</td>
<td>209.46 ± 5.02</td>
</tr>
<tr>
<td>Hyperlipidemic-Atorvastatin (10 mg/kg body weight)</td>
<td>154.82 ± 7.70</td>
<td>189.16 ± 2.48</td>
</tr>
<tr>
<td>Hyperlipidemic-MEFPA-250 mg/kg body weight</td>
<td>125.75 ± 4.65</td>
<td>165.07 ± 4.60</td>
</tr>
<tr>
<td>Hyperlipidemic-MEFPA-500 mg/kg body weight</td>
<td>182.50 ± 6.48</td>
<td>234.38 ± 2.09</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (Standard error of mean), n = 6.
Table 3: Effect of MEFPA on Serum Alanine transaminase (ALT) and Alkaline phosphatase (ALP) after 60 days of treatment

<table>
<thead>
<tr>
<th></th>
<th>Alanine transaminase (ALT) in IU/L</th>
<th>Alkaline phosphatase (ALP) in IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>21.08 ± 1.25</td>
<td>89.72 ± 1.54</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>50.73 ± 1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>317.00 ± 4.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidemic - Atorvastatin (10 mg/kg body wt.)</td>
<td>26.15 ± 1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>184.50 ± 3.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidemic - MEFPA-250 mg/kg body wt.</td>
<td>41.37 ± 1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>257.12 ± 3.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperlipidemic - MEFPA-500 mg/kg body wt.</td>
<td>36.82 ± 1.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>201.48 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (Standard error of mean), n = 6.
<sup>a</sup>Represents values statistically significant w.r.t. Normal control group (p< 0.05).
<sup>b</sup>Represents values statistically significant w.r.t. Hyperlipidemic control group (p<0.05).

Administration of hyperlipidemic diet produced significant elevation in the levels of serum Alanine transaminase (ALT) and Alkaline phosphatase (ALP) with respect to the Normal control group (Table 3). Treatment with standard drug Atorvastatin and the two doses of the MEFPA produced significant reduction in the levels of both ALT and ALP compared to the Hyperlipidemic control group. Atorvastatin treatment led to a greater decline in the serum levels of both enzymes than the declines shown by the groups treated with the extract. The decline shown by the group treated with 500 mg/kg body weight dose of the extract was more than that of the group treated with 250 mg/kg body weight dose.

**In-vivo antioxidant activity:**

MDA (Malondialdehyde) level in hyperlipidemic control group has shown significant rise with respect to the Normal control group (Table 4).
Table 4: Antioxidant enzyme activities in the liver homogenates after 60 days of treatment with selected doses of Methanolic flower extract of *P. acerifolium* (MEFPA)

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal control</th>
<th>Hyperlipidemic Control</th>
<th>Hyperlipidemic - Atorvastatin (10 mg/kg body wt.)</th>
<th>Hyperlipidemic - MEFPA-250 mg/kg body wt.</th>
<th>Hyperlipidemic - MEFPA-500 mg/kg body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nM/g of liver tissue)</td>
<td>1.16 ± 0.07</td>
<td>5.35 ± 0.30</td>
<td>1.72 ± 0.09</td>
<td>3.57 ± 0.05</td>
<td>2.61 ± 0.04</td>
</tr>
<tr>
<td>SOD (units/min/mg protein)</td>
<td>5.18 ± 0.02</td>
<td>2.01 ± 0.12</td>
<td>4.89 ± 0.07</td>
<td>3.37 ± 0.09</td>
<td>3.96 ± 0.15</td>
</tr>
<tr>
<td>Catalase (units/min/mg protein)</td>
<td>13.14 ± 0.55</td>
<td>6.39 ± 0.24</td>
<td>12.07 ± 0.31</td>
<td>7.85 ± 0.29</td>
<td>9.13 ± 0.47</td>
</tr>
</tbody>
</table>

Each value is Mean ± SEM (Standard error of mean), n = 6.

*a* Represents values statistically significant w.r.t. Normal control group (p< 0.05).

*b* Represents values statistically significant w.r.t. Hyperlipidemic control group (p<0.05).

Treatment with standard drug Atorvastatin as well as the two doses of the MEFPA led to the significant decline in MDA levels thus exhibiting the antioxidant activity of the extract. Atorvastatin treated group showed the highest decline in levels of TBARS (Thiobarbituric acid reactive substances), followed by the 500 mg/kg body weight and the 250 mg/kg body weight doses of the MEFPA.

Superoxide dismutase (SOD) activity in hyperlipidemic control group has shown significant decline with respect to the Normal control group (Table 4). Treatment with standard drug Atorvastatin as well as the two doses of the MEFPA led to the significant increase in SOD activity levels thus demonstrating the antioxidant activity of the extract. Atorvastatin treated group showed the highest increase in SOD activity, followed by the 500 mg/kg body weight and the 250 mg/kg body weight doses of the MEFPA.
Catalase activity has also shown a significant decrease in the hyperlipidemic control group (Table 4). Treatment with the standard drug Atorvastatin and the two doses of the MEFPA led to a significant increase in Catalase activity. Atorvastatin treated group showed the highest increase in catalase activity, followed by the 500 mg/kg body weight and the 250 mg/kg body weight doses of the MEFPA.

Discussion

Plants have been used as a source of medicine by man since ancient times. More than 16,000 species of higher plants occur in India, of which approximately 7500 are used as medicine by various ethnicities this country [24]. Plant based medicines have also been found to improve patient tolerance even on long term use [25]. Also, due to their low cost, herbal drugs are increasingly being found effective in reaching poor populations in the developing world where many still do not have access to high price synthetic medicines. World Health Organization has suggested the evaluation of the potential of plants as effective therapeutic agents, especially in areas where there is lack of safe modern drugs [26].

The preceding phytochemical tests indicated the presence of glycosides, terpenes and flavonoids in the crude methanolic flower extract. Several such compounds are known to possess potent antioxidant activity [27]. MEFPA also showed significant antihyperlipidemic activity in diet induced hyperlipidemic model of rats, which was less than that of the standard Atorvastatin drug used in treatment. These actions may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptors and their protection etc. resulting in an increased removal of LDL-C from blood and its increased degradation and catabolism of cholesterol from the body. All these events either individually or in combination lead to decreased serum LDL-C levels which may have also reduced serum total cholesterol (TC) levels during the treatment with test extract [28].

MEFPA treatment also increased serum HDL-C levels in the hyperlipidemic rats though the increase was less compared to the
Atorvastatin treated group. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as "reverse cholesterol transport", thus increased serum HDL-C levels may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process. This is supported by the fact that MEFPA treatment led to a decrease in cardiac risk indicator –"Atherogenic Index" values due to decrease in Total cholesterol levels along with increase in HDL-C [29]. The MEFPA also led to a decline in the percentage body weight gain compared to the hyperlipidemic control group though Atorvastatin was found to be more effective in decreasing percentage body weight gain. This may be attributed to the antihyperlipidemic action of the MEFPA.

Rats treated with both doses of MEFPA (250 and 500 mg/ kg body weight)and Atorvastatin (10 mg/ kg body weight) caused significant decrease in the levels of ALT and ALP activities with Atorvastatin exhibiting the highest response, compared to diet induced hyperlipidemic control group. These results suggest the hepatoprotective activity of the MEFPA. This activity may be due to the antioxidant properties of the plant [29].

Many diseases owe their origins to free radicals and hyperlipidemia and its consequences are no exception. They occur when free radicals are generated in excess or when the cellular antioxidant defense system is defective, they can stimulate chain reactions by interacting with proteins, lipids and nucleic acids causing cellular dysfunction and even death. The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT). These block the initiation of free radical chain reactions [30].

TBARS (Thiobarbituric acid reactive substances) are produced in the body as a result of lipid peroxidation. Hence they are measured in order to determine the extent of oxidative stress occurring inside the body. 99 % of TBARS is a compound known as Malondialdehyde. Reactive oxygen species (ROS) degrade polyunsaturated lipids, forming malondialdehyde [31]. The levels of Malondialdehyde were found to be significantly increased
in the hyperlipidemic control group compared to the normal control group indicating an increase in oxidative stress. Both doses of MEFPA led to a significant decrease in Malondialdehyde levels though less than that of the decrease exhibited by Atorvastatin treatment. This may be due to the free radical scavenging activity of the MEFPA.

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen [32]. SOD activity decreases in the body due to oxidative stress. The hyperlipidemic control group showed significantly decreases SOD activity compared to the normal control group, while both doses of MEFPA and Atorvastatin led to the significant increase in SOD activity compared to the hyperlipidemic control group. Atorvastatin showed a better increment in SOD activity than the MEFPA. This result may have been due to the in-vivo antioxidant activity of the MEFPA.

Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers of all enzymes; one catalase enzyme can convert 40 million molecules of hydrogen peroxide to water and oxygen each second [33]. Catalase activity diminishes in disease conditions involving oxidative stress like hyperlipidemia. In this study the catalase activity was found to be significantly decreased in the hyperlipidemic diet control group compared to the normal control group. Both doses of MEFPA resulted in significant increase in the Catalase activity compared to the hyperlipidemic control group though the rise was less than that exhibited by the Atorvastatin treated group. This result may similarly be due to the free radical scavenging activity of the MEFPA.

**Conclusion**

Both doses (250 and 500 mg/ kg body weight) of MEFPA (Methanolic extract of flowers of *P. acerifolium*) showed significant antihyperlipidemic activity in animal experiments following a dose-dependent relationship. The extract also showed significant in-vivo anti-oxidant activity. Thus, the plant’s antidiabetic activity found in
earlier works and the antihyperlipidemic activity found in this study indicate that the antioxidant properties of the plant may be responsible for these activities. Even though, All the studies were done with crude methanolic extract of the flower and further detailed studies need to be carried out using the fractions containing the active constituents responsible for particular activities to exactly pinpoint on the active principle responsible for antihyperlipidemic activity.

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