ANTIHYPERTENSION ACTIVITY OF HEIMIDESEMS INDICUS BIOFRACTION IN POLOXAMER-407 INDUCED HYPERLIPIDEMIC RATS

Mehul G. Rana*, Dr. Ashvin V. Dudhrejiya¹ and Rachana V. Katbamna²

*¹²S.J. Thakkar Pharmacy College, Opp. NRI Bungalow, Avadh Road, Rajkot, Gujarat.

¹²B.K. Mody Government Pharmacy College, Polytechniq Campus, Aji Dam, Rajkot, Gujarat.

ABSTRACT

The various bio-fractions of water extract of roots of *Hemidesmus indicus* (family: Asclepiadaceae) was evaluated for anti-hyperlipidemic activity in poloxamer 407 induced hyperlipidemic rat. Hyperlipidemia was induced in rat by i.p. injection of poloxamer 407 (30% w/w in distilled cool water; 400 mg/kg) and 1 hours before the administration of P-407, the rat of reference group were administered with atorvastatin (50 mg/kg p.o.) and finofibrate (FF 65 mg/kg p.o.) while test groups received the various bio-fractions (Benzene, Ethyl acetate, Methanol, Water) of water extract of plant (100 mg/kg p.o and 200 mg/kg p.o of each bio-fractions). After 3, 6 and 24 hour of treatment, serum lipid profiles were investigated. The administration of the ethyl acetate fractions of root extract significantly (p < 0.05) reduced the serum levels of total cholesterol, triglycerides (TG) and very low density lipoprotein (VLDL) as well as the atherogenic index (A.I.) the P-407 induced hyperlipidemic control rats after 6 h of treatment. After 24 h of treatment, the ethyl acetate fractions of extract induced a significant reduction (p < 0.001) in serum total cholesterol (TC), VLDL, low density lipoprotein (LDL) as well as the atherogenic index and significant increase in HDL levels, when compared to P-407 control group. All these effects were comparable to those of the reference standard, atorvastatin and finofibrates. The results of the investigation was found to be that the ethyl acetate fraction water extract of *Hemidesmus indicus* has potential...
antihyperlipidemic activity and might be used for the prevention of hyperlipidemia associated disorders.

**KEYWORDS:** atherogenic index, hyperlipidemia, *Hemidesmus indicus*, biofractions, poloxamer 407.

**INTRODUCTION**

Hypelipidemia is a metabolic disorders which is recognized as elevated lipid i.e. low density lipoproteins (LDL), cholesterol (esters derivatives) and triglycerides, level in blood. These lipids are associated with blood plasma proteins and remain in the dissolved state in the blood. The primary reason for hyperlipidemia is genetic abnormalities followed by defect in lipid metabolism which is caused by the defect in lipoprotein lipase activity\(^1\) or the absence of the surface Apoprotein C-II\(^1\), formation of reactive oxygen species\(^3\)\(^-\)\(^4\) and environmental factors.\(^1\) These factors are initiation of atherosclerosis and development of cardiovascular diseases. The primary treatment in patients with hyperlipidemia is lowering of LDL levels thereby reducing the risk of developing ischemic heart disease or the occurrence of further cardiovascular and cerebrovascular diseases.\(^1\)\(^-\)\(^5\)

Various antihyperlipidemic drugs have been found effective in lowering elevated serum low-density lipid levels, but they have been many side effects. e.g. statins, 3-hydroxy-3-methylglutaryl coenzyme A(HMG CoA) reductase inhibitors. HMG CoA reductase enzyme plays a central role in the production of cholesterol in the liver. Statins have rare but severe adverse effects.\(^6\) Also, the other agents like Niacin, Fibrates, Cholesterol absorption inhibitors, bile acid sequestering resins shows the adverse effects like flushing, pruritis, GI Irritaion, exacerbation of gout, impairment in the absorption of fat soluble vitamins and also they are devoid of antioxidant property.\(^1\)

Currently, the use of complementary and alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long tern use.\(^7\)

Evidently, many natural products or plants have gain attraction as hypolipidemics which are antioxidant but have no side effects in many studies.\(^8\)\(^,\)\(^9\) These natural drugs can better manage the hyperlipidemia associated disorders which are usually accompanied by increased
oxidative stress. Recent study has shown that medicinal plants intake results in an increase of antioxidant enzymes activity and HDL cholesterol and a decrease in malondialdehyde, which may reduce the risk of heart disease. Accordingly, traditional herbal formulae or medicinal plants would be valuable as new drugs for patients suffering from blood lipid disorders.

Considering all the above factors, it is worthwhile investigating plant drug which have been in existence though traditional system of medicine and believe to have wide biological activities, higher safety margin than synthetic drug. Hence studying the activity of the herb, which is claimed to possess antihyperlipidemic activities suggesting its usefulness in the treatment of hyperlipidemia may pave way for newer and better therapies.

*Hemidesmus indicus var. indicus* (family: Asclepiadaceae) is a widely distributed medicinal plant in India, known as Anantmul in gujarati, Nannari in Tamil and Indian sarsaparilla in English has been extensively used in Ayurvedic system of medicine. It is a medicinal, perennial, prostrate and twining shrub. In Ayurvedic literature of India, root and root bark have been recommended as a remedy for various ailments like biliousness, blood diseases, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism. A number of active chemical constituents from this plant have been identified which include essential oils and phytosterols, like hemidesmol, hemidesterol, and saponins and nine pregnane glycosides viz. Desinine, Indicine, Hemidine, Indicusin, Hemidescine, Emidine, Medesdine, Hemisine and Demicine from *H. indicus*.

Evidently, this plant have been reported to be used as hypoglycemic, hypolipidemic, antioxidant, antithrombotic, tranquilizer, anti-inflammatory, anti-pyretic, antiulcerogenic, cardioprotective, hepatoprotective, renoprotective, genotoxic and anti-genotoxic, radioprotective, neutralization of viper venom, anti-bacterial and larvicidal. The objective of the present research work was to investigate the antihyperlipidemic activity of biofraction of water extract of root of *H.indicus* by studying in vivo effect on poloxamer p-407 induced hyperlipidemia in rats.
MATERIALS AND METHODS

Plant Material
Roots of *Emidesmus indicus* procured from local market of Rajkot and were identified by Dr. Rajesh Raviya, Professor of Botany, Department of Biology, MVM Science and Home Science College, Rajkot. Flowers were washed with water, air dried for several days. Dried plant material was ground into coarse powder and stored into an air tight container till further usage. Voucher specimen (SJTPC/2/2014) was deposited for further reference.

Preparation of crude extract
Powder was exhaustively extracted with water in soxhlet apparatus at controlled temperature (40°C) for 72 hours. Resulting solutions were filtered through Whatman filter paper (No.42). The filtrates so obtained were concentrated in a water bath at low temperature (40°C). The dried weight of crude extracts was determined and designated as HIWE. HIWE (25 g) was subjected to solvent-solvent successive partitioning with solvents (3×200 ml for each solvent type) of increasing polarity- benzene, ethyl acetate, methanol and water designated as WB, WEA, WM and WA were stored in a sealed jar at 4°C until further use. For dosing, the extract and fractions were uniformly suspended in 0.5% Carboxy Methyl Cellulose (CMC) dissolved in water and administered orally (p.o).

Preliminary phytochemical screening
The bio-fractions of water extract of roots of *Emidesmus indicus* were subjected to preliminary phytochemical screening to check the presence of flavonoids, tannins and phenolic compounds, alkaloids, glycosides, terpenoids, steroids, carbohydrates and proteins by using their respective chemical tests.\[37\]

Experimental Animals
Healthy male and female Sprague Dawley rats weighing between 250–280 g were procured from the Zydus Cadila Research Centre, Ahmadabad. Animals were housed at animal house facilities of S. J. Thakkar Pharmacy College, Rajkot, Gujarat. Animals were housed in polypropylene cages and maintained in a regulated standard environment (12 hr light/dark cycle, 22 ± 2°C and 55 ± 5% relative humidity). They were fed with standard rat pellet diet and water *ad libitum*. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for the
care and use of laboratory animals. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), S. J. Thakkar Pharmacy College, Rajkot. (Ref. no.SJT-75/2013).

**In vivo antihyperlipidemic activity**[^38]

Antihyperlipidemic activity of bio-fractions of water extract of roots of *Hemidesmus indicus* was examined in Poloxamer-407 induced hyperlipidemic rats[^38]

Male Sprague dawley rats were randomly divided into 12 groups as follows-

Group 1: Vehicle control group in which rats were administered vehicle i.e. 0.5% w/v sodium CMC suspension and normal saline, i.p.

Group 2: Diseases control group in which rats were administered with single dose of Poloxamer 407 (500 mg/kg i.p.).

Group 3& 4: Standard control in which rats were administered Atorvastatin (AS 50 mg/kg) and Fenofibrate (FF 65 mg/kg body wt), p.o. and P-407, i.p, respectively.

Group 5: Test group of low dose in which rats were administered with 100 mg/kg body wt. benzene fraction of HIWE (WB– 100), p.o. and P-407, i.p.

Group 6: Test group of high dose in which rats were administered with 200 mg/kg body wt. benzene fraction of HIWE (WB– 200), p.o. and P-407, i.p.

Group 7: Test group of low dose in which rats were administered with 100 mg/kg body wt. ethyl acetate fraction of HIWE (WEA– 100), p.o. and P-407, i.p.

Group 8: Test group of high dose in which rats were administered with 200 mg/kg body wt. benzene fraction of HIWE (WEA – 200), p.o. and P-407, i.p.

Group 9: Test group of low dose in which rats were administered with Received 100 mg/kg body wt. Methanol fraction of HIWE (WM– 100), p.o. and P-407, i.p.

Group 10: Test group of high dose in which rats were administered with Received 200 mg/kg body wt. Methanol fraction of HIWE (WM– 200), p.o. and P-407, i.p.

Group 11: Test group of low dose in which rats were administered Received 100 mg/kg body wt. aqueous fraction of HIWE (WA– 100), p.o. and P-407, i.p.

Group 12: Test group of high dose in which rats were daily administered with Received 200 mg/kg body wt. aqueous fraction of HIWE (WA– 200), p.o. and P-407, i.p.

All test extracts/reference drugs/vehicle were administered by oral gavage at 24 hr and 1 hr prior to i.p. injection of 30% (w/v) P-407. Food was withheld in all groups till 6th hr blood samples collection and animals allowed access only to water.
Blood sampling and Biochemical estimation

Blood samples were collected at 3, 6 and 24 hr after P-407 injection from retro-orbital plexus under the influence of ether anesthesia. The blood samples were centrifuged (6000 rpm for 10 min at 4°C) and serum was used for lipid analysis. The supernatant clear serum thus obtained was transferred carefully with help of micropipette into small test tubes for estimation. The serum concentration of Total cholesterol (TC), HDL-C, Triglycerides (TG), LDL-C were measured by standard procedures using an autoanalyzer using commercially available kits (Span Diagnostics Ltd, Surat, India). Very low-density lipoprotein-cholesterol (VLDL-C) was calculated by Friedwald formula\(^{(39)}\): 
\[
\text{VLDL-C} = \frac{\text{TG}}{5}
\]
Low density lipoprotein-cholesterol (LDL-C) was calculated using Modified Friedwald formula (MMF) proposed by Chen \textit{et al} (2010)\(^{(40)}\) because original Friedwald’s formula is not valid when triglycerides levels are more than 400 mg/dl.

MFF: 
\[
\text{LDL-C} (\text{mg/dl}) = \{\text{Non-HDL-C} \times 90\% \} - \{\text{TG} \times 10\% \}
\]
Where; 
\[
\text{Non-HDL-C} = \text{TC} - \text{HDL-C}
\]
Atherogenic index (A.I) = \[
\frac{\text{VLDL-C} + \text{LDL-C}}{\text{HDL-C}}
\]

STATISTICAL ANALYSIS

Data was expressed as mean ± SEM. Statistical analysis by one-way ANOVA with Tukey’s posttest was performed as required using GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego, CA, USA. \(p<0.05\) was considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening revealed that biofraction of HIWE showed the presence of triterpenoids, glycosides, phenolic acids, flavonoids, sterols and tannins.

\textit{In vivo} antihyperlipidemic activity

The serum TC, TG, LDL-C and VLDL-C levels were significantly \((p < 0.001)\) increased in the hyperlipidemic P-407 control group at 3h (Table 1), 6hr (Table 2) and 24 h (Table 3), when compared with the normal control group. But ethyl acetate fraction of water extract of \textit{hemidesmus indicus} was found to be effective in significantly reducing serum TC, TG and VLDL \((p < 0.001)\) levels when compared to the P-407 induced hyperlipidemic control rat (disease control), after 6 and 24 h of treatment at a dose of 200 mg/kg p.o. (Table 2 and Table 3). After 3 h of treatment, WEA 200 reduced serum TC level but not significantly, but it reduced
TG and VLDL level. After 6 h of treatment, WEA 200 significantly reduced serum TC, TG and VLDL-C levels but after 24 h of treatment, WEA 100 and WEA 200 were reduced significantly TC, TG, VLDL-C (p < 0.001). WEA 200 also increased the serum HDL-C levels significantly (p < 0.05) after 24 h of treatment, when compared to the P-407 control group. The most useful finding was that WEA 200 significantly lowered the atherogenic index (A.I.) after 6 h (p < 0.05) and 24 h (p < 0.001) of treatment. All these effects were comparable to those of the reference standard, atorvastatin and fenofibrate.

**Table 1: Effect of biofractions of water extract of root of *Hemidesmus indicus* on serum lipid profile 3 h after poloxamer 407 -induced acute hyperlipidemia in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>102.82 ± 6.69</td>
<td>86.01 ± 3.12</td>
<td>17.20 ± 0.62</td>
<td>36.40±1.99</td>
<td>51.17±5.35</td>
<td>1.43±0.15</td>
</tr>
<tr>
<td>DC</td>
<td>169.05±19.6</td>
<td>617.57±54.43</td>
<td>123.51±10.89</td>
<td>21.92±1.58</td>
<td>70.66±11.88</td>
<td>3.19±0.38†</td>
</tr>
<tr>
<td>WB-100</td>
<td>162.53±5.50‡</td>
<td>578.40±27.52</td>
<td>115.68±5.50</td>
<td>27.77±1.25</td>
<td>63.45±6.78</td>
<td>2.33±0.30</td>
</tr>
<tr>
<td>WB-200</td>
<td>147.68±8.14‖</td>
<td>556.40±21.69</td>
<td>111.28±4.34</td>
<td>29.86±1.12</td>
<td>50.40±7.53</td>
<td>2.12±0.08</td>
</tr>
<tr>
<td>WEA-100</td>
<td>154.51±8.16⁺</td>
<td>523.70±20.09</td>
<td>104.74±4.02</td>
<td>30.91±2.32</td>
<td>58.87±7.26</td>
<td>2.26±0.11</td>
</tr>
<tr>
<td>WEA-200</td>
<td>144.83±5.95‖</td>
<td>498.17±22.36</td>
<td>99.63±4.47</td>
<td>31.80±2.38</td>
<td>51.91±4.54</td>
<td>2.04±0.40</td>
</tr>
<tr>
<td>WM-100</td>
<td>159.43±10.9‡</td>
<td>573.73±20.51</td>
<td>114.75±4.10</td>
<td>25.93±1.26‡</td>
<td>62.78±9.75</td>
<td>2.51±0.50</td>
</tr>
<tr>
<td>WM-200</td>
<td>149.87±4.97‖</td>
<td>545.84±15.38</td>
<td>109.17±3.08</td>
<td>29.14±1.64</td>
<td>54.08±9.44</td>
<td>2.29±0.35</td>
</tr>
<tr>
<td>WA-100</td>
<td>175.19±7.02‖</td>
<td>586.17±15.58</td>
<td>117.23±3.12</td>
<td>27.56±1.86</td>
<td>74.25±7.54</td>
<td>2.83±0.42</td>
</tr>
<tr>
<td>WA-200</td>
<td>173.91±5.88‖</td>
<td>577.49±11.99</td>
<td>115.50±2.40</td>
<td>29.07±2.04</td>
<td>72.60±5.22</td>
<td>2.62±0.37</td>
</tr>
<tr>
<td>AS 50</td>
<td>115.13±0.98‡</td>
<td>416.70±22.28‖</td>
<td>83.34±4.46⁻</td>
<td>35.15±2.21</td>
<td>30.31±11.52</td>
<td>2.00±0.15</td>
</tr>
<tr>
<td>FF 65</td>
<td>126.08±7.97</td>
<td>370.48±15.45‖</td>
<td>74.10±3.09‖</td>
<td>38.63±3.33⁻</td>
<td>41.66±8.27</td>
<td>2.00±0.08</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M. (n = 6). One way ANOVA followed by Tukeys test. †, ‡, Φ compared to normal control group (p < 0.05, P<0.01, P<0.001), respectively; *,**,# compared to P-407 control group (p < 0.001); ***compared to P-407 control (p < 0.05, P<0.01, P<0.001), respectively. TC: total cholesterol, TG: triglyceride, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, A.I.: atherogenic index.

**Table 2: Effect of biofractions of water extract of root of *Hemidesmus indicus* on serum lipid profile 6 h after poloxamer 407 -induced acute hyperlipidemia in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>94.02 ± 4.35</td>
<td>74.36 ± 3.15</td>
<td>14.87 ± 0.63</td>
<td>36.45±2.26</td>
<td>44.37±4.98</td>
<td>1.27±0.19</td>
</tr>
<tr>
<td>DC</td>
<td>191.80±16.69‖</td>
<td>694.30±39.06&quot;</td>
<td>138.86±7.81&quot;</td>
<td>46.18±2.64ns</td>
<td>61.63±14.57</td>
<td>3.22±0.13&quot;</td>
</tr>
<tr>
<td>WB-100</td>
<td>186.85±7.31&quot;</td>
<td>684.47±17.21</td>
<td>136.89±3.44</td>
<td>44.50±2.12</td>
<td>59.67±4.14</td>
<td>2.35±0.09</td>
</tr>
<tr>
<td>WB-200</td>
<td>175.49±8.58&quot;</td>
<td>674.30±15.45</td>
<td>134.86±3.09</td>
<td>42.93±2.69</td>
<td>51.87±8.47</td>
<td>2.25±0.21</td>
</tr>
<tr>
<td>WEA-100</td>
<td>156.50±7.55‡</td>
<td>581.45±22.36</td>
<td>116.29±4.47</td>
<td>39.37±2.14</td>
<td>47.28±8.47</td>
<td>1.76±0.28</td>
</tr>
<tr>
<td>WEA-200</td>
<td>136.60±7.39</td>
<td>548.58±17.03</td>
<td>109.72±3.41</td>
<td>37.37±2.87</td>
<td>34.45±7.31</td>
<td>1.38±0.23</td>
</tr>
</tbody>
</table>
All values are expressed as mean ± S.E.M. (n = 6). One way ANOVA followed by Tukeys test. †, ‡, ‡, Φ compared to normal control group (p < 0.05, P<0.01, P<0.001), respectively; *,**, # compared to P-407 control group (p < 0.001); ***compared to P-407 control (p < 0.05, P<0.01, P<0.001), respectively. TC: total cholesterol, TG: triglyceride, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, A.I.: atherogenic index.

Table 3: Effect of biofractions of water extract of root of Hemidesmus indicus on serum lipid profile 24 h after poloxamer 407 -induced acute hyperlipidemia in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>108.30 ± 4.18</td>
<td>93.02 ± 3.99</td>
<td>18.60 ± 0.80</td>
<td>39.80±2.08</td>
<td>52.35±4.56</td>
<td>1.35±0.17</td>
</tr>
<tr>
<td>DC</td>
<td>377.08+21.33a</td>
<td>837.41+41.04</td>
<td>167.48±8.21a</td>
<td>61.80±3.06a</td>
<td>200.01±20.34a</td>
<td>3.26±0.36a</td>
</tr>
<tr>
<td>WB-100</td>
<td>312.23±5.80</td>
<td>788.77±21.87</td>
<td>157.75±4.37</td>
<td>56.03±5.29</td>
<td>151.70±5.30</td>
<td>2.87±0.35</td>
</tr>
<tr>
<td>WB-200</td>
<td>300.23±10.8b</td>
<td>711.41±21.95a</td>
<td>142.28±4.39a</td>
<td>53.23±3.62a</td>
<td>151.16±9.26</td>
<td>2.91±0.29</td>
</tr>
<tr>
<td>WEA-100</td>
<td>286.84±13.30a</td>
<td>696.64±18.49a</td>
<td>139.33±3.70a</td>
<td>47.05±2.19</td>
<td>146.15±12.98</td>
<td>1.85±0.05</td>
</tr>
<tr>
<td>WEA-200</td>
<td>246.55±7.47a</td>
<td>564.10±24.47a</td>
<td>112.82±4.89a</td>
<td>42.51±2.78</td>
<td>127.22±8.70</td>
<td>1.37±0.10</td>
</tr>
<tr>
<td>WM-100</td>
<td>329.99±13.10a</td>
<td>794.09±20.29a</td>
<td>158.82±4.06a</td>
<td>45.43±4.04</td>
<td>176.69±11.81</td>
<td>4.07±0.49</td>
</tr>
<tr>
<td>WM-200</td>
<td>311.53±17.88a</td>
<td>762.14±13.59a</td>
<td>152.43±2.72a</td>
<td>46.07±3.06</td>
<td>162.70±17.96</td>
<td>3.74±0.64</td>
</tr>
<tr>
<td>WA-100</td>
<td>333.79±21.09a</td>
<td>768.50±16.94a</td>
<td>153.70±3.39a</td>
<td>55.98±3.73a</td>
<td>173.18±18.06</td>
<td>3.15±0.33</td>
</tr>
<tr>
<td>WA-200</td>
<td>314.07±17.48a</td>
<td>728.84±16.93a</td>
<td>145.77±3.39a</td>
<td>53.33±2.83</td>
<td>161.79±13.24</td>
<td>3.03±0.18</td>
</tr>
<tr>
<td>AS 50</td>
<td>202.20±8.78a</td>
<td>559.90±24.64a</td>
<td>111.98±4.93a</td>
<td>40.43±3.11</td>
<td>89.60±6.74a</td>
<td>1.09±0.04</td>
</tr>
<tr>
<td>FF 65</td>
<td>226.35±17.88a</td>
<td>512.82±27.22a</td>
<td>102.56±5.44a</td>
<td>43.87±4.33</td>
<td>112.95±15.68a</td>
<td>1.31±0.12</td>
</tr>
</tbody>
</table>

DISCUSSION

Hyperlipidemia characterized by abnormally elevated serum triacylglycerol (TG), total cholesterol (TC), LDL-C and VLDL-C, is an established risk factor for the development of coronary artery disease (CAD).[41] The P-407 treated rodent is a well established animal
model of dose dependent hyperlipidemia and atherosclerosis.\cite{42} P-407 has been utilized in the hyperlipidemic model due to its convenience, reproducibility and the lack of undesirable underlying pathological conditions.\cite{42} Increased serum total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) accompanied by reduced HDL-C levels, are important risk factors for atherosclerosis development\cite{43,45} In particular, many studies have found LDL-C to be the most dangerous among the serum lipids, and the oxidation of LDL leads to its increased penetration of arterial walls.\cite{46,47} When there is excess LDL in the blood, it is deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. Moreover, in animal experiments, the LDL of hypercholesterolemic rabbits was more susceptible to oxidative modification than that of normal lipidemic rabbits.\cite{48} This oxidative modification of LDL is causally involved in the initiation and promotion of atherosclerosis.\cite{49} Thus, an increased cholesterol level can be a significant predictor of the development of coronary artery disease and serum LDL-C levels should be used as the basis for initiating and monitoring treatment of patients with elevated blood cholesterol.\cite{50,51} According to these studies, lowering serum TC and LDL-C levels is important for reducing the risk of atherosclerosis. In the present study, administration of ethyl acetate fraction of water extract of roots of the plant significantly reduced the serum TG, TC, VLDL and LDL levels (Tables 2 and 3) and the results imply that the ethyl acetate fraction have beneficial effects on serum lipid profile by reducing the lipid levels among the others.

HDL carries cholesterol and cholesterol esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids.\cite{52} This pathway plays a very important role in reducing cholesterol levels in the blood and peripheral tissues and in inhibiting atherosclerotic plaque formation in the aorta.\cite{53} The elevated levels of HDL-C exhibit an anti-atherogenic effect by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism.\cite{54} Our results show that ethyl acetate fraction of water extract of roots of the plant significantly increased HDL-C level concentrations when compared with the poloxamer 407-induced hyperlipidemic control rats. These results suggest that ethyl acetate fraction of \textit{H.indicus} is an effective lipid lowering agent and may protect against cardiovascular diseases that result from hyperlipidemia.

The A.I., the ratio of LDL to HDL, is commonly used as an index for atherosclerosis.\cite{55} The A.I. is believed to be an important risk factor for atherosclerosis, and was significantly
lowered in the poloxamer 407-induced hyperlipidemic rats administered with fraction of water extract of *hemidesmus indicus*. This decrease in the A.I. is yet another positive change resulting from the treatments. In particular, the ethyl acetate fraction showed a stronger lipid-lowering effect than the other fractions, as well as a high antiatherogenic potential, with atherogenic index (A.I.) values of less than 2 after 24 hrs. Treatment of rat with ethyl acetate fraction of water extract of *hemidesmus indicus* significantly reduced the atherogenic index (A.I.) compared to P-407 induced hyperlipidemic control suggesting the potential ability of the fraction in reducing the risk of atherosclerosis (Table 2 and 3). The results imply that oral administration of ethyl acetate fraction of water extract of *hemidesmus indicus* have potential ability to reduce the risk of atherosclerosis.

Since Johnston and Plamer[39] and Johnston[42] have demonstrated that the increase in triglycerides (TG) seen following P-407 ip. injection results primarily due to an inhibition of TG degradation, where P-407 directly inhibits capillary lipoprotein lipase (LPL) responsible for plasma TG hydrolysis. The serum TG lowering activities of ethyl acetate fraction of water extract of *hemidesmus indicus* can be attributed to the ability of the extract to increase the lipoprotein lipase activities.

The mechanism responsible for the elevation of serum cholesterol following i.p. injection of poloxamer 407 solution to rats may be due to stimulation of 3-hydroxy-3- methylglutaryl-Co-enzyme A (HMG-CoA) reductase activity in the liver by the poloxamer vehicle.[42,43] Besides, Atorvastatin, used as positive control in this study is a HMG-CoA reductase inhibitor which is the rate-limiting enzyme in the biosynthesis of cholesterol. Thus, the cholesterol lowering effects of the fraction might be related partly to the decreased activity of hepatic HMG-CoA reductase.

The roots of *H.indicus* contains different tannins such as ellagic acid and gallic acid[48-50] as well as different flavonoids such as 2-hydroxy-4-methoxy-benzoic acid, Hyperoside, Rutin, Isoquercitin etc.[51-54] Besides, roots also contains different triterpenoids: Hexadecanoic acid, hexatriacontane, 6 lupeol, octacosanoate (a new ester), β-amyrin acetate, lupeol acetate, α-amyrin, β-amyrin and sitosterol.[54,55] The observed anti hyperlipidemic activity of the ethyl acetate fraction of water extract of roots of *H.indicus* may be due to the presence of the above mentioned flavonoids, tannins and triterpenoids as it has been reported that flavonoids,[56] tannins and terpenoids[57] possess anti hyperlipidemic activity.
In summary, the present study demonstrated that biofractions of *H.indicus* had hypolipidemic effects in poloxamer 407-induced hyperlipidaemic rats, where the ethyl acetate fraction significantly lowered serum TC, TG and LDL-C concentrations, elevated HDL-C levels and decreased serum A.I. values. These results suggest that the plant extracts may be beneficial in preventing atherosclerotic cardiovascular diseases and further investigations are warranted to examine the mechanism of action as well as to isolate the active constituents responsible for these activities.

ACKNOWLEDGEMENTS
Authors are thankful to S.J. Thakkar Pharmacy College, Rajkot for providing facilities to carried out experiments. Also thankful to our colleagues for their kind help and support during the course of present study.

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