INTRODUCTION

Hyperlipidemia a predisposing factor to the development of atherosclerosis, coronary artery disease (CAD) and several cardiac manifestations such as myocardial infarction (MI), ischemia, and angina and leads to morbidity and mortality. Hyperlipidemia specifically characterized by alterations occurring in serum lipid and lipoprotein profile i.e. increased concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglyceride (TG) with a decrease in the concentrations of high density lipoprotein cholesterol (HDL-C). It has been reported that abdominal obesity, impaired postprandial lipid metabolism and insulin resistance are all interrelated risk markers for coronary heart diseases. Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated risk in diabetes mellitus. Thus, in this condition there is a possibility of increase in the blood glucose level. Allopathic hypolipidemic drugs are largely available in the market but due to the side-effects and contraindications of these drugs have marred their popularity. Now-a-days herbal hypolipidemic products have gained importance to fill the lacunae created by the allopathic medicines. In recent scientific practice, statins are the most trusted lipid lowering agent. Statins (mainly atorvastatin) demonstrate the beneficial role by competitive inhibition of hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoAR) which blocks cholesterol biosynthesis, which in turn stimulates the synthesis of LDL receptors present on hepatic cell and thus lowers the plasma LDL cholesterol concentration. Statins with the beneficial role associated with some side effects like gastrointestinal disturbance, liver dysfunction, cholestasis, insomnia and myalgia. Herbal remedies which were used traditionally now significantly documented for the safety profile and as a therapy for some of the pathological conditions. By observing the synergistic activity of phytochemicals, recently it has been advocated to use polyherbal formulation. In recent trend to achieve the maximum benefit of herbal therapy researcher combining a set of herbs in the form of specific formulation which is expected to deliver maximum potency compared to a single herb. Divya Methipachak (DMP) is marketed polyherbal formulation, each 100g containing methi dana (Trigonella foenum graecum) - 75mg, sajjikshar -3mg, trikatu churn (Zingiber officinalis, Piper nigrum, Piper longum) - 3mg, nimbu sat (Citrus limon) - 2mg, kalajeera (Cuminum cyminum) - 2mg, sendha namak (sandhav lavan) - 15mg. The presence of potential phytoconstituents such as alkaloids, flavonoids, saponins, carbohydrates, limonene, gallic acid, ellagic acid, ferulic acid and flavonols, monoterpenes, zingiberol, gengerol, pipeline, volatile oil (caryophyllene), piperidine, piperlonguminone, limonene, terpinene, geraniol, bergamotene, citronellal and linalool and this marketed polyherbal formulation claiming to possess anti-hyperlipidemic activity, antiabetic activity, beneficial role in the treatment of coronary artery disease. But till now there is no scientific data has been documented. Hence, the present study was carried out to evaluate the anti-hyperlipidemic activity of DMP a polyherbal formulation on triton induced hyperlipidemia in rats.

MATERIALS & METHODS

Experimental animals:

Laboratory bred Wistar albino rats (180-200 g) of either sex were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. The animals had free access to standard food pellets (Amrut Laboratory Animal feed, Maharashtra, India) containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, and water ad libitum. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Drug and dosage:

The dried granules of the polyherbal preparation, Divya Methipachak were supplied by the Patanjali chikitsalaya, manufactured by Divya Pharmacy Ltd., India. The formulation was administered at doses of 150 mg/kg p.o and 300 mg/kg p.o in the form of solution prepared in water. The doses were selected based on the human dose mentioned in the Ayurvedic literature. Triton X-100 (a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from...
National chemicals, Vadodara, Gujarat. HDL-C, TG, TC, and blood glucose kits were procured from Robonik India Pvt Ltd, Mumbai. Other chemicals used were obtained from SD Chemicals Ltd. (Mumbai, India). All chemicals used in the present study were of analytical grade.

**Triton Induced Hyperlipidemia:**

**Experimental Design:**

The animals were randomly divided into 5 groups of 6 each. From group II to V, Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h. The group II to V received a single dose of triton administered at a dose of 100 mg/kg, i.p. After 72 h of triton injection, group III received a daily dose of standard drug (10 mg/kg, p.o) and group IV and V received a daily dose of DMP (p.o) for 7 days. The group I was served as normal control. The different groups were assigned as described below.

- **Group I:** Vehicle control (1% Tween 80)
- **Group II:** Positive control (Triton X-100)
- **Group III:** Atorvastatin (10 mg/kg, p.o) + Triton X-100
- **Group IV:** Low dose of Methipachak (150 mg/kg, oral) + Triton X-100
- **Group V:** High dose of Methipachak (300 mg/kg, oral) + Triton X-100

**Biochemical analysis:**

On 8th day after fasting for 18 h the animals were anesthetized with ether and blood was withdrawn by retero orbital sinus puncture. Serum was separated by centrifugation of blood at 3000 rpm/10 min for estimation of biochemical parameters such as TC, TG, HDL-C, LDL-C, VLDL-C, serum blood glucose and atherogenic index (AI) were calculated.

Triglycerides and cholesterol were measured with enzymatic kits. HDL-cholesterol was determined after precipitation of VLDL and LDL with phosphotungstic acid and magnesium chloride. The VLDL- and LDL-cholesterol concentrations were calculated from the Friedewald’s equation:

\[ \text{VLDL-C} = \frac{\text{TG}}{5} \]

\[ \text{LDL-C} = \text{TC} - \text{HDL-C} - \left( \frac{\text{TG}}{5} \right) \]

AI was calculated by using the formula of Schulpis:

\[ \text{Atherogenic Index (AI)} = \frac{\text{TC} - \text{Total serum HDL-C}}{\text{Total serum HDL-C}} \]

**Histopathological studies:**

At the end of the treatment period, animals from all the four groups were sacrificed and liver was dissected out, washed, 5 μm thick section slides were prepared and stained with haematoxylin – eosin and examined by light microscopy (Fig. 1).

**Statistical analysis**

Results are expressed as mean± S.E. statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P<0.05 was considered significant.

**RESULTS**

**Effect on TC, TG, HDL-C and blood glucose -**

The experimental results revealed that there was a significant increase in serum TC, TG and blood glucose level with triton X-100 treated group compared to normal control whereas HDL-C value indicated a significant decrease with triton X-100 compared to normal control (Table 1). Prophylactic treatment with Atorvastatin (10 mg/kg), low (150 mg/kg) and high (300 mg/kg) dose of DMP demonstrated significant fall in serum TC, TG and blood glucose level compared to triton X-100 treated group. In case of HDL-C a significant fall was observed compared to triton X-100 treated group (Table 1).

**Effect on LDL-C, VLDL-C and Atherogenic Index -**

Group II (triton X-100 treated group) exhibited a significant raise in LDL-C and VLDL-C when compared with normal group animals. Administration of DMP low dose (150 mg/kg) and high dose (300 mg/kg) shows significant decrease in LDL-C and VLDL-C when compared with group II animals (Table 2). Decreased in AI was observed in all the treated groups (group III to group V) compared to triton X-100 treated group (Table 2).

**Effect on histological score:**

In the histopathological study, Triton treated group shows fatty infiltration and granular degeneration as compared to normal control. Treatment with Divya Methipachak of dose 150 mg/kg and 300 mg/kg shows mild cytoplasmatic fatty infiltration and mild granular degeneration as compared to cafeteria diet fed rats. Atorvastatin shows negligible cytoplasmatic fatty infiltration and granular degeneration.

**DISCUSSION**

The aim of the present study was to elucidate the role of the polyherbal formulation DMP during hyperlipidemia induced by triton in rats. DMP was found to be potential anti-hyperlipidemic agent in a dose dependent manner in alleviating abnormal conditions induced by triton.

Hyperlipidemia was induced in the rats using triton X-100. Triton X-100 increase the cholesterol levels by 47% and serum triglycerides by 88% compared with values of control group after 24 h. Triton-induced hyperlipidemia may be considered a simple and rapid test for an evaluation of compounds potentially active on serum cholesterol. Systemic administration of triton X-100 resulted in a biphasic elevation of plasma cholesterol and triglycerides. Concentration of serum cholesterol levels increases after 24 h in phase I. The triton X-100 acts as surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues resulting into increased blood lipid concentration and also it increases hepatic synthesis of cholesterol and the induced hyperlipidemia decreases nearly to control levels within next 24 h in phase II1. Atorvastatin is most trusted anti-hyperlipidemic c drug. It is competitive antagonist of HMG-CoAR. It demonstrate the beneficial role by competitive inhibition of HMG-CoAR which blocks cholesterol biosynthesis, also stimulates the synthesis of LDL receptors present on hepatic cell and thus lowers the plasma LDL cholesterol concentration1. The polyherbal formulation DMP in both and high dose provides protection against hyperlipidemia by decreasing the TC, TG, LDL-C, VLDL-C level and increasing HDL-C level. The results of experimental data was further supported by histopathological analysis. The HDL-C is considered to have anti-atherogenic properties; HDL-C gives protection against many cardiac problems and obesity7.

As mentioned earlier, DMP contains 8 different constituents and the formulation is described in the ancient ayurvedic literature. A survey on the activities of the constituents revealed that Trigonella foenum graecum12, Zinger officinale, Piper nigrum and Piper longum13 are reported to be effective in experimental hyperlipidemia. Apart from the presence of potential phytoconstituents such as steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin), alkaloids (mainly trigonelline), zingiberol, piperine, piperlongumunine and free amino acids justify the potential of this herbal formulation. This may be due to the
combination and complementary actions of the individual components of the polyherbal formulation.

ACKNOWLEDGEMENTS

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REFERENCES


TABLE 1: EFFECT OF DIVYA METHI-PACHAK – A POLYHERBAL FORMULATION ON SERUM TOTAL CHOLESTEROL, TRIGLYCERIDES, HDL-C AND GLUCOSE LEVEL IN TRITON INDUCED HYPERLIPIDEMIA IN RATS.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>19.35±0.71</td>
<td>20.66±0.49</td>
<td>18.66±0.12</td>
<td>61.84±1.23</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>52.66±1.20</td>
<td>36.95±0.95</td>
<td>3.63±0.25</td>
<td>140.29±0.03</td>
</tr>
<tr>
<td>3</td>
<td>Std 10 mg/kg (ATR)</td>
<td>23.00±0.57</td>
<td>22.73±0.08</td>
<td>17.06±0.07</td>
<td>101.84±0.38</td>
</tr>
<tr>
<td>4</td>
<td>LD DMP 150 mg/kg</td>
<td>25.00±0.25</td>
<td>23.88±0.06</td>
<td>16.90±0.05</td>
<td>103.07±1.55</td>
</tr>
<tr>
<td>5</td>
<td>HD DMP 300 mg/kg</td>
<td>23.62±0.22</td>
<td>22.86±0.12</td>
<td>17.20±0.15</td>
<td>98.03±2.38</td>
</tr>
</tbody>
</table>

Values are expressed as mg/dl, mean ± SEM; n=6. *p<0.05, **p<0.01, ***p=0.001 when compared to control, p=0.05, **p=0.01, ***p=0.001 when compared to positive control.

TABLE 2: EFFECT OF DMP ON SERUM LDL-C, VLDL-C AND Atherogenic INDEX IN TRITON INDUCED HYPERLIPIDEMIA IN RATS.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Atherogenic Index (AI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>4.82±0.03</td>
<td>4.13±0.09</td>
<td>15.97±0.35</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>56.42±0.39</td>
<td>7.39±0.37</td>
<td>61.29±5.70</td>
</tr>
<tr>
<td>3</td>
<td>Std 10 mg/kg (ATR)</td>
<td>10.49±0.1</td>
<td>4.55±0.03</td>
<td>14.05±0.71</td>
</tr>
<tr>
<td>4</td>
<td>LD DMP 150 mg/kg</td>
<td>12.87±0.06</td>
<td>4.78±0.02</td>
<td>10.4±0.52</td>
</tr>
<tr>
<td>5</td>
<td>HD DMP 300 mg/kg</td>
<td>10.99±0.07</td>
<td>4.57±0.03</td>
<td>7.08±1.34</td>
</tr>
</tbody>
</table>

Values are expressed as mg/dl, mean ± SEM; n=6. *p<0.05, **p<0.01, ***p=0.001 when compared to control, p=0.05, **p=0.01, ***p=0.001 when compared to positive control.

Figure 1: Histopathology of liver

1) Normal control group showing normal architecture; 2) Toxic control (Triton treated group) showing fatty infiltration and granular degeneration; 3) Atorvastatin + Triton treated group showing negligible cytoplasmic fatty infiltration and granular degeneration; 4) DMP+ Triton treated group (150mg/kg) showing mild to moderate cytoplasmic fatty infiltration and granular degeneration. 5) DMP + Triton treated group (300mg/kg) showing mild cytoplasmic fatty infiltration and mild granular degeneration

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