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Antihyperlipidemic effect of ambrex, a polyherbal formulation against experimentally induced hypercholesterolemia in rats

Jamuna Devi* and Johanna Rajkumar

Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, India-602105

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Ambrex, a polyherbal formulation was tested for its antihypercholesterolemic effect against experimentally induced hypercholesterolemia in rats. Alteration in the levels of serum marker enzymes, alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and general biochemical parameters glucose, protein, urea and creatinine were tested in both treated and untreated groups. Rats treated with high fat diet for four weeks exhibited hypercholesterolemia. Oral administration of ambrex at a dose of 40 mg/kg body weight significantly decreased serum total cholesterol, triglycerides and low density lipoproteins (LDL) levels and there was a concomitant increase in serum high density lipoprotein (HDL) levels. Atherogenic index of the ambrex treated group showed marked improvement compared to other groups. Studies established that ambrex might be a safe drug that exhibits negligible toxic effects on liver and kidney functions under experimental condition. The histopathological observation of liver tissue also confirms the same. The results shows the ambrex possess considerable antihypercholesterolemic activity compared with standard drug, atorvastatin.

Key words: Hypercholesterolemia, amber, Withania somnifera, atorvastatin.

INTRODUCTION

Hypercholesterolemia is one of the major causes of cardiovascular diseases, such as atherosclerosis, myocardial infarction or hypertension and is a primary cause for death (Robbins, 1991; Gerhardt and Gallo, 1998; Gomes et al., 1998; Sheyla et al., 2005). Hypercholesterolemia is generally characterized by elevated levels of serum cholesterol, triglycerides and low density lipoproteins (LDL) cholesterol and decreased high density lipoprotein (HDL) cholesterol level. Management of increased levels of serum LDL cholesterol would possibly lead to retardation of the progression of atherosclerotic lesions (Altschul, 1964; Annie and Kurup, 1986). Hypolipidemic drugs such as fibrates, statins and bile acid sequestrants used for the treatment show toxic side effects (Chattopadhyaya et al., 1996). The consumption of these synthetic drugs results in diarrhoea, nausea, gastric irritation and abnormal liver function (Kumar et al., 2008). Even though the modern allopathic drugs are effective, they are costly and associated with side effects (Grundy et al., 2004). Therefore, there emerges an urgent need to identify a better hypolipidemic drug with fewer side effects.

About 60% of the world’s population relies on herbal medicine for primary healthcare (Modak et al., 2007). The goal of health for all cannot be achieved without herbal medicines. Nowadays, herbal medicines are gaining prime importance all over the world. Hence, the present work is focused on determination of the hypolipidemic activity of ambrex, a polyherbal formulation which is a cocktail of four indigenous herbs Withania somnifera.

*Corresponding author. E-mail: jamunapadhu@gmail.com. Tel. 9840234882
(Ashwagandha), Orchis mascula (Roomimastagi), Cycas circinalis (Madanakamappu) and Shorea robusta (Shalamishri) with Pon amber. Ambrex has also been reported for its hepatoprotective and antiulcerogenic activities (Devi et al., 2003; Mallika and Devi, 2004). The hypolipidemic effect of Ambrex has not yet been explored. In the current study, ambrex was further investigated for its hypolipidemic activity in experimental animals.

MATERIALS AND METHODS

Polyherbal formulation

Ambrex (Care and Cure Herbs Limited, Chennai) is a marketed soft gelatin capsule and the composition of the formulation in a 250 mg capsule is W. somnifera (100 mg), O. mascula (25 mg), C. circinalis (62.5 mg), S. robusta (25 mg) with amber, a resin from Pinus succinifera (37.5 mg). The contents are processed and formulated according to the principles of Ayurveda which are aimed at enhancing efficacy.

Experimental protocol

Twenty-four male Wistar rats weighing about 120-130 g were used for the present study. The animals were fed with standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water ad libitum. The animal experiments were conducted as per the guidelines and approval of the Institutional Animal Ethical Committee (IAEC No. Biotech REC.001/10). The high fat diet consisted of 1% cholesterol, 10% egg yolk powder, 5% lard and 0.5% cholic acid (Huanxin et al., 2009). The animals were fed high fat diet for 30 days to induce hypercholesterolemia (75% Increase). Test drug, ambrex, and standard drug, atorvastatin, were dissolved in water and administered once a day through tube feeding for the next 15 days. The rats were divided into four groups of six animals each as follows: Group A: Control rats receiving standard diet; Group B: rats receiving high fat diet; Group C: rats receiving high fat diet and ambrex (40 mg/kg b.w ); The dosage was fixed based on previous studies (Mallika and Devi, 2004). Group D: rats receiving high fat diet and standard drug, atorvastatin (10 mg/kg b.w).

Assay kits and reagents

Cholesterol (SRL) & Cholic acid (LOBA) were obtained from Ganesh Scientific Suppliers, Chennai. The assay kits for serum glutamate-ornithine transaminase (SGOT) (Erba), serum glutamate pyruvate transaminase (SGPT) (Erba), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), glucose, total protein, urea, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglycerol (TG) and cholesterol (Merck make) were purchased from VKM Scientific Products, Chennai. All other reagents used were of analytical grade.

Biochemical analysis

Blood serum was used for the analysis of total cholesterol, triglyceride, HDL-C, LDL-C, SGOT, SGPT, ALP, LDH, glucose, total protein, urea and creatinine using standard enzymatic assay kits supplied by Merck India Limited, Mumbai, India. The atherogenic index was calculated using the formula:

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\text{Atherogenic index} = \frac{\text{Total cholesterol}}{\text{Total HDL cholesterol}}
\]

Protection % = \( \frac{\text{Atherogenic index of control group} - \text{Atherogenic index of treated group} \times 100}{\text{Atherogenic index of control group}} \)

Histopathological studies

At the end of the experimental period, portions of liver tissue were fixed in 10% formalin. The tissue samples were ultra-sectioned, stained with hemotoxylin and eosin and then viewed under light microscope for histopathological changes.

Statistical analysis

Statistical significance of data was analysed by one-way analysis of variance (ANOVA) using Tukey’s Multiple comparison as post hoc test using the Graph pad prism software package for windows (Version 5). The results are expressed as mean ± SEM/SD. p values <0.05 are considered as significant.

RESULTS

Body and organ weights analysis

Administration of ambrex to the high fat diet fed rats decreased the body weight when compared to untreated high fat diet group animals (Figure 1a). The present study reveals that there were no significant changes in the relative organ weights of kidney, heart and lungs, but liver showed a significant increase in high fat diet group in comparison to other groups which correlates with the fat deposition on hepatic tissues (Figure 1b).

Serum lipid profile

Total cholesterol, triglycerides, HDL-cholesterol and LDL cholesterol in the different groups of animals are presented in Table 1a and b. It details the serum lipid profile of various groups before and after the treatment protocol. From the data presented, it is evident that high fat diet group animals showed significant rise in the serum total cholesterol, triglycerides, LDL cholesterol levels and marked decrease in the HDL cholesterol levels. Administration of ambrex resulted in statistically
significant decrease in serum total cholesterol (22.45%), triglycerides (27.49%) and low density lipoprotein cholesterol level (14.10%) and significant augmentation in HDL cholesterol (7.42%) level as compared to untreated hypercholesterolemic rats. In addition, ambrex treatment showed a better decrease in atherogenic index and increased percentage of protection when compared to atorvastatin treatment (Table 2).

**General Biochemical parameters & Kidney function test**

Table 3 depicts the effect of ambrex treatment on blood glucose, total protein, urea and creatinine. Both ambrex treated and untreated hypercholesterolemic rats showed significant increase in the blood glucose level when compared to normal control animals. However, ambrex treated group showed slight decrease in serum urea and creatinine when compared to untreated control. The high fat diet group animals showed marked elevation in total protein, urea and creatinine levels as compared to treated groups.

**Liver function test**

In relation to liver function test, alanine amino transferase (ALT), aspartate amino transferase (AST), ALP and LDH were assessed and the results are given in Table 4. It is observed that ALT, AST, LDH and ALP levels were found elevated in high fat diet group (Group B) animals. The activities of ALT, AST, ALP and LDH in ambrex treated group are markedly lower than those of the high fat diet group animals.

**Histopathological observation**

Microscopic examination of liver section of untreated high
Fat diet group animal showed marked vascular congestion, fatty deposition and foamy degeneration of hepatocytes (Figure 2b). In contrast, normal architecture of hepatic cells was seen in the ambrex treated group (Figure 2c). In atorvastatin treated group, the liver section showed microvesicular hepatocytes and fatty changes (Figure 2d). The morphology of liver section evidenced that ambrex possess hepatoprotective activity.

**DISCUSSION**

Cholesterol is an integral component of cell membrane and acts as a precursor for the biosynthesis of bile acid, steroid hormones and Vitamin D (Panneer et al., 2011). It is well established that hypercholesterolemia is an important risk factor for the development and progression of atherosclerosis and cardiovascular diseases (Deepa and Varalakshmi, 2005; Prasad and Kalra, 1993). Elevation in serum cholesterol and triglycerides levels would definitely lead to the development of coronary atherosclerosis. Hence, maintenance of cellular cholesterol homeostasis is an important criterion in the management of hypercholesterolemia. The clinical manifestations of atherosclerosis could be diminished when serum lipid concentration is reduced by hypolipidemic agents (Yang et al., 2006). Reports have indicated that currently used hyperlipidemic drugs lead to adverse effects such as myalgia, diarrhoea, dyspepsia, nausea, pain in extremity, liver enzyme abnormalities, arthralgia and urinary tract infection. Therefore, the prevailing status warrants researchers to investigate efficient and safe hyperlipidemic drugs from natural resources (Ram, 1996).

In recent years, research on herbal medicines is gaining prime importance in the treatment of various metabolic disorders due to their efficacy and safety. Hence, the present study is designed to investigate the hypolipidemic potential of ambrex, a polyherbal formulation. The current study substantiates the hypolipidemic property of a polyherbal formulation, ambrex.
Atherogenic index of control and experimental animals.

<table>
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<tr>
<th>Group</th>
<th>Atherogenic index</th>
<th>Protection %</th>
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<tbody>
<tr>
<td>A</td>
<td>2.70</td>
<td>-</td>
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<tr>
<td>B</td>
<td>4.67</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>2.87</td>
<td>163.70</td>
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<td>D</td>
<td>2.88</td>
<td>163.33</td>
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In relation to liver function test, ambrex treatment resulted in slight decrease in the enzyme activities of serum AST, ALT and ALP compared to high fat diet fed rats. This was an indication that administration of ambrex to the rats prevents leakage of these enzymes and restores the integrity of cell membrane in the liver.

The reason behind the decline in glucose level in Atorvastatin treated group animals was uncertain but it could be that high serum cholesterol elevates the level of glucan-like peptide-1 which augments insulin secretion from the β cells of pancreas that results in hypoglycemia (Xu et al., 1949; Prasad, 2008). Table 3 shows that urea and creatinine are reduced in treated animals as compared to Group B animals. This was especially interesting as there was minimal impairment noticed in renal function test. However, total protein level augmented in Group B animals when compared to treated animals.

The medicinal properties of ambrex may be due to the combined effect of catechins, flavonoids, withanolides and alkaloids in W. somnifera (Nadia et al., 2011); succinic acid in amber (Norbert, 2009); triterpenes and tannic acid in S. robusta (Poornima, 2009); phytosterols in O. mascula and flavanoids in C. circinalis (Gurav et al., 2013). These compounds are known to exhibit antioxidant properties, prevent free radical generation, increase HDL cholesterol and reduce LDL cholesterol thus preventing the risk of cardiovascular diseases in humans (Saleem et al., 2011). Moreover, the histopathological observations show reversal of all parameters in the ambrex treated group towards the control value.
Findings revealed that there was an increased lipid infiltration in the hepatocytes of high fat diet group animals, but in ambrex treated animal, restoration of the normal morphology of hepatocytes was observed. Overall, the present study provides a preliminary scientific basis for hypolipemic effects of ambrex, a polyherbal formulation. The results are encouraging enough for further studies to decipher the possible mechanism of action and elucidation of bioactive components.

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ABBREVIATIONS

LDL, Low density lipoproteins; HDL, high density lipoprotein; SGOT, serum glutamate-ornithine transaminase; SGPT, serum glutamate pyruvate transaminase; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; AST, aspartate amino transferase; ALT, alanine amino transferase.

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