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RESEARCH ARTICLE PHARMACEUTICS

"EVALUATION OF THE ANTI-HYPERLIPIDAEMIC ACTIVITY OF PREMNA INTEGRIFOLIA ON NICOTINE INDUCED HYPERLIPIDAEMIA IN RATS"

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ABSTRACT

Nicotine caused significant increase in the serum Cholesterol, Triglyceride, VLDL, LDL & significant reduction in HDL level. *Premna integrifolia* & Atorvastatin treatment showed significant prevention in increased serum Cholesterol, Triglyceride, LDL as compared to Nicotine control (NC) group. While HDL level was significantly increased in treated & standard group as compared to Nicotine control (NC) group. Materials and methods used for research are as: male albino rats weighing 200-250 gm were divided into 4 groups viz. Normal control; Nicotine control (NC); Nicotine control (NC) & *Premna integrifolia (Agnimantha)* (500 mg/ kg) treated; Nicotine control (NC) & Atorvastatin treated (standard control). Blood samples were collected after 7 days, for lipid estimation. From the above results we conclude that *Premna integrifolia is* effective as an anti-hyperlipidemic agent.

KEY WORD

hyperlipidaemia, *Premna integrifolia*

INTRODUCTION

Nicotine is an alkaloid found in products such as cigars, cigarettes and coffee, but mainly isolated from tobacco leaf called *Nicotiana tabacum*. The free base is a liquid, but the alkaloid is usually found in the hydrogen trtrate or sulphate. Nicotine, in its pure form is odorless and on exposure to air becomes dark brown in colour and takes the characteristic smell of tobacco. It is considered to be the most widely used stimulant next to caffeine (Effraim K.D et al., 2000).

Atherosclerosis is a complex multifactorial process that involves inflammatory and fibro proliferative responses to various stimuli acting on the vascular wall. The process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall; however, it is much more than that as the lesions of atherosclerosis represents a series of highly specific cellular and molecular responses (Ross et al, 1999).

The American Heart Association has identified the primary risk factor associated with the atherosclerosis to be the elevated levels of cholesterol and triglyceride in the blood. Therefore the therapist considers the treatment of hyperlipidemia to be one of the major approaches of the atherogenic process (Ghatak et al, 1995).

Lipids are essential for membrane synthesis, maintenance of membrane integrity, as an energy source, as hormone precursors, and as signaling molecules. To aid transport through the relatively aqueous blood, lipids are transported as cholesteryl esters or triglycerides within the lipoproteins. However, increased levels of lipoproteins in the circulation are associated strongly with atherosclerosis (Robert, 2002).

In some literature there are described various use of Premna integrifolia like rheumatisum, neuralgia, carminative, galactogogue, stomachic, laxative, stomachic, tonic etc.

In the light of the above reports the present investigation was undertaken to study the potential of *Premna integrifolia* (500 mg/kg) in the treatment of hyperlipidemia using experimental animals.

MATERIAL AND METHODS

Plant material:

Root barks of premna intigrifolia were obtained from a commercial supplier of Ahmedabad. Methanolic extract of above identified root bark were prepared.

Preparation of methenolic extract:

The coarse powder (1 kg) of root barks of *Premna integrifolia* was extracted exhaustedly in round bottom flask with methanol for 48 hours with vigorously shake at the regular intervals. The Methanolic extract was filtered and solvent removed under vacuumed to yield a dry extract. The extract was stored in airtight container in cool and dry place and used throughout the project.

Preliminary phytochemical screening:

Preliminary phytochemical screening of the root barks of *Premna integrifolia* extract was carried out for the detection of the various plant constituents (Khandelwal et al, 2004).

Selection of animals:

All animals were housed at ambient temperature (22±1^{0}C) and relative humidity (55±5%) with fixed 12h/12h light/dark cycle.

Animals had free access to Standard pellet diet and water given *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Treatment Protocol

Male albino rats weighing 200-250 gm allocated 4 groups of 6 rats each. Group I (normal control) received standard normal diet. Whereas Group II (Nicotine control), Group III (test) Group IV (standard) were fed on high fed diet (contains 2% Cholesterol, 1% sodium cholate and 2.5% Cocconut oil) for 30 days. Group III & Group IV received Premna integrifolia (500 mg/kg, p.o.) & Atorvastatin (15 mg/kg, p.o.) in added to HFD respective for 30 days.

Estimation of biochemical parameters:

After 30 days blood samples were drawn by retro-orbital puncture using a fine sterile capillary tube and the plasma used for the estimation of lipid profile viz. serum cholesterol, serum triglyceride, LDL, VLDL and HDL levels.

Serum lipid profile

Experimental protocol was approved by IAEC as per guidelines of CPCSEA.

Statistical Analysis:

Results are presented as mean ± SEM. Statistical difference between the means of the various groups were analyzed using one-way analysis of variance. Data were considered statistically significant at P≤0.05 and highly significant at P≤0.001. Statistical analysis was performed using Sigma state statistical software.

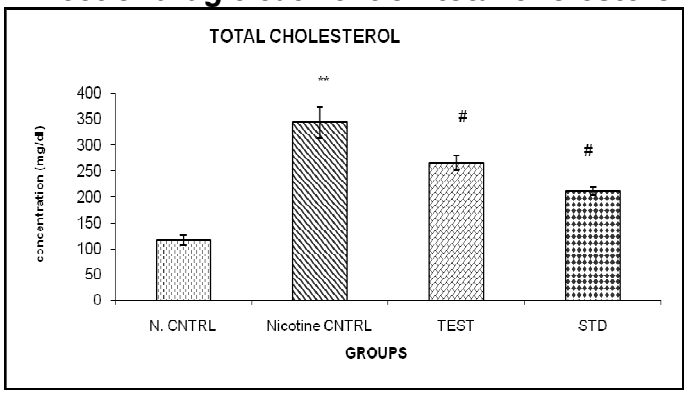
RESULTS

Physical observation:

There was significant increase in the body weight (gms), serum cholesterol, serum triglyceride, LDL, VLDL and decreased HDL levels of rats in group II- Nicotine control (NC) compared to group I- Normal control. But in group III- HFD & *Premna integrifolia* (500mg/kg) treated and group IV- HFD & Statin (15mg/kg) treated there was significant decreased in the body weight (fig 1), serum cholesterol(fig 2), serum triglyceride(fig 3), LDL(fig 4), VLDL(fig 5) and increased HDL(fig 6) levels compared to cholesterol control.

Figure 1

Effect of drug treatment on total cholesterol



Values are expressed as Mean + S.E.M. of n=6 observation.
**** P<0.05 (Compared with normal control)***
***** P<0.001 (Compared with normal control)***
 # P<0.05 (Compared with Nicotine control)
P<0.001 (Compared with Nicotine control)

Figure 2
Effect of drug treatment on serum Triglyceride level

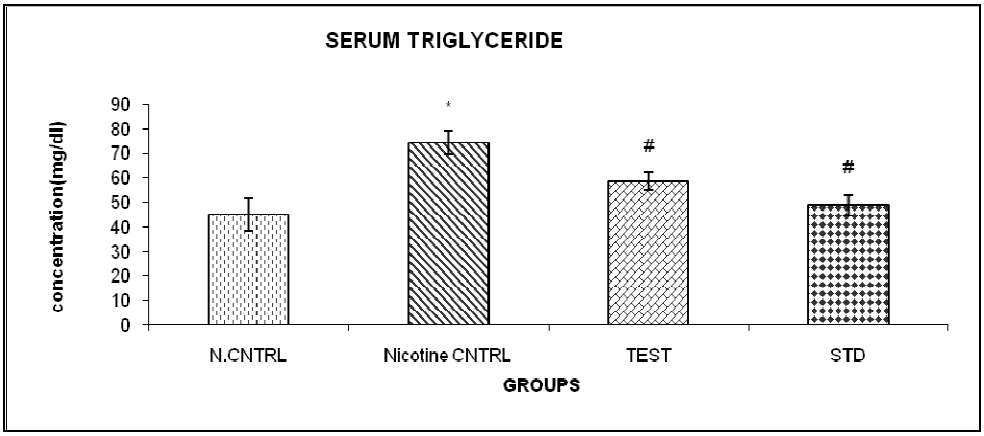


Figure 3
Effect of drug treatment on serum LDL level

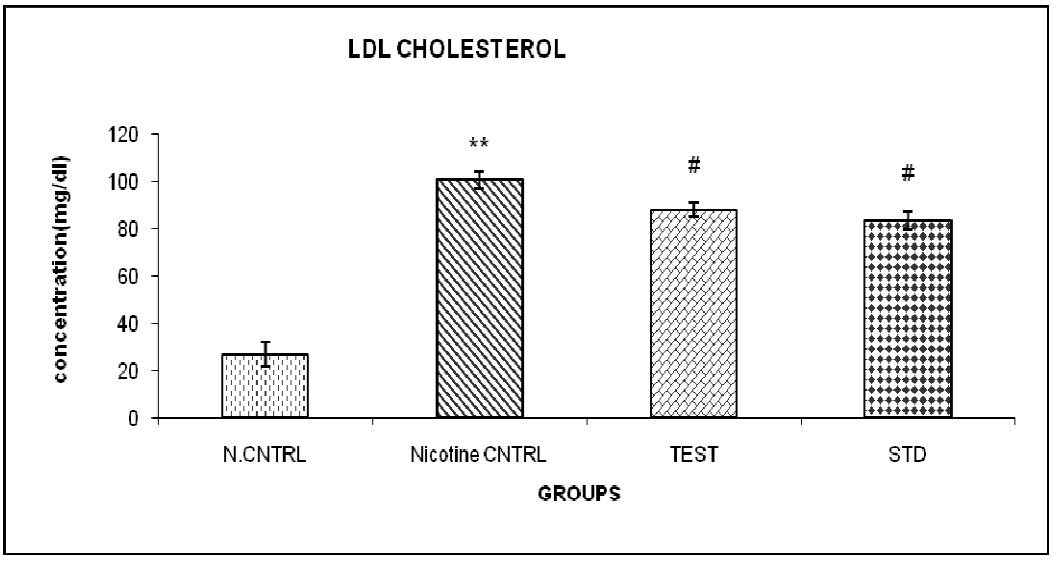


Figure 4
serum VLDL level

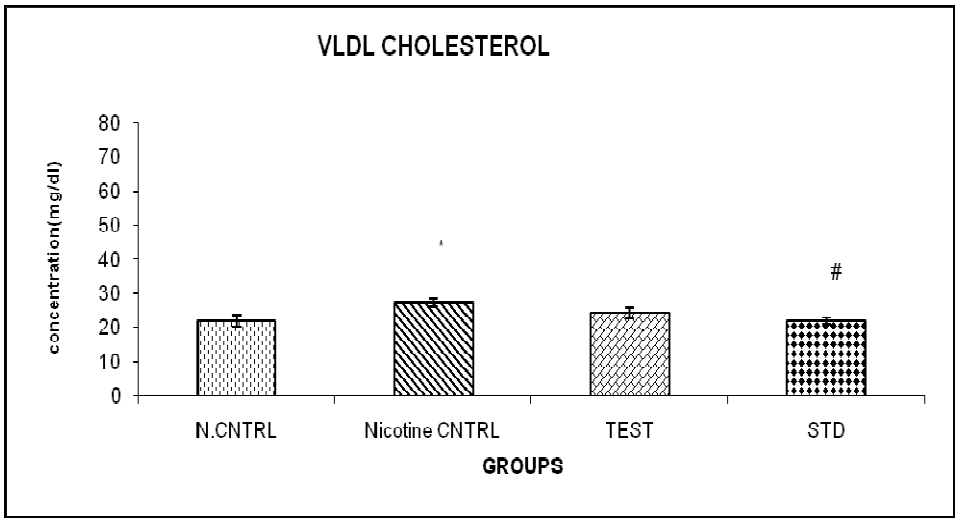
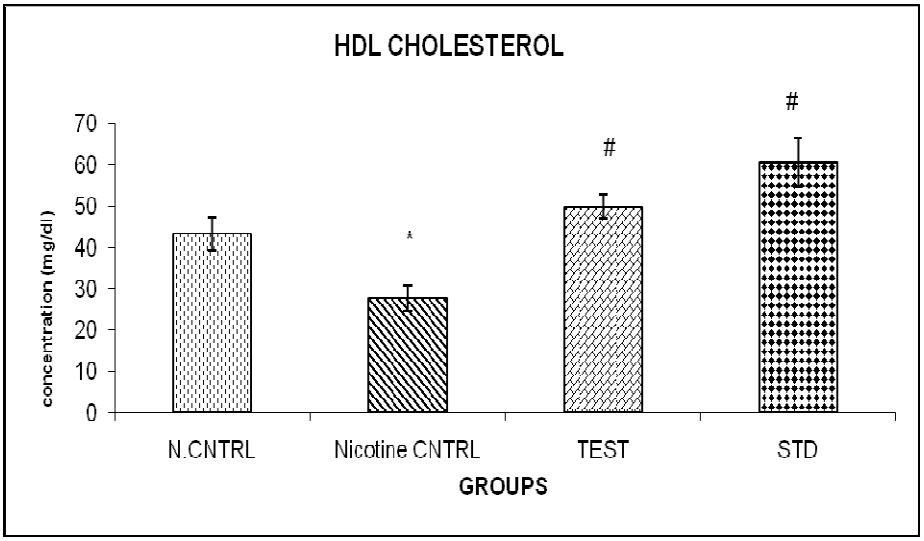


Figure 5
Effect of drug treatment on serum HDL level



DISCUSSION

Nicotine a drug that is dependence producing elicits an increase in numbers of nicotine binding sites in the brains of chronically treated animals. This increase in binding is due to an increase in receptor density with no increase in affinity. Because nicotine receptors are subject to profound and prolonged desensitization on exposure to the drug resulting in functional blockage. It has been suggested that this underlies the mechanism of agonist – induced up regulation. In the present study, the administration of 1mg/kg of nicotine to rats produced profound central nervous system effects which manifested as decreased food intake, transient convulsion, artificial paralysis and loss of weight. These changes may be

explained in the light of up-regulation of central nicotine receptor binding sites leading to initial stimulation and later diminished responsiveness or tolerance. According to Kritchevesky and Balfour nicotine also indirectly affects the satiety center (Effraim K.D et al., 2000).

Administration of nicotine (1mg/kg) to the animals also raised the serum cholesterol, triglycerides and glucose levels. This is consistent with the reports of Dusek and Girdano and Schienalbein that nicotine causes the elevation of plasma free fatty acids which may serve as building blocks for the synthesis of both cholesterol and triglycerides. The hyperglycaeamia recorded may be due to the stimulation of adenylceclase enzyme in tissues resulting in the production of camp. Increased cAmp levels in blood stimulates glycogenolysis thus increasing the levels of glucose in the blood.

Hyperlipidemia is one of the major risk factor for CAD. Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides.

Earlier studies have shown a significant elevation in the plasma lipid parameters in response to high fat diet. Erlier reported significant increase in serum cholesterol, serum triglyceride, serum LDL and serum VLDL levels because of high fat diet in rats. As well as it resulted in decrease in serum HDL level. Similarly in the present study, we found significant elevation in various lipid levels (Total cholesterol, VLDL, LDL, Triglyceride) and significant decrease in HDL level in HFD group animals as compare to normal control animals (Chandar et al, 1996).

Whereas serum cholesterol was significantly reduced when NC rats were treated with *Premna integrifolia* (500 mg/ kg).

Triglycerides are independently related with coronary heart disease. In one study showed low concentration of triglyceride level in Nicotine control rats treated with anti-hyperlipidemic drug (El hazmi et al, 2001). Nicotine control rats treated with anti-hyperlipidemic drug showed decrease activity of lipoprotein lipase in adipose tissue. Decreased in the triglyceride level may be related to increase in the endothelium bound lipoprotein lipase activity that hydrolyses the triglycerides into fatty acids (Srinivasan et al, 1989).

Study suggests that Lipoprotein lipase is the key enzyme, which catalyses the hydrolysis of triacylglycerol rich lipoproteins (chylomicrons and VLDL) and increased activity of this enzyme causes increased clearance of triacylglycerides from the blood. The higher the activity of these enzymes in rats correlates with the lower levels of serum triglycerides (Mini et al, 2004).

Similarly in present study, there was decrease in serum triglyceride of Nicotine control rats when treated with *Premna integrifolia* (500 mg/ kg) and atorvastatin as compared to Nicotine control. But decrease in the triglyceride level with *Premna integrifolia* (500 mg/ kg) was not significant.

LDL is dangerous because it can penetrate the endothelial wall and contribute to the creation of lipid foam, which forms the core of a plaque deposit. Oxidized LDL cholesterol also triggers within the endothelium an inflammatory process that accelerates atherosclerosis. LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. It is known that VLDL is highly rich in triglycerides and is involved in the transport of triglycerides from liver to extra hepatic tissues, whereas LDL is mainly formed from VLDL in presence of heparin releasable lipoprotein lipase, an enzyme present in the endothelial cells of the blood vessel walls.

Reduction in LDL cholesterol and increase in HDL cholesterol concentration are significantly related to lipid lowering therapy. Reduced levels of LDL on Nicotine control rats treated with anti-hyperlipidemic drug may be possibly due to increase with catabolism of LDL (High fat diet plus cholesterol feeding) (Muthu et al, 2005).

Similarly, in present study there was increased in concentration of VLDL and LDL were observed in the serum of Nicotine control rats when compared with the control. Treatment with *Premna integrifolia* (500 mg/ kg) and atorvastatin reduced VLDL and LDL. Thus, decrease in LDL and VLDL may be due to increase in catabolism of LDL.

HDL is synthesized mainly in intestine and liver. It has high phospholipids content and is involved in reverse cholesterol transport. HDL is considered to be a beneficial lipoprotein as it has an inhibitory effect in the pathogenesis of atherosclerosis. Low level of HDL is associated with high risk of coronary artery disease (Boden et al, 2000). Increase in HDL concentration in plasma has a preventive role in the development of atherogenesis (Muthu et al, 2005).

Similarly, in the present study, HDL level in both serum and tissue was significantly increased when HFD rats treated with *Premna integrifolia (*500 mg/kg) and atorvastatin.

Above data suggests the role of *Premna integrifolia (500mg/kg)* as anti-hyperlipidaemic agent.

CONCLUSION

Thirty days treatment of methanolic extract of root bark of *Premna integrifolia (*500 mg/kg) in rats significantly decreased serum total cholesterol, LDL, VLDL, triglyceride and significantly increased HDL. So, *Premna integrifolia (*500 mg/kg) can be developed as anti-hyperlipidaemic agent.

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