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ORIGINAL ARTICLE

Effect of Amlodipine, Cilnidipine and Diltiazem on lipid profiles of hypertensive rats fed with high fat diet: A comparative study

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KEYWORDS

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Abstract *Objective:* The present study was aimed to compare the effect of calcium channel blocker (Amlodipine, Cilnidipine and Diltiazem) on lipid profiles of hypertensive rats fed with high fat diet for four weeks.
Methods: Hypertensive rats were randomly allocated into four groups and except hypertensive rats remaining all groups received high fat diet for 4 weeks. At the end of protocol blood pressure was measured by tail cuff method and blood is withdrawn from the retro-orbital puncture, separated serum is used for the assessment of various biochemical parameters. Finally liver and aorta isolated for histological changes.
Results: Calcium channel blocker significantly reduces the lipid levels raised in hypertensive rats fed with high fat diet and also restore the pathological changes of aorta and liver tissues.
Conclusion: These results indicate that they have a lipid lowering effect due to effect on different stages of metabolism of lipids.

1. Introduction

Calcium channel blockers (CCBs) are commonly used for various cardiovascular diseases like hypertension and ischaemic heart disease.[1] They are chemically classified into phenylalkylamines, dihydropyridines, and benzothiazepines.[2] Even though hypertension participated in the progression of atherosclerosis, the exact mechanism hasn't been ruled out yet.[3] Hence the effect of calcium antagonists on serum lipids and lipoproteins is of increasing interest. Current therapeutic interventions for the prevention of cardiovascular disease are directed at established risk factors such as hypertension, dyslipidemia (elevated low density lipoprotein [LDL]-cholesterol, triglycerides as well as decreased high-density lipoprotein [HDL]-cholesterol), and hyperglycaemia.[4]

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Over the past 30 years, considerable experimental and clinical evidence has accumulated to support the suggestion that calcium channel blockers (CCBs) have significant anti-atherosclerotic effects that are independent of their hypotensive effects.[5]

The accumulation of information in the last decade has suggested several cellular mechanisms for CCBs which may influence atheroma;[6] (a) Inhibition of vascular smooth muscle cell proliferation and migration (b) inhibition of calcium influx and deposition in the arterial wall (c) inhibition of synthesis and deposition of extracellular matrix (d) enhanced removal and degradation of cholesterol rich lipoproteins (e) protection of LDL from oxidation (f) inhibition of platelet activation and (g) preservation of endothelial function and hemodynamic effect.

The present study investigates the comparative effect of Amlodipine, Diltiazem and Cilnidipine on the lipid profile of rat fed with high cholesterol diet.[7] Amlodipine is a widely used drug for hypertension and Cilnidipine is a new calcium channel blocker approved for treatment of hypertension, whereas Diltiazem is used for various other cardiovascular diseases. Studies also include a histopathological investigation to assess cellular damage and improvement after the drug treatment in different groups.

2. Materials and methods

2.1. Chemicals

Cholesterol, sodium cholate, cocoa butter and coconut oil were purchased from national chemicals, Baroda. All other reagents and chemicals obtained were of analytical grade. Amlodipine was dissolved in 0.5% DMSO solution and diluted to required volume with water. Diltiazem was freely dissolved in distilled water; Cilnidipine was prepared in a 0.5% DMSO solution.

2.2. Animals

All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of Pharmacy Department, The M.S. University of Baroda and with permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy Wistar rats of either sex weighing between 200 and 300 g were housed in polypropylene cages and maintained under standardized conditions (12-h light/dark cycle, 24 ± 2 °C, 35 to 60% humidity) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt. Ltd., Pune) and purified drinking water *ad libitum*. All animals were acclimatized for one week before the experiment started.

2.3. Induction of hypertension: DOCA-salt-induced hypertension

Hypertension was induced experimentally in Male Wistar rats (200–250 g) by unilateral nephrectomy.[8] Rats were anaesthetized with ketamine (100 mg/kg body weight), and a lateral incision was made in the area overlying the kidney. The renal blood vessel was ligated with fine sterile silk thread and the kidney was removed. The incision was sutured and closed with Michel clips. All operated rats received an injection of ampicillin (10 mg/kg, i.p.) daily for 5 days. Neosporin powder (polymyxin B sulfate BP, zinc bacitracin BP, neomycin sulfate IP) was applied locally to prevent infection. One week later, Deoxycorticosterone acetate (DOCA) (20 mg/kg, twice a week; s.c., for 4 weeks) dispersed in olive oil was injected into uninephrectomized rats. A solution of 1% saline + 0.2% KCl *ad libitum* was given instead of drinking water. In sham-operated control animals, a similar procedure was performed except the treatment with DOCA.

2.4. Composition of high cholesterol diet and its preparation

Cholesterol (0.5%), Sodium cholate (0.1%), Cocoa butter (10%), Coconut oil (10%) and Pellet powder (79.4%)

2.5. Preparation of diet

79.4 gm. of pellet powder was mixed with 100 mg of sodium cholate separately. Simultaneously 500 mg of cholesterol and 10 gm. of cocoa butter were dissolved in 10 ml of warm coconut oil. This oil solution of cholesterol was added slowly into the powdered mixer to obtain a soft homogenous cake. This cake was molded into pellets.

2.6. Experimental design

Rats were randomly divided into the following groups. Each group consists of six animals.

Group 1: Sham control: They were fed with standard laboratory diet and water *ad libitum* for four weeks.

Group 2: DOCA induced hypertensive rats: They were fed with high cholesterol diet +0.5% DMSO[9] for four weeks.

Group 3: DOCA induced hypertensive rats + Amlodipine: They were administered Amlodipine (5 mg/kg, p.o) for four weeks along with high cholesterol diet.

Group 4: DOCA induced hypertensive rats + Diltiazem: They were administered Diltiazem (30 mg/kg, p.o) for four weeks along with high cholesterol diet.

Group 5: DOCA + Cilnidipine: They were administered Cilnidipine (10 mg/kg, p.o) for four weeks along with high cholesterol diet.

2.7. Preparation of serum sample

At the end of 4th week, animals were fasted overnight and hemodynamic study was carried out, then serum and tissue were subjected to different biochemical analysis & histoarchitecture study.

The blood samples were withdrawn from the retro-orbital plexus under light ether anesthesia and allowed to clot for 10 min at room temperature. It was centrifuged at 2500 RPM for 20 min. The serum obtained was kept at 4 °C until used.

After blood collection, all the animals were sacrificed by ether anesthesia followed by spinal dislocation. Liver and aorta were removed in a chilled condition and immediately placed in 10% buffered formalin, embedded in paraffin, cut

to 5 μm sections for slides and stained with hematoxylin and eosin. The slides were examined with a Magnus microscope. The sections were observed under 40× and 100× magnifications.

2.8. Hemodynamic and biochemical parameters

2.8.1. Hemodynamic study

Arterial blood pressure was measured by non-invasive (indirect or tail-cuff) method. Rats were trained for at least one week until the blood pressure was steadily recorded with minimal stress and restraint. The first cardiovascular parameters were discarded and the mean of five or six subsequent measurements were recorded. Cardiovascular parameters (systolic, diastolic, mean blood pressure, and heart rate) were measured at the end of treatment by using Letica 5002 Storage Pressure Meter (Panlab Blood Pressure Recorder model LE 2002 N, Barcelona, Spain).

2.8.2. Serum parameters

Serum was used for measurement of Total cholesterol, High Density Lipoprotein (HDL) cholesterol, triglycerides by using kits supplied by Span Diagnostic Ltd. whereas low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were calculated by Friedewald's formula.[10]

2.9. Statistical analysis

All the data were expressed as mean ± SEM. Statistical significance between more than two groups were tested using a one-way ANOVA followed by the Bonferroni multiple comparison test as appropriate using computer based fitting program (Prism, Graph-pad). Differences were considered statistically significant when $P < 0.05$.

3. Results

Administration of high cholesterol diet for four weeks showed a significant increase ($P < 0.001$) in body weight in the sham group compared to DOCA induced hypertensive rats. The calcium channel blockers treatment showed a significant decrease in body weight($P < 0.05$, $P < 0.01$) after four weeks of treatment as compared to DOCA induced hypertensive rats (Fig. 1).

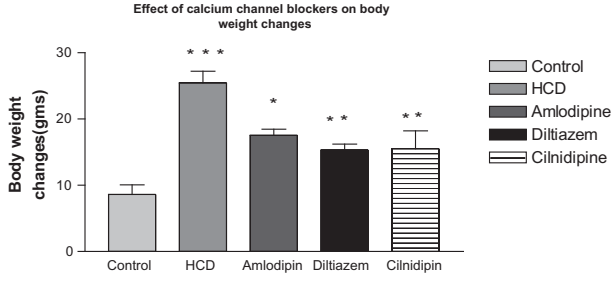

Figure 1 Comparative study of change in body weight in control, high cholesterol diet and treated groups (Values are expressed as mean ± SEM for six rats, NS = non-significant; ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

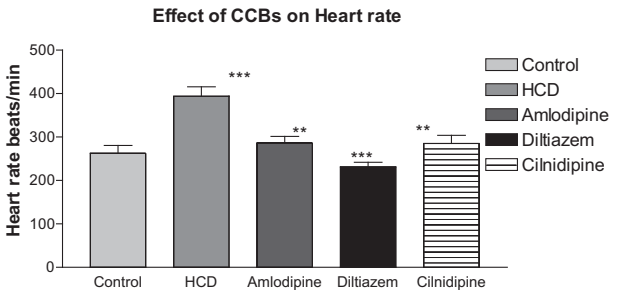

Figure 2 Effect of calcium channel blockers on heart beats of hypertensive rats fed with high cholesterol diet. (Values are expressed as mean ± SEM for six rats, NS = non-significant; ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

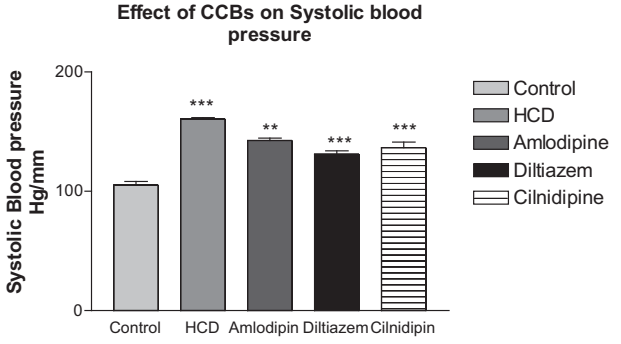

Figure 3 Effect of calcium channel blockers on systolic blood pressure of hypertensive rats fed with high cholesterol diet. (Values are expressed as mean ± SEM for six rats, NS = non-significant; ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

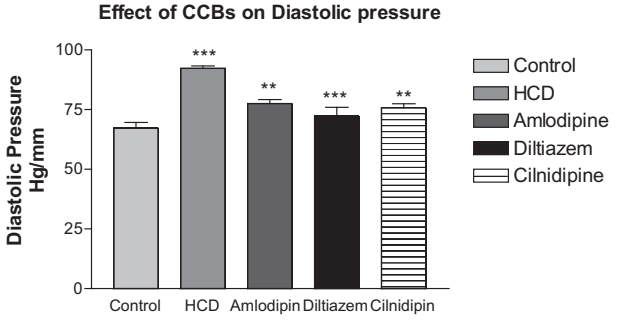

Figure 4 Effect of calcium channel blockers on diastolic blood pressure of hypertensive rats fed with high cholesterol diet. (Values are expressed as mean ± SEM for six rats, NS = non-significant; ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Heart rate, systolic and diastolic blood pressure were significantly increased in hypertensive rats fed with high fat diet. Amlodipine, Cilnidipine and Diltiazem treated groups showed a significant decrease compared to HCD control (Figs. 2–4). Total serum cholesterol was significantly ($P < 0.001$)

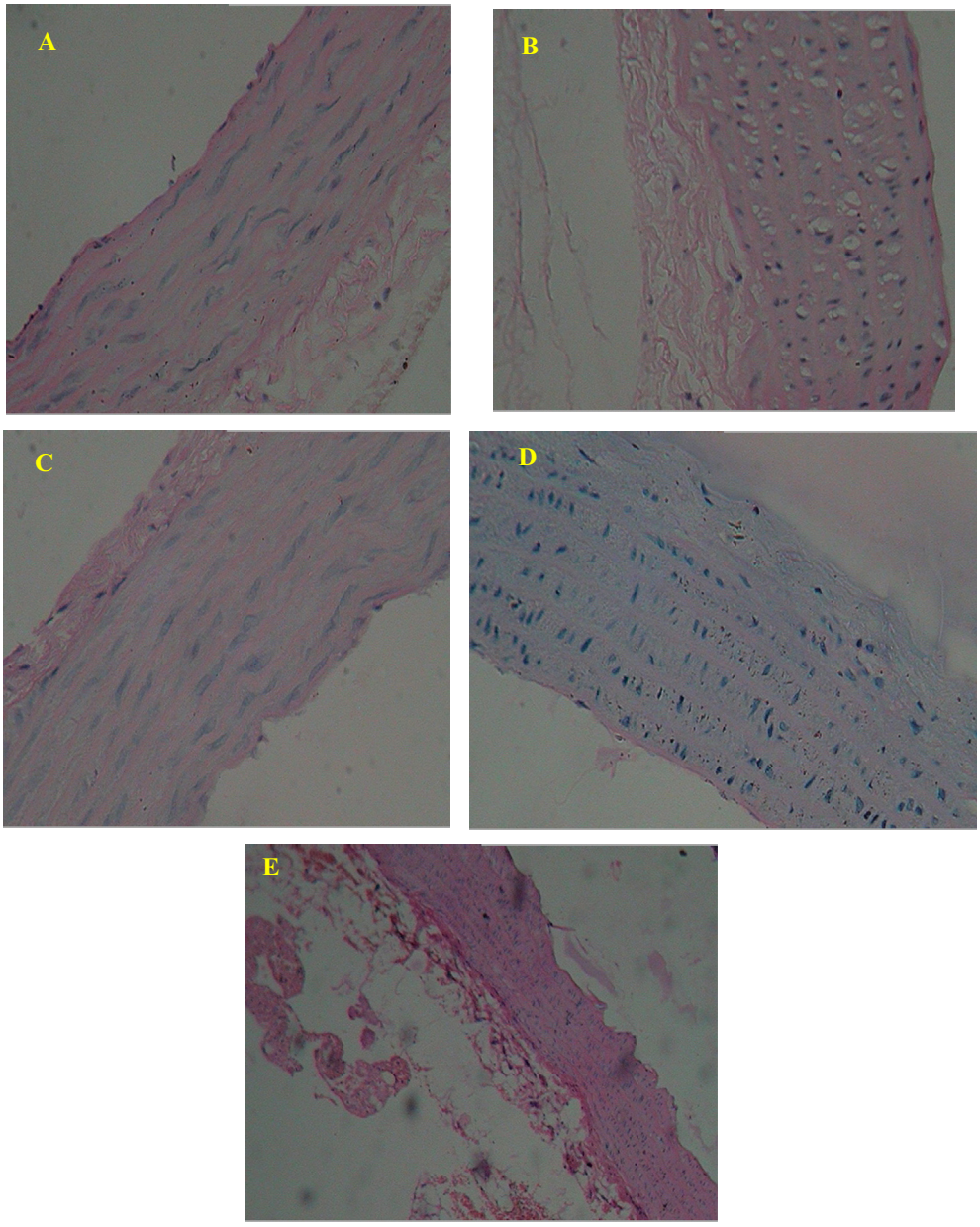

Figure 5 Histopathology of cross section of aorta wall stained by hematoxylin–eosin staining of hypertensive rat fed with high cholesterol diet.

increased after four weeks of treatment with high cholesterol diet in DOCA induced hypertensive rats compared to the sham group. Amlodipine, Diltiazem and Cilnidipine treated groups showed a significant ($P < 0.05$, $P < 0.01$) decrease in serum total cholesterol after four weeks compared to DOCA induced hypertensive rats.

Serum triglyceride levels were significantly ($P < 0.001$) increased in DOCA induced hypertensive rats after four weeks of treatment with high cholesterol diet compared to the sham group. Treatment with Amlodipine, Diltiazem and Cilnidipine showed a significant decrease ($P < 0.05$, $P < 0.01$, $P < 0.001$) in TG level as compared to DOCA induced hypertensive rats. Serum LDL-C levels were increased significantly ($P < 0.001$) after four weeks of treatment with high cholesterol diet in DOCA induced hypertensive rats compared to the sham group. Amlodipine, Diltiazem and Cilnidipine treated group showed a significant decrease ($P < 0.05$, $P < 0.001$) in LDL-C level after four weeks as compared to DOCA induced hypertensive rats. VLDL-C showed a significant ($P < 0.001$) increase in the HCD group compared to the control. However, Amlodipine treatment does not show significant changes whereas, Diltiazem and Cilnidipine treated groups showed a significant ($P < 0.01$) decrease in VLDL-C level after four weeks compared to the HCD control.

Serum HDL-C was decreased significantly ($P < 0.001$) after four weeks of treatment with high cholesterol diet in DOCA induced hypertensive rats compared to the sham group. Significant increase in HDL-C ($P < 0.01$) was observed with Amlodipine Cilnidipine treated groups whereas, Diltiazem treated groups showed no significant change after four weeks of treatment compared to DOCA induced hypertensive rats.

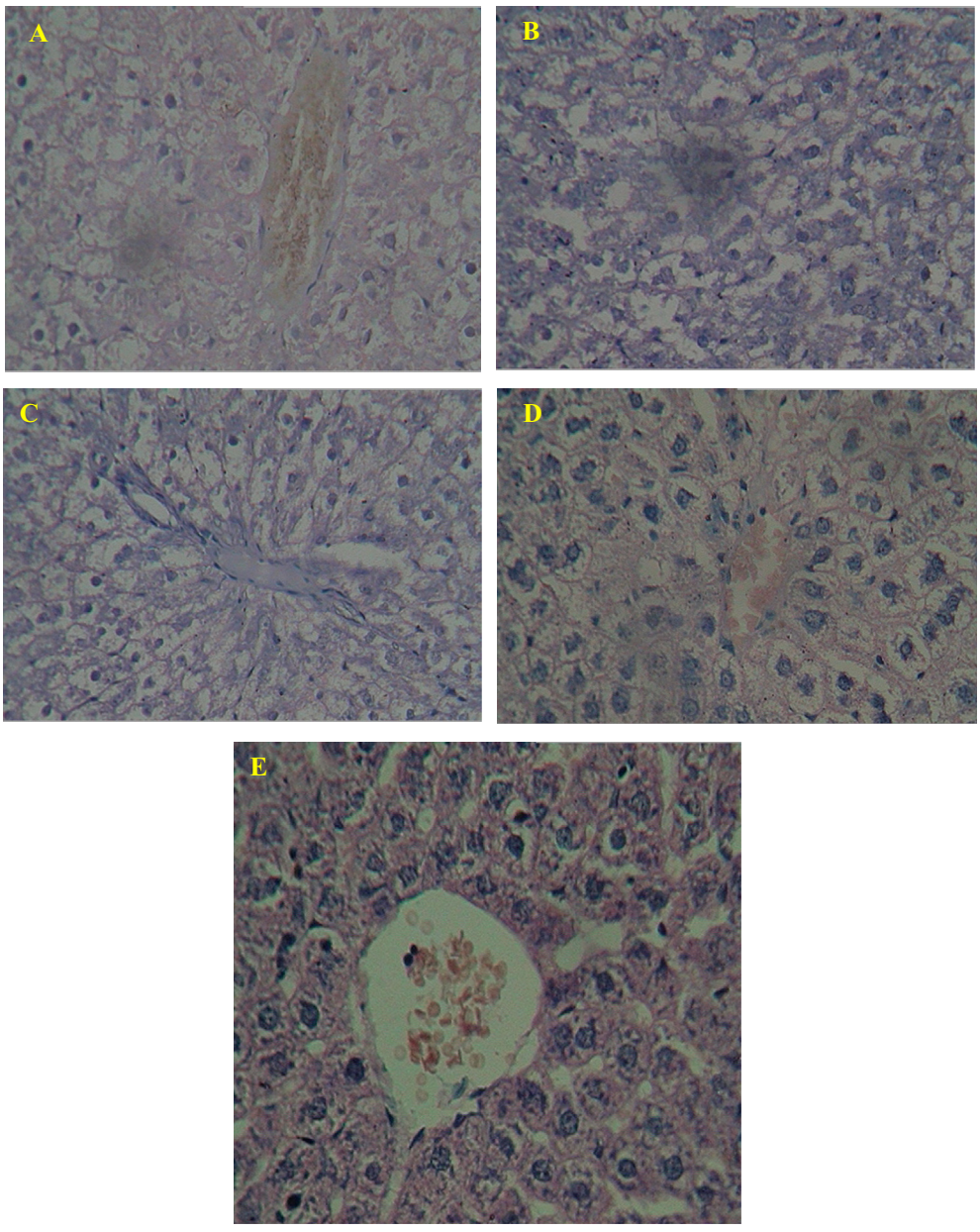

Figure 6 Histopathology of cross section of liver stained by hematoxylin–eosin staining of hypertensive rat fed with high cholesterol diet.

3.1. Histopathological study

3.1.1. Aorta: (Fig. 5)

HCD showed the increase in lipid deposition and endothelial dysfunction compared to the control after four weeks. Amlodipine, Diltiazem and Cilnidipine treated groups showed decreased lipid deposition and endothelial dysfunction compared to HCD control.

3.1.2. Liver: (Fig. 6)

HCD showed the increase in intracellular space compared to the control after four weeks. Amlodipine, Diltiazem and Cilnidipine treated groups showed decreased intracellular space compared to HCD control.

4. Discussion

Today, in most of the developed and developing countries hyperlipidaemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths.[11,12] In 1984 it was demonstrated for the first time that there exists a link between serum cholesterol levels and risk of coronary heart disease (CHD).[13] Worldwide, it causes deaths almost twice as many as those caused by cancer and 10 times as many as those caused by accidents.

In recent years the role of calcium antagonists is expanding into long term therapy of cardiovascular diseases like hypertension and ischemic heart diseases. Hypertension is a driver of the development of atherosclerosis underlying cardiovascu-

Table 1 Effect of calcium channel blockers on lipid profiles of hypertensive rats fed with high cholesterol diet. (Values are expressed as mean ± SEM for six rats, NS = non-significant; ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

	Control	HCD	Amlodipine	Diltiazem	Cilnidipine
TC mg/dl	40.11 ± 1.999	122.2 ± 5.608***	93.01 ± 5.715**	99.76 ± 3.981*	90.12 ± 4.395**
TG mg/dl	42.54 ± 4.182	230.6 ± 23.20***	164.1 ± 12.51*	145.1 ± 3.930**	134.1 ± 5.786***
LDL mg/dl	17.24 ± 1.301	65.73 ± 2.871***	31.15 ± 12.00*	35.30 ± 6.090*	14.49 ± 1.878***
VLDL mg/dl	8.503 ± 0.8359	53.42 ± 8.012***	39.39 ± 3.698NS	27.02 ± 2.244**	26.06 ± 1.277**
HDL mg/dl	53.05 ± 5.627	21.67 ± 0.8436***	48.21 ± 3.889**	41.13 ± 5.628NS	48.83 ± 3.024**

lar disease, although the molecular mechanisms and associations have not been resolved.[14] Hence the effect of calcium antagonists on serum lipids and lipoproteins is of increasing interest. The present study reported the influence of various calcium antagonists on the lipid profile of hypertensive rat fed with high cholesterol diet, using a single experimental animal model. This is the best animal model for studying related to intermediary metabolism.[15]

A specially prepared diet was used for induction of hypercholesterolemia in rats with a duration of four weeks for maintenance of sustained hypercholesterolemia.

Body weight was significantly increased in the HCD group whereas, Amlodipine, Cilnidipine and Diltiazem treated groups showed a decrease when compared to the HCD group.

Serum total cholesterol levels were increased in the HCD group.[16] These changes are associated with a phenomenon that excessive load of cholesterol in the liver above the acceptable level of its normal process causes the system to be unable in metabolize the lipids resulting in high cholesterol return in the circulating blood.[17] Serum total cholesterol was significantly decreased by Amlodipine, Cilnidipine and Diltiazem treatment compared with the HCD group. This effect may be due to the activation of more than one pathway, like activation of lysosomal cholesterol ester hydrolase and prevention of induction of HMG CoA gene so as to reduce cellular cholesterol biosynthesis[18] (see Table 1).

Decreased serum LDL-Cholesterol might be due to the increased expression of LDL–receptors on the cell surface leading to increase in uptake and metabolism of LDL-Cholesterol following calcium channel blocker therapy.[19,20]

HDL-C mediates the process termed reverse cholesterol transport. Cholesterol in peripheral cells is transported from the plasma membranes of peripheral cells to the liver by an HDL-C.[21] Thus, HDL-C causes removal of cholesterol from the body to the liver where it gets excreted and reduced the risk of atherosclerosis.[22] Significant change was observed in HDL-C in the HCD group compared to the control. Amlodipine and Cilnidipine only show a significant increase in HDL-C but in the remaining treatment groups Diltiazem treatment showed no significant change in serum HDL-C.

Dihydropyridines increase the triglyceride lipase to cause a rapid breakdown of triglycerides and mobilization of free fatty acids; thereby leading to a fall in levels of serum triglycerides.[23] Alternatively calcium channel antagonists could remove triglycerides by vasodilation and expanding the capillary bed where the enzyme exerts its activity.[24] Serum VLDL-C levels were increased in the HCD group. Amlodipine treatment did not show significant changes but Diltiazem and Cilnidipine treated groups showed a decrease in serum VLDL-C.

Findings of the present study correlate with previous studies and it was found that there is a similarity between the studies.[25,26] In previous studies high cholesterol diet altered the lipid profile in normal rats whereas calcium channel blockers decreased the lipid profile of patients. In our study there is a correlation between lipid profile, hemodynamic parameters and calcium channel blockers of hypertensive rats and the main aim is a comparative study of the three different classes of calcium channel blocker with the evidence of histopathological examination of the aorta and liver.

5. Conclusion

This study explains the comparative study of CCB (Amlodipine, Diltiazem and Cilnidipine) on the lipid profile of rats fed with high cholesterol diet. CCB treatment decreases serum cholesterol, triglyceride, LDC-C, VLDL-C and increases the HDL level. Cilnidipine showed more favorable effect on lipid level of rats when compared to Amlodipine and Diltiazem.

Finally, the new generation of dihydropyridines has more advantage in favor of lipid lowering effect besides antihypertensive property. The more salient question of whether these effects are clinically relevant to patients taking therapeutic doses of CCBs is only now beginning to be addressed. Although our study showed Dihydropyridines are more beneficial in reducing lipid levels than other Benzothiazepines like Diltiazem.

Conflict of interest

The authors state that there is no conflict of interest.

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