



Investigation of HMG Co A Reductase Inhibitory Activity of Antihyperlipidemic Herbal Drugs *In Vitro* Study

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ABSTRACT

Hyperlipidemia (Elevated cholesterol or triglyceride) is a major cause of atherosclerosis and atherosclerosis associated conditions such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease. Lipid metabolism is regulated by various enzymes. HMG Co A reductase is a key enzyme involving in rate limiting step of cholesterol biosynthesis. Various herbal drugs are potent antihyperlipidemic like Andrographis paniculata, Anthocephalus indicus, Ocimum sanctum, Picrorrhiza kurroa, Plumbago zeylanica, Terminalia arjuna. Their mechanism of action is still unclear. They might be acting through HMG Co A Reductase. Liver microsomal preparation was prepared from high fat diet fed wistar rats. Oxidation of NADPH was measured at 340 nm for HMG Co A reductase activity after addition of 3 different concentration (1 µg/ml, 10 µg/ml, 100 µg/ml) of herbal drugs. Andrographis paniculata (IC₅₀-2.959 µg/ml), Anthocephalus indicus (IC₅₀-5.310 µg/ml) and Terminalia arjuna (IC₅₀-4.526 µg/ml) were found to possess HMG Co A reductase inhibitory activity. From the study it can be concluded that Andrographis paniculata, Anthocephalus indicus and Terminalia arjuna possess HMG Co A reductase inhibitory activity. While Ocimum sanctum, Picrorrhiza kurroa and Plumbago zeylanica do not possess HMG Co A Reductase inhibitory activity.

KEYWORDS: Hyperlipidemia, HMG Co A reductase, NADPH

INTRODUCTION

The lipid metabolism is regulated in many different ways. Enzymes are major regulators of lipid metabolism [1]. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is one of the enzymes involved in cholesterol biosynthesis [2]. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an endoplasmic reticulum (ER) protein, catalyses the rate-determining reductive deacylation of HMG-CoA to mevalonate in cholesterol biosynthesis [3]. The reducing equivalents are supplied by NADPH. In mammals, only one gene has been found to encode HMG-CoA reductase [4]. While in yeast, fungi, and plants more than one gene encode the enzyme. Yeast contains two functional genes for HMG-CoA reductase, HMG-CoA reductase 1 (HMG1) and HMG2. HMG1 and HMG2 are differently expressed and when HMG1 is deleted, HMG2 can replace the function of HMG1 [4, 5]. HMG Co A Reductase is found in cytosol and microsomal fractions of the cell [6]. Inhibition of HMG CoA reductase prevents and reverses MYC-induced lymphomagenesis [7]; treats Adenocarcinomas of the prostate, stomach, lung, breast and colon and Ewing's sarcomas [8]; promotes anti-inflammatory pathways in vascular wall [9] and inhibits cholesterol biosynthesis [6].

There are several antihyperlipidemic drugs available in market like statins, fibrates, niacin etc. Various herbal drugs are also potent antihyperlipidemic but their mechanism of action is still unclear. Some of these drugs are *Andrographis paniculata* [10], *Anthocephalus indicus* [11], *Ocimum sanctum* [12], *Picrorrhiza kurroa* [13], *Plumbago zeylanica* [14] and *Terminalia arjuna* [15]. They might be acting through inhibition of HMG Co A Reductase.

MATERIALS AND METHODS**Animals**

Male Wistar rats were housed in cages with free access to high fat diet and water *ad libitum* and acclimatized to the surroundings for three week prior to the experiment.

Herbal Drug collection and identification

All six plants were obtained from Vanchetna botanical garden, Gandhinagar and their botanical identification were confirmed by the Department of botany, M.G. science institute, Ahmedabad.

Herbal Drug Extract Preparation

Andrographis paniculata Leaf Extract: [10]

The leaves were oven-dried at 50° C and ground into powder-form with a grinder. For the preparation of aqueous extract of this plant, powder was extracted using water at the ratio of 1:5. They were left in the water bath at 55° C for 3 hrs. The liquid obtained was then filtered and evaporated by rota evaporator. (6.3 % yield).

Anthocephalus indicus Root Extract: [11]

The roots were dried under shade and made into fine powder using grinder. Powder was extracted thrice with 150 ml portions of 95% ethyl alcohol by cold maceration at room temperature. Time allowed for each extraction was 4 hrs. The root extract obtained after third extraction was colorless. All the extracts were mixed (450 ml); alcohol was evaporated at room temperature. (4.53 % yield).

Ocimum sanctum Leaf Extract: [12]

Fresh leaves were collected, cleaned, air-dried and powdered in a grinder. Powder was extracted overnight with 400 ml water with magnetic stirrer. The water extract was separated and the residue was re-extracted with water. The combined water extract was evaporated by rota evaporator. (13.33% yield).

Picrorrhiza kurroa Root Extract: [13]

The roots were oven-dried at 50° C and ground into powder-form with a grinder. For the preparation of aqueous extract of this plant, Powder was extracted using water at the ratio of 1:5. They were left in the water bath at 55° C for 3 hrs. The liquid obtained was then filtered and evaporated by rota evaporator. (6.25% yield).

Plumbago zeylanica Root Extract: [14]

The roots were powdered. Power was taken and subjected to soxhlation in 50% v/v ethanol for 5 hrs. The liquid obtained was filtered and evaporated by keeping in an oven at 40° C to obtain a solid residue. (8.25% yield).

Terminalia arjuna Bark Extract: [15]

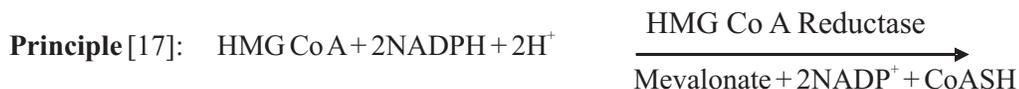
The stem bark was powdered and subjected to soxhlation in 50% v/v ethanol for 16 hrs. The The liquid obtained was filtered and evaporated by keeping in an oven at 40° C to obtain a solid residue. (7.5 % yield).

Enzyme Assay

HMG Co A Reductase Assay

Liver microsomes preparation : [16]

Male wistar rat was sacrificed by decapitation. Liver was removed and placed into ice-cold 0.25 M sucrose solution to remove blood. The liver was homogenized (after mincing with scissors) in 4 ml/g tissue wet weight of ice-cold 0.25 M sucrose solution using a homogenizer. The crude homogenate was centrifuged for 20 min at 11,500 RPM and the pellets were discarded. Supernatant was collected and 0.1 ml 88 mM CaCl₂ was added per ml of supernatant. Supernatant was placed on ice for 5 min. with occasional shaking. The supernatant was centrifuged at 13,500 RPM for 35 min. The pellets were resuspended in 10 ml of 0.1 M tris buffer, at pH 7.4 by homogenization, and it was stored at -20° C until use.



HMG Co A and NADPH are converted to Mevalonate and NADP⁺ respectively with the help of HMG Co A Reductase. Rate of oxidation of NADPH to NADP⁺ is measured at 340 nm continuously at 30 sec intervals for 5 min. Inhibition of HMG Co A Reductase decreases the rate of oxidation of NADPH.

Procedure [18, 19]: All reagents were prepared in 0.1 M Tris HCL buffer. Procedure was carried out at 25° C. 50µM HMG Co A and 10 mM Dithiothreitol (DTT) were preincubated for 1.5 hrs. For test, drug was incubated for 5 min with 0.1 M Tris HCL buffer, 1 mM Disodium EDTA, 75 mM NaCl, 50µl ml microsomes at 25° C. To this 0.1 mM NADPH and preincubated 50µM HMG Co A & 10 mM Dithiothreitol (DTT) were added and oxidation of NADPH was immediately recorded at 340 nm continuously at 30 sec intervals for 5 min. Control reading was taken by omitting

Investigation of HMG Co A Reductase.....Herbal Drugs – *In Vitro* Study H.D. Patel *et al* drug. To determine the relative oxidation of NADPH by other enzymes present in homogenate whole system excluding HMG CoA was measured. Rate of oxidation of NADPH was calculated using $6200 \text{ M}^{-1} \text{ cm}^{-1}$ as an extinction coefficient.

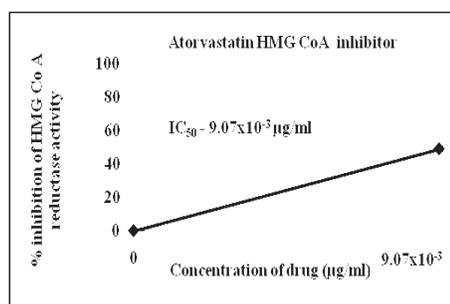
RESULTS

Oxidation of NADPH for HMG Co A reductase activity was found out for *Andrographis paniculata*, *Anthocephalus indicus*, *Ocimum sanctum*, *Picrorrhiza kurroa*, *Plumbago zeylanica* and *Terminalia arjuna* and their data are shown in table-1. IC_{50} values were derived by linear regression data analysis ($Y=mX+c$). IC_{50} values for HMG Co A reductase activity of *Andrographis paniculata*, *Anthocephalus indicus* and *Terminalia arjuna* are shown in Fig. 2, 3 and 7 respectively. Atorvastatin was taken as a standard for HMG Co A reductase inhibitor.

Table: 1 Rate of oxidation of NADPH in HMG Co A Reductase assay

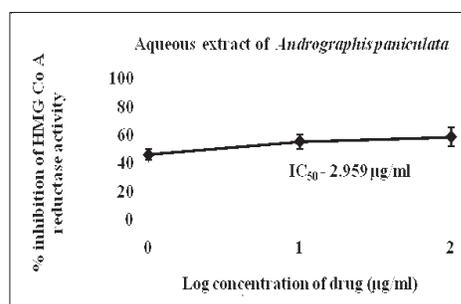
Drugs	Rate of oxidation (nmoles/min)			
	Control	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
<i>A. paniculata</i> leaves	$39.10 \pm 6.45^{\#}$	24.25 ± 3.32	$20.83 \pm 4.80^*$	$15.53 \pm 1.79^*$
<i>A. indicus</i> roots	$35.47 \pm 4.71^{\$}$	$23.23 \pm 0.99^*$	$15.84 \pm 1.96^*$	$5.42 \pm 2.25^*$
<i>O. sanctum</i> leaves	$39.10 \pm 6.45^{\#}$	31.44 ± 4.32	23.88 ± 2.84	24.15 ± 3.03
<i>P. kurroa</i> roots	$39.10 \pm 6.45^{\#}$	36.1 ± 6.78	31.65 ± 5.59	27.54 ± 3.80
<i>P. zeylanica</i> roots	$21.48 \pm 1.46^{@}$	20.90 ± 0.83	19.76 ± 0.75	17.64 ± 0.38
<i>T. arjuna</i> stem bark	$21.48 \pm 1.46^{@}$	18.45 ± 1.13	$4.39 \pm 1.18^*$	$1.71 \pm 0.75^*$

Values are Mean \pm SEM. * $P < 0.05$ when compared with respective control groups.
[# Water as a control; \$ Methanol as a control; @ DMSO (50%) as a control]



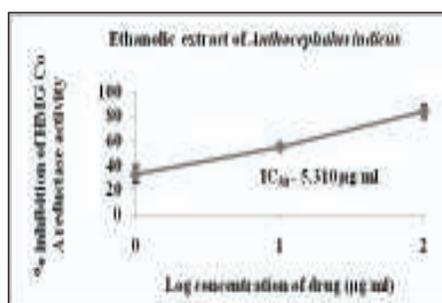
Values are means \pm SEM

Fig 1 Effect of Atorvastatin on HMG Co A reductase activity.



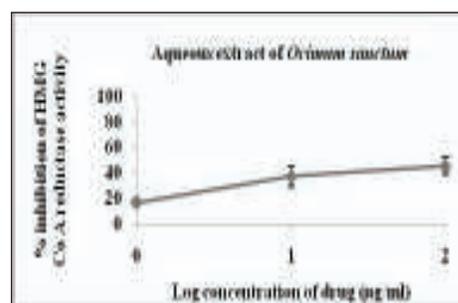
Values are means \pm SEM. IC 50 Value was derive by linear regression data analyses ($y=mX+c$)

Fig 2 Effect of aqueous extract of *Andrographis paniculata* leaves on HMG Co A reductase activity.



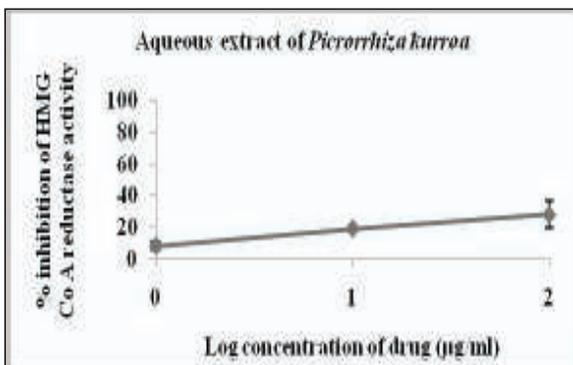
Values are means \pm SEM. IC 50 Value was derive by linear regression data analyses ($y=mX+c$)

Fig 3 Effect of ethanolic (95%) extract of *Anthocephalus indicus* roots on HMG Co A reductase activity.



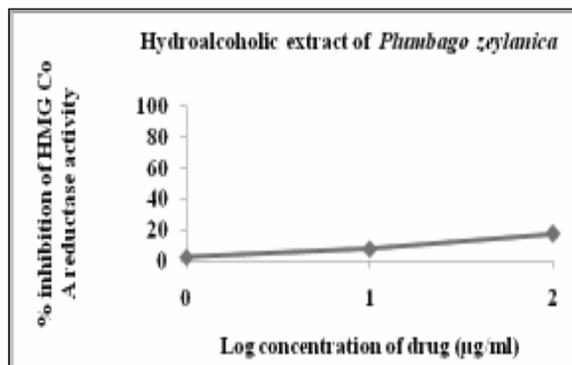
Values are means \pm SEM

Fig 4 Effect of aqueous extract of *Ocimum sanctum* leaves on HMG Co A reductase activity



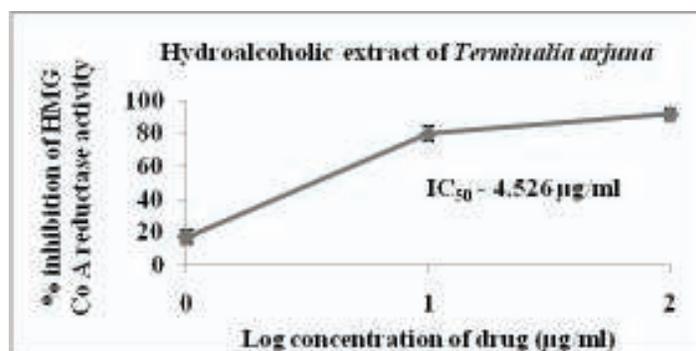
Values are means \pm SEM

Fig 5 Effect of aqueous extract of *Picrorrhiza kurroa* roots on HMG Co A reductase activity.



Values are means \pm SEM

Fig 6 Effect of hydroalcoholic extract of *Plumbago zeylanica* roots on HMG Co A reductase activity.



Values are means \pm SEM. IC 50 Value was derived by linear regression data analyses ($y=mX+$)

Fig 7 Effect of hydroalcoholic extract of *Terminalia arjuna* stem bark on HMG Co A reductase activity.

DISCUSSION

The lipid metabolism is regulated in many different ways. Enzymes are major regulators of lipid metabolism. From various enzymes involved in lipid metabolism HMG Co A reductase is the important enzyme which is involved in cholesterol [2].

Many herbal drugs are reported to possess antihyperlipidemic activity. These drugs includes *Andrographis paniculata* [10], *Anthocephalus indicus* [11], *Ocimum sanctum* [12], *Picrorrhiza kurroa* [13], *Plumbago zeylanica* [14] and *Terminalia arjuna* [15]. Since their mechanism of action are not known. We thought to screen these herbal drugs for inhibitory effects on HMG Co A reductase enzyme.

Aqueous extract of *Andrographis paniculata* leaves at 100 and 200 mg/kg has been reported to normalize the increase in plasma total cholesterol (TC) and low density lipoproteins (LDLs) in high cholesterol fat diet fed rats [10]. In our study, this extract was found to inhibit HMG Co A reductase isolated from liver isolated from high fat diet fed wistar rat with IC_{50} 2.959 μ g/ml. (Fig.2) This enzyme inhibition was comparable to that observed with standard drug, atorvastatin (IC_{50} - 9.071×10^{-3} μ g/ml \sim 7.5 nM [3]) (Fig.1) as HMG Co A reductase inhibitor. Bitter water soluble lactone andrographolide is major chemical constituent of *Andrographis paniculata* leaves (2.5%) [20]. so from our data we can say that andrographolides might be responsible for strong inhibition of HMG Co A reductase.

Ethanollic extract (95%) of *Anthocephalus indicus* at 500 mg/kg in rats has been reported to revert the increase in plasma level of total cholesterol (TC), phospholipids (PL), triglycerides (TGs), and total proteins (TP) due to triton WR-1339. In addition, the extract reactivates the post heparin lipolytic activity (PHLA) [11]. The authors have proposed the stimulation of lipases as the possible mechanism of action of this drug. In addition, antioxidant activity

of this extract may give added advantage. In contrast to above, our results demonstrate that HMG Co A reductase enzyme was strongly inhibited by this extract. IC₅₀ value for HMG Co A reductase inhibition was 5.310 µg/ml. (Fig.3) Thus the said extract is likely to work at different levels in controlling the rise in plasma lipid levels. Since the extract is multicomponent one, different constituents like saponins, terpenes, glycosides and alkaloids [11] might be having different mechanism of action. The strong antihyperlipidemic activity can thus be due to effect of both endogenous and exogenous pathways. Screening based fraction may reveal the exact chemical constituents responsible for inhibitory activity of the enzyme.

Aqueous extract of *Ocimum sanctum* leaves at 200 mg/kg has been reported to decrease plasma total cholesterol (TC), low density lipoproteins (LDLs) and triglycerides (TGs) which were induced by streptozotocin [12]. No potent inhibition of HMG Co A reductase was observed in our study. (Fig.4)

Aqueous extract of *Picrorrhiza kurroa* roots at 50, 100 and 200 mg/kg has been reported to decrease serum total cholesterol (TC), low density lipoproteins (LDLs) and triglycerides (TGs) in high fat diet treated mouse [13]. This extract did not show any significant inhibition of HMG Co A reductase (Fig.5) in our study.

Hydroalcoholic extract of *Plumbago zeylanica* root at 500 mg/kg has been reported to reduce serum total cholesterol (TC), low density lipoproteins (LDLs), triglycerides (TGs), TC/HDL and LDL/HDL ratios in rabbits fed with atherogenic diet [14]. In our study inhibition of HMG Co A reductase was less marked. (Fig.6)

Hydroalcoholic extract of *Terminalia arjuna* stem bark at 100 and 500 mg/kg has been reported to decrease serum TC, TC/HDL, LDL/HDL ratios and LDL Cholesterol. It did not show any change in serum triglycerides and HDL cholesterol levels. This extract has been reported to decrease fat content in liver and heart. This indicates a decreased availability of fats to these organs. This could be due to decreased absorption of fats from intestine [15]. In our study, hydroalcoholic extract inhibited HMG Co A reductase with IC₅₀ 4.526 µg/ml. (Fig.7). This extract contains fibres, β-sitosterol, saponins and tannins [15]. These chemical constituents might be responsible for HMG Co A reductase inhibition.

CONCLUSION

Andrographis paniculata, *Anthocephalus indicus* and *Terminalia arjuna* possess HMG Co A reductase inhibitory activity. While *Ocimum sanctum*, *Picrorrhiza kurroa* and *Plumbago zeylanica* do not possess HMG Co A Reductase inhibitory activity. In future study will be required to isolate active constituents that are responsible for this activity.

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