COMPARATIVE EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF SOME MARKETED HERBAL FORMULATIONS IN TRITON INDUCED HYPERLIPIDEMIC RATS

KANUNGO S. K[1*], PANDA D. S[1], SWAIN S. R[1], BARIK B. B[2] AND TRIPATHI D. K[3]

[1]Institute of pharmacy and Technology, Salipur, Cuttack-754202, E-mail: sunil.kanungo@rediffmail.com
[2]University Department of Pharmaceutical sciences, Utkal University, Bhubaneswar,
[3]Bengal School of Technology, Sugandha, Hoogly,

Summary

A comparison was made between the hypolipidemic activities of five different marketed herbal formulations along with a prepared formulation in triton induced hyperlipidemic rats. Various parameters such as serum Cholesterol and Triglycerides were determined before 24 hours of triton administration and in two different phases (synthesis and excretory) after 24 and 48 hours respectively. HDL cholesterol was determined only in the excretory phase. There is a significant decrease in cholesterol and triglyceride levels in all the treated animals without much variation and an increase in the HDL level was seen in all the treated animals after 48 hours of triton administration in excretory phase.

Key words: Hypolipidemic; Herbal formulation

Introduction

Many investigators have demonstrated a correlation between raised serum lipids and the incidence of coronary heart disease (CHD) and atherosclerosis. In the serum lipids, a high concentration of total cholesterol (TC), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL) and triglycerides (TG), and a low concentration of high-density lipoprotein cholesterol (HDL) are mainly concerned in the relationship. Increased interest in the prevention and management of CHD and atherosclerosis and the identification of hyperlipidaemia as risk factor has stimulated the study of drugs which prevent or reduce the risk.

Recently, several herbomineral drugs have been advocated for their hypolipidemic action. In this study a comparison was made between the hypolipidemic activity of 5 different marketed herbal formulations and one prepared formulation in relationship to their composition (table-1).

Herbs have been used since ancient times for reducing body lipids. Reports on all garlic studies performed, found cholesterol was lowered by an average of 9-12% over a one-to-four month period(1). Guggul, a mixture of substances taken from the plant *Commiphora mukul*, is an approved treatment for elevated cholesterol in India and has been a mainstay of the Ayurvedic approach in preventing atherosclerosis. One trial studying the effects of guggul reported that serum cholesterol dropped by 17.5 % (2). In another report comparing guggul to the drug clofibrate, average fall in serum cholesterol was slightly greater in the

guggul group while HDL cholesterol rose in 60% of people responding to guggul, while clofibrate treatment did not elevate HDL (3). *Commiphora mukul,* the natural product and its components guggulsterone E & Z known to have promising cardio-protective & hypolipidemic activity possibly by inhibiting the oxidative modification of LDL, involvement in lipid peroxidation and acts as an antagonist of bile acid receptor. A significant hypocholesterolaemic effect of *Trigonella foenum-graecum* has been well documented by several authors from animal and clinical study. In some studies of animals and humans with both diabetes and high cholesterol levels, fenugreek lowered cholesterol levels as well as blood sugar levels(4).

Asphaltum punjabium (Shilajit) is hot, bitter and mostly reduces Kapha, but is beneficial for Vata and Pitta as well according to Ayurvedic concept. It is an adaptogen or rasayana. It is called Yogavahi, which means it strengthens and enhances all other herbs and processes in the body. It is a known free radical scavenger, anti-stress agent and a powerful adaptogen (5). *Zingiber officinale*, *Piper nigrum* and *Piper longum* in combination is called as trikatu in Ayurveda. Besides their therapeutic potential these three ingredients mostly used in herbal formulation as bioavailability modifier. Ginger possesses antioxidant, antiobesity and lipid peroxidation activity (6, 7). Green pepper and long pepper proved to have antibacterial and antioxidant activity(8) . Piperine is a potent inhibitor of drug metabolism and inhibits lipid peroxidation *in vivo*(9).

TABLE 1: COMPOSITION OF DIFFERENT HERBAL FORMULATIONS

O.B Powder	Trim fit	sleek	B-Slim	Lasuna capsule	Prepared formulation
Cyamposis psoraliodes	Guggulu pure	Muli beej	Garcinia cambogia	Allium sativum	Zingiber officinale
Una Qua dodium Chloride	Asphaltum punjabium	Sudh shilajeet	Trigonella foenum graecum		Piper nigrum
Zingiber officinale	Boerhaavia diffusa	Trimurti ras	Glycyrrhiza glabra		Piper longum
Cuminium cymimumi	Embelia ribes	Madohar guggulu	Calcined ash		Commiphor a mukul
Piper nigrum	Triphala (AFI)	Loh Bhasam			Trigonella foenum graecum
Sodi chloridum	Trikatu (AFI)	Vidangadi lauha			Asphaltum punjabium
	Eugenia jambolana				
	Aegle marmalos				
	Terminalia arjuna				

AFI- Ayurvedic formulary of India

Methods

Preparation of Drug Suspension

The commercial and prepared formulations were triturated with equal quantities of gum acacia and suspended in water.

Preparation of Triton Solution

A 20%w/v solution of Triton WR1339 (isooctyl-polyoxyethylene phenol) was prepared in phosphate buffer (pH 7.2; 0.05M).

Hypolipidemic studies

Male Wistar rats (84) weighing 200- 350 g body weight were divided into 14 groups of 6 animals each as given in table-1. The animals were maintained at $22^0 \pm 3^0$C and 30-70% relative humidity with 12: 12 hr L: D cycle. Animals were starved throughout the experimental period, but water was provided ad libitum. The animals were injected intraperitonially with 200 mg/kg Triton WR 1339 (isooctyl-polyoxyethylene phenol).

TABLE 2: TREATMENT ANIMAL GROUPS

Group No.	**Treatment**
1	Untreated control
2	Triton control
3	Triton treated + Sleek capsule (50mg/kg)
4	Triton treated + Sleek capsule (50mg/kg)
5	Triton treated + B-Slim tablet (50mg/kg)
6	Triton treated + B-Slim tablet (50mg/kg)
7	Triton treated + O.B.Powder (50mg/kg)
8	Triton treated + O.B.Powder (50mg/kg)
9	Triton treated + Trim Fit capsule (50mg/kg)
10	Triton treated + Trim Fit capsule (50mg/kg)
11	Triton treated + Lasuna capsule (50mg/kg)
12	Triton treated + Lasuna capsule (50mg/kg)
13	Triton treated + Prepared formulaton (50mg/kg)
14	Triton treated + Prepared formulaton (50mg/kg)

Rats in group 1 were maintained as the untreated control. Triton was administered, intraperitonially to the rats of group 2- 14 at a dose of 200mg/ kg body weight. Animals in group 3, 5, 7, 9, 11, 13 received the drug suspension at a dose of 50mg/ kg body weight by oral intubation immediately after the

administration of triton (synthesis phase). Rats in groups 4, 6, 8, 10, 12, 14 received the drug suspension at a dose of 50mg/ kg body weight, 22 hours after the administration of triton. Group 2 was left without dosing after triton administration. Blood was collected from the retro orbital sinus in heparinized vials 24 hours before the start of the experiment and then 24 and 48 hours after triton administration (Burstein et al. 1982). Cholesterol, Triglyceride and HDL Cholesterol were estimated in the blood using an auto analyzer. The animals were euthanised (intraperitoneal administration of thiopental sodium at a dose of 100mg/kg body weight) following the last blood collection.

Statistical analysis

All the results obtained were expressed as means $\pm$ SEM, and were analyzed using student's't' test. A p value < 0.05 is considered significant

Results

The 14 groups comprising of 6 animals in each, followed fairly uniform dietary patterns during the course of the study. Similar treatments were given to two groups of animals one for synthesis phase and another for excretory phase, keeping the untreated control and triton control common for both. Triton treatment increased the cholesterol level from 45.68 ± 3.30 mg/dl to 251.32 ± 6.81 mg/dl after 24 hours and from 40.57 ± 2.99 to 100.87 ± 8.89 after 48 hours. Similarly the triglyceride levels increased from 69.35 ± 2.32 mg/dl to 1436.68 ± 193.37 after 24 hours and from 53.19 ± 10.77 to 218.92 ± 55.58 mg/dl after 48 hours.

In the synthesis phase:

The cholesterol level was significantly reduced without much variation among all the groups treated with different formulations. The lowering of cholesterol was found to be maximum in the group treated with the prepared formulation from 251.32 ± 6.81 mg/dl to 140.26 ± 27.81 mg/dl after 24 hours and from 100.87 ±

8.89 mg/dl to 71.45 ± 6.85 mg/dl after 48 hours. Similar results were observed in case of triglyceride, the group under treatment with sleek capsule showed maximum reduction from 1436.68 ± 193.37 mg/dl to 905.12 ± 90.57 mg/dl after 24 hours and from 218.92 ± 55.58 mg/dl to 69.34 ± 11.42 mg/dl after 48 hours.

Table 3: LEVELS OF CHOLESTEROL AND TRIGLYCERIDES IN SYNTHESIS PHASE

Groups	**Cholesterol in mg/dl**			**Triglycerides in mg/dl**		
	-24 hr	24 hr	48 hr	-24 hr	24 hr	48 hr
1	59.07 ± 3.76	45.68± 3.30	40.57 ± 2.99	146.44 ± 16.04	69.35 ± 2.32	53.19 ± 10.77
2	46.34 ± 3.39	251.32 ± 6.81	100.87 ± 8.89	171.28 ± 19.56	1436.68 ± 193.37	218.92 ± 55.58
3	45.77 ± 4.84	140.73 ± 29.43*	72.42 ± 6.94*	202.71 ± 20.03	905.12 ± 90.57*	69.34 ± 11.42*
5	45.69 ± 4.85	143.08 ± 29.22*	74.28 ± 6.39*	186.08 ± 14.59	931.90 ± 87.33*	76.50 ± 6.31*
7	46.91 ± 4.25	144.54 ± 29.31*	74.80 ± 6.2*	179.95 ± 9.98	975.72 ± 49.91*	78.36 ± 5.49*
9	45.92 ± 3.82	142.09 ± 28.57*	73.89 ± 5.16*	204.43 ± 19.10	917.72 ± 86.96*	71.84 ± 9.98*
11	47.56 ± 4.52	143.08 ± 29.49*	73.55 ± 6.84*	183.51 ± 12.12	970.94 ± 32.46*	73.36 ± 5.45*
13	47.5 ± 4.65	140.26 ± 27.81*	71.45 ± 6.85*	177.75 ± 11.77	964.64 ± 33.87*	70.76 ± 5.43*

Each value represents the mean±SEM of six observations; *$p < 0.05$ is significant

In the excretory phase:

The cholesterol level was significantly reduced without much variation among all the groups treated with different formulations. The lowering of cholesterol was found to be maximum in the group treated with B-Slim tablet from 251.32 ± 6.81 mg/dl to 178.87 ± 18.39 mg/dl after 24 hours and the group treated with O.B powder from 100.87 ± 8.89 mg/dl to 99.21 ± 6.18 mg/dl after 48 hours. Similar results were observed in case of triglyceride, the group under treatment with prepared formulation showed maximum reduction from 1436.68 ± 193.37 mg/dl to 977.18 ± 15.05 mg/dl after 24 hours and the group trated with Lasuna capsule from 218.92 ± 55.58 mg/dl to 157.42 ± 16.55 mg/dl after 48 hours. The HDL level increased in all groups significantly, however maximum increase was

observed in the group treated with Sleek capsule from 20.34 ± 0.94 mg/dl to 29.01 ± 2.16 mg/dl after 48 hours.

Table 4: LEVELS OF CHOLESTEROL AND TRIGLYCERIDES AND HDL IN EXCRETORY PHASE

Groups		**Cholesterol in mg/dl**		**Triglycerides in mg/dl**		**HDL in mg/dl**	
	-24 hr	24 hr	48 hr	-24 hr	24 hr	48 hr	48 hr
1	59.07 ± 3.76	45.68± 3.30	40.57 ± 2.99	146.44 ± 16.04	69.35 ± 2.32	53.19 ± 10.77	20.44 ± 0.54
2	46.34 ± 3.39	251.32 ± 6.81	100.87 ± 8.89	171.28 ± 19.56	1436.68 ± 193.37	218.92 ± 55.58	20.34 ± 0.94
4	48.75 ± 2.08	183.16 ± 19.07*	110.07 ± 6.04	153.32 ± 14.31	979.36 ± 42.18*	157.71 ± 27.92*	29.01 ± 2.16*
6	48.49 ± 2.46	178.87 ± 18.39*	111.23 ±7.9	147.48 ± 10.66	1003.32 ± 20.93*	157.84 ± 18.88*	28.69 ± 2.33*
8	49.54 ± 3.26	188.78 ± 17.68*	99.21 ± 6.18	155.38 ± 10.73	998.08 ± 17.87*	160.21 ± 12.50*	28.59 ± 2.26*
10	48.98 ± 1.8	185.08 ± 18.08*	110.77 ± 7.52	155.59 ± 14.20	984.93 ± 23.78*	160.22 ± 19.24*	28.71 ± 2.64*
12	48.84 ± 2.74	183.49 ± 18.22*	109.39 ± 5.88	155.76 ± 11.11	993.27 ± 24.33*	157.42 ± 16.55*	28.78 ± 2.22*
14	48.92 ± 1.86	182.66 ± 18.75*	109.99 ± 6.00	154.97 ± 11.79	977.18 ± 15.05*	159.63 ± 10.13*	28.30 ± 2.23*

Each value represents the mean±SEM of six observations; *$p < 0.05$ is significant

Discussion

The biphasic nature of triton induced hyperlipidemia is helpful in understanding the mode of action of hypolipidemic agents. Drugs interfering with lipid biosynthesis or uptake will be active in the synthesis phase, while drugs interfering with lipid excretion and metabolism will be active in the excretory phase. The present study revelaed that the different formulations are active both in synthesis and excretory phase.Triton induces hyperlipidemia by increasing the hepatic synthesis of cholesterol and triglycerides (10).

In the excretory phase of triton induced hyperlipidemia, the breakdown of lipids occurs. The drugs effective in the excretory phase could be assumed to increase the metabolism or excretion of lipids. The reduction in lipid levels may be by virtue of increased bile acid synthesis and increased degradation of cholesterol to fecal bile acids and neutral sterols.

Ample of evidence exists with respect to the fact that HDL cholesterol is inversely related to total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atheroscelerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall (11).

High serum cholesterol is regarded by many as the main cause of coronary arherosclerosis (12). Several cholesterol lowering interventions have reduced coronary heart diseases (CHD) events in primary and secondary prevention clinical trials(13,14). Even expert panels in Europe and USA have therefore recommended dietary changes and, if necessary, addition of drugs to reduce high cholesterol concentrations especially low-density-lipoprotein (LDL) cholesterol(15) in patients with CHD. No statistically or clinically significant changes were seen in weight, blood pressure, serum blood glucose levels, uric acid levels, or findings of other routine biochemical tests. Until the mid-1990s, the importance of dyslipidaemia as a risk factor for coronary heart disease was controversial, as was the use of lipid lowering treatment. However, after publication of the 4S trial in 1994, four other trials confirmed significant reductions in fatal and non-fatal cardiovascular events when statins were used in both primary and secondary prevention.

Many guidelines have been recommended for reducing levels of total cholesterol, triglycerides and low-density lipoproteins to decrease risk for coronary heart disease. Most cardiologists agree that adherence to these guidelines would reduce rates of morbidity and mortality from heart disease. There is little doubt that elevated cholesterol levels increases the risk for coronary heart disease. Observational research indicates that a linear relation exists. A 20% increase in risk for coronary heart disease is associated with a 10% increase in

serum cholesterol levels. This dose-response effect occurs at any cholesterol level and is apparent in both men and women and in both black and white persons.

It is found that all the herbal formulations screened, have the unique ability to lower serum cholesterol levels with lowering of serum triglyceride levels and increase in HDL level.

The method of hyperlipidemia induction using triton is artificial and not very similar to hyperlipidemia in humans, especially hypertriglyceridemia.

References

1. Holzgartner J, Schmidt U, Kuhn U. Comparison of the efficacy of garlic preparation vs bezafibrate. Arzneim-Forsch Drug Res.1992; 42: 1473-77.

2. Agarwal RC, Singh SP, Saran RK.Clinical trial of guggul lipid new hypolipidemic agent of plant origin in primary hyperlipidaemia. Indian J Med Res. 1986; 84**:** 626-34.

3. Nityanand S, Srivastava JS, Asthana OP. Clinical trials with gugulipid – A new hypolipidemic agent. J Assoc Phys India. 1989; 37: 323-28.

4. Xue WL, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ. Effect of Trigonella foenum- graecum (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. Asia Pac J Clin Nutr. 2007; 16: 422-426.

5. U.C.Datta and G. King, Materia Medica of the Hindus. Machine Press, Calcutta, India, 1877; pp.33-37.

6. Ali Taghizadeh Afshari, Alireza Shirpoor, Amirabbas Farshid, Ramin Saadatian, Yousef Rasmi, Ehsan Saboory, Behrooz Ilkhanizadeh and Abdolamir Allameh. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry, 2007; 101: 148-153

7. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (Zingiber officinale) in streptozotocin-induced diabetic rats.Br J Nutr. 2006; 96: 660-666.

8. Suchandra Chatterjee, Zareena Niaz, S. Gautam, Soumyakanti Adhikari, Prasad S.

Variyar and Arun Sharma. Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*).Food Chemistry. 2007; 101: 515-523.

9. Rakesh K. Johri, Neelima Thusu, Annu Khajuria and Usha Zutshi. Piperine-mediated changes in the permeability of rat intestinal epithelial cells : The status of γ-glutamyl transpeptidase activity, uptake of amino acids and lipid peroxidation .*BiochemicalPharmacology*. 1992; 43: 1401-1407.

10. Vogel H G and Vogel W H. Drug Discovery and Evaluation Pharmacological assays,Springer Verlag, Berlin, 1997; pp.598.

11. Miller G J and Miller N E. Plasma- HDL Concentration and development of Ischaemic Heart Disease, Lancet. 1975; 1**:** 16-19.

12. Gotto AM Jr, LaRosa JC, Hunninghake D. The cholesterol facts. A summary of the evidence relating dietary facts, serum cholesterol and coronary heart disease, Circulation. 1990; 81: 1721-33.

13. Leren P. The Oslo. Diet Heart Study: eleven-year report. Circulation. 1970; 42: 935- 42.

14. Buchwald H, Varco RL, Matts JP. Effect of partial ileal bypass on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia-report of the program on the Surgical Control of Hyperlipidaemias (POSCH). N Engl. J. Med. 1990; 323: 946-55.

15. Tiwari, A.K., Agrawal, A., Gode, J.D. and Dubey, G.P.(1991) "A prospective randomised crossover study of propranolol and Abana in hypertensive patients: Effect of lipids and lipoproteins. The Antiseptic. 1991; 88: 41.