ASSESSMENT ON POLYHERBAL FORMULA'S ANTI-HYPERLIPIDEMIC MOVEMENT IN ALBINO WISTAR RATS

[1]KRISHNAMURTHY HEMAMALINI, [2]VOBULAPURAM NISHANTHA, [3]THOTAPALLY LALITHA, [4]B.BHAVANI

[1]Professor, [2,3]Assistant Professor, [4]UG Student, [1,2,3,4]Department of Pharmacy, Brilliant Grammer School Educational Society Group of Institutions-Integrated Campus, Hyderabad, India.

ABSTRACT

The investigation of the anti-hyperlipidemic properties of an ethanolic extract of a herbal preparation made from the leaves of three Indian medicinal plants—Azadirachta indica, Curcuma longa, and Zingiber officinale—against Triton WR-1339-induced hyperlipidemia in male Wistar rats is included in this article. The goal of the preclinical investigation was to see whether poly herbal formulations may reduce hyperlipaemia caused by Triton WR-1339. The five groups of six animals used in the current investigation include normal control, diseased control, Triton and herbal treatment group (PHF 300), Triton and herbal treatment group (PHF 600), and Triton and Atorvastatin treatment group. In With a single intravenous injection of 2ml/kg or 200mg/kg of Triton WR1339 in normal saline (0.9% Nacl), blood total cholesterol (TC), triglyceride (TG), LDL-cholesterol, and VLDL were elevated in overnight-fasted wistar rats. To the triton induced hyperlipaedimic rats, the poly herbal formulation (300 and 600 mg/kg/b.w/p.o) and Atorvastatin (10mg/kg/b.w/p.o) was administered orally for seven days. There is a significant reduction in LDL-C,VLDL, Serum total cholesterol, triglycerides ,Atherogenic index, and there is a increase in HDL-C levels in poly herbal formulation treated grouped when compared to Triton treated group. With the standard drug Atorvastatin (10 mg/kg/b.w. /p.o.), in treatment of hyperlipidemia the poly herbal formulation treated grouped exhibited competitive potential exhibiting a alternative natural therapeutic agent .

Key words: *Azadirachtaindica,curcuma longa ,Zingiberofficinale*, Atherogenic index, Poly herbal formulation, hyperlipidemia

INTRODUCTION:

The result of several interconnected variables, such as metabolic, genetic, and lifestyle factors that affect the metabolism of plasma lipoprotein, is hyperlipidemia. (1) Lowering lipid and cholesterol levels by dietary changes or medication can lower the risk of coronary heart disease. (2). By employing various methods, the well-known anti hyperlipidemic medications, including bile acid binding agents, statins, and fibrates, control lipid metabolism. (3) As a result, there is a rising need for innovative herbal medications that can inhibit or control The herbal medications are in great demand since they can lower blood TC and TG values. Nowadays, the search is concentrated on plants with hypolipidemic action due to higher availability and better absorption in the body. [(4)]

A polyherbal formulation containing ethanolic extracts of the three herbs The leaves of plants,*Azadirachtaindica*,*Curcuma longa* ,*Zingiberofficinale*was reported to caontainFlavanoids, Alkaloids, Saponins, tannins, phenols, anthocyanins, terpenoids, sterolsEugenol, Eugenal, Carvacol, Linalool, Limatrol, Caryophyllene, Anthocyans, *Azadirachtaindicawas reported to contain*ϒ-sitosterol, aegelin, lupeol, rutin, marmesinin, β-sitosterol, flavone, glycoside, Oisopentenylhalfordiol, marmeline and phenylethylcinnamamides.*Curcuma longa*was reported to contain gallic acid,cyanidin, glycoside, glycoside-5-amboline,triterpenoids, tannins,gallotannins, essential oils, beta sitosterol,myricyl alcohol ,myricetin.[(5)(6).] *Zingiberofficinalewas reported to contain*ϒ-sitosterol, aegelin, lupeol, rutin, marmesinin, β-sitosterol, flavone, glycoside, Oisopentenylhalfordiol, marmeline and phenylethylcinnamamides.[(7)]

*Zingiberofficinale*was reported to contain gallicacid,cyanidin, glycoside, glycoside-5-amboline,triterpenoids, tannins,gallotannins, essential oils, beta sitosterol,myricyl alcohol ,myricetin.

Due to the presence of steroids,saponins,phenols,tannins,flavanoids ,they have previously reported to have anti hyperlipidemicand hypoglycemic activities and the and the ability of PHF to reduce serum cholesterol,triglycerides and lipids have beenstudied.[(8),(9).]The present study was undertaken to investigate the anti-hyperlipidemic effect of ethanolicextractpolyherbal formulation prepared using the three medicinal plants leavesof*Azadirachtaindica*

,*Curcuma longa* ,*Zingiberofficinale*.Our aim is to find out the potential of ethanolic extract of poly herbal formulation in bringing about various changes in parameters of lipid profile TG,TC,LDL-C VLDL-C ,HDL-C. The poly herbal formulation consisting of ethanolic extracts of *Azadirachtaindica*

,*Curcuma longa* , *Zingiberofficinale*was selected for studying its hypoglycemic activityaganistTriton WR-1339 induced Hyperlipidemia in male *Wistar* rats.

Plant collection, authentication and extraction:

Polyherbalethanolic extract preparation.

The leaves of plants *Azadirachtaindica*,*Curcuma longa* ,*Zingiberofficinale*(were collected from the local area .Plant leaves were collected and shade dried for 15 days, and then grinded powdered ,each fraction containing equal portion of plant powder was subjected to cold maceration using 90% ethanol, and then subjected to vaccumfilteration and filterate was subjected to steam distillate and the concentrate was subjected to rota evaporator.

Dose Selection:

Plant extracts ofleaves of plants*Azadirachtaindica*

,*Curcuma longa* ,*Zingiberofficinale*have anti hyper lipaedimic ,anti microbial ,anti oxidant activity at200 to 600 mg/kg body weight .Polyherbalethanolic extract in proportion of,i.e 300Mg/kg(PHF 300)and 600 mg/kg (PHF 600)were prepared. To attain uniform consistency , in a clock wise direction each extract was weighed and mixed thoroughly with the help of mortar and pestle.(10) For invitroand invivo study the final formulations were stored at 4^{o}c.According to their effective doses the poly herbal formulation containing three herbs was prepared .At doses of 300 and 600 mg/kg ,p.o at a constant volume of 0.5ml/100g of body weight.Phyto chemical analysis revealed the presence of Flavanoids,phenols,Saponins.By using Folin-Ciocalteau method total phenolic content was determined.(11)

Chemicals and reagents

Triton WR-1339 andFolin-Ciocalteau reagent obtained from commercial sources,Triglycerides,total cholesterol were determined using E-coline diagnostic kits,Atorvastation 10 mg tablets were used as standard drugs.

Animals:

Male *Wistar* rats (250-300 g) were used in the study supplied by Vignan institute of Pharmaceutical technology. By using standard pellet chow feed and water ad libitum supplied for animals as food. with The experiments and study protocol were approved by institutional animal ethical committee.,Vignan institute of Pharmaceutical technology. ((Reg. No. 2003/ PO/RE/S/18/CPSCEA)

Materials and methods

Hyperlipidemia Induced by Triton WR-1339

Triton WR-1339 induced hyperlipidemia:

- Male *Wistar* rats (250-300) gm. weight, were fasted for 18 hours and then injected intravenously freshly prepared solution of 200 mg/kg Triton WR-1339 (Isooctyl- Polyoxy-Ethylene Phenol) in normal saline.
- Serum cholesterol levels increase sharply 2-3 times after 24 h .The animals were administered,Polyherbalethanolic extract in proportion of 300Mg/kg (PHF 300) and 600 mg/kg (PHF 600), Standard drug Atorvastatin (10 mg/k.g/b.w. /p.o.) and solvent for the controls orally by Intragastic tube once daily for seven consecutive days.
- On 8th day, the animals were fasted for 18 hrs (had only access to water) and Triton WR 1339 was injected intravenously.
- Serum cholesterol and triglycerides analyses were made 24 hours after Triton injection.

The systemic administration of the surfactant Triton to mice or rats results in a biphasic elevation of plasma cholesterol and triglycerides

Male *Wistar* rats (250-300) gm. weight, were fasted for 18 hours and then injected intravenously freshly prepared solution of 200 mg/kg Triton WR-1339 (Isooctyl- Polyoxy-Ethylene Phenol) in normal saline

Freshly prepared solution of 200 mg/kg Triton WR-1339 (Isooctyl- Polyoxy-Ethylene Phenol) in normal saline was injected intravenously, to the Male *Wistar* rats (250-300) gm. weight , which were previously fasted for 18 hours.

After 24 h Serum cholesterol levels increase sharply 2-3 times.

To the animals Polyherbalethanolic extract in proportion of 300Mg/kg (PHF 300) and 600 mg/kg (PHF 600), Standard drug Atorvastatin (10 mg/k.g/b.w. /p.o.) and solvent for the controls administered orally by Intragastic tube once daily for seven consecutive days

The animals were fasted for 18 hrs On 8th day and Triton WR 1339 was injected intravenously.

24 after Triton injection.Serum cholesterol and triglycerides analyses were made.

Experimental Procedure:

- Male *Wistar* rats were divided into Fivegroups of six animals in each group and were treated with single dose/day (*p.o.*) of standard drug or extracts.
- **Normal control group:** The first group (Normal Control) received normal saline 0.9% w/v orally for one week.
- **Triton treated group:** The second group (Triton Positive Control) received Triton WR-1339 dissolved in 0.9% w/v saline (200 mg/kg, b.w.) by i.v. route.
- **Triton + PHF1 treated group:** The third groups was administered300Mg/kg b.w. /p.o. (PHF 300), once in a day for one week.
- **Triton + PHF2 treated group:** The fourth groups was administered 600 mg/kg b.w. /p.o. (PHF 600), once in a day for one week

- **Triton + Atorvastatin:** The fifth group was treated with Atorvastatin 10 mg/kg/b.w. once in a day for one week (Served as Standard). Saline, polyherbal extract and Atorvastatin were administered orally by Intragastic tube once daily for seven consecutive days.
- On the 8th day, the animals were starved for 18 hrs (had only access to water) and then injected intravenously freshly prepared solution of 200 mg/kg/b.w Triton WR-1339 (Isooctyl- Polyoxy-Ethylene Phenol) in normal saline, to all the four groups rats.
- Serum cholesterol levels increase sharply 2-3 times after 24 h (Phase-I) Triton injection.
- Blood was collected at 24 hr after Triton injection by Retro - Orbital Sinus Puncture, under mild Ether anaesthesia.
- The collected blood samples were centrifuged using semi ultra-cooling centrifuge at 3000 rpm for 10 minutes at room temperature and were directly used for estimating various biochemical tests (Serum Cholesterol, Triglycerides, LDL-C and HDL-C).
- All samples were stored at 4°C until analysis.

Table No.1 Grouping of animals

S.NO.	Groups	
1.	GroupI	**Normal control group**
2.	Group2II	**Triton treated group(Disease control)**
3.	GroupIII	**Triton + (PHF 300)treated group**
4.	GroupIV	**Triton + (PHF 600)treated group**
5.	GroupV	**Triton + Atorvastatin(STANDARD)**

Statistical Analysis

Data were analyzed using one way analysis of variance (ANOVA) followed by Turkey test and $P<0.05$ was considered significant. Values are expressed as Mean ± SEM for six rats per group.

RESULTS

Acute Toxicity Studies

No (Mortality) death was observed till the end of study. Body weight was recorded at 0, 7th and 14 day. The ethanolic extract of PHF was found to be safe up to 3000mg/kg body weight.

ANTI HYPER LIPIDEMIC ACTIVITY

Table No.2Effect of hydroalchoholic extract of PHF on Serum Cholesterol, Triglyceride, HDL-C, LDL-C and VLDL-C on TritonWR-1339 induced Hyperlipidemic Rats

Groups	Total Cholesterol (mg/dL)	% Change	Triglyceride (mg/dL)	% Change	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I Control (Normal Saline 0.9% w/v)	82.8 ± 6.70	0.88 ±0.16	68.8 ± 4.22	1.025±0.22	44.17±1.14	64.91±2.49	15.9±0.27
II Triton Treated (Triton 200 mg/kg, i.v.)	284.4±17.18	--------	364.2 ± 23.73	------	26.77±1.12	167±4.51	28.6±0.35
III Triton + PHF (300mg/kg/p.o.)	209.4±26.632**	27.40±3.22**	283.3± 27.65**	16.78 ±2.42*	36.26±0.82***	129± 2.30***	25.38±0.55**
IV Triton + PHF (600 mg/kg/p.o.)	158.6±23.48**	48.30±2.66**	208.4±25.65**	42.16±2.30**	39.67±0.51**	116.6±2.13***	22.7±0.42**
V Triton + Atorvastatin 10mg/kg/p.o)	147.9±21.31**	45.29±1.61**	204.5±26.55**	41.17±2.28**	47.06±0.76***	112±2.14***	20.9±0.41**

TRI- Triton WR 1339 (200 mg/kg; i.v.); PHF – Poly Herbal Formulation
% change was calculated using formula % Change = [(Tt - Tc)) / Tc)] × 100
Where in Tt = values of treated group and Tc = values of respective control group.
% change of PHF group is calculated with respect to control (Normal Saline) group,

While % change of TRI + PHF groups is calculated with respect to TRI group.
All the values are expressed as mean ± SEM, n=6, value ***p< 0.001, **p< 0.01 and *p< 0.05 Drug and PHF treated group compared to Toxic control (Triton) group. **Table No.10 depicts the variation of body weight andTable Table No.3Atherogenic Index of various groups of animals.**

Treatment Groups	Body Weight (gm)	Atherogenic Index (A.I.)
I. Normal Control	185.9±1.10	2.02±0.27
II. Triton Treated (Triton 200 mg/kg, i.v.)	205.2±1.53	7.02±0.38
III. Triton + PHF 300 (300mg/kg/p.o.)	197.1±0.92*	3.14±0.01**
IV. Triton + PHF 600(600 mg/kg/p.o.)	195.2±1.77**	2.83±0.06***
V. Triton + Atorvastatin (10mg/kg/p.o)	184.6±0.98***	2.43±0.05***

All the Values are expressed as mean ± SEM, n=6, value ***p< 0.001, **p< 0.01 and *p< 0.05 Compared to Drug and PHF treated groups to vs. Toxic Control (Triton)

Effect of hydroalchoholic extract of PHF on Serum Cholesterol, Triglyceride, HDL-C, LDL-C and VLDL-C in TritonWR-1339 induced Hyperlipidemic Rats

Total cholesterol and triglycerides levels were significantly increased in Triton WR-1339 injected rats as compared to normal control rats. Triton injected rats showed significance increase (P<0.0001) the levels of TC, TG, LDL & VLDL compared to normal control group. Treatment with Poly Herbal Formulation, at different doses (300 mg/kg, 600mg/kg), significantly (P<0.001) prevented the elevation of these parameters when compared to Triton induced hyperlipidemic rats. Treatment with PHF (300 and 600 mg/kg) caused a dose dependent changes in Total Cholesterol and Triglycerides (Table-9)

Effect of hydroalchoholic extract of Polyherbal Formulation on Body Weight and Atherogenic Index on Triton WR-1339 induced Hyperlipidemic rats.

Triton treated group showed significance increase (P<0.0001) the levels of body weight and Atherogenic Index compared to normal group. Post-treatment with PHF and Atorvastatin at different doses (300mg/kg, 600mg/k.g. /b.w.) and 10 mg/k.g/b.w., significantly (P<0.001) prevented the elevation of these parameter when compared to hyperlipidemic rats (Table-10).

Discussion

Hyperlipidemia and Hypertriglyceridemia are important risk factors for diabetes-accelerated atherosclerosis...

The present study includes the screening test for hypolipidemic drugs in which the agents were administered orally to rats immediately following and 20 hr after intravenous injection of Triton, and activity was determined by measuring serum cholesterol and Triglycerides 43 hr post –Triton .

In our study, this model gave similar plasma lipid profile changes, at 24 h after Triton WR-1339 injection in rats. This result demonstrates the feasibility of using Triton induced hyperlipidemic rats as an experimental model to investigate the hypolipidemic effect of polyherbal extracts.

The reduction of total cholesterol by the ethanolic extract of polyherbal formulation was associated with a decrease of its LDL fraction in serum and liver, which is the target of several hypolipidemic drugs. This study suggests that cholesterol lowering activity of the ethanolic extract of polyherbal formulation could be the result of the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids.

The ethanolicextract of our polyherbal formulation also increased HDL-cholesterol levels thus exhibiting antihyperlipidemic action. Atherosclerotic index (A.I) is believed to be an important risk factor for diagnosis of atherosclerosis. The ethanolic extract of our polyherbal formulation reduced atherogenic index which is one of the most important risk factors of atherosclerotic plaques.

A polyherbal formulation containing ethanolic extracts of the three herbs The leaves of plants O. sanctum ,*Azadirachtaindica* ,*Curcuma longa* ,*Zingiberofficinale*was reported to caontain Flavanoids, Alkaloids, Saponins, tannins, phenols, anthocyanins, terpenoids, sterols[17]Eugenol, Eugenal, Carvacol, Linalool, Limatrol, Caryophyllene, Anthocyans, *Aeglemarmelos was reported to contain*ϒ-sitosterol, aegelin, lupeol, rutin, marmesinin, β-sitosterol, flavone, glycoside, Oisopentenylhalfordiol, marmeline and phenylethylcinnamamides.*Syzygiumcumini*was reported to contain gallic acid,cyanidin, glycoside, glycoside-5-amboline,triterpenoids, tannins,gallotannins, essential oils, beta sitosterol,myricyl alcohol ,myricetin.

Thus, the presence of Flavanoids, Alkaloids, Saponins, tannins, phenols, glycoside, essential oils,Rutin, , beta sitosterol in polyherbal formulation maybe the contributing factor towards its anti-hypercholesterolemia effect and justifies the folkloric use of these plants.

Conclusion

The present study indicated that the ethanolic extract of polyherbal formulation at doses of 300mg & 600mg, post treatment decreases the Triton Wr-1339 induced increased levels of total cholesterol, TGs, LDL, VLDL and increases HDL Levels. Among these two doses 600mg, decreases the lipid parameters more significantly than the 300 mg, hence the higher dose is more protective than the lower dose. Lowering high cholesterol levels significantly reduce the risk of heart attacks, strokes and death.

References

1. Robert WM, Thomas PB. Drug therapy for hypercholesterolemia and dyslipidemia. The pharmacological basia of therapeutics. 2006;12:182-5.
2. Moss JN, Dajani EZ. Antihyperlipidemic agents. Screening methods in pharmacology. 2016 Jan 26;2:121-43.
3. Kumar A, Mazumder A, Saravanan V. Antihyperlipidemic activity of Camellia sinensis leaves in Triton WR-1339 induced albino rats. Pharmacognosy magazine. 2008;4(13):60.
4. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Natural product reports. 2000;17(3):215-34.
5. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *curcuminlonga*Linn. Phytomedicine. 2000 Mar 1;7(1):7-13.
6. Pattanayak P, Behera P, Das D, Panda SK. AegelLinn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy reviews. 2010 Jan;4(7):95.
7. Yadav NP, Chanotia CS. Phytochemical and pharmacological profile of leaves of *Azadirachtaindica*Linn. The Pharmaceutical Reviews. 2009 Nov;11:144-50.
8. Shrikant Baslingappa S, Nayan Singh J T, Meghatai M P, Parag M H. Jamun (*zingeberofficinalis*(L.)): a review of its food and medicinal uses. Food and Nutrition Sciences. 2012 Aug 28;2012.
9. Guo Y, Wu G, Su X, Yang H, Zhang J. Antiobesity action of a daidzein derivative on male obese mice induced by a high-fat diet. Nutrition Research. 2009 Sep 1;29(9):656-63.
10. .Han LK, Kimura Y, Kawashima M, Takaku T, Taniyama T, Hayashi T, Zheng YN, Okuda H. Anti-obesity effects in rodents of dietary teasaponin, a lipase inhibitor. International Journal of Obesity. 2001 Oct;25(10):1459-64.
11. Sadasivam S, Manickam A. Biochemical methods for agricultural sciences. Wiley eastern limited; 1992.