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Research Article

CARDIOPROTECTIVE AND ANTI-HYPERLIPIDEMIC ACTIVITY OF RESVERATROL

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ABSTRACT

Resveratrol is a natural polyphenol present mainly in grapes. It has been shown to offer Cardioprotective and Anti-hyperlipidemic in animal models due to the ability to maintain antioxidant level. Our aim was to see effect of Resveratrol against cholesterol rich diet induced Hyperlipidemia and Isoproterenol induced Myocardial Ischemia. Wister rats were pretreated with Resveratrol(100 and 200 mg/kg/day.p.o) and Atorvastatin (10mg/kg/day.p.o)for a period of 26 days and from 15th day to 26th day. Another group of winter rats was pretreated with Resveratrol(100 and 200 mg/kg/day.p.o), propranolol(40mg/kg/day.i.p) for 7 days, on 8thand 9th Isoproterenol(5mg/kg/day.s.c) was co-administered to induce Myocardial Ischemia. Resveratrol produced significant inhibition of high cholesterol diet hyperlipidemia and Isoproterenol induced Myocardial Ischemia which was clearly shown by histopathological study. Hence it can be concluded that resveratrol, an herbal nutritional supplement, better against Myocardial Ischemia and Hypercholesterolemia.

Keywords: Resveratrol(RES), Isoproterenol(ISO), Propranolol, High cholesterol Diet(HCD), Atorvastatin.

INTRODUCTION

Hypercholesterolemia is a major obligation faced by the many countries in the world. Clinical studies indicated that hypercholesterolemia is an essential risk factor for coronary artery disease (CAD), where low-density lipoprotein (LDL) cholesterol plays a major role in the atherosclerosis and pathogenesis of CAD. several studies noted that hyperlipidemia induces oxidative stress and the oxidative modification of lipoproteins in vessel walls might play a key role in atherogenesis (Wittenstein et al., 2002). Cardiovascular problems are most common reasons for the deaths in world wide. Changes in the levels of lipoprotein and abnormalities in lipid metabolism stands for best understood risk factor for atherosclerosis and cardiovascular diseases[1]. Myocardial infraction is ischemic necrosis of portion of myocardium due to sudden occlusion of branch of coronary artery[2]. It is the one of serial cause of the death in US and other developed countries. Main risk factors for the MI is the atherosclerosis of coronary artery, calcium reduction, generation of free radicals, oxidative metabolism of catecholamines, these oxidative products impact on the cardiac myocyte membrane and also depress the cardiac contractile function, prior to which damage in the mitochondria, sarcotubular system and contractile functions[3].Therefore, it is an independent and major risk factor for cardiovascular morbidity and mortility. Excess production of reactive oxygen species (ROS) under pathophysiological conditions; prevail over the antioxidant defences which lead to oxidative stress. Under oxidative stress the cellular components get injured considerably[4,5]. Although we see revolution in the field of cardiovascular disease (CVD) therapy in the last many years due to the development of a large number of drugs, yet the deaths due to CVD still accounts for 25%[6]. The increasing risk factor non-compliance to drug therapy and unabated disease pathology may be the reason behind it. A worldwide attempt is continuously being made to look for newer effective drugs in terms of cost, safety, and effectiveness. The success however achieved is still limited. Traditional medicinal herbs are enthusiastically being explored now-a-days by the researchers and information on their preclinical and clinical efficacy is being worked out. Thus herbal medicines are greatly emerging as effective alternative or adjuncts to modern medicine. In this connection, we also tried to look for an herbal drug which can prove to be a better Cardioprotective agent and anti hyperlipidemic agent. The Southern French have a very low mortality rate due to coronary heart disease (CHD) despite having a high-fat diet and smoking habits. This so-called 'French paradox' has been attributed in part to wine consumption, particularly red wines[7]. Resveratrol (3,5,49-trihydroxy-stilbene), a polyphenol present in red wine, has been thought to be responsible for the cardiovascular benefits associated with moderate wine consumption[8]. In purified or synthetic form, resveratrol has been shown to subreduce the synthesis of lipids in rat liver[9], to inhibit the synthesis of eicosanoids in rat leukocytes, to interfere with arachidonate

metabolism, to inhibit platelet activation / aggregation, to inhibit the activity of some protein kinases[10], to exert a strong inhibitory effect on reactive oxygen species produced by human polymorphonuclear leukocytes[11], and is an antioxidant more powerful than vitamin E in preventing LDL oxidation. However because of its unpredictable effect on other organ systems, it is unwise to recommend a glass or two of wine to someone with a known predisposition to CHD.

MATERIALS AND METHODS

Drugs:

Resveratrol was purchased from Zenith Nutrition's Pvt. Ltd., Bangalore, India. Atorvastatin was received as a gift sample from Dr. Reddy's Laboratories. Isoproterenol (ISO) was purchased from Samarth Pharma Pvt. Ltd as brand name Isolin. Propanalol was purchased from Abbott Ind Ltd.

Experimental animals:

In-house laboratory bred healthy male albino rats of Wistar strain weighing 150-220g will be included for the study. Animals will be housed in polypropylene cages on clean paddy husk bedding. Animals will be maintained under controlled temperature at 25°C±2°C with 12 hr light/dark cycle. All animals will have a free access to food and water *ad libitum.*

Acute Oral Toxicity Study

Group of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg (exceptionally an additional fixed dose of 5000 mg/kg may be considered). The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified. Then the main test was terminated. High dose and low dose were selected by 1/10th and 1/20th of the 2000mg/kg.

EXPERIMENTAL PROCEDURE:

Wister rat of either sex, weighing of 100-150 gm was procured from Central Animal House Facility, Karnataka college, Bangalore. The animals were kept in polypropylene cages (five rats in each cage) under standard laboratory conditions and had a free access to commercial pellet diet and tap water. Rats were randomized into the following five groups for each study:

Anti Hyperlipidemic study:

Male Wistar rats are divided into the 5 groups each group have six animals

Group 1: Normal saline was administered for 26 days.

Group 2: Hyperlipidemic control (High Cholesterol Diet)was administered for 26 days.

Group 3: Animals are treated with High Cholesterol Diet + standard drug (atorvastatin10 mg/kg) once a day starting on 15th day till 26th day.

Group 4: Animal are treated with High Cholesterol Diet +Resveratrol (100mg/kg) once a day starting on 15th day till 26th day.

Group 5: Animal are rats treated with High Cholesterol Diet + Resveratrol (200mg/kg) + once a day starting on 15th day till 26th day.

On 27th day, animals were sacrificed and various biochemical parameters in serum and

Tissues were estimated. Hearts were also examined for histological changes and infarct size in all the groups.

Serum parameters:

Total cholesterol(TC), total protein(TP), triglyceride(TG), HDL cholesterol, LDL cholesterol, VLDL cholesterol were estimated using commercially available kits. (Reckon Diagnostic ,Baroda , india)

CARDIOPROTECTIVE STUDY:

Male Wistar rats are divided into the 5 groups each group have six animals.

Group1: Normal control.

Group2: Animals are treated with isoproterenol.

Group3: Animals are treated with standard drug propranolol (10 mg/kg).

Group4: Animals are treated with Resveratrol drug (100mg/kg).

Group5: Animals are treated with Resveratrol (200mg/kg).

All the grouped rats will be pretreated with the extract and standard either s.c. or orally for 1 week. Then, they will be given 5.25 and 8.5 mg/kg isoproterenol s.c. on two consecutive days.

Forty-eight hours after the first isoproterenol administration, the rats are sacrificed and autopsied. The hearts are removed and weighed, and frontal sections embedded for histological examination.

Serum parameters:

The serum was separated immediately by cold centrifugation and used for determination of the myocardial infarction marker enzymes LDH, CK-MB, AST, ALT, and ALPusing commercially available kits. (Reckon Diagnostic, Baroda, India)

Statistical analysis:

Statistical analysis was carried out using Graph pad prism 3.0 (graph pad software San Diego, CA, USA). All results were expressed as mean ± Standard error of the mean.Groups of data were compared with the analysis of variance (ANOVA), followed by Dunnett's t-test.

RESULTS

Diet induced hypercholesteremia

Table 1: Effect of *Resveratrol* on plasma total cholesterol, TG, Total protein, LDL, VLDL, HDL, levels in cholesterol diet (CD) induced hypercholesterolemia in rats.

Treatment group	TP	TG	HDL	LDL	VLDL	TC
Normal	6.13±0.039***	72.98±0.344***	51.57±0.64***	180.2±1.56***	14.59±0.68***	205.1±1.628**
Control	3.190±0.27***	122.1±1.081***	28.07±1.74***	235.6±1.13**	24.42±0.21***	265.5±1.120***
Standard atorvastatin10 mg/kg	6.96±0.25***	79.55±1.135***	45.87±1.83***	191.2±4.07***	15.90±0.22***	217.5±4.23***

Low dose. RES 100mg/kg	5.46±0.08***	94.21±1.127***	35.75±0.6389***	219.5±1.48***	18.86±0.18***	245.6±1.41***
High dose. RES 200mg/kg	6.025±0.072**	84.87±1.056**	40.40±0.2159***	181.6±1.53***	17.32±0.22***	208.3±0.984* **

Data was analysed using onewayANOVA followed by Dunnett'st test.
*****P<0.001, **P<0.01,*P<0.05.n = 6**

ISO induced myocardial necrosis.

Table 2: Effect of Resveratrolon plasma LDH, CK-MB, AST, ALP levels in ISO induced myocardial necrosisin rats.

Treatment group	LDH	CK-MB	AST	ALT
Normal control	231±0.86	178.9±0.88	90.31±0.29	87.52±0.93
Isoproterenol 8.5 mg/kg	645.6±1.35$^{\#\#}$	438.9±0.54$^{\#\#}$	230.4±0.75$^{\#\#}$	344.59±0.96$^{\#\#}$
Propranolol 10 mg/kg	565.5±1.33***	437.0±0.55***	205.0±0.12***	136.91±0.861***
RES100 mg/kg +ISO	287.2±1.15***	194.0±0.51***	115.5±1.27***	163.96±0.88***
RES 200 mg/kg +ISO	212.7±0.87***	187.8±0.41***	89.8±6.42***	127.24±0.65***

Data represents mean ±SEM of six rats per group. Data were analysed using ANOVA followed by Dunnett'st-test.
$^{\#\#}$P<0.01 normal versus ISO induced, ***P<0.01and*** P<0.05

ISO induced versus treated groups.

Antioxidant study-

DPPH test

The extent of scavenging effect, Resveratrol was tested for antioxidant activity using 1,1-diphenyl- 2-picryl hydrazyl (DPPH) free radical. Resveratrol has showed antioxidant activity (Table: 98, 9 and Figure 22). Resveratrol showed 11,12,13,14 and 16 µg/ml IC50 as compared with standard 24 µg/ml.

Table 3: DPPH % Radical Scavenging activity of Resveratrol.

Sl. No.	Concentration µg/ml	% RSA±SEM (test)	% RSA±SEM(Standard)
1	10	16.89±0.11	21.93±0.15
2	20	23.89±.10	27.18±0.07
3	30	19.04±0.32	31.89±0.19
4	40	26.36±0.17	35.92±0.14
5	50	25.4±0.07	38.7±0.28

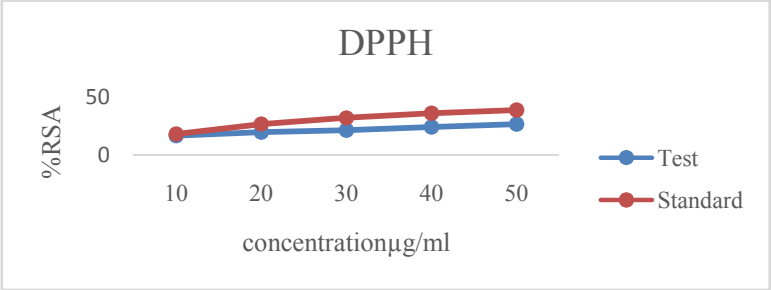

Figure 1: Graph for% Radical Scavenging activity of Dpph against concentration.

Table 4: Antioxidant activity expressed in IC50

SAMPLE	DPPH Scavenging activity IC50(±SEM)µg/ml
Test	40
Standard	15

HYDROXYL RADICAL TEST

The extent of scavenging effect, Resveratrol was tested for antioxidant activity using hydroxyl radical free radical. Resveratrol has showed antioxidant activity. It showed 15,12,13,14 and I7 µg/ml IC50 as compared with standard 25 µg/ml ascorbic acid

Table 5: % Radical Scavenging activity of Resveratrol

Sl. No.	Concentration µg/ml	% RSA±SEM (test)	% RSA±SEM(standard)
1	10	17.11±0.42	19.32±0.10
2	20	22.74±0.31	27.14±0.13
3	30	23.97±0.11	32.04±0.06
4	40	24.17±0.63	35.8±0.26
5	50	26.77±0.10	38.8±0.2

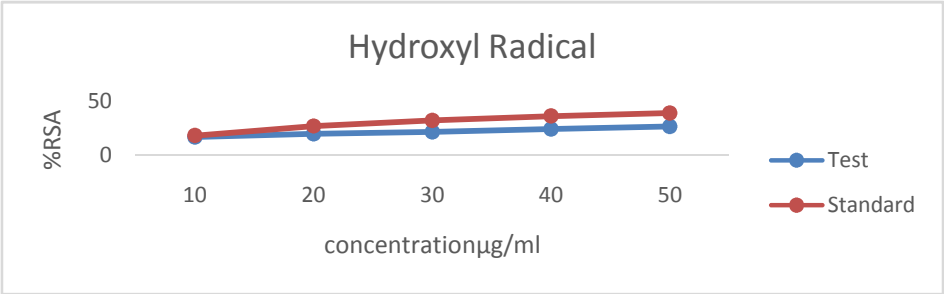

Figure 2: Graph for % Radical Scavenging activity of hydroxyl radical against concentration.

Table 6: Antioxidant activity expressed in IC50

SAMPLE	Scavenging activity IC50(±SEM)µg/ml
Test	41
Standard	17

Hydrogen peroxide test

The extent of scavenging effect, Resveratrol was tested for antioxidant activity using Hydrogen peroxide free radical. Resveratrol has showed antioxidant activity. It showed 16,11,15,14 and I7 µg/ml IC50 as compared with standard 25 µg/ml ascorbic acid.

Table 7: % Radical Scavenging activity of Resveratrol

Sl No.	Concentration µg/ml	% RSA±SEM (test)	% RSA±SEM(standard)
1	10	16.74±0.06	18.04±0.05
2	20	22.82±0.31	26.74±0.17
3	30	15.46±0.46	32.03±0.06
4	40	19.23±0.20	35.99±0.09
5	50	14.58±0.32	38.79±0.30

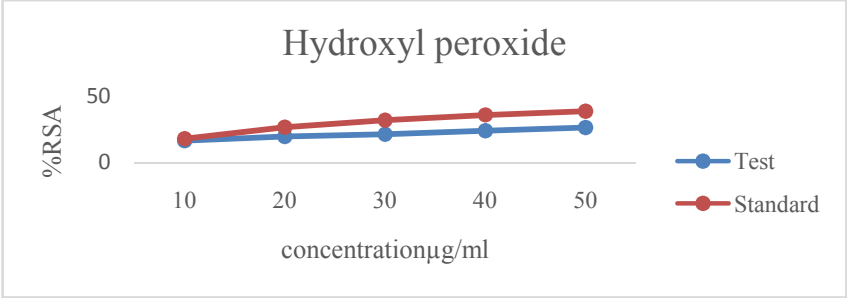

Figure 3:.Graph of Hydrogen peroxide % Radical Scavenging activity of Resveratrol

Table 8: Antioxidant activity expressed in IC50

SAMPLE	Scavenging activity IC50(±SEM)µg/ml
Test	43
Standard	18

Lipid peroxidation

The extent of scavenging effect Resveratrol was tested for antioxidant activity using **Lipid peroxidation** free radical. Resveratrol has showed antioxidant activity. It showed 16,11,12,13 and I6 µg/ml IC50 as compared with standard 25 µg/ml curcumin.

Table 9: % Radical Scavenging activity of Resveratrol

Sl. No.	Concentration µg/ml	% RSA±SEM (test)					% RSA±SEM(standard)
		NOR	CON	STD	LD	HD	
1	10	18.08±0.19	22.08±0.19	17.74±0.79	22±0.31	14.08±0.37	19.04±0.34
2	20	25.15±0.27	25.02±0.53	24.23±0.32	25.14±0.37	16.92±2.28	27.07±0.13
3	30	33.85±0.65	34.16±0.65	26.17±0.20	28.18±0.48	24.19±0.28	32.02±0.09
4	40	39.22±0.48	39.32±0.50	32.81±0.55	32.06±0.51	28.97±0.27	35.92±0.29
5	50	42.05±0.08	45.78±0.5	35.31±0.42	36.3±0.42	34.01±0.09	38.87±0.24

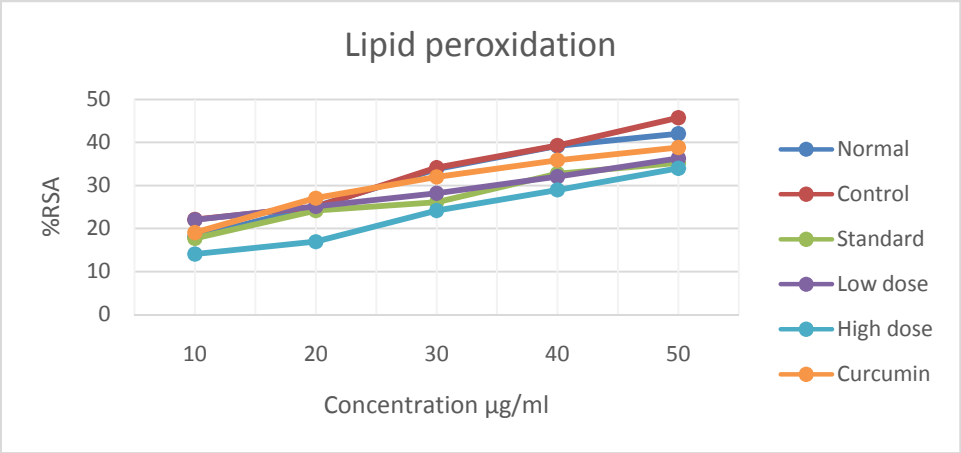

Figure 4: Graph of Lipid peroxidation % Radical Scavenging activity of Resveratrol

Table 10: Antioxidant activity expressed in IC50

SAMPLE	Scavenging activity IC50(±SEM)µg/ml
Normal	20
Control	21
Standard	25
Low dose(RES 100mg/kg)	19
High Dose(RES 200mg/kg)	32
Curcumin	17

RESULTS AND DISCUSSION

Resveratrol act as antioxidants. Based on these assumptions on Resveratrol was used to study the anti-atherosclerotic and Cardioprotective activity.

Cholesterol diet induced hypercholesterolemia in rats

In this study, rats fed with High Cholesterol Diet containing 700 ml peanut oil and 300 ml lardoil, 100g cholesterol, 30g propyl-thio-uraciland100g cholic acid was given orally. The high cholesterol diet (CD) was given for 26 days along with *Resveratrol.*

Animals fed with cholesterol diet (CD) treated group showed significant increase in total cholesterol, triglycerides, LDL-cholesterol levels respectively where as HDL-cholesterol level showed significant decrease when compared to the control TG, TC,LDL-cgroup.

Animals fed with CD and treated with atorvastatin (ATR) significant decrease in total cholesterol, triglycerides, LDL-c levels where as HDL-c, level was significantly increased when compared to the CD treated group. Animals fed with CD and treated with Resveratrol (100mg/kg,b.w. p.o) treated group showed significant decrease in total cholesterol, triglycerides, LDL-c levels respectively whereas HDL-clevel showed significant increase when compared to the CD treated group. And the results were comparable with that of the standard drug Atorvastatin.

Animals fed with CD and treated with Resveratrol (200mg/kg, b.w. p.o) treated group showed significant decrease in total cholesterol, triglycerides, LDL-clevels respectively whereas HDL-c level was significantly increased when compared to the cholesterol diet treated group. And the results were comparable with that of the standard drug Atorvastatin. Histopathological study was done there was increase in the size of the tunica intima in all CD treated groups in aorta of all rats. There was reduction in the thickness of the wall of aorta treated with Resveratrol (100gm/kg, 200mg/kg).

Isoproterenol induced myocardial necrosisinrats

The present study showed that the administration of ISO (5 mg/kg/day,s.c) to the Winstar rats produced significant cardiotoxicity, which is evident by the increased levels of serum marker enzymes LDH, AST,ALP, CK-MB. Histopathological observations showed cytoplasmic vacuolation, infiltration of chronic inflammatory cells, disarrangement in myocardium cells thereby confirmed the damage by ISO in rats.

In ISO treated rats compared to the normal rats showed significant increase in cardiac markers.

Resveratrol100mg/kg decreased LDH, AST,ALP, CK-MB at200mg/kg Resveratrol shows more significant levels LDH, AST,ALP, CK-MB.

Antioxidant property

Resveratrol has shown dose dependent increase in anti-radical activity. However, the anti-radical activity of *Resveratrol* was much less when compared to ascorbic acid. Cardioprotective activity of *Resveratrol* may be partially due to its free radical scavenging activity.

CONCLUSION

This study demonstrates the cardioprotective and antihyperlipidimic nature of chronicresveratrol treatment at a dose that mimics the resveratrol content achievable through moderate red wine intake and a second dose that mimicks resveratrol supplementation. Resveratrol treatment protects against a depression in cardiac function, and reduces infarct

size caused by ischemic injury. The cardioprotective and antihyperlipidimic effects may have been through resveratrol's ability to act as an anti-oxidant, anti-inflammatory, or anti-apoptotic signaling agent, though further research is required to elucidate the cellular mechanisms by which resveratrol exerts these effects in the current hypertensive model. Treatment with the resveratrol at the dose level of 100 and 200mg/kg significantly lowered the serum level total cholesterol, TG, Total protein, Albumin, LDL, VLDL, HDL, AST, ALT, LDH, CK-MB of when compared with the control group.

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