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A Polyherbal Extract Formulation Lowers the Cardiovascular Disease Risk Factors Concurrently with Systemic Oxidative Status in Normocholesterolemic Rats

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Abstract: The study was designed to evaluate the effect of oral administration of the polyherbal extract, consisting of *Emblica officinalis*, *Terminalia chebula*, *Terminalia belerica*, *Camellia sinensis* and *Ganoderma lucidium*, on the atherogenic lipid profile and cardiac risk in association with fecal cholesterol level and systemic oxidative status in normocholesterolimic rats. Sixteen normocholesterolemic Long Evan rats were divided into control (Cont) and polyherbal extract fed (PHG) rats. The PHG rats were treated with the polyherbal extract (10 ml of 20% w/v extract / kg body weight daily), while the control rats (n=8) were treated with normal water. After treatment regimen for six weeks, the plasma atherogenic lipid profile, cardiac risk ratio (CCR), hepatorenal function-related parameters, fecal cholesterol levels, lipid peroxide (LPO) levels of hepatorenal tissues and systemic oxidation status in RBC membranes were assessed. The oral administration of polyherbal extract significantly ($p < 0.05$) lowered the plasma levels of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C), concurrently with significant ($p < 0.05$) rises of the fecal excretion of cholesterol in the PHG rats. Oral administration of the polyherbal extract also lowered the cardiac disease risk by 7-30%. The systemic oxidative stress, as evaluated in the RBC membranes, was reduced significantly ($p < 0.05$) due to oral administration of polyherbal extract. The amelioration effect of polyhedral feeding forgoes any deleterious effect on hepatorenal performance. Thus, cardiovascular disease preventive effect of polyherbal extract was reflected by a simultaneous amelioration of CCR and systemic oxidation status in the present study.

Key words: Hypercholesterolemia · Cardiovascular Disease · Polyherbal Extract

INTRODUCTION

Hypercholesterolemia is considered as one of the prominent and well-established biochemical risk factors for priming and progression of cardiovascular diseases (CVDs) [1,2]. Conventional treatment strategies of hypercholesterolemia always involve dietary modification, life style change and pharmacotherapy. A therapeutic dietary regimen and controlled life style can provide a modest decrease in cholesterol levels through decreasing mildly elevated cholesterol by at most 10-15% [3]. Pharmacotherapeutic approach often relies on the use of recent lipid lowering drugs like statins, fibrates, nicotinic acid and cholestyramine etc. Statins are vastly used to treat hypercholesterolemia if diet is ineffective [4], however, there are inconsistencies in therapeutic response in man and women [5] and therapeutic success in mortality benefit in high-risk population [6]. Additionally, intolerance to statin or fibrate [7] and side effects like statin myopathy [8], statin myalgia [9] and hepatotoxicity [10] are also reported.

Herbal medicines are popularly being used as remedies against a large number of diseases worldwide from prehistoric time. The herbal medicines are being favorites due to its safety and pharmacological efficiency. According to 2007 National Health Interview Survey, the prevalence of complementary and alternative medicine use was 38% in American adults with more frequent use of

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herbal medicines [11]. Different herbs have been tested in clinical trials and some herbs are claimed to be effective in lowering cholesterol and encouraging safety profiles [12]. Drawing attention to the herbal medicine could be a possible natural tool to overcome the limitation of the conventional pharmacotherapeutic options against hypercholesterolemia and associated cardiovascular risk factors.

The Ayurvedic material 'Triphala' is a combination of three powdered fruits: Amalaki (*Emblica officinalis*), Haritaki (*Terminalia chebula*) and Bibhitaki (*Terminalia belerica*) [13]. *Camellia sinensis* and the mushroom *Ganoderma lucidium* have a long history of therapeutic uses in traditional Chinese medicine. The polyherbal mixture used in this experiment was formulated by modifying the 'Triphala' with *C. sinensis* and *G. lucidum.* Triphala, C. *sinensis* and *G. lucidium* have separately been reported to have hypolipidemic effects upon hypercholesteremic situation [14-16]. But study lacks to report the hypocholesterolemic effect of such ingredients in normocholesterolemic condition that pertinently confirm the true value of a medication in the prevention or treatment of CVD factors. Therefore, we were interested to evaluate the effect of this polyherbal formulation on cardiovascular risk factors in normocholesterolemic rats.

MATERIALS AND METHODS

Plant Materials Collection and Extraction: Dry *Terminalia chebula* fruit, *Terminalia belerica* fruit, *Emblica officinalis* fruit, *G. lucidium* fruit body and *C. sinensis* leave were collected from local market and authenticated by the Department of Botany, Jahangirnagar University, Savar, Dhaka. The polyherbal formulation was prepared by mixing 20% of each item in powdered form homogenously. This polyherbal formulation was then subjected to hot water extraction. Hot water extract was filtered and stored at 4°C and used in subsequent experiments.

Experimental Animal and Maintenance: Sixteen ~22 weeks old in-bred Long Evan rats (180-210g) were used in the present experiment. The rats were housed in plastic cages under controlled conditions of 12-h dark-light cycle and maintained on laboratory chow diet. The rats were divided into two groups: control group (Cont) and Polyherb-administered group (PHG). The rats of the PHG group were orally administered to water extract of the polyherbs at a dose of 10 ml (20% w/v)/kg body weight daily. The Cont group rats received normal water. All the rats in this study were cared for and sacrificed in accordance with the ethical norms approved by Bangladesh Association for Laboratory Animal Science.

Separation of Serum and Erythrocytes: After 24 hours of the last administration, overnight fasted rats were sacrificed under light anesthesia (100 mg ketamine/kg body weight) and blood was collected into a heparinized tubes. Blood was then centrifuged at 1000Xg for 10 minutes at 25°C to separate the plasma and erythrocytes. The plasma and erythrocytes samples were stored at-20 °C up to further analyses. The liver and kidneys were excised immediately and perfused with physiological saline. The tissues were minced and homogenized with ice-cold phosphate buffer (25mM, pH 7.4). These homogenates were used to assess the tissue levels of lipid peroxide (LPO) and total protein.

Analyses of the Plasma Biochemical Indices: The plasma concentration of trirglycerides (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were analyzed spectrophotometrically using commercial kits (Randox Laboratories Ltd, UK), whereas low density lipoprotein-cholesterol (LDL-C) was calculated from Friedewald formula [17].

The Cardiac Risk Ratio (CRR), namely LDL-C/HDL-C and TC/HDL-C ratios, was also calculated by the equation reported by Ikewuchi and Ikewuchi [18] and Fernandez and Webb [19]. The plasma level of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), plasma urea and creatinine were also analyzed colorimetrically using commercial diagnostic kit (Randox Laboratories Ltd, UK) to estimate hepatorenal functional status.

Analysis of Fecal Cholesterol Level: Dried and powdered rat feces were subjected to fecal cholesterol analysis following the methods of Hossain *et al*. [20] and expressed as mg/g of dry stool.

Analyses of Hepatorenal Lipid Peroxide (LPO): The levels of lipid peroxide (LPO) in the hepatorenal tissues were analyzed by the estimation of the Thiobarbituric Acid Reactive Substances (TBARS) of the liver and kidney tissue homogenates following the methods of Hossain *et al*. [20, 21] and Uddin *et al*. [22]. The levels of LPO were expressed as nmol/mg of protein of the tissue homogenate against 1, 1, 3, 3-tetraethoxypropane as standard.

Total protein was estimated by the method of Lowry *et al*. [23].

Analyses of Systemic Oxidation Status: The analyses of systemic oxidation status were performed by the estimation of the level of lipid peroxide of plasma and erythrocyte membranes. The levels of lipid peroxide of plasma and erythrocyte membranes were determined through the estimation of the TBARS of plasma and erythrocyte membranes [20, 21]. The levels of LPO were expressed as nmol/mg of protein of the tissue homogenate against 1, 1, 3, 3-tetraethoxypropane as standard.

Statistical Analysis: The results are expressed as mean ± SEM (Standard error of mean). All parameters for inter-group differences were analyzed by unpaired student's *t*-test. Correlation was evaluated by simple regression analysis. A level of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Polyherbal Extracts on Plasma Lipid Profile and Cardiac Risk Ratio (CCR): The oral administration of polyherbal extract lowered the plasma levels of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) significantly ($p < 0.05$) in the polyherbal extract-fed (PHG) rats, as compared to those of the control rats ($p < 0.05$). But the plasma levels of high density lipoprotein-cholesterol (HDL-C) and triacylglycerol (TG) of PHG group rats were similar to those of the control rats (Table 1). The administration of the Polyherbal extract significantly ($p < 0.05$) reduced the Cardiac Risk Ratio (CCR; TC/HDL-C and LDL-C/HDL-C) in the extract-treated PHG group rats, when compared to those of the control rats (Table 1).

Results are mean ± SEM (Standard error of mean). Rats of the Cont (control) group (n=8) were administered with normal mineralized water while the rats of the polyherbal extract-fed group (PHG) (n=8) were administered with polyherbal extract. Here, TG = Triglyceride, TC = Total cholesterol, LDL-C = Low density lipoprotein cholesterol, HDL-C = High density lipoprotein cholesterol, ALT= Alanine transaminase, AST= Aspartate Transaminase, ALP = Alkaline Phosphatase. Asterisk (*) indicates statistical significant difference with respect to control group ($p < 0.05$). Data were analyzed by unpaired student's *t*-test.

Table 1: Effect of oral administration of polyherbal extract on the plasma lipid profile, cardiac risk ratio (CCR) and fecal cholesterol level

Parameters	Cons	PHG
Plasma lipid profile		
TC (mg/dl)	47.5 ± 1.0	32.4 ± 4.2*
TG (mg/dl)	127 ± 23	127 ± 21
HDL-C (mg/dl)	3.8± 0.40	3.70 ± 0.25
LDL-C (mg/dl)	19.5 ± 2.0	6.4 ± 0.40*
Hepatorenal functional parameters		
ALT (U/L)	31.3 ± 5.4	21.0 ± 2.3
AST (U/L)	83.5 ± 3.0	82.22 ± 5.5
ALP (U/L)	210 ± 27	126 ± 17*
Creatinine (mg/dl)	4.6 ± 0.4	5.8 ± 0.3
Urea (mg/dl)	47.2 ± 2.5	51 ± 1.8
Cardiac risk ratio (CCR)		
LDL-C/HDL-C	5.90 ± 0.70	1.85 ± 0.02*
TC/HDL-C	13.4 ± 1.30	9.4 ± 1.15*
Fecal cholesterol level		
(mg/g dry stool)	10. 8 ± 2.10	33.4 ± 1.30*

Table 2: Effect of oral administration of polyherbal extract on hepatorenal lipid peroxide and systemic oxidation status

Lipid Peroxide (nm/mg of protein)	Cont	PHG
Liver	9.0 ± 0.8	9.4 ± 0.7
Kidney	19 ± 1.2	11.9 ± 1.0*
Plasma	0.24 ± 0.01	0.24 ± 0.02
Erythrocyte Membrane	21.5 ± 5.5	9.3 ± 2.4*

Effect of Polyherbal Extracts on Hepato-renal Function: The plasma level of alkaline phosphatase (ALP) was significantly ($p < 0.05$) decreased in the extract fed PHG rats, whereas the level of alanine aminotransferase (ALT) and aspartate transaminase (AST) were similar as compared to those of the control rats (Table 1). But there was a slight rise, albeit insignificantly, of plasma creatinine and urea levels in the PHG group rats than those of the control rats (Table 1).

Effect of Polyherbal Extracts on Fecal Cholesterol Level: The levels of excretory cholesterol through feces in the PHG rats rose significantly ($p < 0.05$), when compared to those of the control rats as a result of oral administration of polyherbal extract (Table 1).

Effect of Polyherbal Extract on the Levels of Hepatorenal Lipid Peroxide (LPO): The oral administration of polyherbal extract significantly ($p < 0.05$) decreased the levels of LPO of renal tissue in extract-fed PHG rats with respect to those of the control group rats. The levels of LPO of hepatic tissue of extract-fed test group rats remained similar to those of control group rats (Table 2).

Effect of Polyherbal Extracts on Systemic Oxidation Status: The oral administration of polyherbal extract significantly ($p < 0.05$) decreased the levels of LPO in the erythrocyte membranes but not of the plasma of the PHG rats in comparison with those of the control rats (Table 2).

Results are mean ± SEM (Standard error of mean). Rats of the Cont (control) group (n=8) were administered with normal mineralized water while the rats of the polyherbal extract-fed group (PHG) (n=8) were administered with polyherbal extract. Asterisk (*) indicates statistical significant difference with respect to the control group ($p < 0.05$). Data were analyzed by unpaired student's *t*-test.

Analysis of Statistical Relationship: The regression analysis revealed a significant negative correlation ($r = -0.73$; $p < 0.05$) between serum total cholesterol (TC) and fecal cholesterol levels. The analysis of regression also revealed a significant positive ($r = 0.64$; $p < 0.05$) correlation between TC levels and LDL-C level.

DISCUSSION

We demonstrated the effects of the oral administration of polyherbal extract on the atherogenic lipid profile and cardiac risk in association with fecal cholesterol level and systemic oxidative status in this study. We also studied the effects of polyherbal extract on hepatorenal functional performance-related biochemical parameters and tissue oxidation level.

The plasma levels of TC and LDL-C were decreased significantly in the polyherbal extract-fed rats than those of the control group rats while plasma TG and HDL-C remained unchanged. The decreased levels of the plasma TC and LDL-C were consistent with the significant rises of the levels of fecal cholesterol of the polyherbal extract-treated rats. A moderate significant positive correlation ($r = 0.64$; $p < 0.05$) was also demonstrated between plasma TC and LDL-C level. We thus speculate that increased fecal excretion of dietary cholesterol might contribute to the lower level of plasma TC and LDL-C at least to some extent. A significant negative correlation ($r = -0.73$; $p < 0.05$) between plasma TC levels and fecal cholesterol level also supported our speculation. Rises in blood cholesterol, particularly TC and LDL-C, are always considered as major risk factors for coronary artery disease [24]. Increased circulatory cholesterol level also has prognostic rule in cardiovascular diseases [25]. Reduced of LDL-C indicates increased clearance of LDL-C from circulation [26]. The possible mechanism of this clearance may be due to either increased excretion of dietary cholesterol or decreased hepatic cholesterol synthesis [27]. But within our experimental limit, increased excretion of dietary cholesterol through feces was the possible mode of circulatory LDL-C clearance.

The effect of polyherbal extract on the entire physiological status was studied by the evaluation of hepatorenal-performance-related enzyme level as well as hepatorenal tissue oxidation status. The oral administration of the polyherbal extract did not interfere with the hepatorenal performance rather ameliorated the hepatobilliary function, as indicated by decreased plasma alkaline phosphate (APL) level in test (PHG) group rats (Table 1). The results of hepatorenal tissue oxidation status suggested an amelioration of renal tissue oxidation without any deleterious effect (Table 2). Therefore, ameliorative effect of polyherbal extract feeding on atherogenic lipid profile forgoes any anomaly of physiological status.

In case of cardiac risk ratio (CRR), polyherbal extract treatment lead to significant reduction of cardiovascular risk in the test group rats, as compared to those of the control group rats. The oral administration of polyherbal extract significantly ($p < 0.05$) decreased the levels of lipid peroxide (LPO) in the erythrocyte membrane and resulted in an unperturbed plasma lipid peroxide levels (Table 2). Our results are qualitatively consistent with the fact that each constituent of the polyherbal extract used in the present investigation, such as *Emblica officinalis* (28), *Terminalia chebula* (29), *Terminalia chebula* (30), *Cemellia sinensis* (31), *Ganoderma lucidum* (32) has antioxidant properties. We speculate that reduced systemic oxidation status, as reflected by the decreased levels of LPO in the erythrocyte membranes, is the manifestation of decreased LDL-oxidation that plays a crucial role in atherogenesis. In other words, reduced systemic oxidation status demonstrates lower risk of cardiovascular risk. The proposition is supported by the significant positive correlation ($r = 0.64$; $p < 0.05$) between plasma TC levels and the levels of LPO in the erythrocyte plasma membrane. Cardiac risk ratio (CCR), a measure for the accurate predictors of major cardiovascular events such as stroke and myocardial infarction through reduction of total blood cholesterol, has been clearly related to a reduction in the cardiovascular death [33]. TC/HDL-C is considered as the best single predictor of major acute cardiovascular event while LDL-C/HDL-C is the most powerful measure of cardiovascular disease risk in the elderly people [33, 34]. Oxidative stress is thought

to be linked to certain cardiovascular diseases. Systemic oxidative stress is believed to play a major role in the initiation and progression of atherosclerotic disease whereas cardiovascular and cerebrovascular complications are secondary to atherosclerosis [35]. Systemic oxidation of lipoproteins plays an important role in the development of atherosclerosis [36]. A coherent improvement of cardiac risk ratio and systemic oxidation status is thus more pragmatic. Indeed, cardiovascular disease preventive effect of polyherbal extract was reflected by a simultaneous amelioration of cardiac risk ratio and systemic oxidation status in the present study.

CONCLUSION

We demonstrated the effect of oral administration of polyherbal extract on the atherogenic lipid profile and associated cardiac risk in association with fecal cholesterol level and systemic oxidative status in this experiment. Present study suggested an ameliorative effect of oral administration of polyherbal extract with the clearance of plasma total cholesterol by fecal excretion and simultaneous amelioration of cardiac risk ratio and systemic oxidation status. However, the exact mechanism is still to be revealed that obviously demands further study with disease-related therapeutic interventions.

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