Studies on antihyperlipidemic and endothelium modulatory activities of polyherbal formulation (POL_4) and its ingredients in high fat diet-fed rats

Abdul Malik[1,2], Malik Hassan Mehmood[1]*, Muhammad Shoaib Akhtar[2], Ghulam Haider[1] and Anwarul Hassan Gilani[1,3]

[1]Natural Product Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi, Pakistan

[2]Faculty of Pharmacy, University of Sargodha, Sargodha, Pakistan

[3]Pakistan Council for Science and Technology, G-5/2, Islamabad, Pakistan

Abstract: A compound herbal formulation (POL_4) is used traditionally in interior parts (Distt. Badin) of Sindh, Pakistan, for the treatment of metabolic disorders like diabetes and hyperlipidemia. This study is aimed to determine the effectiveness of POL_4 and its ingredients in hyperlipidemia and associated endothelial dysfunction and hypertension. POL_4 is composed of equal proportion of *Nigella sativa*, *Cichorium intybus*, *Trigonella foenum graecum* and *Gymnema sylvestre* mixed in powdered form. Chronic (6 to 7 weeks) administration of POL_4 and its ingredients mixed in diet caused a notable attenuation in total cholesterol, low density lipoprotein cholesterol, triglycerides, atherogenic index, C-reactive protein and glucose, while it has increased high density lipoprotein levels. POL_4 intervention markedly ($p<0.01$) reduced systolic blood pressure in rats to 127±1.92 vs. 145.4±1.07 mm of Hg using tail-cuff method and significantly ($p<0.05$) improved endothelium-dependent relaxation (75±2.88 vs. 82.75±1.22%) to acetylcholine in isolated aortae of rats in treatment groups using force transducer and PowerLab system. Similar activities were assessed on the part of ingredients of POL_4. These findings indicate that POL_4 and its ingredients possess antihyperlipidemic, endothelium-dependent modulatory and antihypertensive activities, thus providing an evidence to the vernacular use of POL_4 in hyperlipidemia and hypertension.

Keywords: Poly herbal formulation, antihyperlipidemic, antihypertensive, endothelial modulatory.

INTRODUCTION

Persistent hyperlipidemia is a major contributing factor precipitating cardiovascular disorders (CVDs) such as, hypertension, myocardial infarction, peripheral artery and coronary artery diseases (Orsó *et al*., 2009). Hyperlipidemia expedites the process of atherosclerosis and its consequences of developing multi-vessels disease, heart failure and stroke. Controlled lipid levels achieved by non-pharmacological or pharmacological interventions are known to protect from cardiovascular events (Scicchitano *et al*., 2014). It is well documented that pre-hyperlipidemia is highly prevalent in developing (around 80%) countries including Pakistan compared to the developed countries (Dodani *et al*., 2008), which might be associated with sedentary life style and unhealthy dietary habits. The available treatment options for hyperlipidemia include statins, fibrates, bile acid sequestrants and niacin, however, their prolonged use is associated with multiple adverse effects like rhabdomyolysis, cardiomyopathy, myopathy, osteoporosis, constipation, intolerance and poor compliance (Last *et al*., 2011; Casula *et al*., 2012). In Asian countries including Pakistan, the alternative approaches, especially the use of herbal remedies and supplements are becoming very popular nowadays for their medical uses (Kajal *et al*., 2016).Herbal remedies have gained maximum attention in current era because of the presence of millions of constituents offering diverse range of activities with inbuilt potential to nullify associated adverse effects (Salvamani *et al*., 2014). One of the oldest traditional systems of the world, Greeco-Arab/Tibb-e-Unani medicine is commonly practiced in South Asian countries. In our settings due to cultural acceptance, people prefer using medicinal plants or herbal formulations for various chronic ailments like constipation and diarrhea (Mehmood *et al*., 2015), hypercholesterolemia (Naz *et al*., 2016), hypertension and vascular resistance (Aziz *et al*., 2013; 2009). Similarly, formulations like a combination of *S. reticulate, C. zeylanicum, L. speciosa, C. sinensis* and *G. sylvester* for diabetes (Baig *et al.,* 2014), Angiosifa(Parasuraman *et al*., 2013) and Itrifal Saghir (Kamali *et al*., 2012) are popular for their therapeutic use as antihyperlipidemic and anti-obesity, respectively. A compound herbal formulation (POL_4) is popular in our system of traditional medicine for its medicinal utility in cardiometabolicdisorders (table 1), however no report is available in the literature to rationalize its medicinal uses in hyperlipidemia and hypertension.

**Corresponding author:* e-mail: hassan.mehmood@aku.edu; malikhassan.mehmood@gmail.com

This study is aimed to validate the medicinal use of the parent formulation and its ingredients in hyperlipidemia, endothelial dysfunction and hypertension using high fat diet-induced hyperlipidemic rat model.

MATERIALS AND METHODS

Identification and preparation of polyherbal (POL_4) formulation

The ingredients [*N. sativa* (Ns), *C. intybus* (Ci), *T. foenum graecum* (Tfg) and *G. sylvestre* (Gs)] of polyherbal (POL_4) formulation were procured from Rehmania Pinsar Store, Sargodha and samples were identified by Dr. Muhammad Amin Ullah Shah, Taxonomist, Department of Botany, University of Sargodha, Sargodha, Pakistan. The individual samples were deposited in the faculty herbarium with following voucher numbers; *N. sativa* (Malik-632), *C. intybus* (Malik-633), *T. foenum graecum* (Malik-634) and *G. sylvestre* (Malik-635). All the ingredients were powdered and mixed in equal parts to compose POL_4. For further studies, the parent formulation and its ingredients were mixed in the diet of different HFD-fed animal groups.

Animals

Sprague-Dawley rats (180-250g) of either sex, kept at the "Animal House" of Aga Khan University, Karachi, were used in this study. The animals were maintained at moderate temperature (23±5°C), acceptable relative humidity (55±5%), 12-hr light/dark periods and were kept in plastic cages containing sawdust (changed after two days). Animals were given normal diet and tap water. All the animals were grouped at random in desired number of groups by numbering method and were acclimatized for 5 to 7 days before starting the experiment. The experimental protocol were approved from institutional ethics committee at University of Sargodha, Sargodha, Pakistan.

Chemicals

Cholesterol, cholic acid, atorvastatin, acetylcholine and phenylephrine were sourced from Sigma Chemicals, St. Louis, MO, USA. Isoflurane was obtained from the Pharmacy of Aga Khan University Hospital, Karachi, Pakistan. Butterfat was procured from United King Bakers, Bahadurabad, Karachi, Pakistan. The reagents for the assessment of serum total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein-cholesterol, triglycerides, glucose and C-reactive protein levels were purchased from Roche Diagnostic Karachi, Pakistan, while their estimation was carried out using Cobas c111 autoanalyzer diagnostic system (Roche Diagnostics International Ltd., Switzerland).

Preparation of different diets and intervention

Different diets were used in this study. *Normal diet* (ND) was developed at the animal house of Aga Khan University. ND consists of (gram/kilogram): fiber 380, flour 380, molasses 12, powdered milk 150, sodium chloride 5.8, vegetable oil 38, potassium metabisulphate 1.2, nutrivet-L 2.5, fish meal 170. A total of 56 rats were randomly divided into 8 groups. Animals in group 1 (n=7) were administered the normal diet. *High fat diet* (HFD) was prepared using combination of cholesterol, cholic acid and butter fat (2, 0.5 and 5% w/w), respectively, an addition to normal diet components for 6-7 weeks (Aziz *et al*., 2013).Animals in groups 2-8 (n=7 in each group) were administered HFD. The animals in groups 4-8 were

Table 1: Showing description of the ingredients of compound herbal formulation

Ingredients of POL_4(Botanicals)	Vernacular names/part used	Family	Medicinal uses	Phytochemical Constituents
Nigella sativa, L.	Black seed, Kalonji /seeds	Ranunculaceae	Hypocholesterolemic, hypoglycemic, cardiodepressant(Razavi and Hosseinzadeh, 2014)	Flavonoids, saponins, thymoquinone and tocopherols (Khan and Afzal, 2016)
Cichorium intybus, L.	Chicory, Kasni / seeds	Asteraceae	Hypocholesterolemic, hypoglycemic, cardiodepressant, cardiotonic (Nadkarni, 1986; Street *et al*., 2013).	Tannins, flavonoids, saponins and caffeoylquinic acid (Street *et al*., 2013)
Trigonella foenum graecum, L.	Fenugreek, Methi /seeds	Fabaceae	Hypocholesterolemic, antidiabetic, hypotriglyceridemic (Duke *et al*., 2002; Chaturvedi *et al*., 2013).	Flavonoids, diosgenin, trigonelline and 4-hydroxyisoleucine (Khorshidian *et al*., 2016)
Gymnema sylvestre, R.Br.	Gurmar booti / leaves	Asclepiadaceae	Hypocholesterolemic, cardiotonic, hypoglycemic, antiobesity (Nadkarni, 1986; Tiwari *et al*., 2014).	Flavonoids, saponins, tannins, gymnemic acid and deacylgemnemic acid (Tiwari *et al*., 2014)

administered POL_4 and its ingredients mixed in diet at a dose of 6% w/w, respectively. The animals in group 3 were administered atorvastatin (a standard lipid lowering drug), at 10mg/kg/day dissolved in distilled water and given through oral gavage (Belagali *et al*., 2013). All measures were ensured for uniform mixing of POL_4 and its ingredients with normal diet.

Assessment ofnon-invasive blood pressure in non-anaesthetized rats

Non-invasive blood pressure was recorded at day 0 and at week 6 and/or 7 of the study using tail cuff plethysmography (Model 92, IITC Inc., Woodland Hills, USA) joined with PowerLab (4/25) data attainment system linked to a computer with installed software of Chart 5.3 (AD Instruments, Sydney, Australia). Prior to the study protocol, the animals were given training skills for easy blood pressure measurement. As soon as the animals got acclimatized for these procedures, 4–6 measurements, within time interval of 4-8 min, of systolic blood pressure in non-anaesthetized rats were attained and the mean values were calculated accordingly. Possible experimental variables like body temperature at 27°C, respiration, body motion and noise intensity were made minimum to get quality results (Aziz *et al*., 2009).

Measurement of endothelium-dependent vasorelaxation in rat aortic preparations

At terminal day of the experimental protocol, rats fasted for 12-16 hr were euthanized followed by anesthesia with isoflurane (2-5% v/w) by inhalation in a closed chamber until achievement of deep anesthesia. Dissection was performed at earliest to remove thoracic aortae. All the aortae were shifted to Kreb's solution with ingredients in mmol/L: [NaCl (118.4), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4$ (1.20, $NaHCO_3$ (25), glucose (11) and $CaCl_2$ (2.5) with pH 7.4], cleaned of attached fatty tissues and were sliced into rings of 2 to 3 mm length. Afterward aortic rings were immersed in tissue bath assembly loading Kreb's solution at 37°C, bubbled with carbogen (95% O_2 and 5% CO_2), connected with a force transducer (50-7905, Harvard Apparatus, USA) and PowerLab data recording system (Model ML845, AD Instruments, Australia). The aortic rings were stabilized for 45 min at normal tension of 2g, with refreshing physiological salt solution at every 15 min. After stabilization, acetylcholine concentration response curves (ACh; 1×10^{-8} to 10^{-4}mol/L) were constructed on phenylephrine (P.E; 1×10^{-6}mol/L)-induced contractions (Amin *et al*., 2015).

Biochemical estimations

Estimation of lipid profile, glucose and CRP

The rats were exposed to isoflurane (2-5% v/w by inhalation) in a closed chamber until fully anesthetized. The blood samples were withdrawn in vacutainer by cardiac puncture from overnight fasted rats. The blood samples were centrifuged at 3000 rpm for 10min (Beckman Coulter Allegra X-22, USA) to extract serum. The estimation of serum total cholesterol (TC), low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides, glucose and C-reactive proteins was carried out using Cobas c111 auto analyzer. Body weights were also measured for the determination of weight variations. Atherogenic index (AI) and coronary risk index (CRI) were calculated using formula: AI= TC-HDL-C/HDL-C and CRI= TC/HDL-C.

STATISTICAL ANALYSIS

The data was presented as means ± S.E.M. Student's *t*-test was applied for comparison between two groups, , while One-way ANOVA (analysis of variance) was applied to compare means in multiple groups by Dunnett's and/or Tukey's post-test. *P* value of less than 0.05 was accounted significantly different. All graphs were constructed using GraphPad Prism software (GraphPad Software, California, USA).

RESULTS

Effects of treatment on serum parameters in high fat diet-induced hyperlipidemic rats

Chronic administration of high fat diet (HFD) caused a significantly increase in serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), triglycerides (TG), C-reactive proteins (CRP), atherogenic index (AI) and coronary risk index (CRI), while it has decreased the high density lipoprotein-cholesterol (HDL-C) compared to normal diet control. Administration of POL_4 and its ingredients to HFD-fed rats at a dose of 6% w/w reversed the raised parameters including TC, LDL-C, TG, AI, CRI and CRP, while this intervention has also cause a significantly rise in HDL-C levels compared to HFD-fed rats without any treatment. Similar effects have been observed on the part of positive control of atorvastatin (10 mg/kg/day, oral gavage), a standard lipid lowering agent. The body weight was decreased due to slight reduction in daily feed intake in treatment groups compared to control. The comparative effect of different groups on selected serum parameters has been presented in table 2.

Effect of different treatments on systolic blood pressure

The chronic administration of POL_4 and its ingredients to high fat diet (HFD)-fed rats significantly reduced systolic blood pressure to 127±1.92 vs. 145.4±1.07mm of Hg (mean ± SEM; n=6–7). The comparative systolic blood pressure lowering effect in different groups has been presented in fig. 1.

Effect of treatment on endothelium-dependent reactivity

Administration of POL_4 and its ingredients to HFD-fed rats caused significant improvement in endothelium-dependent relaxation by augmenting acetylcholine (ACh)-mediated relaxation to 75.0±2.88 vs. 82.75±1.22% (n=6–7). The HFD administration to rats resulted in

Table 2: Effect of different treatments on serum parameters of HFD-fed rats

Parameters	ND	HFD	HFD + Atorva	HFD + POL_4	HFD + *N. sativa*	HFD + *C. intybus*	HFD + *T. foenum graecum*	HFD + *G. sylvestre*
TC (mg/dL)	73.5 ± 7.75	317.2 ± 11.82$^{###}$	143.8 ± 5.29***	169.2 ± 15.47***	164.4 ± 11.05***	156.5 ± 8.42***	175.4 ± 6.63***	177.0 ± 8.00***
LDL-C (mg/dL)	38.7 ± 4.66	267.6 ± 8.47$^{###}$	97.8 ± 5.25***	121.2 ± 10.05***	133.6 ± 9.46***	105.8 ± 6.72***	145.2 ± 7.33***	164.8 ± 9.12***
HDL-C (mg/dL)	33.2 ± 1.32	20.2 ± 1.45$^{###}$	25.2 ± 1.26^{ns}	28.4 ± 3.65**	27.9 ± 2.05**	26.9 ± 2.14*	27.2 ± 1.15*	26.1 ± 3.94*
TG (mg/dL)	64.7 ± 4.95	83.5 ± 5.56$^{###}$	75.2 ± 1.25*	74.8 ± 3.76**	75.7 ± 3.52*	76.4 ± 2.45*	74.3 ± 3.58**	75.9 ± 5.90*
AI	1.35 ± 0.17	14.66 ± 3.35$^{###}$	4.72 ± 1.40**	4.94 ± 1.29**	4.80 ± 0.96**	5.0 ± 1.63**	5.23 ± 1.46**	6.73 ± 0.95*
CRI	2.3 ± 0.36	15.7 ± 2.02$^{###}$	5.6 ± 1.14***	5.7 ± 0.95***	5.8 ± 1.07***	6.1 ± 0.75***	6.3 ± 1.02***	5.1 ± 1.24***
Glucose mg/dL)	109.7 ± 5.62	119.4 ± 4.17$^{##}$	112.2 ± 2.12^{ns}	81.2 ± 4.64***	83.6 ± 5.53***	86.8 ± 7.12***	73 ± 4.34***	62.2 ± 5.15***
CRP (mg/L)	0.026 ± 0.016	0.153 ± 0.83$^{###}$	0.147± 0.027^{ns}	0.144 ± 0.034*	0.145 ± 0.092*	0.146 ± 0.042^{ns}	0.144 ± 0.052*	0.147 ± 0.062^{ns}
Weight variation (%age)	32.2 ± 1.98	47.8 ± 2.22$^{###}$	40.2 ± 1.05*	37.2 ± 1.56**	36.4 ± 2.33**	39.2 ± 1.46*	37.7 ± 1.68**	38.4 ± 2.11**

Values shown are means ± SEM, n=7, $^{###}p$<0.001 and $^{##}p$<0.01 compared to ND (unpaired student's t-test), while ns (non-significant),*p<0.05, **p<0.01 and ***p<0.001 compared to HFD (One way-ANOVA followed by Tukey's post-test).
POL_4 (poly herbal formulation); Ns (*N. sativa*); Ci (*C. intybus*); Tfg (*T. foenum graecum*); Gs (*G. sylvestre*); ND (normal diet); HFD (high fat diet); TC (total cholesterol); LDL-C (low density lipoprotein-cholesterol); HDL-C (high density lipoprotein-cholesterol); TG (triglycerides); CRP (C-reactive protein); AI (atherogenic index) and CRI (coronary risk index).

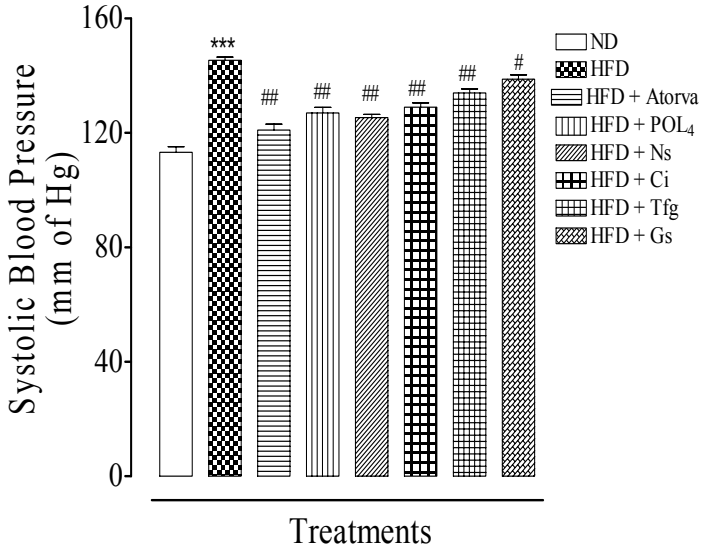

Fig. 1: Effect of different treatments on systolic blood pressure in HFD-fed rats
Data represent the means ± SEM of 6 to 7 determinants. ***p<0.001 shows a comparison of ND vs. HFD (unpaired student's t-test), while ns (non-significant), $^{#}p$<0.05 and $^{##}p$<0.01 show a comparison of treatment groups vs. HFD (one-way ANOVA followed by Dunnett's test). POL_4 (polyherbal formulation); Ns (*N. sativa*); Ci (*C. intybus*); Tfg (*T. foenum graecum*); Gs (*G. sylvestre*); ND (normal diet); HFD (high fat diet); Atorva (atorvastatin).

endothelium dysfunction which was evident by resistance to ACh-mediated inhibitory response in the isolated aortic rings as shown in fig. 2. The comparisons of ACh concentration-response curves in the aortic preparations of different treatment groups were presented in fig. 2.

DISCUSSION

In view of the folkloric medicinal use of ingredients of POL_4 and the combined formulation in cardiometabolic disorders like hyperlipidemia and hypertension(Duke *et*

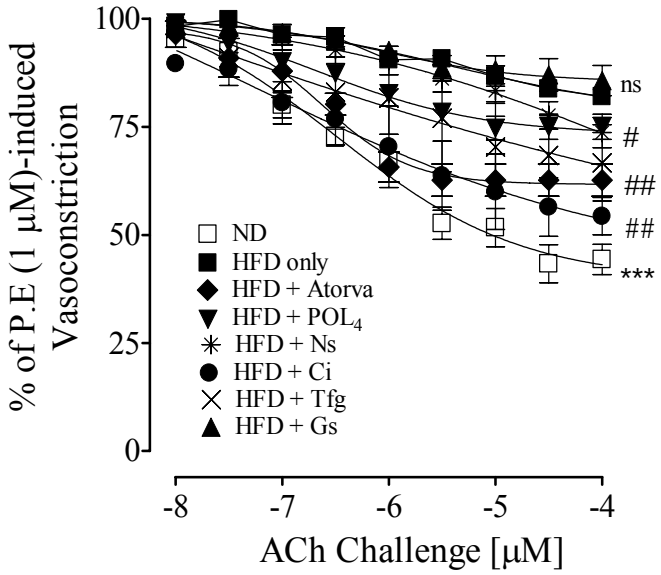

Fig. 2: Effect of different treatments on endothelium-dependent relaxation in isolated aortae of HFD-fed rats.
Data show the means ± SEM of 6 to 7 sets of experiments. ***p<0.001 shows a comparison of HFD with ND (Student's t-test), while ns (non-significant), #p<0.05 and ##p<0.01 show a comparison of all treatments with HFD (One way-ANOVA followed by Tukey's post-test). POL_4 (polyherbal formulation); Ns (*N. sativa*); Ci (*C. intybus*); Tfg (*T. foenum graecum*); *Gs* (*G. sylvestre*); ND (normal diet); HFD (high fat diet); Atorva (atorvastatin).

al., 2002), POL_4 and its individual components [*N. sativa, C. intybus, T. foenum graecum,G. sylvestre*] mixed in diet were administered to HFD-fed rats. These interventions prevented raised serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG), C-reactive proteins (CRP), atherogenic index (AI), coronary risk index (CRI) and fasting blood glucose. Similarly, atorvastatin (suspended in distilled water and administered through oral gavage) also reduced the raised lipid profile. The treatment with POL_4 and its ingredients also significantly increased high-density lipoprotein-cholesterol (HDL-C) compared to only HFD-fed rats. The body weight was compromised accompanied by a reduction in daily feed intake in intervention groups compared to HFD fed rats. These findings have clearly suggested that POL_4 affects cholesterol/lipoprotein distribution and plays a part in uptake of HDL-C, thus resulting in an up-regulation of LDL-C hepatic receptors known to catabolize increased LDL-C(Krause *et al*., 1984). This may be due to the presence of a potent antioxidant, ascorbic acid or tocopherols in POL_4 (Razavi and Hosseinzadeh, 2014; Vanamala *et al*., 2012), while presence of polyphenols, 4-hydroxyisoleucine, saponins and soluble fibers in *T. foenum graecum,* known for lipid lowering effect, may have a contributing role in observed antihyperlipidemic effects (Chaturvedi *et al*., 2013; Madar and Stark, 2002).

High fat diet containing cholesterol, cholic acid and butterfat at 2, 0.5 and 5% w/w, respectively, is known to alter lipid peroxidation and antioxidant enzymes resulting in increased oxidative stress leading to development of obesity, vascular dysfunction and hypertension in experimental animals (Tobert, 2003; Olusi, 2002). Increased body weight and/or adipocyte hypertrophy, hepatic steatosis and visceral adiposity are the outcomes of chronic use (Jin *et al*., 2013). Prolonged use of HFD enhances serum TC, very low density lipoprotein-cholesterol ($_V$LDL-C), LDL-C, AI and CRI (Mopuri and Meriga, 2014). The cholic acid is well known to help cholesterol and fat assimilation and also blocks the conversion of cholesterol into bile acid, hence causing increase in the pool of cholesterol in serum(Ando *et al*., 2005). Thus, the test material(s) offering protection against hyperlipidemia in HFD-fed animal model may be because of its inhibitory influence on cholesterol and fat absorption from dietary source as observed on the part of POL_4 and its ingredients.

Oxidative stress, one of the characteristic features of HFD administration, is a major culprit in development of hypercholesterolemia, endothelium-dependent dys-function and atherosclerosis. Triggered oxidative stress along with hypercholesterolemia has synergistic role to accelerate the development of vascular resistance, hypertension and associated complications like coronary artery disease, myocardial ischemia and myocardial infarction in animals and humans (Tobert, 2003; Lind, 2002). When assessed for effect on systolic blood pressure (SBP) and endothelial function, the parent formulation and its components have decreased SBP and improved the endothelium-dependent relaxation by

causing an increase in ACh-induced relaxation in following orders: Ci>Tfg>POL_4Ns. This study reports first time the blood pressure lowering and endothelial modulating properties of POL_4 and *C. intybus* in HFD-fed rats, however, *G. sylvestre* has been found relatively the least effective as antihypertensive which is also in line with previous studies for its antihypertensive effect in different animal models (Preuss *et al*., 1998; Bhansali *et al*., 2013). Similarly, the weak endothelial modulatory activity has been observed on the part of *G. sylvestre* indicating that its exclusion from current form of the formulation may be suggested if used for the treatment of hypertension.

Numerous studies have reported that high fat diet significantly increases body weight by causing accumulation of adipose tissue (Hariri and Thibault, 2010; Reuter, 2007). The present study has demonstrated that HFD intake induces obesity which was evident by the significant weight gains in HFD-fed animals. The administration of POL_4 and its components to HFD-fed rats have exhibited beneficial effects on weight gain and food intake as compared to HFD-fed rats without any treatment. This contribution might be because of the existence of some of the secondary metabolites in *N. sativa* like thymoquinone and tocopherols and in *G. sylvestre* like gymnemic acid and deacylgemnemic acid which are known for their antiobesity activities (Hasani-Ranjbar *et al*., 2013; Pothuraju *et al*., 2014; Manish *et al*., 2011).

CONCLUSION

This study has shown that POL_4 and its ingredients possess antihyperlipidemic, endothelium- modulating and antihypertensive properties. The antihyperlipidemic property may be attributed to the inhibitory influence of POL_4 on absorption and synthesis of lipids. Thus, this study provides an evidence to the empirical medicinal use of POL_4 and its constituents in cardiovascular disorders like hyperlipidemia and hypertension.

ACKNOWLEDGEMENTS

The study was carried out during elective rotation of Mr. Abdul Malik, research volunteer, working under supervision of Dr. Malik Hassan Mehmood and Prof. Dr. Anwarul Hassan Gilani at Department of Biological and Biomedical Sciences (DBBS). We would like to thank Animal Facility staff members for their help in animal experiments. Our sincere gratitude to Department of BBS for funding under the cover of Faculty Research start up fund awarded to Dr. Malik Hassan Mehmood. We are also thankful to Faculty of Pharmacy, University of Sargodha, Sargodha for providing necessary chemicals to carry out this study. We would like to thank Dr Nauman Aziz for his continuous guidance and helping us to provide test material.

REFERENCES

Amin F, Gilani AH, Mehmood MH, Siddiqui BS and Khatoon N (2015). Coadministration of Black seeds and Turmeric shows enhanced efficacy in preventing metabolic syndrome in fructose-fed rats. *J. Cardiovac. Pharmacol.*, **65**(2): 176-183.

Ando H, Tsuruoka S, Yamamoto H, Takamura T, Kaneko S and Fujimura A (2005). Regulation of cholesterol 7α-hydroxylase mRNA expression in C57BL/6 mice fed an atherogenic diet. *Atherosclerosis.*, **178**(2): 265-269.

Aziz N, Mehmood MH and Gilani AH (2013). Studies on two polyherbal formulations (ZPTO and ZTO) for comparison of their antidyslipidemic, antihypertensive and endothelial modulating activities. *BMC complement. Altern. Med.*, **13**(1): 1.

Aziz N, Mehmood MH, Mandukhal SR, Bashir S, Raoof S and Gilani AH (2009). Antihypertensive, antioxidant, antidyslipidemic and endothelial modulating activities of a polyherbal formulation (POL-10). *Vascul. Pharmacol.*, **50**(1): 57-64.

Baig IA, Muhammed S and Jahan N (2014). Toxicity studies on herbal formulation used in diabetes mellitus. *Pak. J. Pharm. Sci.,* **27**(6).

Belagali Y, Ullal, SD, Shoeb A, Bhagwath V Ramya K and Maskeri R (2013). Effect of vanillin on lipid profile in a model of hyperlipidemia, a preliminary study. *Indian J. Exp. Biol.,***51**: 288-291.

Bhansali S, Shafiq N, Pandhi P, Singh AP, Singh I, Singh PK, Sharma S and Malhotra S (2013). Effect of a deacyl gymnemic acid on glucose homeostasis & metabolic parameters in a rat model of metabolic syndrome. *Indian J. Med. Res.*, **137**(6): 1174.

Casula M, Tragni E and Catapano AL (2012). Adherence to lipid-lowering treatment: the patient perspective. *Patient Prefer Adherence*, **6**: 805-814.

Chaturvedi U, Shrivastava A, Bhadauria S, Saxena JK and Bhatia G (2013). A mechanism-based pharmacological evaluation of efficacy of *Trigonella foenum graecum* (fenugreek) seeds in regulation of dyslipidemia and oxidative stress in hyperlipidemic rats. *J. Cardiovasc. Pharmacol.*, **61**(6): 505-512.

Dodani S, Kaur R, Reddy S., Reed GL, Navab M and George V (2008). Can dysfunctional HDL explain high coronary artery disease risk in South Asians?. *Int. J. Cardiol.*, **129**(1): 125-132.

Duke JA, Bogenschutz-Godwin MJ, Celliar Du and Duke PAK J (2002). Handbook of Medicinal Herbs. 2nd edn., CRC Press, Boca Raton. pp.296-294.

Hariri N and Thibault L (2010). High-fat diet-induced obesity in animal models. *Nutr. Res. Rev.*, **23**(02): 270-299.

Hasani-Ranjbar S, Jouyandeh Z and Abdollahi M (2013). A systematic review of anti-obesity medicinal plants-an update. *J. Diabete Metab. Disord.*, **12**(1): 1.

Jin D, Xu Y, Mei X, Meng Q, Gao Y, Li B and Tu Y (2013). Antiobesity and lipid lowering effects of the

aflavins on high-fat diet induced obese rats. *J. Funct. Foods.*, **5**(3): 1142-1150.

Kajal A, Kishore L, Kaur N, Gollen R and Singh R (2016). Therapeutic agents for the management of atherosclerosis from herbal sources. *Beni-Suef Uni. J. Basic Appl. Sci*., **5**(2): 156-159.

Kamali SH, Khalaj AR, Hasani-Ranjbar S, Esfehani MM, Kamalinejad M, Soheil O and Kamali SA (2012). Efficacy of 'Itrifal Saghir', a combination of three medicinal plants in the treatment of obesity; A randomized controlled trial. *DARU J. Pharm. Sci.*, **20**(1): 1.

Khan MA and Afzal M (2016). Chemical composition of *Nigella sativa*. *Inflammopharmacol.*, **24**(2-3): 67-79.

Khorshidian N, Yousefi Asli M, Arab M, Mortazavian AM and Adeli Mirzaie A (2016). Fenugreek: Potential Applications as a Functional Food and Nutraceutical. *Nutr. Food Sci. Res.*, **3**(1): 5-16.

Krause BR, and Hartman AD (1984). Adipose tissue and cholesterol metabolism. *J. Lipid Res.*, **25**(2): 97-110.

Last AR, Ference JD and Falleroni J (2011). Pharmacologic treatment of hyperlipidemia. *Am. Fam. Physician.*, **84**(5): 551.

Lind L (2002). Lipids and endothelium-dependent vasodilation – a review. *Lipids.*, **37**(1): 1-15.

Madar Z and Stark AH (2002). New legume sources as therapeutic agents. *Br. J. Nutr*., **88**(S3): 287-292.

Manish K, Aditi K, Renu A, Gajraj S and Poonam M (2011). Antiobesity property of hexane extract from the leaves of *Gymnema sylvestre* in high fed cafeteria diet induced obesity rats. *Int. Res. J. Pharm*., **2**(8): 112–116.

Mehmood MH, Munir S, Khalid UA, Asrar M and Gilani AH (2015). Antidiarrhoeal, antisecretory and antispasmodic activities of *Matricaria chamomilla* are mediated predominantly through K^+-channels activation. *BMC Complement. Altern. Med.*, **15**(1):1.

Mopuri R and Meriga B (2014). Anti-Lipase and anti-obesity activities of *Terminalia paniculata* bark in high calorie diet-induced obese rats. *Glob. J. Pharmacol.*, **8**(1): 114-119.

Nadkarni KM (1986). Indian Materia Medica 3rd revised and enlarged edn. Popular Prakashan, Bombay, India.

Naz R, Anjum FM and Butt M.S (2016). Dietary supplementation of bitter gourd reduces the risk of hypercholesterolemia in cholesterol fed sprague dawley rats. *Pak. J. Pharm. Sci.*, **29**(5).

Olusi SO (2002). Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. *Int. J. Obes. Relat. Metab. Disord.*, **26**(9).

Orsó E, Ahrens, N and Schmitz G (2009). Familial hypercholesterolemia and lipoprotein (a) hyperlipidemia as independent and combined cardiovascular risk factors. *Atheroscler. Suppl.*, **10**(5): 74-78.

Parasuraman S, Babuji SSH, Thing GS, Kumari KS, Yoganishalini A, Lian CW, Kumutha M, Kassim T and Dhanaraj SA (2013). Antihyperlipidemic effect of Angiosifa, a polyherbal formulation, in Sprague–Dawley rats. *Phcog. J.*, **5**(5): 221-227.

Pothuraju R, Sharma RK, Chagalamarri J, Jangra S and Kumar Kavadi P (2014). A systematic review of *Gymnema sylvestre* in obesity and diabetes management. *J. Sci. Food Agric.*, **94**(5): 834-840.

Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S and Anderson RA (1998). Comparative effects of chromium, vanadium and *Gymnema sylvestre* on sugar-induced blood pressure elevations in SHR. *J. Am. Coll. Nutr.*, **17**(2): 116-123.

Razavi BM and Hosseinzadeh H (2014). A review of the effects of *Nigella sativa* L. and its constituent, thymoquinone, in metabolic syndrome. *J. Endocrinol. Invest.*, **37**(11): 1031-1040.

Reuter TY (2007). Diet-induced models for obesity and type 2 diabetes. *Drug Discovery Today: Disease Models*, **4**(1): 3-8.

Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA and Shukor MY (2014). Antiartherosclerotic effects of plant flavonoids. *BioMed Res. Int.*, **2014**.

Scicchitano P, Cameli M, Maiello M, Modesti PA, Muiesan ML, Novo S, Palmiero P, Saba PS, Pedrinelli R, Ciccone MM and di Studio Ipertensione G (2014). Nutraceuticals and dyslipidaemia: Beyond the common therapeutics. *J. Func. Foods.*, **6**: 11-32.

Street RA, Sidana J and Prinsloo G (2013). *Cichorium intybus*: Traditional uses, phytochemistry, pharmacology, and toxicology. *Evid.Based Complement. Altern. Med.*, **2013**.

Tiwari P, Mishra BN and Sangwan NS (2014). Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. *BioMed Res. Int.*, **2014**.

Tobert JA (2003). Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nat. Rev. Drug Discov.*, **2**(7): 517-526.

Vanamala J, Kester AC, Heuberger AL and Reddivari L (2012). Mitigation of obesity-promoted diseases by *Nigella sativa* and thymoquinone. *Plant Foods Human Nutr.*, **67**(2): 111-119.