Research Paper

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Antioxidant And Antihyperlipidemic Profile of A Polyherbal Formulation in Patients With Coronary Artery Disease: a Double Blind Placebo Controlled Clinical Study With an Additional Actives Identification by Lcms

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KEYWORDS : ayurveda; coronary artery disease; paraoxonase 1; polyherbal preparation.

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ABSTRACT

Aims

The present study explores the anti-atherogenic, antioxidant property and the safety profile of the polyherbal preparation with coronary artery disease (CAD) patients of eastern India. The study will also identify the active ingredients of the herbal preparation.

Material and Patients

The study was double-blinded, randomized, placebo controlled, and was approved by the ethical committee of the R. G. Kar Medical College, Kolkata, India. They were randomized and given either the polyherbal preparation or the identical looking placebo at a dose of 2 tablets twice daily for a period of 3 months. Constituents of the polyherbal preparation were identified by LCMS analysis. Patients were assessed for their routine serum parameters and oxidative stress.

Results

The chosen drug was found to elevate plasma HDL-C and Apolipoprotein A1 level, and Paraoxonase 1 activity, without altering the antioxidant status, compared to the patients treated with placebo and at the time of entry. Presence of active principles such as Coumaric acid, catechin, caffeic acid, quercetin, naringin and ferulic acid were identified in this polyherbal preparation. The drug was safe and well tolerated among patients.

Conclusion

In view of these results as well as its non-toxicity, the polyherbal formulation appears to be a potentially safe and affordable drug for managing CAD.

Introduction

Coronary artery disease (CAD) is the end result of the accumulation of atheromatous plaques within the walls of the coronary arteries that supply oxygen and nutrients to the myocardium[1]. Despite early onset, no evidence of CAD is seen for years, making the diagnosis difficult. After decades of progression, some of these atheromatous plaques may rupture and start limiting blood flow to the heart muscle, and often lead to a sudden heart attack. Oxidative stress that results in oxidation of circulating low density lipoprotein (LDL) is known to be involved in the pathogenesis of atherosclerosis [2]. High blood pressure and increased heart rate can increase consumption of oxygen by blood, causing increased production of free radicals and hence enhanced oxidative stress. Oxidative damage to the heart muscle can result if the body's antioxidant system does not respond to quench these increased levels of free radicals [3].

Well designed epidemiological studies carried out over the decades have defined the prognostic significance of low HDL-cholesterol (HDL-C) and high LDL-C [4] in CAD. The resulting ox-LDL is linked to the initiation and pathogenesis of atherosclerosis, and ultimately to cardiovascular disease [5]. LDL particles transport cholesterol into the artery wall, retain there by arterial proteoglycans and attract macrophages which engulf the LDL particles and start the formation of plaques. Increased levels of LDL, associated with atherosclerosis accumulate in the artery wall where they are oxidized and taken up by ox-LDL-generated foam cells. This leads to development and progression of atherosclerosis [6]. On the other hand, the HDLs oppose atherosclerosis directly by removing cholesterol from foam cells, inhibiting the oxidation of LDLs, and limiting the inflammatory processes of atherosclerosis [7]. HDLs also have antithrombotic properties. Thus, HDL-C interrupts the process of atherogenesis at several stages. Apolipoprotein A1 (APO A1) constitutes 70% of HDL and helps to clear cholesterol from arteries. Paraoxonase 1 (PON1), an enzyme associated at HDL surface is classified into three individual phenotypes, isozymes A, AB and B corresponding to the genotypes Q, QR and R respectively [8]. Human serum PON1 contains two common polymorphic sites, the one at position 55 (Leu/Met), affects the PON1 activity. The Leu55 isozyme shows about 30% higher activity than the Met55 isozyme. In contrast, the second polymorphic site at position 192 (Arg/Gln) determines qualitative properties of the enzyme, substrate specificity patterns of the two isozymes, and their distinctive affinities for many different substrates. It plays a major role in the antioxidant, anti-inflammatory cardio-protective actions of HDL, and the genetic deletion of PON1 is associated with increased atherosclerosis in animal models [9]. It can prevent oxidative damage of lipoproteins by hydrolyzing lipid hydroperoxides and inhibiting lipoproteins oxidation [10]. PON1 protects against macrophage-mediated LDL oxidation, and increases HDL binding to macrophages, which in turn, stimulates the ability of HDLs to promote cholesterol efflux. These major anti-atherogenic properties of HDL and PON1 require macrophage binding sites for the

HDL-associated PON1.

Oxitard, a standardized polyherbal preparation contains *Mangifera indica* (mango), *Withania somnifera* (Ashwagandha), *Daucus carota* (carrot), *Glycyrrhiza glabra*, *Vitis vinifera* (grape), *Emblica officinalis* (amla), *Syzygium aromaticum* (clove), *Triticum sativum* (wheat) and Yashad Bhasma (specially processed zinc), and is promoted to prevent CAD. The drug is available from a reputed Indian herbal pharmaceutical company in the form of tablets. Amongst the herbs, *W. somnifera* possesses revitalizing and antidepressant properties, and also helps to overcome nervous exhaustion [11]. *D. carota* containing flavones has hypoglycaemic activity [12]. Likewise, mangiferin, a naturally occurring glucosylxanthone present in *M. indica* has gastroprotective and hepatoprotective effects [13], while glabridin from *G. glabra* is a neuroprotector [14]. Glycyrrhizin, a constituent of *Glycyrrhiza glabra* has anti-inflammatory activity [15]. *Vitis vinifera* seed extract is used in atherosclerosis due to its free radical scavenging activity [16]. *Emblica officinalis* enhances natural killer cell activity and antibody dependant cellular cytotoxicity [17,18]. *Syzygium aromaticum* has antioxidant, anti-inflammatory, cytotoxic and anaesthetic properties [19]. *Triticum sativum* is reported to decrease VLDL and triglycerides [20].

The objective of the present study was aimed to evaluate the efficacy of Oxitard in normalizing the of lipid profile of the CAD patients and investigate the antioxidative role of the preparation scavenging in the process when the patients are treated with the polyherbal preparation.

2. Materials and methods

2.1. Composition of the polyherbal formulation

The Oxitard tablets were procured from the manufacturer, The Himalaya Drug Company. Each tablet of polyherbal preparation contained *M. indica* – 94 mg, *W. somnifera* – 71 mg, *D. carota* – 47 mg, *G. glabra* – 29 mg, *V. vinifera* – 12 mg, *E. officinalis* – 141 mg, *S. aromaticum* – 29 mg, *T. sativum* – 6.5 mg and Yashad Bhasma – 2.5 mg.

2.2. Chemicals and Materials

Methanol, Acetonitrile, Dichloromethane and water were procured from the Merck (LC-MS grade). Ammonium acetate was purchased from Sigma Aldrich chemical. Glacial Acetic acid & Formic acid (90%) was purchased from the RFLC Limited or the highest purity available commercially.

2.3 Study design

The study was double-blind, randomized, placebo controlled and was approved by the ethical committee of R. G. Kar Medical college. The study group comprised of three hundred patients attending the C.T.V.S. outpatient department (OPD) of R. G. Kar Medical College. Patients were enrolled for the study after informing and explaining the study protocol and objective in presence of witnesses, obtaining written consent. The patients were allocated to interventions by those assessing outcomes. The participants were assigned to interventions as per a random number table. The study was conducted in complete accordance with the Declaration of Helsinki and GCP guidelines issued by the Ministry of Health, Government of India. In order to ensure appropriate and consistent quality of medicinal plant/herbal substances, good agricultural and collection practice (GACP) was followed during the collection, harvesting, manufacture, processing, and packaging of the herbal formulation. Botanical identification and ayurvedic criteria of the desired quality were in accordance with the guidelines of pharmacopoeial standards of ayurvedic formulations (1987). The patients baseline demographic and clinical characteristics for each group of patients on entry are given in the table 1. The trial was registered with registration number Memo No. RKC/4184 dated 16/10/09.

2.4. Inclusion criteria

CAD patients including post CABG, CAD waiting for surgery and CAD unsuitable for surgery of either sex were included in the study. CAD were confirmed by coronary angiography as well as ECG, ECHO, chest X-ray and other relevant investigations that showed significant (75% or more luminal obstruction of coronary artery) CAD involving one or more vessels.

2.5. Exclusion criteria

All acute cases with active infection and very sick patients were excluded. Subjects not willing to sign the consent and comply with the study were also excluded from the study.

2.6. Study procedures

A total of 300 patients were screened, of which 255 fulfilling the inclusion criteria qualified for the study. The patients were enrolled only when they signed the informed written consent. All the patients received standard medication (statin and ecosprin) for CAD during the entire study period. The patients were randomized and divided into test and placebo groups. The test group received additional two tablets of the herbo-mineral preparation daily, while for the placebo group was given two identical-looking tablets without containing the test drug for a period of three months. The patients were free to withdraw from the study, if desired. Monthly follow-up of the patient status was carried out. The clinical parameters were assessed before and after three months for both the patient groups. The antioxidant status of the patients was determined by assaying the enzymatic [superoxide dismutase (SOD), catalase, glutathione peroxidise (GPx), glutathione reductase (GR)] and non-enzymatic (reduced glutathione, GSH_{red}) antioxidants, as well as malondialdehyde (MDA) levels in the hemolysate. The liver function test, renal function test, total lipid profile, blood sugar profile were also carried out to assess the drug safety. The clinical picture obtained after treatment were compared with the pre-treatment patients to evaluate the drug efficacy. The patient's characteristics on entry are given in Table 1.

2.6.1 MDA and antioxidant status assay

The packed erythrocyte obtained from heparinised blood was washed with saline, diluted with distilled water, and centrifuged at 10000 rpm for 10 min at 4 ºC to obtain the hemolysate. The MDA level in the hemolysate formation was measured [21]. Following reported procedures, GSH_{red} level [22], and the activities of SOD [23] as well as catalase [24] were assayed.

2.6.2 Assay of other biochemical parameters

The serum was prepared by centrifuging blood at 1000 g for 15 min at 4 °C. The serum total cholesterol, triglyceride, HDL-C and APO A1 were measured using the respective commercial kits (Merck, Mumbai, India) using a semi-automated clinical chemistry analyser (Microlab 300). The LDL-C and VLDL was calculated from the results. The serum total bilirubin (STB), conjugated bilirubin (SCB), unconjugated bilirubin (SUB), glutamic pyruvate transaminase (SGPT), glutamic oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) levels were measured using commercial kits (Merck, Mumbai, India) using a semi-automated clinical chemistry analyzer.

2.6.3 Assay of paraoxonase1 activity

Following reported procedure, the paraoxonase 1 activity was assayed spectrophotometrically using paraoxon as the substrate [25].The paraoxonase activity is expressed in unit per litre of serum, where 1 unit equals 1 μmol of substrate hydrolyzed per minute.

2.6.4 Assay of total antioxidant response

The total antioxidant response in hemolysate was measured spectrophotometrically [26].

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2.7. Follow-up and assessment
Patients underwent clinical examination on entry, and at the end of three months. Adverse effects, if any were noted down.

2.7.1 Primary and secondary outcome measures
Primary predefined outcomes were clinical recovery as assessed from the lipid profile and liver function test, normalisation or management of oxidative stress and chronic fatigue, assessed from their antioxidant status, and also symptomatic recovery. The secondary end points were safety profile of tested polyherbal formulations

2.7.2 Adverse effects
The drug safety was assessed primarily on the basis of adverse event profiles and changes in hematological parameters. The adverse effects as volunteered by the patients were noted in the case report forms (CRFs).

2.8. Statistical analysis
The values expressed as mean ± SEM. The results were analyzed by using Student's't' test for unpaired data. $P < 0.001$ was considered as significant. Statistical analysis was conducted to evaluate both the safety and the efficacy of polyherbal preparation compared to placebo and at the time of entry.

2.9. Extraction procedure and sample preparation:
Weighed accurately about 2g of powdered sample in a 250ml clean beaker. Add 60ml of 12% v/v glacial acetic acid solution and sonicated for 10minutes. Keep it aside for settling for 2-5 minutes and filtered the supernatant liquid through Whatman filter paper in a 250ml separating funnel. Add 20ml 12%v/v glacial acetic acid to the residue. Sonicated for 5 minutes and filtered the solution into the same separating funnel. Add 15ml of strong ammonia slowly (the solution should be made basic, check the p^H of the solution with the help of p^H meter, it should be about p^H 8).

Fractionated the solution with 100ml of dichloromethane (HPLC grade or equivalent) in a separating funnel and allowed the layers to separate. Filtered the dichloromethane extract through whatman filter paper in evaporating dish/beaker. Repeated the same process with another 100ml of dichloromethane and filtered. Evaporated the dish/beaker to dryness and reconstituted the residue with 5ml methanol (HPLC grade) and filtered through 0.45µm syringe filter prior to the 20µl of column loading.

2.10. Chromatographic conditions:
A Shimadzu LC-20AD series pump and DUG-20A3 series shimadzu degasser and chromatographic run by using Luna C18 (250 X 4.6mm, 5um) phenomenex column. The mobile phase consisting of water (Merck, maker) with 10mM ammonium acetate and 0.1% formic acid in pump A and acetonitrile (Merck, maker) in pump B. A linear gradient program was: 0-5 min of 3% to 9% of acetonitrile (binary), 5-15 min of 9% to 16% acetonitrile (binary), 15-45 min of 16% to 50% acetonitrile (binary), 45-48 min of 50% to 90% acetonitrile (binary), 48-51 min of 90% acetonitrile (linear) followed by 51-60 min of 90% to 3% acetonitrile (binary), delivered at a flow rate of 0.6ml/min with splitter and run time was about 60 min. The injection volume 20µl was injected through SIL-HTC shimadzu auto sampler and the ambient temperature was achieved through CTO-10 AS VP column oven at 40 dec.

2.11. Mass spectrometric conditions (MS):
The API 2000 (Applied biosystem/MDS SCIEX, Canada) mass spectrometer coupled with ESI (Electron spray ionization) source and a chromatographic system. Batch acquisition and data processing was controlled by Analyst 1.5 version software.

The MS parameters optimization was carried out with 2mg/ml of test solution prepared in methanol (Merck, maker). Intensity response was checked in both positive and negative ionization mode. It was found good intense response in the negative mode and other parameters like declustering potential (DP) -20v, nebulizing gas (GS1 and GS2)55 and 65psi, curtain gas (CUR) 25psi, focusing potential (FP) -300v, Entrance potential (EP) -2v and source temperature (TEM) 420·C and Collision energy (CE) for fragmentation of precursor to product ions were optimized by multiple run through LC until to obtain the each of the most intense precursor to product ion transition state. Acquisition was performed by setting the precursor and product ions of the individual with appropriate Multiple Reaction Monitoring (MRM) scan mode. Collision energy (CE) was optimized with respect to ionization intensity response.

3. Results
Three hundred cases were screened out of which two hundred fifty five fulfilled all the inclusion criteria qualified for the study. Out of the 128 patients treated with Oxitard tablet, 101 patients reported on the third month. Amongst the 127 patients in the placebo group, 100 patients reported on the third month. The sample size calculation was conducted through a pre-study power analysis.

Analysis of the polyherbal preparation
To generate the LCMS chromatograms, Oxitard-A capsules were treated with successive acid –base extraction and the extracted solution was injected in to the LCMS instrument through SIL-HTC shimadzu auto sampler and the ambient temperature was achieved through CTO-10 AS VP column oven at 40 ^{0} C. The LCMS chromatogram is shown in the figure 1. The various peaks identified are Coumaric acid (Rt= 34.15 min), Catechin (Rt= 31.42 min), Caffeic acid (Rt= 49.74 min), Quercetin (Rt= 44.49 min), Naringin (Rt= 54.83min) and Ferulic acid (Rt= 53.01 min).

Effect on the lipid profile of the CAD patients
Parameters such as total cholesterol (TC), HDL, LDL, triglyceride (TG), very low density lipoprotein (VLDL) and APO A1 were included in the lipid profile. Amongst these, only the plasma HDL and APO A1 levels were significantly rejuvenated in the treated group (Table 4), compared to the placebo group. The plasma HDL and APO A1 levels in the placebo group did not change much compared to that at the entry time.

Effect on the Paraoxonase 1 activity
Despite not showing any antioxidant activity, the polyherbal formulation increased the activity of the antioxidant enzyme, PON1 in the treatment group (Table 6). However, the placebo group and/ or patients at the time of entry (0 month) did not show any PON1 activity. Paraoxonase activity at the baseline and in the placebo group showed no activity. In treatment group paraoxonase activity was appreciated at both salt stimulated as well as basal stimulation which represented in Table 6.

During the period of treatment (3 months), no significant change was found in any of the antioxidant parameters (Table 5) of the treated and placebo groups of CAD patients. Likewise, the liver, renal and hematological profiles, and the blood sugar level were also unaltered in both the groups during the study (Table 3). No clinically significant drug-related adverse events were reported or observed for the treatment group during the entire study period.

4. Discussion
The antioxidant and anti-atherogenic activity in the CAD patients before and after the intake of a polyherbal formulation were evaluated in the present study. We used RBC as a physiologically important model for investigating the antioxidant property. Results did not reveal any detectable change in the plasma

antioxidant status between the treatment and placebo groups during study period. The chosen drug did not alter the activities of the antioxidant enzymes. It contains several natural antioxidant phenolics, which exhibits no free radical scavenging activity as evident from the MDA status in the hemolysates of both the placebo and drug treated groups.

Results showed a significant rise in the plasma HDL-C level in the treatment group *vis-à-vis* placebo control that correlated with the increased Apo A1 level in the former group. The antioxidant enzymes is perhaps responsible for the rejuvenation of HDL and Apo A1 levels. Apo A1 is present in virtually all HDL particles that help to clear cholesterol from arteries [27]. Earlier, mice over-expressing Apo AI have been found to have reduced atherosclerosis [28,29]. Taken together, the present results strongly suggested potential benefit of the drug for the treatment of CAD patients. However, surprisingly despite the increase of Apo A1 in the treatment group, the TC level remained unaltered when compared to placebo group. The change in HDL/LDL ratio of drug treated group clearly indicated from the results obtained. The observation clearly hinted at the beneficial role of the polyherbal preparation in CAD patients.

PON 1 is an important enzyme, and low activity of PON 1 is an independent risk factor for CAD [30]. Low PON1 activity may render HDL atherogenic [31], and patients susceptible to developing coronary diseases [32]. Moreover, PON1 activity dictates the antioxidant property of HDL [33]. Knockout mice lacking serum PON1 have an increased susceptibility to atherosclerosis [34], with a 42% excess in stenosis [35].

In our studies, we found no detectable PON1 activity in patients at the time of entry or in the placebo group. The treatment group showed significant PON1 activity suggesting prevention of HDL to atherogenic modifications such as glycation and homocysteinylation [36]. It appears from the present study that PON1 activity would prevent the compositional and functional changes of HDL in the patients, treated with Oxitard. The plasma homocysteine (Hcy) concentrations is correlated negatively with HDL-C and APO A1 in patients with CAD [37]. The increased HDL-C and APO A1 in the Oxitard-treated patients may help in hydrolysis of homocysteine thiolactone by HDL-PON. This may prevent hyperhomocysteinemia, a proven independent risk factor of CAD.

The positive effect of the polyherbal preparation is possibly due to the synergistic action of all its ingredients. Besides the health benefits of its constituent herbs, the mineral ingredient, Yashad Bhasma containing zinc is essential for a large number of enzymes, responsible for the synthesis and degradation of the major metabolites. Oxitard may replenish the daily loss of zinc from our body. The limited antioxidant effect of the drug as found during the study is not explainable. Nevertheless, its ability to elevate HDL-C, Apo A1 along with PON 1 is expected to be useful for the CAD patients. The duration of treatment was small. Long time of the drug may exibit antioxidant potency in CAD patient.

Successful trials of this formulation with longer time will provide an inexpensive and safe treatment for atherosclerosis, and may point a better insight in the oxidant hypothesis of atherosclerosis [30].

Conclusion

Common therapies for CAD include drugs such as statins, fibrates, etc. All of these have side effects like kidney failure, nausea, amnesia, homicidal impulses, cramping in legs, etc. Our results showed significant rejuvenation of the HDL, Apo A1 level and the antioxidant enzyme PON 1 in the CAD patients treated with the herbo-mineral preparation as compared to patients treated with placebo and the untreated group (at the time of entry). The hematological, liver, renal and antioxidant profiles were found to be unaltered in all patients, treated with Oxitard. The drug also did not show any adverse side effects. These results are encouraging and strongly advocate further clinical trials with Oxitard. This would provide an alternative treatment strategy of atherogenic conditions through a novel mechanism of HDL up-regulation.

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Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Table 1: Demographic data of patients on entry

Parameters	Patients
Group A: Group B	128:127
Age (years) (mean±SD)	57±10
Diet (veg: non veg)	69:186
Sex Ratio (male: female)	162:83
Smokers (no. of patients)	75
Social Drinkers (no. of patients)	50

Both the groups are comparable.

Table 2: Effect of Oxitard treatment on various parameters in CAD patients[a]

Hematological Profile

	At entry	At the end of treatment(At 3 months)	
Parameter	Patients	At the end of treatment group	Placebo group
Hb (g%)	12.1±0.95	12.0±0.76	12.3±0.81
Total WBC count(/mm^3)	8794±1680	9023±1512	10182±8873
Neutrophils	53.2±10.78	51.9±9.76	54±10.4
Lymphocyte	29.2±5.77	30.0±5	28.2±6
Eosinophil	6.5±3.27	7±2	6.6±1.6
Basophil	0.3±0.51	1±1	0.3±0.52
Monocyte	1.0±0.82	0	1.2±0.82

Table 3: Biochemical Profile

Parameter	At entry	At the end of the treatment	
	Patients	Treatment group	Placebo group
Fasting blood sugar (mg/dl)	134.6±54.07	133.5±21.33	134.0±25.7
Liver Function Test			
Total serum protein (g/dl)	6.4±0.28	6.0±0.3	6.0±0.31
Albumin (g/dl)	3.9±0.2	3.9±0.21	4.0±0.21
Globulin (g/dl)	2.4±0.2	2.4±1.8	2.4±0.16
Albumin/Globulin	1.6±0.13	1.6±0.12	1.6±0.12
AST (U/L)	32.9±2.42	32.6±2.73	32.6±2.74
ALT (U/L)	35.6±3.69	35.3±3.77	36.0±3.6
Renal function test			
blood urea nitrogen (mg/dl)	35.4±5.27	35.9±6.1	34.6±4
Serum creatinine (mg/dl)	1.1±0.25	2.0±0.25	1.0±0.24

Table 4: Lipid Profile

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Parameter	Patients (0 months)	Treatment group	Placebo group
Total cholesterol (mg/dl)	189.4± 36.68	187.2 ± 28.34	188.3 ± 24.39
Triglyceride (mg/dl)	185.5 ± 37.11	187.1 ± 27.67	182.3 ± 34.08
HDL-C (mg/dl)	32.7 ± 8	35.6 ± 7.18*	32.8 ± 9
LDL-C (mg/dl)	119.6 ± 34.78	112.8 ± 30.22	116.0 ± 23.69
VLDL-C (mg/dl)	37.1 ± 7.4	36.4 ± 5.53	36.4 ± 6.81
Apo A1 (mg/dl)	113.4 ± 15.8	118.8 ± 12.58*	113.3 ± 14.5

Table 5: Antioxidant Profile

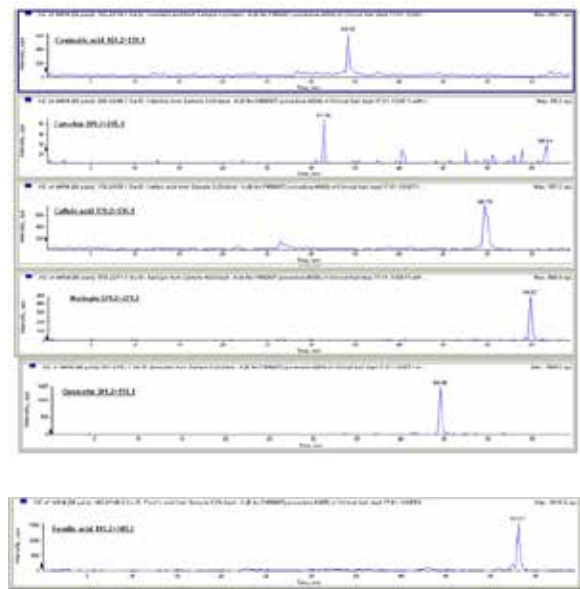
Parameter	Patients (0 months)	Treatment group	Placebo group
SOD (Unit/min/mg of prot.)	1.6 ± 0.37	1.7 ±0.38	1.6 ± 0.35
Catalase (µmol/mg of prot.)	256.0± 37.54	260.4 ± 34	255.2 ± 39.28
GPx (nmol NADPH oxidized/mg of prot.)	12.6 ± 1.56	12.4 ± 1.38	12.8 ± 1.2
GSHred (nmol/mg of prot.)	61.6 ± 23.11	67.2 ±23.4	59.9 ± 23.4
GR (nmol NADPH oxidized/mg of prot.)	4.1 ± 0.7	4.5 ± 0.62	4.6 ± 0.48
MDA (nmol/mg of prot.)	1.0 ± 0.31	0.9 ± 0.29	1.0 ± 0.34
TBAR (mmoles trolox equiv/l)	1.7 ± 0.18	1.1 ± 0.23	1.2 ± 0.19

#All the CAD patients received statin and ecosprin during the entire study period. The treatment group received additional two tablets of Oxitard daily, while the placebo group was given two identical-looking tablets, but no Oxitard. After three months, the parameters were assayed as described in Materials and methods. *P<0.001 was considered significant.

Table 6: Paraoxonase activity status

Patient Group	PON activity (salt stimulated) (U/ml)	PON activity (basal) (U/ml)
0 month	No activity	No activity
Treatment Group	0.7 ± 0.16	1.5 ± 0.16*
Placebo Group	No activity	No activity

Fig. 1 Compound identified in Oxitard-A capsules by liquid chromatography tandem mass spectrometry (negative ionization mode)



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