Chapter 2

Ameliorative Potential of Dimethoxycurcumin: Effect on Lipid Profile and Changes in Tissue Fatty Acid Composition in Arsenic Intoxicated Rats

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Abstract

Arsenic is a well known toxic metalloid which induces a broad spectrum of toxicological effects, biochemical dysfunctions and dyslipidemia, constituting serious hazard to health. DiMC is sound recognized for its antioxidant, anti-cancer, anti-inflammatory and anti-apoptotic actions. Hence the present study was designed to examine the antihyperlipidemic effect of Dimethoxycurcumin (DiMC) against arsenic induced dyslipidemia in rats. Oral administration of As (5 mg/kg of body weight (BW)/d) for 4 weeks showed significant (p<0.05) elevated levels of TC, LDL-C, VLDL-C, FFA, PL, and TGs, and the activity of HMG-CoA reductase in the plasma and liver with significant (p<0.05) reductions in levels of HDL-C hepatic ubiquinones and activities of LCAT and LPL in the plasma and liver. Pre-administration of DiMC (80 mg/kg of BW) for 4 weeks in As intoxicated rats significantly (p<0.05) reduced plasma and liver TC, FFA, TGs, VLDL-C, and LDL-C levels, and the activity of HMG-CoA reductase, and significantly (p<0.05) increased the activities of LCAT and LPL and the levels of HDL-C and ubiquinone in the livers of rats. The result of the present study indicates that DiMC showed an antihyperlipidemic effect in addition to its antioxidant effect in experimental rats.

Keywords

Arsenic; Antihyperlipidemic; Dimethoxycurcumin; Rats
Introduction

Lipoprotein abnormalities are the main leading point for confirming the disorder called dyslipidemia [1]. This disorder occurs metabolically with increase levels of lipoprotein in both serum and plasma. Among the risk factors for heart disease, it is one of the important factors to deal seriously.

Arsenic the metalloid and 33rd element of the periodic table is a dangerous toxic metal known worldwide for its various toxicity types. Arsenic has been reported for it potentiality to induced dyslipidemia in rats [1]. In addition, arsenic has been reported to increased plasma lipids, plasma TL, cholesterol, TG, bilurubin, LDL-C and decreased the HDL levels, attributing to the effect of it's on the permeability of the liver cell membrane, and liver dysfunction [1,2]. The major metabolic pathway of inorganic arsenic in humans is its methylation in the liver. Arsenite salt may exert its, toxicity through reactions with thiols in cells especially vicinal dithiols. On the other hand, recent results suggest that arsenic may also exert its toxicity through the generation of reactive oxygen species [3]. Previous literature illustrated that the plant constituents with antioxidant and antihepatotoxic potential, a number of medicinal measures proved to reveal a defensive effect against As stimulated varied toxicity [3].

Naturally occurring curcumin is the chief bioactive constituent extracted from the rhizome of the turmeric plant (Curcuma longa) Zingiberacea family. DiMC [1,7-bis(3,4-methoxyphenyl)-1,6-heptadiene-3,5-dione] is an analogue of curcumin obtained by the methylation of both free phenolic groups in the parent compound [4]. DiMC are well known for its antioxidant, antimitogenic and anticarcinogenic effects as well as antihepatotoxic and antibacterial properties. Hence, the present study was carried out to assess the ameliorating effects (antihyperlipidemic, antiatherosclerosis) of DiMC on plasma, serum, and liver lipid levels and lipoprotein levels in arsenic intoxicated rats.

Materials and Methods

Chemicals

Arsenic, 1,1′,3,3′-tetramethoxy propane, bovine serum albumin and were purchased from Sigma Chemical Co., St. Louis, MO, USA. Dimethoxycurcumuin was procured from Cayman chemicals, USA. All other biochemicals, chemicals and solvents were of certified analytical grade and purchased from S.D. Fine Chemicals, Mumbai or Himedia Laboratories Pvt. Ltd., Mumbai, India. Reagent kits were obtained from span Diagnostics, Mumbai, India.

Animals

Male albino Wistar rats with the body weight of 180–190 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University were used in this
study. The animal treatment and protocol employed were approved by the Institutional Animal Ethics Committee, Annamalai University (Registration Number: 853/2011/CPCSEA). The animals were housed as six animals per each polypropylene cage bedded with husk. The animals were fed on a pellet diet (Lipton India Ltd., Mumbai, India) and allowed to drink water ad libitum. During the study period, changes in body weight, diet and water ingestion were recorded weekly.

**Experiment Protocol**

In the present study, As was administered intragastrically at a dose of 5 mg/kg body weight/day for 4 weeks and DiMC was administered 90 minutes before the administration of As. The DiMC was dissolved in 0.5% of carboxy methyl cellulose, CMC) alone. In the experiment, a total of 24 rats were used. The rats were divided into six groups (n=6) and treated orally for 4 weeks: group 1, control rats treated with normal saline and CMC solution for 28 days; group 2- treated with As (5 mg/kg BW) in normal saline for 28 days; group 3- treated with DiMC (80 mg/kg BW) + As (5 mg/kg BW); group 4- treated with with DiMC (80 mg/kg BW) administered orally for 28 days.

**Sample Preparation**

At the end of the experimental duration, rats were fasted overnight and sacrificed by decapitation. Blood samples were collected and both serum and plasma were separated and used for lipid analysis. The liver tissue was dissected out, washed in ice-cold saline, and patted dry and weighed. The liver tissue was used for the lipid extraction.

**Extraction of Lipids and Estimation of Lipids from Plasma**

Lipids were extracted from plasma, serum and liver tissues within 24 h [5]. The aliquots obtained at the end of the extraction process were taken for the analysis of lipids. The level of total cholesterol [6] and triglycerides in plasma [7] were estimated by using a reagent kit from Sigma diagnostics (I) Pvt. Ltd. (Baroda, India) and the values were expressed as mg/dL in plasma. Phospholipids in plasma were estimated using the following procedure that is to 0.1 mL of plasma 1 mL of 5N H2SO4 and con HNO3 were added and digested to a colorless solution [8]. The inorganic phosphorous content in the extract was determined by using a diagnostic kit from Sigma Diagnostics (I) Pvt. Ltd (Baroda, India) [9] and the values was expressed as mg/dL in plasma. Free fatty acids in plasma were estimated [10] and the values were expressed as mg/dL in plasma.

**Estimation of Lipids from Serum and Liver Tissue**

From the lipid extract of liver and serum, the levels of total cholesterol [6] and TGs [7] were estimated by using diagnostic commercial kits (Qualigens Diagnostics, Mumbai, India) according to the manufacturers’ procedures.
The level of FFA was estimated by and the absorbance was read at 550 nm immediately [10]. The PL level was estimated, in which the conversion of organic phosphorus to inorganic phosphorus was involved [8]. The phosphorus content in this solution was also determined [9].

**Cholesterol in the Lipoprotein Fractions of Plasma**

HDL-cholesterol fraction was separated from the plasma sample by the precipitation techniques [11]. The very low density lipoprotein-cholesterol (VLDL-C) and LDL cholesterol concentrations were calculated from the Friedewald's equation. LDL Cholesterol = Total cholesterol - (HDL cholesterol + VLDL cholesterol) and VLDL cholesterol = Triglycerides/5. The values were expressed as mg/dL in plasma.

**Serum Lipoproteins**

Serum Lipoproteins were fractionated by a dual precipitation technique [12].

**Assessment of Activity of the Lipid Metabolic Key Enzymes**

The activity of HMG-CoA reductase was assayed indirectly by assessing the ratio of HMG-CoA to mevalonate in both plasma and liver [13]. The activity of lecithin cholesterol acyl transferase (LCAT) in plasma was assayed [14] and the amount of cholesterol present in the protein free filtrate was estimated and the value expresses the cholesterol present in the test sample at zero time [5]. The activity of the LCAT was assayed by incubating the reaction mixture to different time period (60, 120 and 180 min), the reaction was arrested and the cholesterol content was estimated and expressed as a function of the disappearance of free cholesterol during the incubation period with the unit of μmoles of cholesterol/ hr/ ml of plasma. Plasma lipoprotein lipase (LPL) activity was also assayed [15] and the values were expressed as μmoles of glycerol liberated / hr / ml of plasma.

**Assay of Lecithin Cholesterol Acyltransferase (LCAT) Activity in Liver**

The activity of LCAT in liver was assayed and it was expressed as micro mol of cholesterol per hour per milligram protein [14].

**Neutral Lipid Extraction and Separation**

One milliliter of liver homogenate was mixed with 18 ml methanol and 12 ml petroleum ether (b.p. 40–60°C) and ubiquinone-6 was added as internal standards. After intensive vortexing, the phases were separated by centrifugation and the upper organic phase was collected and dried under nitrogen. For the determination of total ubiquinone, ubiquinone-9 and -10, the neutral lipid fraction was dissolved in 100 ml of CM (2:1, v:v) and injected into an HPLC system equipped with a reversed-phase col-
umn (Hewlett-Packard Hypersil ODS 3 mm). A convex
gradient with methanol:water (9:1, v:v) as solvent A and
methanol:isopropanol:hexane (2:1:1, v:v) as solvent B was
used with a program time of 46 min. The flow rate was 1.5
ml:min and the absorption of the evaluate at 210 nm was
monitored [16].

Statistical Analysis of the Data

Values are given as mean ± S.D. for six rats in each
group. The data for various biochemical parameters were
analyzed by analysis of variance (ANOVA) using SPSS
version 16.0 (SPSS, Cary, NC, USA) and DMRT was use
to obtained individual comparison. A value of p < 0.05 was
considered to indicate a significant difference between
groups.

Results

Effect of DiMC on Morphological Changes

Figure 1A and 1B depicts the effect of As and DiMC
on food and water intake, body weight gain (%) in control
and experimental rats. In As treated condition, water and
pellet diet consumption were significantly decreased (p <
0.05) with decrease in body weight gain. Pre-administra-
tion of DiMC in As intoxicated rats significantly reversed
the changes near normal. DiMC treated alone rats didn't
shows any significance changes when compared to con-
trol.

Activities of the enzymes HMG-CoA reductase, LPL
and LCAT in plasma and liver of control and experimen-
tal rats were shown in Figure 2A and 2B. Activity of the
rate limiting enzyme HMG-CoA reductase was signifi-
cantly elevated in the plasma and liver of As administered
rats along with the decreased activities of LPL and LCAT
in plasma and decreased activities of LCAT in liver when
compared to the control rats. Pretreatment of DiMC to
As exposed rats, shows significant (p < 0.05) decrease in
the activity of HMG-CoA reductase with the increase in
the activities of LPL and LCAT in plasma and liver of rats
when compared to As alone treated rats. No significant
different were observed between control and DiMC alone
treated rats.
Effect of As and DiMC on the Amount of Ubiquinone in Liver of Control and Experimental Rats

The lower content of total ubiquinone content in the rat liver was observed in As treated rats when compared with the control rats (Figure 3). When total ubiquinone amounts (reduced plus oxidized forms) were determined, both forms of these lipids, ubiquinone-9 and -10 were diminished in the rat liver with As treatment compared to control (Figure 3). Pretreatment with DiMC revive the lower content of ubiquinone in As intoxicated rats.

Effect of As and DiMC on Changes in the Levels of Lipids and Lipoproteins in Plasma of Control and Experimental Rats

Plasma TC, TGs, and FFA and phospholipids and the changes in the levels of lipoprotein cholesterol (HDL-C, LDL-C and VLDL-C) of control and experimental rats were shown in Figure 4A and 4B. A significant (p < 0.05) elevation in the levels of TC, TGs, and FFA and phospholipids in plasma of As treated rats were observed. Administration of DiMC in As intoxicated rats significantly decreased these elevated plasma lipids towards near normal. Significant (p<0.05) increase in the levels of LDL-C and VLDL-C with decreased level of HDL-C in plasma was observed in As treated rats. In the case of DiMC treatment in As intoxicated rats, variations observed were significantly changed to near normal. There is no significant different between DiMC alone treated rats and control rats.
Effect of As and DiMC on Changes in the Levels of Lipids and Lipoproteins in Serum of Control and Experimental Rats

Serum total cholesterol, triglyceride and FFA levels were significantly increased (P<0.05) and that of PL level was decreased significantly (P<0.05) in As administered rats compared to that of control animals. In rats pretreated with DiMC along with As the PL level was significantly increased (P<0.05), whereas serum cholesterol, triglyceride and FFA levels were significantly reduced (P<0.05) compared to rats treated with As alone (Figure 5A). Figure 5B shows the levels of serum lipoproteins of control and experimental rats. We observed a significant (P < 0.05) increase in the levels of LDL-C, VLDL-C, and a significant (P < 0.05) decrease in the levels of HDL-C in the serum of As-treated rats when compared with control rats. Pretreatment with DiMC in As-intoxicated rats shows a significant (P < 0.05) decrease in the levels of plasma LDL-C and VLDL-C, with a significant (P < 0.05) increase in the level of HDL-C were observed when compared with As-treated rats. There was no significant between the rats treated with DiMC alone when compared to the control.

Effect of As and DiMC on Changes in the Levels of Lipids in Liver of Control and Experimental Rats

Figure 6 depicted the significant decrease level of PL and increase levels of TC, TGs, and FFA in the liver tissue of As treated rats when compared with control rats (P < 0.05). DiMC pretreatment significantly (P < 0.05) decreased the levels of TC, TGs, and FFA, and significantly (P < 0.05) increased the PL in the liver tissue when compared with As alone treated rats (P < 0.05) (Figure 6). There was no significant difference between DiMC alone treated rat and control rats (Figure 6).
**Discussion**

Arsenic induces wide toxicological effects and biochemical dysfunctions with serious hazard to health. Lipoprotein abnormalities resulting in the distraction of serum and cellular lipid levels account for the origin of cardiovascular diseases. Dysfunction of liver by increased gathering of As can disturbed lipid homeostasis in addition to glucose homeostasis as liver play a major role for maintaining lipid profiles. In our study, significant increase in the levels of plasma, serum and liver lipids (TC, TG and FFA), lipoproteins (LDL-C and VLDL-C) and free cholesterol with the common symptoms of As toxicity includes the decreased food and water intake, body weight gain and increase liver weight were observed in As treated rats. It might be due to the increased degeneration of lipids and proteins and also the decreased protein synthesis and it is accordance with the finding of Miltonprabu and Sumedha [1]. DiMC clearly revealed the free radical scavenging activity of DiMC which could be due to its lipophilic nature, whereas in the donation of H-atom from the 3rd and 5th identical β-diketone in heptane moiety to a lipid alkyl or lipid peroxyl radicals enhances the scavenging ability of DiMC and ameliorate As induced multidysfunction.

Increase in the activity of HMG-CoA reductase leads to the excessive production and accumulation of cholesterol and initiate the development of atherosclerosis [1,17]. Decrease in the ratio of HMG-CoA/mevalonate indicates increased activity of the enzyme HMG-CoA reductase. In the present study, we have found that As intoxication caused a significant decrease in the activity of HMG-CoA/mevalonate showing increased activity of the enzyme HMG-CoA reductase. Increased activity of the plasma and liver enzyme HMG-CoA reductase in As treated rats may be due to the use up of HMG-CoA enzyme by the As because of its affinity to sulfydryl group [18] as the enzyme HMG-CoA contain sulfydryl group. While pre-treatment of DiMC to rats decreased the activity of HMG-CoA reductase near to control when compared with As intoxicated rats. This may be due to the ability of DiMC to protect the SH groups from the oxidative damage through the inhibition of peroxidation of membrane lipids and stabilizes the membrane [19]. Lecithin cholesterol acyl transferase (LCAT) is the key enzyme responsible for the esterification and transesterification of cholesterol moiety in between the lipoprotein fractions like HDL, VLDL and LDL present in circulation. Lipoprotein lipase (LPL) plays vital role in catabolism of TGs and releases the FFA from chylomicrons and VLDL, thereby regulating the level of TGs in circulation. In our study, reduction in the activity of LPL may contribute the hypertriglyceridemia in plasma and inducts the organization of LDL, aggregation and the oxidation of LDL potentiates the atherogenisis in similar way reported by Taskinen [20]. In our study decreased activity of LCAT and LPL in both plasma and liver were noted in As intoxicated rats, promoting the accumulation of free cholesterol and of remnant lipoprotein in both
plasma and liver which may accelerate the atherogenesis [1]. Treatment of DiMC in As toxic rats prevents the accumulation of cholesterol by activating the expression of LCAT and catabolic enzymes of cholesterol degradation pathway [19]. Administration of DiMC activates the activity of LPL there by increasing the HDL and decreasing the LDL through the reduction of TGs [4].

Ubiquinone is considered to be the major lipid soluble antioxidant in the cell and present in all cellular membranes and its core function at these locations is to supply as a lipid soluble antioxidant proved to be highly competent in exasperating the effects of free radicals and reactive oxygen metabolites [1]. Ubiquinone has a high amount of carbon double bonds and therefore a higher reducing potential than the vitamin C or vitamin E. Thereby it is the first line of defense against free radicals mediated oxidative stress. Interestingly pretreatment of DiMC restored the amount of ubiquinone in the liver of rats intoxicated with As. Restoring activity of DiMC may be due to its membrane protective effect, mitochondrial membrane potential protective capability and its free radical scavenging activity [19].

Cholesterol is a lipid that is an important constituent of body cells and so widely distributed throughout the body. Cholesterol exists in three forms in the blood: high-density lipoproteins (HDLs) which are believed to protect against arterial disease and a low-density version (LDLs) and very low density type (VLDLs), these latter two were being risk factors. In this study to As significantly rises the plasma, and serum lipoproteins LDL, VLDL and TG and fall in HDL, and it may be due to changes in gene expression in hepatic enzyme like HMG-CoA reductase (hydroxy-3-methyl glutaryl-CoA), which in turn depresses LDL-receptor gene expression [1]. An increase in plasma and serum LDL-C and VLDL-C fractions along with a decrease in HDL-C was observed in As treated rats, because liver is one of the susceptible organs of As. Pre-administration of DiMC significantly reduced the levels of VLDL and LDL and elevated the level of HDL. The results indicate that DiMC could provide an antihyperlipidaemic activity by activating the 3-Hydroxy-3-methyl glutaryl-Coenzyme A reductase, plays a major role in the regulation of cholesterol metabolism and a rate limiting enzyme in the pathway of cholesterol biosynthesis.

In As treated rat a significant increase in the cholesterol and triglyceride values followed by a significant decrease in the phospholipid level was observed in the plasma, serum and liver tissue and it show the brutal potential of As in inducing hyperlipidemia. As induced alterations in hepatic lipid profiles were reported earlier by Muthumani and Milton Prabu [2] and Miltonprabu and Sumedha [1]. The increased concentration of FFA in plasma, serum and liver could be due to As persuaded disturbance of mitochondrial task which pilots to the inhibition of β-oxidation and elevated accretion of FFA in the liver,
serum and plasma. Generally, the surplus of FFA in circulation endorses the conversion of FFA into PL [2]. Decline activity of LPL observed in As intoxicated rats may contribute the hypertriglyceridemia in plasma and serum and initiates the union of LDL, aggregation and the oxidation of LDL and potentiates the atherogenesis. Pre-administration of DiMC may trigger LPL thus increasing the HDL and decreasing the LDL cholesterol through the diminution of TGs in As toxicity [19].

**Conclusion**

In conclusion, the current exploration disclosed that the pre-administration of DiMC in As intoxicated rats distorted the plasma, serum and hepatic lipids by regulating the lipid metabolizing enzymes to near ordinary levels. It can be affirmed that, the DiMC has favorable effects (antihyperlipidemic, antiatherosclerosis) on plasma, serum and liver lipids and lipoproteins in arsenic intoxicated rats.

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