RESEARCH ARTICLE

Received on: 26/11/2015
Published on:20/12/2015

Corresponding Author
Ashishkumar V. Kyada,
Department of Pharmacology,
S. J. Thakkar Pharmacy College,
Rajkot, Gujarat, India.
Email: kyadashish@rediffamil.com

QR Code for Mobile users

Conflict of Interest: None Declared !

In-vitro low-density lipoprotein protective and lipid metabolism modulatory effects of *Calotropis procera* root bark in poloxamer-407-induced hyperlipidemia

Devdas D. Santani[1], Ashishkumar V. Kyada[2]*,
[1]Department of Clinical Pharmacy Practice, Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India
[2]Department of Pharmacology, S. J. Thakkar Pharmacy College, Rajkot, Gujarat, India

ABSTRACT

Atherogenic hyperlipidemia is a metabolic syndrome characterised by abnormal increase in the circulatory lipids and lipoprotein levels and important cause of cardiovascular diseases worldwide. A variety of plants have been claimed to have antihyperlipidemic effect based upon their folklore use in Indian traditional system of medicine. However, many of such plants are not scientifically validated on the basic of phytochemicals present. Therefore, in the present investigation, antihyperlipdemic activity of *Calotropis procera* root bark aqueous extract and its different solvent fractions have been investigated in poloxamer-407, a surfactant-induced hyperlipidemia in rats. Administration of crude extract, ethyl acetate and aqueous fraction of the plant has significantly reduced total serum cholesterol, triglycerides, very low density lipoprotein when compared to disease control group. Low-density lipoproteins were also reduced, but not statistically significant in these animal groups. Moreover, extract and fractions also demonstrated protective effect against copper-catalyzed human plasma LDL oxidation, suggesting beneficial role of plant in retarding atherogenic pathobiology.

Keywords: Hyperlipidemia, LDL oxidation, Poloxamer-407, HMG-CoA reductase, *Calotorpis procera*

Introduction

Cardiovascular diseases (CVD) are widely recognized as the leading cause of premature death and a major cause of disability in most developed and developing countries[1]. Atherogenic Hyperlipidemia is the predictor of Coronary Artery Disease (CAD). Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic impasse[2,3]. Atherosclerosis, a major manifestation of the pathophysiology of CVDs, constitutes the single most important contributor to this growing burden of this disease. Epidemiological studies have identified numerous risk factors that contribute to the pathogenesis of atherosclerosis disease and subsequent CVDs includes endothelial dysfunction, lipid abnormalities, hypertension, diabetes, smoking, immunological and inflammatory factors, oxidative stress and genetic susceptibility[4,5]. Therefore, the prime consideration in delaying arteriosclerosis is to reduce elevated serum/serum levels of lipids. Oxidized LDL has been implicated in the early progression of athersoclerosis and have definite role in producing characteristic lesions of atherosclerosis in the arterial wall[6].

Thus, the management of dyslipidemia is still a major challenge with respect to patients who are intolerant to the adverse effects of these classic hypolipidemic drugs[7]. Although these drugs have brought about remarkable success in hyperlipidemia and atherosclerosis therapy, the efficacy of these drugs is still debatable as pathophysiology of atherosclerosis involves multifactorial, highly complex and integrated pathobiological mechanisms. Considering these views, there is a great need ever for the natural products with pleotropicity and led to exploration of natural

therapeutic agents as an effective alternative therapy for hyperlipidemia.

Calotropis Procera (Ait.) R. Br. is a xerophytic shrub, belonging to Asclepiadaecae family, has a wide presence throughout Asia and Africa. Literature suggests that plant has been extensively investigated for phytochemicals and biological activities. Reported constituents of *Calotropis procera* in the literature include cardiac glycosides, cardenolides, tritepenoids, flavonoids, sterols and fatty acids[8,9]. Medicinal values of this plant have been recognized in the light of scientific studies conducted to establish the credentials of *Calotropis procera* as a valuable in alternative system of medicine. From literature review, it was observed that *Calotropis procera* root bark possesses α-amyrin, β-amyrin, β-sitosterol, lupeol[10,11]. However, antihyperlipidemic potential of *Calotropis procera* root bark has not been reported. Therefore, present investigation was undertaken to evaluate the efficacy of *Calotropis procera* (Ait.) R. Br. root bark aqueous extract and different solvent fractions in hyperlipidemic rats.

MATERIALS AND METHODS

Plan collection and processing:

Roots of *Calotropis procera* (Ait.) R. Br. were collected from the bank of Bhadar dam, Dhoraji, Rajkot and identified by a taxonomist. Roots were washed with water; bark was peeled off using knife and air dried for several days. Dried plant material was chopped, ground into coarse powder and stored into an air tight container till further usage.

Extraction method:

Root bark (100 g) was exhaustively extracted with distilled water in soxhlet apparatus at controlled temperature (40 °C) for 72 hours. Resulting solution was filtered through Whatman filter paper (No.42). The filtrate so obtained was concentrated in a water bath at low temperature (40 °C). The dried weight of crude extracts was determined and designated as *Calotropis procera* Root Water Extract (CPRWE) and stored at 4 °C in an air tight container till further usage.

Preparation of fractions:

Fractionation was preformed following the protocol designed by Kupchan *et al* and modified by vanWagenen *et al*[12]. Briefly, dried crude extract (20 g) was subjected to solvent-solvent partitioning with solvents (3 × 200 ml for each solvent type) of increasing polarity- n-hexane, dichloromethane, ethyl acetate, and methanol. The combined solvent portion of respective fractions was evaporated to yield hexane fraction (HF), dichloromethane fraction (DMF), ethyl acetate fraction (EAF) and methanol fraction (MF) respectively. At each step of solvent fractionation, 40 ml of distilled water was added. Methanol insoluble residues were considered as aqueous fraction (AF). All fractions were evaporated to dryness at low temperature of 40 °C and stored.

Preliminary phytochemical investigation:

CPRWE and solvent fractions were subjected to qualitative phytochemical screening for the presence of various secondary metabolites[13].

Animals:

Healthy male and female Sprague Dawley rats weighing between 250–280 g were procured from the Zydus Cadila Research Centre, Ahmadabad. Animals were housed at our Institute's animal house facilities. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for the care and use of laboratory animals. The study protocol was approved by Institutional Animal Ethics Committee (IAEC), S. J. Thakkar Pharmacy College, Rajkot. (Ref. no. SJT-73/2012).

Acute toxicity study:

The acute oral toxicity test was performed according to OECD guidelines (423, adopted 17th December, 2001)[14] for limit test. Overnight fasted female rats were divided into control and test groups (n=3). Control group received 0.5 ml Carboxymethylcellulose (0.5% CMC) suspension by oral gavage while each test group received a single high dose of 2000 mg/kg body wt (weight) of crude extract and solvent fractions (DMF, EAF, MF and AF). All crude extracts were suspended in 0.5% CMC prior to administration. Animals were allowed access to food (starting from 1 h after drugs administration) and water *ad libitum* throughout experimental period. After the administration of extracts, animals in each group were observed individually at least once during first 30 mins after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days for any signs of toxicity or mortality. During initial 2 hr, animals were observed mainly for physical sings, behavioral profile, neurological profile and

autonomic profile[15]. If toxic signs or lethality is not observed after 48 h, then 1/5th, 1/10th parts of the maximum tolerated dose for CPRWE and 1/10th, 1/20th parts for solvent fractions were selected for subsequent investigation.

Induction of experimental hyperlipidemia:

Poloxamer-407 (P-407), a surfactant, was used as the inducing agent. Needles and syringes to be used for administration were also cooled to prevent gelation within the syringe during injection[16].

Experimental Design:

After 1 week of acclimation to the laboratory conditions, overnight fasted male Sprague Dawley rats were randomly divided into 14 groups (n=6); Group I served as normal control (NC) and received 0.5% CMC, Group II as disease control (DC) and administered with 0.5% CMC prior to P-407 i.p. injection. Groups III-XIV served as treatment groups. Among treatment groups, Group III and IV were treated with 200 and 400 mg/kg body wt CPRWE respectively while Group V-XII received DMF, EAF, MF and AF at a dose of 100 and 200 mg/kg body wt prior to P-407 i.p. administration. Group XIII and Group XIV received atorvastatin, 50 mg/kg body wt and fenofibrate, 65 mg/kg body wt respectively before P-407 injection. The DC and all treatment groups were challenged with a single dose of 30% (w/v) of P-407 intraperitoneally (i.p.) at a dose of 500 mg/kg body weight. NC group were injected with saline i.p. instead of P-407.

All test extracts/reference drugs/vehicle were administered by oral gavage at 24 hr and 1 hr prior to i.p. injection of 30% (w/v) P-407. Food was withheld in all groups till 6th hr blood samples collection and animals had allowed access only to water. Blood samples were collected at 3, 6 and 24 hr after P-407 injection from retro orbital plexus under the influence of ether anasthesia. The blood samples were centrifuged (6000 rpm for 10 min at 4 ^{o}C) and serum was used for lipid analysis.

Measurement of serum lipid profile:

The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated in serum using commercially available kits (Span Diagnostics Ltd, Surat, India). Very low-density lipoprotein-cholesterol (VLDL-C) was calculated by Friedwald formula[17]:

VLDL-C = TG/5.

Low density lipoprotein-cholesterol (LDL-C) was calculated using Modified Friedwald formula (MMF)[18] because original Friedwald formula is not valid when triglycerides levels are more than 400 mg/dL.

MFF: LDL–C (mg/dl) = {Non -HDL-C × 90%} – {TG ×10%}

Where; Non -HDL-C = TC – HDL–C

Isolation of human Serum LDL:

Isolation of LDL was performed through a heparin–citrate buffer precipitation method developed by Wieland and Seidel[19]. Five millilitres of plasma were vortexed with 50 ml of heparin– citrate buffer (prepared by adding 5000 IU/L heparin to 100 ml 0.064 M trisodium citrate, pH 5.05 adjusted with 5 M HCl) and incubated for 10 min at room temperature. Resultant white precipitate were separated by centrifugation at 3500 rpm at 4 ^{o}C and resuspended in 1 ml of 10 mM phosphate-buffered saline (PBS, pH 7.4). Protein content of LDL-C suspension was measured according to Lowry method modified by Pomory using bovine serum albumin as standard[20].

Induction of LDL oxidation:

LDL oxidation was performed following the method described by Khunawat *et al*[21]. An aliquot of LDL (containing 200 µg LDL) in 10 mM phosphate buffered saline (PBS, pH 7.4) was incubated with 100 µl of different concentrations (25, 50, 100, 200, 500, 1000, 1500 µg/ml) of CPRWE and its different solvent fractions in a total volume of 1.5 ml for 30 min at 37 ^{o}C. Copper-mediated LDL oxidation was initiated by adding 10 µl of freshly prepared 0.167 mM $CuSO_4$ to all tubes and again incubated again at 37 ^{O}C for 6 h. At the end of incubation period, oxidation kinetic was terminated by adding 10 µl EDTA (10 mM).

Measurement of Malondialdehyde in LDL:

LDL oxidation preventive capacity of extracts was assayed by measuring the amount of malondialdehyde (MDA) formed as per the method described by Okhawa *et al* and modified by Pulla and Lokesh[22]. Briefly, 0.5 ml of incubated LDL was mixed with 1 ml of (1.15 M) KCl and 2 ml of chilled thiobarbituric acid (TBA) reagent (0.25 M HCl, 15% trichloroacetic acid, 0.38% TBA + 0.055% butylated hydroxy toluene). The reaction mixtures were kept in a boiling water bath for 60 min at 100 ^{o}C and absorbance was measured at 570 nm. The amount of MDA (thiobarbituric acid reactive material) was

calculated using molar extinction coefficient 1.56 X 10^5 $M^{-1}cm^{-1}$ and expressed as nmoles of MDA/mg LDL-protein.

Statistical analysis:

Data was expressed as mean ± SEM. Statistical analysis by one-way ANOVA with Tukey's multiple comparison test performed using GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego, CA, USA. $p< 0.05$ was considered statistically significant.

RESULTS

Extraction and fractionation:

Table 1 highlights the extractable yield of CPRWE and different solvent fraction. The n-hexane fraction showed negligible yield, and therefore it is not tested in subsequent investigation.

Preliminary phytochemical investigation:

Table 1 shows the presence of secondary metabolites in CPRWE and solvent fractions such as alkaloids, carbohydrates, glycosides, saponins, flavonoids, triterpenoids, sterols, phenolic compounds/tannins, proteins and amino acids in variable proportions depending upon the polarity of solvents used for fractionation

Table 1: Extract yield* of *Calotropis procera* root bark and solvent fractions

	Extract/Fractions				
	CPRWE	**DMF**	**EAF**	**MF**	**AF**
Yield (%)	14.32	2.87	7.46	26.77	20.63
Phytochemicals					
Alkaloids	+	-	-	+	-
Carbohydrates	+	-	-	+	+
Glycosides	+	+	+	+	+
Triterpenes	+	-	+	+	+
Sterols	+	+	+	-	-
Flavonoids	+	+	+	+	+
Phenolics/Tannins	+	-	+	+	+
Saponins	+	-	-	+	+
Proteins	+	-	-	+	+

Footnote: *Yield (%) is a percentage of the weight of the extract in relation to the weight of the raw material. CPRWE: *Calotropis procera* root bark water extract; DMF: dichloromethane fraction; EAF: ethyl acetate fraction; MF: methanol fraction; AF: aqueous fraction

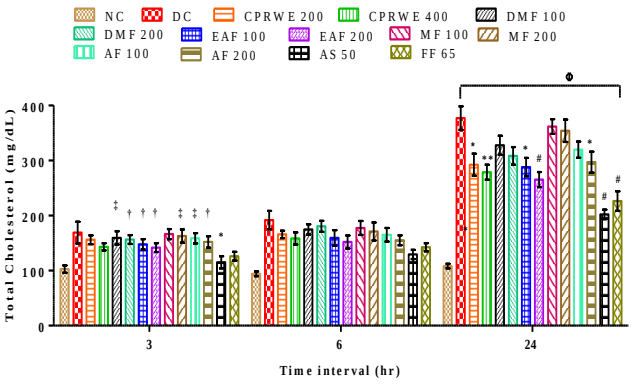
Oral acute toxicity study:

In acute toxicity study, a single dose of 2000 mg/kg body wt in female Sprague Dawley rats did not cause any death after 48 h and 14 day post-treatment in rats. There was no change in general behavior or other physiological activities.

Effects of CPRWE and fractions on serum lipids:

In the present investigation, hyperlipidemia was induced as per method described by Jonston and Palmer by administration of a single P-407 i.p. at a dose of 500 mg/kg body wt. Fig. 1 shows the effect of CPRWE and different solvent fractions on serum TC levels. The DC group animals injected with P-407 showed a rise in serum TC levels at 3 ($p<0.1$) and 6 ($p<0.01$) when compared to that of NC group at same time periods. CPRWE and solvent fractions showed a significant reduction at 24 hr ($p<0.05$ for CPRWE 200, EAF 100 and AF 200, $p<0.01$ for CPRWE 400 and $p<0.001$ for EAF 200) and was comparable to that standard groups. Statistical significance for AS 50 and FF 65 at 24 hr was found to be $p<0.001$.

Figure 1: Effects of CPRWE and different solvent fractions on serum TC level

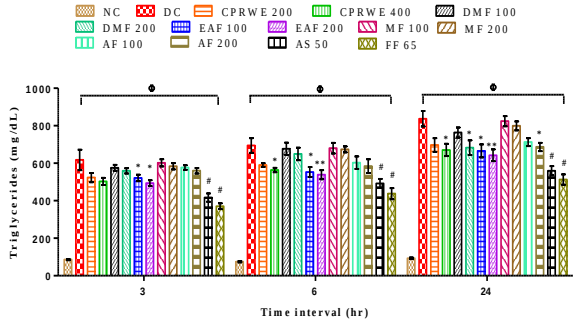


Footnote: Serum TC levels are measured at an interval of 3, 6 and 24 hr post P-407 administration. Values are expressed as mean (mg/dl) ± SEM (n=6). †$p<0.01$, ‡$p<0.01$, Φ$p<0.001$ when compared with NC group. *$p<0.05$, **$p<0.01$, #$p<0.001$ when compared with DC group. NC: normal control; DC: disease control; CPRWE: *Calotropis procera* root bark water extract (200

and 400 mg/kg body wt); DMF: dichloromethane fraction (100 and 200 mg/kg body wt); EAF: ethyl acetate fraction (100 and 200 mg/kg body wt); MF: methanol fraction (100 and 200 mg/kg body wt); AF: aqueous fraction (100 and 200 mg/kg body wt); AS: atorvastatin (50 mg/kg body wt) and FF: fenofibrate (65 mg/kg body wt).

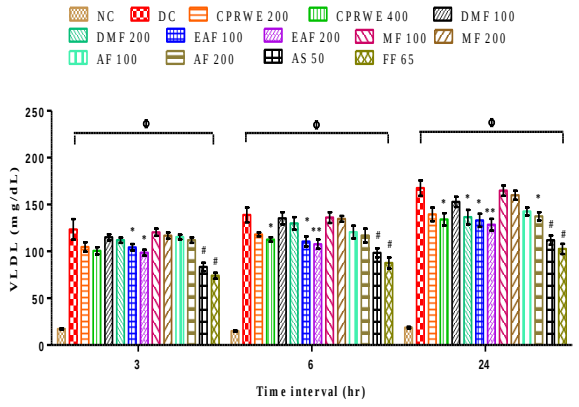
P-407 i.p. injection in animals resulted in significantly higher levels of TG and VLDL (Fig. 2 & 3) at all time intervals tested in all groups ($p<0.001$) when compared with saline injected NC group. At 3 hr, only CPRWE 400 ($p<0.05$), EAF 200 ($p<0.05$), AS 50 and FF 65 ($p<0.001$ for both standards) showed a significant protection against TG elevation when compared with that of DC group. Potency of plant extract and different solvent fractions was more evident at 24 hr ($p<0.05$ for CPRWE, DMF 200, EAF 100 and AF 200, $p<0.01$ for EAF 200) and was found to be comparable to that of standards.

Figure 2: Effects of CPRWE and different solvent fractions on serum TG level



Footnote: Serum TG levels are measured at an interval of 3, 6 and 24 hr post P-407 administration. Values are expressed as mean (mg/dl) ± SEM (n=6). †p<0.01, ‡p<0.01, Φp<0.001 when compared with NC group. *p<0.05, **p<0.01, #p<0.001 when compared with DC group. Abbreviations of experimental groups are explained under fig. 1.

Figure 3: Effects of CPRWE and different solvent fractions on serum VLDL



Footnote: Serum VLDL levels are measured at an interval of 3, 6 and 24 hr post P-407 administration. Values are expressed as mean (mg/dl) ± SEM (n=6). †p<0.01, ‡p<0.01, Φp<0.001 when compared with NC group. *p<0.05, **p<0.01, #p<0.001 when compared with DC group. Abbreviations of experimental groups are explained under fig. 1.

In the present investigation, we observed a characteristic pattern in HDL levels after P-407 i.p. injection (Table 2). The DC and MF treatment groups showed a significant decline at 3 hr ($p<0.05$) when compared with that of NC group. At 24 hr, HDL levels in DC and MF groups were significantly increased when compared to the NC group animals. Whereas, animals treated with CPRWE, EAF, AF and standard drugs produced elevation in serum HDL, though levels remained within normal range, comparable to NC group animals.

Table 2: Effects of CPRWE and different solvent fractions on serum HDL

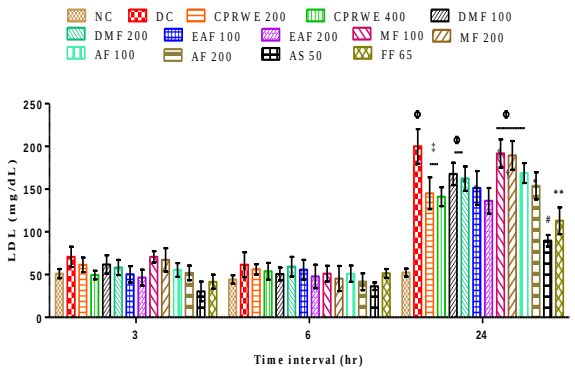
Groups	HDL cholesterol (mg/dl)		
	Time interval (hr)		
	3	6	24
NC	36.40 ± 1.99	36.45 ± 2.26	39.80 ± 2.08
DC	21.92 ± 1.58†	46.18 ± 2.64	61.80 ± 3.06Φ
CPRWE 200	29.78 ± 1.69	37.88 ± 1.24	53.85 ± 3.91
CPRWE 400	32.40 ± 1.53	36.23 ± 1.88	47.45 ± 2.56
DMF 100	26.95 ± 3.29	43.07 ± 2.62	56.40 ± 3.10†
DMF 200	29.53 ± 1.49	42.45 ± 3.36	52.32 ± 2.55
EAF 100	33.90 ± 3.77	35.97 ± 1.59	46.15 ± 3.34
EAF 200	35.37 ± 3.18	38.98 ± 2.74	42.73 ± 2.70
MF 100	20.85 ± 2.64	45.18 ± 2.01	57.12 ± 3.57†
MF 200	23.18 ± 2.61	46.00 ± 2.43	54.68 ± 3.22
AF 100	32.98 ± 4.93	41.47 ± 2.45	52.92 ± 2.51
AF200	32.05 ± 1.96	43.77 ± 3.80	49.60 ± 4.49
AS 50	35.15 ± 2.21	34.37 ± 2.16	40.43 ± 3.11
FF 65	38.63 ± 3.33	36.70 ± 4.25	43.87 ± 4.33

Footnote: Serum HDL levels are measured at an interval of 3, 6 and 24 hr post P-407 administration. Values are expressed as mean (mg/dl) ± SEM (n=6). †p<0.01, ‡p<0.01, Φp<0.001 when compared with NC group. *p<0.05, **p<0.01, #p<0.001 when compared with DC group. Abbreviations of experimental groups are explained under fig. 1.

In our study, we did not observe a significant increase in serum LDL till 24 hr in all groups after P-407 injection (Fig. 4). Among the treatment groups, only AS 50 and FF 65 significantly prevented increase in serum LDL cholesterol ($p<0.001$ for AS 50 and $p<0.05$ for FF 65). However, CPRWE 400 and EAF 200 have shown reasonable reduction in serum LDL level, but numerically not significant.

Figure 4: Effects of CPRWE and different solvent fractions on serum LDL.

.

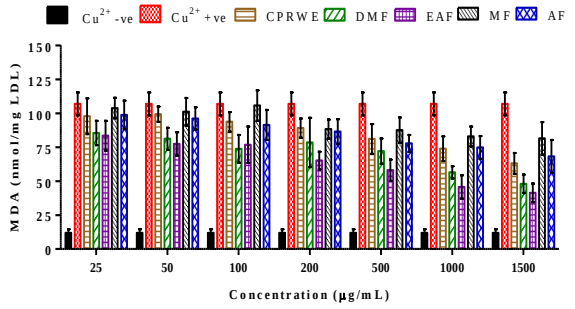


Footnote: Serum LDL levels are measured at an interval of 3, 6 and 24 hr post P-407 administration. Values are expressed as mean (mg/dl) ± SEM (n=6). †p<0.01, ‡p<0.01, Φp<0.001 when compared with NC group. *p<0.05, **p<0.01, #p<0.001 when compared with DC group. Abbreviations of experimental groups are explained under fig. 1.

Effect of CPRWE and fractions on *in vitro* LDL oxidation:

Fig. 5 we shows the protective effect of CPRWE and different solvent fractions on the copper-induced LDL oxidation in the present study. The inhibitory efficacy of the CPRWE and fractions on LDL modification is in following order: EAF>DCM>CPRWE>AF>MF. Among the fractions, EAF fraction significantly reduced the formation of MDA adducts in a dose-dependent manner after 6 hr incubation when compared with positive control while DCM and CPRWE required higher concentrations to produce the same effect observed with EAF. The MF was least effective among the fractions and did not decrease MDA levels even at 1500 µg/ml concentration.

Figure 5: Effects of CPRWE and different solvent fractions on MDA production from *in vitro* copper-induced plasma LDL oxidation



Footnote: Test extract and fractions are tested at 25, 50, 100, 200, 500, 1000, 1500 µg/ml in triplicate. Before addition of $CuSO_4$, LDL was incubated with test extracts for 30 min. Values are expressed as mean ± SEM (n=3). p<0.05 for CPRWE and AF; p<0.01 for DMF and EAF. Statistically significant cumulative mean difference between test groups and positive control was 21.54, 22.15, 36.15, and 42.95 at 95% confidence interval for CPRWE, AF, DMF and EAF respectively after ANOVA followed by Tukey's multiple comparison test. NC: negative control group (only native LDL, without Cu^{2+} and treatment); PC: positive control group (native LDL with Cu^{2+} only); CPRWE: *Calotropis procera* root bark water extract; EAF: ethyl acetate fraction; MF: methanol fraction; AF: aqueous fraction.

DISCUSSION

Elevated serum lipids may primarily be due to genetic defect or secondarily to diet, drugs or diseases and potential risk factor in the development of CVDs including atherosclerosis and ischemic heart diseases[23]. In contrast to Triton WR-1339, P-407 (non-ionic synthetic copolymer surfactant), provides an attractive means of inducing hyperlipidemia within 24 h of its intraperitoneal (i.p.) injection because of its rapid onset, convenience, reproducibility and lack of undesirable toxicity[16,24]. Therefore, P-407 was used in this study to develop hyperlipidemic animal model.

In the present investigation, a single dose of P-407 demonstrated a significant rise of serum TC, notably at 6 hr and 24 hr as compared to that of NC groups. The observed effect of P-407 was in accordance with the previously reported studies[25,26]. Animals treated with CPRWE, EAF and AF have shown significant reduction in serum TC levels. From the results, it was observed that EAF at 200 mg/kg body wt showed highest TC lowering effect, comparable to that of fenofibrate, but less than atorvastatin. It has been reported that P-407 is similar to Triton WR-1339 with regards to their effect on lipid metabolism *in vivo*. P-407 also elevates TC in experimental animals by indirectly stimulating hepatic HMG-CoA reductase, a rate limiting enzyme in cholesterol biosynthesis[27]. However, another study by same researcher reports that the increase in serum cholesterol following P-407 i.p. administration may be due to inhibition of cholesterol α-hydroxylase and not due to HMG CoA reductase activation[28]. From these observations, it appears that various phytochemicals present in CPRWE, EAF and AF might have suppressed HMG CoA reductase enzyme, similar to atorvastatin and fenofibrate[29], thereby contributing to the antihyperlidemic potential of *Calotropis procera* root bark.

P-407 has been reported to have predominant effect on the hepatic triglyceride production. Published data in literature suggests that the probable mechanism behind P-407-induced

hypertriglyceridemia is inhibition of plasma lipoprotein lipase (LPL), a capillary bound enzyme. LPL is responsible for hydrolysis and clearance of triglycerides from the circulation[26]. In the current study, we observed a statistically significant increase in TGs upon P-407 injection intraperitoneally. This explains that P-407 inhibiting LPL resulted in the accumulation of TGs in systemic circulation. Another study has reported that the concentration of TG in rats following a single 300 mg i.p. injection of P-407 resulted in a marked increase in serum TG with a maximum rate of accumulation (5.74 mg/dl/min) occurred between 2 and 4 hr post injection. Moreover, they also demonstrated that P-407 causes more than 95% suppression of LPL activity 3 hr post-injection[16]. From the results of this study, we decided to measure serum lipid levels at an interval of 3, 6 and 24 hr post P-407 administration in rats. It is, therefore postulated that active phytochemicals of crude extract, ethyl acetate and aqueous fraction of *Calotropis procera* root bark may have ability to accelerate TG breakdown by activating LPL and reduce the secretion of VLDL in circulation.

The pro-atherogenicity of TG is indirect and related to its lipoprotein transport and metabolism abnormality in terms of reduced clearance of VLDL and LDL which are highly atherogenic[30]. Therefore, it is evident that the reduction in serum TG levels could be a key aspect in delaying cardiovascular complications in hyperlipidemic patients. From our study results, we suggest that treatment with *Calotropis procera* root bark may have beneficial effect in preventing long term cardiovascular complications by virtue of their ability to reduce elevated TG levels.

Elevated LDL-C levels play a crucial role in the development of atherosclerosis lesions that progress from fatty steaks to ulcerated plaques as LDL-C is known to accumulate in the extracellular sub-endothelial space of arteries and to be toxic to arterial walls[31]. Treatment with CPRWE, EAF, AF afforded reasonable protection by decreasing serum LDL at 24 hr, however, reduction was not statistically significant. Only standard treatment groups showed a tremendous reduction in LDL levels at 24 hr HDL-C is believed to have an anti-atherogenic effect by counteracting LDL-C oxidation and facilitating the translocation of cholesterol from peripheral tissue such as arterial walls to the liver for catabolism[32]. In our study, a significant decrease at 3 hr and increase[25] at 24 hr in HDL levels in DC group as compared with normal animals. However, in treatment groups, CPRWE, EAF, AF, atorvastatin, fenofibrate didn't allow fluctuation in HDL levels and remained within normal range.

As previously discussed, elevated blood LDL and subsequent oxidative modification accelerates the atherosclerotic lesion formation[6,32]. We also studied the effect of CPRWE extract and its solvent fractions on the copper-catalysed human LDL oxidation, assayed by formation of thiobarbituric acid reactive substance (TBARS). We found that the CPRWE, DCM and EAF significantly counteracted the formation of TBARS. It has been assumed that the copper ion binds to lipoprotein and causes degradation of lipid hydroperoxides[33,]. The results obtained from present study indicate that CPRWE, DMF, EAF and AF are capable of affecting the rate of LDL oxidation at the end of 6 hr incubation. This could be due to the ability of the extracts to chelate or interfere with copper[33,34]. We observed that EAF showed maximum inhibition of LDL oxidation. This could probably due to high amount of lipid soluble components of EAF which might specifically interact with some lipogenic radicals generated during copper-catalyzed oxidative processes within the hydrophobic core of LDL[34,35].

Taken together, the lipid lowering potential of *Calotropis procera* root bark aqueous extract and its different solvent fractions, mainly EAF have shown a predominant anti-hyperlipidemic activity in terms of reducing serum TC, TG and LDL levels in P-407 induced acute hyperlipidemia in rats. Moreover, plant extract and fractions also demonstrated protective effect against copper-catalysed LDL oxidation, an extensively studied biochemical modification and its implication in atherosclerosis. These observed therapeutic effects can be attributed to the presence of phytochemicals in *Calotropis procera* root bark, most notably, α- and β- amyrin, lupeol and β-sitosterol as previously reported in literature[10,11]. The underlying mechanism of this activity is not elucidated by the present study. However, many researchers have demonstrated various mechanisms by which these phytochemicals produce lipid lowering effect. It is, therefore, presumed that the observed effect of root bark could

be due to inhibition HMG CoA reductase, stimulation lipolytic action of serum LPL and hepatic lipase (HL) enzymes, enhanced elimination of cholesterol through bile and faeces, and increased LDL receptor expression on hepatocytes.

In conclusion, ethyl acetate fraction of *Calotropis procera* root bark aqueous extract could be a potential candidate in the management of hyperlipidemia and prevention of atherosclerosis.

REFERENCES

1. Glass CK, Witztum JL. Atherosclerosis-the road ahead. Cell. 2001;104:503–16.
2. Goldstein JL, Schroot HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease 11: genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest. 1973;52:1544–68.
3. Harrison D, Kathy KG, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. American J Cardiol. 2003;91:7A–11A.
4. Yokoyama M. Oxidant stress and atherosclerosis. Curr Opin Pharmacol. 2004;4:110–15.
5. Hansson GK Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685–95
6. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. Proc Natl Acad Sci U S A. 1990;87:5134–8
7. Toth PP. Drug treatment of hyperlipidaemia: a guide to the rational use of lipid-lowering drugs. Drugs. 2010;70:1363–79.
8. Silva MCC, Batista da Silva A, Teixeira FM, Pereira de Sousa PC, Rondon RMM, Júnior JERH, Sampaio LRL, Oliveira SL, Holonda ANM, Mendes de Vasconcelos SM. Therapeutic and biological activities of *Calotropis procera* (Ait.) R. Br. Asian Pa J Trop Med. 2010;3:332-6
9. A K Meena, Yadav A, Rao MM. Ayurvedic uses and pharmacological activities of *Calotropis procera* Linn. Asian J Tradit Med. 2011;6:45-53
10. Saber AH, Maharan GH, Rizkallah MM. Sterols and pentacyclic triterpenes of 18. *Calotropis procera.* Bull Fac Pharm Cairo Univ. 1969;7:91-104.
11. Saxena VK, Saxena YP. Isolation and study of triterpenoids from *Calotropis procera.* J Res Indian Med Yoga Homeopathy. 1979:14:152-4.
12. VanWagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC, Swithenbank C. Ulosantoin, a potent insecticide from the Sponge *Ulosa ruetzleri.* J Org Chem. 1993;58:335-7
13. Khandelwal KR. *Practical Pharmacognosy.* 19th ed. Pune, India:Nirali prakashan;2009:149-56
14. OECD (organization for economic co-operation and development) Guidelines for the Testing of Chemicals (No. 423) "Acute Oral Toxicity-Acute Toxic Class Method" (Adopted on 17 December 2001). https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
15. Lemos M, Santin JR, Mizuno CS, Boeing T, Barreto de Sousa JP, Nanayakkara D, Bastos JK, Faloni de Andrade S. *C langsdorffii*: evaluation of potential gastroprotective of extract and isolated compounds obtained from leaves. Rev. Bras. Farmacogn. 2015;25: 238–45
16. Johnston TP, Palmer WK. Mechanism of poloxamer 407 induced hypertriglyceridemia in the rat. Biochem Pharmacol. 1993;46(6):1037–42
17. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in serum without use of preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
18. Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, Zou Y, Ge J, Chen H. A modified formula for calculating low-density lipoprotein cholesterol values. Lipids Health Dis. 2010;9:52
19. Wieland H, Sheidel D. A simple special method for precipitation of LDL. J Lipid Res. 1983;24;904-9.
20. Pomory CM. Color development time of the Lowry protein assay. Anal Biochem. 2008;378:216-7.
21. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-ngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KS. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. Leaves. J Ethnopharmacol. 2008;116:439–46
22. Pulla RAC, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol Cell Biochem.1992;111:117-24
23. Montori VM, Brito JP, Ting HH. Patient-centered and practical application of new high cholesterol guidelines to prevent cardiovascular disease. JAMA. 2014;311:465–66.
24. Wasan KM, Subramanian R, Kwong M, Goldberg IJ, Wright T, Johnston TP. Poloxamer-407 mediated alterations in the activities of enzymes regulating lipid metabolism in rats. J Pharm Pharm Sci. 2003;6:189-97.
25. Lee YS, Kim YW, Kim SG, Lee I, Lee MG, Kang HE. Effects of poloxamer 407-induced hyperlipidemia on the pharmacokinetics of carbamazepine and its 10,11-epoxide metabolite in rats: Impact of decreased expression of both CYP3A1/2 and microsomal epoxide hydrolase. Eur neuropsychopharm. 2012;22:431–40,
26. Porter JA, Carter BL, Johnston TF, Palmer WK. Effects of Pravastatin on Serum Lipid Concentrations in Poloxamer 407-Induced hyperlipidemic rats. Pharmacotherapy. 1995;15:92-8

27. Johnston TP, Palmer WK. Effect of poloxamer 407 on the activity of microsomal 3-hydroxy-3-methylglutaryl CoA reductase in rats. J Cardiovasc Pharmacol. 1997;29:580-5.
28. Johnston TP, Nguyen LB, Chu WA, Shefer S. Potency of select statin drugs in a new mouse model of hyperlipidemia and atherosclerosis. Int J Pharm. 2001;229:75-86
29. Lee JJ, Jin YR, Yu JY, Munkhtsetseg T, Park ES, Lim Y, Kim TJ, Pyo MY, Hong JT, Yoo HS, Kim Y, Yun YP. Antithrombotic and antiplatelet activities of fenofibrate, a lipid-lowering drug. Atherosclerosis. 2009;206:375–82
30. John KS, Kathiravan MK, Somani RS, Shishoo CJ. The biology and chemistry of hyperlipidemia. Bioorg Med Chem 2005;15:4674-99.
31. Andersen P. Hypercoagulability and reduced fibrinolysis in hyperlipidemia: Relationship to the metabolic cardiovascular syndrome, J Cardiovas Pharmacol. 1992;20:S29-S31.
32. O'Keefe Jr JM, Lavie Jr CJ, McCallister BD. Insight into the pathogenesis and prevention of coronary artery disease. May Clinic Proceeding. 1995;70:69-79.
33. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002;30:620–50.
34. Visavadiya NP, Soni B, Dalwadi N. Free radical scavenging and antiatherogenic activities of *Sesamum indicum* seed extracts in chemical and biological model systems Food and Chemical Toxicology.2009;47:2507–15
35. Brown JE, Khodr H, Hider RC, Rice-Evans CA. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. Biochem J. 1998;330:1173–8.

Cite this article as:

Devdas D. Santani, Ashishkumar V. Kyada. *In-vitro* low-density lipoprotein protective and lipid metabolism modulatory effects of *Calotropis procera* root bark in poloxamer-407-induced hyperlipidemia. Asian Journal of Pharmacology and Toxicology, 03(12), 2015, 34-42.