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Artigo

Clerodendron glandulosum Coleb., Verbenaceae, ameliorates high fat diet-induced alteration in lipid and cholesterol metabolism in rats

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RESUMO: "*Clerodendron glandulosum* Coleb., Verbenaceae, melhora a dieta rica em gordura induzida por alteração no metabolismo de lipídios e colesterol em ratos." Este estudo foi realizado para avaliar a eficácia do extrato liofilizado das folhas de *Clerodendron glandulosum* Coleb., Verbenaceae (FECG), em alterar o metabolismo de lipídios e colesterol em ratos hiperlipidêmicos alimentados em uma dieta rica em gordura. Plasma e perfil lipídico hepático, lipídeos e enzimas que metabolizam o colesterol em tecidos-alvo e o conteúdo de lipídeos fecais totais e ácidos biliares foram avaliados em ratos normolipidêmicos e hiperlipidêmicos tratados com FECG. Os resultados foram comparados com a droga sintética hipolipemiante Lovastatina (LVS). Os resultados indicam que FECG foi capaz de regular positivamente a hiperlipidemia induzida experimentalmente por alteração significativa no perfil lipídico do plasma e tecidos. Estes resultados podem ser atribuídos à absorção reduzida, a eliminação efetiva e catabolismo aumentado de lipídeos e colesterol, possivelmente devido ao alto teor de saponina e fitoesterois em *C. glandulosum* Coleb. Uso de extrato de *C. glandulosum* como um possível agente terapêutico contra a hipercolesterolemia e hipertrigliceridemia é indicado.

Unitermos: *Clerodendron glandulosum,* Verbenaceae, hiperlipidemia, hipercolesterolemia.

ABSTRACT: The present study was undertaken to evaluate the efficacy of freeze dried extract of *Clerodendron glandulosum* Coleb., Verbenaceae, leaves (FECG) on alteration in lipid and cholesterol metabolism in high fat diet fed hyperlipidemic rats. Plasma and hepatic lipid profiles, lipid and cholesterol metabolizing enzymes in target tissues and fecal total lipids and bile acid contents were evaluated in FECG treated normolipidemic and hyperlipidemic rats. These results were compared with synthetic hypolipidemic drug Lovastatin (LVS). Results indicate that FECG was able to positively regulate induced experimental hyperlipidemia by significant alteration in plasma and tissue lipid profiles. These results can be attributed to reduced absorption, effective elimination and augmented catabolism of lipids and cholesterol possibly due to high content of saponin and phytosterols in *C. glandulosum*. Use of *C. glandulosum* extract as a potential therapeutic agent against hypercholesterolemia and hypertriglyceridemia is indicated.

Keywords: *Clerodendron glandulosum,* Verbenaceae, hyperlipidemia, hypercholesterolemia.

INTRODUCTION

Diets containing high amount of fats or cholesterol lead to both hypercholesterolemia and hypertriglycedemia which are major prognosis for cardiovascular diseases (Reiner & Tedeschi-Reiner, 2006); and leading causes of death in developing and developed countries (Yokozawa et al., 2003) The World Health Organization (WHO) estimates that sixty per cent of the world's cardiac patients will be Indians by 2010 (Radhakrisnan, 2008). In view of the adverse effects of synthetic lipid-lowering drugs, the search for natural products with lipid-lowering potential and with minimal or no side effect is recommended (Adaramoye et al., 2008). In recent times research interest has focused on various herbs that possess hypolipidemic property that may be useful in reducing the risk of cardiovascular disease (Craig, 1999). Because of their perceived effectiveness, minimal side effects and, relative low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathian, 1998). North eastern states of India (biodiversity hotspots) house a treasure-trove of plants with novel medicinal properties (Albert & Kuldip, 2006). These plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their beneficial effects (Sudhir, 2002). *Clerodendron glandulosum* Coleb., Verbenaceae, is a herb

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Clerodendron glandulosum.Coleb extract ameliorates high fat diet/fatty acid induced lipotoxicity in experimental models of non-alcoholic steatohepatitis ☆

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ABSTRACT

This study evaluates the protective role of *Clerodendron glandulosum*.Coleb (CG) aqueous extract against high fat diet/fatty acid induced lipotoxicity in experimental models of non-alcoholic steatohepatitis (NASH). Supplementation of NASH mice with CG extract (1% and 3% in high fat diet for 16 weeks) prevented high fat diet induced elevation in liver enzymes, plasma and hepatic lipids, mitochondrial oxidative stress and compromised enzymatic and non-enzymatic antioxidant status and histopathological damage to hepatocytes. Furthermore, results from *in vitro* study indicates, addition of CG extract (20–200 μg/ml for 24 h) to HepG2 cells minimizes oleic acid induced lipid accumulation, higher lipid peroxidation, cytotoxicity and reduced cell viability. These *in vivo* and *in vitro* studies suggest that CG extract has the potential of preventing high fat/fatty acid induced NASH.

1. Introduction

Non-alcoholic steatohepatitis is a pathological condition characterized by accumulation of lipids in the liver of non-alcoholic individuals and consequent oxidative stress leading to cirrhosis of liver in the long run (Duvnjak et al., 2007). It has received clinical importance only recently after a long lag phase of ignorance mainly due to its asymptotic nature, lack of relevant diagnostic tests and erroneous misinterpretation with hepatitis (Dabhi et al., 2008). Incidence of NASH in USA is 30% of the adult population (Bellentani and Marino, 2009). Even, in Asian countries, number of individuals being diagnosed with NASH is on the rise (Amarapurkar et al., 2007). Obese individuals are at maximum risk of developing NASH (Duvnjak et al., 2007) and a strong positive correlation exists between insulin resistance (IR) and development of NASH (Chitturi et al., 2002), and the same has been validated for animals as well (Chidambaram and Venkatraman, 2010).

Most of the synthetic lipid lowering drugs are efficient in management of hyperlipidemia and obesity but, are of negligible therapeutic value against NASH (Nakamoto et al., 2009). Hence, it is a major challenge for pharmaceutical industry to develop a combination therapy that is effective against NASH in obese and IR individuals. Herbal medicines are becoming increasingly popular and being looked up to for management of hyperlipidemia, obesity and IR, primarily because of their minimal side effects and their multiple mode of action in controlling lipid metabolism. Recently, phytochemical extract of *Alisma orientalis*, *Acanthopanax senticosus*, *Sida rhomboidea* Roxb, etc. have been shown to be effective in controlling these metabolic disorders including NASH, mainly due to their potent hypolipidemic and antioxidant properties (Hong et al., 2006; Park et al., 2006; Thounaojam et al., 2010a). World Health Organization has also recommended use of herbal medicines in lieu of synthetic counterparts in keeping with their fewer side effects, low cost and multiple therapeutic uses (Calixto, 2000).

Clerodendron glandulosum.Coleb (Fam: Verbenaceae), a herb found in north eastern states of India (Albert and Kuldip, 2006), is a folklore medicine consumed by urban and tribal populace of Manipur against diabetes, obesity and hypertension (Jadeja et al., 2009a). A series of evaluations conducted with CG extract in our

Abbreviations: NASH, non-alcoholic steatohepatitis; IR, insulin resistance; CG, *Clerodendron glandulousm*.Coleb extract; HepG2, Human hepatoblastoma cell line; TG, triglycerides; FFA, free fatty acids; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DCF-DA, 2′,7′-dichlrofluorescin diacetate; MDA, malonaldehyde; GSH, reduced glutathione, SOD, superoxide dismutase; CAT, catalase; GST, glutathione-Stransferase; GR, glutathione reductase; GPx, Glutathione peroxidase; OA, oleic acid; HFD, high fat diet; ROS, reactive oxygen species; VLDL, very low density lipoproteins; ApoB, apolipoprotein B; .O2, superoxide radical; H2O2, hydrogen peroxide radical; .OH, hydroxyl radical; LOOH, lipid hydroperoxide; LDH, Lactate dehydrogenate; LPO, lipid peroxidation.

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Anti-obesity potential of *Clerodendron glandulosum*.Coleb leaf aqueous extract

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ABSTRACT

Ethnopharmacological relevance: *Clerodendron glandulosum*.Coleb leaf aqueous extract (CG) is traditionally used by people of North-East India to alleviate symptoms of diabetes, obesity and hypertension. Previous study from our laboratory have documented anti-diabetic and anti-hypertensive properties of CG extract but, till date there are no pharmacological studies available on its anti-obesity potential. This inventory investigates the underlining molecular mechanism/s of CG induced regulation of *in vivo* HFD induced obesity and *in vitro* adipocyte differentiation.

Aim: To evaluate effects of CG extract on (i) expression of genes regulating visceral adiposity and (ii) *in vitro* adipocyte differentiation and LEP release.

Materials and methods: Body weight, lee index, plasma lipids and LEP, mRNA expression of PPARγ-2, SREBP1c, FAS, CPT-1 and LEP in epididymal adipose tissue of control and experimental groups were evaluated. Also, potential of CG extract on *in vitro* adipocyte differentiation and LEP release was assessed.

Results: Supplementation of CG extract to HFD fed mice significantly prevented HFD induced increment in bodyweight, lee index, plasma lipids and LEP, visceral adiposity and adipocyte hypertrophy. Also, CG extract supplementation resulted in down regulation of PPARγ-2, SREBP1c, FAS and LEP expression along with up-regulation of CPT-1 in epididymal adipose tissue compared to HFD fed mice. *In vitro* study recorded significant anti-adipogenic effect of CG extract that resulted in decreased adipogenesis, TG accumulation, LEP release, G3PDH activity along with higher glycerol release without significantly altering viability of 3T3L1 pre-adipocytes.

Conclusions: *Clerodendron glandulosum*.Coleb extract prevents adipocyte differentiation and visceral adiposity by down regulation of PPARγ-2 related genes and Lep expression thus validating its traditional therapeutic use in controlling obesity.

1. Introduction

Obesity has attained grave proportion worldwide (WHO, 2003) and is a causative factor for several life threatening diseases such as diabetes, hypertension and cardiovascular ailments (Aronne and Isoldi, 2007; Shah et al., 2008). Due to undesirable side effects of synthetic anti-obesity drugs use of traditional herbal medicines for controlling weight gain is *in vogue*.

Clerodendron glandulosum.Coleb (CG; Fam: Verbenacea) is an herb endemic to North-Eastern region of India and commonly known as "*Kuthab laba*". Leaves of CG are used by *Apatani* and *Nyishi* tribes of North-East India as a therapeutic agent against hypertension (Kala, 2005; Deb et al., 2009). Rural and urban people of Manipur (India) grow CG in kitchen garden and the leaves are sold in the market. Traditionally, cross sections of people across Manipur consume decoction of CG leaves for treating diabetes, obesity and hypertension (Jadeja et al., 2009). Recent studies from our laboratory have shown that, CG aqueous extract can improve fructose induced insulin resistance and hypertension (Jadeja et al., 2010a), regulate high fat diet induced hyperlipidemia in rats (Jadeja et al., 2010b) and prevent HFD induced hepatic steatosis (Jadeja et al., 2010c). In addition, safety evaluation of CG aqueous extract in Swiss albino mice has revealed its LD_{50} indices to be higher than 3000 mg/kg (Jadeja et al., 2011).

Since, there are no pharmacological reports on anti-obesity potential of CG extract to date and due to promising preliminary results obtained in our laboratory, the present work was undertaken to decipher the influence of CG extract on the expression

Abbreviations: LEP, leptin; PPARγ-2, peroxisome proliferator-activated receptor gamma isoform 2; SREBP1c, sterol regulatory element binding proteins isoform 1c; FAS, fatty acid synthase; CPT-1, carnitine parmitoyltransferase 1; HFD, high fat diet; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; EDTA, ethylenediaminetetraacetic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate buffer saline; DMSO, dimethyl sulphoxide.

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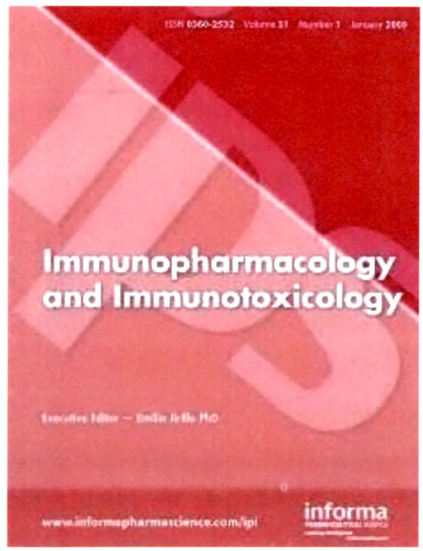

Clerodendron glandulosum.Coleb leaf extract attenuates in vitro macrophage differentiation and expression of VCAM-1 and P-selectin in thoracic aorta of atherogenic diet fed rats

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Clerodendron glandulosum.Coleb extract prevents *in vitro* human LDL oxidation and oxidized LDL induced apoptosis in human monocyte derived macrophages

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ABSTRACT

This study reports the protective role of *Clerodendron glandulosum* (CG) extract against *in vitro* LDL oxidation and Ox-LDL induced macrophage apoptosis using various experimental models. Effect of CG extract on Cu^{2+} mediated LDL oxidation kinetics and formation of various intermediary products and its ability to prevent human monocyte derived macrophage mediated LDL oxidation have been investigated. Ox-LDL induced macrophage apoptosis was evaluated by nuclear condensation, cell cycle analysis, and annexin V-FITC/PI staining in presence or absence of CG extract. Results recorded in the present study clearly suggest the protective role of CG extract against LDL oxidation and Ox-LDL induced macrophage oxidative stress, mitochondrial dysfunction, and apoptosis. This is the first report on the protective role of CG extract on two key events of atherosclerosis portending its possible therapeutic use as an anti-atherogenic herbal medicine.

1. Introduction

Oxidation of LDL is crucial in plaque formation and onset of atherosclerosis (Steinberg, 1997), a cardiovascular disorder posing as the leading cause of death in developed as well as developing countries (Stachura and Pierzynowski, 2009). A number of effects like inhibition of motility of resident macrophages, induction of monocyte differentiation into macrophages, promotion of its uptake by macrophages through scavenger receptors, and causation of subsequent cytotoxicity have all been reported for Ox-LDL (Young and McEneny, 2001). Asmis and Begley (2003) have reported cytotoxicity of Ox-LDL in the form of apoptosis of monocyte derived macrophages. Apparently, macrophage apoptosis plays a key role in development of atherosclerotic lesion as several studies have demonstrated occurrence of macrophage apoptosis in human atheroma (Isner et al., 1995; Kolodgie et al., 1999). Natural antioxidants and lipid lowering interventions such as food supplements, spices, and herbs find extensive application in prevention of atherosclerosis because of their ability to prevent *in vitro* LDL oxidation (Chang et al., 2006; Chu et al., 2009) and plaque formation (Ho et al., 2010).

Clerodendron glandulosum.Coleb (CG; Fam: Verbenaceae) is an herb endemic to North-Eastern region of India and commonly known as "*Kuthab laba*". Apatani and Nyishi tribes of North-East India use leaves of CG as a therapeutant against hypertension (Kala, 2005; Deb et al., 2009) and, a cross sections of people across Manipur traditionally consume a decoction of CG leaves for treating various ailments like diabetes, obesity and hypertension (Jadeja et al., 2009a). Recent studies from our laboratory have demonstrated many favorable effects of CG aqueous extract against fructose induced insulin resistance and hypertension (Jadeja et al., 2010a), high fat diet induced hyperlipidemia in rats (Jadeja et al., 2010b) and hepatic steatosis (Jadeja et al., 2010c) and obesity (Unpublished observations) in C57BL/6J mice, besides its free radical scavenging activity (Jadeja et al., 2009b). Moreover, safety evaluation of CG aqueous extract in Swiss albino mice has revealed its LD_{50} indices to be higher than 3000 mg/kg (Jadeja et al., 2011).

These reports have established the therapeutic property of CG extract in combating metabolic disorders and cardiovascular diseases and, the present study in continuation investigates the efficacy of CG aqueous extract as an anti-atherosclerotic agent using *in vitro* experimental models.

Abbreviations: LDL, low density lipoprotein; Ox-LDL, oxidized LDL; HMDMs, human monocyte derived macrophages; PBS, phosphate buffer saline; MDA, malonaldehyde; LHP, lipid hydroperoxide; CD, conjugated diene; PC, protein carbonyl; ApoB, apolipoprotein B100; nLDL, native LDL; EDTA, ethylene diamine tetra acetic acid; BHT, butylated hydroxytoluene; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulphoxide; DCF-DA, dichlorodihydrofluorescein diacetate; REM, relative electrophoretic mobility; PUFA, poly unsaturated fatty acid.

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