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Protective effects of the leaf extract of *Artemisia campestris* in the damage induced by lipotoxicity in podocytes

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Abstract:

Artemisia campestris of the Asteraceae family is a plant used in traditional Tunisian medicine for its anti-venom, anti-inflammatory, antirheumatic, antimicrobial and antidiabetic properties. Palmitic acid (PA) treatment of podocytes produces inflammation, insulin resistance, oxidative stress, and endoplasmic reticulum stress. The aim of this study was to analyze the protective role of Artemisia extract and their different components in podocyte injury induced by PA. Immortalized conditional mouse podocytes were treated with PA, with or without Artemisia total extract and different components. Analysis of the pro-inflammatory cytokine IL-6, CHOP and the inducible Cox-2 mRNA expression levels showed an increase in podocytes treated with PA that was significantly prevented by the addition of Artemisia extract. Each components of extract showed different protective effects. However, a lost in Akt phosphorylation signal (pAkt) was observed in PA-treated podocytes in the presence of insulin. In contrast, similar PA-treated podocytes with Artemisia extract showed a significant increase of pAKT in the presence of insulin. Conclusion: A. campestris leave extract prevented PA-induced injury in mouse podocytes by anti-inflammatory and anti-oxidative effects and recovered the insulin signaling pathway. This study suggests a potential therapeutic role of A. campestris in the pathogenesis of renal complications associated to obesity.

Keywords:

- Podocyte
- Lipotoxicity
- Artemisia campestris
- Poliphenols.

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Introduction

WHO is supporting ethnobotanical studies and pharmaceutical research on traditional medicinal herbs in order to optimize their use and apply them in health systems. Artemisia campestris is a plant used in traditional Tunisian medicine for its anti-venom, anti-inflammatory, anti-rheumatic, antimicrobial and anti-diabetic properties due to the presence of different components such as flavonoids, chromones, acetophenones, coumarin and essential oils [1]. In recent years, the Reno protective effect of this plant has been seen in different pathologies that affect the kidney [2-7]. Recently, it has been described how extracts from the leaf of another Artemisia genus, specifically Artemisia absinthian L., are able to suppress the growth of liver carcinoma cells through the induction of apoptosis [8]. Currently, obesity is reaching epidemic levels worldwide. The excess of lipids accumulates in non-adipose tissues favoring the damage of these organs in a process called lipotoxicity [9]. The kidney, one of the organs affected by this process. [10-12], begins to accumulate lipid drops, the mechanisms involved being oxidative and endoplasmic reticular stress, inflammation, and the development of insulin resistance [10]. Podocytes, key cells in maintaining the integrity of the glomerular filtration barrier and in the development of insulin resistance, are affected to a large extent by this lipotoxic process [13,14]. Studies conducted in our laboratory have shown that palmitic acid (PA) is a saturated fatty acid that contributes to renal dysfunction associated with the development of the Metabolic Syndrome [8,9]. The aim of the present study was to analyze the protective effect of A. campestris leaf extract on renal damage produced by PA in mouse immortalized podocytes.

Material and methods

Extraction of the leaves of the plant, fresh leaves of A. campestris - were collected in the region of Kssour Essef in the city of Mahdia (Tunisia), cleaned and air dried for several days in a ventilated, dark and temperature-controlled room. Then they were crushed. The dry powder extract was macerated in pure methanol three times. The macerate was filtered and evaporated in a rotary evaporator at 40 ° C. The dry residue was weighed and stored at 4 ° C until use. This extract was dissolved in methanol / H2O (10:90, v / v) and a liquid-liquid partition was performed with solvents of increasing polarity: hexane, to remove lipophilic pigments and residual fat, dichloromethane to separate the components of low polarity, ethanol-acetic to extract the constituents of medium polarity and n-Butane to obtain the polar components and the majority of glycosides. The resulting fractions were concentrated, a rotary evaporator was dried under reduced pressure and weighed to determine its mass. The butanoic fraction was used in all experiments.

Cell culture

Immortalized mouse podocytes obtained in the laboratory of Prof. Richard Coward of the University of Bristol (UK), and were treated as described in Martinez et al., 2015 [11]. The differentiated podocytes were treated with 500 µM of PA for 24 h, with or without n-butanoic fraction at concentrations of 100, 50 and 25 µg / ml.

For the insulin stimulation test, the podocytes were maintained in serum-free medium for 18 h. Then, the cells were incubated with or without PA for 24 h, washed and insulin was added for 5-10 min at a final concentration of 100 nM.

Cell viability against leaf extract of A. campestris was analyzed with propidium iodide by flow cytometry (Cytomics FC500, Beckman Coulter).

qRT-PCRL RNA extraction and quantitative RT-PCR (q) were performed as previously published [11,14]. The specific sequences of oligos used in this study are collected in Table 1 and 2.

Table 1: List of specific primers and probes used in RT-PCR using the reactive Taqman.

	Primer Forward	Primer Reverse	TaqMan-Probe
β- actina	GCTCTGGCTCCTAGCACCAT	GCCACCGATCCACACAGAGT	ATCAAGATCATTGCTCCTCCTGAGCGC
COX-2(Ptgs2)	CCCTGAAGCCGTACACATCA	GTCACTGTAGAGGGCTTTCAATTCT	TTGAAGAACTTACAGGAGAGAAGGAAATGGCTG
CHOP(DDIT3)	CCACCACACCTGAAAGCAGAA	AGGTGAAAGGCAGGGACTCA	CTGGTCCACGTGCAGTCATGGCA
GLUT-4	ACTCATTCTTGGACGGTTCCTC	CACCCCGAAGATGAGTGGG	TGGCGCCTACTCAGGGCTAACATCA

Table 2: List of specific RTD primers used in RT-PCR

IDT	IDT Reference	Gen_Bank ID
IL6	Mm.PT.53.17354790	NM_031168.1

Western blots

The cells were washed with PBS and lifted in a RIPA buffer with protease inhibitors. The protein concentration was measured by the Bradford method. The protein samples were separated on 10% SDS-PAGE gels and transferred to PVDF membranes (BioRad). The membranes were blocked and incubated with the antibodies: antiphospho-AKT (Thr308) (Cell Signaling) and anti-total AKT (Santa Cruz Biotechnology, INC). The bands were densitometrated using the Image 1.45 program (National Institutes of Health, Bethesda, MD). The amount of protein of the control condition was assigned a value of 100% and the rest was relativized to it.

Statistical analysis

The results have been expressed as an average SEM. To determine if there were significant differences, the analysis of variance (ANOVA) was used and, in cases in which the significance reached values of $p < 0.05$, the Kruskal-Wallis

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test was applied, using the statistical program GraphPad-InstatGrafic (GraphPad Software, Inc)

Results

The extract of A. campestris prevents inflammation and endoplasmic reticular stress caused by palpitate.

The podocytes showed a similar cellular viability when exposed to the major components of the butanoic extract of A Campestris or to the way in which these extracts were dissolved (data not shown).The podocytes treated with PA showed a significant increase in mRNA expression of inflammation markers such as Interleukin-6 (IL-6) and Cox-2 (inducible Cyclooxygenase-2), which was significantly decreased by the addition of A. Campestris extract, in dose-dependent dose. In addition, an increase in endoplasmic reticular stress markers such as CHOP (CCAAT / enhancer-binding protein homologous protein) and GRP78 / BiP (endoplasmic reticular chaperone and signaling regulator) is also observed (Fig. 1).

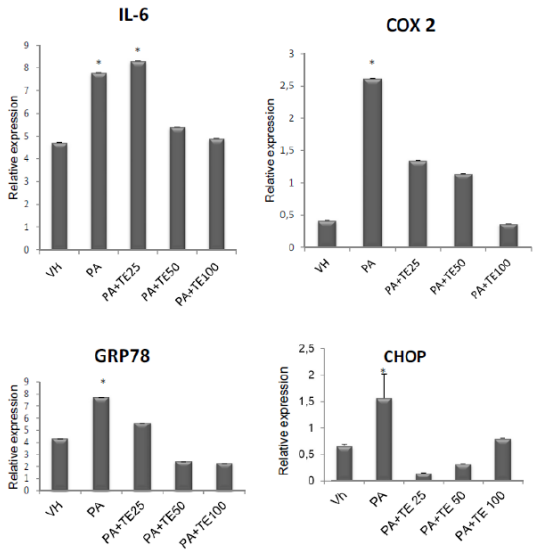

Figure 1: The leaf extract of A. campestris prevents inflammation, oxidative stress and endoplasmic reticular and produces PA in podocytes. A) Levels of mRNA of genes related to inflammation, such as interleukin 6 (IL-6) and cyclo-oxygenase 2 (COX-2); B) Gene levels mRNA related to endoplasmic reticular stress such as: glucose regulated protein 78 (GRP78) and CHOP in podocytes treated with PA and with different doses of A. campestris extract. VH: Vehicle; PA: treated with 500m of PA; PA + TE25: Treatments with PA and extract of A. campestris a25µg / ml; PA + TE25: Treated with PA and extract of A. campestris at 50µg / ml; PA + TE100: Treatments with PA and extract of A. campestris at 100µg / ml. *: P <0.05PAvs. VH N = 3 4 experiments

The extract of A. campestris prevents the insulin resistance of the podocytes treated with palmitate. The extract of A. campestris retrieves the signaling of the markers via Insulin. The podocytes treated with PA showed a loss of phosphorylation of PI3K / PKB (or Akt) in the presence of insulin, previously described by our laboratory [11] that was prevented with the addition of Artemisia extract ($p \leq 0.05$) at similar levels of the podocytes treated via insulin (Fig.2).Also, the analysis of the expression of genes with glucose metabolism, such as GLUT-4 glucose transporter (Fig.2)

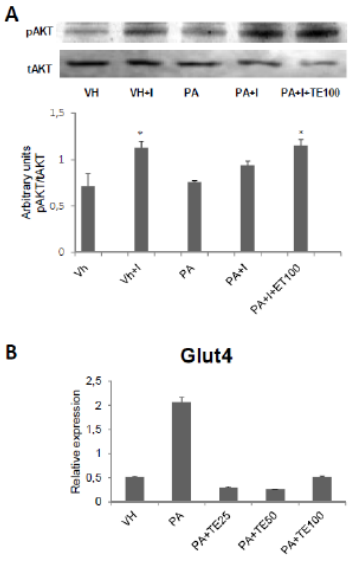

Figure 2: The leaf extract of A. campestris prevents the insulin resistance produced by palmitic acid (PA) in podocytes. A) A representative Western blot and the quantification of pAkt (Ser 473) in podocyte proteins: with conveyance (VH); treated with PA; treated with PA and insulin (PA + I); treated with PA, insulin and 100µg / ml of A. campestris extract (PA + I + TE); The values were normalized with total protein kinase B (tAkt), (pAkt / tAkt); * p <0.05 Vhvs. Vh + I and Vh.vs. PA + I + TE; B) GLUT4.VH glucose conveyor mRNA expression: Conveyor; PA: treated with 500m of PA; PA + TE25: Treatments with PA and extract of A. campestris at 25µg / ml; PA + TE25: Treated with PA and extract of A. Campestris at 50 µg / ml; PA + TE100: Treatments with PA and extract of A. campestris at 100 µg / ml. *: P <0.05 PAvs. VH N = 4 experiments

Discussion

In recent years the development of kidney disease associated with obesity is an emerging concept. In this context, the podocyte, a key piece in the development of kidney disease associated with obesity and the Metabolic Syndrome, is the cell type that is affected by lipotoxicity, causing inflammation, oxidative stress and endoplasmic reticular, cytoskeletal alterations and finally, the cell death [9.6].

The administration of the A. Campestris leaf extract has shown that it has to renoprotective effects [2-7] due to the richness in flavonoids and polyphenols [1,10]. In our study we have shown the anti-inflammatory effect that this extract confers to podocytes, as we have shown by the decrease in basal levels of an inflammatory marker such as interleukin 6 (IL-6) and cyclooxygenase 2 (COX-2) in a dose-dependent way.

The PA produces reticular stress, as indicated by the increase in Chaperone expression (Grp78) and one of the main transcription factors that mediates the stress of RE (CHOP) and, whose last effect is apoptosis [6]. The results of

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the present study show that the extract of A. Campestris prevents this effect because the expression levels of both GRP78 and CHOP return to basal levels when treated with the extract of A. Campestris. In previous studies in our laboratory, lipotoxicity has been shown to be associated with the development of insulin resistance in the kidney and more specifically in podocytes [8,9]. In the present work, the ability of the extract of A. Campestris to avoid the loss of phosphorylation of protein kinase B (PKB or Akt) induced by the treatment with PA is revealed, and therefore, it reflects that the path of Insulin signaling is not blocked at that level, as a consequence of lipotoxicity. In this sense, it has been seen that when A. Campestris extract is administered to diabetic rats, it significantly improves biochemical parameters, decreases proteinuria and oxidative stress [7]. Our study also shows that GLUT4 glucose conveyor expression levels, raised by the effect of the PA treatment, return to baseline values when even low doses of A. Campestris extract are administered.

Conclusions

The leaf extract of Artemisia campestris prevents the PA induced damage in mouse podocytes for its anti-inflammatory effects and for preventing endoplasmic reticular stress. Also, the extract of A. Campestris prevents the loss of the insulin signaling pathway. This study suggests a potential therapeutic role of the extract of A. campestris in the pathogenesis of renal complications associated with lipotoxicity in obesity.

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