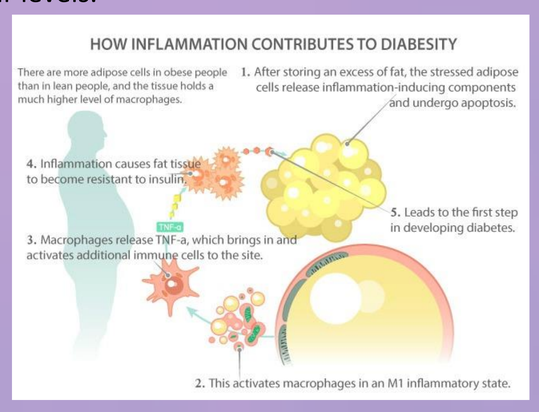
Anti-inflammatory and anti-insulin resistance activities of Andrographolide from Vietnamese *Andrographis paniculata*

Introduction

Diabetes is a lifelong (chronic) disease and is a group of metabolic disorders characterized by high levels of sugar in blood (hyperglycemia). More than 230 million people worldwide are affected, and it is expected to reach 350 million by 2025. It is caused due to deficiency of insulin or resistance to insulin or both. Insulin is secreted by β-cells of pancreas to control blood sugar levels.



Andrographolide from *Andrographis panicula* has been reported widely for multifarious pharmacological activities, some of them being for its antioxidative, antihyperglycemic, immunomodulatory anticancer and α-glucosidase inhibitory effects.

Our aims:

+ Isolate Andrographolide from Vietnamese Andrographis

+ Explore novel functions of Andrographolide for insuliin receptor on 3T3-L1 cell

Materials and methods

Isolation of Andrographolide from *Vietnamese andrographis* (Ho-Young Park et al. 2010)

BMDMs were treated as indicated and processed for analysis by sandwich ELISA, as previously described (Yang et al. 2009).

The levels of cytokines secreted by cell culture and serum were analysed by ELISA reagent (BD Pharmingen).

Results

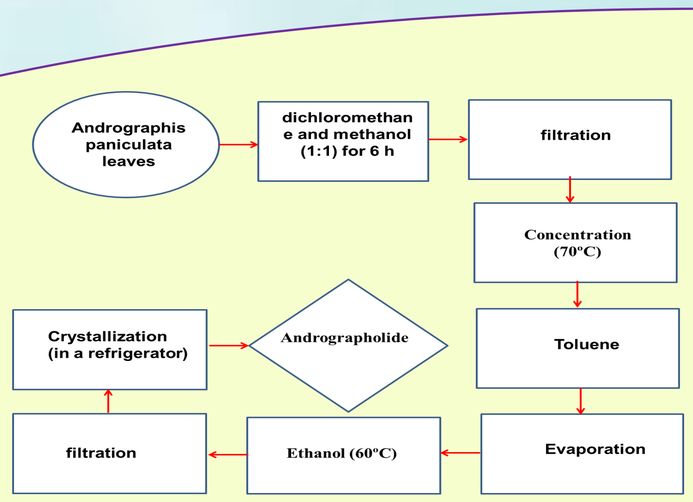


Figure 1. The isolation of Andrographolide from *Andrographis*

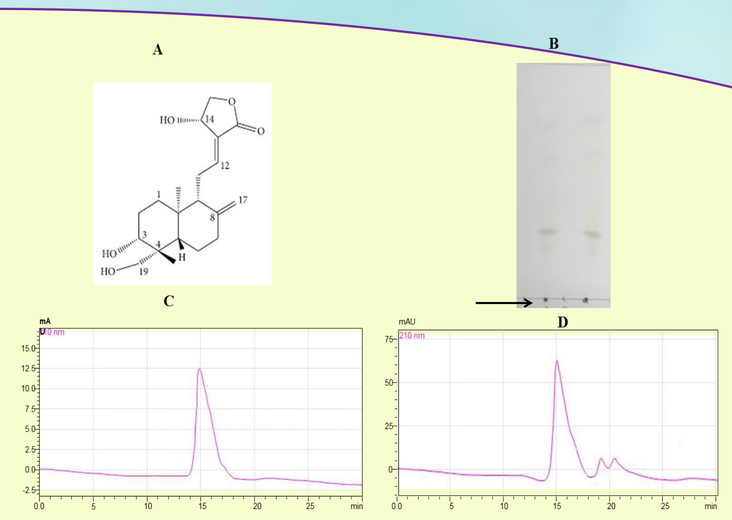


Figure 2. TLC and HPLC for Standard and sample Andrographolide

A) Structure of Andrographolide
B) 1: Standard Andrographolide, 2: Sample Andrographolide
C) HPLC for standard Andrographolide.
D) HPLC for sample Andrographolide.

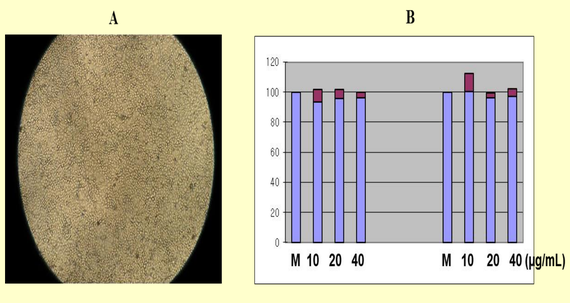


Figure 3. Effect of andrographolide on cell viability

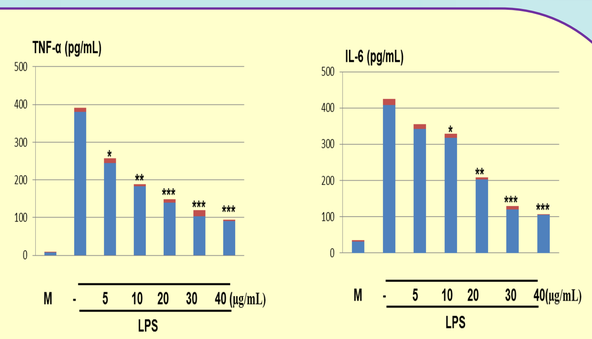


Figure 4. Effect of andrographolide for anti-inflammatition on cells

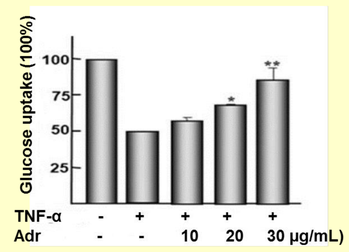


Figure 5. Anti-insulin resistance activity of andrographolide in TNF-α-treated 3T3-L1 adipocytes. * p<0.05, ** p<0.01. Adr, Adrographolide.

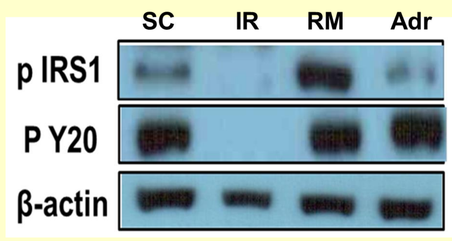


Figure 6. Expression levels of phosphorylated insulin receptor Substrate 1 (p-IRS1) and phosphorylated tyrosine (pY20), in groups treated with adrographolide (Adr) and rosiglitazone maleate (RM) in comparison to the normal and insulin-resistant (IR) groups. β-actin was used as a loading control. The experiment was repeated thrice

A. Raw 264.7 were seeded at a density of 1×10^6 cells/well. After 5 days, the cells were treated with andrographolide (10, 20, 40 μg/mL) for the time indicated. Cell viability was assessed after incubation for indicated time in the presence of CCK-8. SC, solvent control. Data are presented as the mean ± SD of three independent experiments.

B. 3T3-L1 were seeded at a density of 1×10^6 cells/well. After 5 days, cells were treated with andrographolide (10, 20, 40 μg/mL) for the time indicated. Cell viability was assessed after incubation for indicated time in the presence of CCK-8. SC, solvent control. Data are presented as the mean ± SD of three independent experiments.

LPS-induced- TNFα, IL-6 production are inhibited by andrographolide . Raw 264.7 were treated with andrographolide (5, 10, 20, 30, 40 μg/mL) or solvent control (0.1% DMSO) for 20 min before LPS (1 μg/mL) for 18 h .The supernatants were harvested after 18 h and assessed for cytokine production by ELISA. The results are expressed the mean ± SD of five experiments. Significant differences (***P < 0.001), compared with control cultures; SC, solvent control.

Conclusions

Andrographolide (30 μg/ mL) significantly reduced anti-inflammatory production in the lipopolysaccharide (LPS)-treated Raw 264.7. The LPS-induced TNF-α were suppressed by Andrographolide.

Andrographolide (30 μg/mL) exerted anti-insulin resistance activity as it significantly improved the glucose uptake in tumor necrosis factor (TNF)-α treated 3T3-L1 adipocytes.

Enhancement of glucose uptake by andrographolide (30 μg/mL) was accompanied by increased expression of p-IRS1 and pY20.

The results of our study suggest the potential use of andrographolide as anti-inflammatory, anti-insulin resistance agents for prevention of chronic diseases.

References

1. Meenu Sharmz and R.G. Sharma (2013). *Identification, purification and quantification of Andrographolide from Andrographis paniculata (Burm.F.) nees by HPLC at different stages of life cycle of Corp. J. Curr. Chem. Pharm*. Sc: 3(1), 2013, 23-32.
2. Yuta Sugiyama, Yukari Hiraiwa, Yuichiro Hagiya, Motowo Nakajima, Tohru Tanaka and Shun-ichiro Ogura (2018). *5-Aminolevuilinic acid regulates the immune response in LPS-stimulated Raw 264.7 macrophages*. BMC Immunology: 19:41