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Original Article

Lipolysis assay in adipocytes acacia leaf In-Vitro study

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ABSTRACT

Introduction: Lipolysis plays a central role in the regulation of energy balance which is defined as the process of hydrolysing the triglycerides (TG) into glycerol and free fatty acids. Free fatty acids (FFA) are released through this process into the bloodstream with the aim of traveling to other tissues and accordingly exerting other effects throughout the body or re-esterifying by the adipocyte. Methods and Materials: Cell culture- 3T3L1 pre-adipocytes were obtained from NCCS, Pune. Substances were extracted from Acacia leaves (known as the wattles or acacias, belonging to the subfamily Mimosoideae of the pea family Fabaceae) by boiling in 50% v/v alcohol/water for 3 hours. Following centrifugation at 2,000g for 10 minutes, the extracted substances were concentrated and different concentrations were prepared. Results: The addition of extract increased the glycerol content significantly ($p<0.001$) at concentrations 1µg/ml, 10µg/ml and 100µg/ml. The response of acacia extract was in concentration dependent manner. Thus, acacia leaf extract promotes lipolysis in differentiated adipocytes. Conclusions: Acacia leaf extract promotes lipolysis in differentiated adipocytes. It is a natural, effective and non-toxic treatment for obesity and diabetes mellitus among others.

Keywords: Lipolysis; acacia leaf, glycerol, adipocytes, diabetes mellitus.

Introduction

Lipolysis is the biochemical pathway responsible for the catabolism of triacylglycerol (TAG) stored in cellular lipid droplets. The hydrolytic cleavage of TAG generates non-esterified fatty acids, which are subsequently used as energy substrates, essential precursors for lipid and membrane synthesis, or mediators in cell signalling processes. Consistent with its central importance in lipid and energy homeostasis, lipolysis occurs in essentially all tissues and cell types, it is most abundant, however, in white and brown adipose tissue [1].

Agents of natural origin with very little side effects are required as substitute for the chemical therapeutics. A vast range of these natural products and medicinal plants, including crude extracts and isolated compounds from plants can be used to regulate carbohydrate metabolism [2].

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Noorul Huda, Lakshmi Thangavelu, Anitha Roy. Lipolysis assay in adipocytes acacia leaf-in vitro study. J Adv Pharm Edu Res 2018;8(1):32-35.
Source of Support: Nil, Conflict of Interest: None declared.

Increases in adipose tissue mass and adipocyte volume have other broad metabolic consequences including reduced mitochondrial function, increased ER stress, impaired insulin signalling, and higher rates of basal lipolysis [3-6].

A large proportion of the Indian population for their physical and psychological health needs depend on traditional system of medicine. Medicinal plants have become the focus of intense study in term of conservation as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. Herbal medicines are free from side effects and less costly when compared to synthetic drugs [7]. Use of various plant parts like leaves, bark, flowers, fruits, seeds, roots or the whole plant as such for medicinal purpose has a long tradition in different culture. The diverse culture of our country is a rich source of traditional medicines, many of which are of plant origin. Scientific data on such plant derivatives could be of clinical use [8,9,10].

As the prevalence of obesity and Diabetes mellitus are very common in our society, research on plants with antidiabetic and antihyperlipidaemic action has great value in modern therapeutics [9,11,12,13].

Herbs, which are powerful healing agents, must be used appropriately. Medicinal plants contain active ingredients that may interact negatively with prescribed medications or other remedies [10,14,15].

Acacia catechu, commonly known as catechu, cachou and black cutch is an important medicinal plant and an economically important forest tree [11,16,17]. Acacia Catechu belongs to a family

Mimosaceae. It has been traditionally used for the treatment of diarrhoea, dysentery, ulcers, skin eruptions [12,18,19]. The diverse culture of our country is a rich source of traditional medicines, many of which are of plant origin. Scientific data on such plant derivatives could be of clinical use [13,20,21]. Catechu or cutch (Katha in Hindi and Manipuri), the extract prepared from the hard wood of Acacia catechu [14,22,23]. Catechu contains catechuic acid, catechutannic acid (25%-33%), acacatechin (10%-12%), catechu red, quercetin, catechin (2%-12%), epicatechin, phlebotanin (25%-33%), gummy matter, quercitrin, quercitin and moisture. Quercitin is a phe-nolic flavonoid and catechu of acacia is a pseudotanin. Catechu and epicatechin usually accompany other flavonoids [14, 15,24]. It is reported that Acacia catechu has hypoglycaemic activity [16,25].

Catechu was used in the treatment of diarrhoea and throat infection [17,26] because the tannin and polyphenols present in it impart astringent activity. Considering the above facts, the present study was undertaken to evaluate the lipolytic effects of Acacia Catechu on adipocytes.

Materials and Methods

Cell culture

3T3L1 pre-adipocytes were obtained from NCCS, Pune and maintained in DMEM high glucose media with 10% FBS supplemented with penicillin (5units/mL), streptomycin (5µg/mL). Two days' post confluence, 3T3L1 pre adipocytes were switched to differentiation media containing 0.5mM of IBMX, 0.25µM of DEX and 1mg/l of insulin in DMEM medium with 10% FCS. After three days of induction differentiation media was replaced with maintenance medium containing 1mg/mL insulin alone which was replaced with fresh culture medium (DMEM with 10% FCS) after 2 days.

3T3L1 differentiation

3T3L1 pre-adipocytes were cultured in 48 well plate. Two days' post-confluence switched to differentiation medium containing 0.25µM DEX, 0.5 mM IBMX and 1 mg/L of insulin in DMEM medium with 10% FBS to induce differentiation (day 0). After 72hrs of induction, the differentiation medium was replaced with maintenance medium containing 1 mg/mL insulin for 48hrs (day 5). The medium was subsequently replaced again with fresh culture medium (DMEM with 10% FBS) after 2 days. Alternatively, pre adipocytes were maintained with fresh growth every other day throughout the experiment.

Preparation of Acacia leaf extract

Substances were extracted from Acacia leaves by boiling in 50% v/v alcohol/water for 3 hours. Following centrifugation at 2,000g for 10 minutes, the extracted substances were concentrated and different concentrations were prepared.

Measurement of lipolysis

Lipolytic activity was measured by assaying glycerol released from cells into incubation buffer. Briefly, on days 8 to 10, the medium was removed and the differentiated adipocytes were incubated for 10 minutes in serum-free medium with 1% BSA and Krebs- Ringer phosphate (KRP) buffer (128 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 1.35mM MgSO4, and 10mM disodium hydrogen phosphate [Na2HPO4] pH 7.4) containing 1 U/mL adenosine deaminase. Various concentrations of acacia leaf extract in KRP buffer at concentrations of 0.001, 0.01, 0.1, 1, 10, 100 and 1000µg/mL were then added to cells for 6 hours. The incubation mixture was aspirated and used to assay glycerol.

Glycerol assay

Glycerol was assayed enzymatically as described previously. The glycerol assay reagent contained 5mM MgSO4, 0.9mM ATP, 0.9mM Phospho Enol Pyurvate, 6 U pyruvate kinase, 2 U lactate dehydrogenase, and NADH to a final absorbance at 340 nm of 0.9 to 1.0 in potassium phosphate buffer, pH 7.0. The absorbance at 340nm was proportional to the concentration of glycerol.

Results

The physiological effect of acacia leaf extract on lipid storage in differentiated adipocytes was analysed. Ten days after differentiation was induced, 3T3-L1 adipocytes were cultured in the presence of acacia leaf extract (0.001–100µg/mL). After incubation with acacia extract for 24h, the amount of glycerol released into the culture medium was measured. The addition of extract increased the glycerol content significantly ($p<0.001$) at concentrations 1µg/ml, 10µg/ml and 100µg/ml. The response of acacia extract was in concentration dependent manner. Thus, acacia leaf extract promotes lipolysis in differentiated adipocytes.

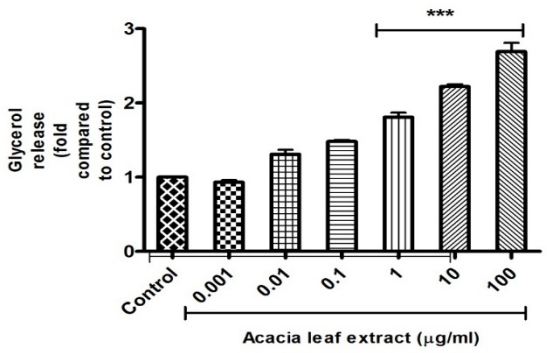

Figure 1

Values are expressed as Mean ± SEM. ***P<0.001 considered significant Control Vs. Acacia extract (figure 1)

Discussion

The results of this study showed that the extracts at different concentrations released glycerol into the culture medium.

The main finding in this study was that as the Acacia leaf extract concentration was increased, the glycerol released was also increased. When the concentration of Acacia leaf extract was 0.001µg/mL the glycerol released into the culture medium was almost equal to the control which is seen to be less than 1. There is a gradual increase in the amount of glycerol released into the culture medium when the Acacia leaf extract concentration was increased upto 100µg/mL. The largest amount of glycerol released was when the concentration of acacia leaf extract was 100µg/mL.

Medicinal plants contain diversity of phytochemicals that serve to protect them against pathogens. These phytochemicals have a proven history in the treatment of various diseases in humans and have served as a source for proven templates for drug

development [3]. Phenols are a diverse group of polar compounds attributed to the treatment and management of various conditions including; haemorrhagic shock, ageing, ischemia, Alzheimer, Parkinson's disease, arthritis, gastrointestinal disorders, carcinogenesis, atherosclerosis and most importantly diabetes mellitus [27-29].

In a study conducted by [30], it was shown that NP induces a lipolytic effect in subcutaneous fat cells and ANP increases lipid mobilization in obese women. However, hypocaloric diet promoted both an increase of NP-induced lipolysis in fat cells and of ANP-mediated lipid mobilization. This shows that natriuretic peptides have a lipolytic effect [30].

In another study, the methanol leaf, root/bark extracts of Acacia nilotica, Sida cordifolia Tinospora cordifolia, Withania somnifera and Ziziphus mauritian showed the activity against B. subtilis, E. coli, P. fluorescens, S. aureus, X. axonopodis pv. malvacearum, A. flavus, D. turcica and F. verticillioides and Plant based products have been effectively proven for their utilization as source for antimicrobial compounds [31].

Literature review on Acacia catechu shows that it has antipyretic, Antidiahorreal, Hyperglycemic, Hepatoprotective, Immunomodulatory, and Anti mycotic activities [32-37].

Conclusion

Acacia leaf extract promotes lipolysis in differentiated adipocytes. It is a natural, effective and non-toxic treatment for obesity and diabetes mellitus among others. More research is indicated to determine the full potential that alternative medicine has to offer in the management of Obesity. With the increasing numbers of patients suffering from obesity and conventional medicine failing to effectively control the problem, alternative therapies can be effectively helpful.

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