S T A T E M E N T

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SUBJECT: Dissertation entitled: *"Mechanisms of modulation of obesity processes in an in vitro model of human adipocytes by the application of biologically active molecules"*, submitted for **the award of the educational and scientific degree "Doctor" in the field 5.11. Biotechnology (Technology of biologically active substances), of mag.-farm. Martina Stoyanova Savova, with scientific supervisor: Prof. Dr. Milen Georgiev.**

Based on Order No. I-64/29.05.2023, I am appointed to prepare an opinion according to the requirements of the Academic Staff Development Act in the Republic of Bulgaria and the Regulations on the Conditions and Procedure for Acquisition of Scientific Degrees and for Occupation of Academic Positions at IMicB-BAS.

Brief biographical data

Education

Martina Stoyanova Savova graduated with a Master of Pharmacy in 2019 from the Faculty of Pharmacy at MU-Plovdiv.

Scientific production

Author and co-author of 176 publications.

She participates as a researcher in 3 international projects.

He has 14 participations in scientific conferences and congresses.

H-Index = 7 - EXTREMELY HIGHLY APPROPRIATE FOR A DOCTORATE.

Work experience

2019-Present - Working as a biologist at the Center for Plant Systems Biology and Biotechnology.

Relevance of the topic

The prevalence of obesity has reached epidemic proportions in recent decades, leading to an increased incidence of co-morbidities such as type 2 diabetes, atherosclerosis, hypertension, and dyslipidemia and a corresponding increase in healthcare costs.

Current recommendations for obesity control include, first and foremost, changes in diet and exercise. However, highly restrictive calorie-restricted diets often pose a major challenge for patients in the long term and, after returning to normal calorie intake, the body makes up for the weight loss. Combining lifestyle changes with drug therapy aids the weight loss process. The approved anorexigenic agents currently available to medicine are limited in both number and mechanism of action. Revolutionary, in terms of weight loss achieved, is the introduction in 2020 of incretin mimetics (originally developed for type 2 diabetes) and in particular the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide. Despite the high efficacy shown by semaglutide in obese patients in terms of metabolic profile and body mass index (BMI), there appears to be a significant percentage of patients who do not respond to this biological therapy. Therefore, the discovery of a safe and effective anti-obesity therapy is of the utmost necessity, taking into account the serious drawbacks and adverse side effects accompanying the drugs approved so far.

Structure of the dissertation

The dissertation submitted in my opinion is structured following the requirements of the Academic Staff Development Act and has all the qualities of, EVEN EXCEEDS THEM, a complete scientific product.

The **literature review** discusses in detail the issues related to the key role and modulation of key signaling pathways related to adipogenesis, energy metabolism, and intercellular and intracellular communication as a possible new approach for the prevention of overweight and obesity. Potential molecular targets for influencing obesity include the major adipogenic transcription factors CCAAT/enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ), as well as their regulated molecular players in lipogenesis such as sterol regulatory binding protein 1 (SREBP1), fatty acid synthase (FASN) and acetyl-CoA carboxylase. In addition, stimulation of lipolysis is a parallel mechanism by which excess weight can be controlled. Another key signaling pathway is that of the phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT) cascade, which is involved in multiple cellular functions, including differentiation and insulin-stimulated glucose uptake in adipocytes.

The purpose of the work is clearly and specifically stated and follows from the conclusions of the literature review. To achieve the goal, 7 specific tasks are formulated.

The method set includes a wide variety of modern in vitro/in vivo methods such as:

- NMR-based metabolic profiling;
- Real-time polymerase chain reaction of miRNAs and microRNAs;
- Western blot analysis of key transcription factors involved in lipolysis;
- Confocal fluorescence microscopy;
- *In vivo* model to study the phenotype and lipid accumulation of obesity in *Caenorhabditis elegans* nematodes;

allowing high reliability of the results obtained.

The establishment of this *in vivo* model of obesity on *Caenorhabditis elegans* nematodes is in line with the requirement to reduce the number of experimental animals, the so-called 3R (reduce, replace, refine) rule.

The results are original, duly, correctly, and comprehensively illustrated. The objective has been met successfully and at an extremely high scientific level.

The obtained results establish in detail the effect of plant extracts and their metabolites from *Polygonum aviculare, P. hydropiper*, and *Z. jujuba* on the function and physiology of adipose cells, as major structural units of adipose tissue, and modulating their inflammatory response. A constellation of methods and models has been developed to reveal the possible molecular mechanism of action of the investigated plants, their extracts, and metabolites. The results of the experiments performed in the present work provide, for the first time, data on the mechanism of action of horsetail leaf extract in a model of obesity in human adipocytes. Inhibition of the PI3K/AKT signaling pathway was identified as the major molecular mechanism of the effect of quinap extract, apigenin, and betulinic acid on adipogenesis. Furthermore, the observed influence of PPARγ and C/EBPα expression upon treatment with quinap extract and selected molecules indicates the involvement of these transcription factors in the mechanism of action of the established anti-adipogenic effect. In conclusion, the thesis builds and successfully defends the hypothesis that all plant secondary metabolites investigated (apigenin, betulinic and olive acids) are involved in the anti-adipogenic effect via PI3K/AKT signaling, which appears to be their common molecular mechanism.

In **discussing** the results obtained, the dissertation has been able to summarize the data obtained and compare them with those available in the literature.

The **conclusions** are stated succinctly and are in line with the aim and objectives. They fully reflect the results of the research.

I agree with the **contributions** made, namely:

✓ Scientific and fundamental:

1. The anti-adipogenic activity of leaf extracts of *Z. jujuba* and aerial parts of *P. aviculare* and *P. hydropiper* in an *in vitro* model of obesity in human adipocytes.
2. The molecular pathways involved in the mechanism of anti-adipogenic action of apigenin, betulinic and butyric acid in adipocytes were determined.
3. The effect of rosmarinic acid in SGBS adipocytes on adipogenesis and lipolysis as well as on the expression of inflammatory factors during adipocyte differentiation was characterized.
4. The inhibitory effect on lipid depots by administration of betulinic acid (10 μM) was shown to be associated with stimulation of nhr-49 and acs-2 expression, whereas at a concentration of 50 μM betulinic acid affected genes related to lipid hydrolysis and the action of desaturases in *C. elegans*.
5. The effect of betulinic acid on the expression of microRNAs whose target genes are involved in lipolysis and lipogenesis in *C. elegans*.

✓ Scientific and applied:

1. An *in vitro* model of adipocyte differentiation was introduced and optimized as a screening platform for the anti-adipogenic potential of plant extracts and natural molecules.
2. A model was adapted to study the phenotype and lipid accumulation in obesity in nematodes of *C. elegans*, which provides an in vivo platform to evaluate the anti-obesogenic potential of molecules of different origins.
3. The data obtained on the molecular mechanism of action of betulinic acid may serve as a basis for the development of product(s) for weight control and obesity prevention.

I would like to express my excellent impression of the detailed design of the study and the overall reading of the development, which confirms the personal contribution of the dissertation to the present work. The dissertation is a logically planned and competently executed research study.

It is noteworthy that the dissertator has applied 6 scientific publications related to the dissertation, of which 4 are in journals with impact factor and Q1, from which 3, the impact

factor is above 7. This repeatedly exceeds the quantitative criteria for obtaining the educational and scientific degree "DOCTOR".

CONCLUSION

The development of mag. Martina Stoyanova Savova is a completed dissertation scientific work, THAT EXCEEDS the requirements of the Academic Staff Development Act in the Republic of Bulgaria. The developed problem is very actual, scientifically substantiated, and dissectable. The results and conclusions of the research are of significant scientific contribution.

Based on the above I give an excellent evaluation of the development and vote POSITIVE for the award of the educational and scientific degree "DOCTOR" in the field 5.11. Biotechnology (Technology of biologically active substances), of mag.-farm. Martina Stoyanova Savova.

11.07.2023
Sofia

Prepared opinion:
/Prof. Magdalena Spasova Kondeva-Burdina, PhD, ERT/