Life Sciences 237 (2019) 116914

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Oral gallic acid improves metabolic profile by modulating SIRT1 expression in obese mice brown adipose tissue: A molecular and bioinformatic approach

Alanna Fernandes Paraíso[a,b], Jaciara Neves Sousa[b], João Marcus Oliveira Andrade[a,b], Eloá Santos Mangabeira[b], Deborah de Farias Lelis[b], Alfredo Mauricio Batista de Paula[b], Andréia Maria Eleutério Barros-Lima Martins[b], William James Nogueira Lima[e], André Luiz Sena Guimarães[b], Geraldo Aclécio Melo[c], Michaela Schwarz[d], Sérgio Henrique Sousa Santos[b,e,*]

[a] Departament of Nursing, Faculdades Santo Agostinho, Montes Claros, Minas Gerais, Brazil
[b] Postgraduate Program in Health Science, Universidade Estadual de Montes Claros (Unimontes), Montes Claros, Minas Gerais, Brazil
[c] Postgraduate Program in Biology, Universidade Estadual de Montes Claros (Unimontes), Montes Claros, Minas Gerais, Brazil
[d] Department of Transplantation Surgery and Section for Surgical Research, Medical University of Graz, Austria
[e] Institute of Agricultural Sciences (ICA), Food Engineering, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Gallic acid
Sirtuins
Obesity
Thermogenesis
Metabolism
Bioinformatic

ABSTRACT

Aims: The aim of the presente study was to examine the effects of oral gallic acid (GA) administration on the brown adipose tissue of obese mice fed with high-fat diet. New mechanisms and interactions pathways in thermogenesis were accessed through bioinformatics analyses.

Main methods: Swiss male mice were divided into four groups and fed during 60 days with: standard diet, standard diet combined with gallic acid, high-fat diet and high-fat diet combined with gallic acid. Body weight, food intake, and blood parameters (glucose tolerance test, total-cholesterol, high-density low-c, triglyceride and glucose levels) were evaluated. Brown and subcutaneous white adipose tissue histological analysis were performed. SIRT1 and PGC1 − α mRNA expression in the brown adipose tissue were assessed.

Key findings: Our main findings showed that the gallic acid improved glucose tolerance and metabolic parameters. These results were accompanied by bioinformatics analyses that evidenced SIRT1 as main target in the thermogenesis process, confirmed as increased SIRT1 mRNA expression was evidenced in the brown adipose tissue.

Significance: Together, the data suggest that the gallic acid effect in brown adipose tissue may improve body metabolism, glucose homeostasis and increase thermogenesis.

1. Introduction

The brown adipose tissue is characterized by multilocular lipid structures and high mitochondria content which is mainly found in the interscapular region of rodents, being responsible for energy transference via UCP1 thermogenic activity, which is localized in the inner mitochondria membrane [1–3]. The brown adipocytes and beige cells (derived from the subcutaneous adipose tissue), are metabolically beneficial due to their unique thermogenic properties [4] (Fig. 1).

Thermogenesis is essential for body temperature maintenance in rodents, as it is a natural biological process of heat generation and energetic homeostasis control, via balance between energy expenditure and intake [4].

Studies have demonstrated gallic acid anti-obesity role in metabolic disorders [5]. Gallic acid, a polyphenol found in red wine and grapes, has a number of therapeutic properties, including anti-inflammatory, anti-oxidant and anti-viral effects contributing in cardioprotection [6,7]. In addition, an animal study showed an anti-obesity effect, improving dyslipidemia in hypercholesterolemic animals [5].

In addition to molecular and mechanistic experiments, the

* Corresponding author. Institute of Agriculture Science, Universidade Federal de Minas Gerais, Av. Universitária, 1000 - JK, Montes Claros, Minas Gerais, 39404 – 547, Brazil.
E-mail address: sergiosousas@hotmail.com (S.H.S. Santos).

https://doi.org/10.1016/j.lfs.2019.116914
Received 19 July 2019; Received in revised form 18 September 2019; Accepted 25 September 2019
Available online 14 October 2019

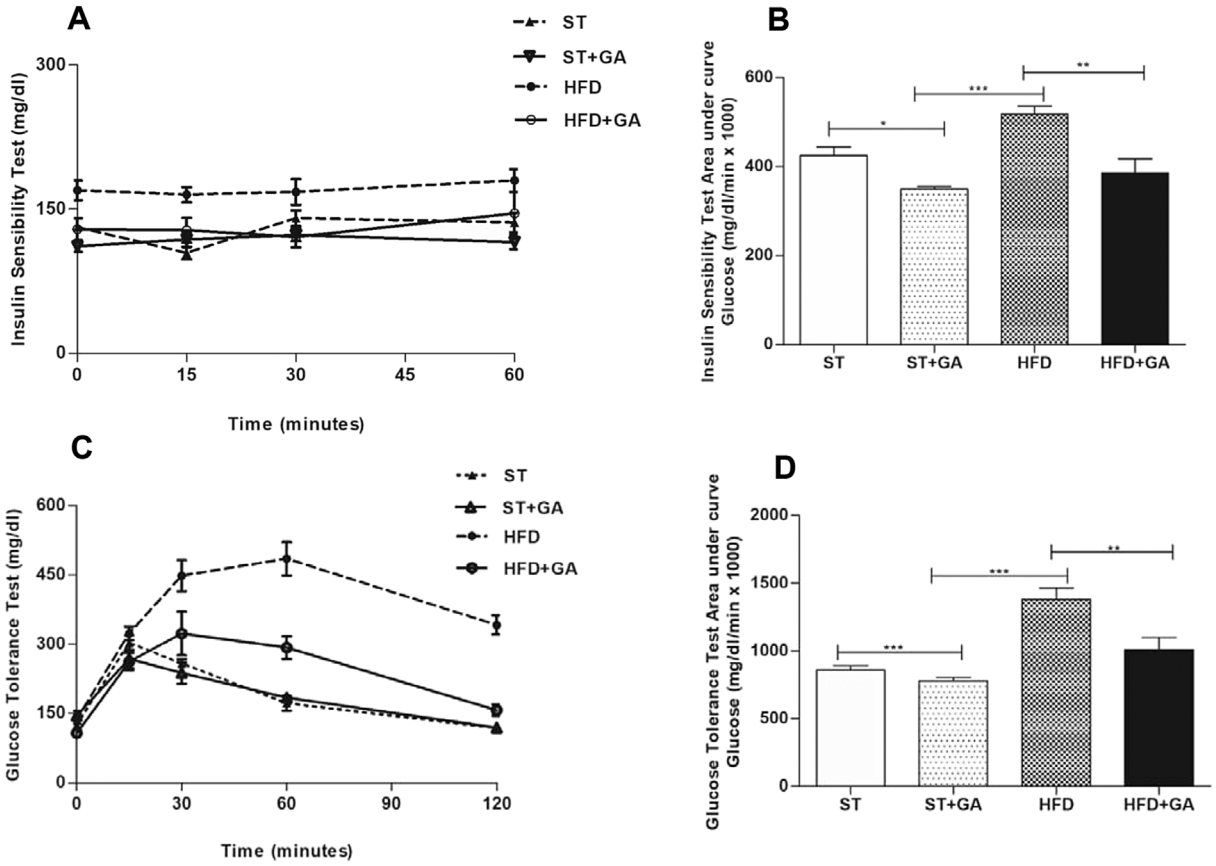

Fig. 1. Gallic acid oral administration effects on insulin sensitivity, glucose tolerance and fasting glucose levels in mice fed standard or high-fat diet. **(A)** Intraperitoneal Insulin Sensitivity Test (IPIST) and IPIST insulin area under the curve (mg/dL) **(B)** Intraperitoneal glucose tolerance test (IPGTT) and IPGTT glucose area under the curve. **(C)**. Fasting Glucose (mg/dL). Abreviations: Standard diet (ST), standard diet plus gallic acid, (ST + GA), high-fat diet (HFD), HFD plus gallic acid (HFD + GA). Values represent an average ± SD (n = 5). Significance was assessed using test Two-Way Anova $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) levels of probability.

bioinformatics approach, a cluster of computational techniques, aimed to organize and clarify the interactions associated to biological macromolecules is a valuable tool to confirm or guide biomolecular investigations. Bioinformatic analysis were already performed to investigate several pathological process such as oral lichen planus, skin carcinoma, pressure ulcer, radicular cyst and periapical granuloma [8–11], and thus might be an useful tool to explore the gallic acid pathways interactions and new action mechanisms involved between this natural compound and thermogenesis and obesity.

The gallic acid molecular mechanisms and effects are still poorly elucidated. Therefore, the present study aimed to evaluate the gallic acid effects on thermogenesis in the brown adipose tissue of high-fat fed mice. Additionally, we propose to investigate the differential involvement of protein-coding genes in thermogenesis, through bioinformatics analyses.

2. Material and methods

2.1. Diets

Obesity was induced in male Swiss mice by high-fat diet (24.55% of carbohydrate, 14.47% of protein, and 60.98% of fat, presenting a total of 5.28 kcal/g of diet) (Rhoster® LTDA, São Paulo, Brazil). The control group was fed the standard diet (ST) (50.30% of carbohydrate, 22.0% of protein, and 7.80% of fat with a total of 2.18 kcal/g of diet), as previously described [10].

2.2. Experimental design

Male Swiss mice (two months old) were obtained from Universidade Estadual de Montes Claros (UNIMONTES). The animals were housed under a 12 h light-dark cycle, and the temperature was maintained at 23.0 ± 2.0 °C. Mice had free access to food and water for the sixty day treatment period. The mice were randomly divided into four groups ($n = 8$each) and respectively fed with a standard diet (ST) (Purina -Labina®), standard diet plus gallic acid, high-fat diet (HFD), and high-fat diet plus gallic acid (GA) (100 mg/kg/body weight) (Sigma Aldrich) [12]. The study was approved by the ethics committee for animal experimentation of the Universidade Estadual de Montes Claros, Minas Gerais, Brazil, by the process nº092 /2015.

2.3. Measurements of body weight, food intake and biochemical investigation

Food intake and body weight gain (BW) were measured twice per week during treatment. At the end of the experimental period, the mice were subjected to a glucose-tolerance test, where D-glucose (2 g/kg of body weight) was intraperitoneally injected into overnight fasted mice. Glucose levels from tail blood samples were obtained at 0, 15, 30, 60 and 120 min after injection. The insulin sensitivity test was performed on overnight-fed mice, after insulin intraperitoneal injection (0.75 units/kg body weight; Sigma–Aldrich®, St. Louis, USA). Tail blood samples were taken at 0, 15, 30, and 60 min after injection. Blood glucose levels were determined using an Accu-Check glucometer (Roche Diagnostics Corp.®, Indianapolis, Indiana, USA) [10].

Blood samples were centrifuged 3200 rpm for 10min, at 4 °C, (Centrifuge Excelsa II, Mod. 206 BL) and the plasma separated for determination of total cholesterol, High Density Lipoprotein (HDL) cholesterol, triglycerides and glucose levels, using enzymatic tests (Wiener Lab., Argentina) and Wiener lab BT3000 analyzer [10,13].

2.4. Histological analyses

The brown and subcutaneous adipose tissue were excised and fixed in 4% buffered-formalin solution and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin. The slides were evaluated under a conventional light microscope (Axioskop40). Images of tissues areas (× 10 ocular and × 40 objective lenses) were captured with an Axio Cam MRcZeis camera. The representative images from mice per group were shown.

2.5. Bioinformatics analysis procedures

Initially, we performed a bioinformatic analysis of human genes related to “thermogenesis”, “gallic acid”, “sirtuin” and “obesity” on the GeneCards database and NCBI (https: //www.ncbi. Nlm.nih.gov/mesh/) [14]. The initial gene list was then expanded using the Web-available software STRING(version 10.0) and STITCH (version 5.0) [15,16] and predicted associations with a higher level of confidence (results with a score ≥0.9) between each gene and all the other genes involved in the analyzed process were obtained. The network obtained was expanded only once, revealing new possible genes associated with the searched mechanisms. The combined score for each gene was adjusted by multiplying by 1000 to obtain a single score, called the Weighted Number of Links (WNL) [9,17,18].The Total Interaction Score (TIS) represents all gene interactions in the entire STRING database.

2.6. Topological and ontological analysis

Topological and closeness analyzes were carried out with Cytoscape. Ontological analysis, and BinGO was applied (Shannon et al., 2003). All two structures (molecular functions and cellular components) controlled vocabularies were used to describe the gene products. The results suggest the molecular pathways involved in the process.

2.7. Reverse transcription and RT-PCR

To confirm the *in silico* analysis of genes from the bioinformatics findings, we performed a real time PCR of the leader genes associated with the thermogenesis process in the adipose tissue. After a 12-h-fasting period, the mice were decapitated and brown and subcutaneous adipose tissue samples (BAT) were collected, weighed and immediately stored at −80 °C.The BATs mRNA were prepared in Trizol reagent (Invitrogen Corp. ®, San Diego, California, USA) and treated with DNAse (Promega). Reverse transcription was carried out with M-MLV (Invitrogen Corp. ®) using random hexamer primers.

Gene expression was normalized to the endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The SIRT-1 and PGC1-α mRNA levels were determined by qRT-PCR and amplified using specific primers and SYBR green reagent (Appllied Biosystems®, USA) in Quant Studio 6 flex, 96 wells equipment (Applied Biosystems®). The expression analyzes were performed using the $2\Delta\Delta^{CT}$method [19].

2.8. Statistical analysis

All data was transferred to Graph Pad Prism software (Version 5.0®, San Diego, USA) and submitted to specific tests with a statistical confidence of 95% ($p < 0.05$). Data are expressed as the mean ± SEM. The statistical significance of the differences in mean values between mice groups were assessed by one-way ANOVA or two-way ANOVA (glucose tolerance and insulin sensitivity tests) and the Tukey post-test.

Bioinformatics analyzes were performance using SPSS (Version 18.0,1BM, New York, NY, USA). Genes were ranked according to this parameter in clusters, by the clustering method K-means [10,11,20]. The differences between the various classes in WNL terms were assessed using Kruskal-Wallis tests ($p < 0.001$). WNL and TIS values are used for different purposes [10,11,18,20,21]. The TIS is associatedwithgreat interactionswhile WNL is relatedto specific network interactions [11]. The categories with higher both WNL and TIS were chosento identify the genes that have more interactions.

3. Results

3.1. Gallic acid treatment improved metabolic parameters in HFD fed mice

Body weight was significantly decreased in ST + GA (54.8 ± 5.972) and HFD + GA (49.0 ± 1.834) when compared with the control group ($p < 0.01$). Adiposity was significantly increased in ST (0.040 ± 0.014) as compared with ST + GA (0.022 ± 0.011) and HFD (0.075 ± 0.009) as compared with HFD + GA (0.039 ± 0.010). The energy intake per body weight were similar between treatments submitted to the same diet type (Table 1).

The serum high-density lipoprotein levels were not different among groups. Although there were no significant differences, the HFD + GA group showed an increase in HDL when compared to the control group. We observed a significant decrease in cholesterol showing a statistical association with ST + GA (101.5 ± 9.29) as compared to HFD (157 ± 18.29) and HFD + GA (154 ± 33.74) groups ($p < 0.0042$). However there were no statistical differences between groups HFD and HFD + GA. The triglycerides were reduced in animals fed standard diet plus gallic acid (147 ± 7.57) and HFD + GA (144 ± 8.71) as compared to HFD (183 ± 5.03) ($p < 0, 02$).

The glucose levels in fasting assessed during the insulin sensitivity test (Fig. 1B) were markedly increased in the experimental group fed high-fat diet (170.6 ± 6.45) as compared to the HFD + GA group (130.9 ± 10.4) ($p < 0,05$). There was also a decrease in glucose levels in the ST + GA group when compared to the control group. In the glucose tolerance test, the area under the curve was larger in the HFD group compared to the HFD + GA and ST + GA treated groups (Fig. 1D).

Table 1
Gallic acid effects on metabolic parameters.

Groups	ST	ST + GA	HFD	HFD + GA
Energy Intake	0,27 ± 0,11	0,2856 ± 0,11	0,36 ± 0,19	0,38 ± 0,16
Initial Body Weight	23,30 ± 1,57	22,9 ± 0,77	22,20 ± 2,32	22,30 ± 1,20
Final Body Weight	63,20 ± 6,06	54,8 ± 5,97*	76,20 ± 5,34	49,00 ± 1,83*
High-Density Lipoprotein	65,95 ± 11,06	75,95 ± 24,63	88,05 ± 15,06	112,6 ± 20,13
Total Cholesterol	117,0 ± 6,000	101,5 ± 9,292	157,0 ± 18,29	154,0 ± 33,74
Triglycerides	160,7 ± 22,30	147,3 ± 7,572*	183,3 ± 5,033	144,0 ± 8,718*
Glucose	90,00 ± 20,4	85,50 ± 13,10	79,50 ± 13,80	92,00 ± 7,211

ST, standard diet; ST + GA, standard diet plus gallic acid; HFD, high-fat diet; HFD + GA, high-fat diet plus gallic acid. $p < 0.05$

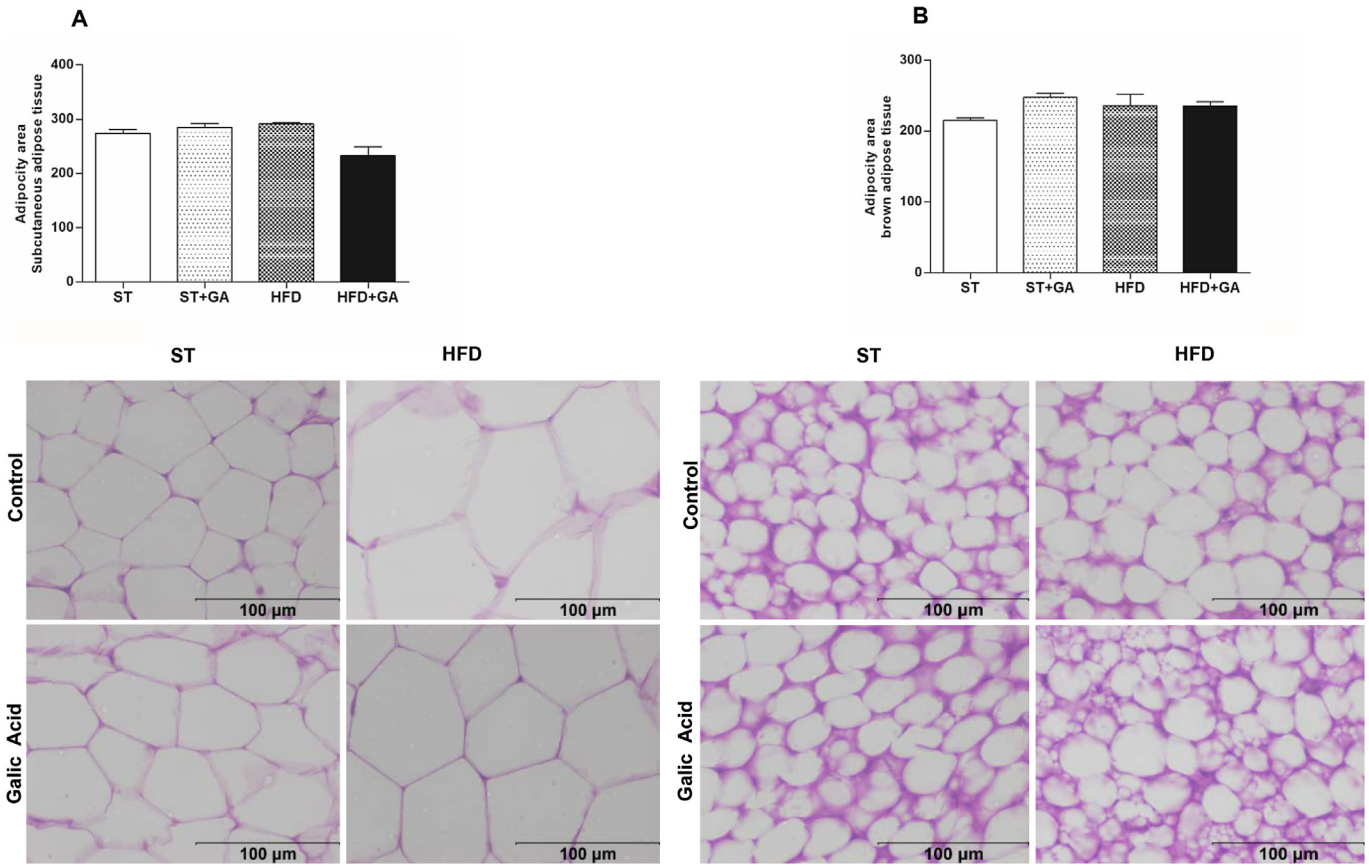

Fig. 2. Gallic acid oral administration effects on adipocity area subcutaneous and brown adipose tissue in mice fed standard or high-fat diet. **(A)** Subcutaneous adipocity area (bar graph). **(B)** Brown adipocity area (bar graph). **(C)** Histological section of adipose tissue stained with Hematoxylin/eosin staining. Scale bar, 100 µm. Abreviations: Standard diet (ST), standard diet plus gallic acid, (ST + GA), high-fat diet (HFD), HFD plus gallic acid (HFD + GA). Values represent an average ± SD (n = 5). Significance was assessed using test Two-Way Anova $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) levels of probability. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The glucose area under the curve (AUC) graphs based on the IPIST (Fig. 1B) and IPGTT (Fig. 1D), evidenced that an eight week drug treatment decreased the glucose AUC in treated mice. The glucose levels were higher in HFD animals as compared to HFD + GA. Gallic acid treatment in both control and HFD groups improved insulin sensitivity and glucose tolerance in mice.

3.2. Brown and subcutaneous adipose tissue morphologic analyzes

Histological adipose tissue analysis were performed to examine the gallic acid effects on thermogenesis. The histological analysis were based on the adipocyte size (Fig. 2A–B). Brown and subcutaneous adipose tissue analysis did not showed statistically differences between groups of treatment and control. However, a numerical decrease in adipocyte area (WAT) can be observed in the obese group after treatment with GA (Fig. 2B).

Bioinformatics analyses reveal the leader genes potentially modulated by Gallic acid during thermogenesis in brown adipose tissue.

With the aim at investigating the mechanisms associated with “thermogenesis”, “gallic acid”, “sirtuin” and “obesity”, an *in silico* analysis were performed using the software platform cytoscape for visualization of molecular interaction networks. All topological analyses were carried out with Cytoscape and Biological Networks Gene Ontology (BINGO) tool. The protein interaction map obtained in the STRING software is displayed in Fig. 3A–C. Regarding the keywords used”, the preliminary query in gene cards suggested 5 genes (UCP1, LEP, PPARGC1A, NAMPT, NR1H2). One expansion was performed in order to expand to 5 more interactions (Fig. 3A). Analyzes obtained from STITCH also guided our actions in choosing the genes to be evaluated in this gallic acid study (Fig. 3B).

The network exhibited the following power law behavior: correlation: 0.960; R2:0.964 (Fig. 4A) in agreement with the scale-free theory of network. In this context, closeness analysis, which measures the grade of proximity of a node to the rest of nodes, was performed.The larger the value, the faster the information spreads through this node (correlation: 0.886; R2:0.765) (Fig. 4B). Considering genes that presented a combination of higher WNL and lower TIS, sirtuin 1 (SIRT1) was considered the main target for the thermogenesis process (Fig. 4C and D). The heat map was built based on WNL and TIS values (Fig. 4E).

The ontological analysis demonstrated that regulation of molecular function (transcription regulator activity) and cellular component (nucleoplasm) (Fig. 5A and Fig. 5B), are the main biological processes involved. The protein interaction network and the leader gene are presented in Fig. 5C.

The mice group treated with gallic acid displayed increased SIRT1 and PGC1-α mRNA expression in the brown adipose tissue of high-fat fed mic treated with as compared to the control group (Fig. 6A–B). Additionally, PGC1-α expression was increased in the HFD + GA group as compared to the group ST + GA (Fig. 6A).

4. Discussion

In the present study, we evaluated for the first time the thermogenic effects produced by oral gallic acid in a model of metabolic stress induced by high-fat diet. We observed a reduction in body weight, and triglycerides serum levels in animals treated with gallic acid

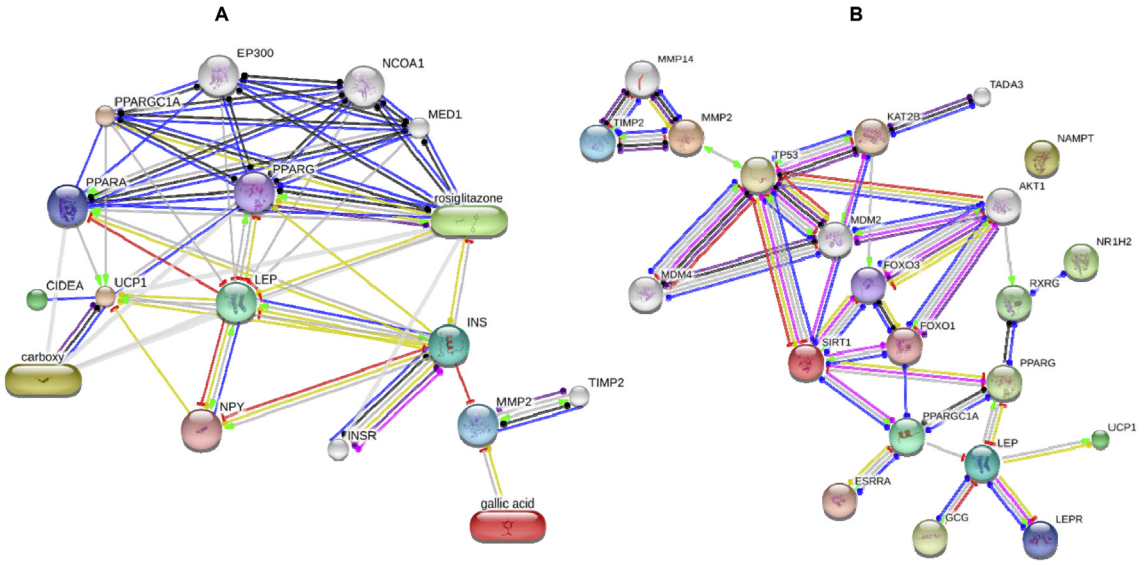

Fig. 3. Data was derived from STRING and STITCH (confidence level > 0.9). Down-regulation is a red bar and up-regulation is a green arrow. Yellow circle represents the interaction direction, although it is not possible to assure if it is down or up regulated (e.g., if it is up- or down-regulated). Black circle at both ends means interaction between two proteins, but we do not know whether it is down or upregulated. In deep blue:binding; in blue: phenotype; in indigo blue: catalysis; in violet: post translation; in black: reaction; and in yellow: expression. **(A)** Gene interaction map and up- and down-regulated genes involved in the following conditions: "thermogenesis", "gallic acid", "sirtuin" and "obesity" **(B)** Gallic acid protein interaction network for Stitch. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

accompanied along with increased glucose tolerance, insulin sensibility and thermogenic process. More importantly, we showed for the first time, through bioinformatics analyzes, new possible mechanisms by which the gallic acid modulated thermogenesis, where sirtuin 1 (SIRT1) was considered the main target.

Corroborating our data, Hsu & Yen also demonstrated the reduction in liver TAG and cholesterol levels in the HFD + GA animals [28]. The treated animals exhibited a significantly decreased body weight gain with steady food intake. Doan et al. corroborates these findings by showing that gallic acid was able to induce a body weight reduction and prevent metabolic disorders without changing the food intake [12]. New studies have shown the high-fat diet prejudicial effects on metabolic performance. A study performed by Latha and Daisy corroborates our data by evidencing a beneficial metabolic role of this compound and its anti-obesity effects such as decreased visceral fat, demonstrating the gallic acid potential modulate body composition [5].

Latha & Daisy also showed that gallic acid lowers blood glucose with a simultaneous increase in plasma insulin [5]. A study have reported that this chemical compound has antidiabetic or anti-hyperglycemic activity. Prasad and collaborators, through in vitro studies showed that gallic acid induces GLUT4 translocation with subsequent stimulation of glucose uptake, thus exerting antidiabetic effects [22]. We have also reported that the improved glucose tolerance observed in our treatment model indicated a greater insulin release during hyperglycemia.

Our study proposed to explore through bioinformatics analyzes, the molecular mechanisms to explain the gallic acid signal effectors and its potential role in thermogenesis. Based on our findings we have outlined a proposed molecular pathway whereby SIRT1 (Fig. 3A) has emerged as critical for the molecular basis of thermogenesis. Employed by browsing networks for genes of interest, inspecting interaction evidence and performing interactive clustering, the SIRT1 gene was obtained as leader.

Our study is the first study that associated bioinformatics analyses with the gallic acid on obesity and thermogenesis. Bioinformatic analysis are performed using public databases, scientific publishing databases and gene database, to add knowledge about genes and molecular mechanisms involved in disease pathogenesis and to point to possible therapeutic targets. In this study, SIRT1 was identified as the main gene involved in the gallic acid effects in the thermogenesis process, based in the literature search and laboratory experiments.

Some metabolic benefits described for different models and drug tests were previously attributed to increased SIRT1 expression, which is an important regulator of metabolic disorders. Initially described in yeasts, the sirtuins comprise a 7 component enzymes group (each type with specific functions and locations) that deacetylates histones in mammals. The SIRT1 is located in the nuclei and is associated to epigenetics regulation, especially of histones and transcription factors [23].

Corroborating these findings, Doan and cols showed that gallic acid regulates mitochondrial gene expression via SIRT1-PGC1-α through the AMPK in brown adipose tissue in animals in dietary stress conditions and in vitro experiments. This was the only study that reported the gallic acid effects on thermogenesis. Interestingly, and correlating with our findings, PGC1-α is considered a central inducer of mitochondrial biogenesis necessary for the thermogenic process, thus being considered an important marker [12].

Ramadori et al. reported that SIRT1 gene deletion affects leptin signaling by reducing energy expenditure. These observations suggest a pivotal role of the sirtuins in fat mass control and adipokine regulation [24]. The SIRT1 increased expression in white adipose tissue and pancreatic β cells improves glucose tolerance and increases insulin secretion in response to glucose, while the SIRT1 deletion impairs insulin secretion and glucose uptake [25]. Several studies reported that SIRT1 is involved in different metabolic processes. In mammals, SIRT1 is an important regulator of energy homeostasis, playing a role in glucose and lipid metabolism [26].

Other studies demonstrated the SIRT1 involvement in protecting against metabolic damage induced by high-fat diet and inflammation in adipose tissue [27,28]. Our results showed an association between gallic acid and increased SIRT1 expression in brown adipose tissue.

Moreover once again, the present study is the first to show interaction networks between protein-coding genes, leader genes and molecular pathways using bioinformatics analyzes, associating gallic acid treatment, thermogenesis and obesity. The TIS is associated with extensive interactions while WNL is related to specific network

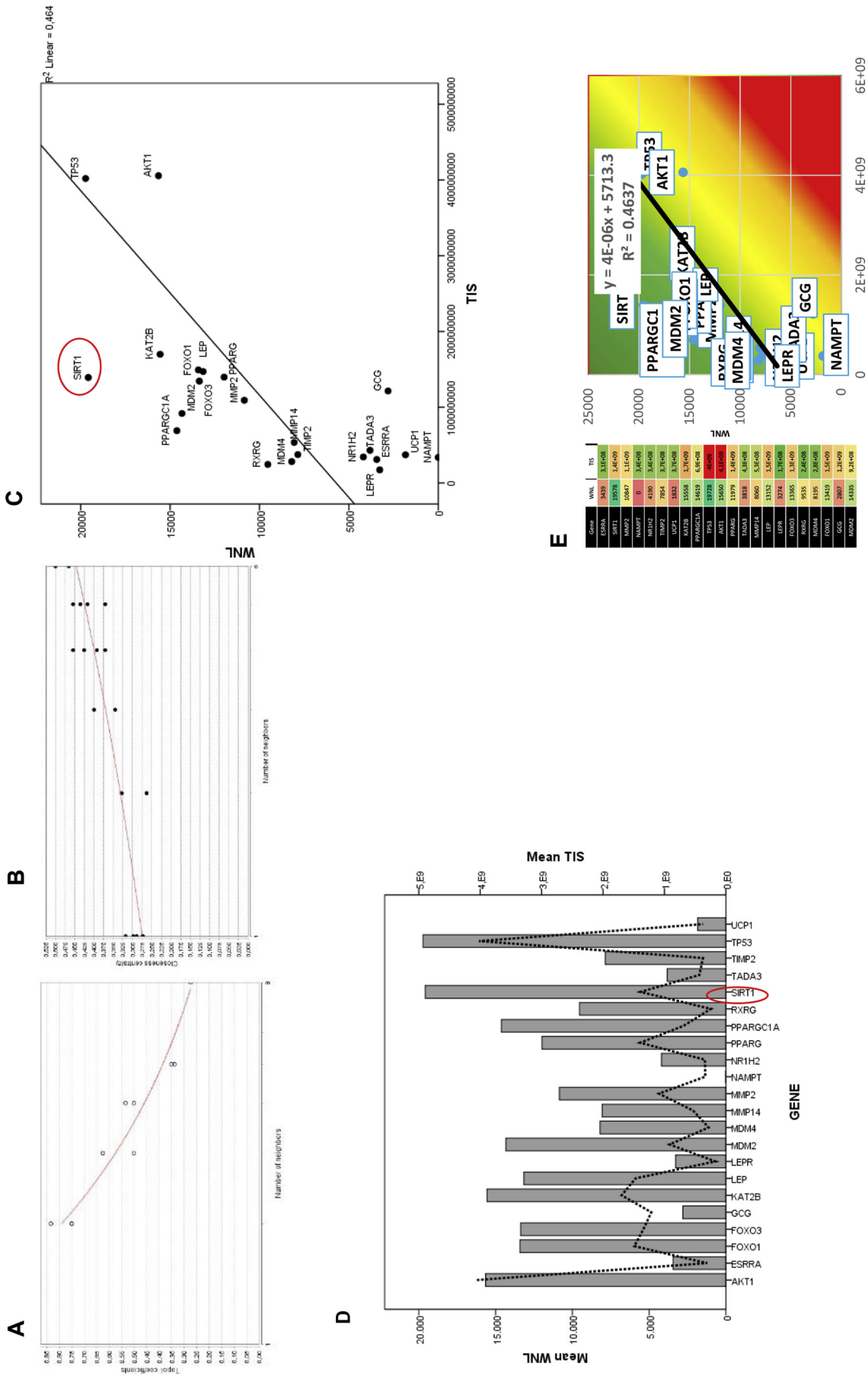

Fig. 4. Data analysis genes involved in the studied conditions: "Thermogenesis", "gallic acid", "sirtuin" and "obesity" **(A)**. Network exhibited the following power law behavior (correlation: 0.960; R2:0.964). **(B)** Closeness centralities (correlation: 0.886; R2: 0.765) **(C)**. Scatter diagrams showing condition-related connectivity (WNL, weighted number of links) versus the global connectivity (TIS Interactions Total Score). **(D)** Relationship between WNL and TIS means. **(E)** Heat map, the green area represents higher WNL and lower TIS. (For the reference interpretation in this figure legend, the reader is referred to assess the web version of this paper). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

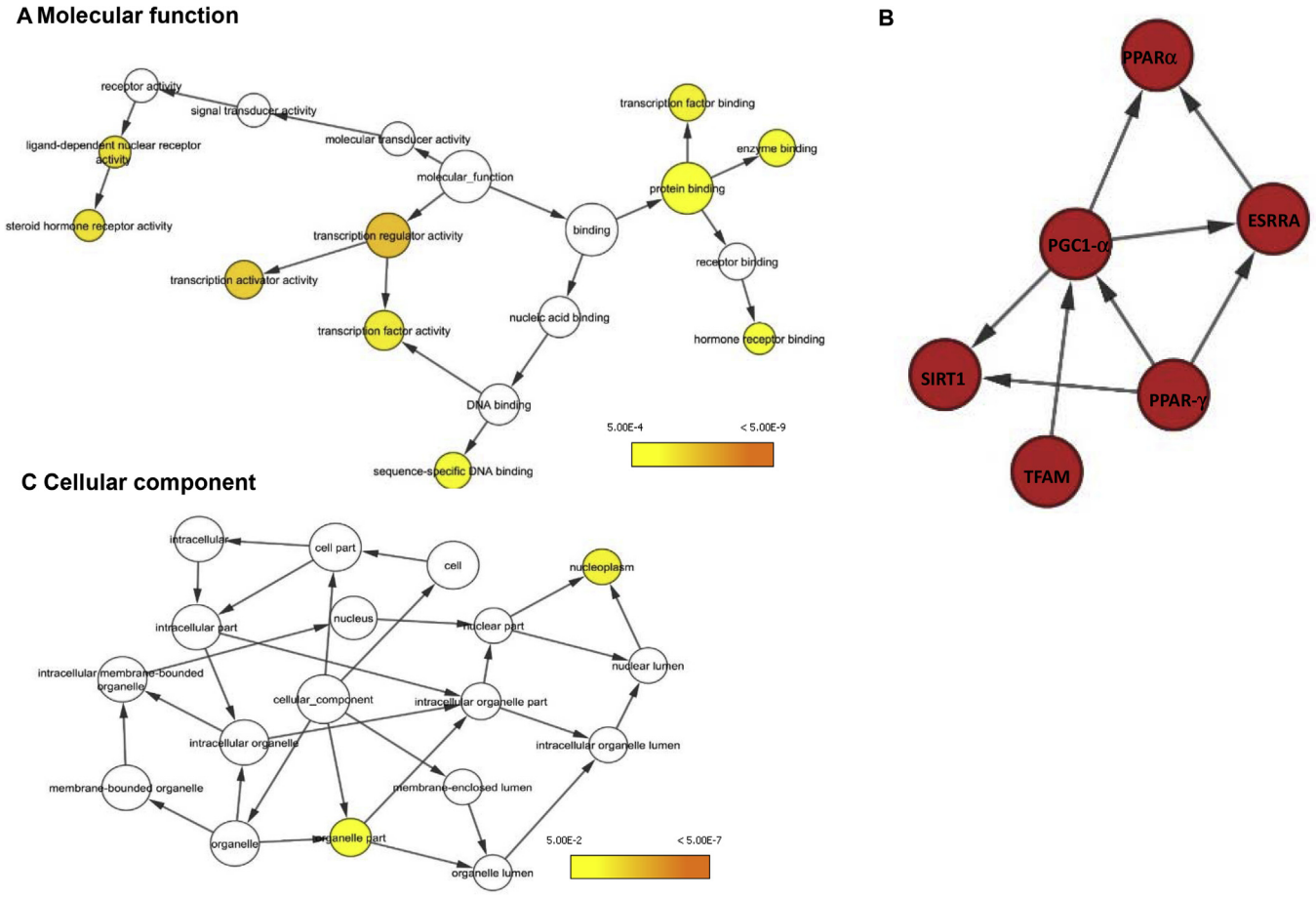

Fig. 5. Network ontological analyzes showing the most important pathways over-represented in the graph versus whole set annotation, carried out with BinGO software (p-value < 0.0001, Benjamini-Hochberg correction, hypergeometric clustering). **(A)** Molecular function and **(B)** Cellular component. (**C)** Pathway network leader gene Mcode.

interactions [11]. Considering only WNL values for gene clustering might be a confusion factor. Since genes with higher TIS a value acts in manifoldness biological process. The WNL and TIS values combined for the gene clustering is critical to identify targets genes [11].

Bioinformatics analyses for a specific phenomenon can potentially disclose knowledge about protein-protein, direct or indirect, interactions, cellular processes and molecular mechanisms. Our data shows a gene interaction map considering both direct and indirect physical functional protein linkages. For this reason further experimental tests and target therapies may be planned.

Previous studys reported the involvement of AMPK in the therapeutic effects of GA [12] and also resveratrol/Sirtuins modulation oh thermogenesis [29]. In the current case AMPK is known as an important metabolic second messenger despite it is not specific (which made association between TIS and WNL suggested not to include AMPK).

5. Conclusion

In conclusion, the present study contributes to elucidate the gallic acid metabolic role by inducing SIRT1 and PGC1-α high expression in group treated with high-fat diet, which is a leader gene demonstrated

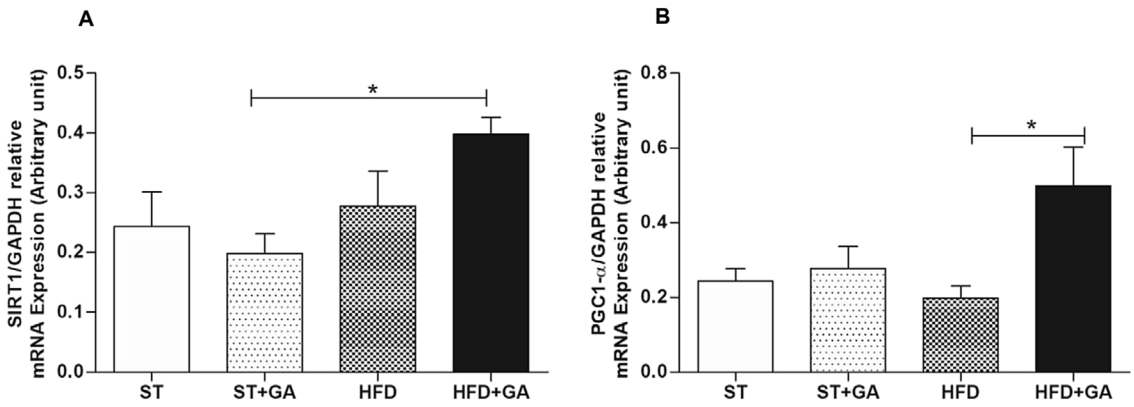

Fig. 6. 8-week gallic acid effects on SIRT1 and PGC1-α expression in the brown adipose tissue of high fat fed mice. **(A)** SIRT1. **(B)** PGC1-α

by the bioinformatic analyzes and might be responsible for thermogenesis activation under a high-fat diet. Furthermore, these results support the hypothesis that the gallic acid might be used as a potential therapeutic agent for the prevention of obesity-related disorders. However, more studies are necessary to elucidate the pathways and mechanisms by which this acid modulates the metabolism.

Declaration of competing interest

The authors have no competing interests.

Abbreviations

AUC	Area under the curve
BAT	Brown adipose tissue
BW	Body weight gain
CPT-1	Carnitine Palmitoyltransferase 1
GA	Gallic acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT4	Glucose Transporter type 4
HDL	High Density Lipoprotein
HFD	High-fat diet
LEP	Leptin
NAMPT	Nicotinamide phosphoribosyltransferase
NR1H2	Nuclear receptor subfamily 1 group H member 2
PGC1-α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα	Peroxisome proliferator-activated receptor
SIRT1	Sirtuin 1
ST	Standard diet ()
TIS	Total Interaction Score
WNL	Weighted Number of Links
UCP1	Uncoupling Protein-1

Funding

This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES and Fundação de Amparo à pesquisa do Estado de Minas Gerais (FAPEMIG). Dr Santos, Dr Guimarães e Dr De Paula are research fellows of CNPq.

References

[1] B. Cannon, J. Nedergaard, Brown adipose tissue: function and physiological significance, Physiol. Rev. 84 (2004) 277–359.
[2] S.M. Labbe, A. Caron, D. Lanfray, B. Monge-Rofarello, T.J. Bartness, D. Richard, Hypothalamic control of brown adipose tissue thermogenesis, Front. Syst. Neurosci. 9 (2015).
[3] R. Oelkrug, E.T. Polymeropoulos, M. Jastroch, Brown adipose tissue: physiological function and evolutionary significance, J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 185 (2015) 587–606.
[4] A. Bartelt, J. Heeren, Adipose tissue browning and metabolic health, Nat. Rev. Endocrinol. 10 (2014) 24–36.
[5] R.C. Latha, P. Daisy, Insulin-secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from Terminalia bellerica Roxb in streptozotocin-induced diabetic rats, Chem. Biol. Interact. 189 (2011) 112–118.
[6] H.N. Graham, Green tea composition, consumption, and polyphenol chemistry, Prev. Med. 21 (1992) 334–350.
[7] S.H. Kim, C.D. Jun, K. Suk, B.J. Choi, H. Lim, S. Park, et al., Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells, Toxicol. Sci.: Off. J. Soc. Toxicol. 91 (2006) 123–131.
[8] B. Orlando, N. Bragazzi, C. Nicolini, Bioinformatics and systems biology analysis of genes network involved in OLP (Oral Lichen Planus) pathogenesis, Arch. Oral Biol. 58 (2013) 664–673.
[9] F.O. Poswar, L.C. Farias, C.A. Fraga, W. Bambirra, M. Brito-Junior Jr., M.D. Sousa-Neto, et al., Bioinformatics, interaction network analysis, and neural networks to characterize gene expression of radicular cyst and periapical granuloma, J. Endod. 41 (2015) 877–883.
[10] T.A. Pinheiro, A.S. Barcala-Jorge, J.M.O. Andrade, T.A. Pinheiro, E.C.N. Ferreira, T.S. Crespo, et al., Obesity and malnutrition similarly alter the renin-angiotensin system and inflammation in mice and human adipose, J. Nutr. Biochem. 48 (2017) 74–82.
[11] F.O. Poswar, L.I. Santos, L.C. Farias, T.A. Guimarães, S.H.S. Santos, K.M. Jones, et al., An adaptation of particle swarm clustering applied in basal cell carcinoma, squamous cell carcinoma of the skin and actinic keratosis, Meta Gene 12 (2017) 72–77.
[12] K.V. Doan, C.M. Ko, A.W. Kinyua, D.J. Yang, Y.H. Choi, I.Y. Oh, et al., Gallic acid regulates body weight and glucose homeostasis through AMPK activation, Endocrinology 156 (2015) 157–168.
[13] A.S. Jorge, G.C. Jorge, A.F. Paraiso, R.M. Franco, L.J. Vieira, A.M. Hilzenderger, et al., Brown and white adipose tissue expression of IL6, UCP1 and SIRT1 are associated with alterations in clinical, metabolic and anthropometric parameters in obese humans, Exp. Clin. Endocrinol. Diabetes 125 (2017) 163–170.
[14] M. Rebhan, V. Chalifa-Caspi, J.D.L. Prilusky, GeneCards: integrating information about genes, proteins and diseases, Trends Genet. 13 (1997) 163.
[15] M.C. Von, L.J. Jensen, B. Snel, S.D. Hooper, M. Krupp, M. Foglierini, et al., STRING: known and predicted protein-protein associations, integrated and transferred across organisms, Nucleic Acids Res. 33 (2005) D433–D437.
[16] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, et al., STRING v10: protein-protein interaction networks, integrated over the tree of life, Nucleic Acids Res. 43 (2015) D447–D452.
[17] U. Covani, S. Marconcini, L. Giacomelli, V. Sivozhelevov, A. Barone, C. Nicolini, Bioinformatic prediction of leader genes in human periodontitis, J. Periodontol. 79 (2008) 1974–1983.
[18] N.L. Bragazzi, V. Sivozhelezov, C. Nicolini, LeaderGene: a fast data-mining tool for molecular genomics, J. Proteom. Bioinform. 4 (2011) 083–086.
[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.
[20] E.M.S.H. Sobrinho-Santos, I.S. Dias, S.H.S. Santos, A.M. de Paula, J.D. Feltenberger, A.L.S. Guimarães, L.C. Farias, Bioinformatics analysis reveals genes involved in the pathogenesis of ameloblastoma and keratocystic odontogenic tumor, Int. J. Cell Mol. Med. 5 (2016) 199–219.
[21] T.A. Guimaraes, L.C. Farias, C.A. Fraga, J.D. Feltenberger, G.A. Melo, R.D. Coletta, et al., Evaluation of the antineoplastic activity of gallic acid in oral squamous cell carcinoma under hypoxic conditions, Anti Canccer Drugs 27 (2016) 407–416.
[22] C.N. Prasad, T. Anjana, A. Banerji, A. Gopalakrishnapillai, Gallic acid induces GLUT4 translocation and glucose uptake activity in 3T3-L1 cells, FEBS Lett. 584 (2010) 531–536.
[23] S. Michan, D. Sinclair, Sirtuins in mammals: insights into their biological function, Biochem. J. 404 (2007) 1–13.
[24] G. Ramadori, T. Fujikawa, M. Fukuda, J. Anderson, D.A. Morgan, R. Mostoslavsky, et al., SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity, Cell Metabol. 12 (2010) 78–87.
[25] G. Kelly, A review of the sirtuin system, its clinical implications, and the potential role of dietary activators like resveratrol: part 1, Alternative medicine review, J. Clin. Ther. 15 (2010) 245–263.
[26] P.T. Pfluger, D. Herranz, S. Velasco-Miguel, M. Serrano, M.H. Tschop, Sirt1 protects against high-fat diet-induced metabolic damage, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 9793–9798.
[27] M.P. Gillum, M.E. Kotas, D.M. Erion, R. Kursawe, P. Chatterjee, K.T. Nead, et al., SirT1 regulates adipose tissue inflammation, Diabetes 60 (2011) 3235–3245.
[28] C.L. Hsu, Y. Gow-Chin, Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats, Br. J. Nutr. 98 (4) (2007) 727–735.
[29] J.M.O. Andrade, A.S. Barcala-Jorge, G.C. Batista-Jorge, A.F. Paraíso, K.M. Freitas, D.F. Lelis, et al., Effect of resveratrol on expression of genes involved thermogenesis in mice and humans, Biomed. Pharmacother. 112 (2019) 108634.