The Effect of Berberine on Obesity Through Browning of the Inguinal White Adipose Tissue of Male Rats

Original Article

Reham A. Abdelrahman[1], Doaa A. Abdelmonsif[2], Eman F. Farghaly[1], Maha W. Abounazel[1] and Eiman I. Zaki[1]

[1]Department of Histology and Cell Biology, [2]Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt

ABSTRACT

Background: Obesity increases the risk of many diseases. White adipose tissue (WAT) browning is being targeted in the treatment of obesity. Berberine (BR) is a plant alkaloid, used mainly in treatment of diarrhea. Studies revealed that BR improves some metabolic disorders in obese but the browning effect of BR on WAT is not fully investigated.

Aim of the Work: Testing the possible impact of BR on browning of inguinal WAT (iWAT) of adult male albino rats, and to study its possible ameliorating effect on the obesity associated disturbed lipid profile.

Materials and Methods: Forty-eight adult male albino rats were divided into two main groups; normally fed diet group (NFD) and high fat diet group (HFD). Normal fat diet group was subdivided into three subgroups. Normal fat diet control group (NFDc) received no treatment. Normal fat diet-berberine low dose (NFD+BRL) group received 5mg/kg body weight (b.w) BR, and normal fat diet-berberine high dose (NFD+BRH) group received 10mg/kg b.w berberine. High fat diet group was subdivided into three subgroups: High fat diet control group (HFDc), high fat diet-berberine low dose (HFD+BRL) group and high fat diet-berberine high dose (HFD+BRH) group.

Results: Berberine promoted adipocyte browning in the iWAT, where the beige adipocytes were revealed. In addition, browning markers expression in iWAT; UCP1, PGC-1α and CIDEA increased. Also, BR ameliorated the disturbed lipid profile in HFD group.

Conclusion: Berberine showed to be a safe browning agent which also improved the dyslipidemia occurred in HFD group. Berberine high dose showed better results than the lower dose. Thus, we believe that BR has excellent potential as an effective safe anti-obesity agent.

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Corresponding Author: Eiman Ibrahim Zaki, PhD, Department of Histology and Cell Biology, Faculty of Medicine, Alexandria University, Alexandria, Egypt, **E-mail:** eimaniazaki@gmail.com

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INTRODUCTION

Obesity being a multifactorial chronic disease, causing major health problems, has fueled an attention to adipose tissue. Many diseases as metabolic and cardiovascular diseases are related to obesity, which advantage a lowered quality of life[1]. Obesity is also accused to increase susceptibility to infections, which may raise a risk for COVID-19 and its related mortality. In COVID-19 thrombotic incidents were an exacerbating cause of death. Thromboembolic risk is higher in obese. Thus, reasonably follows, obesity may raise death from COVID-19. Moreover, it is assumed that COVID-19 is related to human angiotensin converting enzyme 2 (ACE2) which is the presumed receptor for the access of COVID-19 into cells. It is expressed in adipose tissue making adipose tissue a target to COVID-19. Obese persons have more adipose tissue, thus more ACE2-expressing cells are present in their bodies, rendering them more vulnerable to COVID-19 infection[2].

Obesity results when the energy intake tops the energy loss. The available approaches of obesity treatment are based on either decreasing the caloric intake as through diet regimens, pharmacological approaches and bariatric surgery or increasing energy loss as by exercise. Understanding the types of adipose tissue, raised up the interest to increase energy expenditure by alternative ways. White adipose tissue (WAT) is known to store excess energy, while brown adipose tissue (BAT) disperses energy in the form of heat. This is done through uncoupling protein 1 (UCP1) or thermogenin. UCP1 is a protein found in the inner membrane of the mitochondria which reduces ATP production and allows the cell to release energy in form of heat[3]. It is now reported that this protein can appear in WAT making these cells function as brown adipocytes. These transferred cells are called 'beige' or 'brite' adipocytes. They took these names as their function is between that of the brown and white adipocytes[4]. Thus, there is now an increasing attention to use therapeutic approaches for obesity, which aim to stimulate these thermogenic adipocytes, to increase the energy disbursement[3].

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There are theories for the origin of beige adipocytes, as it is assumed that it originates by trans-differentiation from mature white adipocytes, or from adipogenic progenitor cell. Appearance of beige adipocytes within WAT is called browning. This can be stimulated by different stimulants like cold exposure or β-adrenergic agonists[5]. Nowadays, interest has been raised to test different pharmaceuticals to induce browning.

Berberine (BR) is a plant alkaloid, it is part of several Chinese herbal medicines, mainly used in treatment of diarrhea. Studies have also revealed that BR improves some metabolic disorders, like insulin resistance and hyperlipidemia[6].

Other studies showed that BR decreased body weight and the fat content in non-alcoholic fatty liver. Nevertheless, the effect of BR on browning of WAT and its mechanism is not fully investigated[6]. Thus, our aim was to examine the possible impact of BR on browning of inguinal WAT (iWAT) of adult male albino rats, in addition to study its possible ameliorating effect on the obesity associated disturbed lipid profile.

MATERIALS AND METHODS

Drugs and chemicals

Berberine (BR) (berberine chloride; yellow powder, purity ≥97%) was obtained from new test Egypt chemical company imported from LKT laboratories. Gene JET RNA Purification Kit for extracting RNA was obtained from (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Kits for lipid profile analysis were obtained from (Spectrum Diagnostics, Hannover, Germany). Other chemicals were bought from local commercial suppliers.

Experimental animals

Forty-eight adult male albino rats, 6-8 weeks old, weighing 150-200 gm were grown up in the Animal House in the Department of Physiology, Faculty of Medicine, University of Alexandria, Egypt. The research ethics code for this experiment was approved by the Research Ethics Committee, Faculty of Medicine, University of Alexandria, Egypt (serial number 0105505).

Animals were distributed randomly into two main groups; normally fed diet group (NFD); fed standard rat chow and high fat diet group (HFD); fed HFD for twelve weeks to induce obesity.

Normal fat diet group (NFD) was further subdivided into three subgroups. Normal fat diet control subgroup (NFDc) which received no treatment. Normal fat diet-berberine low dose (NFD+BRL) subgroup and normal fat diet-berberine high dose (NFD+BRH) subgroup, received daily for four weeks, intraperitoneal injection (i.p) of 5mg/kg body weight (b.w),[7] and 10mg/kg b.w berberine,[8] respectively. High fat diet group (HFD) was further subdivided into three subgroups after finishing the twelve weeks of HFD regimen. High fat diet control subgroup (HFDc) which received no treatment. High fat diet-berberine low dose (HFD+BRL) subgroup and high fat diet-berberine high dose (HFD+BRH) subgroup, received daily for four weeks, intraperitoneal injection (i.p) of 5mg/kg body weight (b.w),[7] and 10mg/kg b.w berberine,[8] respectively. All rats' weights were taken at the beginning of the study, then every week during the experiment and before being sacrificed.

General appearance and survival

The general appearance and mortality rate were assessed for all animals in all subgroups during the study period. At the end of the study, the inguinal fat depot of all subgroups were examined for the changes in amount and color.

Body weights

The rats' weights of NFD and HFD groups were recorded before and after BR administration.

Inguinal fat depots weights

The weights of the inguinal fat depots of all subgroups were recorded at the end of the study.

Histological assessment

Inguinal white adipose tissue (iWAT) specimens were taken for histological examination. For the light microscopic examinations, part was fixed in 10% formal saline and processed to get 4-6 μm thick paraffin sections to be stained by Haematoxylin and Eosin (H&E) stain, and another part was cut into small pieces about 2 mm^3 then immersed immediately in osmium tetroxide stain for staining lipid droplets[9]. For transmission electron microscopic (TEM) examination, specimens were cut into small pieces about 0.5-1 mm^3 and immersed immediately in 3% phosphate buffered glutaraldehyde fixative and were processed for assessment[10].

Morphometric analysis

Digital images from H&E-stained sections were taken using (Olympus DP20) digital camera connected to (Olympus BX41) microscope. The images of each subgroup were used to determine the size of the white adipocytes by measuring the area occupied by each cell. Results were measured using NIH Image j (v1.49) software.

Lipid profile assessment

Blood samples were taken from the aorta to obtain the serum by centrifugation at 3000 rpm for 15 min. Cholesterol, triglycerides, high density lipoproteins cholesterol (HDL-ch) and low density lipoproteins cholesterol (LDL-ch) levels were measured in rats' serum using Spectrum assay kits (Spectrum Diagnostics, Hannover, Germany)[11–13].

Quantitative reverse transcription PCR (qRT-PCR)

Inguinal white adipose tissue (iAT) specimens were washed in ice-cold saline, weighed, and stored at (-80ºC) for determination browning markers; uncoupling protein 1 (UCP1), cell death inducing DNA fragmentation factor

alpha like effector A (CIDEA) and peroxisome proliferator activated receptor co-activator 1-alpha (PGC-1α) gene expression[14].

Total RNA extraction

Total RNA was extracted from inguinal adipose tissue (iAT) samples under RNAse-free conditions and following the manufacturer's protocol of Gene JET RNA Purification Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). After assessment of the concentration and purity of the extracted RNA samples via NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA), RNA was re-suspended in RNAse-free DEPC (Diethyl-pyro-carbonate)-treated water and stored at (-80ºC) until further processing[15].

Quantitative RT-PCR

Reverse transcription was done in 20 µL reaction volume with 100 ng of total RNA and High Capacity cDNA Reverse Transcription Kit (In*vitro*gen, USA). The quantification of UCP1, CIDEA and PGC-1α cDNA was done using the StepOne reverse transcription PCR system (Applied Biosystems, USA). Amplification of the synthesized cDNAs was performed in duplicates in a 25 µl reaction volume containing 2X Maxima SYBR Green qPCR Master Mix (Applied Biosystems, USA). Specific primer pair for UCP1 was the sense primer AGTGTACCCAGCTGTGC AATGACCA and the antisense primer AAACATGATGACGTTCCAGGATCCG (Gen Bank NM_012682.2)[16]. As for CIDEA, specific primer pair was the sense primer TCAGACCCTAAGAGACAACACA and the antisense primer CATTGAGACAGCCGAGGA (GenBank NM_001170467.1)[16]. Concerning PGC-1 α, specific primer pair was the sense primer GACCCCAGAGTCA CCAAATGA and the antisense primer GGCCTGCAGTTCCAGAGAGT (GenBank NM_031347.1)[11]. The amplification system consisted of one cycle at 95˚C for 10 min followed by 40 cycles of denaturation at 95˚C for 15 sec, an annealing step for 30 sec, and an extension step at 72˚C for 30 sec. Moreover, the annealing temperature used was 62°C for UCP1 and 65°C for each of CIDEA and PGC-1α. PCR amplification was followed by a melting curve analysis where the identity of the PCR product was verified. With every PCR a no-template control was run to assess the specificity of the reaction. The data analysis was done using StepOne™ Software v2.3 where the level of gene expression was determined by the comparative CT method for gene expression relative to the housekeeping gene beta-actin.

Statistical Evaluation of the Data

The data was analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov was utilized to validate the normality of distribution of variables. Student t-test was used to compare between two studied groups. ANOVA test to compare between more than two groups and Post Hoc test (Tukey) for pairwise comparisons. Significance of the obtained results was considered at the 0.05% level.

RESULTS

General appearance and survival

High fat diet was well tolerated by the rats in HFD group, with a noticed increase in body weight. Berberine administration, showed an evident decline in food intake in rats of the four subgroups received BR. No mortality was detected in all subgroups during the study period. Examining the dissected iWAT depots showed an increase in the amount in the HFDc subgroup compared to that of the NFDc subgroup. Meanwhile, the four BR treated subgroups; with either dose, showed an observed decrease in the amount of iAT and a change in color of such depot, being dark yellow to brown (Figure 1).

Body weights

Comparing the rats' weights of NFD and HFD groups before BR administration, revealed an increase in the rats' weights of HFD group compared to that of NFD group (Figure 2a). While comparing the different subgroups by the end of the study, after BR administration, revealed that NFD group showed a decrease of the weight values in BR treated subgroups compared to the NFDc subgroup. The mean weight value of NFD+BRH subgroup was less than that in NFD+BRL subgroup received the low dose of BR. For the HFD group the mean values of HFDc subgroup was higher than that of the NFDc subgroup. In addition, a remarkable weight loss was noticed in both subgroups received BR compared to the HFDc subgroup. Moreover, the mean weight of HFD+BRH subgroup was less than HFD+BRL subgroup. On the other hand, mean values of both subgroups, HFD+BRL and HFD+BRH subgroup, were still higher than NFDc subgroup (Figure 2b).

Inguinal fat depots weights

Assessing the weights of inguinal fat depots at the end of the study demonstrated that, in NFD subgroup a remarkable reduction in the weights of subgroups received BR, compared to the NFDc subgroup, was recorded. At the same time, the inguinal fat depots' weights of NFD+BRH was less than that in NFD+BRL subgroup. Comparing the mean values of HFDc to that of the NFDc subgroup an increase in values were recorded. Moreover, a decrease in mean values of weights of inguinal fat depots was noticed in both subgroups received BR in comparison to the HFDc subgroup. Furthermore, comparing HFD+BRH subgroup to HFD+BRL subgroup, lower values were noticed. On the other hand, when comparing to NFDc subgroup, HFD+BRL subgroup showed equivalent results, while HFD+BRH subgroup showed lower values (Figure 2c).

Histological assessment

Light microscopic results

Examination of the iWAT of H&E-stained sections of NFDc subgroup, revealed the classical histological structure of the closely packed polyhedral unilocular

adipocytes with flattened peripherally located nuclei and scattered blood vessels in between. On the other hand, examination of white adipose tissue of H&E-stained sections of HFDc subgroup, showed a noticeable increase in the size of unilocular adipocytes in comparison to NFDc subgroup with intense mononuclear cellular infiltration noticed within the WAT. While examination of NFD+BRL and NFD+BRH sections revealed, islands of beige adipose tissue interspersed between unilocular white adipocytes associated with many blood vessels. The small beige adipocytes showed central rounded nuclei and small multiple lipid droplets in the cytoplasm. Few areas of cells with hypereosinophilic cytoplasm, dark nuclei together with the appearance of hyalinized material were seen within the islands. In addition, collagen fibers and few foci of mononuclear cellular infiltration were encountered. Beige islands are noticed more in NFD+BRH compared to NFD+BRL subgroup. In addition, in NFD+BRH subgroup, the surrounding unilocular white adipocytes appeared distorted and small sized. Mast cells were noticed adjacent to the islands in NFD+BRH subgroup. Similar findings were seen in BR treated HFD subgroups. Noticing that unilocular adipocytes were much smaller than that in HFDc subgroup. Furthermore, hypereosinophilic cells, hyalinized material and mononuclear cellular infiltration were more depicted in such subgroups compared to BR treated NFD subgroups (Figures 3,4).

Osmium tetroxide-stained sections revealed large black stained lipid droplets of unilocular adipocytes in NFDc subgroup. While examination of iAT of HFDc subgroup revealed larger black stained lipid droplets in comparison to that of NFDc subgroup. All subgroups received BR showed multiple aggregations of small lipid droplets of the beige adipocytes, scattered in between the large black lipid droplets of unilocular adipocyte. These small lipid droplets are more frequent with the high BR dose compared to that of the low one (Figure 5)

Electron microscopic results

Electron microscopic examination of the inguinal white adipose tissue of NFDc and HFDc subgroups showed, white adipocytes filled with a single large lipid droplet and having a thin rim of cytoplasm. The nucleus is oval, flattened and is pushed to one pole of the cell (Figure 6). For subgroups received BR, electron microscopic examination of the iAT of NFD+BRL and NFD+BRH subgroups, revealed multiple beige adipocytes. Each cell showed, a central nucleus surrounded with lipid droplets of variable sizes and densities. Some adipocytes with intermediate characteristics between white and beige adipocytes having a flattened nuclei and multiple lipid droplets were noticed. Besides, adipocytes with indented nuclei were revealed. In addition, multiple mitochondria, lysosomes, rER and sER were seen in the cytoplasm. Mast cells were encountered within the sections of both subgroups and eosinophils were revealed in NFD+BRH subgroup. Additionally, striking feature was depicted in NFD+BRH subgroup which was the appearance of multiple desmosomes joining adjacent cells together (Figures 7,8). Similarly, HFD subgroups received BR revealed evident browning of the unilocular adipocytes as multiple beige adipocytes appeared in the sections. The appearance of multiple electron lucent lipid droplets among the electron dense ones was a feature noticed in these subgroups but not in NFD subgroups received BR. In addition, some cells showed degenerating features as perinuclear cisternal dilatation, dense nuclei, dilated rER, bizarre shaped and swollen mitochondria (Figures 9,10).

Morphometric analysis

Cell size of white adipocytes

Comparing white adipocytes cell sizes revealed that NFD+BRL and NFD+BRH subgroups showed a decrease in the mean values compared to the NFDc subgroup. In addition, NFD+BRH subgroup values were lower than that of NFD+BRL subgroup. Meanwhile, a rise in cell size of HFDc subgroup with the comparison to the NFDc subgroup was recorded. Similarly, HFD subgroups received BR showed a remarkable reduction in mean values compared to HFDc subgroup. Besides, HFD+BRH subgroup cell sizes were lower than that of HFD+BRL subgroup. Interesting, both HFD groups received BR showed values lower than NFDc group (Figure 11a).

Biochemical results

Lipid profile parameters

Normal fat diet (NFD) subgroups received BR by either dose showed a decrease in serum total cholesterol, TG, LDL with an increase in serum HDL levels compared to NFDc subgroup. Meanwhile, higher dose of BR had a superior effect on the lipid profile compared to the low dose. Regarding HFDc subgroup, there was an increase in serum total cholesterol, TG, LDL with lower HDL as compared to NFDc subgroup. Interestingly, in HFD groups a significant improvement was shown in serum lipid profile parameters after BR injection as compared to HFDc. Similarly, high dose of BR showed better results compared to the low dose. However, in subgroup received the low dose of BR this improvement was still not equivalent to the NFDc subgroup. While the subgroup received high dose of BR the results were comparable to NFDc except for LDL still showed higher levels with a significant difference (Figure 11b).

Browning Biomarkers

In order to confirm browning of iWAT after i.p injection of BR, the expression of UCP1, PGC-1α and CIDEA mRNAs was determined in the studied groups. In NFD groups, PCR results showed a significantly enhanced mRNA expression of browning biomarkers after different BR doses compared to NFDc subgroup. Meanwhile, superior results were recorded with the higher dose compared to the lower one.

Similarly, in HFD subgroups received BR all browning biomarkers were significantly increased compared to HFDc subgroup. Likewise, the group received the higher dose of BR showed a significantly superior mRNA expression of browning biomarkers compared to the group received the lower dose (Figure 11c).

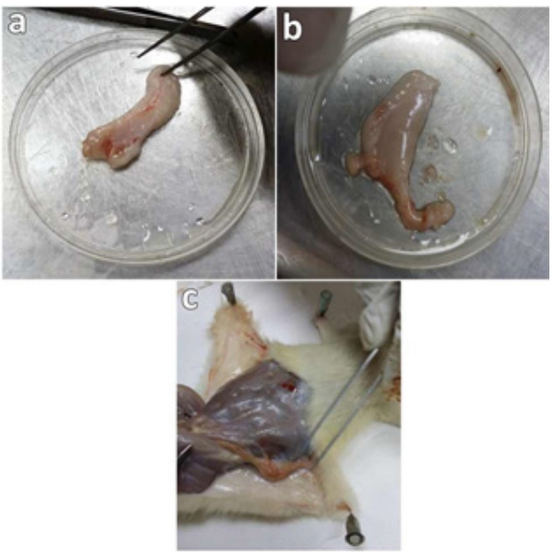

Fig. 1: (a&b); Dissected iWAT depots of control rats. a; NFDc subgroup, b; HFDc subgroup. Notice the increased amount in the HFDc subgroup. (c); Site of dissection of the inguinal fat depots in a BR treated subgroup. Notice dark yellow to brown color of inguinal fat.

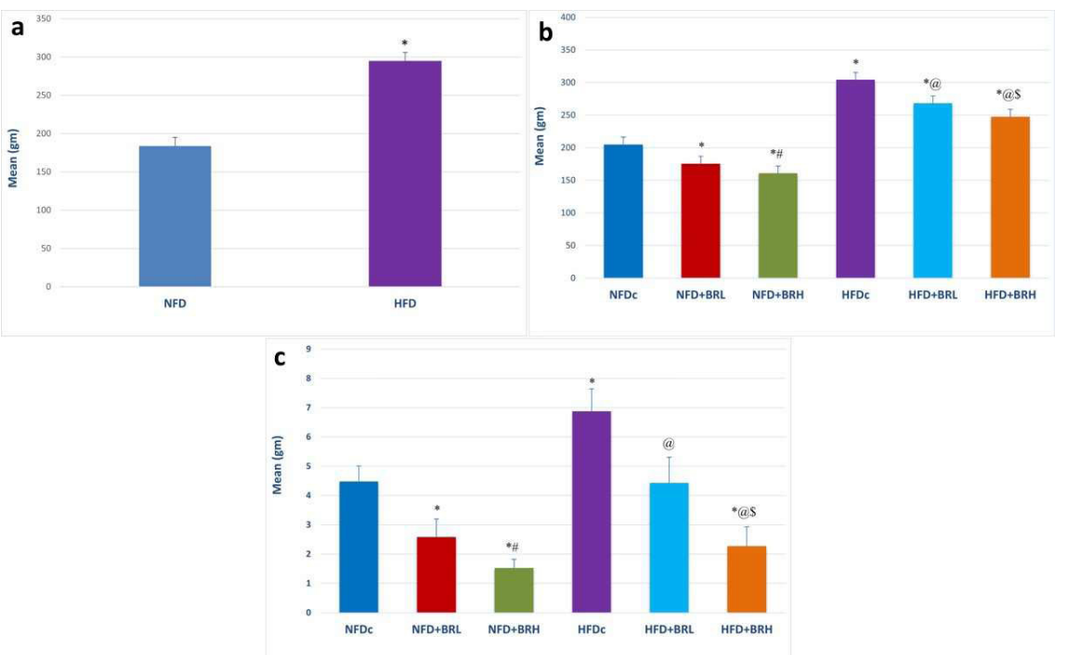

Fig. 2: Comparison between the two studied groups according to, a; rats' weight before BR administration. n=24. b; rats' weight at the end of the study. n=8. c; Inguinal fat depots' weights. n=8. Data expressed as mean ± SD. Statistically significant at $p \leq 0.05$. *: Significant with NFDc. #: significant with NFD+BRL. @: significant with HFDc. $: significant with HFD+BRL.

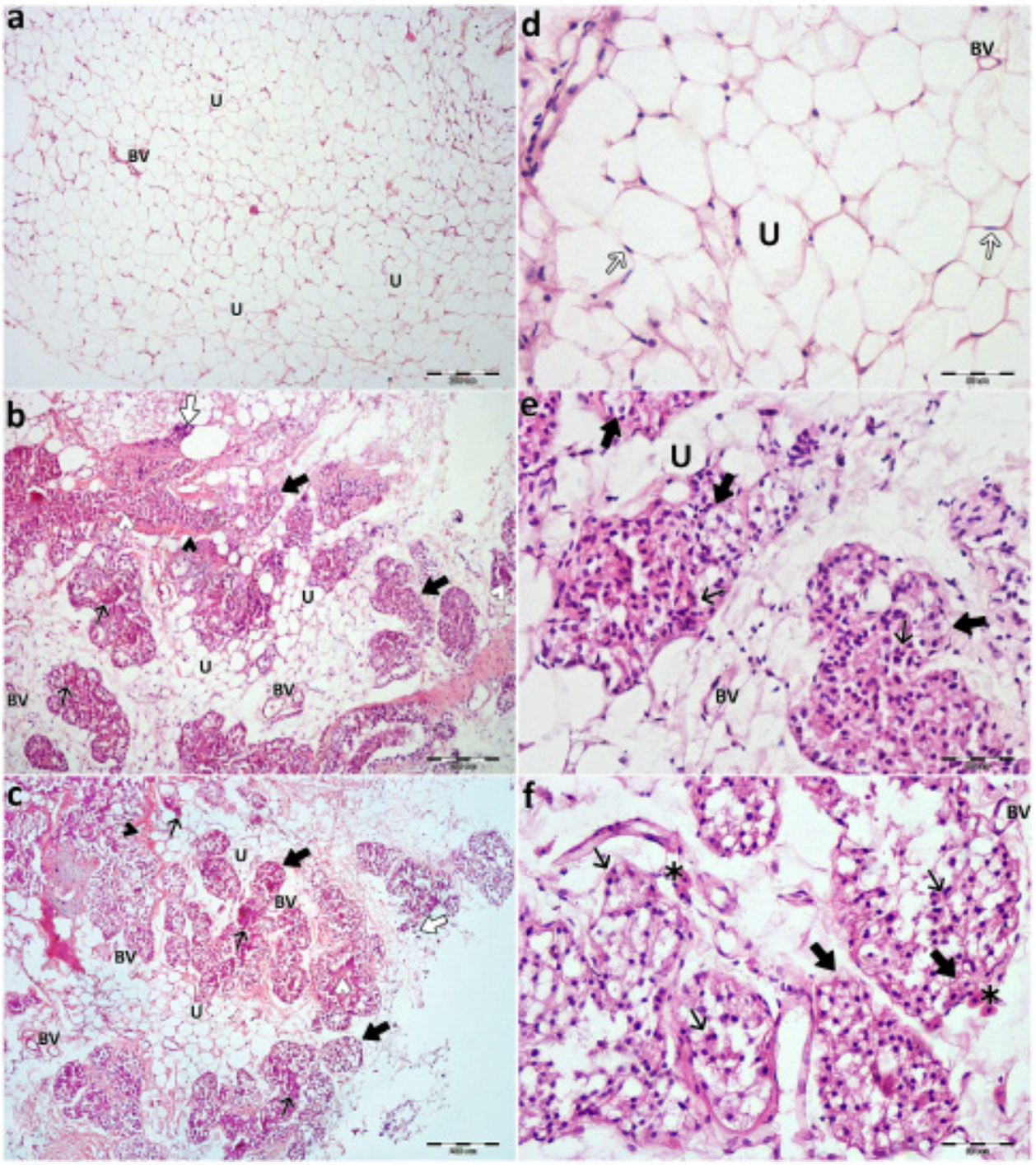

Fig. 3: Photomicrographs of iAT of NFD group. (a-c); (H&E stain, Mic. Mag ×100) a: Photomicrograph of NFDc subgroup showing, closely packed polyhedral unilocular adipocytes (U) with scattered blood vessels (BV) in between. b&c: Photomicrographs of NFD+BRL and NFD+BRH subgroup respectively showing, islands of beige adipose tissue (thick black arrow) interspersed between unilocular adipocytes (U). Cells with hypereosinophilic cytoplasm and dark nuclei (thin black arrow) were revealed. Notice collagen fibers (black arrowhead), hyalinized material (white arrowhead), mononuclear cellular infiltration (thick white arrow) and increased blood vessels (BV) within the islands. (d-f); (H&E stain, Mic. Mag ×400) d: Photomicrograph of NFDc subgroup showing, unilocular adipocytes (U) with flattened nuclei (thin white arrow) and scattered blood vessels (BV) in between. e&f: Photomicrographs of NFD+BRL and NFD+BRH subgroup respectively showing, beige adipocytes appearing as rounded cells with central nuclei having multiple small lipid droplets (thick black arrow). Notice cells with hypereosinophilic cytoplasm (thin black arrow) and mast cells (*).

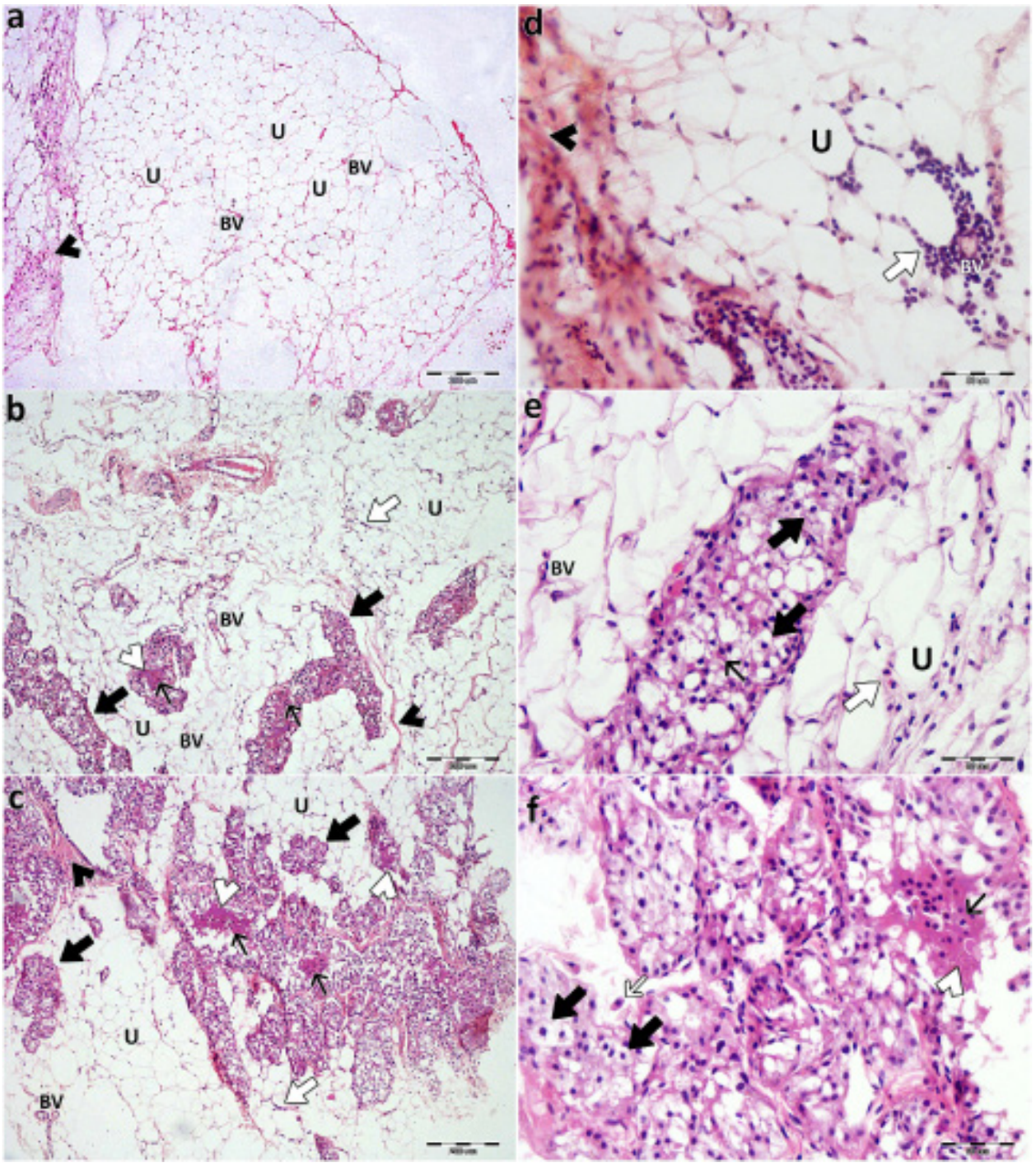

Fig. 4: Photomicrographs of iAT of HFD group. (a-c); (H&E stain, Mic. Mag ×100) a: Photomicrograph of HFDc subgroup unilocular adipocytes (U). b&c: Photomicrographs of HFD+BRL and HFD+BRH subgroups respectively showing, islands of beige adipose tissue (thick black arrow). Many cells with hypereosinophilic cytoplasm and dark nuclei (thin black arrow) were revealed. Black arrowhead; collagen fibers. White arrowhead; hyalinized material. Thick white arrow; mononuclear cellular infiltration. BV; blood vessels. (d-f); (H&E stain, Mic. Mag ×400) d: Photomicrograph of HFDc subgroup showing, large unilocular adipocytes (U). Notice mononuclear cellular infiltration (thick white arrow) and collagen fibers (black arrowhead). e&f: Photomicrographs of HFD+BRL and HFD+BRH subgroups, respectively. Thick black arrow; beige adipocytes. Thin black arrow; cells with hypereosinophilic cytoplasm. White arrowhead; hyalinized material. Thick white arrow; mononuclear cellular infiltration. Thin white arrow; mast cell.

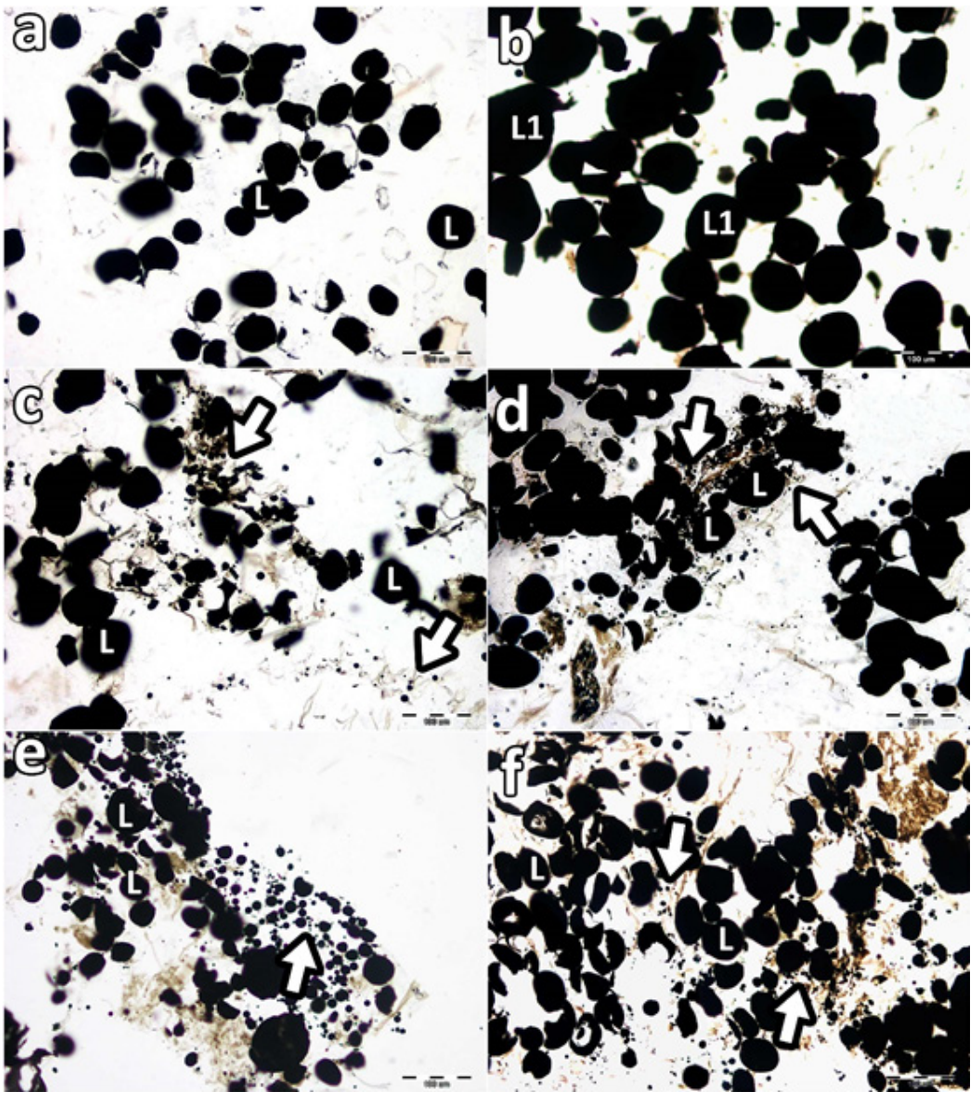

Fig. 5: Photomicrographs of osmium tetroxide-stained sections of iAT showing, a: NFDc subgroup with large black stained lipid droplets (L). b: HFDc subgroup with larger sized black stained lipid droplets (L1) compared to NFDc subgroup. c-f: Photomicrographs of iAT showing, aggregations of small sized black lipid droplets (white arrow) scattered in between large black lipid droplets (L). c: NFD+BRL subgroup. d: HFD+BRL subgroup. e: NFD+BRH subgroup. f: HFD+BRH subgroup. (Osmium tetroxide stain, Mic. Mag. ×200).

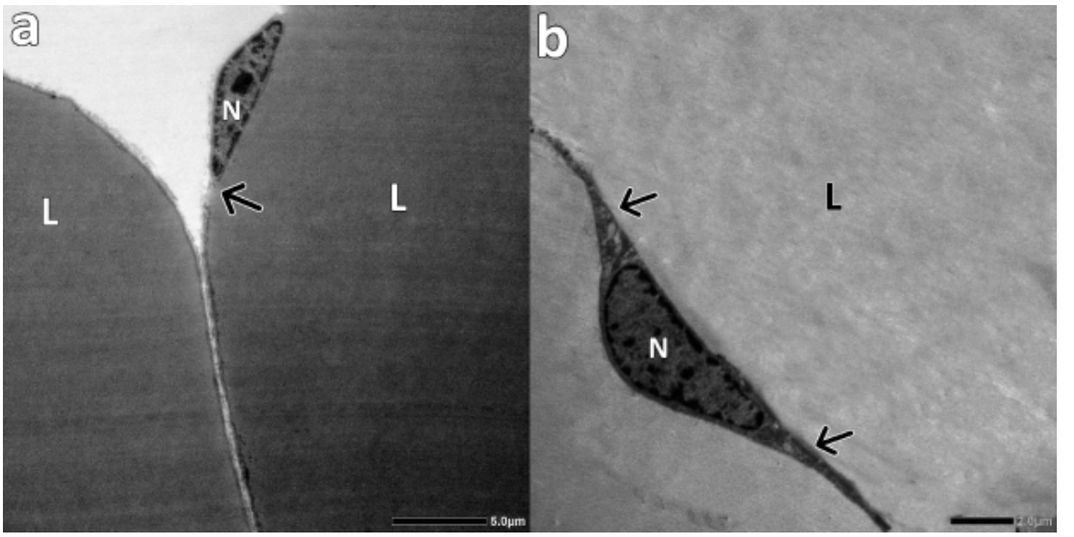

Fig. 6: Electron micrograph of inguinal white adipose tissue of control subgroups. a: Electron micrograph of iWAT of NFDc subgroup (Uranyl acetate/ lead citrate stain, Mic. Mag. ×1200). b: Electron micrograph of iWAT of HFDc subgroup (Uranyl acetate/ lead citrate stain, Mic. Mag. ×2000). Notice that each white adipocyte shows, a flattened nucleus pushed to one pole of the cell (N), thin rim of cytoplasm (thin black arrow) with a single large lipid droplet (L).

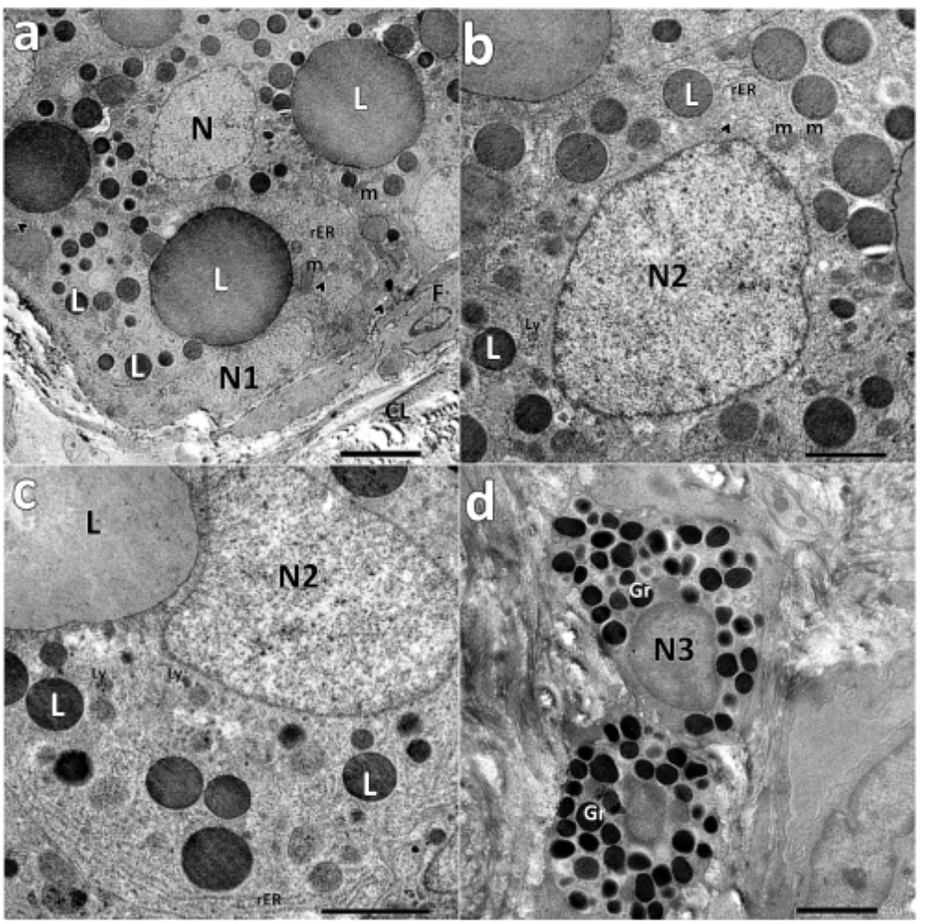

Fig. 7: Electron photomicrographs of iAT of NFD+BRL subgroup showing, (a-c): Multiple beige adipocytes, each cell showed a central nucleus (N) surrounded by variable sized lipid droplets (L) with different densities. Smooth endoplasmic reticulum (black arrowhead), rough endoplasmic reticulum (rER), lysosomes (Ly) and multiple mitochondria (m) were revealed. Adipocytes with intermediate characteristics between white and beige adipocytes having a flattened nucleus (N1) and multiple lipid droplets were noticed and others showed an indented nuclei (N2). (F); Fibroblasts. (CL); Collagen fibrils. (d): Mast cells with central nucleus (N3) surrounded by numerous membrane bound granules with uniform density (Gr). (Uranyl acetate/ lead citrate stain, Mic. Mag. a:X1200. b:X3000. c: X4000 d:X3000)

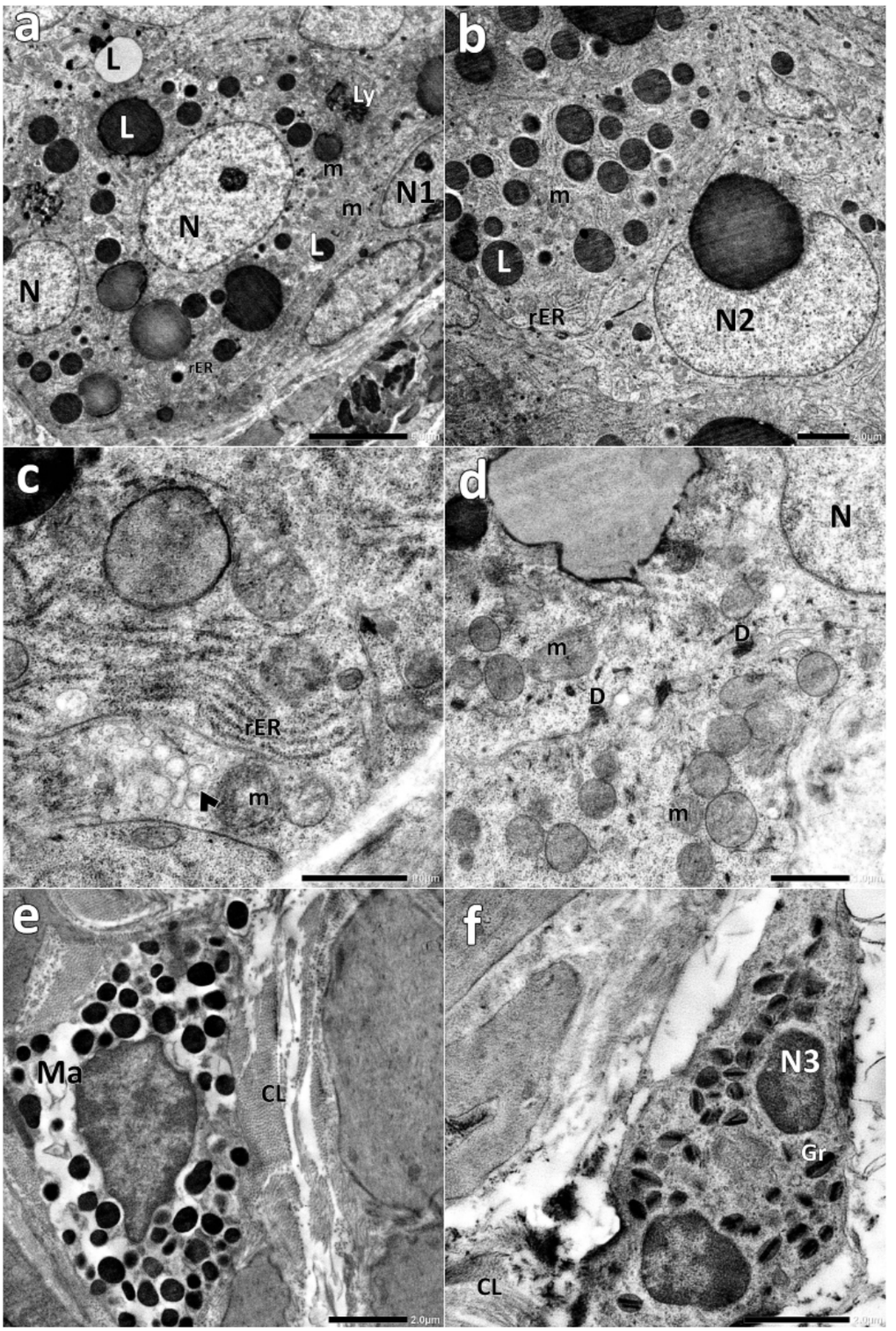

Fig. 8: Electron photomicrographs of iAT of NFD+BRH subgroup revealed, (a-c): Multiple beige adipocytes, having a central nucleus (N) surrounded by lipid droplets (L) with variable sizes and densities. Notice adipocytes with flattened nuclei (N1) and others with an indented nuclei (N2). (d): Desmosomes (D) between adjacent cells. (e): Mast cells (Ma) with its characteristic granules. (f): Eosinophil with bi-lobed nuclei (N3) and characteristic granules (Gr) with crystalline core. m; mitochondria. rER; rough endoplasmic reticulum. Black arrowhead; smooth endoplasmic reticulum. Ly; lysosomes. (CL); collagen fibrils. (Uranyl acetate/ lead citrate stain, Mic. Mag. a:X1500. b:X2000. c:X8000 d:X6000 e:X3000. f:X4000).

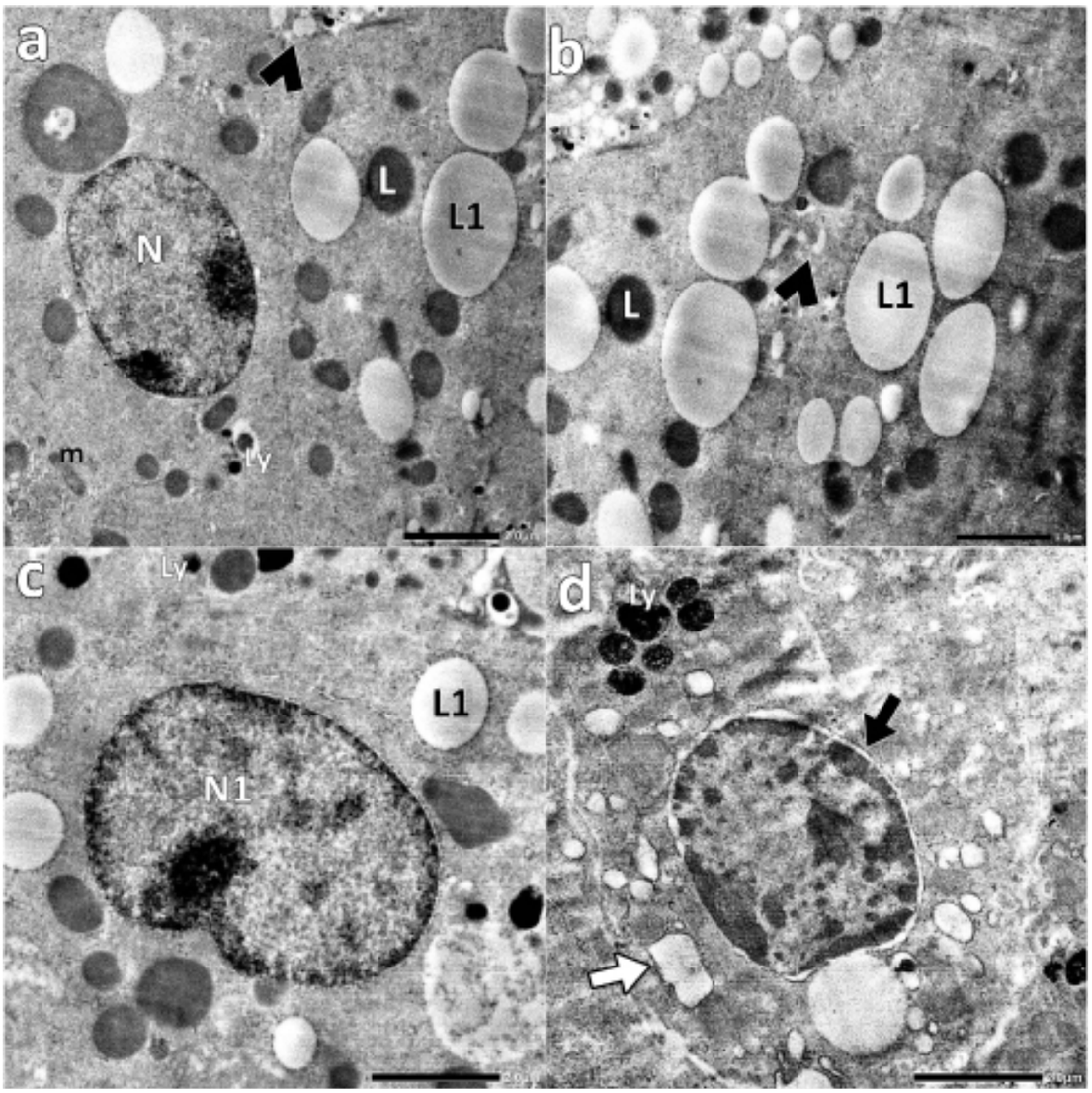

Fig. 9: Electron photomicrograph of iAT of HFD+BRL subgroup showing (a-c): Beige adipocytes with a central nucleus (N) surrounded by lipid droplets (L) many of them appeared electron lucent (L1). Notice adipocytes with indented nuclei (N1). (d): Degenerating beige adipocyte with perinuclear cisternal dilatation (black arrow) and dilated rough endoplasmic reticulum (white arrow). black arrowhead; smooth endoplasmic reticulum. m; mitochondria. Ly; lysosomes. (Uranyl acetate/ lead citrate stain, Mic. Mag. a:X3000. b:X3000. c:X4000 d:X4000)

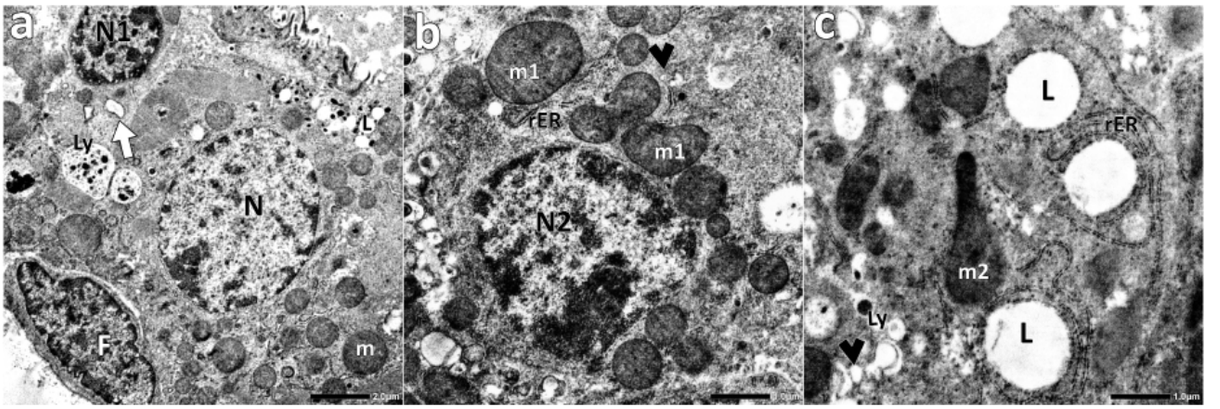

Fig. 10: Electron photomicrograph of iAT of HFD+BRH subgroup showing, (a): beige adipocytes with a central euchromatic nucleus (N) and small electron lucent lipid droplets (L). A degenerating cell with central electron dense nucleus (N1) and dilated rough endoplasmic reticulum (white arrow) is revealed. (b) Adipocyte with indented nucleus (N2) and swollen mitochondria (m1) is shown. (c): Electron photomicrograph showing a beige adipocyte with electron lucent lipid droplets (L) and bizarre shaped mitochondria (m2). F; Fibroblast. rER; rough endoplasmic reticulum. black arrowhead; smooth endoplasmic reticulum. m; mitochondria. Ly; Lysosomes. (Uranyl acetate/ lead citrate stain, Mic. Mag. a:X2500. b:X5000. c: X5000.)

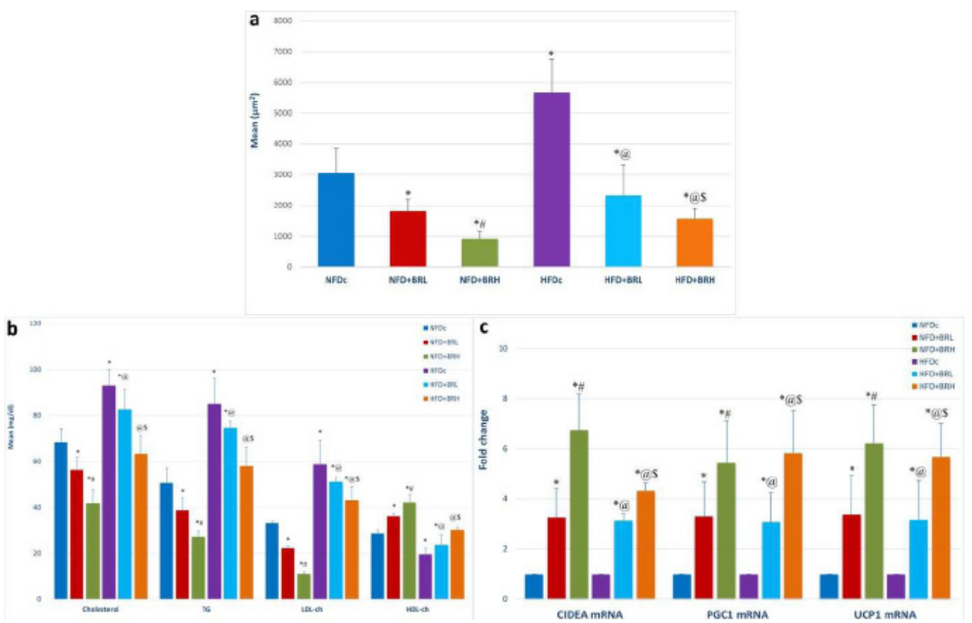

Fig. 11: Comparison between the studied subgroups according to, a; white adipocytes cell size. n=30. b; lipid profile. n=8. c; browning biomarkers. n=8. Data expressed as mean ± SD. Statistically significant at $p \leq 0.05$. *: Significant with NFDc. #: significant with NFD+BRL. @: significant with HFDc. $: significant with HFD+BRL.

DISCUSSION

Obesity is now considered as a pandemic, which raised the necessity to try to find new therapeutic approaches to counteract energy imbalances. Hence, new interventions that induce browning of WAT could enhance adipocyte physiology by improving glucose uptake and lipid disposal. Using natural agents or herbal medicines to stimulate the induction of beige adipocytes; such as BR, has raised a concern to be used in treatment of obesity[3].

Berberine, one of the major ancient Chinese phytotherapy, used mainly as antidiarrheal drug. Recent studies reported that BR efficiently prevents obesity through many mechanisms like the modification of gut microbiota, intestinal permeability and regulation of some genes. BR is likewise found to be efficient in decreasing blood glucose by inhibiting α-glycosidase enzyme, causing that only restricted amount of glucose is absorbed, preventing of being overweight[17].

Berberine is also assumed to cause browning of WAT[5]. Browning, britening and beiging are all terms used to define the appearance of beige adipocytes within WAT, this happens when WAT is exposed to different stimuli. This process is of great interest because of its prospective use in fighting obesity and its related disorders. Brown and beige adipocytes are named thermogenic adipocytes, because they can disintegrate chemical energy in form of heat via mitochondrial UCP1. Thus, they increase energy expenditure which might result in a significant body weight loss. In addition, animal studies revealed that such cells can oxidize serum free fatty acids leading to the improvement of the hyperlipidemia[14].

Despite the importance of such an issue, only few studies were done using histopathological and ultra-structural examination to clarifying the induced beige adipocytes. In view of the previously mentioned findings, it has become quite essential to investigate the changes caused by BR on the iWAT, to examine its possible role in WAT browning. Moreover, the present work examined the effect of BR on improving the disturbed lipid profile associated with obesity owing to confirm the anti-hyperlipidemic properties of BR.

In the present study HFD regimen significantly increased the rats' weight and the weights of inguinal fat depots in HFD group compared to the NFD group, these results agreed with other experiments which used similar diet regimens[18]. Incidentally, BR administration did not exhibit any signs of illness or mortality throughout the study period, indicating the safeness of such drug. Interesting, BR significantly reduced the food intake by rats, together with a significant weight loss and a decrease in the weights of the dissected inguinal fat depots. Noticing that this decrease was more prominent in subgroups received the high dose of BR (10 mg/kg body weight) compared to the subgroups received the low dose (5 mg/kg body weight). A noteworthy finding is that a brownish color change was noticeable in the inguinal fat depots dissected from BR treated rats. These results were in accordance with previous studies reported by Zhang *et al*. and Hu Y *et al*[19,20].

Light microscopic examination of iWAT of NFDc subgroup, stained by H&E-stain, revealed the classical histological structure of closely packed polyhedral unilocular adipocytes with flattened peripherally located nuclei and scattered blood vessels in between. Meanwhile the adipocytes in HFDc subgroup appeared larger than adipocytes of the NFDc subgroup, this was confirmed by morphometric analysis. In accordance with these results, He *et al*.,[21] postulated that HFD intake increases the size of adipocytes.

The administration of BR was associated with smaller adipocyte size compared to the control subgroups. Observing that this reduction in size was more in subgroups received the high dose of BR compared to the subgroups received the low one. These results agreed with others that demonstrated that BR decreased the size of adipocytes[22].

Intraperitoneal injection of BR for four weeks, by either dose resulted in marked histological changes in the iWAT, which was more prominent in subgroups received the higher dose of BR. Light microscopic examination in H&E-stained histological sections, showed islands of beige adipose tissue interspersed between small sized unilocular white adipocytes associated with blood vessels scattered in between. The beige adipocytes showed central rounded nuclei surrounded with small lipid droplets. These results agree with Zhang *et al*.,[7] who stated that BR stimulates the formation of brown-like adipocytes in iWAT.

Examination of H&E-stained sections of HFDc subgroup revealed evident mononuclear cellular infiltration, which confirm the state of inflammation induced by HFD. Chronic inflammation of adipose tissue with the appearance of proinflammatory cells, is related to over feeding[23].

Alternatively, light microscopic examination of H&E-stained sections in BR treated groups showed, few areas of small sized hypereosinophilic cells with dark pyknotic nuclei together with a hyalinized material within the islands of beige adipocytes. Such cells are considered degenerating, metabolically inactive and in some instance dying or dead cells[24]. These changes were previously seen accompanied lipolysis[25]. It is worth mentioning that Yang *et al*.,[26] clarified that BR induces lipolysis in adipocytes. Lipolysis may induce a transient inflammatory response, which was assumed by Babaei *et al*.,[27] to stimulate beige adipogenesis. It is also reported that lipolysis induced by adrenergic signaling stimulates adipocyte progenitors to form beige cells[28].

Berberine is believed to cause browning by different mechanisms. One is that BR causes trans-differentiation of white adipocytes to beige ones, another is by stimulating new beige adipocyte formation from adipogenic progenitor cells[29].

Lipid droplets were stained with osmium tetroxide stain. In the subgroups received BR, osmium tetroxide-

stained sections revealed aggregations of small sized black lipid droplets, of beige adipocytes, scattered in between large sized black lipid droplets of unilocular adipocyte. There was a noticeable increase of the aggregations of small sized lipid droplets in subgroups received (10mg/kg body weight) BR. These results reflect successful browning effect of BR[30].

Ultra-structural results were in accordance with the light microscopic ones. Where the TEM sections of NFDc and HFDc subgroups showed the unilocular white adipocytes with single lipid droplet. The BR treated subgroups revealed evident browning with typical trans-differentiation of white adipocytes into beige cells which were more manifested in the higher dose of BR. This trans-differentiation was evidence as some adipocytes; transdifferentiating adipocytes, showed intermediate characteristics between white and beige adipocytes, showing flattened nuclei with rounded mitochondria and multiple lipid droplets, indicating that they are still in the transformation phase[31].

Electron lucent lipid droplets appeared in HFD subgroups; this finding may be due to the increased content of the saturated fatty acids in it, after the HFD regimen. As the density of the lipid droplets correlates to the degree of saturation of fatty acids present, high content of saturated fatty acids, which does not bind osmium devotedly causing the lipid droplet appear electron lucent. On the other side, the greater the unsaturated fatty acid content, the more electron dense lipid droplet will appear[32].

A striking feature was depicted in NFD+BRH subgroup, which is the appearance of multiple desmosomes between adipocytes, this could be explained that desmosomes conduct differentiation in some tissues[33].

Mast cells and eosinophils with their characteristic granules were also encountered in TEM examination of NFD subgroups received BR. It is reported that histamine secreted by mast cells is present in high levels in BAT, which affects thermogenesis. Furthermore, cold exposure, which might induce browning, is stated to cause the recruitment of mast cells to WAT. Mast cells secrete histamine and other elements that stimulate UCP1 expression and beiging of WAT[34,35]. Moreover, an increase in adipose tissue eosinophils was detected in previous reports that used exposure to cold temperature as an inducer of browning[36]. IL-4 present in adipose tissue is mainly secreted by eosinophils. Eosinophils and IL-4 has a role in browning of WAT, as their lack was related to impair browning induced by cold exposure[37].

In HFD subgroups received BR, some cells showed degenerating features in the form of, perinuclear cisternal dilatation, dense nuclei, dilated rER, bizarre shaped and swollen mitochondria[38]. With the prevalent inflammatory status, this causes endoplasmic reticulum (ER) stress where impairment in rER function occurs, resulting in the accumulation of unfolded or misfolded proteins. To overcome this, the adipocyte expands their endoplasmic reticulum volume[39]. While the mitochondrial swelling may result from impairment of mitochondrial metabolism, diminished ATP generation and failure of the mitochondrial cation pump. These changes may occur because, in obesity, the adipose tissue has a higher capacity to secrete pro-inflammatory cytokines and to become a hypertrophic cell prone to macrophage infiltration. This possibly explains the appearance of degenerative changes encountered in HFD group. Another assumption for these changes is the intensified secretion of reactive oxygen species (ROS), as BR is reported to increase ROS in some cells[40].

In our study a disturbed lipid profile is recorded in HFDc subgroup. This was in consonant with Li X *et al*.,[41] that reported that dyslipidemia accompanied HFD regimen. Conversely, BR administration caused improvement in these profiles, where this improvement is better with the higher dose of BR. This effect was returned to the ability of BR to increase the expression of some enzymes that control the hydrolysis of TG. Additionally, theses enzymes, stimulate lipolysis in mature adipocytes which may also partially account for the weight loss effect of BR[42,43]. Others assumed that BR also reduces the levels of cholesterol in blood, by reducing its absorption from the intestine[44]. All these characteristics make BR a potential suppressor of adiposity via the regulation of lipid levels and treating dyslipidemia associated with obesity.

This study was also targeted to test beige genes (browning biomarkers); (UCP1), (PGC-1α) and (CIDEA) expression in the iWAT by (qRT-PCR) to confirm the browning effect of BR[14]. Interestingly, there was a marked expression of browning biomarkers in BR treated subgroups compared to the control subgroups.

These proteins are known to affect the function of BAT. UCP1 is present in the inner mitochondrial membrane in brown adipocytes and has an essential role in heat generation, interceded by its proton transport function[45]. During the process of white to beige adipose tissue conversion, UCP1 rich beige adipocytes appears within WAT[46]. Our results came in accordance with Lu X *et al*.,[47] who stated that BR stimulates the expression of UCP1. Moreover, PGC-1α is considered as one of the main inducers of brown adipocyte development, the expression of PGC-1α in white adipocytes make them acquire the BAT features, as it regulates the expression of UCP1[48]. For CIDEA, a lipid droplet associated protein in adipocytes,[49] its expression increase with britening and it was also reported that it has a regulatory function on UCP1[50]. Thus, the high expression of these biomarkers with BR administration, reveals its potentials in obesity control through browning of WAT.

CONCLUSION

Berberine in our study showed to be a safe browning agent, as it did not exhibit any signs of illness or mortality throughout the study period. Additionally, to the color the inguinal pad that appeared browner in BR-treated rats, BR not only decreased the weights of rats and the inguinal

fat depots' weights, but also transformed large unilocular adipocytes into smaller ones and make the beige adipocytes with their histological features to be noted. Likewise, the browning biomarkers; UCP1, PGC-1α and CIDEA genes expression were increased in the iWAT to confirm the browning effect of BR. Furthermore, BR was effective in treating the dyslipidemia associated with the obese rat model. Thus, we believe that BR has excellent potential as an effective safe anti-obesity agent.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير البربرين على السمنة من خلال تحويل النسيج الدهني الأبيض الأربي لجرذان الذكور إلى النسيج الدهني البني

ريهام عبد الله محمد عبد الرحمن[١]، دعاء علي عبد المنصف[٢]، إيمان فؤاد بيومي فرغلي[١]، مها وجدي أبو نازل[١]، إيمان إبراهيم أحمد زكي[١]

[١]قسم الهستولوجيا وبيولوجيا الخلية، [٢]قسم الكمياء الحيوية ـ كلية الطب ـ جامعة الأسكندرية.

المقدمة: السمنة تزيد من خطر الإصابة بالعديد من الأمراض. فقد تم استهداف تحويل الأنسجة الدهنية البيضاء (WAT) الى انسجة بنية في علاج السمنة. البربرين (BR) هو نبات قلويد طبيعي ، يستخدم بشكل رئيسي في علاج الإسهال. كشفت الدراسات أن BR يحسن بعض الاضطرابات الأيضية لدى البدناء ، , و لكن تأثير BR على تحويل الأنسجة الدهنية البيضاء WAT الى انسجة بنية لم يتم التحقيق فيه بشكل كامل.

الهدف من العمل: هو اختبار التأثير المحتمل لعقار البربرين على تحول لون WAT الأربي (iWAT) لذكور الجرذان البيضاء إلى اللون البني ، بالإضافة إلى دراسة تأثيره المحتمل في علاج الشحوم المضطربة المصاحبة للسمنة.

الطريقة وخطة العمل: تم تقسيم ٤٨ من ذكور الجرذان البيضاء البالغة إلى مجموعتين رئيسيتين تتغذيان بشكل طبيعي, الأولى على مجموعة نظام غذائي للدهون العادية (NFD) ومجموعة نظام غذائي عالي الدهون (HFD) . تم تقسيم مجموعة النظام الغذائي للدهون العادية (NFD) إلى ثلاث مجموعات فرعية. مجموعة التحكم في النظام الغذائي للدهون الطبيعية (NFDc) التي لم تتلق أي علاج. النظام الغذائي العادي للدهون - مجموعة جرعة البربرين المنخفضة (NFD + BRL) التي تلقت ٥ مجم / كجم من وزن الجسم (وزن الجسم) BR ، ومجموعة جرعة عالية من البربرين (NFD + BRH) التي تلقت ١٠ مجم / كجم من وزن الجسم بربارين. تم تقسيم مجموعة النظام الغذائي عالي الدهون (HFD) إلى ثلاث مجموعات فرعية: مجموعة التحكم في النظام الغذائي عالي الدهون (HFDc) ومجموعة النظام الغذائي عالي الدهون - مجموعة جرعة منخفضة من البربرين (HFD + BRL) ومجموعة النظام الغذائي عالي الدهون - مجموعة جرعة عالية من البربرين (HFD + BRH).

النتائج: اثرالبربرين بشكل فعال في الخلايا الشحمية في iWAT ، حيث لوحظ ظهورالخلايا الشحمية البيج. بالإضافة إلى ذلك ، تم زيادة التعبير عن علامات اللون البني في iWAT ، مثل UCP1 و PGC-1α و CIDEA بشكل ملحوظ. و قد حسن البربرين أيضًا من الشحوم المضطربة المسجلة في مجموعة HFD.

الأستنتاج: أظهر البربرين أنه عامل آمن لتحويل النسيج الدهني الأبيض إلى النسيج الدهني البني و قد أدى أيضًا إلى تحسين الشحوم المضطربة التي حدثت في مجموعة HFD. كما أظهرت الجرعة العالية نتائج أفضل من الجرعة الأقل. وبالتالي ، فإننا نعتقد أن BR لديه إمكانات ممتازة كعامل فعال وآمن لمكافحة السمنة.