Effect of *Brassica rapa* var. *rapifera* on Insulin Resistance and Inflammatory Analysis of Visceral Adipose Tissue of Obese Wistar Rats Under Glucolipotoxicity Condition

Efector de *Brassica rapa* var. *rapifera* sobre la Resistencia a la Insulina y el Análisis Inflamatorio del Tejido Adiposo Visceral de Ratas Wistar Obesas en Condiciones de Glucolipotoxicidad

Leila Smail1; Nadjiha Hamlat1; Sihem Berdja1; Saliha Boumaza1; Samia Neggazi1; Ghouti Kacimi2; Lynda Boudarene3 & Souhila Aouichat Bouguerra1


SUMMARY: Obesity-related pathophysiologies such as insulin resistance and the metabolic syndrome show a markedly increased risk for type 2 diabetes and atherosclerotic cardiovascular disease. This risk appears to be linked to alterations in adipose tissue function, leading to chronic inflammation and the dysregulation of adipocyte-derived factors. *Brassica rapa* have been used in traditional medicine for the treatment of several diseases, including diabetes. This study aimed to investigate the effect of nutritional stress induced by a high-fat and high-sucrose diet on the pathophysiology of visceral adipose tissue and the therapeutic effect of *Brassica rapa* in male Wistar rats. We subjected experimental rats to a high-fat (10 %) high-sucrose (20 %) for 11 months and treated them for 20 days with aqueous extract Br (AEBr) at 200 mg/kg at the end of the experiment. At the time of sacrifice, we monitored plasma and tissue biochemical parameters as well as the morpho-histopathology of visceral adipose tissue. We found AEBr corrected metabolic parameters and inflammatory markers in homogenized visceral adipose tissue and reduced hypertrophy, hyperplasia, and lipid droplets. These results suggest that AEBr enhances anti-diabetic, anti-inflammatory and a protective effect on adipose tissue morphology in type 2 diabetes and obesity.

KEY WORDS: *Brassica rapa*; AEBr; Diabetic; Glucolipotoxicity; Visceral adipose tissue.

INTRODUCTION

Overweight and obesity are defined as abnormal or excessive accumulation of body fat that can impair health (Keys *et al.*, 1972). Accumulation of fat in the abdominal region during obesity is strongly implicated in the development of insulin resistance, a necessary condition for the development of type 2 diabetes. In this situation, the action of insulin is impaired, resulting in extensive lipolysis and the release of free fatty acids. These used by the liver and muscle, to the detriment of glucose, favor the installation of glucolipotoxicity, marked by an overproduction of hepatic glucose, increased synthesis of hepatic and muscular triglycerides, and a reduction in the consumption of glucose by the liver and muscular. Visceral adipose tissue is also characterized by low-grade inflammation, marked by the recruitment of immune cells (Finkel & Holbrook, 2000). Thus, at the stage of insulin resistance, visceral adipose tissue presents morphological (infiltration of inflammatory cells, increase in the size of adipocytes) and functional signs (altered adipokine secretion profile with an increase in pro-inflammatory hormones and cytokines such as TNF-α, IL-6, and leptin, and decreased levels of proteins with anti-inflammatory and insulin-sensitizing properties such as adiponectin) established during obesity and type 2 diabetes (Wennen & Hotamisligil, 2003).

A variety of natural products have been proposed as pharmacological treatments for type 2 diabetes and metabolic syndromes. In traditional medicine, *Brassica rapa* or “turnip”...
Phytochemical study of aqueous extract of Brassica rapa (AEBr): the preparation of AEBr has been described (Smail et al., 2017).

**Diet.** The composition of the standard laboratory diet proposed by National Livestock Feed Office (ONAB) https://www.onabnutrition.dz. A rat’s daily intake is 20 g equivalent of 9 calories.

**High fat sucrose diet (HFSD):** this diet consists of a standard diet enriched with egg yolk (5 g equivalent of 45 calories) and sucrose (9 g equivalent of 36 calories) daily estimate of 90 calories.

**Ethical approval.** The present study was approved by the institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (DGRSDT; https://www.dgrsdt.dz) and Use Committee of the University of Bab Ezzouar (Algiers, Algeria; Permit number for the present research project: F00220110048) and has been achieved according to the Executive Decree no.10 n90 completing the executive Decree no. 04-82 of the Algerian government, establishing the terms and modalities of animal welfare in animal facilities.

**Animals.** Forty adult male albino rats were housed in a temperature controlled room and provided water and food ad libitum. They were acclimatized to laboratory conditions for one week prior to the experiments. The rats were randomly divided into four groups.

The first group (control) (n = 10 normal animals) and the second group (Control+AEBr) (n = 10 normal animals treated with AEBr 200 mg/kg for the 20 last days of experimentation) by intraperitoneal injection).

The third group was fed a high-fat high-sucrose diet (HFSD) (n = 10 animals) and the fourth group (HFSD+AEBr) (n = 10 animals induced by HFSD and treated with AEBr 200 mg/kg during the 20 last days of experimentation) was fed for 11 months.

**Biological study**

**Analytical methods.** The animals were bled from the retroorbital venous plexus. This technique eliminates the use of anesthetic agents that affect the measurement of biochemical parameters. Blood was collected in dry tubes, centrifuged at 3000 rpm for 10 min, and stored at -20°C. Blood glucose, triglyceride, total cholesterol, LDL-ch, and HDL-ch were measured using an enzymatic colorimetric method with a biosystem test kit. Blood insulin levels were determined by radioimmunoassay using the CIS test kit (ORIS INDUS) CPK. In addition, creatinine and LDL levels were determined using an automated CKL, 0-323.

**Isolation of tissue.** At the end of the experiment, the animals were euthanized with anesthesia with urethane (25 %) at a rate of 0.4 mL/100 g. Visceral adipose tissue was immediately excised, frozen in liquid azote, and stored at -80°C. The tissue was extracted in ice-cold solubilization buffer by using a motor-driven Potter homogenizer (20 mM L HEPES, 8 mM L EDTA, 0.2 mM L Na3VO4, 10 mM L Na4P2O7, 2.5 mM L phenylmethylsulfonyl fluoride, 1 mg/mL aprotinin, 2.5 mg/mL benzamidine, 2.5 mg/mL peptatin, 2.5 mg/mL leupeptin, 160 mM L NaF, 2 mM L dichloroacetic acid, and 1 % Triton X-100, pH 7.4. After 20 min at 4°C, the samples were centrifuged at 20000 ¥ g, and the supernatants were stored at -80°C.

The total lipid. Content was determined according to the method of Folch (1957).

**Inflammation markers**

**Determination of Nitrogen Monoxide (NO).** The measured concentration represents the sum of nitrite and nitrate. The conversion of nitrate to nitrite is based on a cadmium reduction reaction described in Grand et al. (2001).

**Tumor Necrosis Factor-α (TNF-α), Monocyte Chemotactic Protein-1 (MCP1), and Nuclear Factor Kappa B (NF-κB):** Assessment was performed using an immunoenzymatic assay. Invitrogen ELISA Kits were used to measure the levels of different markers in the visceral adipose tissue of all groups. The absorbance was measured...
at 450 nm using an ELISA plate reader (BioTek Instruments).

**Insulin Receptor Substrates (IRS1); Apoptosis Signal-regulated kinase 1 (AKT):** Assessment was performed using Invitrogen ELISA.

**Histology of visceral adipose tissue:** After fixation in Bouin’s aqueous, the specimens of visceral adipose tissue was dehydrated, embedded in paraffin, and cut at 3 µm. The sections were stained with Masson’s trichrome.

**Inclusion and Sectioning of Samples in the O.C.T:** Fragments of visceral adipose tissue were placed horizontally on a cryostat sample holder at -20 °C. The samples were then coated evenly with O.C.T (Tissue Cryostat, Sakura). After a few minutes allowing the O.C.T to attach to the tissue and dry, the sample holder can then be positioned vertically in order to make 3 µm sections. Tissue sections were fixed in formalin for 10 min and stained with Nile blue (Sigma).

**Culture of Adipocytes by Enzymatic Digestion:** Visceral adipose tissue was immediately immersed in a sterile tube containing trypsin 1 % (Gibco, USA) and placed in a water bath at 37 °C for 30 min. Trypsin (1 %) facilitated cell separation, and the resulting suspension contained separate adipocytes.

**Morphological, Histochemical, and morphometric analyses:** A drop of the cell suspension was spread on a glass slide (26 × 76 mm) placed on a hot plate, and the adipocytes were fixed by heating on a glass slide. The cells were stained for 10 min with a solution of May Grunwald Giemsa (v/v, 1/1, Fluka). To identify neutral lipids, histochemical analysis was performed by staining with Nile blue (Sigma) for 10 min. Observations were performed using an Olympus light microscope. Cell counts were performed for 100 cells in different fields on several slides.

**Statistical analysis:** Data were analyzed by ANOVA using Graph pad version 8. The results are expressed as the mean ± standard deviation. Differences were considered statistically significant at P ≤ 0.05.

**RESULTS**

**Biochemical Analysis:** The diet administered to animals in this study induced obesity and type 2 diabetes. In our experiment, we noted a significant increase in glycemia, triglyceridemia, and cholesterolemia as well as a decrease in insulinemia in animals subjected to HFSD compared to the corresponding control signs of diabetes mellitus, accompanied by an increase in atherogenic markers, LDL-cholesterol and a decrease in cardioprotective lipid HDL-cholesterol (Figs.1 and 2). Treatment with AEBr corrected the metabolic disorders by reducing glycemia, triglyceridemia, cholesterolemia levels and increasing the levels of insulin compared to diabetic animals (Figs.1 and 2), protected the animals’ vascular complications by decreasing atherogenic markers, and increased the levels of cardioprotective lipids (Fig. 2).

**Total Lipid:** The estimation of the total lipid levels in visceral adipose tissue as well as its triglyceride content indicated a significant increase in an animal group HFSD compared to the corresponding controls, this increase is 79.41 ± 14.29 vs 59.76 ± 9.92 mg / 100 g tissue of total lipids (P < 0.0001) (Fig. 3). Administration of AEBr to animals subjected to HFSD significantly decreased total visceral adipose lipid content by reducing lipid accumulation (Fig. 3).

**Inflammation Markers:** The estimation of inflammatory states in visceral adipose tissue by rates of NO, MCP1, TNFα, and NF-kB showed significantly an increase in HFSD animal group compared to controls (Fig. 4). AEBr treatment significantly reduced the levels of these markers (Fig. 4).

**Fig. 1.** Effect of AEBr in insulin-resistance after the administration of high fat-sucrose diet in *Rattus norvegicus*. Control: *Rattus norvegicus* control; Control + AEBr: *Rattus norvegicus* control treated with aqueous *Brassica rapa* extract; HFSD: *Rattus norvegicus* received a high-fat (10 %) and high-sucrose (20 %) diet; HFSD + AEBr: *Rattus norvegicus* received a high-fat sucrose diet and was treated with an aqueous extract of *Brassica rapa*. IRS1 p [S312]: Insulin Receptor Substrates; AKT p [S473]: Apoptosis Signal-regulated kinase 1

Data are expressed as the mean ± S.E.M; (n =10), ****p < 0.0001 (HFSD vs control); +++p < 0.001, ++++p < 0.0001 (HFSD + AEBr vs HFSD);
Insulin resistance markers: The evaluation of IRS1 Ps 312 and AKT showed a large increase in IRS1 and a decrease in AKT in visceral adipose tissue in the HFSD group compared to the control (Fig. 1). AEBr treatment significantly corrected the levels of IRS1 Ps 312 and AKT.

Histopathology and histochemical analysis of Visceral Adipose Tissue: Topographic staining (Masson's trichrome) showed normal organization of adipose tissue, characterized by lobules in which fat cells were limited by relatively dense connective tissue (Figs. 6 and 7).
In addition, we observed structural alterations were marked mainly by an increase in the size of the adipocytes, indicating hypertrophy, and the presence of hemorrhagic and inflammatory foci such as fibrosis within adipose tissue (Fig. 6).

Histochemical analysis (Nile blue) revealed neutral lipids in pink and fatty substances with an acidic character (blue). Compared with the corresponding controls, we observed structural alterations marked mainly by adipocyte hypertrophy (Fig. 7) and accumulation of neutral lipids within adipocytes (Fig. 8).

**Morphological and cytochemical studies of the adipocytes:** The adipocytes of rats subjected to a HFSD stained with MGG showed cellular hypertrophy, an increase in the number of adipocytes indicating hyperplasia, and the appearance of budding of the membrane plasma were observed compared to their corresponding controls (Fig. 8). Cytochemical examination by Nile blue staining of the same cells revealed pinkish coloration of the lipid droplets, highlighting neutral lipids.

**DISCUSSION**

Long-term administration (11 months) of a HFSD causes metabolic disorders and alterations in visceral adipose tissue in rats. Our results confirmed the work of (Smail et al., 2017) on *Psammomys obesus*. In our study, we observed hyperglycemia accompanied by hypoinsulinemia after 6 months of a high-carbohydrate diet. Likewise, we noted the appearance of insulin resistance marked by an increase in the levels of IRS phosphorylated on serine residues and a decrease in the levels of AKT p S473, indicating an inhibition of the insulin receptor.
signaling pathway, characteristic of type 2 diabetes (Smail et al., 2017). Treatment with AEBr (200 mg/kg) during the last 20 days of the experiment improved hyperglycemia and insulin resistance in diabetic rats by decreasing blood glucose and IRS1 PS312 levels, and re-balanced the level of insulin and AKT P473. Our results are in agreement with those of (Mohajeri et al., 2011) in alloxan-diabetic rats treated with Brassica rapa (200 mg/kg). The antidiabetic effect of Brassica juncea seed extract has been attributed to stimulation of glycogen synthesis, resulting in increased hepatic glycogen content and suppression of glycogen phosphorylase and other glyconeogenic enzyme activities (Khan et al., 1995). Our results showed the occurrence of dyslipidemia marked by an increase in triglyceridemia, LDL-Ch, and a decrease HDL-Ch, accompanied by a hypertrophy and infiltration of inflammatory cells into lipid droplets of visceral adipose tissue. This situation of exacerbation of glycemia and lipidemia would be a marker of glucotoxicity and lipotoxicity resulting in lipolysis induced by hyperleptinemia (Daryoush et al., 2011); the administration of AEBr improves the metabolic disorder. Brassica rapa induces a hypoglycemic effect by decreasing glucose levels, improving insulin levels, and correcting dyslipidemia associated with increased HDL-Ch. Our results showed a reduction in triglyceridemia in lipid droplets in visceral adipose tissue after the administration of Brassica rapa in diabetic rats, which is consistent with numerous studies showing the effect Brassica antihypertriglyceridemic agent in diabetic rats (Daryoush et al., 2011). Structural alterations were observed in the adipose tissue of rats subjected of a HFSD. Staining of sections with Masson’s trichrome and Nile blue sulfate revealed a phenomenon a significant adipocyte hypertrophy and hyperplasia, with fat cells from obese rats much larger than those from controls, polyhedral in appearance and giant lipid droplet occupying ther entire cytoplasmic space. Very small adipocytes with microdroplets inside were also observed in these sections. According to Virtue & Vidal-Puig (2010) in the case of obesity, there is an increased demand for lipid storage and to meet this demand and accommodate excess lipids, adipose tissue increases its storage capacity by increasing the size of its adipocytes (hypertrophy) and/or increasing their number (hyperplasia). Lipid storage and mobilisation are dynamically regulated at the level of the lipid droplet making it more than just a place of desposition (Paar et al., 2012). Lipid droplet remodeling in a pathophysiological case such as obesity can be explained either by the addition of neutral lipids to the pre-existing droplet (Murphy et al., 2010). Numerous studies have shown that in humans and rodents, obesity-associated inflammation is manifested by macrophage infiltration in adipose tissue, with macrophages constituting up to 50 % of the cells in fat (Paar et al., 2012). Adipose tissue is a source of increased levels of TNF in obese macrophages and fat cells (Murphy et al., 2010). Although macrophages are potent sources of pro-inflammatory cytokines, the triggers of adipose tissue inflammation are not fully understood but may involve the secretion of cytokines or chemokines by adipocytes or endothelial cells. Hypertrophy and necrosis may also occur (Paar et al., 2012). TNF inhibits the action of insulin in vitro and in vivo by altering the expression or activity of several proteins in the insulin signaling pathway in cells. TNF decreases insulin receptor stimulation by insulin as well as phosphorylation...
of insulin receptor substrate (IRS) in cultured cells and tissues (Kang et al., 2022). TNF is an endocrine and paracrine mediator of insulin resistance in obesity. Induced hyperglycemia in T lymphocytes increases the synthesis of pro-inflammatory cytokines, which are responsible for the increase in NO by activation of inducible nitric oxide synthase (iNOS) (Stentz et al., 2005). Glucolipotoxicity and insulin resistance activate the transcription factor NF-κB and play a role in inducing pro-inflammatory gene expression and promoting the inflammatory process in vascular tissue. The initial event of inflammation in vascular tissue begins with the recruitment of monocytes to lesions, where MCP-1 plays a key role (Dagre et al., 2005). NF-κB coordinates the induction of a wide range of genes encoding pro-inflammatory cytokines (interleukins 1, 2 and 6 and TNFα), chemokines (IL-8 and MCP1), adhesion molecules, acute phase proteins, immune receptors, growth factors, and inducible enzymes such as vascular endothelial growth factor, cyclooxygenase-2 (COX-2), matrix metalloproteinases, iNOS, all molecules involved in inflammation other than angiogenesis, cell proliferation, adhesion, migration and invasion (Mahmoud et al., 2013). Brassica ameliorates inflammatory conditions. This result is consistent with a previous study showing an improvement in inflammatory states with the presence of quercetin, kaempferol, epigallocatechin gallate and curcumin reducing inflammatory markers (NO, MCP-1, TNFα and NF-κB) in experimental diabetics models (Mahmoud et al., 2013). The current study showed that a diet high in fat and sucrose increased the level of IRS1-p-serine and impaired AKT activity in the visceral adipose tissue. Brassica normalized IRS1 and AKT levels in diabetic rats. Hyperglycemia leads to the activation of several serine kinase cascades. There are several potential targets for these kinases in the insulin signaling pathway, including the insulin receptor (IR) and insulin receptor substrate (IRS) proteins. Increased phosphorylation of IRS at serine sites, instead of tyrosine, indicates impaired insulin activity. In fact, IRS1 serine phosphorylation is less able to associate with downstream target molecules, particularly phosphatidylinositol 3-kinase/AKT, because of impaired insulin action and glucose transport. This state promotes hyperinsulinemia and development of insulin resistance (Kim et al., 2006).

These results suggest that the naturally occurring AEBr may have important implications for the prevention and early treatment of T2DM and its complications.

ACKNOWLEDGEMENTS

Team from the anatomy pathology laboratory of the CHU of BENI MESSOUS

REFERENCES


**Corresponding author:**

Dr. Leila Smail  
Faculty of Biological Sciences  
University of Sciences and Technology Houari Boumediene, (USTHB)Algers ALGERIA  
E-mail: leila84.smail@gmail.com