Glucotoxicity, lipotoxicity, and glucolipotoxicity are secondary phenomena that are proposed to play a role in all forms of type 2 diabetes. The underlying concept is that once the primary pathogenesis of diabetes is established, involving probably both genetic and environmental forces, hyperglycemia and very commonly hyperlipidemia ensue and thereafter exert additional damaging or toxic effects on the $\beta$ cells. Elevations of plasma fatty acid levels that often accompanying insulin resistance (1, 2) may also play a pathogenic role in the early stages of the disease as glucose levels begin to rise outside of the normal range, as well as their contribution on the deterioration of $\beta$ cell function after the onset of the disease (3).

The abnormal metabolic medium created by hyperglycemia or the diabetic state initiates series of dysfunctional responses culminating in the development of premature cardiovascular disease (4, 5). The oxidative stress pathway serves, as a common element, to link all the major pathways implicated in diabetic vasculopathy (6). Hyperglycemia induces oxidative stress in vascular cells by enhancing the production of reactive oxygen species (ROS). These results in damage to cellular proteins, membrane lipids, DNA and activation of nuclear factor-$\kappa$B (NF-$\kappa$B) (5).

Fruits and vegetables are primarily food sources providing essential nutrients for sustaining life. They also contain a variety of phytochemicals such as phenolics and flavonoids providing important health benefits. Thus, regular consumption of fruits and vegetables is associated to reduced risks of chronic diseases, such as cancers and cardiovascular disease (7).
Brassica Rapa (Br) has active biological compounds such as flavonoids (isorhamnetin, kaempferol and quercetin glycosides), phenylpropanoids derivatives (8), indole alkaloids and sterol glucosides (9). Indole fraction from Brassica rapa roots is known to possess anti-inflammatory potential via inhibition of pro inflammatory mediators such TNFα and interleukin-6 (10), antidiabetic effect in diabetes mellitus type 2 (11, 12). We found out that it was interesting to induce a glucolipotoxicity in vivo by a high fat high sucrose diet, in Psammomys obesus and analyze the Brassica rapa therapeutic effect.

EXPERIMENTAL

Biological material

The present study was approved by the Institutional Animal Care 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. 10–90 completing the Executive Decree no. 04–82 of the Algerian Government, establishing the terms and modalities of animal welfare in animal facilities.

Psammomys obesus gerbils also known as the “fat sand rat” were captured in the Algerian Sahara. During two week acclimation period, the animals were fed with the halophilic plants, rich in water and mineral salts (13), that they normally eat in the desert. They were put in individual cages under controlled temperature and lighting conditions, with free access to food and water. They were divided into four groups. The first group (n = 8 normal control), the second group (n = 8 normal treated with Br 200 mg/kg during 20 days by oral gavage) were fed, during 9 months, by natural plants from the same halophilic family during 9 months, but growing along the edge of sea (salicornia; composition per 100 g: water 80.8 g; mineral salts 6.9 g; lipids 0.4 g; proteins 3 g; carbohydrates 8.4 g; and 45-50 kcal/100 g). The third group (n = 8 diabetic control) and the fourth group (n = 8 diabetic treated with Br 200 mg/kg during 20 days by oral gavage) received during 6 months a high-fat (10%) and high sucrose (20%) diet, comprising the salicornia plants plus the daily addition of half of cooked egg yolk and 20 g of saccharose per day (composition of egg per 100 g: water 40-60 g; proteins 13.5 – 17.5 g; carbohydrates 0.2 g; lipids 30 – 31 g; cholesterol 1.2 – 1.3 g; and 370 – 400 kcal/100 g).

Preparation of aqueous extract of Brassica rapa var rapiferra

Fresh roots of B. rapa var. rapiferra were collected from Algeria’s markets in February 2012. Voucher specimen (INA/P/No 6) has been kept in the herbarium of the Department of Botany, National Institute of Agronomy (INA), Algiers, Algeria. The roots were separated from turnip tops, each cut into small slices and dried in shade till complete drying. The plant material was pulverized into powdered form. The aqueous extract was prepared by decocting powdered roots (100 g) three times till complete exhaustion, then the collected aqueous extract was lyophilized (Cryodos 80, -75°C, 5 m3/h) to obtain extract in yield of 0.15%. The extract was stored in sealed glass vials at ± 4°C prior to be tested and analyzed.

Chemical study

Total phenolic content

Total phenolic contents of the extract were determined using Folin–Ciocalteu reagent according to the method of (14), using gallic acid as a standard, and as modified by (15). An aliquot (0.2 mL) of extract solution containing 1000 µg of extract was mixed with 46 mL of distilled water and 1 mL of Folin–Ciocalteu reagent in a volumetric flask. After spending 3 min in the dark, 3 mL of sodium carbonate solution (7.5%) was added. Absorbance at 740 nm was measured in a spectrophotometer (Shimadzu 1800, Mulgrave, Victoria, Australia) after shaking and spending an additional 2 h in the dark. The total phenolic content was evaluated from a standard calibration curve of gallic acid, and results were expressed as microgram of gallic acid equivalents per milligram of extract (µGAE/mg).

<table>
<thead>
<tr>
<th>Extract / Standards</th>
<th>Total phenolic content (µg GAE/mg)</th>
<th>Total flavonoids (µg QE/mg)</th>
<th>DPPH (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>9.41 ± 0.18</td>
<td>1.01 ± 0.09</td>
<td>2100 ± 13</td>
</tr>
<tr>
<td>BHA</td>
<td>n.a</td>
<td>n.a</td>
<td>21.28 ± 0.12</td>
</tr>
<tr>
<td>BHT</td>
<td>n.a</td>
<td>n.a</td>
<td>12.76 ± 0.08</td>
</tr>
</tbody>
</table>

Each value is expressed as the means ± standard deviations for triplicate experiments. n.a: not applied.
Therapeutic effect of *Brassica rapa var. rapifera* in type...

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**Determination of total flavonoids**

The total flavonoids were determined according to the Dowd method described by (15). 4 mL of diluted solution of extract was mixed with 4 mL of aluminum trichloride solution (2% in methanol). After 15 min, the absorbance was measured at 415 nm. Quercetin was used as reference compound to produce the standard curve. The results are expressed as µgQE/mg.

**Antioxidant activity: Scavenging effect on DPPH radical**

The DPPH free radical scavenging assay was carried out as described by (16). The aqueous extract was dissolved in methanol. Sample of 25 µL of each concentration (100, 200, 400, 600, 800 and 1000 µg/mL) were added to the DPPH methanol solution (60 µM, 975 µL). After 30 min of incubation at 25°C, the absorbance at 517 nm was measured by using UV spectrophotometer (Jasco, V-530). Ascorbic acid and α-tocopherol were used as reference compounds. The radical scavenging activity was then calculated from the equation: % of radical scavenging activity = [(Abs sample – Abs control)/Abs control] × 100, where Abs sample is the absorbance of the tested extract.

**Biological study**

**Analytical methods**

The animals were bled from the retro-orbital venous plexus; this technique eliminates the use of anesthetic agents affecting measurements of biochemical parameters. Blood, collected in dries tubes, was centrifuged at 3000 rpm for 10 min and stored at -30°C. Blood glucose, triglyceride, cholesterol and protein were measured by enzymatic colorimetric method using a test kit of Biosystem. Blood insulin was determined, by radioimmunoassay, using CIS test kit (ORIS INDUS) CPK. In addition, uric acid was determined by the automate CKL, 0-323.

**Isolation of tissue**

At the end of the experiment, animals were killed after anesthesia with urethane (25%). The aorta and heart tissues were 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 Na2P04, 2.5 mM/L phenylmethyisulfonfyl 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, 1% Triton X-100, pH 7.4. After 20 min at 4°C, the samples were centrifuged at 20000 × g, the supernatants were stored at -80°C.

**Determination of malondialdehyde (MDA)**

The MDA was evaluated after reaction with thiobarbituric acid (TBA) (Sigma) (17). The samples were centrifuged at 10000 × g for 20 min at 4°C in buffered (Na2HPO4/NaH2PO4) 0.2 M, pH 6.5. The MDA contained in the supernatant in the presence of 10% TCA reacts with TBA and causes the formation of a complex read at 532 nm.

**Catalase activity determination (CAT)**

CAT activity was determined by monitoring the disappearance of H2O2 at 240 nm. The reaction mixture contained 50 mM K-phosphate buffer (pH 7) 0.33 mM H2O2 and enzyme extract (18).

**Measurement of inflammation insulin pathway markers**

The assessment was determined by immunoenzymatic assay. Invitrogen ELISA Kits were used for measuring the levels of different markers in the tissue. IRS 1 [S312], AKT [S p 473], and NF-xB p65. The estimation is made by Elisa reader at 450 nm (BioTek Instruments).

**Statistical analysis**

Data were analyzed with ANOVA using STATISTICA version 6 and completed with HSD Tukey test. The results were expressed as the mean ± standard deviation.

The differences at level p < 0.05 were considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Chemical study**

**Total phenolics and flavonoids contents**

The aqueous extract showed a weak amount of phenolic and flavonoid compounds with values of 9.41 ± 0.18 µg GAE/mg and 1.01 ± 0.09 µg QE/mg, respectively (Table 1).

**Antioxidant activity**

The principle antioxidant activity was based on the availability of electrons to neutralize any free radicals. In the present study, the antioxidant activity was evaluated using scavenging DPPH free radicals assay. The results of the aqueous extract and those controls (BHA and BHT).

The investigated aqueous extract of *Brassica rapa* showed a weak antiradical activity with IC50...
value of \(2100 \pm 13 \mu g/mL\), which was extremely lower than the high antioxidant effect of BHA and BHT (\(IC_{50} = 21.28 \pm 0.12 \mu g/mL\) and 12.76 \(\pm 0.08 \mu g/mL\), respectively) (Table 1).

**Effect of Br on biochemical parameters of Po**

As concerns animals submitted to natural diet supplemented with high fat-sucrose diet (\(P\) diabetic) during 9 months of experiment, we noted a high increase of serum glucose, proteins and dyslipidemia which was marked by TC, TG, VLDL, LDL and decreased HDL in \(P\) diabetic. Therefore, a highly significant increase of cardiac marker CPK and LDH and kidney marker creatinine were observed in \(P\) diabetic rats compared to \(P\) control. The treatment with aqueous extract of *Brassica rapa* (\(Br\))ameliorated the deregulation observed under a high fat high sucrose diet (Table 2a).

**Effect of \(Br\) redox statues of Po**

The elevation of plasma and erythrocyte MDA level in the group of \(P\) diabetic was highly significant but reversed with the Cat in this condition. The treatment with of \(Br\) prevented this oxidative stress (Table 2b).

The evaluation of insulinema showed an increase after 3 months and a decrease after 9 month of experiment (Fig. 1).

**Effect of \(Br\) on inflammatory parameters and redox statues of \(Po\)**

In both aorta and heart tissue, the AKT level was decreased while the NFκB level was increased in \(P\) diabetic. The treatment with \(Br\) modulated these parameters. The evaluation of redox status parameters showed an increase of MDA and a decrease of Cat levels in aorta and heart of \(P\) diabetic compared to \(P\) control, and reversed with treatment of \(Br\) in \(P\) diabetic +\(Br\) (Table 3 and 4).

This study emphasized on the protective effect in vivo of aqueous extract of *Brassica rapa* (\(Br\)) in a high fat-sucrose diet treated diabetic rats.

Total phenolics and flavonoids contents were determined according to their importance as an

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**Table 2a. Effect of aqueous extract of \(Br\) in glucose, lipid profile, CPK and protein level after the administration of high fat-sucrose diet in *Psammomys obesus* (**Po**).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(P) control</th>
<th>(P) control + (Br)</th>
<th>(P) diabetic</th>
<th>(P) diabetic + (Br)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g/L)</td>
<td>0.65 ± 0.05</td>
<td>0.62 ± 0.06</td>
<td>1.81 ± 0.12****</td>
<td>1.15 ± 0.13++</td>
</tr>
<tr>
<td>Triglyceride (TG) (g/L)</td>
<td>0.38 ± 0.08</td>
<td>0.37 ± 0.09</td>
<td>3.46 ± 167****</td>
<td>1.68 ± 0.44++</td>
</tr>
<tr>
<td>Cholesterol total (TC)(g/L)</td>
<td>0.9 ± 0.06</td>
<td>0.81 ± 0.02</td>
<td>4.88 ± 0.22****</td>
<td>2.35 ± 0.53++</td>
</tr>
<tr>
<td>LDL-chol. (mM/L)</td>
<td>0.54 ± 0.26</td>
<td>0.49 ± 0.01</td>
<td>27.55 ± 3.18****</td>
<td>0.64 ± 0.14++++</td>
</tr>
<tr>
<td>HDL- chol. (mM/L)</td>
<td>1.89 ± 0.21</td>
<td>1.71 ± 0.1</td>
<td>1.05 ± 0.08**</td>
<td>5.37 ± 0.67++++</td>
</tr>
<tr>
<td>Creatinine (U/L)</td>
<td>28 ± 1.41</td>
<td>25 ± 0.01</td>
<td>114 ± 17.61****</td>
<td>44.75 ± 14.08+++</td>
</tr>
<tr>
<td>CPK (U/L)</td>
<td>4 ± 0.1</td>
<td>4 ± 0.5</td>
<td>642.5 ± 114.9****</td>
<td>90.75 ± 7.41++++</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>4 ± 2.82</td>
<td>3 ± 1.13</td>
<td>92 ± 10.10****</td>
<td>13.75 ± 4.27+++</td>
</tr>
<tr>
<td>Protein (mg/dL)</td>
<td>0.67±0.03</td>
<td>0.66 ±0.06</td>
<td>0.87 ± 0.00**</td>
<td>0.66 ± 0.03++</td>
</tr>
</tbody>
</table>

**Table 2b. Evaluation of the oxidative stress parameters in the blood of **Po submitted at high fat-sucrose diet and treated with aqueous extract of \(Br\).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(P) control</th>
<th>(P) control + (Br)</th>
<th>(P) diabetic</th>
<th>(P) diabetic + (Br)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (U/L)</td>
<td>0.17 ± 0.01</td>
<td>0.35 ± 0.03</td>
<td>0.07 ± 0.00****</td>
<td>0.61 ± 0.01++</td>
</tr>
<tr>
<td>Catalase eryth. (U/L)</td>
<td>1.43 ± 0.26</td>
<td>2.19 ± 0.08</td>
<td>1.20 ± 0.08</td>
<td>2.36 ± 0.64++++</td>
</tr>
<tr>
<td>MDA (µM/L)</td>
<td>72.8 ± 5.31</td>
<td>71.2 ± 3.65</td>
<td>149.8 ± 2.60****</td>
<td>71.4 ± 0.89+++</td>
</tr>
<tr>
<td>MDA eryth (µM/L)</td>
<td>87 ± 1.41</td>
<td>80 ± 0.56</td>
<td>108 ± 1.58****</td>
<td>92.2 ± 3.96+++</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M; (n = 8). **p < 0.001, ****p < 0.0001 (\(P\) diabetic vs \(P\) control), +p < 0.001, ++++p < 0.0001 (\(P\) diabetic + \(Br\) vs \(P\) diabetic); \(P\) control: *Psammomys obesus* control; \(P\) control + \(Br\): *Psammomys obesus* control treated with extract aqueous of *Brassica rapa*; \(P\) diabetic: *Psammomys obesus* received a high-fat (10%) and high sucrose (20%) diet; \(P\) diabetic + \(Br\): *Psammomys obesus* received a high fat-sucrose diet and treated with extract aqueous of *Brassica rapa*.**
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Antioxidant compounds. In fact, there is a relationship between the antioxidant ability and the total phenol contents. Phenolic antioxidants are products of a secondary metabolism in plants, and the antioxidant activity is mainly induced in their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (19). The principle antioxidant activity is based on the availability of electrons to neutralize any free radicals. In the present study, the antioxidant activity was evaluated by determining IC₅₀, using scavenging DPPH free radicals. The value of IC₅₀ was used in the *in vitro* study. This activity could be

![Figure 1. Effect of aqueous extract of *Br* in insulinemia after the administration of high fat-sucrose diet in *Po*. Data are expressed as the mean ± S.E.M; (n = 8). ***p < 0.0001, **p < 0.01 (P diabetic vs. P control), NS, ++++p < 0.0001 (P diabetic + Br vs. P diabetic).](image)

Table 3. Effect of aqueous extract of *Br* in the inflammation-oxidative stress parameters of the aorta after the administration of high fat-sucrose diet in *Po*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P control</th>
<th>P control + Br</th>
<th>P diabetic</th>
<th>P diabetic + Br</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (pM/100 g)</td>
<td>2.68 ± 2.27</td>
<td>2.65 ± 2.00</td>
<td>179.75 ± 44.8****</td>
<td>3.03 ± 1.04++++</td>
</tr>
<tr>
<td>Catalase (UI/min/g protein/100 g)</td>
<td>35.93 ± 5.74</td>
<td>33.8 ± 2.6</td>
<td>6.74 ± 139****</td>
<td>24.38 ± 8.10++++</td>
</tr>
<tr>
<td>AKT (pg/mL)</td>
<td>1.15 ± 0.53</td>
<td>1.04 ± 0.43</td>
<td>0.41 ± 0.29****</td>
<td>1.01 ± 0.04++++</td>
</tr>
<tr>
<td>NFkB (ng/mL)</td>
<td>9.80 ± 0.63</td>
<td>9.76 ± 0.54</td>
<td>92.70 ± 0.63****</td>
<td>16.3 ± 0.59++++</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M; (n = 8). ***p < 0.0001 (P diabetic vs. P control), ++++p < 0.0001 (P diabetic + Br vs P diabetic)

Table 4. Effect of aqueous extract of *Br* in the inflammation-oxidative stress parameters of the heart after the administration of high fat-sucrose diet in *Po*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>P control</th>
<th>P control + Br</th>
<th>P diabetic</th>
<th>P diabetic + Br</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (pM/100 g)</td>
<td>1.21 ± 0.27</td>
<td>1.18 ± 0.10</td>
<td>1.98 ± 0.08**</td>
<td>0.89 ± 0.20+++</td>
</tr>
<tr>
<td>Catalase (UI/min/g protein/100 g)</td>
<td>17.63 ± 11.52</td>
<td>16.33 ± 0.69</td>
<td>10.72 ± 3.53**</td>
<td>30.80 ± 24.46</td>
</tr>
<tr>
<td>AKT (pg/mL)</td>
<td>0.15 ± 0.03</td>
<td>0.14 ± 0.02</td>
<td>0.01 ± 0.00****</td>
<td>0.16 ± 0.01++</td>
</tr>
<tr>
<td>NFkB (ng/mL)</td>
<td>4.22 ± 1.32</td>
<td>4.20 ± 8.84</td>
<td>5.92 ± 0.08</td>
<td>4.06 ± 1.78</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± S.E.M; (n = 8). **p < 0.001, ****p < 0.0001 (P diabetic vs. P control), ++p < 0.001, (P diabetic + Br vs. P diabetic)
explained by the presence of the phenolics compounds which are related to different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion-chelation, and acting as substrates for radicals such as superoxide and hydroxyl (20).

In our study, we found a hyperglycemia associated to hypertriglyceridemia and hypercholesterolemia characteristic of type 2 diabetes (21-23). After 20 days of treatment with aqueous extract of *Brassica rapa* (200 mg/kg) ameliorated this biochemical parameters in diabetic rats. Our results were according to (24) in alloxan diabetic rats and treated with *Brassica rapa* (200 mg/kg), in streptozotocin diabetic rats and treated with different dose of *Brassica nigra* (25) and in diabetic rats treated with *Brassica oleracea* (26).

The hypoglycemic effect of the seed extract of *Brassica juncea* was attributed to the stimulation of glycogen synthesis leading the increase of hepatic glycogen content and the suppression of glycogen phosphorylase and other glyconeogenic enzymes activity (27). Of the same, oral administration of *BNO* (*Brassica Nigra Oil*) 1000 mg/kg body weight significantly increased the hepatic glycogen levels in STZ diabetic rats, possibly because of reactivating the glycogen synthase system as a result of the presence of H2O2 (41, 42). There is a clear link between hyperglycemia and active oxygen/nitrogen species in experimental and clinical types of diabetes (43). Accumulation of reactive oxygen species (ROS) due to oxidative stress is also instrumental in the expression of cell death as ROS can easily react with and oxidize vital cellular components such as lipids, proteins and DNA (44).

In our study, we showed a compensatory hyperinsulinemia associated with hyperlipidemia after 3 months of a high fat-sucrose diet indicator of insulin resistance. (45) suggested that impaired blood lipids were the characteristic of subjects with insulin resistance, especially circulating FFAs (46). Fatty acid mobilization from adipose tissue is sensitive to insulin. Insulin is most potent acting in the suppression of adipose tissue lipolysis. A rise of plasma insulin concentration of only 5 IU/mL inhibits lipolysis by 50%, whereas a reduction in basal insulin levels results in a marked acceleration of lipolysis (47). Our treatment ameliorates these disorders. In (28) it was demonstrated that BNO increased plasma insulin concentrations in diabetic rats. Insulin levels higher than those of the control group may result an inhibition of lipolysis and decreased plasma triglyceride and cholesterol levels. These observations were supported by a recent evidence that FFAs activate directly macrophage to secrete pro-inflammatory cytokines that render muscle cells insulin resistant (48). Meanwhile, the role of the presence of the phenolics compounds which are related to different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion-chelation, and acting as substrates for radicals such as superoxide and hydroxyl (20).
of pro-inflammatory cytokines in regulating insulin sensitivity has been suggested by several lines of evidence. To this effect, subjects with T2DM exhibited higher serum levels of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6 (49). Moreover, FFAs contribute on the increased production of reactive oxygen species and lead to the activation of stress-sensitive signaling pathways under hyperglycemic status (50). The polyphenols of quercetin had anti-oxidative and anti-inflammatory activities (12).

CONCLUSIONS

In conclusion, the present study has demonstrated the potency of aqueous extract of Brassica rapa (Br) to ameliorate hyperglycemia, hyperlipidemia and insulin resistance in diabetic rats. Brassica extract suppressed oxidative and inflammation stress in vivo in plasma and aorta and heart tissue in rats fed a high fat-sucrose diet. These results suggest that aqueous extract of Brassica rapa (Br) may have an important implications for the prevention and early treatment of T2DM.

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Received: 18. 10. 2016