

1 **Berberine chloride ameliorated PI3K/Akt-p/SIRT-1/PTEN signaling**
2 **pathway in insulin resistance syndrome-induced rats**

3 Short running title:

4 **Berberine chloride targeting PI3K/Akt-p/SIRT-1/PTEN pathway in HFD-**
5 **treated rats**

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18 **Abstract:**

19 Insulin resistance is one of dangerous factors as it leads to numerous metabolic
20 disorders such as non-insulin dependent diabetes mellitus. It affects most tissues
21 mainly adipose tissue, liver and muscle. Nowadays, berberine has several medical
22 applications against diseases. The current study was carried out to identify the effect
23 of berberine chloride (BER-chloride) on phosphatidyl inositol-3-kinase/
24 phosphorylated protein kinase B/ sirtuin type 1/ phosphatase and tension homologue
25 (PI3K/Akt-p/SIRT-1/PTEN) pathway during insulin resistance phenomena. Insulin
26 resistance model was performed in experimental rats by using high fat diet. Plasma
27 glucose, serum insulin, lipid profiles, hepatic oxidative stress markers were estimated.
28 Serum transaminases activities and kidney function tests were determined. Further,
29 hepatic PI3K, AKt-p, SIRT-1; PTEN levels were assayed. The concentration of
30 adiponectin in serum, hepatic tissue and white adipose tissue was determined.
31 Moreover, fold change in hepatic insulin, insulin receptor and retinol binding protein-
32 4 (RBP4) at molecular level was performed. Histopathological study of white adipose
33 tissue was also determined. The results showed increase the rats' body weights, blood
34 glucose, homeostatic model assessment, glycated hemoglobin, insulin and lipid
35 profiles levels in group of rats fed on high fat diet for eight weeks and this elevation
36 was decreased after administration of BER-chloride for two weeks. Further, BER-
37 chloride administration exhibited improvement of oxidative stress parameters, PI3K,
38 AKt-p, SIRT-1 and PTEN. This was associated with down-regulation of RBP4.
39 According to these data we conclude that, BER-chloride mediated several insulin
40 signaling pathways that could be of therapeutic significance to insulin resistance.

41 **Key words**

42 Berberine chloride, insulin resistance, adiponectin, insulin receptor.

43 **1. Introduction**

44 The increased prevalence of obesity between individuals has become a serious health
45 problem worldwide. Under normal conditions, β -cell of pancreas maintains the
46 normal glucose tolerance by increasing insulin release to overcome the reduction of
47 insulin efficiency. One of the predisposing risk factor to obesity is the amount of fat in
48 the diet due to modern life styles. Obesity usually accompanied by insulin resistance
49 and hyperglycemia [1]. Insulin resistance defined as a disease condition in which
50 insulin is secreted from β -cell of pancreas but its function is impaired in peripheral
51 tissues such as liver, adipose tissue and skeletal muscle. Insulin resistance usually
52 associated with metabolic disorders such as hyperlipidemia, type II diabetes mellitus,
53 non-alcoholic fatty liver and cardiovascular disease and early mortality is considered
54 one of insulin resistance prognosis in some individuals [2]. Phosphatidyl inositol-3-
55 kinase/ phosphorylated protein kinase B (PI3K/Akt) pathway is one of the most
56 important signaling pathways which involved in metabolic effect of insulin [3].
57 Therefore any treatment strategy of insulin resistance should be associated with
58 targeting of insulin signaling pathway complications. Nowadays, using of herbal
59 compounds occupied a huge importance in medical field.

60 Berberine (BER) is a natural isoquinoline alkaloid isolated from different plants such
61 as *Berberis vulgaris* [4]. BER is a strong base which is usually unstable when present
62 in free form so it usually accompanied with chloride ion in form of BER-chloride [5,
63 6]. BER has several pharmacological activities, it acts as anticancer [6, 7], anti-
64 inflammatory [7, 8], antileishmanial [8, 9] and anti-human immunodeficiency virus
65 [5, 10]. BER can be used for improving some cardiac diseases and intestinal
66 infections especially bacterial diarrhea [9, 10]. Furthermore, recently it is used as a

67 neuroprotective agent against some neurodegenerative diseases such as *Alzheimer's* and
68 *Parkinson's* diseases as it has the ability to pass the blood brain barrier [4].
69 To investigate whether BER-chloride has a protective effect on insulin resistance, we
70 set up *in vivo* model for insulin resistance by High fat diet (HFD) feeding. The effects
71 of BER-chloride on various insulin signaling pathway were investigated.

72 **2. Materials and methods:**

73 **1.1. Materials:**

74 BER-chloride was obtained from Sigma-Aldrich Chemical Co. (USA). Kits and
75 reagents for the assay of blood glucose level (BGL), protein, lipid profiles [total
76 cholesterol (TC), triacylglycerol (TG) and high density lipoprotein-cholesterol (HDL-
77 c)] and glycated hemoglobin (HbA1C), as well as both kidney function tests
78 (creatinine and urea) and liver enzymes [alanine aminotransferase (ALT) and
79 aspartate aminotransferase (AST)] were obtained from Spinreact (Spain), Human
80 (Germany), Biosystem (Egypt), and Biolabo (France), respectively. Ribonucleic acid
81 (RNA) extraction kit, Maxime reverse transcription (RT) premix kit, 2x Taq master
82 mix, deoxyribonucleic acid (DNA) Ladder, ribonuclease (RNase)-free water, and the
83 primer sequences of β -actin, insulin, insulin receptor (IR) and rat retinol binding
84 protein-4 (RBP4) were obtained from Qiagen (Germany), Intron Biotechnology (Korea)
85 and Fermentas, Thermo fisher scientific (Germany), respectively.

86 Enzyme linked immunoassay (ELISA) kit of insulin, PI3K, AKt-P, sirtuin type 1
87 (SIRT-1), phosphatase and tension homologue (PTEN) and adiponectin were
88 purchased from DRG (USA), Wuhan Fine Biological Technology Co. (China), Ray
89 Biotech (Georgia), MyBiosource (USA), Abcam (USA), Bioscience (USA),
90 respectively. Foline reagent, thiobarbituric acid (TBA), reduced glutathione (GSH),
91 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), cumene hydroxide, methyle green,
92 sodium pentobarbital and poly ethylene glycol (PEG) were obtained from Sigma-
93 Aldrich Chemical Co. (St. Louis, Mo, USA). Organic solvents; ethanol 95% and
94 methanol were of high pressure liquid chromatography (HPLC)-grade and brought
95 from Merck (USA). Other reagents were obtained with high grade.

96 **1.2. Experimental animal protocol and samples**

97 **preparation:**

98 Female albino Sprague-Dawley rats (*Rattus norvegicus*), of body weight (130 – 150)
99 g and aged (10 - 12) weeks old, were obtained from the experimental animal house of
100 Medical Research Institute, Alexandria University, Egypt. Rats were housed in
101 polycarbonate cages in groups of six rats per cage. They were kept under conventional
102 conditions of temperature and humidity with a 12-h photoperiod. Food and water were
103 supplied *ad libitum*. The experimental animals were conducted in accordance with the
104 National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH
105 1996). This study was carried out in strict accordance with the recommendations in
106 the Guide for the Care and Use of Laboratory Animals. The protocol was approved
107 according to the Ethics of Animal House in Medical Technology Center, Alexandria
108 University, Egypt.

109 All the animals were acclimatized for one week before the start of the experiment.
110 After that, thirty animals were divided into five groups (n = 6 per group), Group 1
111 (Sham control group) that were healthy and free from any disease, rats of this group
112 were fed standard diet for 10 weeks, Group 2 (Control vehicle) fed low fat diet (LFD)
113 for eight weeks then received 20% PEG by intragastric tube (10 ml/kg Bwt) for two
114 weeks [11, 12]. Group 3 (Control BER-chloride) fed LFD for eight weeks then orally
115 given BER-chloride dissolved in 20% PEG (100 mg/kg Bwt) for two weeks. Group 4
116 (Induction group) fed HFD for eight weeks then orally given 20% PEG (10 ml/kg
117 Bwt) for two weeks [11, 12]. Group 5 (Induction treated group) fed HFD for eight
118 weeks then orally given BER-chloride dissolved in 20% PEG (100 mg/kg Bwt) for
119 two weeks [13].

120 Rat's body weights were recorded at the first and last week of treatment. Blood
121 sampling and animal scarification were performed under sodium pentobarbital anesthesia, and
122 all efforts were made to minimize suffering. At the end of the study, animals were fasted
123 overnight after 8-h, blood samples were collected in sodium fluoride tubes for
124 assessment of fasting BGL. After full fasting period (12-h), blood samples were
125 collected, and then centrifuged at 3000 rpm for 10 min. The obtained serum was kept
126 at -20°C until analyzed. Hepatic tissues and white adipose tissue from control and
127 experimently groups were exised immediately and washed with ice-cold saline.
128 Homogenization was carried out in 0.1M sodium phosphate-buffer, pH 7.4 (for
129 hepatic tissue) and in 0.15M potassium chloride (for white adipose tissue). The
130 homogenate was centrifuged at 4000 rpm for 15 min at 4°C and supernatant was
131 stored at -80°C until analysis [14, 15]. In each group, part of liver was preserved in
132 liquid nitrogen, and stored at -80°C for total RNA isolation and polymerase chain
133 reaction (PCR) analysis and part of white adipose tissue was fixed in 10% neutral
134 buffered formalin solution for histopathological examination.

135 **1.3. Biochemical, molecular, histopathological studies** 136 **and statistical analysis:**

137 **1.3.1. Biochemical and molecular studies:**

138 Glucose levels in all groups were measured by a glucose assay kit that is dependent on
139 glucose oxidase-peroxidase method. Serum insulin of all groups was assayed by DRG
140 insulin ELISA kit. The insulin resistance was evaluated by calculating the
141 homeostatic model assessment-insulin resistance (HOMA-IR) as previously
142 described [16]. HbA1C % was determined by Biosystem kit. Total protein
143 concentration was determined spectrophotometrically using Beirut assay based kit.
144 Serum lipid profile (TC, TG, and HDL-c), kidney function tests (urea and creatinine)

145 and liver function tests (ALT and AST) were carried out according to commercial kits
146 manufacturer's instructions. LDL-c and very low density lipoprotein cholesterol
147 (vLDL-c) levels were calculated by using a specific formula [17]. Standardized
148 methods were used to determine the level of thiobarbituric acid reactive substances
149 (TBARS) [18] and GSH [19] in liver. In addition, hepatic activities of xanthine
150 oxidase (XO) [20], glutathione peroxidase (GPx) [21, 22] and adenine triphosphatase
151 (ATPase) [23] were carried out.

152 PI3K, Akt-P, SIRT-1 and PTEN levels in liver homogenate and adiponectin level in
153 serum, liver homogenate and white adipose tissue were determined by using ELISA
154 kits. These assays employ the quantitative sandwich enzyme immunoassay technique.
155 Primers used for PCR technique were designed using the known sequences for the
156 respective genes (Table 1). Programs are given as denaturation temperature/
157 denaturation times/ annealing temperature/ annealing times/ extension temperature/
158 extension times/ number of cycles. The primers were run on Mini Cyclor (Eppendorf,
159 Labcaire, Germany).

160 Total RNA was extracted from hepatic tissue by using total RNA extraction Kit and
161 processed according to kit manufacturer's instructions. After that the concentration of
162 total RNA was measured by spectrophotometer at 260 and 280 nm. One microgram of
163 the isolated RNA was reverse transcribed into single-strand complementary DNA
164 (cDNA) using reverse transcriptase (Maxime RT Pre-Mix kit, Fermentas, EU). For
165 gene expression, the gene specific primers were used and the programs (Table 1) were
166 optimized for each primer pair and all programs started with a 30s period at 95°C and
167 ended with a 60s extension at 72°C. The PCR products were resolved on 1.5%
168 agarose gel. Gels were stained with ethidium bromide, visualized by 30 nm
169 Ultraviolet Radiator (Alpha-Chem. Imager, USA), and photographic record was

170 made. The optical density and the microgram content of bands were calculated by the
171 UVIBAND MAX software program.

172 **Table 1. Primer sequences and PCR conditions**

Gene	Primer sequence		PCR conditions			Number of cycles
			Denature (°C)	Anneal (°C)	Extend (°C)	
β-actin	F	5'-CAT CAC TAT CGG CAA TGA GC-3'	95°C/30 s.	52.5	72°C/60 s.	40
	R	5'-GAC AGC ACT GTG TTG GCA TA-3'				
RBP4	F	5'-TTTTCTGTGGACGAGAAGGGT-3'	95°C/30 s.	51.5	72°C/60 s.	40
	R	5'-TGGTCATCGTTTCCTCGCTTG-3'				
IR	F	5'-TGA CAA TGA GGA ATG TGG GGA C-3'	95°C/30 s.	50	72°C/60 s.	40
	R	5'-GGG CAA ACT TTC TGA CAA TGA CTG-3'				
Insulin	F	5'-TTC TAC ACA CCC AAG TCC CGT C-3'	95°C/30 s.	52	72°C/60 s.	40
	R	5'-ATC CAC AAT GCC ACG CTT CTG C-3'				

173 PCR conditions (denaturation temperature/ denaturation times/ annealing temperature/ annealing times/ extension temperature/ extension times/

174 number of cycles of PCR program) and gene specific primers which used in PCR program were illustrated in Table 1.

175 **2.3.2. Histopathological preparation of white adipose tissue:**

176 White adipose tissue of each rat from each group was excised and immediately fixed
177 at 10% neutral buffered formalin solution after washing with ice cold normal saline.
178 The resultant fixed tissue samples were used for histological examination in the
179 Histopathology Laboratory of Medical Technology Center, Alexandria University,
180 using the routine procedures developed in the respective laboratories. The tissue was
181 cut at 3 mm thick, and the blocks were embedded in paraffin. Using a rotary
182 microtome, sections of 8 μ m thickness were cut. The sections were stained with
183 hematoxylin and eosin and examined under Olympus microscope (Olympus, Tokyo,
184 Japan) at (40X) magnification for any histopathological changes.

185 **2.2.3. Statistical analysis:**

186 Data were analyzed using Primer of Biostatistics software program (Version 5.0) by
187 one-way analysis of variance (ANOVA). Significance of means \pm SD was detected
188 groups by using multiple comparisons Student-Newman-keuls test at $p < 0.05$.
189 Adiponectin correlation was analyzed by SPSS (Version 20.0) software program,
190 using person coefficient (r).

191 **3. Results:**

192 **3.1. Body weight, BGL, insulin resistance, lipid** 193 **profiles, oxidative stress markers, serum** 194 **transaminases activity and kidney function tests**

195 **3.1.1. Body weight, BGL and insulin resistance parameters**

196 Feeding of HFD for 8-weeks leads to increase the body weight of the rats than control
197 level. Fasting BGL, HOMA-IR and HbA1C were also elevated 1.4, 3.7 and 45.2-

198 folds, compared to sham control rats. Moreover, elevation of insulin level was
199 reported in HFD group 1.0-fold in serum and 72.3-fold in hepatic tissue.
200 Administration of BER-chloride leads to decrease fasting BGL, serum insulin,
201 HOMA-IR and HbA1C to 0.5, 0.2, 0.6 and 0.2-folds, respectively compared with
202 HFD rats (Table 2). Moreover, HFD up regulated insulin gene expression in liver
203 tissue and the treatment with BER-chloride for two weeks did not showed any
204 positive effect on insulin expression (Fig 1).

205 **Table 2. Effect of BER-chloride on body weight, BGL, serum insulin, HOMA-IR and HbA1C after the treatment of diabetic induced**
 206 **experimental animals.**

	Body weight (g)	BGL (mg/dl)	Serum insulin (μIU/ml)	HOMA-IR (mg/dl)	HbA1C (%)
Sham control	141.0 \pm 7.4 ^a	76.0 \pm 1.4 ^a	10.0 \pm 0.2 ^a	1.91 \pm 0.061 ^a	4.2 \pm 0.4 ^a
Control vehicle	142.2 \pm 8.04 ^a	73.33 \pm 2.6 ^a	10.1 \pm 0.6 ^a	1.9 \pm 0.13 ^a	4.4 \pm 0.42 ^a
Control BER-chloride	144.0 \pm 6.07 ^a	75.2 \pm 3.31 ^a	10.45 \pm 0.33 ^a	1.92 \pm 0.12 ^a	4.0 \pm 0.24 ^a
Induction	202.3 \pm 5.39 ^b	180.1 \pm 4.38 ^c	20.17 \pm 2.93 ^c	8.97 \pm 1.37 ^c	6.1 \pm 0.39 ^c
Induction treated with BER-chloride	200.5 \pm 8.02 ^b	97.7 \pm 5.61 ^b	15.67 \pm 2.42 ^b	3.76 \pm 0.48 ^b	5.1 \pm 0.18 ^b

207 Values represent the mean \pm SD of six rats. ANOVA (one way) followed by Student-Newman-keuls test.

208 Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

209 **3.1.2. Lipid profiles parameters**

210 Lipid profile in this study showed significant increase in HFD group than that of sham control where TC increased 1.2-fold and TG, LDL-c and
 211 vLDL-c were 0.9-fold increase, while HDL-c was significantly decreased by 43%. The disturbance which occurred in lipid profiles was partially
 212 repaired after using BER-chloride as treatment for two weeks (Table 3).

213 **Table 3. Effect of BER-chloride on lipid profiles after the treatment of diabetic induced experimental animals.**

	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	vLDL-c (mg/dl)
Sham control	130.5 ± 2.79 ^a	110.3 ± 3.6 ^a	59.33 ± 3.14 ^a	110.33 ± 3.62 ^a	22.1 ± 0.7 ^a
Control vehicle	127.3 ± 4.41 ^a	110.0 ± 4.47 ^a	64.3 ± 3.01 ^a	41.0 ± 3.74 ^a	22.3 ± 1.2 ^a
Control BER-chloride	129.3 ± 2.25 ^a	111.2 ± 2.40 ^a	63.2 ± 2.56 ^a	43.9 ± 4.24 ^a	21.77 ± 0.5 ^a
Induction	282.2 ± 3.31 ^c	210.5 ± 3.73 ^c	33.3 ± 2.88 ^c	206.7 ± 2.78 ^c	42.9 ± 0.4 ^c
Induction treated with BER-chloride	164.5 ± 4.14 ^b	141.5 ± 2.26 ^b	42.8 ± 3.37 ^b	93.4 ± 6.20 ^b	28.133 ± 0.7 ^b

214 Values represent the mean ± SD of six rats. ANOVA (one way) followed by Student-Newman-keuls test.

215 Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

216 **3.1.3. Oxidative stress markers, serum transaminases activity**
217 **and kidney function tests**

218 Experimental HFD rats showed elevation of TBARS and XO (1.5 and 0.8-folds)
219 compared to sham control. Both TBARS and XO were decreased nearly 0.4 and 0.2
220 folds after two weeks from BER-chloride treatment. On the other hand, the GSH, GPx
221 and ATPase were decreased by 50.9%, 41.9% and 35.2% in HFD group compared
222 with sham control and after administration of BER-chloride; those previous
223 parameters were increased by percentage 42.9, 33.7 and 26.1 respectively (Table 4).
224 Also, HFD intake increased both liver function parameters and kidney function test
225 comparing to sham control one. Administration of 100 mg/kg Bwt BER-chloride in
226 HFD rats for two weeks leads to reduction of liver enzymes activities and kidney
227 function tests nearly 0.3 and 0.2-folds (Table 5).

228 **Table 4. Effect of BER-chloride on hepatocyte prooxidants/antioxidants status after the treatment of diabetic induced experimental**
 229 **animals.**

	TBARS (nmol/mg protein)	GSH (mg/mg protein)	XO (IU/mg protein)	GPx (U/mg protein)	ATPase (μmol/pi/min/mg protein)
Sham control	2.1 \pm 0.02 ^a	0.285 \pm 0.05 ^a	128.0 \pm 1.6 ^a	7.06 \pm 0.3 ^a	0.71 \pm 0.02 ^a
Control vehicle	2.03 \pm 0.04 ^a	0.29 \pm 0.06 ^a	130.17 \pm 4.26 ^a	7.02 \pm 0.55 ^a	0.73 \pm 0.01 ^a
Control BER-chloride	2.12 \pm 0.28 ^a	0.29 \pm 0.05 ^a	129.67 \pm 4.46 ^a	7.17 \pm 0.22 ^a	0.71 \pm 0.01 ^a
Induction	5.25 \pm 0.35 ^c	0.14 \pm 0.02 ^c	231.5 \pm 7.56 ^c	4.1 \pm 0.33 ^c	0.46 \pm 0.05 ^c
Induction treated with BER-chloride	3.15 \pm 0.24 ^b	0.20 \pm 0.03 ^b	171.3 \pm 3.83 ^b	5.48 \pm 0.33 ^b	0.58 \pm 0.03 ^b

230 Values represent the mean \pm SD of six rats. ANOVA (one way) followed by Student-Newman-keuls test.

231 Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

232 **Table 5. Effect of BER-chloride on serum transaminases activities and kidney function tests after the treatment of diabetic induced**
 233 **experimental animals.**

	ALT (U/ml)	AST (U/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Sham control	33.5 ± 2.1 ^a	46.0 ± 3.2 ^a	33.4 ± 2.3 ^a	0.71 ± 0.1 ^a
Control vehicle	34.2 ± 4.6 ^a	49.3 ± 1.8 ^a	31.83 ± 4.6 ^a	0.72 ± 0.04 ^a
Control BER-chloride	32.2 ± 1.94 ^a	49.5 ± 3.78 ^a	29.67 ± 2.16 ^a	0.71 ± 0.04 ^a
Induction	58.5 ± 5.09 ^c	85.7 ± 5.68 ^c	56.17 ± 2.14 ^c	0.90 ± 0.05 ^b
Induction treated with BER-chloride	40.8 ± 3.71 ^b	62.5 ± 6.80 ^b	39.83 ± 3.49 ^b	0.75 ± 0.06 ^a

234 Values represent the mean ± SD of six rats. ANOVA (one way) followed by Student-Newman-keuls test.

235 Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

236 **3.2. Insulin signaling pathway parameters and** 237 **adiponectin concentration**

238 **3.2.1. Insulin signaling pathway parameters**

239 Marked significant reduction of PI3K, AKt-p and SIRT-1 by 57.1%, 42.9% and
240 61.9%, was reported in HFD rats compared to sham control. The treatment of rats
241 with BER-chloride leads to increase PI3K, AKt-p and SIRT-1 to 84.4%, 49% and
242 120%, respectively. However, PTEN was increased in HFD rats by 66.6% and
243 decreased to 27.5% after BER-chloride treatment (Table 6). Also, RBP4 was
244 decreased from 1.5-fold to 0.4-fold after BER-chloride treatment. However, BER-
245 chloride failed to affect the HFD adverse effect on IR expression (Fig.1).

246 **Table 6. Effect of BER-chloride on PI3K, Akt-p, SIRT-1 and PTEN levels in hepatocyte after the treatment of diabetic induced**
 247 **experimental animals.**

	PI3K (pg/g)	Akt-p (pg/g)	SIRT-1 (ng/g)	PTEN (pg/g)
Sham control	493.0 ± 12.3 ^c	350.0 ± 23.5 ^c	105.0 ± 10.2 ^c	1200±28.9 ^a
Control vehicle	486.0 ± 12.0 ^c	335.0 ± 19.3 ^c	110.0 ± 6.9 ^c	1250.0 ± 25.3 ^a
Control BER-chloride	491.0 ± 23.5 ^c	342.0 ± 18.9 ^c	109.0 ± 9.7 ^c	1150.0 ± 65.3 ^a
Induction	211.5 ± 21.5 ^a	200.0 ± 12.1 ^a	40.0 ± 1.2 ^a	2000.0 ± 28.9 ^c
Induction treated with BER-chloride	390.0 ± 31.2 ^b	298.0 ± 14.2 ^b	86.0 ± 8.5 ^b	1450.0 ± 23.5 ^b

248 Values represent the mean ± SD of six rats. ANOVA (one way) followed by Student-Newman-keuls test.

249 Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

3.2.2. Adiponectin concentration

Adiponectin percentage in serum, liver and white adipose tissue of HFD rats was reduced (52%, 80% and 45%), and this percentage was elevated after two weeks of BER-chloride treatment (48%, 385% and 65.3%), respectively (Table 7).

Table 7. Effect of BER-chloride on adiponectin level in serum, liver and white adipose tissue homogenates after the treatment of diabetic induced experimental animals.

	Adiponectin (ng/ml)		
	Serum	Liver	White adipose tissue
Sham control	1.42 ± 0.49 ^a	11.03 ± 0.97 ^a	21.42 ± 1.65 ^a
Control vehicle	1.39 ± 0.49 ^a	10.67 ± 0.97 ^a	22.97 ± 1.12 ^a
Control BER-chloride	1.59 ± 0.26 ^a	10.77 ± 1.15 ^a	23.43 ± 1.51 ^a
Induction	0.682 ± 0.11 ^b	2.0 ± 0.7 ^b	11.78 ± 1.89 ^b
Induction treated with BER-chloride	1.01 ± 0.18 ^a	9.7 ± 0.9 ^a	19.47 ± 1.67 ^a

Values represent the mean ± SD of six rats. SPSS (Version 20.0).

Means with letters (a), (b) and (c) were statistical represented compared to sham control group as follow: a= p < 0.001, b= p < 0.01, c= p < 0.05.

257 **3.3. Histological results**

258 The biochemical results were confirmed by the histological studies in white adipose
259 tissue (Figs. 2A-2E). Control rat's white adipose tissue revealed normal tissue (Fig
260 2A). Both PEG and BER-chloride administrated groups after feeding LFD were
261 similar to control rats (Fig 2B and 2C). However, adipose tissue of HFD rats revealed
262 multiple fibrosis and degeneration for the architecture of the adipocytes (Fig 2D).
263 Treatment of HFD rats with BER-chloride for two weeks lead to regeneration of the
264 cells and reduction of the lipids droplets inside it (Fig 2E).

265 **4. Discussion:**

266 In recent years insulin resistance has a huge challenge, it represented one of the risk
267 metabolic conditions, and in many cases it occurs due to bad dietary habits such as
268 junk foods which is characterized by huge percentage of fats [24] or due to some
269 diseases like non-insulin dependent diabetes mellitus [25]. In present study HFD
270 feeding for eight weeks induced hyperinsulinaemia which is attributed to inability of
271 liver to utilize the secreted insulin although the normal function of pancreatic β -cell
272 [26]. During this period the rats become more obese compared to LFD controls due to
273 elevation of insulin level which inhibit fatty acid oxidation so fats accumulated
274 mainly in liver because it is the main organ of oxidation process [27]. Elevated levels
275 of fasting insulin, glucose, and HOMA-IR index confirming the state of insulin
276 resistance. Furthermore increasing of HbA1c is another indicator of insulin resistance
277 and correlated with renal function parameters elevation [28].

278 Additionally, results of current study showed up regulation of RBP4 expression in
279 HFD rats. From previous studies RBP4 i: elevates the process of gluconeogenesis in
280 liver so hyperglycemia is occurred [29], ii: hindered the insulin signaling in muscle

281 and iii: decreased the uptake of glucose by reduction the activity of PI3K [30].
282 Defects in PI3K demonstrated in other findings [31]. Previous studies showed highest
283 level of RBP4 are associated with body mass index increase, insulin resistance and
284 hypertriglyceridemia [32].
285 Study of PTEN in this research had a significant importance. PTEN is lipid
286 phosphatase inhibits insulin signaling by dephosphorylating phosphatidylinositol
287 (3,4,5) triphosphate (PIP₃) to phosphatidylinositol (4,5) diphosphate (PIP₂) [33].
288 Hence, PTEN is antagonizing the action of PI3K and inhibits Akt as appear in current
289 results, where HFD rats showed elevation in PTEN level and reduction of PI3K [34].
290 It is known that, SIRT1 is a key regulator of lipid mobilization through its action
291 together with adenosine monophosphate (AMPK) by increasing fatty acid metabolism
292 [35]. So, reduction of SIRT1 concentration in HFD rats is linked by hyperlipidemia
293 and insulin resistance due to decrease in phosphorylated and/or activated AMPK
294 resulting in lipid synthesis increase [36]. Results of current study are in accordance
295 with previous studies [37]. Further, SIRT1 has several roles in insulin signaling
296 pathway it i: regulates secretion of insulin from β -cell of pancreas by reduction the
297 expression of uncoupling protein-2 (UCP2) and improvement the depolarization in β -
298 cell of pancreas [38] and ii: regulates the insulin signaling pathway by deacetylation
299 of insulin receptor substrate-2 (IRS2) and activation of Akt in cells [39]. From those
300 mentioned mechanisms of SIRT1 and PTEN, the current study showed reduction of
301 Akt-p concentration in hepatic cells of HFD rats.
302 Moreover adiponectin has an important role in insulin resistance pathway; it is a
303 member of adipocytokines which secreted by adipocytes and has a regulating effect
304 on insulin sensitivity [40]. It was reported that disturbance in lipid metabolism and
305 excessive fat deposition leads to abnormal synthesis of adipocytokines [41]. HFD rats

306 associated with reduction of adiponectin level in serum, hepatic and adipose tissues
307 which may be attributed to disruption of both adiponectin receptor-1 and 2 leading to
308 elevation of glucose level and reduction the activity of peroxisome proliferator
309 activated receptor α -signaling pathways respectively, and finally insulin resistance
310 occurs [42]. Further, there are some studies suggest the role of SIRT1 in regulation of
311 adiponectin secretion from the adipocytes by deacetylating of fork head transcription
312 factor O1 (FOXO-1) protein and enhancement the transcription of gene that encodes
313 adiponectin in adipocytes [43]. Hence, reduction of SIRT1 effect on adiponectin
314 secretion.

315 Role of SIRT1 is extended to control the production of reactive oxygen species (ROS)
316 [44] as SIRT1 is considered one of the important proteins that protect cells from stress
317 damage [37]. Under normal condition the hepatocyte balance the oxidative stress by
318 the action of antioxidant enzymes such as GPx which converts hydrogen peroxide
319 (H_2O_2) to water [45]. Rats suffer from insulin resistance have low GPx activity so
320 H_2O_2 accumulated and hepatic cells damaged. These results were confirmed by
321 elevation of liver enzymes (ALT and AST). H_2O_2 accumulation also affected on renal
322 tissue which confirmed by increase both urea and creatinine levels. Another cause of
323 elevation of ROS in case of insulin resistance is attributed to the dysregulated
324 production of adipocytokines where plasma adiponectin concentration is inversely
325 correlated with systemic oxidative stress [46].

326 In recent years with regard to the adverse effects of synthetic drugs, increasing
327 attention has been paid by researchers to herbal medicines. BER is a major form of
328 isoquinoline alkaloid isolated from several herbal plants and it has several biological
329 effects [47]. Nowadays, BER is manufactured by chemical synthesis, chloride or
330 sulfate salt of BER is used for clinical purposes [48].

331 Our *in vivo* study revealed that treatment with BER-chloride has negative effect on
332 insulin resistance by activating two proteins involved in several physiological
333 processes, SIRT-1 and AMPK [49]. Those two proteins able to activate each other,
334 AMPK activates SIRT-1 by elevation the level of nicotinamide
335 phosphoribosyltransferase and SIRT-1 stimulates AMPK through deacetylation of
336 serine-threonine kinase LKB1 [50, 51]. Also, BER has the ability to improve insulin
337 resistance through other mechanisms, where it i: protects β -cell of islet of *Langerhans*
338 from damage, ii: allows glucose uptake of skeletal muscle, iii: improves hepatic
339 gluconeogenesis and iv: decreases the level of lipids in blood [52, 53].

340 As a result of SIRT-1 and AMPK pathways activation, adiponectin level was restored
341 after BER-chloride administration in our study which similar to results obtained by
342 [54]. Elevation of adiponectin level is linked by regulation of β -oxidation of fatty
343 acids and glucose metabolism [55, 56]. Hence, treated rats with BER-chloride showed
344 significant reduction of lipid profiles and BGL. Also, BER-chloride ameliorates
345 hyperlipidemia results from insulin resistance via different mechanisms i: BER lowers
346 blood cholesterol levels through inhibiting cholesterol uptake and absorption in the
347 intestine [57], ii: BER reduces the secretion of cholesterol from enterocytes into the
348 blood by down regulation acetyl CoA transferase II enzyme [58] and lastly iii: BER
349 increases the regulation of LDL-receptor and hence, BER decreases the level of LDL-
350 c [59]. Further, BER chloride is able to improve glucose control by stimulation the
351 glycolysis in peripheral tissue [60], inhibition of FOXO-1 and hepatic nuclear factor
352 4, lead to suppression of glucose-6-phosphatase and phosphoenolpyruvate
353 carboxykinase enzymes which responsible for liver gluconeogenesis [61, 62] and
354 activation of glucose transport-1 (GLUT1) [63].

355 The current study showed BER-chloride has the ability to increase the level of
356 antioxidant enzymes (GSH, GPx and XO) by reducing the elevated level of lipid
357 peroxidation [64]. Also it was reported that BER has the ability to prevent
358 nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which is a major
359 source of ROS production [65]. These results in accordance with previous studies
360 which proved that BER is a strong antioxidant molecule due to its ability to scavenge
361 free radicals [66]. Moreover, BER-chloride exerts protective effect against ROS
362 through SIRT-1 activation where SIRT-1 able to modulate NOX4/NADPH oxidative
363 subunit [67]. Reduction of ROS production lead to decrease the level of liver enzymes
364 in group of rats administrated BER-chloride.

365 In current study, BER-chloride down regulates RBP4 which acts as an effective
366 insulin sensitizing function [68]. From previous studies, it was reported that reduction
367 the level of RBP4 is related to elevation of HDL-c and decrease TG levels in some
368 patients [69]. Also as result of reduction of SIRT1 in HFD rats, elevation of TBARS
369 and XO and reduction of GPx and GSH was noticed and this was improved after
370 BER-chloride administration.

371 **5. Conclusions:**

372 Berberine chloride can be considered one of therapeutics used to decrease insulin
373 resistance through its effect on several insulin signaling pathways. A schematic
374 representation was designed (Fig 3) to summarize the modification of insulin
375 resistance by BER-chloride.

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581 **Figure Legends**



582 **Fig 1.** Effect of BER-chloride on the fold change of insulin, IR and RBP4 genes.

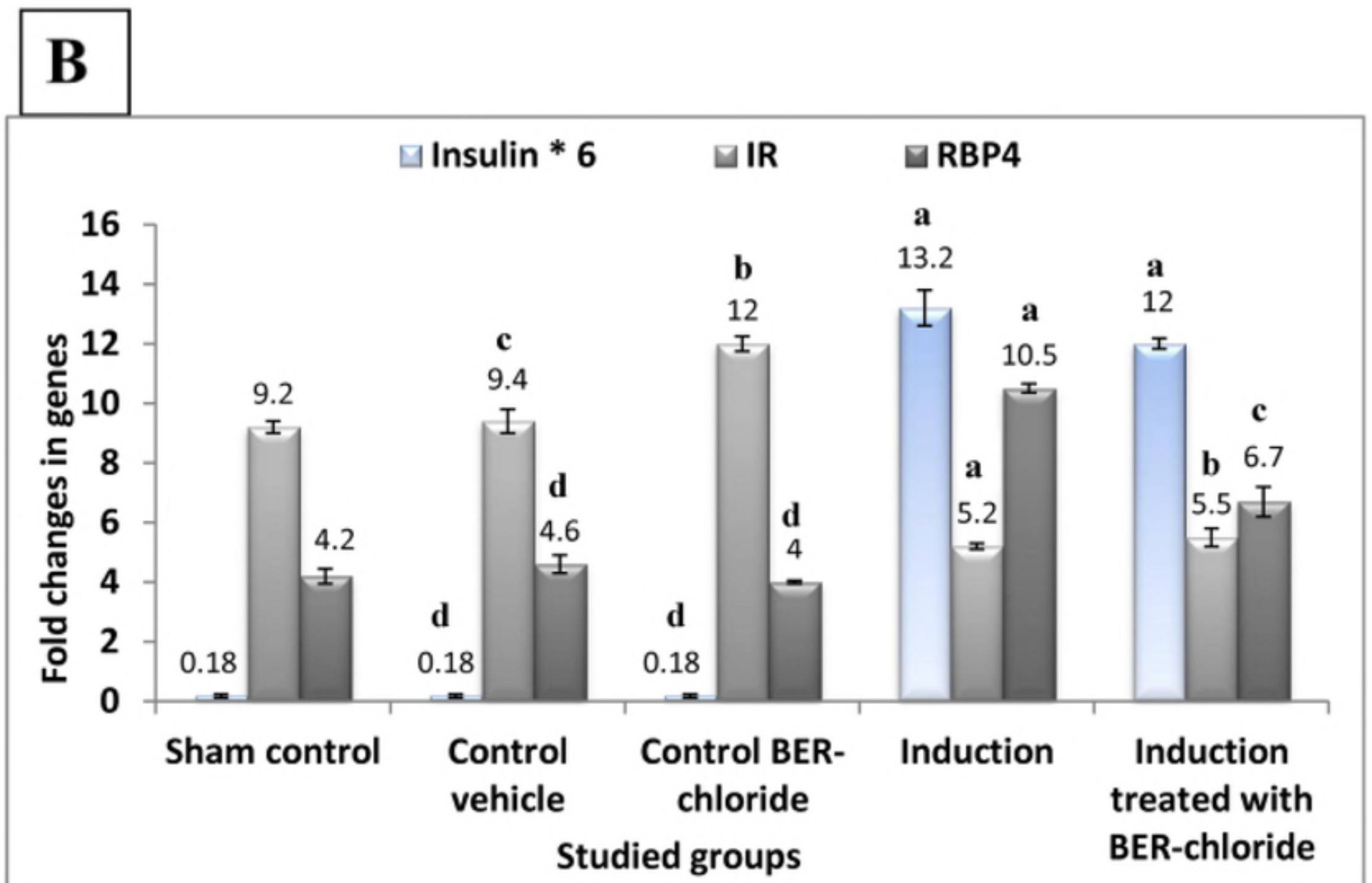
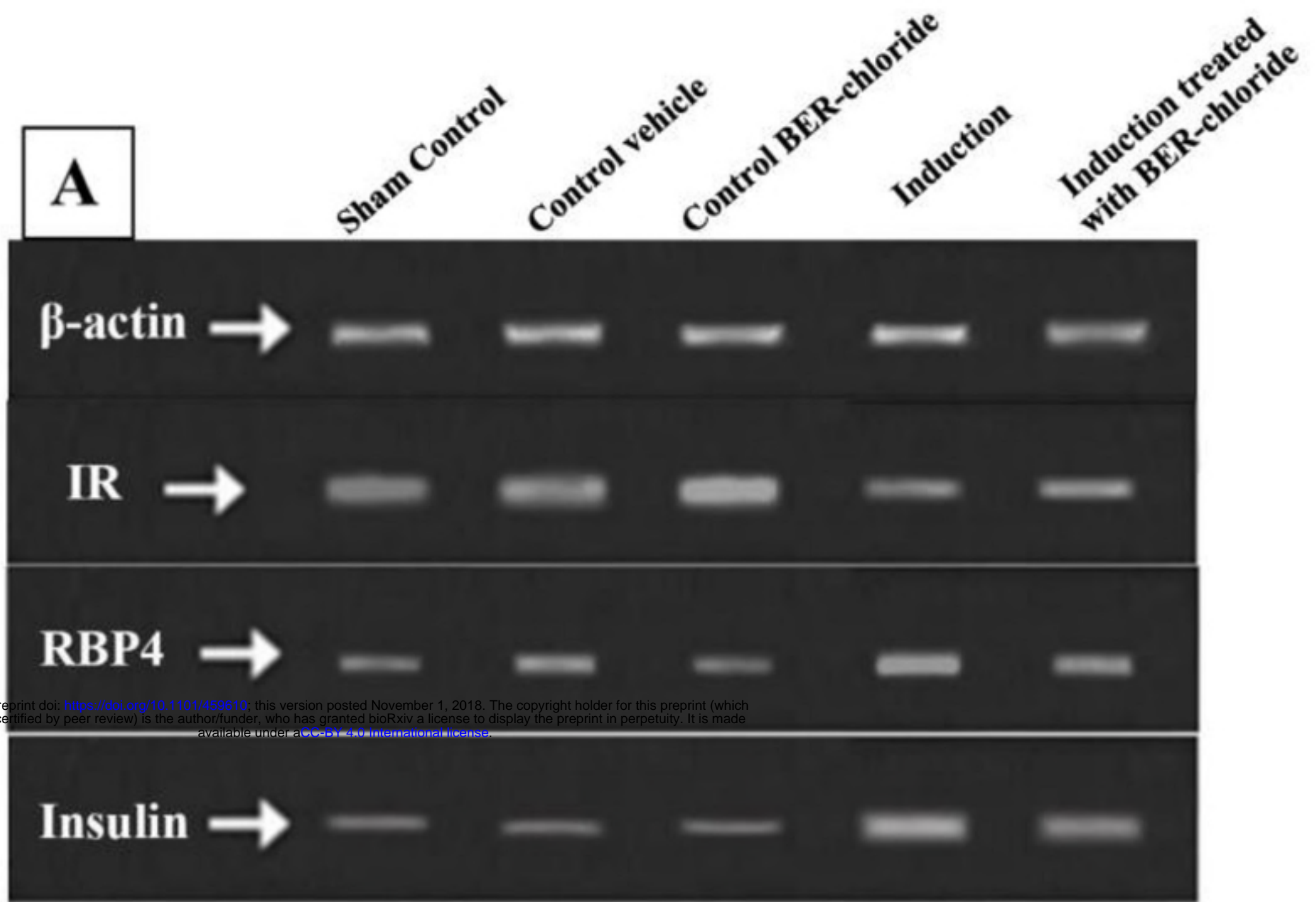
583 (A) Agrose gel electrophoresis of gene expression of insulin (293 bp), IR (129 bp) and
584 RBP4 (392 bp) compared to β -actin (300 bp). (B) Fold change of gene expression in
585 liver homogenate after the treatment of diabetic induced experimental animals
586 represented as 6 rats \pm SE. ANOVA (one way) followed by Student-Newman-keuls
587 test. Means with letters (a), (b), (c) and (d) were statistically represented compared to
588 sham control group as a at $p < 0.001$, b at $p < 0.01$, c at $p < 0.05$ and d at $p > 0.05$.

589 **Fig 2.** White adipocyte sections pictures in the different groups of rats, stained with
590 hematoxelin and eosin.

591 (A) Sham control rats, (B) Control vehicle (PEG) rats, (C) Control BER-chloride, (D)
592 HFD-fed rats and (E) HFD-fed rats and treated with BER-chloride ($X = 400$).

593 **Fig 3.** Schematic diagram for the effect of BER-chloride on insulin signaling in HFD-
594 insulin resistance induced rats.

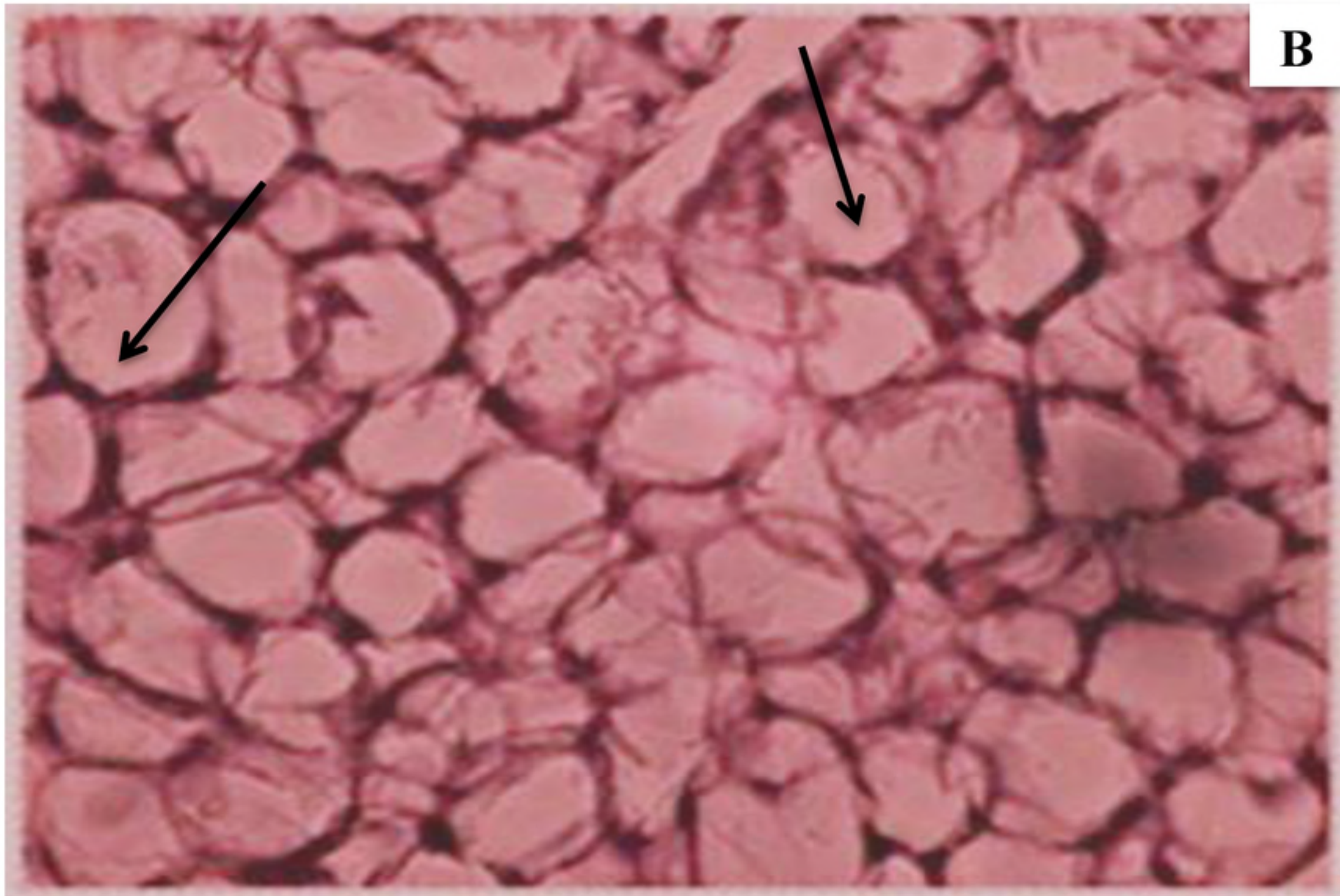
595 Blue arrows  means inhibition and violet arrows  means activation.



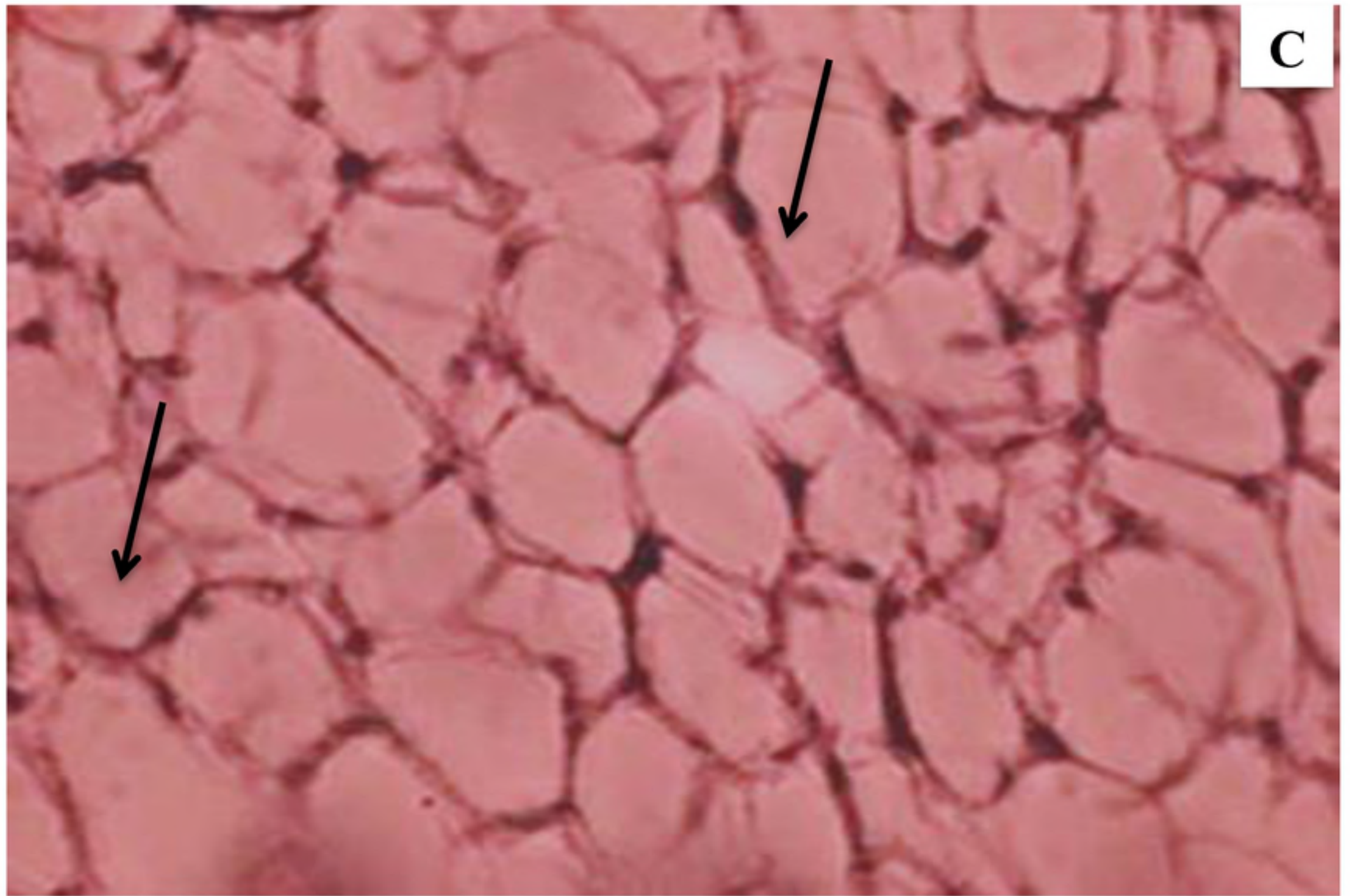
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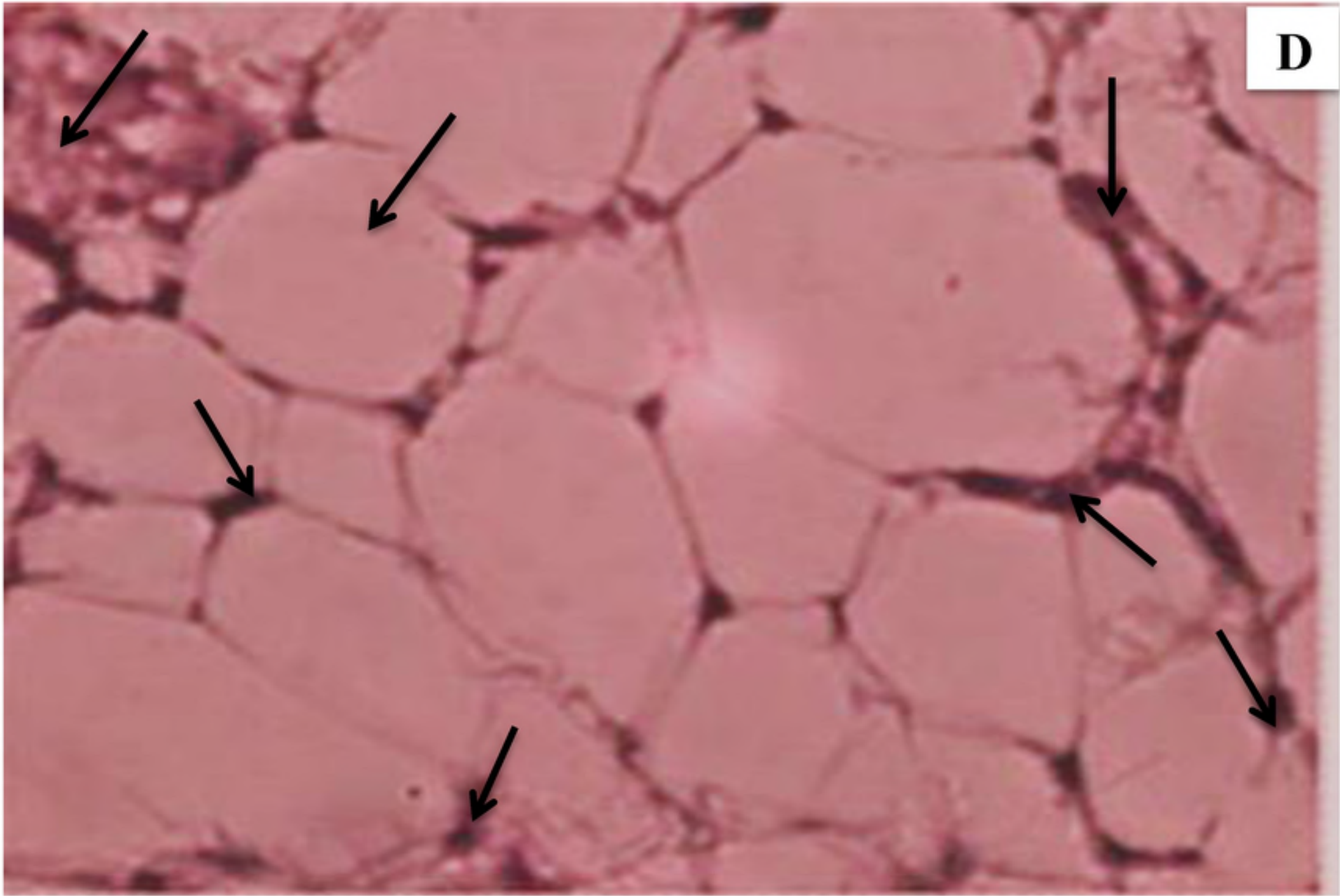
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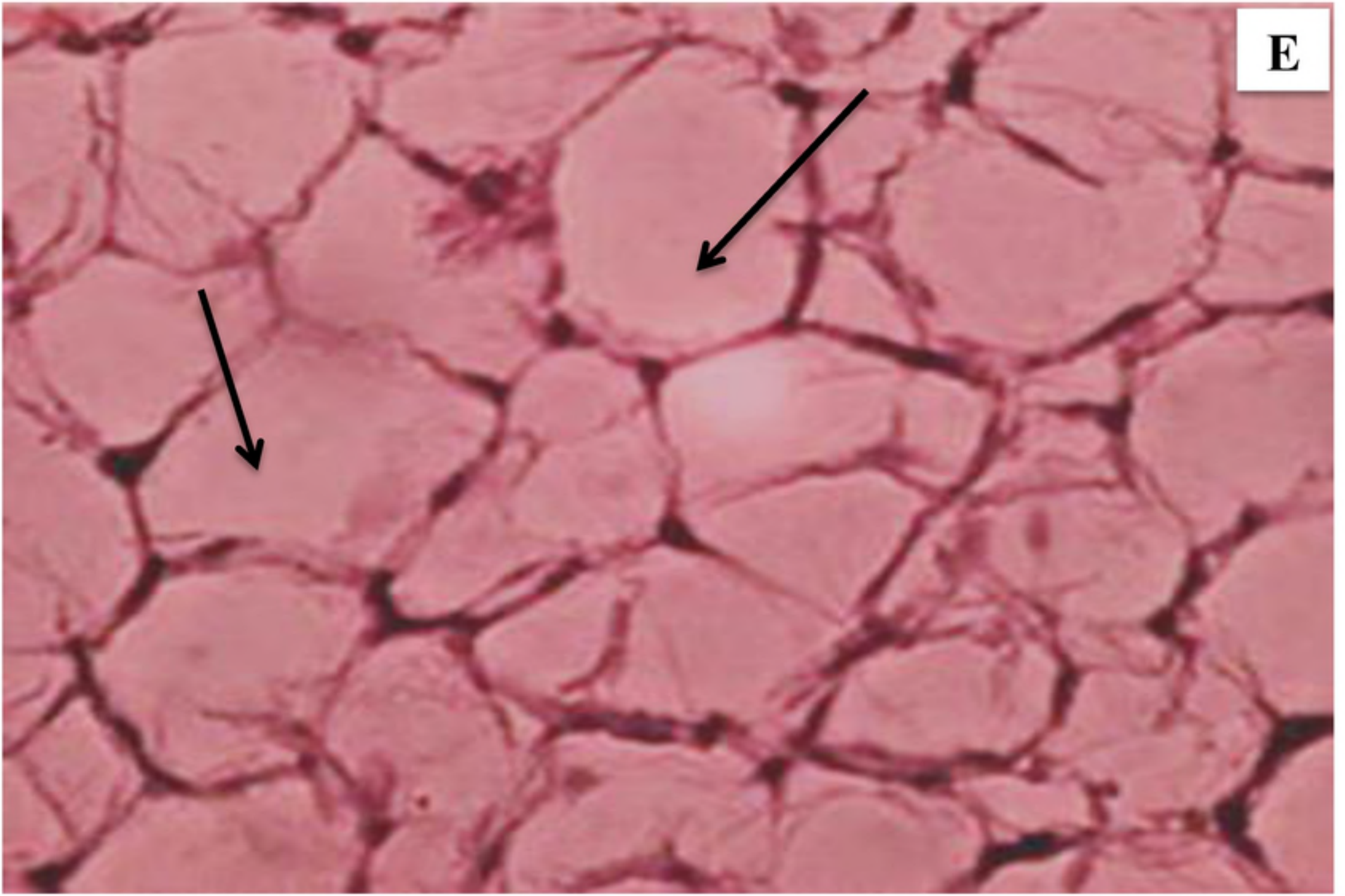
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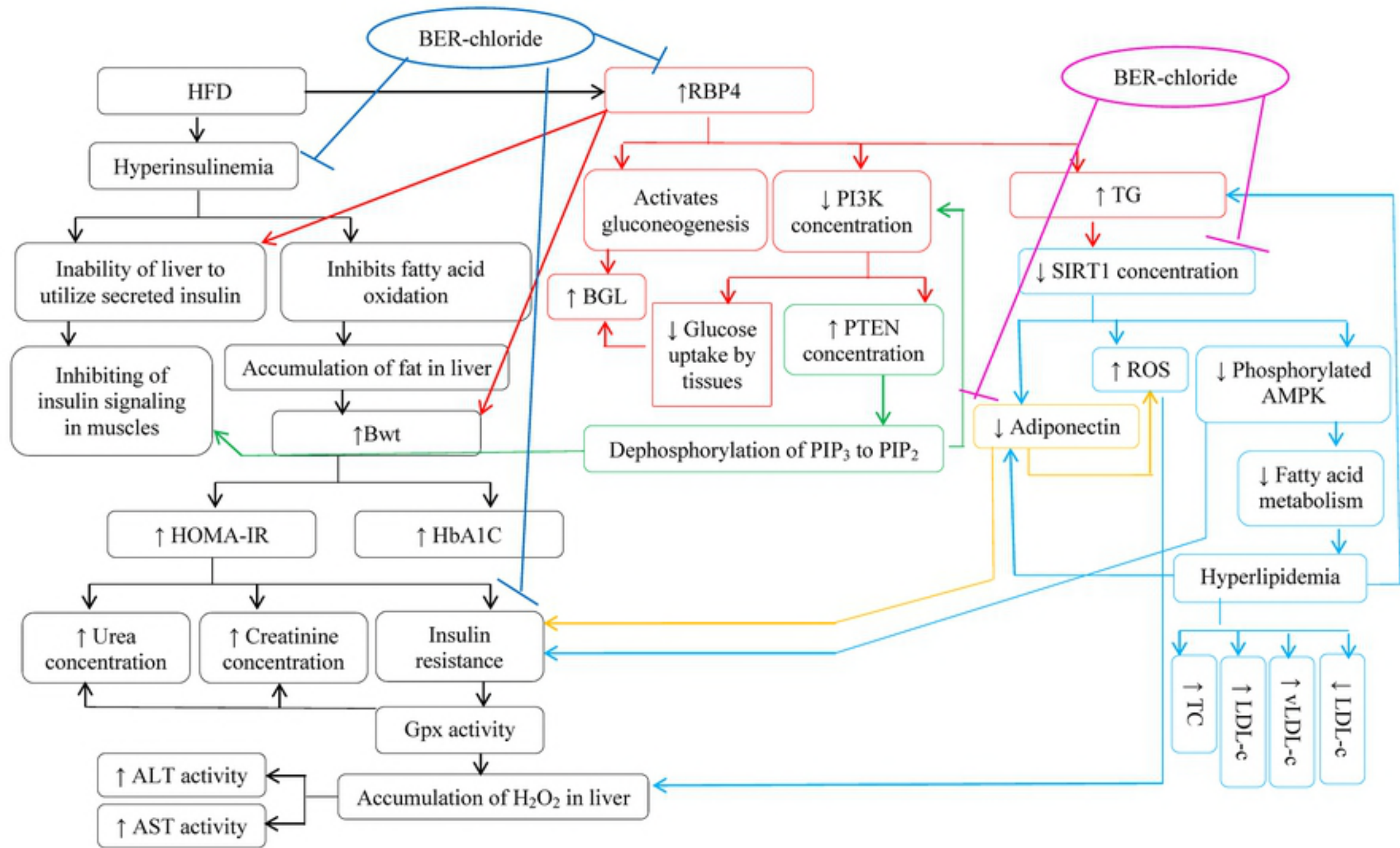
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