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RESEARCH ARTICLE

Betaine alleviated hepatic and renal injury in diabetic pregnant rats: biochemical and histopathological evidences

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Abstract

Purpose Pregnancy is the most intense physiological alteration in energy metabolism that women experience in their lifetime. Liver and kidney are the two most susceptible organs to energy metabolism. Diabetes is well-defined as a syndrome interfering with energy metabolism triggered by impaired blood glucose adjustment. Herein, protective effects of betaine on liver and kidney were evaluated in animal model of diabetic pregnancy.

Methods 32 dams were assigned into 4 equal groups: Control (C), Betaine (B, 1.5% *w*/w of total diet daily), Diabetic pregnancy (D), and Diabetic pregnancy treated with betaine (D + B). After physiological delivery, HbA1c concentration in whole blood, serum hepatic and renal biomarkers such as AST, ALT, ALP, urea and creatinine were measured. Also, liver and kidney tissue samples were examined under a light microscope.

Results Diabetic pregnancy was found to be accompanied by increased HbA1c level, concentration of hepatic and renal biomarkers in blood samples, and a gamut of alterations such as apoptotic cells, biliary hyperplasia, sinusoidal dilation, basement membrane thickening, and Bowman's capsule dilation as observed in histopathological sections of the D group. Betaine supplementation significantly decreased AST, ALT, urea and creatinine in the D + B group compared to D group. Also, most of pathologic microscopic alterations were attenuated under betaine treatment in D + B group compared to D group.

Conclusion Findings of the current paper, for the first time, provided evidence regarding protective effects of betaine on liver and kidney function against maternal diabetes in an animal model of STZ-induced diabetic pregnancy.

Keywords Diabetic pregnancy · Streptozotocin · Hyperglycemia · Liver damage · Kidney damage · Betaine

Introduction

Multiple physiological and anatomical changes are triggered after conception thereby influencing every organ in mother's body to cope with increased metabolic demands because of developing fetus and preparation of childbirth. Pregnancy is the most intense physiologically altered energy metabolism that women experience in their lifetime. In this regard, sustaining balanced energy metabolism as the most decisive point to reach a normal pregnancy is crucially required [5, 31, 47]. Liver has a central role in adjustment of energy metabolism in physiological and pathological states, as it regulates the most important pathways in glucose metabolism such as glycogenesis, glycogenolysis, and gluconeogenesis [14]. On the other hand, after heart, kidney has the most mitochondrial content and energy demand [6]. Thus, it is logical that, subsequent to any alteration in mother's energy metabolism, liver and kidney are being influenced more than any other organs during this stage, and their dysfunction is associated with maternal-fetal morbidity [21, 46]. Diabetes is well-defined as a

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syndrome interfering with energy metabolism, triggered by impaired blood glucose adjustment while influencing lipids and protein metabolism in downstream. According to reports by international organizations, global rate of diabetes incidence is increasing rapidly [8]. In parallel, increased number of women at childbearing age suffering from diabetes is more challenging, as diabetes is suggested as one of main causes of adult diseases with fetal origins [28, 33]. Diabetes during pregnancy can be divided into clinical diabetes and gestational diabetes both of which have been shown to be associated with renal and hepatic dysfunction characterized by biochemical and histopathological abnormalities [27]. Since hyperglycemia is generally accepted as main driving force behind diabetes disorders, in pregnancies complicated by diabetes (clinical or gestational), mothers receive insulin/oral hypoglycemic agents (OHAs) or both to retain normal energy metabolism [12]. However, these treatments are accompanied with some unfavorable effects. Insulin administration through subcutaneous injection is an invasive method and has some disadvantages such as hypoglycemia and infection [19]. On the other hand OHAs have been reported to pose hematological disturbances as well as both hepatic and renal side effects [48]. Furthermore, some studies reported failure of these methods to satisfyingly protect liver and kidney against diabetes induced pathological changes [34, 42]. In addition, the use of OHA during pregnancy has not been recommended over the past few years due to possible fetal adverse outcomes [39]. Nowadays, there is a growing global interest in the use of therapeutic drugs derived from natural sources, exclusively herbal medicine because of limited side effects [38].

Betaine is a double source metabolite, which can be obtained either endogenously in mitochondria by choline dehydrogenase activity or exogenously from some foods such as sugar beet, spinach, and whole grains [45]. It has been reported that betaine consumption is safe at a dose of 9–15 (g/day) and 0–5% (daily diet) for human and rat, respectively [16]. Betaine plays a pivotal role in cellular homeostasis as it contributes in critical pathways of metabolism including osmoregulation, methylation, and redox balance. In particular, betaine accumulates in the liver and kidney of mammals where betaine-homocysteine methyl transferase as its specific enzyme is expressed for initiating of betaine influx into one carbon (1C) metabolism [49]. Previously betaine was approved for marketing by FDA to reduce plasma homocysteine as a component of 1C cycle [15]. In recent years, it has been demonstrated that excess urinary excretion and low plasma level of betaine both are associated with diabetes and, liver and kidney dysfunction [8, 10, 18, 22]. Choi et al. [9] and Heidari et al. [18] found that, betaine can mitigate experimental chronic and acute hepatic injury in animal models. Also, in a previous study carried out in our lab, we reported hepato-protective effects of betaine against ethanol-induced liver damage [1]. Moreover, previous studies imply that betaine exerts therapeutic properties in different models of nephrotoxicity. In this case, Fan, et al. [11] showed that betaine supplementation can prevent fructose-induced renal injury. More importantly, existing data suggested that adequate amount of 1C metabolism-related metabolite is very critical in metabolic adaptions [23]. Given the facts mentioned above, we hypothesized that, betaine might be a good candidate to provide hepato-renal protection in pregnancy complicated by diabetes. Hence, in this study, it was attempted to investigate efficacy of betaine supplementation on liver and kidney function in a mouse model of (clinical) diabetic pregnancy. In this regard, effects of betaine were evaluated in terms of biochemical markers and histopathological changes.

Materials and methods

Streptozotocin (STZ) was purchased from Sigma© Chemical Company (St Louis, Missouri, USA). Betaine (Betafin® 96%) was prepared from Biochem© Company (Lohne, Germany). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum urea and creatinine kits were supplied by DIALAB© Company (Vienna, Austria), and HbA1c detection kit was purchased from BioSystems© Company (Barcelona, Spain).

Animals

60 female and 30 male Sprague-Dawley rats (aged 6–8 weeks, weighing between 180 and 200 g) were purchased from Animal laboratory of Razi Herbal Medicine Research Center (Lorestan University of Medical Sciences, Khorramabad, Iran) and were kept at the same place under a controlled environmental conditions (23 ± 1 °C, 50 ± 10% humidity, and 12:12 light–dark cycle) and standard laboratory diet and tap water were available ad libitum.

Cylicity checking and mating

The estrous cycle of rats was characterized, using vaginal lavage obtained between 8:00–10:00 am for 2 weeks. Cyclicity was determined by the methods proposed by Aziz, et al. with some modifications. Briefly, vaginal secretion was collected every morning by inserting fresh plastic Pasteur pipette containing ~1 ml of fresh normal saline up to about 10 mm deep into the vaginal canal and then the pipette was irrigated to flushing the cells from the vaginal lining. One drop of the lavage was smeared on a microscopic slide and was fixed by methanol, and then Giemsa staining was performed and it was viewed under microscope. Rats in estrus stage were allowed to mate overnight with males at a 2:1 ratio. On the following morning, pregnancy was confirmed by presence of vaginal plug (mucus plug) or spermatozoa in vaginal smear

respectively, considered as day 0 of gestation. Each pregnant rat was placed in a separate cage and was followed up until the end of pregnancy [5].

Experimental design

A total of 32 pregnant rats were obtained from preparation steps and were divided into 4 equal groups: Control (C), Betaine (B, 1.5% *w*/w of the total diet daily), Diabetic pregnancy (D), and diabetic pregnancy treated with betaine (D + B). Betaine dose was chosen according to the previous literature [1, 2] and precise dosage of betaine was calculated for each rat per day according to body weight. All dams were feed-deprived for 12 h, diabetes was induced (day 1 of gestation) by intraperitoneally injection of a single dose of STZ (65 mg/kg) [3] freshly dissolved in cold sodium citrate buffer (0.1 M, pH = 4.4) in D and D + B groups, and the other rats only received an equal volume of vehicle. Diabetic state was manifested 48 h after STZ injection by some sign such as polyuria, polydipsia, and polyphagia. Fasting blood sugar level was measured using Glucometer (ACCU-CHEK® Active Glucometer, Roche Diagnostics, Germany) with a drop of blood obtained by tail vein puncture according which rats with blood glucose values more than 16.7 mmol/l were considered as diabetic rats. Once the diabetic state was confirmed, betaine was added to the water (day 3) until the day 21 of pregnancy. Body Weight (BW) of the dams was recorded using an electronic weighing balance (Sartorius, Germany) during pregnancy (end of every week) and Weight Gain (WG) percentage was calculated with respect to pre-gestation weight. Experimental design of the study is briefly demonstrated by a simple graphical scheme (Supplementary Fig. 1).

Biochemical assessment

At the end of experiment, all rats were anesthetized through exposure to light diethyl ether and blood samples were collected through cardiac puncture into heparinized and plain tubes. Whole blood was used to spectrophotometrically measure glycated hemoglobin (as freshly) using ion-exchange chromatography approach, (S2000 UV model; WPA, Cambridge, UK) according to the BioSystems HbA1c detection kit [3]. Plain tubes left to clot for half an hour, then they were centrifuged at 2500 rpm for 12 min and sera samples were drawn and liquated to micro tubes and were kept at −80 °C for further analysis. To assess hepatic function, levels of serum hepatic enzymes including AST, ALT, and ALP were measured. Also, urea and creatinine concentrations in serum samples were evaluated to investigate renal function. In our study, all samples were analyzed using auto analyzer (Alpha-Classic AT plus) at the same time in a single run for each assay to avoid inter-assay variations.

Histopathological analysis

All dams were sacrificed through exposure to overdose of diethyl ether, then their liver and kidney tissue samples were taken and were fixed in 10% formalin solution for 48 h. After routine processing, tissue samples were embedded in paraffin blocks and 5 μm thick sections were prepared. Then, hematoxylin and eosin staining was performed, and tissue samples were examined under light microscope.

Statistical analysis

Statistical analysis was performed using the GraphPad PRISM version 6 (GraphPad Software, San Diego, CA, USA). All results were presented as mean ± S.E.M Statistical differences were determined among all the groups by one-way Analysis of Variance (ANOVA) using Tukey's post-hoc analysis.

Results

Table 1 illustrates weight gain percentage of dams. STZ injection caused a negative weight gain (body weight loss) in D group while there was no statistical difference in the first week of gestation between the other groups. Although weight gain of the dams significantly decreased in D and D + B groups compared to the other groups at the end of second week ($P < 0.0001$ and $P < 0.05$, respectively), however, betaine supplementation significantly increased this parameter in dams of D + B group in comparison with D group ($P < 0.0001$). In third week of pregnancy, dams in D group showed a lower weight gain compared to the other groups ($P < 0.0001$).

Biochemical parameters

Dams in D and D + B groups indicated upper limit of HbA1c values following STZ administration compared to C and B ($p < 0.0001$). Under betaine supplementation, this parameter significantly decreased in dams of D + B group in comparison with D group ($p < 0.0001$) (Fig. 1).

Table 1 Dams weight gain percentage (%) from different groups

Groups	First week	Second week	Third week
C	4.35	14.77	30.40
D	- 3.27	4.63***	18.33 ***
B	4.12	14.44	32.29
D + B	3.67	11.08 * #	27.04

C (control), D (Diabetic pregnancy), B (Betaine), D + B (diabetic pregnancy treated with betaine) * $p < 0.05$ vs. C and B; *** $p < 0.0001$ vs. C and B; # $p < 0.0001$ vs. D

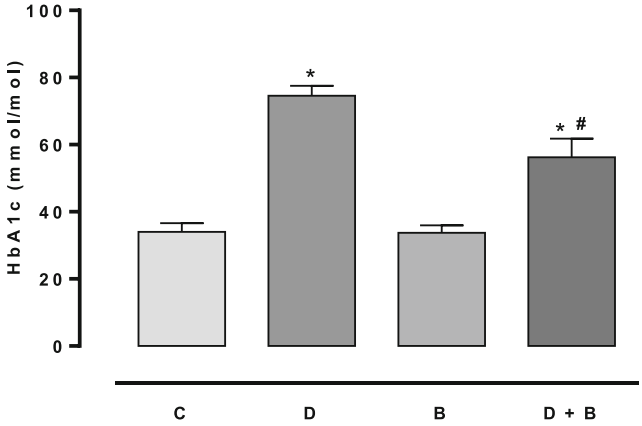

Fig. 1 HbA1c values from different groups. C (control), D (Diabetic pregnancy), B (Betaine), D + B (Diabetic pregnancy treated with betaine). Data are expressed as mean ± S.E.M ($n = 8$) * $p < 0.0001$ vs. C and B, # $p < 0.0001$ vs. D

As shown in Table 2, STZ-induced diabetes in pregnant rats significantly increased serum levels of hepatic enzymes such as AST, ALT, and ALP in D group compared to the other group ($p < 0.0001$). Betaine treatment restored AST and ALT levels back to normal in rats of D + B group compared to D group ($p < 0.0001$). In spite of remarkable effects of betaine on AST and ALT, it failed to decrease ALP level in dams of D + B group compared to D group. Overall, betaine had no effects on AST, ALP, and ALP levels in dams of B group compared to C group (Table 2).

As decipated in Fig. 2, urea and creatinine levels significantly elevated in serum of diabetic pregnant rats in D and D + B groups following STZ injection compared to C and B groups ($p < 0.0001$). Betaine treatment tended to decrease urea levels in dams of D + B group compared to D group, however, it was not statistically different ($p = 0.08$). Creatinine levels were significantly alleviated in dams of D + B group under betaine supplementation compared to D group ($p < 0.05$). No effects of betaine were observed on serum levels of urea and creatinine in dams of B group compared to C group.

Histopathological findings

Examination of the liver sections in both C and B groups showed a normal morphological pattern. Dams in D group indicated an array of pathological changes including regions of distorted liver architecture, dilatation of sinusoid and apoptotic cells (Fig. 3a) as well as moderate infiltration of inflammatory cells, dominantly mononuclear cells exclusively in portal area accompanied by cholangitis, pericholangitis, biliary hyperplasia, and portal vein congestion (Fig. 3c). Betaine treatment restored hepatic normal architecture and decreased number of apoptotic cells (Fig. 3b) also no significant pathological changes were seen in the bile ducts of dams in D + B group. However, a low population of leukocytes were found to be infiltrated in the portal area accompanied with portal vein congestion in this group (Fig. 3d).

Kidney tissue samples of dams in both C and B groups indicated normal architecture, glomerular size, and thickening of basement membrane. Diabetes induced severe damage to the dams kidney such as high degree of tubular necrosis, thickening of glomerular basement membrane, arterioles with arteriolosclerosis, and increased space of Bowman's capsules (Fig. 4a, c). Examination of the sections in the D + B group indicated that betaine supplementation during pregnancy alleviated renal damage characterized by remarkable increase in tubular and glomerular scores (Fig. 4b, d).

Discussion

Herein, for the first time, we mimicked the same condition of pregnant women with uncontrolled clinical diabetes by STZ injection and investigated effects of betaine administration on liver and kidney function using chemical method. In this study, diabetic pregnancy was accompanied by low weight gain, adverse alterations in hepatic and renal serum biomarkers, and structural changes in histopathological samples of dams in the experimental group compared to the control group. Interestingly, betaine supplementation remarkably attenuated most of these alterations in treated dams compared to untreated ones, which is further discussed in detail.

It has been reported that during normal pregnancy ALP increases due to elevated secretion of placenta, although any increase in AST and ALT is always pathological and must be further surveyed [21]. Following increased secretion of relaxin by feto-placental unit, it mediates both endothelin activation

Table 2 Serum hepatic enzymes levels from different groups, postpartum

Groups / Variables	C	D	B	D + B
AST (U/L)	138.4 ± 7.0	307.0 ± 24.56 #	178.1 ± 4.6	192.3 ± 11.52
ALT (U/L)	76.8 ± 4.4	201.6 ± 7.6 #	83.8 ± 3.4	88.7 ± 3.0
ALP (U/L)	436.6 ± 24.56	1317.0 ± 75.48 #	476.9 ± 27.34	1118.0 ± 93.21 #

Diabetes induced by STZ injection and treated with betiane during pregnancy. C (control), D (Diabetic pregnancy), B (Betaine), D + B (diabetic pregnancy treated with betaine). Data are presented as mean ± S.E.M ($n = 8$) # $p < 0.0001$ vs. C and B

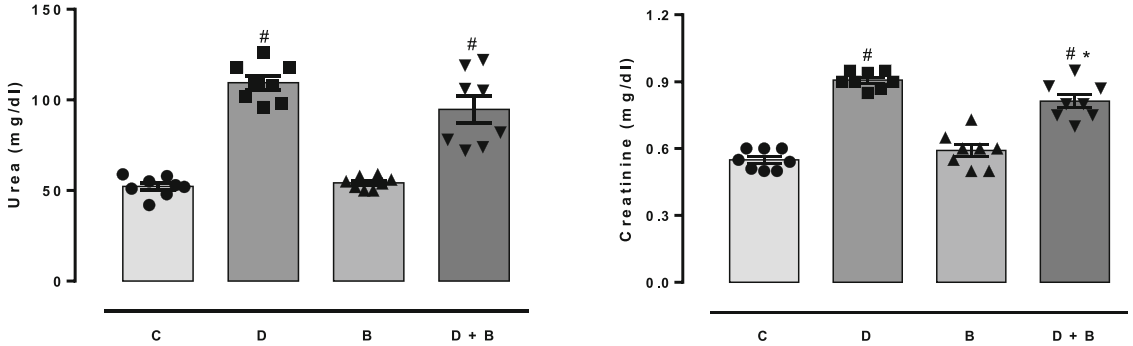

Fig. 2 Serum level of urea and creatinine from different groups. C (control), D (Diabetic pregnancy), B (Betaine), D + B (diabetic pregnancy treated with betaine). Geometrical shapes shows data scattering. Values are presented as mean ± S.E.M (n = 8) $^{\#}$ $p < 0.0001$ vs. C and B; * $p < 0.05$ vs. D

and NO synthesis, which in turn mediates vasodilation of renal arteries subsequently elevating renal plasma flow and Glomerular Filtration Rate (GFR) to sufficiently remove increased waste by-product generated due to development of fetus. As GFR rises in this stage both urea and creatinine decrease compared to non-pregnant women [43]. Since some researchers have claimed that STZ, per se, is responsible for adverse effects on liver and kidney instead of subsequent hyperglycemia, previously there was a discrepancy about main cause of hepatic and renal damages in STZ-induced diabetic models. To date, it has been proved that, hyperglycemia is main cause of these pathological alterations, which is augmented during the pregnancy by physiologically increased insulin resistance as reported in our previous study [7]. It is well-known that, glyco-oxidative stress is a driving force behind most of diabetes disorders, which is thought to occur through several mechanisms, including glucose auto-oxidation, increased flux of polyol pathway, formation of

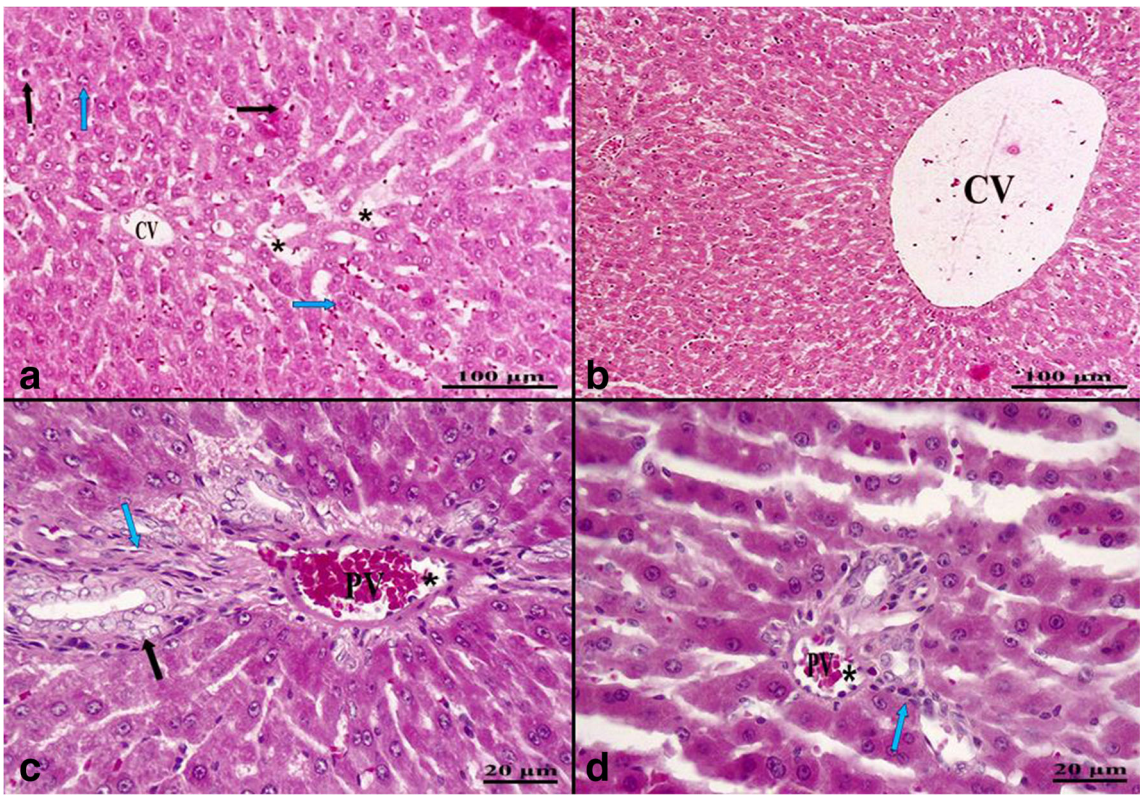

Fig. 3 Photomicrographs of liver sections from diabetic dams, postpartum: (**a**) Note the sinusoidal dilation (black asterisks) around the CV, hepatocytes exhibited apoptotic cells with pyknotic nuclei (black arrows) and vacuolated cytoplasm (blue arrows) in the D group (×100); (**b**) Normal architecture of sinusoids and reduced number of apoptotic cells in the D + B group under betaine treatment (×100); (**c**) Biliary duct hyperplasia (black arrow) and mixed leukocytes infiltration (blue arrow) in portal area accompanied with cholangitis, pericholangitis,and congested portal vein (black asterisk) in the D group (×400); (**d**) Normal bile duct, low infiltration of inflammatory cells (blue arrow) and congested portal vein (black asterisk) in the D + B group (×400)

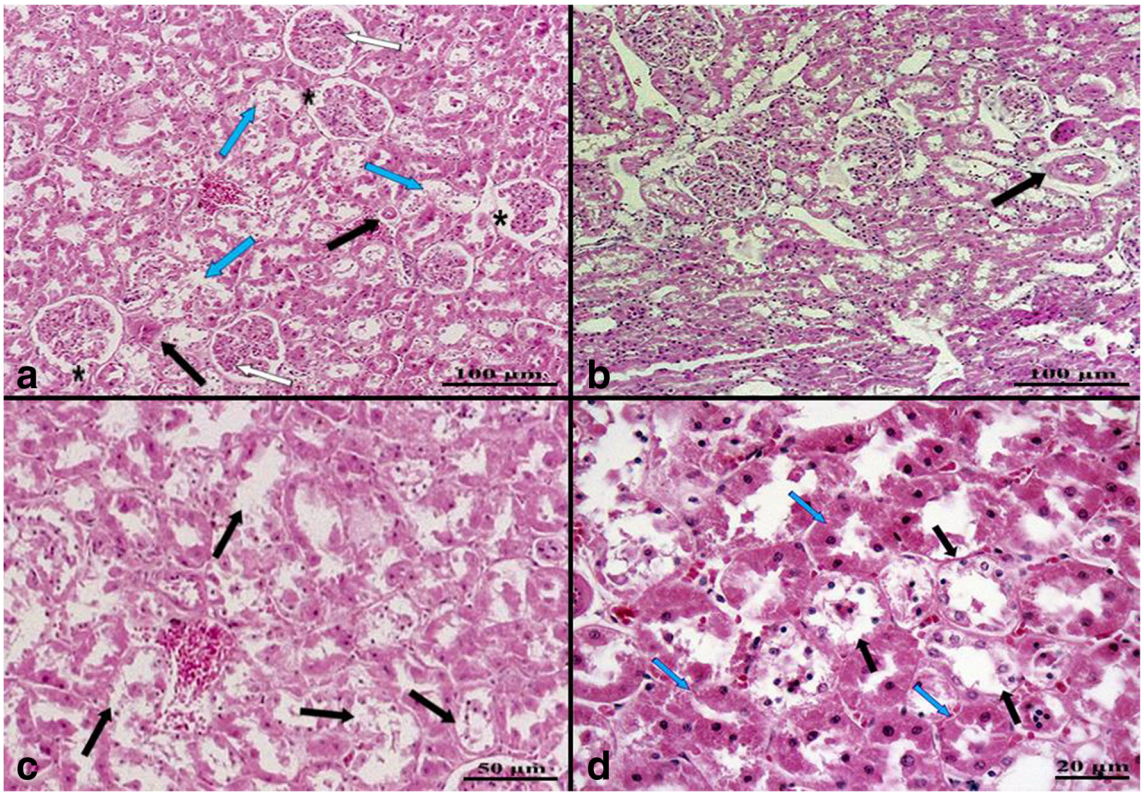

Fig. 4 Photomicrographs of kidney sections from diabetic dams postpartum: (a) Note the loss of brush border and necrosis of proximal tubules (blue arrows), thickened glomerular basement membrane (white arrows), arterioles with narrow lumen and thickened wall (black arrows) and increased space of Bowman's capsules (black asterisk) in the D group (×100); (**b**) Ameliorated renal damage under betaine supplementation characterized by increase in tubular and glomerular scores, as well as arteriole with narrow lumen and thick wall (black arrows) in the D + B group (×100); (**c**) Tubular necrosis and epithelial desquamation (black arrows) in D group (×200); (D) Note the vacuolar degeneration of tubules (black arrows) attenuated under betaine treatment (blue arrows) in the D + B group (×400)

Advanced Glycation End-products (AGEs), activation of protein kinase C, and overproduction of superoxide by mitochondrial electron transport chain [30]. Regarding HbA1c assessment in our report, it provided a double perspective for insulin resistance and redox status of the dams. As HbA1c represents the level of AGEs involving protein glycosylation of antioxidant enzymes, it directly contributes to oxidative stress by inactivation of enzymatic antioxidant system [32]. In this regard, Rodriguez et al. [36] indicated that decreased hepatocytes GSH content well correlates with elevated HbA1c, transaminases and ALP levels in diabetic rats. Also, Mansouri et al. [24] showed a similar connection between decreased hepatic GPx, SOD, and CAT activity with elevated serum levels of ALT, AST, and ALP, 4 weeks after STZ injection. Regarding kidney function, the evidence [40] shows that highly glycosylated Hb and ROS overproduction inhibit NO-mediated relaxation leading to vasoconstriction and decreased GFR, further resulting in increased urea and creatinine concentrations. Also, both in-vivo and in-vitro studies suggested that glyco-oxidative stress triggers necrosis of tubular and glomerular cells resulting in elevated serum levels of urea and creatinine [4]. Moreover, increased urea and creatinine observed in this report indicates amplification of protein and amino acids flux into gluconeogenesis pathway because of insulin deficiency as characterized by low weight gain of the dams [12]. Herein, hepatic and renal serum biomarkers of diabetic dams significantly elevated with HbA1c concentration supporting abovementioned hypothesis. In the current report, betaine supplementation significantly lowered HbA1c concentration along with AST, ALT, creatinine, and tended to decrease urea in diabetic dams. However, ALP levels remained statistically unchanged under betaine treatment, which could be due to its longer half-life in circulation, multiple source of secretion, and physiological stimulus of the pregnancy (by feto-placental unit) compared to transaminases [36]. Several papers including our previous study demonstrated that betaine increases insulin secretion and improves insulin signaling pathways thereby exerting glucose lowering effects [39, 49]. Indeed, betaine supported weight gain in treated dams meaning that, it alleviated gluconeogenesis by decreasing expression of genes encoding phosphoenolpyruvate carboxykinase and glucose 6-phosphatase as previously described [20]. Although, HbA1c concentrations decreased under betaine supplementation, it cannot totally justify our observations, because the level of glycosylated Hb remained much higher than normal controls. Betaine plays a central role

in GSH synthesis as main cellular antioxidant by increasing methionine (Met) availability. In this sense, consistent with several studies reporting betaine as an antioxidant nutrient, many studies carried out in our lab also reveal that betaine augments enzymatic and non-enzymatic antioxidant system [1, 2]. On the other hand, onset of diabetes disorders in liver and kidney has been documented by various researchers in association with disrupted mitochondrial homeostasis and following cellular Adenosine Triphosphate (ATP) depletion as a consequence of redox imbalance [6, 8]. In fact, betaine is a key modulator of mitochondrial homeostasis as it contributes in 1C metabolism particularly in liver and kidney (two main reservoirs of betaine) [49]. The evidence shows that depletion of hepatocytes betaine content and increased betaine urinary excretion are positively correlated with hepatic and renal damage [8, 22]. As mentioned earlier, subsequent to increased Met availability under betaine supplementation, it contributes to more ATP production by providing some intermediate products such as α-ketoglutarate and propionyl-CoA (after conversion to succinyl-CoA) to enter the tricarboxylic acid cycle, which is more important when cells are under the stress due to impairment in energy metabolism [25, 37]. Taking all this into consideration, we adjudge that betaine modulated hepatic and renal serum biomarkers probably in downstream of it's hypoglycemic properties as was evident by attenuated HbA1c values (this could result in improved redox balance due to decreased AGE's).

On the other hand, separate evaluation of alterations in histopathological markers of liver and kidney could shed more lights on underlying mechanism in this study. In addition to apoptotic hepatocytes, sinusoidal dilation and biliary hyperplasia were the most remarkable changes in the liver photomicrographs from diabetic dams in our study. Pregnancy is accompanied with increased gallbladder volume, reduced emptying time, and decreased small intestine motility, which in turn influences biliary system [21]. Indeed, cholestatic liver injury and biliary stones have been identified as causes of biliary hyperplasia [41]. Diabetes in human and animal model is associated with decreased bile flow and development of biliary stones, which is suggested to be related to supersaturated bile by cholesterol (decreased solubility) following diabetic dyslipidemia [35, 41]. In this case, reduced portal inflow defined as one of causes of sinusoidal dilation [13]. Thus, it seems that, biliary hyperplasia lowered portal inflow through physical pressure, which subsequently triggered sinusoidal dilation as observed in this study. In this sense, betaine (trimethylglycine) can provide adequate glycine for conjugation with primary bile acid in order to enhance their hydrophilicity and maintaining normal bile flow [41, 44]. Besides, Hailey et al. [17] remarked that biliary hyperplasia well correlates with increased Peroxisome Proliferator-Activated Receptors (PPARs). It has been reported that betaine could restrict transcriptional activity of PPARs [49]. Therefore, betaine might prevent biliary hyperplasia and sinusoidal dilation

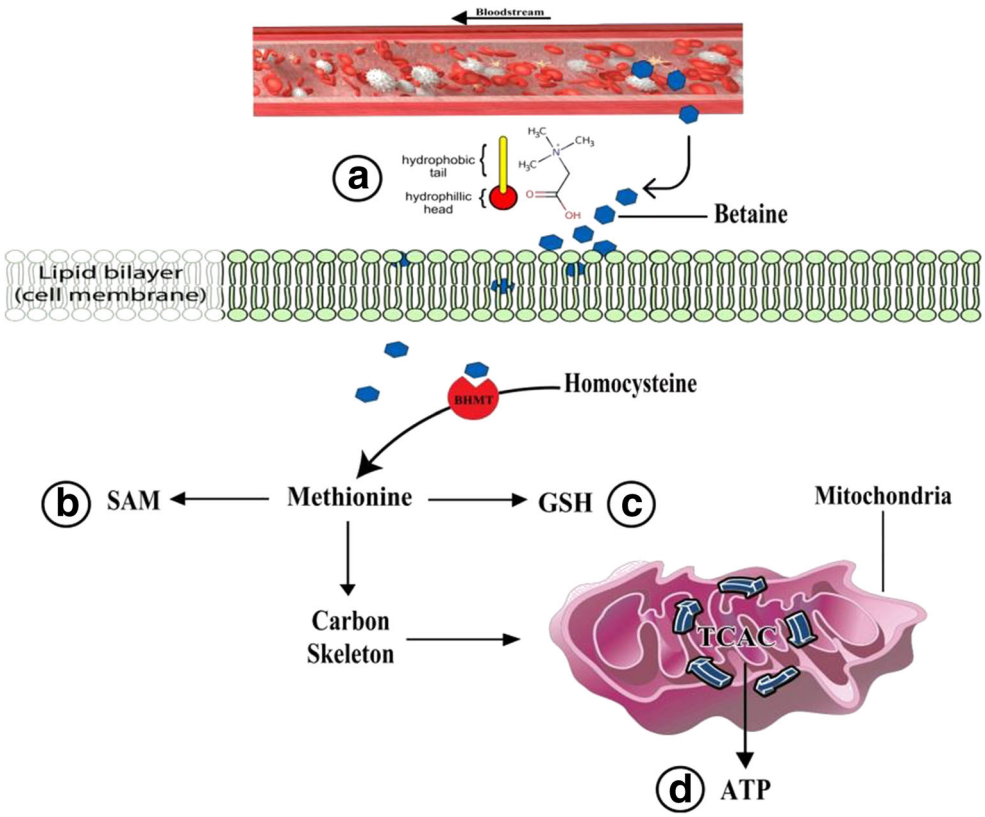

Fig. 5 A simple graphical scheme of betaine protective mechanisms based on existing evidences. A) Forming a protective shield around cells against ROS damages because of it's zwitterionic chemical structure B) Epigenetic regulation of genes including those encoding enzymes involved in glucose metabolism by providing adequate methyl groups as S-Adenosyl Methionine (SAM) C) Improving non-enzymatic antioxidant content of the cell through participating in glutathione (GSH) synthesis as the most important intracellular antioxidant D) Carbon skeleton supply for combusting in tricarboxylic acid cycle (TCAC) followed by production of adenosine triphosphate (ATP)

in downstream of decreased PPARs and increased bile solubility. Regarding renal micrographs, thickening of basement membrane (hall mark of diabetic nephropathy) and increased space of Bowman's capsules has been previously reported in a STZ-induced diabetic model [26]. Exacerbated production of AGEs as evidenced by high concentration of glycosylated Hb in this study, results in AEGs accumulation on basement membrane collagen thereby increases trapped plasma protein which in turn leads to thickening of basement membran [12]. In this case, protective role of betaine has been discussed earlier and it has been found to alleviate HbA1c levels. Also previous papers [29] explained occurrence of increased space of Bowman's capsules in association with diabetes-induced microvascular changes and increased glomerular function, which is intensified due to pregnancy as observed in the present study.

Taken together, biochemical and histopathological findings obviously showed that, protective effects of betaine were more remarkable in liver compared to kidney, which is probably due to dominantly expressed betaine transporters in hepatocytes as main reservoir, as well as, different manners of cellular glucose uptake by hepatocytes (facilitated diffusion by GLUT-2) versus kidney cells (mostly insulin- dependent by GLUT-4) [6, 14]. Overall, it could be concluded that, betaine might exert protective effects mostly associated with its antioxidant, glucose lowering, and ATP providing properties (Fig. 5), however the exact mechanism should be evaluated in future studies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All rats were treated humanely and in compliance with the recommendations of Animal Care Committee for the Lorestan University (Khorramabad, Iran) with approval number: LU.ECRA. 2017.4.

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