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Embryoprotective effect of glycine in a rat model of diabetic embryopathy is due to amelioration of oxidative stress

El efecto embrioprotector de la glicina en un modelo de embriopatía diabética es mediado por mejoría en el estrés oxidativo

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ABSTRACT

The coexistence of pregnancy and diabetes mellitus results in major malformations incompatible with life, developmental delay, pregnancy loss, or maternal-fetal death. Treatment with glycine reverses the harmful effects of glucose, in patients and animal models; as well as the metabolic and biochemical changes caused by diabetes mellitus/hyperglycemia. However, the effect on diabetic pregnancy has not been investigated, so this study was designed. Pregnant rats were randomly assigned to four groups: control, glycine, diabetic, diabetic plus glycine. The subjects were euthanized on day 19 of gestation; fetuses were obtained, as well as liver, kidney, and maternal blood serum. The fetuses were evaluated for malformations, both gross and internal; sections of the fetal liver, kidney, and brain were subjected to histological analysis. Maternal serum was processed to determine glucose, cholesterol, and triglycerides; In the fetal liver, the activities of scavenging enzymes and lipoperoxidation were determined. The administration of glycine improves fetal development and biochemical-clinical parameters, but in healthy rats, it does not affect these parameters or fetal development. Excess glucose can cause oxidative stress, which is partially reversed by glycine, which enhances fetal development impaired by hyperglycemia; however, glycine administration to healthy rats produces a low percentage of malformations, a result hard to explain.

KEY WORDS: Glycine, diabetic embryopathy, embryoprotection, oxidative stress, teratogenesis.

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RESUMEN

La coexistencia de gestación y la diabetes mellitus resulta en malformaciones mayores incompatibles con la vida, retraso de desarrollo, pérdida de gestación, o muerte materno-fetal. El tratamiento con glicina revierte los efectos nocivos de la glucosa, en pacientes y en modelos animales; así como los cambios metabólicos y bioquímicos causados por la diabetes mellitus/ hiperglucemia. Sin embargo, no se ha investigado el efecto sobre el embarazo diabético, por lo que se realizó este trabajo. Se tuvieron ratas preñadas que se asignaron aleatoriamente a cuatro grupos: control, glicina, diabéticos, diabéticos más glicina. Los sujetos se eutanizaron el día 19 de gestación, se obtuvieron los fetos, así como hígado, riñón y suero sanguíneo materno. Los fetos se evaluaron para detectar malformaciones, tanto gruesas como internas; secciones de hígado, riñón y cerebro fetal se analizaron histológicamente. El suero materno se procesó para determinar glucosa, colesterol y triglicéridos; en el hígado fetal se determinaron las actividades de las enzimas depuradoras de radicales libres y la lipoperoxidación. La administración de glicina mejora el desarrollo fetal, y los parámetros bioquímico-clínicos, y en ratas sanas, no afecta éstos parámetros ni el desarrollo fetal. La glucosa en exceso puede causar estrés oxidativo, que es revertido parcialmente por la glicina, lo que mejora el desarrollo fetal alterado por la hiperglucemia, sin embargo, la glicina administrada a ratas sanas produce un bajo porcentaje de malformaciones, resultado que no es fácil de explicar.

PALABRAS CLAVE: Glicina, embriopatía diabética, embrioprotección, estrés oxidativo, teratogénesis.

Introduction

Diabetes Mellitus (DM) is a chronic disease caused by reduced or absent insulin production or peripheric resistance to this hormone. This syndrome, with diverse etiologies, is a public health problem, and in the last decades the number of cases and the prevalence of the disease have dramatically increased. The main manifestation of DM is sustained hyperglycemia, though several other clinical and metabolic parameters also are affected. Long-term complications include diabetic cardiopathy, nephropathy, neuropathy, retinopathy, and vasculopathy. The damage that pregestational DM causes to the embryo during early development is defined as “diabetic embryopathy” (Baker & Piddington, 1993), and it is different from damages caused by gestational DM (GDM).

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Worldwide, approximately 60 million of women in reproductive age have DM, and by the year 2030, this number could double (Centers for Disease Control and Prevention, 2008). DM is one of the most common diseases prevalent in both developed and developing countries. The global incidence of neonates born to diabetic mothers increased from 3.1 per thousand live births in 1998 to 4.7 per thousand in 2004. According to World Health Organization data, about 6 % of neonates (approximately 8 million) are born with congenital defects each year. A relationship between pregestational diabetes and neonates with congenital defects has been demonstrated. In our country, the Guía de Práctica Clínica (2009) published for the Ministry of Health (Secretaría de Salud) recognizes that pregestational diabetes mellitus as a factor in the appearance of neonatal malformations, and proposes as a goal, to reduce these malformations.

Glucose at non-physiological levels has been identified as the main teratogen in DM, with oxidative stress being the primary molecular mechanism. This is a disequilibrium between the reactive oxygen species and the physiological antioxidants (scavenger enzymes, vitamins, cofactors, etc.). Increased reactive oxygen species can damage biomolecules, including proteins, lipids, and nucleic acids. Several molecular mechanisms could produce these reactive molecules, with the hydroxyl radical being harmful for its higher capacity to produce cell damage (Eriksson & Wentzel, 2015).

In support of the idea that diabetic embryopathy is mediated by oxidative stress, antioxidants such as vitamin C and E (alone or combined) (Cederberg *et al*., 2001), lipoic acid (Al-Ghafli *et al*., 2004), resveratrol (Singh *et al*., 2011), N-acetyl cysteine (Wentzel *et al*., 2003), and polyamines (Méndez and Palomar-Morales, 1999), could ameliorate, protect or prevent the harmful effects of DM/hyperglycemia. On the other hand, pioneer studies of Carvajal-Sandoval´s group have demonstrated that glycine treatment improves outcomes of human patients and diabetic male rats (Carvajal-Sandoval *et al*., 1995, 1999 a,b, 2007; Alvarado-Vazquez *et al*., 2003, 2006). Also, it has been demonstrated that glycine reduces teratogenicity of all-trans retinoic acid in in vivo rat model (Martínez-Angoa *et al*., 2006), and in vitro studies, glycine prevents glucose-induced malformations in mouse embryo (Martínez-Galero *et al*., 2008). For these reasons, this study aimed to evaluate the effect of glycine in a rat model of diabetic embryopathy, and the possible participation of improvement of oxidative stress in the embryoprotective effect.

Materials and Methods

Treatments

Twenty-five female Wistar rats, healthy, of 10-12 weeks of age, weighing 220-250 gr, were obtained from the bioterium of the FES Iztacala, and were maintained in the same bioterium in controlled conditions of light:dark cycles, temperature, and humidity, with ad libitum access to food (Enviro S2018 pellets) and purified water. Procedures were realized according to NOM-062-ZOO-1999, with the approval of the bioterium committee. Females were mated by the threesome method overnight with fertile, healthy males, of the same strain, and in the following morning, a vaginal smear was obtained to be visualized in a light microscope Leica DM500.

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Zero day of gestation was assigned when spermatozoids were observed. Pregnant rats were randomly assigned to four groups of five to seven individuals (control, diabetic, glycine, diabetics plus glycine). The individuals of the “diabetic” and “diabetic plus glycine” groups were injected intraperitoneally on the morning of 4th day of gestation with streptozotocin (STZ) (Sigma Chemical Co) at dose of 50 mg/Kg body weight, dissolved in a small volume of citrate buffer (100 mM, pH 3.5), whereas “control” and “glycine” were injected at same time with a comparable volume of the same buffer. Control and diabetic groups remain with ad libitum access to water, but the rats of glycine-treated groups were administered with glycine of alimentary grade, 2 % in drinking water (Martinez-Angoa *et al*., 2006; Paniagua-Castro *et al*., 2006), from days 4th until 18th of pregnancy. Glycine dissolved in water was replaced each third day to avoid contamination.

This schedule of diabetic induction was chosen since pre-mating administration of STZ to rats avoids successful mating in the females of the colony of rats from our institution (Chirino-Galindo *et al*., 2021), a finding that also has been reported by another research group (Al-Ghafli *et al.,* 2004). For this reason, a model of diabetes mellitus induced by STZ when diabetogen is administered in pre-implantational period was established, since it is well known that STZ administration in this period causes hyperglycemia, glucosuria and small fetuses (Golob *et al*., 1970; Sybulsky & Maughan 1971). In addition, the half-life of the agent is very short (Rodrigues *et al*., 1999), and thus, developmental alterations such malformations or resorptions could be principally attributed to diabetes instead of STZ toxicity.

Sample obtention

On the morning of the 19th day, pregnant rats were anesthetized with an intramuscular injection of sodium pentobarbital, at a dose of 60 mg/Kg body weight, and blood was drawn for cardiac punction with a hypodermic syringe. Rats were thus euthanized by cervical dislocation, and uteri were obtained by laparotomy. The kidney and a fraction of the liver were also collected and fixed in formalin. About a third of the liver was homogenized in saline solution, and frozen until use.

Serum analysis

Obtained blood was centrifuged to 3000 rpm for 5-7 min, and serum was recovered, and collected in Eppendorf vials of 2.0 mL of capacity, for storage at -20° C until use. Glucose, cholesterol, and triglycerides were measured, with the aid of commercial kits (Spinreact REF14101, EliTech CHSL-5505, and EliTech TGML-5414, respectively), following the instructions of the manufacturer. Attempts were made to evaluate glycosylated hemoglobin in maternal blood, with the BioSystems 11044 commercial kit. Color changes were monitored in a Jenway 6305 spectrophotometer for glucose, or Sunrise Tecan microplate reader for cholesterol and triglycerides.

Fetal analysis

Uteri were longitudinally cut with scissors, and fetuses were obtained and counted. All the procedure was performed in cold. Fetuses were measured with a Vernier, weighed in a digital

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semi-analytic balance, and fixed in Bouin´s fluid. Two days later, the fluid was removed, and fetuses were rinsed and submerged in ethyl alcohol 70 %, to be subjected to Wilson´s technique modified (Barrow & Taylor, 1969). Morphologic sections were visualized at light microscope. The liver, brain, and kidney were further subjected to histological analysis. In some cases, a small fraction of the liver was obtained before Bouin´s fixation and homogenized in saline solution.

Activity of scavenging enzymes

Crude extracts of the liver of pregnant rats and their fetuses were centrifuged at low speed to eliminate cell debris. In the supernatant, the activity of scavenging enzymes and lipoperoxidation were measured. Catalase activity was determined by the decrease in absorbance at 240 nm due to the conversion of hydrogen peroxide to water (Aebi, 1984). Glutathione peroxidase (GPx) was monitored following the increase in absorbance at 340 nm due to the NADPH formation (Paglia & Valentine, 1967). Glutathione S-transferase (GST) activity was measured by the apparition of the conjugate glutathione-DNCB, which increases absorbance at 340 nm (Tsuchida, 1999). Superoxide dismutase was estimated on the basis of the inhibition of electron transference to NBT from xanthine, catalyzed by the xanthine oxidase, and measured at 560 nm (Beauchamp & Fridovich, 1971). Lipoperoxidation was measured for the reaction of lipoperoxides with thiobarbituric acid, with the use of malondialdehyde as standard (Ohkawa *et al*. 1979). Enzymatic activity was normalized by protein, measured by the method of Lowry et al. (1951). Absorbance changes were monitored in a Jewnway 6305 ultraviolet/visible spectrophotometer.

Histological analysis

Fixed samples of maternal and fetal tissues were removed from fixative (Bouin or formalin), rinsed in water, dehydrated in increased solutions of alcohol, cleared in amyl alcohol, embedded in histologic paraffin, and included. Blocks were cut at 5 µm in a microtome Leica RM2125RTS. Sections were mounted in gelatinized glass slides and stained for the H&E routine procedure. Brain fetal sections were stained with periodic acid-Schiff. The sections were visualized in a light microscope Leica DM500. Photographs were acquired with the Leica EC3 digital camera and analyzed with the Las EZ Leica software.

Statistical analysis

Glucose, cholesterol, and triglycerides levels in sera, as well as litter size, fetal weight, and length, were interpreted by two-way ANOVA, at a confidence level 0.05, in the program Statistica V10 Enterprise®. Resorption or malformation percent were calculated on a litter basis and analyzed for square chi. For the description in the figures and the table, standard error of the mean (SEM) instead of standard deviation was used.

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Results and Discussion

Serum glucose, cholesterol, and triglyceride values observed in healthy, pregnant rats were similar to those previously reported for this species, indicating that treatment with the buffer does not cause any significant metabolic changes. On the other hand, for STZ-treated pregnant rats showed elevated levels consistent with the effects of the diabetogenic agent. Glycine treatment in healthy pregnant female rats does not affect the serum levels: glucose serum concentration in these subjects was higher than in control rats, though the difference was not statistically significant. Considerably, glycine treatment in diabetic female rats is accompanied by a decrease in glucose, cholesterol, and triglyceride values, although the glucose level does not reach the normal values (Figure 1). Results were consistent with the reported by Alvarado-Vasquez *et al*. (2003). Glycosylated hemoglobin shows very low levels in all cases, and without significative difference between groups, and this result was not reported.

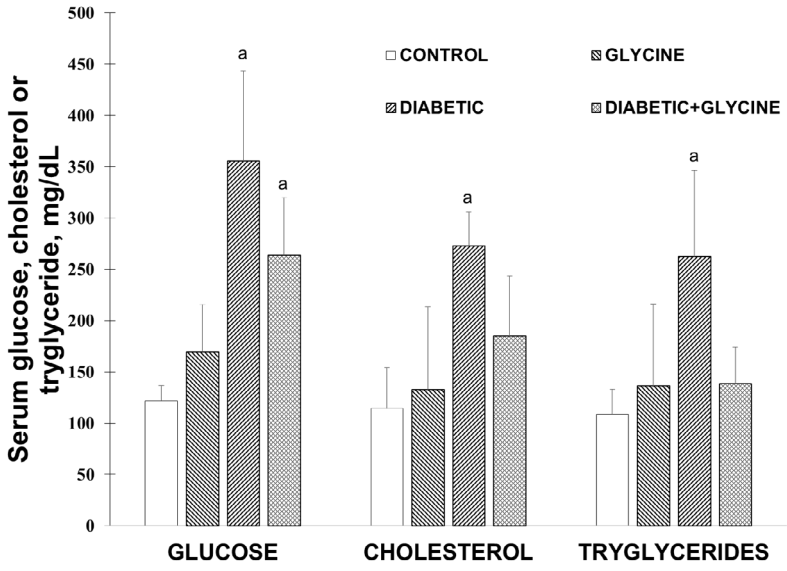

Figure 1. Serum parameters in diabetic rats treated with glycine.

Glucose, cholesterol, and triglycerides were determined in sera of diabetic or control rats with or without treatment with glycine. Mean ± SEM of 5–7 independent determinations. [a]$p < 0.05$ with regard control group.

Glycine administration to normal, healthy rats does not affect fertility of litter size, since this parameter is similar between the two groups of healthy rats. Diabetic induction is accompanied by a non-significant decrease in litter size, with regard control group. Additionally, treatment of pregnant rats with glycine reduces resorption percentage, but the normal values are not reached. Surprisingly, glycine, administered to healthy rats, produces a low malformation percentage (Table 1).

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Buchanan and Kitzmiller (1994) reported that in human and experimental subjects, DM/ hyperglycemia caused a developmental delay and low birth weight. In human beings, and in experimental models, there is also gestation loss for pregestational diabetes mellitus (Freinkel 1980; Freinkel *et al*. 1985), this could explain the higher frequence of embryo resorptions in the present report. In the control group, the frequency of resorptions was low, as has been described for this species (Beaudoin, 1980). In the diabetic plus glycine rats, fetal length and weight are very close to the value found for control group, i.e., glycine appears to restore the diabetes effect over delayed development. Glycine treatment has been demonstrated that reduces the deleterious effect of cadmium over fetal length and size and resorptions percent (Paniagua-Castro *et al*. 2006). However, the frequency of resorptions, although lower that those found for diabetic dams, was higher with regard the value found in control group.

Table 1. Reproductive and fetal parameters in diabetic rats treated with glycine

	Control	Glycine	Diabetics	Diabetics plus glycine
Litter size (n)	12.00 ± 1.22 (5)	11.60 ± 0.60 (5)	9.83 ± 0.70 (6)	11.57 ± 1.90 (7)
Resorption percent (affected litters)	1.19 ± 0.53 (1)	9.08 ± 2.16ab (3)	27.80 ± 5.39a (3)	14.02 ± 4.35ab (3)
Fetal length	2.96 ± 0.04	2.86 ± 0.02a	2.63 ± 0.05a	2.86 ± 0.03ab
Fetal weight	2.80 ± 0.11	2.74 ± 0.03	2.10 ± 0.07a	2.52 ± 0.05ab
Malformations percent (affected litters)	0.0 (0)	3.54 ± 2.20ab (2)	9.79 ± 5.7a (3)	4.08 ± 1.83ab (3)

Values are mean ± EEM of n = 5 to 7 for litter size, and for resorptions and malformation percent, or of all obtained fetuses (58 – 80) and fetal length (cm) and weight (g). Percent resorptions and malformations were calculated on litter size. $^{a}p < 0.05$ with regard control group; $^{b}p < 0.05$ with regard diabetic group.

No malformations were found in the control group fetuses, whereas STZ-induced diabetic rats exhibited a high malformation rate, consistent with previous findings. (Cederberg *et al*., 2001; Al-Ghafli *et al*., 2004; Wentzel & Eriksson, 2005; Wentzel *et al*., 2008; Singh *et al*., 2011). The main malformations found in diabetic rats were exencephaly, hydramnios, and protruded tongue. Exencephaly and hydramnios were detected in whole fetuses, and some other malformations, although generalized, while other generalized malformations were observed only in fetal sections stained using Wilson's modified technique. In the other hand, glycine treatment to rats with induced DM partially reduces both frequence and severity of malformations. Malformations found in fetus of these females were anasarca, local edema and hydramnios (data not shown): Surprisingly, glycine treatment in normal, healthy rats, was accompanied for a low malformation's percentage (anasarca and protruded tongue), higher that the reported value for this species. In Figure 2, two

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fetuses with exencephaly are shown. Ther are not an easy explanation of the small percentage of malformations found in glycine treated rats, in further studies, efforts will be made to verify if this result is casual or produced by glycine. A possible explanation is that glycine, as well as many natural molecules, could have antioxidant and prooxidant properties, depending on the metabolic moment and administration schedule; however, there is no evidence in the literature to support this proposition.

In addition to malformations found in some whole fetuses, it is notorious that fetal liver of diabetic rats, are affected by steatosis, and hemorrhagic zones were observed, when fetuses were subjected to Wilson´s modified technique. These damages were not found in control or glycine-treated fetus (Figure 3). Frequence and severity of malformations found in fetus of diabetic rats are consistent with the literature, and the reduction of the same by glycine administration in supported by the results of Paniagua-Castro *et al*. (2006), who reported that glycine administration reverts cadmium-produced exencephaly in mouse fetuses.

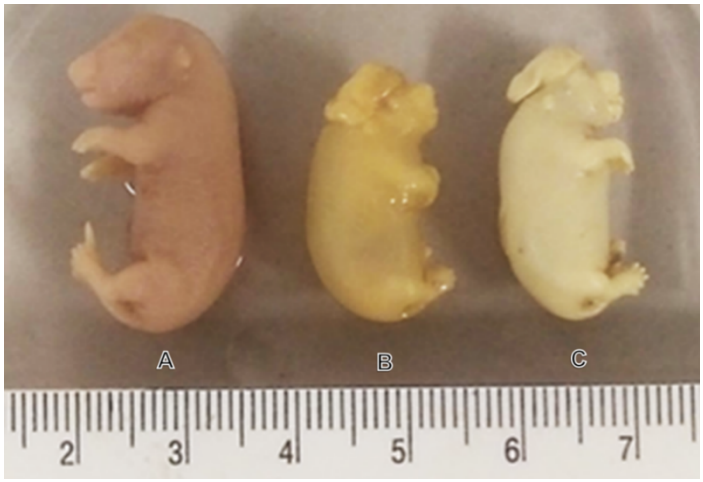

Figure 2. Exencephalic fetuses (B,C) of diabetic females, compared with a normal fetus of a control female. A metric rule is shown for comparison.

Beneficial effects of glycine on human health have been recently reported (Pérez-Torres *et al*., 2017; Razak *et al*., 2017). Glycine administration has been shown that ameliorates main symptoms and signs of DM in human beings and in animal models: glycemia, protein glycation, plasma triglycerides, etc. (Pérez-Torres *et al*., 2017). However, as far as we know, relatively few investigations have been made with regard the embryoprotective or antiteratogenic role of this amino acid over experimental diabetes, but the results of this paper agree with the reversion of

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damage caused by cadmium (Paniagua-Castro *et al*., 2006) or all-trans-retinoic acid (Martinez-Angoa *et al*., 2006) for glycine.

Glycine administration to normal, healthy pregnant rats does not affect reproductive performance, since litter size is not different between the control and glycine groups. Diabetic condition reduces slightly litter size, but this result is not significantly different with regard control group (Table 1). Additionally, treatment of diabetic pregnant females with glycine reduces the percent of resorptions, although control values are not reached. Surprisingly, glycine produces a small percentage of resorptions in normal, healthy rats (Table 1).

Fetuses of normal, healthy rats do not present any malformations. STZ-induced diabetes produces a higher percentage of malformations, more severe than those found in glycine-treated rats. Malformations found in fetus of female rats induced to diabetes with STZ were exencephaly, protruded tongue, and hydramnios. Exencephaly and hydramnios were detected visually in whole fetuses, but other malformations, although generalized, were observed only internally, after the use of modified Wilson´s technique. On the other hand, treatment of diabetic-induced rats with glycine reduces both the severity and the frequency of malformations; and the types of malformations found in these females were anasarca, local edema, and hydramnios. Surprisingly, glycine treatment in normal, healthy rats was accompanied by a slight percentage of malformations (anasarca and protruded tongue). In Figure 2, two exencephaly fetuses are shown.

In maternal liver sections, control pregnant rats show the normal structure of the species: sinusoids, liver cords, and centrolobulillar veins are visible. Diabetic induction is accompanied of a slight disruption of the tissue, whereas treatment of diabetic-induced female rats with glycine appears to restore the structure. Although in sections some empty spaces could be seen, also improvement of hepatic structure could be appreciated. Treatment of glycine in normal, healthy rats does not produce any conspicuous effect with regard to the normal structure of the liver (Figure 4). Similar results were found in male mice, where STZ administration besides producing hyperglycemia and an alteration of lipid metabolism, is accompanied by steatosis, cell tumefaction, and focal necrosis (Flores *et al*., 2006).

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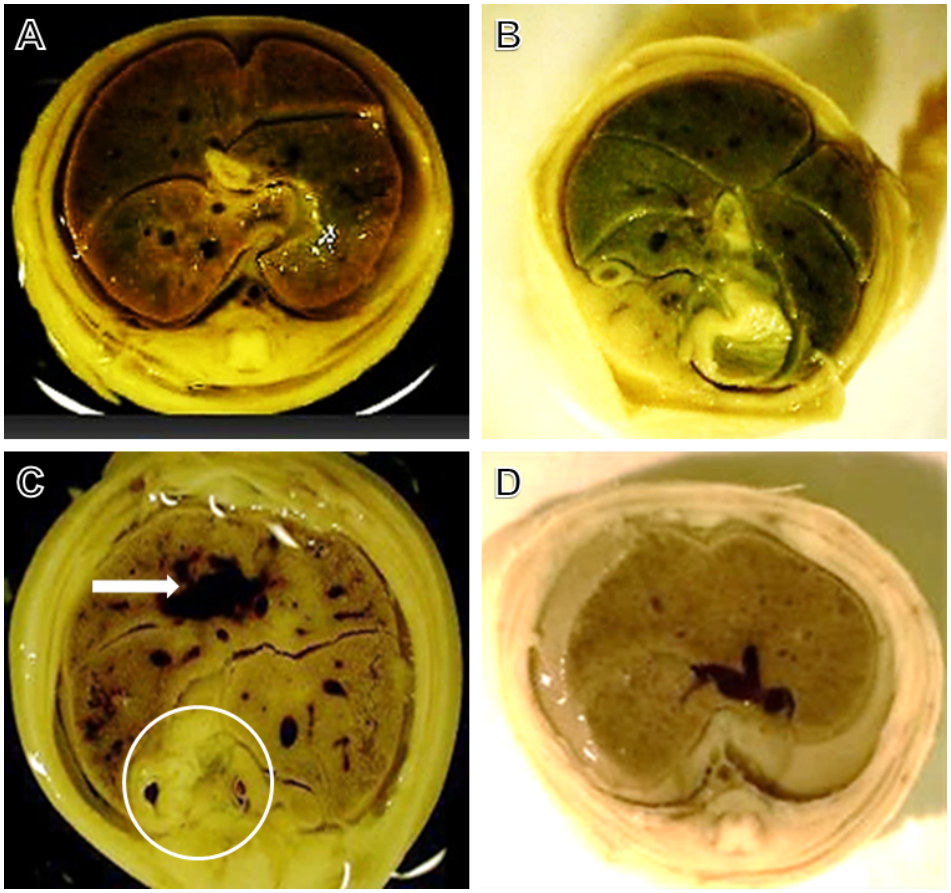

Figure 3. Effect of the treatment of diabetic rats with glycine on fetal liver.

A Wilson´s cut of a representative fetus of a control rat, that shown the normal structure of a fetal liver; B: fetus liver of a rat treated with glycine; C: fetus liver of a diabetic rat; D: fetus liver of a diabetic rat treated with glycine. The arrow shows a hemorrhagic area, and the circle denotes fat deposit.

In the other hand, in maternal kidney of control group, the corpuscles, as well as the proximal and distal tubules, the loop of Henle, and collecting duct appears to have normal structure. In the same form as liver, glycine treatment to normal, healthy pregnant rats do not produces any visible change. Induction of diabetes with STZ profoundly alters the ultrastructure, since empty spaces could be seen in the liver of pregnant STZ-treated rats (Figure 5). These findings support those of Alvarado-Vasquez *et al*. (2003), who reported renal damage due to experimental diabetes, and restauration of normal structure for glycine, in rats.

With regard heart, the fetuses of control groups show the normal structure of this tissue: nucleus, muscle fibers, desmosomes, etc., are of normal appearance. In the hearts of fetuses of glycine-treated mothers, a loss of cardiac fibers could be observed in the interstitial spaces. As in

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maternal tissues, treatment of normal rats with glycine does not affect the structure, but treatment of diabetes-induced rats notably improves the abnormal condition (Figure 6). A similar result is observed in the brain: fetal brains of control group shown the normal structure: pyramidal cells, glial cells, oligodendrocytes in histological sections are normal in appearance, but in fetal brain of the fetuses of STZ-treated rats, there are many empty spaces in histological sections. Fetuses of glycine treated groups, both healthy and diabetic mothers, resemble the observed in fetal control brain (Figure 7).

Finally, in fetal control liver, the structure appears to be normal, as well as the structure of liver fetuses of glycine or diabetics plus glycine treated rats; in contrast, liver of the fetuses of diabetic mothers show profound cell and tissue disorganization (Figure 8).

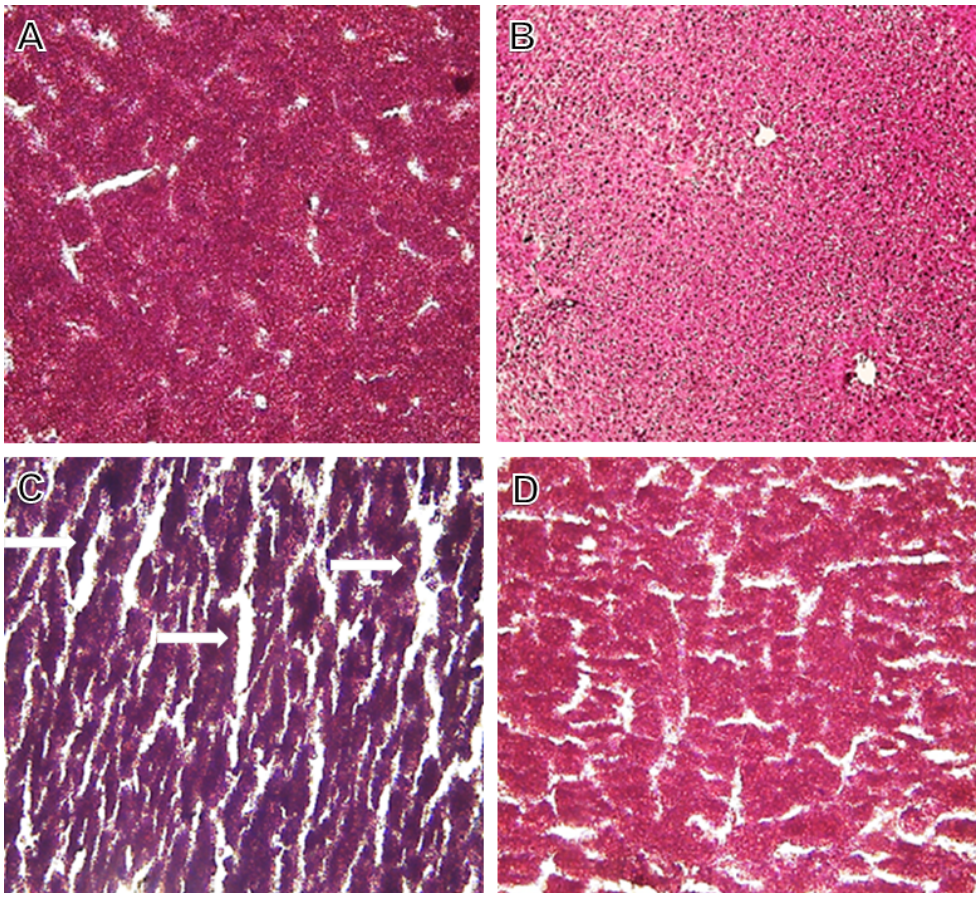

Figure 4. Effect of diabetes and glycine on maternal liver histology..

A: histologic section of liver of control rat; B: histologic section of liver of diabetic rat; C: histologic section of liver of control rat treated with glycine; D: histologic section of liver of diabetic rat treated with glycine. The arrows shown some empty spaces.

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In addition to investigating the effect of glycine on the histological structure of the main organs of pregnant rats and their fetuses, the role of glycine over oxidative stress characteristic of diabetes, was investigated, as has been reported (Chen *et al*., 2018). Scavenging enzymes, measured in crude extracts of maternal and fetal liver, show different responses due to glycine administration or diabetic induction. Catalase is not changed for glycine administration, both in maternal and fetal liver; but surprisingly, the activity is undetectable in maternal or fetal liver from diabetic induced animals. However, higher values, but statistically non different from control group, are shown in glycine-treated diabetic group (Figure 9A).

GPx activity was more variable: Glycine or STZ administration causes an increase in the values in maternal liver, with regard the control, but in diabetic rats the increase is more conspicuous. In fetal liver, control activity is very low, and diabetic induction or treatment with glycine produces a significant increase, in contrast, administration of the amino acid to diabetic rats causes a decrease in activity with regard the diabetic group, without reaching the normal values (Figure 9B).

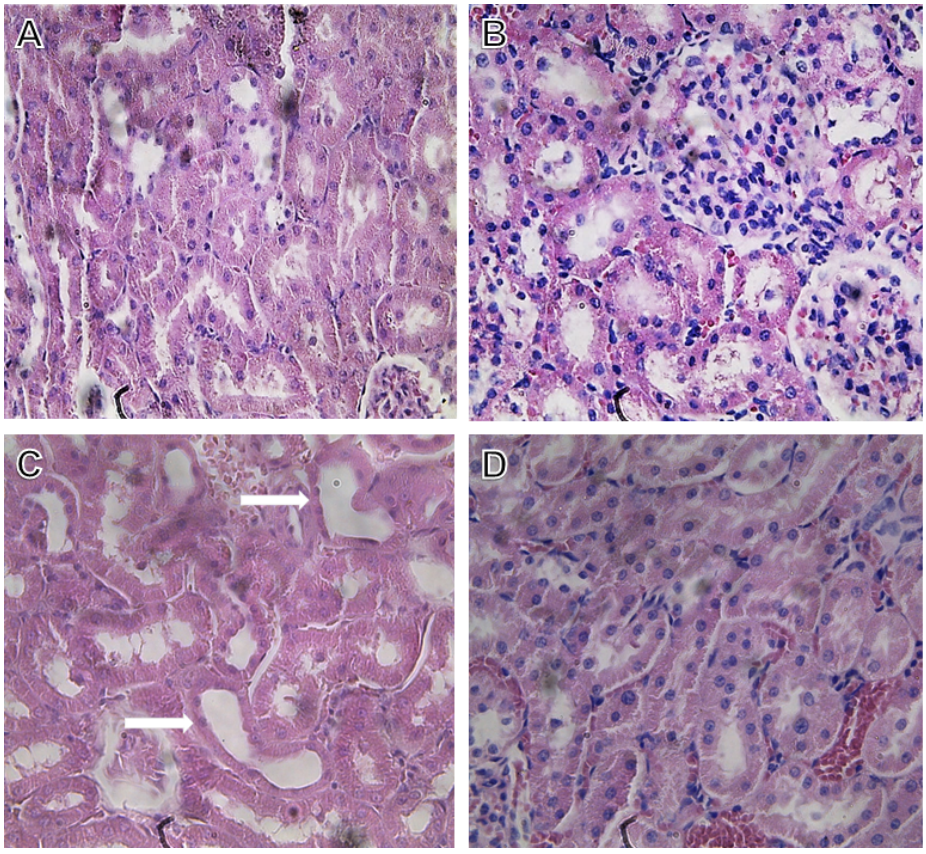

Figure 5. Effect of diabetes and glycine on maternal kidney histology.

A: histologic section of kidney of control rat; B: histologic section of kidney of diabetic rat; C: histologic section of kidney of control rat treated with glycine; D: histologic section of kidney of diabetic rat treated with glycine. Arrows shown empty spaces due to nephron loss.

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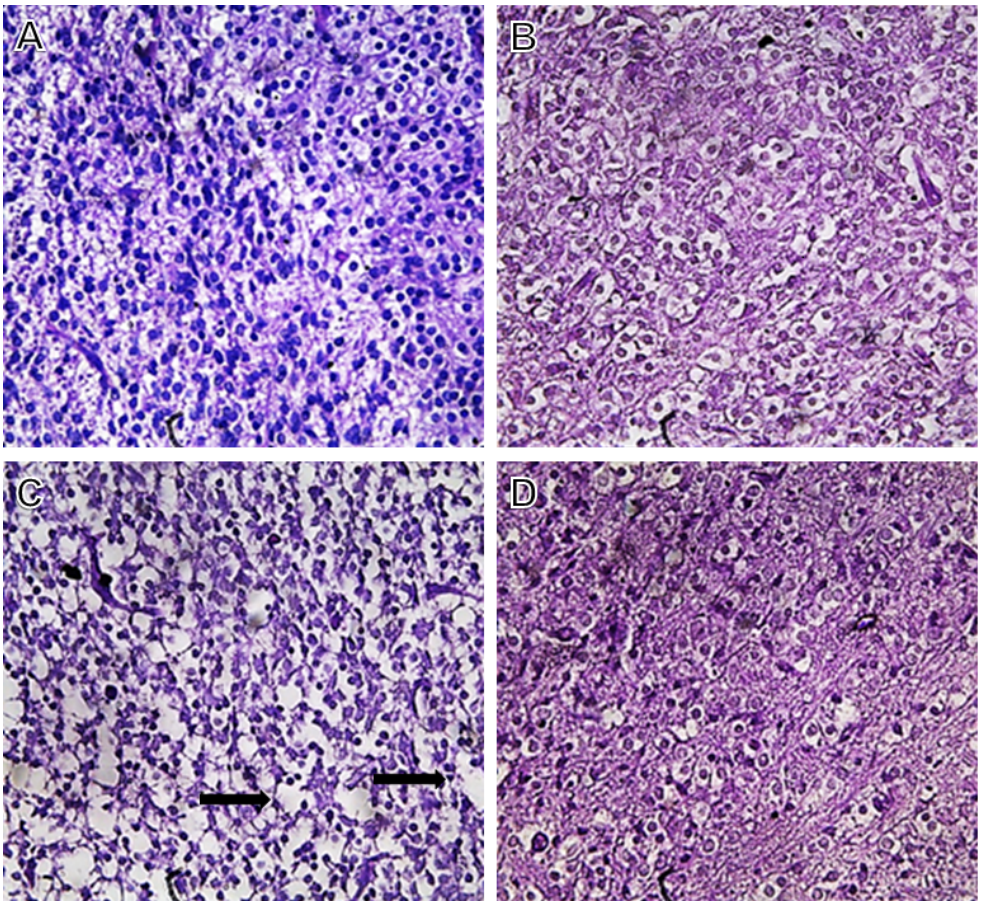

Figure 6. Heart fetal structure by treatment with glycine to pregnant diabetic rats.

A: control fetal heart; B: fetal heart of a fetus of a diabetic rat; C: fetal heart of a control rat treated with glycine; D: fetal heart of a diabetic rat treated with glycine.

The activity of GST was very low, almost to undetectable values, and differences only were seen between the diabetic-induced group and control group, both in maternal and fetal liver (Figure 9C).

In the same way, SOD activity was very low both in maternal and fetal liver of the control group, but diabetic induction does not affect the activity. However, glycine treatment increases the activity significantly; a result similar was found in the dams treated with STZ plus glycine and their fetuses (Figure 9D).

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Finally, lipoperoxidation was increased in liver of diabetic females and their fetuses; similarly, healthy rats treated with glycine and their fetuses shown an increase of lipoperoxidation; administration of the amino acid to diabetic rats only restored the values in maternal liver but not in fetal liver (Figure 10). Glycine reduces oxidative stress and hepatotoxicity caused by lead in rats (Alcaráz-Contreras *et al*., 2011), this explains that glycine treatment reduces lipoperoxidation produced by diabetic induction. Taken together, results shown that DM produces a disequilibrium in scavenging enzymes activity, as well as in maternal and fetal liver (El-Bassiouni *et al*., 2005), and an increase in lipoperoxidation (Cederberg *et al*., 2000, 2001; El-Bassiouni *et al*., 2005; Kamimoto *et al*., 2019): Oxidative stress could be determined indirectly in maternal plasma (Gunet *et al*., 2001) as well as in fetal tissues as heart (Ejdesjó *et al*., 2011; Kumar *et al*., 2012).

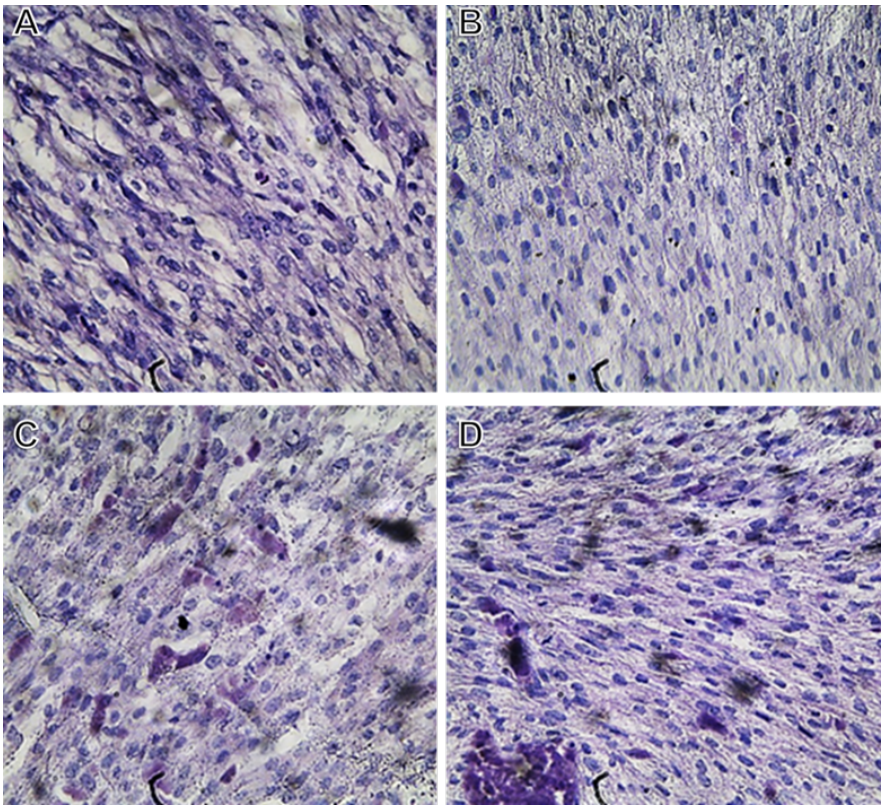

Figure 7. Fetal brain structure by treatment with glycine to diabetic pregnant rats.

A: control fetal brain; B: fetal brain of a fetus of a diabetic rat; C: fetal brain of a control rat treated with glycine; D: fetal brain of a diabetic rat treated with glycine.

On the other hand, in malformed embryos of diabetic rats, the activities of scavenging enzymes also are altered (Ornoy *et al*., 1999; Cederberg *et al*., 2000; Zaken *et al*., 2001), whereas the incubation of postimplantational rodent embryos in hyperglycemic media produces

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increase of lipoperoxidation and shift of scavenging enzyme activity (Zaken *et al*., 2001; Ryu *et al*., 2007). With the use of molecular biology focusing, it has been shown that the gene expression for scavenging enzymes Cat, SOD (and its isozymes) and GPx are altered, an effect linked to diabetic embryopathy (Cederberg *et al*., 2000; Zabihi *et al*., 2007; Wentzel *et al*., 2008; Ejdesjó *et al*., 2011). Supplementation in diet or culture media with vitamins C and E (Cederberg *et al*., 2000,2001), resveratrol (Singh *et al*., 2011), folic acid (Zabihi *et al*., 2007), quercetin (Cao *et al*., 2016) or curcumin (Wu *et al*., 2015) reverts the oxidative stress and malformations linked to diabetic embryopathy. Our group has demonstrated that alpha-linolenic acid and methanolic extract of Buddleja chordata (with great content of verbascoside) reduce lipoperoxidation and dysmorphogenesis (Chirino-Galindo *et al*., 2017, 2021). In this work, activities of scavenging enzymes were determined directly in crude extracts of fetal and maternal liver, since Paniagua-Castro *et al*. (2006) reported that glycine treatment, at the same dose as this work, prevents lipoperoxidation induced by cadmium in pregnant female mice; in addition, glycine, at 100 mg/ body weight, reduces hepatic lipoperoxidation in male rats treated with lead (El-Hafidi *et al*., 2018).

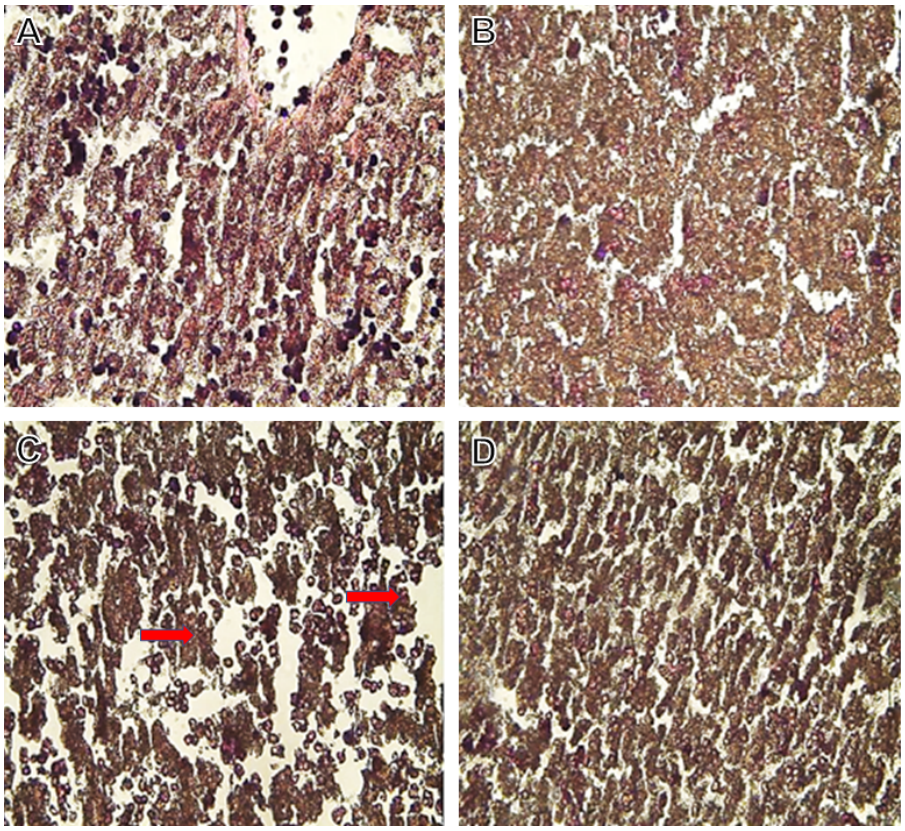

Figure 8. Fetal liver structure by treatment with glycine to pregnant diabetic rats.

A: control fetal liver; B: fetal liver of a fetus of a diabetic rat; C: fetal liver of a control rat treated with glycine; D: fetal liver of a diabetic rat treated with glycine.

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Surprisingly, glycine per se induces an increase in maternal or fetal hepatic lipoperoxidation; maybe this amino acid, like many other antioxidants, could have a prooxidant effect when is administered alone, or could have a biphasic effect, like other molecules (Chirino-Galindo *et al*., 2017).

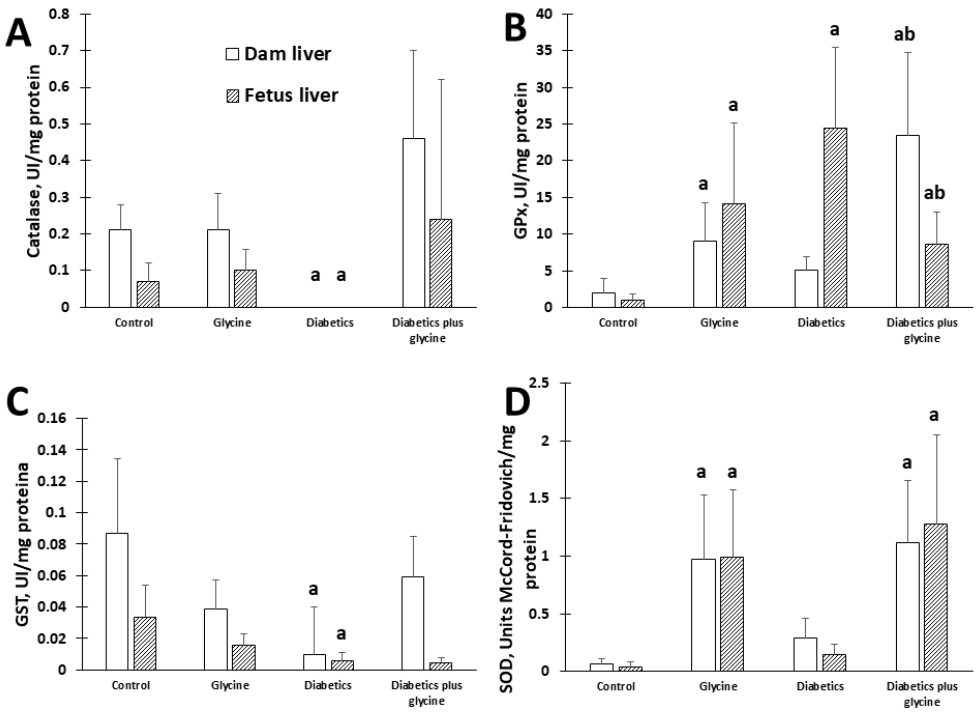

Figure 9. Activity of scavenger enzymes in liver of diabetic rats treated with glycine and their fetuses.

Mean ± SEM of 5–7 independent determinations for maternal liver, or 30–35 determinations in control group. [a]$p < 0.01$ with regard to control group; [b]$p < 0.01$ with regard diabetic group (two-way ANOVA).

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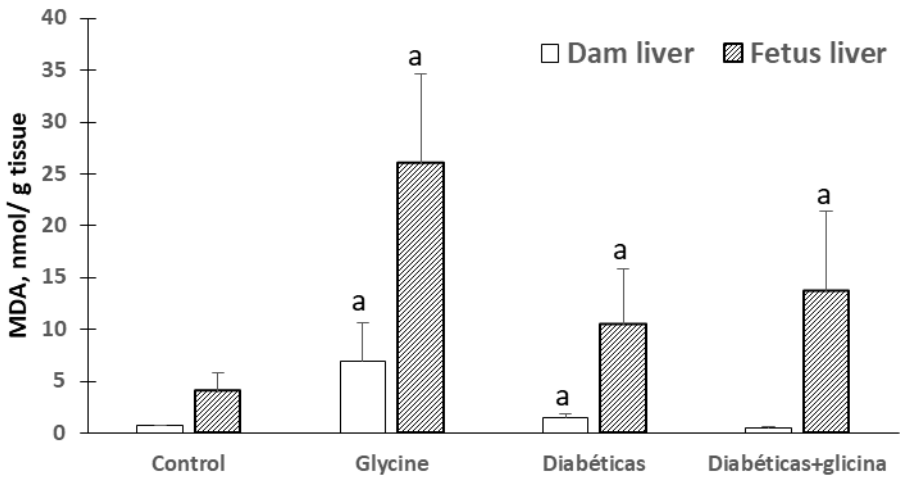

Figure 10. Lipoperoxidation in the liver of diabetic rats treated with glycine.

Mean ± SEM of 30–35 independent determinations. [a]$p < 0.01$ with regard its control (Two-way ANOVA).

Conclusions

Glycine treatment in diabetic pregnant rats prevents developmental alterations caused by the disease, probably to restore normal oxidative state. Nevertheless, when administered alone, it induces slight oxidative stress and a low percentage of malformations and resorptions. Further studies could investigate whether this effect could be mitigated at lower doses and probe the role of glycine overexpression of genes of scavenging enzymes, or molecules involved in oxidative stress, apoptosis, cell cycle, or embryogenesis regulation. This study was limited by the lack of determination of gene expression of scavenging enzymes, apoptosis, or cell cycle genes, that were not determined through immunohistochemistry or PCR; and that inflammation could be placed in dysmorphogenesis. Further studies could focus on investigating these aspects.

Authors contribution

Work conceptualization, ARF, MPM; methodology development, ALRR, GCG; software management, ALRR, GCG, ARF; experimental validation, ARF, MPM; results analysis, ALRR, MPM data management, ALRR, GCG, MPM; manuscript writing and preparation, ARF, MPM; redaction, review and edition, MPM.; project administration, MPM; fund acquisition, MPM.

All the authors of this manuscript read and accepted the published version.

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Ethics

Experimental procedures were realized in accord with NOM-062-ZOO-1999; this study was accepted by the Bioterium Committee of FES Iztacala.

Conflict of interest

The authors declare no conflict of interest.

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