Original Article

Effect of Corn Silk Aqueous Extract on Brown Adipose Tissue of Embryos and Neonates of Diabetic Pregnant Mice: A Histological Study

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Abstract

Context: Many congenital malformations are seen increasingly, due to diabetic mothers causing a burden on health systems. Corn silk (CS) extract has been used as a natural hypoglycemic treatment. However, its teratogenic safety was not studied. **Aims:** Therefore, in this study, we examine the effect of CS aqueous extract on fetuses, offspring of normal and diabetic female mice treated with CS aqueous extract. **Settings and Design:** Pregnant female mice were divided into two groups diabetic and nondiabetic. Then, each of these groups was divided into control and treated. **Subjects and Methods:** A daily dose of 4 g/kg of CS aqueous extract was given orally to the treated groups, control groups were given distilled water. The collection of samples was at day 16.5 of pregnancy, and neonates. Brown adipose tissue (BAT) in the sections of the preserved sample was examined. **Statistical Analysis Used:** BAT areas were measured from 10 samples of each treatment age group in 2 sections. Data were analyzed with one-way ANOVA, then, two-independent sample test (Mann–Whitney) was done to test the significance of differences between groups. **Results:** The BAT areas were negatively affected by diabetes and the extract. Both the extract and diabetes caused an increase in fat accumulation in the adipocytes with varying degrees. **Conclusions:** This study showed for the first time to our knowledge that the use of CS aqueous extract during pregnancy affected BAT organization and area, and that the used dose did not decrease the malformations caused by diabetes. More studies with different doses should be investigated.

Keywords: Brown adipose tissue, corn silk, diabetes, fetus, mouse, neonate

Introduction

Diabetes mellitus (DM) is a number of metabolic disorders, caused by no or insufficient amounts of insulin secretion, body cells' inability to respond to secreted insulin (insulin resistance), or both. In the absence of normal insulin function, cells are unable to obtain glucose, as a source of energy, from the blood. Consequently, blood glucose levels rise abnormally causing hyperglycemia. Untreated hyperglycemia results in long-term damage in several tissues, organs, and systems of the body. For example, nerves, eyes, blood vessels, kidneys, and heart.[1-3] During pregnancy, 2%–5% of women suffer from glucose intolerance and hyperglycemia.[4] This is defined as gestational DM (GDM) if it is diagnosed, for the first time, during the pregnancy. Normally, insulin resistance occurs in pregnancies to facilitate glucose transfer to the fetus. This is to maintain control of glucose, maternal β cell mass, and therefore insulin production increases. Inability to increase insulin secretion, to manage the resistance, causes GDM.[5] Gestational diabetes is associated with risk outcomes for the mother, fetus, child, and adult offspring. For the mother, there might be an increased risk for premature and C section delivery due to a large sized infant, higher possibility of developing GDM in later pregnancies, and type 2 DM later in life. The fetus could be large for gestational age, develop metabolic problems, higher long-term risk for diabetes and obesity, and female offspring susceptibility of GDM in the

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future due to fetal genome epigenetic modifications.[5,6] The causes of gestational diabetes are interactions of genetic and environmental factors, such as maternal obesity, advanced maternal age, previous delivery of macrosomic infant, prior GDM, and high maternal blood pressure during pregnancy. Treatment includes diet, behavioral change, oral antidiabetic agents, and insulin.[4]

Among the countries in the world, IDF named Saudi Arabia the tenth in diabetes prevalence and the highest among the Middle East countries.[7,8] According to the Ministry of Health, 0.9 million diabetic patients were reported in 1992, compared to 2.5 million in 2010. Concern was raised due to this rapid increase in around 2 decades.[9]

Stigma maydis or corn silk (CS) is the thread-like yellow-brown or light green style/stigmas that make the *Zea mays* L.(corn) female flower. It is one of the medicinal herbs traditionally used by the Chinese and the Natives of the Americas for a variety of diseases. CS is used to treat disorders associated with the urinary system including edema, kidney stones, cystitis, prostate disorders, bedwetting, and urinary infections. It relaxes the bladder's lining, increases the secretion of urine (due to its diuretic and kaliuretic properties,[10] and reduces irritation. It is also used for gout, asthma, obesity, and hypertension. CS possesses antidepressant and antifatigue activity,[11] antifungal activity, and contains large amounts of antioxidants. The medicinal properties are attributed to its chemical compounds. It contains vitamins, proteins, carbohydrates, salts (Mg, K, Ca, and Na), volatile and fixed oils, alkaloids, steroids, tannins, saponins, and flavonoids.[12-14]

Adipose tissues in mammals have two different types that defer in function. One is white adipose tissue (WAT), that stores lipids as a source of energy. The other is brown adipose tissue (BAT) that is the site of thermogenesis (heat production), especially in cold conditions. Heat production in BAT is achieved by the oxidation of lipids.[15,16] The functional balance between those adipose tissues maintains the body's energy balance. Histologically, the characteristic cell of WAT is the white adipocyte (WA) containing one large fat vacuole occupying most of the cell size. While the characteristic cell of BAT is the brown adipocyte (BA), which contain multiple small fat vacuoles (or droplets) and is densely packed with mitochondria. The mitochondria in BA contain a unique inner membrane protein called the uncoupling protein-1 that uncouples ATP production from the respiratory chain resulting in heat dissipation if activated.[16-18] BAs have several functions, which include lipolysis, taking up free fatty acids and glucose from the blood stream, and utilizing them as fuel.[17] This involvement in metabolism had drown attention to its role in many metabolic disorders, such as diabetes and obesity, and potential in treatment or mitigation of such disorders.[19] In humans, BAT can be found in the neck and above the clavicle bone. In mice, BAT is present in substantial amounts in the dorsal side of the interscapular and scapular regions, in addition to small depots in the body.[17] The BAT arises from the embryonic mesoderm like skeletal muscles and some WA.[15,19] The tissue can be recognized after E15.5, then it expands until birth. The lipid content in BA during embryonic development is low in the early stages, then increases postnatally.[19]

In China, CS has been used as an antidiabetic treatment.),[12] studied the effect of CS extract on glycemic metabolism. They used 0.5, 1, 2, 4 g/kg body wt. doses of CS aqueous extract on alloxan-induced diabetic mice for 20 days. In mice groups which received the 2 and 4 g/kg doses, blood glucose levels decreased. They concluded that CS affected the glycemic metabolism through increasing insulin secretion and recovering damaged b cells.

Studies showed that Corn silk extract (CSE) has a blood glucose-lowering effect. But, to our knowledge, the safety of CSE on fetuses had not been studied previously. There is a growing number of mothers with PGDM and GDM which raises the rate of diabetes-associated malformations. There is a possibility that maternal treatment with CS extract could reduce the pregestational diabetes teratogenicity. Therefore, the importance of a research on exploring the safety of CS aqueous extract on the fetuses and offspring of diabetic and nondiabetic pregnant mice emerged.

Subjects and Methods

All practical work was approved by the biomedical ethics research committee in King Abdulaziz University (KAU) (reference Number 345 19).

Swiss white rodless adult male and female mice 25–30 g were obtained from KFMRC. The animals were acclimated for a week 22 ± 2°C, normal humidity and 12 h. light/dark cycle having free access to water and food (animal feed). *Stigma maydis* or CS, from *Zea mays* L. (corn) female flower, was harvested from a local farm in Albudaiya (Duba, Tabuk Province, Saudi Arabia). Streptozotocin (STZ) (Sigma S0130-1G) purchased from Bayouni Trading Co. LTD, was used to induce DM in experimental animals.[20] Normal saline, formalin, and diethyl ether were purchased from Al-Rowad Modern supply of medical equipment in Jeddah.

CS was dried and lyophilized then the aqueous extract was prepared according to.[10,12]

Experimental design and dose administration

Experimental animals were divided into the following groups: control group(c) received (0.5 ml) distilled water, treated group (T) received 4 g/kg of CS aqueous extract, diabetic control group (DC) had induced diabetes before mating and received (0.5 ml) distilled water, and diabetic treated group (DT) had induced diabetes before mating and received 4 g/kg of CS aqueous extract. All doses were administered daily from Sunday to Thursday orally using a gavage (24G × 1² animal feeding needle, purchased from Pet Surgical. From E0.5 till delivery. Blood glucose levels and weights were recorded at the beginning of each week.

Diabetes induction

To induce diabetes, 75 mg/kg of STZ in normal saline was injected intraperitoneally in female mice. The injection was done on three consecutive days.[21-23] Then, after 10–14 days, the blood glucose levels of the injected mice were checked, using Accu-chek Performa (Roche Diabetes Care) mice were diabetic, if blood glucose levels were >200 mg/dL.[24-28]

Mating

Mating was done for females in the estrus and proestrus stages according to.[29,30] Where 2 females were put with 1 male and left for 24 h. E0.5 was assigned when clear vaginal plug was seen.

Sample collection

At E16.5, two mothers were anesthetized with diethyl ether. Then, dissected and fetuses were collected. Each fetus was preserved in 10% formalin. Neonates were euthanized (using diethyl ether) and preserved in 10% formalin.

Histological preparations

For histological studies, samples were fixed in 10% formalin solution as soon as they were extracted. Before dehydration, E16.5 fetuses and neonate samples were cut vertically into left and right halves. Blocks were cut into 3 μm sections then stained by hematoxylin and eosin according to.[31]

Sections photography

Stained sections of E16.5 samples were photographed using a slide scanner device [Philips IntelliSite Ultra-Fast Scanner (FMT0225)] at Al Borg Medical Laboratories, using the software (Philips IntelliSite Pathology Solution v3.2, Image Management System 3.3.1). While for the neonate sections, the dissecting microscope (Olympus SZX10) with 6.8x magnification was used in photographing the stained whole neonate sample sections. For higher magnification of the BAT sections, a compound light microscope (Olympus BX51) and (Olympus DP72) camera, at KFMRC was used. For BAT areas, photographs were taken by a dissecting microscope (Nikon SMZ1500) and a (Nikon DS-Fi1) camera, with 7.5x magnification at KFMRC.

To describe the structure of BAT histology, the largest posterior triangular lobe in control fetuses and neonates was examined and compared to the controls in previous literature.[16,32]

Histometric studies

For BAT areas, photographs of E16.5 and neonate sections were processed using NIS Elements Imaging software (v4.13.5) [Figure 1]. The areas were measured from 10 samples of each treatment age group. In most samples, the BAT area was measured in 2 sections.

Statistical analysis

The measurements were statistically analyzed using the SPSS program (IBM SPSS Statistics, v. 1.0.0.1275). Data were analyzed with one-way ANOVA, then, two-independent sample test (Mann–Whitney) was done to test the significance of differences between groups.

Results

General structure of E16.5 fetuses

Brown adipose tissue histological structure in E16.5 fetuses

In normal E16.5 mouse fetus of this study, BAT was seen in the dorsal side of the thorax region. The tissue is polygonal in shape, in some sections a strap of muscles could be seen running through the tissue. The tissue was lobulated with a connective tissue separating the round-edged lobules. The largest posterior triangular lobe was examined [Figure 2a]. The tissue was highly vascularized and appeared loose [Figure 2b]. BA appeared purple with a dark nucleus when stained with hematoxylin and eosin (H and E) stains.

Cells were small, irregular in shape, and contained a relatively large nucleus and numerous small fat vacuoles (droplets). Vacuole sizes could be slightly larger and clearer in different cells [Figure 2c]. Moving closer toward the skin, fat vacuoles appeared larger.

Looking at the BAT area of E16.5 fetuses in general, in the treated group the septa (connective tissue between lobules) appeared to be thicker around large blood vessels compared to the controls.In the Diabetic control group, the septa were the thickest among all treated groups and the lobules were smaller. The thickness of DT septa seemed more than in T due to diabetes but less than DC. It seems that diabetes caused a thickening in the connective tissue between lobules. While treatment with corn silk extract caused thickening in the connective tissue surrounding the large blood vessels, as seen in [Figure 3]. Within the tissue itself, in all treatment groups, BA seemed similar to the controls. A slight variation in fat droplet sizes was noticed. In C droplets were small and mostly unclear, while in T, they were larger and clear. In DC samples, fat droplets were large and slightly more than in T. It was noticed that the tissue seemed to have dilated blood vessels, together with the increased fat content gave a looser appearance. The DT group samples had large droplets similar to T, but not as clear [Figure 4].

Brown adipose tissue histological structure in neonates

In the sagittal sections of normal neonate mice, one of the areas that has the most BAT is interscapular BAT. It is present as a triangle in the dorsal side of the cervical and thoracic vertebrae. This triangle of BAT could include muscle straps depending on the sectioning location. The tissue was lobulated, and a connective tissue (septa) separating the lobes could be seen. In this study, the largest posterior triangular lobe was examined [Figure 5]. The BAT included its characteristic cells, BAs, that were irregular in shape, containing multiple fat vacuoles. With H and E stain, BA took a purple stain with a dark nucleus that could be clearly seen [Figure 5].

Comparing T group to C, BAT appearance in general was similar but septa seemed slightly wider in T [Figure 6]. BAs in T seemed to have larger fat vacuoles than C moving toward the skin. A slight increase in fat was seen in T compared to

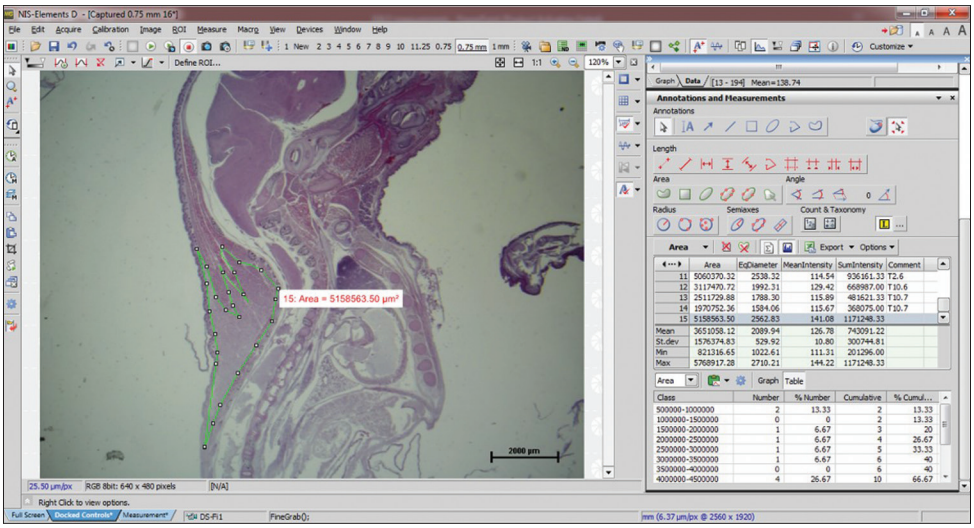

Figure 1: Histometric method used to calculate the brown adipose tissue areas in samples. Green line = the outline of the brown adipose tissue area

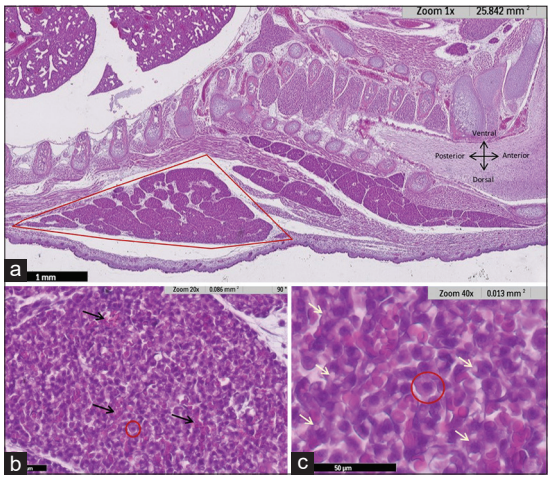

Figure 2: Photomicrographs of BAT in a control E16.5 mouse fetus. (a) The examined BAT lobe in the study indicated with red triangle (×10). (b) Brown adipose tissue with adipocytes (red circle) and blood sinuses (black arrows) (×200). (c) Adipocyte with small fat vacuoles, notice the varying vacuole sizes in deferent cells (white arrows) (H and E, ×400)

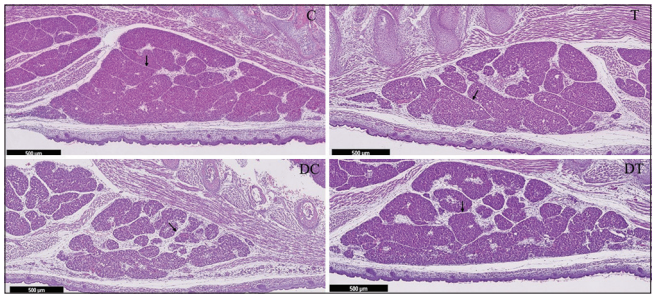

Figure 3: Photomicrographs of E16.5 fetuses' brown adipose tissue, C: Control sample with normal lobules and septa, T: Treated sample with thicker septa especially around blood vessels, DC: Diabetic control sample with thickest septa and smaller lobules, DT: Diabetic treated sample with thick septa. Black arrows = septa (H and E, ×20)

C [Figure 7]. In the DC group, more fat vacuoles within BAs were seen therefore BAT appeared lighter than C. Also, increased amounts of WA within the tissue were noticed compared to C [Figures 6 and 7]. In some samples of DC, large amounts of cells in the tissue appeared with no nuclei, which could indicate inflammation or necrosis [Figure 8]. The DT group seemed to have more lipids and WA within the tissue compared to all groups as seen in [Figure 6].

Histometric studies

Brown adipose tissue area of E16.5 fetuses

Comparing BAT areas of nondiabetic groups of 16.5-day-old fetuses, CSE caused a significant decrease in T ($P = 0.028$) in relation to C. On the contrary, DT increased insignificantly compared to DC. Diabetes, on the other hand, insignificantly decreased BAT area in DC compared to C, but caused a significant increase in DT ($P = 0.026$) compared to T. A near-normal BAT area was noticed when comparing DT to C [Figure 9a].

Brown adipose tissue area of neonates

Both treatments with CSE and diabetes caused a decrease in BAT areas of neonate mice. Comparing T to C and DT to DC, CSE caused insignificant decreases. Also, comparing DC to C and DT to T, diabetes caused insignificant decreases too. However, when the effect of CSE and diabetes were combined, the decrease was significant in DT ($P = 0.016$) compared to C [Figure 9b].

Discussion

A growing number of individuals are diabetic. Diabetes is characterized by high blood glucose or hyperglycemia. This affects many organs leading to several complications. Diabetic pregnant women are prone to those complications in addition to the role of hyperglycemia in increasing the rates of congenital malformations in their offspring.

BAT is a major source of energy in mammal neonates as it helps in adjusting to temperature change after leaving the uterus environment.[33-35]

Regarding the BAT histology in 16.5-day-old fetuses, DM caused an increase in the connective tissue in the areas between

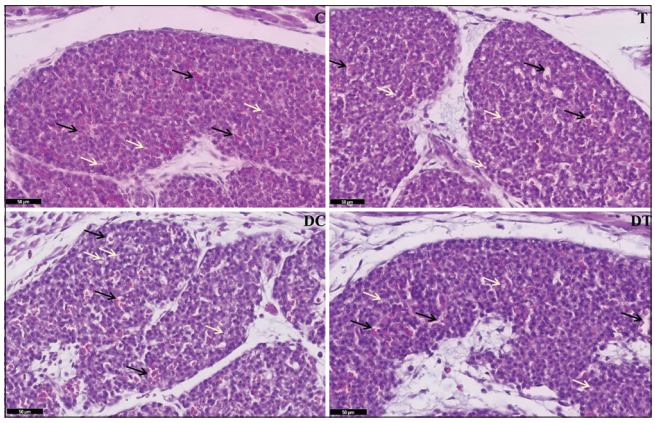

Figure 4: Photomicrographs of E16.5 fetuses' brown adipose tissue, C: Control sample with normal BA, T: Treated sample with clear larger fat droplets within BA, DC: Diabetic control sample with large fat droplets and dilated blood vessels giving a loose tissue appearance, DT: Diabetic treated sample with large unclear droplets. Black arrows = blood vessels, white arrows = fat droplets (H and E, ×200)

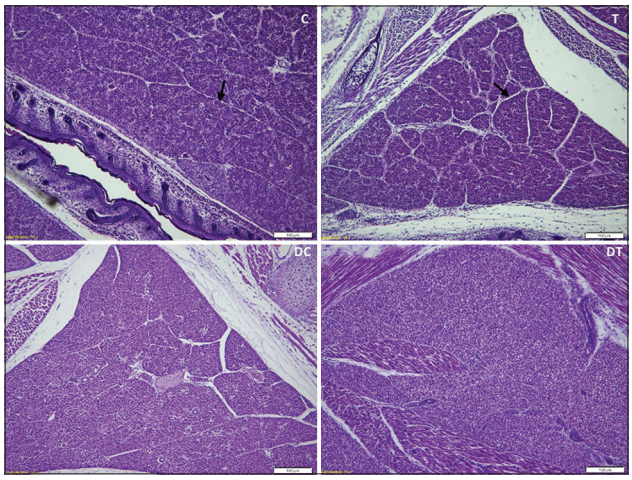

Figure 6: Photomicrographs of brown adipose tissue in neonate mouse: C: Control group, T: Treated group with wider septa (black arrow) compared to C, DC: Diabetic Control group with lighter color due to increased fat content compared to C, DT: Diabetic Treated group which also appear lighter because of the increased fat accumulation (H and E, ×100)

lobules. Blood vessels seemed dilated too which is consistent with previous literature.[36] The dilation in the vessels might have led to leakage of materials into the surrounding tissues causing the increase in the interlobular area,[36] and making the lobules smaller. Treatment with CSE had a similar but milder effect especially around large blood vessels as seen in T. It was noticed that DT septa were wider than C, but narrower than DC. Both diabetes and CSE seemed to cause a slight increase in fat accumulation within the BAs. This could be as an effect of the dilation of blood vessels within the tissue, therefore, increased transport of fatty acids and glucose as suggested by[36,37] provided another explanation, that there might be a reduction in the consumption of lipids by the mitochondria. In their study, they found that maternal hyperglycemia caused a change in BA mitochondrial ultrastructure. The mitochondrial cristae

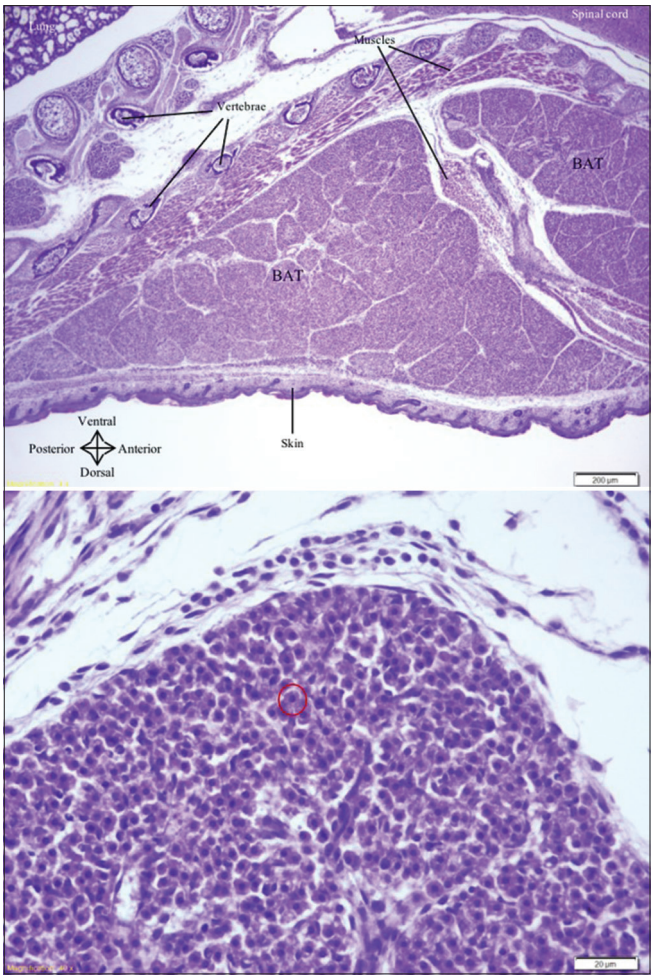

Figure 5: Photomicrographs of brown adipose tissue in a control neonate mouse. Top, brown adipose tissue triangle examined in the study (×40 magnification). Bottom, brown adipose tissue magnified × 400, showing brown adipocytes (red circle) with noticeable fat droplets (H and E, ×40)

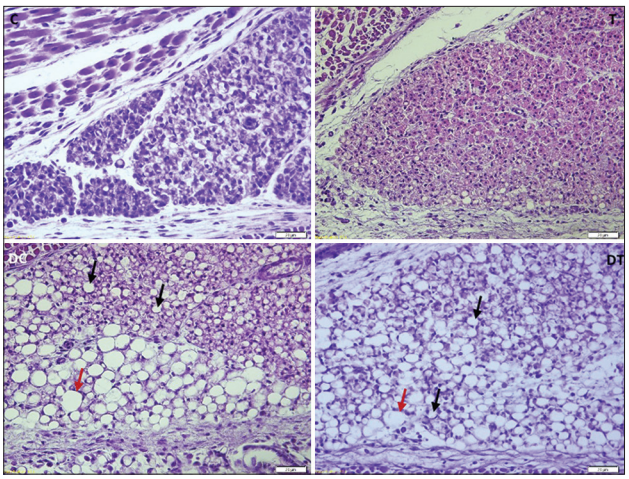

Figure 7: Photomicrographs of neonate brown adipose tissue showing BA cells in the posterior tip of the dorsal brown adipose tissue triangle shown in [Figure 6]: C: Control group, T: Treated group with more fat in BA near the skin, DC: Diabetic Control group with many BA with single fat vacuole (black arrow) and WA (red arrow), DT: Diabetic Treated group comparable to DC with increased fat content (H and E, ×400)

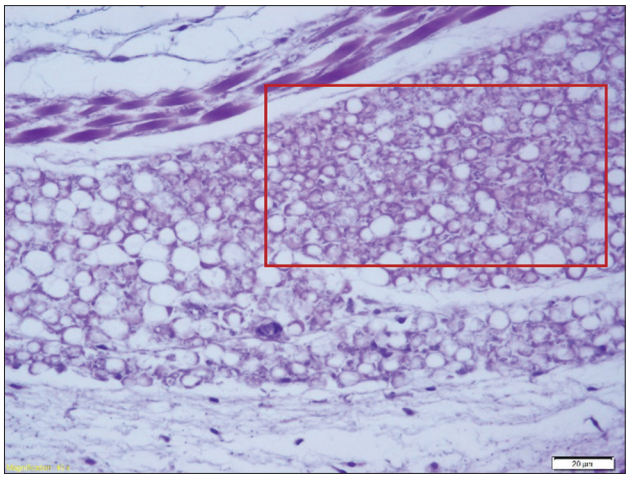

Figure 8: Photomicrograph of the neonate in the DC group showing a large number of cells within brown adipose tissue with no clear nuclei (red triangle) (H and E, ×400)

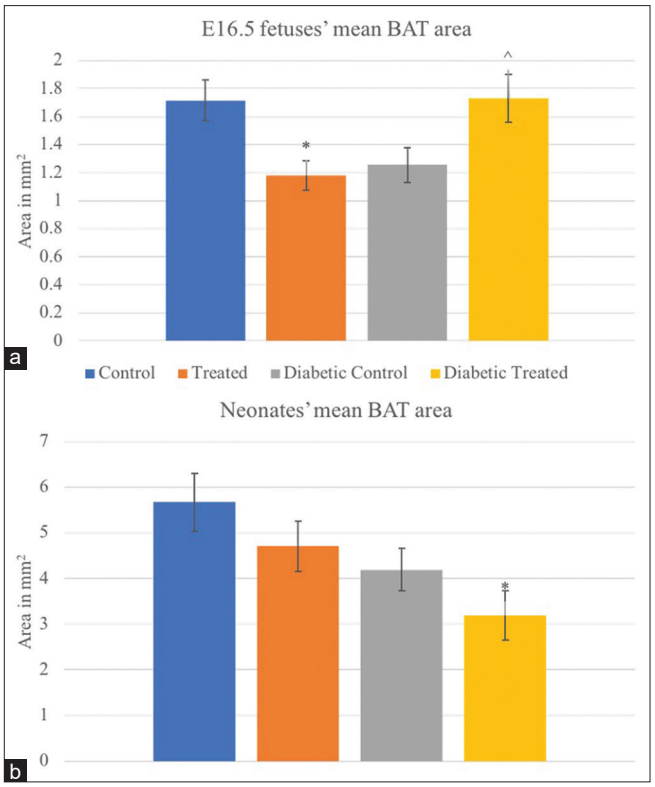

Figure 9: Graph showing the effect of CSE on the brown adipose tissue area of: (a) 16.5-day old mouse fetuses, and (b) neonates. Values are mean ± standard error. *: Significant difference ($P < 0.05$) compared to control group, ^: significant difference compared to the treated group

were fewer and irregular which may have led to a reduction in mitochondrial activity. Therefore, lipid consumption was reduced leading to its accumulation in the cell. They also noticed an alteration in DNA methylation which altered the structure and function of BA.[37] The BAT area data in fetuses of the DC group supported the previous result. Smaller lobules in DC compared to C were manifested in the tissue area reduction. This reduction in size was in agreement with previous literature.[37] In the CSE treatment case, the tissue area was also significantly reduced in T compared to C. But in the DT group, the area increased significantly compared to T, to a similar level of C. The tissue area and size depend on the number of adipocytes and their fat content.[36] It was noticed that lobules in DT BAT were larger than in DC, and the whole area was slightly larger than C. In the neonate age group, CSE resulted in wider septa as seen in T compared to C. But in DC and DT groups, fewer but wider septa were seen. The accumulation of fat within the BAs was slightly higher in T compared to C, but much higher in DC and DT. A turnover of BA to WA was noticed in DC and DT due to increased intracellular fat content. In one sample in the DC group, a large number of adipocytes with no clear nuclei were seen, that could indicate necrosis. Diabetes increases the oxidative stress,[38] and that could be the cause of necrosis. BAT area in the postnatal groups was reduced by both DM and CSE, as expected. The decrease in the amount of BAT area and its transformation to WAT would reduce the amount of energy production available for the fetus or neonate, reducing their ability to acclimate with extrauterine temperature and therefore reducing their survival rate.

This study showed that treating diabetic and normal mice mothers with a daily dose of 4 g/kg of CS aqueous extract affected the BAT area and its histologic structure in E16.5 fetuses and neonates, while it did not reverse the effect of diabetes effect on Bat.

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Conflicts of interest

There are no conflicts of interest.

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