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Anti-diabetic effect of *Cinnamomum zeylanicum* Extract in the cerebrum histomorphometry in old fetus diabetic rats

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ABSTRACT

In pregnant mothers maternal diabetes happens when pancreas cannot produce enough insulin, so blood glucose increases in mother and then in fetus, which results in several hurts in neonates.This study investigated the effect of Cinnamomum zeylanicum extract on Cerebrum Histomorphometry in 18 and 20 days old fetus of diabetic mother. In this experimental study, a total of 16 Sprague Dawley animals were divided randomly into four groups; Normoglycemic control group, Normoglycemic treated group, Diabetic control group, Diabetic treated group with Cinnamomum zeylanicum extract and diabetic treated with Cinnamomum zeylanicum extract. Diabetic groups became diabetic by intraperitoneal injection of streptozotocin (50 mg/kg). Rats in all Groups became pregnant by natural mating. After formation of the nervous system, fetuses were obtained after anesthezing animals on 18th and 20th gestational days; this was euthanized, cerebrum simples were taken and fixed. After produce histological slides, various histological parameters were determined. Data were analyzed using one way ANOVA and Duncan test. Thickness gray matter, the number of cells in gray matter and the ratio of gray matter to white matter of diabetic group In 18 and 20 days old fetus and also the number of cells in white matter in 18 day old fetus was significantly more than that of other groups tested ($p< 0.05$). The results of this study suggest that Cinnamomum zeylanicum extract lowers blood glucose levels in diabetic mothers and their fetuses to prevent damage to the cerebrum.

Key words: *Cinnamomum zeylanicum* extract, Diabetes, cerebrum, Rat.

INTRODUCTION

The incidence of diabetes in the human population has reached epidemic proportions worldwide and it is increasing at the rapid rate.150 million people in 2000, which is predicted to rise to 220 million in 2010[1].

In animals, it can be produced by pancreatectomy; by administration of alloxan, streptozocin, or other toxins that in appropriate doses cause selective destruction of the beta cells of the pancreatic islets; by administration of drugs that inhibit insulin secretion; and by administration of anti-insulin anti-bodies. Strains of mice, rats, hamsters, guinea pigs, miniature swine, and monkeys that have a high incidence of spontaneous diabetes mellitus have also been described [1].

In diabetic mothers during pregnancy, placental transport of glucose and other nutrients will be increased, due to an increased availability at the maternal site, resulting in their increase in fetal and neonatal Macrosmia [2].

Diabetes associated with increasing diseases such as neuropathy and cardiovascular, but it has the reproductive system problems such as abortions, genetic abnormalities, Lack of fetal development and decrease cells sexual in spermatogenesis [3-5]. More Chemical drugs reduced blood sugar in diabetic patients such as glibenclamide [6]. These drugs have side effects and research is essential for a new drug with fewer side effects [7]. Most herbal drugs

cause decreased blood sugar, which demands for herbal products Shows as an anti-diabetic drug with fewer side effects [8].

Cinnamon can be used as a food antioxidant and to enhance food palatability. *Cinnamon* and its extract, irrespective of source, have been associated with a variety of health beneficial effects, including anti-microbial, anti-viral, antioxidant, and insulin-like activities. Many of the corresponding bioactivities are possibly attributed to cinnamaldehyde, a major constituent of the essential oil responsible for the flavor and aroma of whole *cinnamon* [9].

In addition, a number of polymeric polyphenol molecules known as proanthocyanidins are present in the aqueous extract that are likely responsible for the majority of the antioxidant properties of *cinnamon* [9]. While the health-beneficial effects of bio-flavanoids in general are traditionally thought to be due to their antioxidant activity, proanthocyanidins exhibit other properties that may be important for their bioactivities. For example, it has been suggested that related polyphenols can inhibit formation of amyloid fibrils independent of oxidative conditions [10, 11].

One particular extract of *cinnamon*, methyl hydroxy chalcone polymer [MHCP], shows promising data in the area of glucose control. A recent study compared the effect of MHCP in 3T3-L1 adipocytes to that of insulin. [12]. The results from that study support the theory that MHCP triggers the insulin cascade and subsequent transport of nutrients [13, 14]. The study also demonstrated that MHCP treatment stimulated glucose uptake and glycogen synthesis to a similar level as insulin. The study further demonstrated that treatment with endogenous insulin and MHCP resulted in a synergistic effect. Due to these conclusions, it is suggested that MHCP may prove to be a very valuable tool in the fight against diabetes, where insulin is present [13].

In addition to benefiting Type II diabetics, *cinnamon* may benefit individuals with impaired glucose tolerance [i.e., pre-diabetics]. Further, *cinnamon* has been shown to possess antioxidant activities related to lipid peroxidation. [13, 15].

Kim et al, 2006, suggests that compounds in *cinnamon* that increase insulin secretion first as a called insulin-enhancing factor and then described as the methyl hydroxy chalcone polymer [MHCP] [16].

The strong evidence suggests that polyphenols *Cinnamon* has insulin-like activity in animal cells and human [17]. By taking glucose-lowering effect with *cinnamon* and sugar with multiple complications and dangerous diseases in embryos of diabetic patients is ensured, evaluate ways to treat, mitigate and prevent it necessary. The aim of this study was to evaluate changes in the brain tissue of diabetic mothers and healthy fetuses, compared with diabetic controls treated with *cinnamon* and healthy.

MATERIALS AND METHODS

In order to make liquid extract, the *cinnamon* bark was divided into very small pieces. Then they were grounded with a blender. 30 grams of the powder was placated in a sterilized Erlenmeyer and 40 cc of saline was added. This mixture was kept at a cool temperature for 24 hours. Then after a whole day, it was mixed with a shaker for 5 min. At this stage , passing through the Whatmen Paper and calculating the amount of residual soluble extract , *cinnamon* concentration in the final solution was found and the desired dose was prepared .

Animals

Sixteen adult female Sprague-Dawley rats (200-250g weight and 3-4 months old) were acclimatized in an environmentally controlled room (temperature, 22±2^{o}C, and 12h light/12h dark). Food and water were given *ad libitum*. In this study, all experiments conducted on animals were in accordance with the guidance of the Ethical Committee for Research on Laboratory Animals of Shiraz University of medical sciences.

Induction of diabetes mellitus

Adult rats were rendered hyperglycemic by a single intraperitoneal (I.P). Injection of B.W. of stereptozocin (Sigma Chemical Co., USA) (50 mg/kg body weight) (18). Diabetes were identified by polydipsia, polyuria and by measuring non–fasting serum glucose concentration 48h after the injection of STZ, rats with a blood glucose level over 250 mg/dl were considered to be diabetic.

Experimental design

Animals were divided into four identical groups as follows:
(1) Normoglycemic control group (NC): normal rats which received distilled water

(2) Normoglycemic treated group (NCZE): normal rats which received the *Cinnamomum zeylanicum* extract (60 mg/kg B.W)
(3) Diabetic control group (DC): Diabetic rats treated with distilled water.
(4) Diabetic treated group (DCZE): diabetic rats receiving the *Cinnamomum zeylanicum* extract (60 mg/kg B.W)

Female animals of four groups in oestrus stage were caged with male rat for mating. Mating was confirmed by vaginal plug observation (19). Each group included 4 rats and animals were given the extract orally by an intragastric tube once daily for 21 days. The stock solution was prepared for multiple groups, such that 1 mL of extraction was administered per day for each animal.

On day 18 and 20 of pregnancy, two rats of both groups were killed. After obtaining the fetuses, they were immerged in appropriate fixative (bufferd formaline 10% for light microscope). Then the cerebrum was collected from fetus of all rats and the weight of neonates was measured.

Histomorphometric study
All tissue samples were fixed in 10% buffered formalin fixative for histolopathological investigations and subsequently embedded in paraffin. Sections (5 microns thickness) were stained with H&E and Green Masson's trichrome techniques. Sections were observed with Olympus BX51 microscope for evaluation of histomorphometrical parameters such as:

1) Thickness of gray matter (μm), 2) Thickness of white matter (μm), 3) Thickness of molecular layer, 4) The number of cells in the gray matter per unit (mm^2), 5) The number of cells in white matter per unit (mm^2), 6) The ratio of gray matter to white matter.

Thicknesses of gray matter, white matter and molecular layer were measured by ocular micrometer and Olympus BX51 light microscope using Olysia software. The number of cells per unit (mm^2) in both white and gray matters and the ratio of gray matter to white matter was counted by ocular graticule and Olympus BX51 light microscope using Olysia software.

Statistical analysis
All values were expressed as mean ± standard deviation (SD). Significant differences among the groups were determined by one way analysis of variance (ANOVA) followed by Duncan's test to analyze the difference. Using the Statistical Package for Social Sciences (SPSS) 16.0 software package program. Values of $P \leq 0.05$ were taken as statistically significant.

RESULTS

The fetal body weight changes of four groups were shown in Table 1. The mean of body weight in the fetuses of diabetic mothers (FDM) was significantly ($P<0.05$) more than that of the other groups.

Table 2 demonstrates different parameters of cerebrum from fetus of four groups at 18 days old. Table 3 demonstrates different parameters of cerebrum from fetus of the four groups at 20 days old. The thickness of gray matter was decreased significantly ($p<0.05$) in diabetic rat fetuses as compared to that of other groups at days 18 and 20 days old. The number of cells in gray matter and white matter was significantly ($p<0.05$) decreased in diabetic rat fetuses compared with other groups .

The thickness of white matter was decreased in diabetic rat fetuses compared to other groups and this reduction was not significant, while ratio of gray matter to white matter was significantly ($p<0.05$) decreased in diabetic rat fetuses compared with other groups.

DISCUSSION

The prevalence of gestational diabetes is increasing sharply in many developed and developing countries and it causes various anomalies in the fetus (20). One way to reduce the incidence of fetal anomalies, is to control the blood sugar of mothers during pregnancy. The aim of this study was to evaluate the effects of *cinnamon* extract on histomorphometry changes on cerebrum of fetus rat diabetes. The results of this study indicate that maternal diabetes cause changes in the central nervous system of the fetus of diabetic mothers, and this causes to reduce all studied factors except the thickness of the cerebral white matter in fetuses of 18 and 20 day of mothers diabetes control. Water extract of *cinnamon* causes adjustment of aforementioned disorders and in some cases even the amount of studied factors in diabetic groups treated with water extract of cinnamon was close to normal.

On 18 and 20 days of Prenatal the thickness of gray matter and the number of cells in the cerebral gray and white matter of cerebrum had reduced in fetus of diabetic mothers than mothers of other studied groups.

It could be said that gestational diabetes increases the glucose in the fetus brain so that with increasing the concentration of glucose in mothers blood, the glucose goes through the placenta into the fetus blood and eventually the brain (21).This can cause neuropathy and reduce the number of neurons in the fetus and eventually a baby (22) gestational diabetes causes apoptosis of large number of the progenitor cells during the formation of the affected organs. Inadequate expression of genes that regulate life in the progenitor cell is the cause of apoptosis in these cells. Especially gestational diabetes prevents the expression of a gene called Pax3 that this gene states encodes of transcription factor in neural crest cells and neuroepithelial, lack of expression of this gene is induced apoptosis in these cells (23).

Vessels supplying the nerves are also damaged by the effect of diabetes blood. This causes nerve damage which results in nerve cell death (4). Research on diabetic mice shows that pathological changes such as the creation of dark neurons and neuronal loss in the brain, occurs in different areas, especially in the hippocampus. It is believed that hyperglycemia causes exacerbate in ischemia. Diabetes and ischemic cause oxidative stress by the effect of disordering of mitochondrial respiratory chain and this cause of excessive production of reactive oxygen is considered as the main factor in the pathogenesis of cell death. This study also found that gestational diabetes causes changes in the structure and neuronal density in hippocampus so that the density of neurons in this area is reduced (24).

Kodl and colleagues , by examining the integrity of white matter by method of frankshnal of that esotropia (FA) in diabetics , found that this factor has been reduced in many areas, and the results showed that abnormalities of structure can be seen in the white matter of brain(25) by the effects of Type I diabetes.

In our previous study, investigated effect of *Juglans regia leaves* ethanol extract of maternal diabetes on fetus and Offspring's Cerebrum structure and demonstrate mean number of cells in gray matter and white matter increased in diabetic rats' fetus on 18 and 20 pregnancy (26). Also, Results revealed a significant decrease in number of cells in gray matter and white matter at 1, 14 and 28 days after birth and a significant decrease in thickness of gray matter and molecular layer at day 14 post neonatal in cerebrum in offspring of diabetic mothers as compared with other groups (27).

In this study, the comparison between control and treatment groups (normal and diabetic) represents the positive effect of water extract of *cinnamon* in the prevention of diabetes on the fetal cerebrum of diabetic mothers that can be attributed to its antioxidant properties. Studies indicate the existence of antioxidant compounds in *cinnamon* (28). The hypoglycemic activity of *cinnamon* was reported in previous studies and Rafati et al. demonstrated that positive effect of *Cinnamon* Extract on Cerebellum in Diabetic Rats' Fetus (29).

Researchers believe that the effect of *cinnamon* antioxidant is more relevant to two combinations of eugenol and hydroxy methyl of chalcon (MHCP) and suggest that there are more than 50 different compounds in *cinnamon* that *hydroxy methyl of chalcon* is more effective in glucose metabolism(30). This substance, which is soluble in water, increases both oxidation of glucose (31) and insulin secretion and this prevents increasing cellular resistance to insulin (32). And it has also been shown that *cinnamon* can stimulate the uptake of glucose and glycogen synthesis (33).

Hydroxy methyl of chalcon causes the fat cells to show more answers to insulin that this action acts through activating the kinase of insulin receptor and restrain the phosphatase activity of these receptors (30). Anderson and colleagues' study demonstrated that this substance activates the fat cells to insulin by activating the enzyme of insulin receptor and inhibiting the act of insulin _ phosphatase receptor that blocks the action of insulin _ results in phosphorylation of insulin receptor and this consequently increases insulin sensitivity (34).

In experimental studies, it has proven that *cinnamon* extract increases the activity of phosphorylation of β insulin receptor but on the other hand, it reduces tyrosine phosphatase activity and thereby shows the insulin-like properties (35). Some studies have also shown that cinnamon polyphenols, like insulin hormones, stimulates glucose uptake and Glycogen biosynthesis stimulated through the activation of glycogen synthase and inhibition of glycogen synthase kinase action (36). In mice that were under a high-fructose diet to develop insulin resistance in their body, *cinnamon* extract through increasing insulin secretion and increasing glucose uptake decreases insulin resistance (37). Polyphenols in *cinnamon* are made as regulators of insulin receptors of mice fat cells (38).

Brad Hurst and his colleagues confirmed the existence of this factor in *cinnamon* and stated that this combination resulted in a threefold increase of insulin activity in glucose metabolism in epidermal fat cells of mice (39). The results of these studies were consistent with study and the effect of hypoglycemia *cinnamon* extract, to prevent changes in fetuses' cerebrum of diabetic mothers.

Table1: Comparison of means and standard error of the body weight of fetuses of rats at 18 and 20 days of pregnancy

Group	NC	DC	NCZE	DCZE
18	2.95±0.16	3.11±0.95	2.92±0.25	3.07±0.69
20	3.89±0.11	4.48±0.46*	3.72±0.51	4.09±0.48

Table 1, Values are demonstrated with mean± SD. Significant difference between DC and other groups demonstrated with*sign (P<0.05).

Table2: Comparison of means and standard error of the cells number and dimension Cerebrum at 18 days pregnancy

Days	18		18	
Group	NC	DC	NCZE	DCZE
TGM(μ)	402.22±23.31[a]	332.12±22.36[b]	410.61±21.33[a]	384.21±23.11[ab]
TWM(μ)	242.11±14.12 [a]	261.76±16.04 [a]	248.04±18.38 [a]	254.32±13.97 [a]
NGM(n/mm^2)	25731.66±821.01[a]	23885.62±835.11[b]	25831.31±799.19[a]	25339.11±792.61[a]
NWM(n/mm^2)	11976.12±498.12[a]	9976.92±473.81[b]	12014.12±527.92[a]	11172.12±402.72[ab]
GWR	1.72±0.04 [a]	1.65±0.07[b]	1.74±0.06 [a]	1.68±0.09 [a]

Table 2, TGM (Thickness of gray matter), TWM(Thickness of white matter), NGM (Number of cells in gray matter), NWM (Number of cells in white matter), GWR (Ratio of gray matter to white matter), Values are demonstrated with mean± SD. Significant difference between DC and other groups demonstrated with dissimilar sign (P<0.05).

Table 3: Comparison of means and standard error of the cells number and dimension Cerebrum at 20 days pregnancy

Days	20		20	
Group	NC	DC	NCZE	DCZE
TGM(μ)	465.12±32.63	389.46±26.12[b]	464.25±23.72[a]	445.21±27.32[a]
TWM(μ)	302.41±21.41 [a]	322.18±21.84 [a]	298.11±74.26 [a]	312.11±28.44 [a]
NGM(n/mm^2)	25742.61±730.48[a]	23121.62±628.44[b]	25741.38±639.98[a]	24836.32±542.11[a]
NWM(n/mm^2)	10789.64±376.78[a]	9541.21±341.11[b]	10694.91±421.66[a]	10421.64±312.92[a]
GWR	1.69±0.07 [a]	1.55±0.04 [a]	1.68±0.05 [a]	1.65±0.05 [a]

Table 3: TGM (Thickness of gray matter), TWM(Thickness of white matter), NGM (Number of cells in gray matter), NWM (Number of cells in white matter), GWR (Ratio of gray matter to white matter), Values are demonstrated with mean± SD. Significant difference between DC and other groups demonstrated with dissimilar sign (P<0.05).

CONCLUSION

As a result, it can be said that hyperglycemia which occurs in the fetus of diabetics mothers, has harmful effects on the cerebrum, so that diabetes can reduce the number of cells and the thickness of the cerebral gray and white matter of cerebrum. This causes complications and irreversible damage to the nervous system of the fetus, which continues to grow years.

According to the results of biochemical and previous studies of histology, and the results of this study, it can be concluded that *cinnamon* extract with antioxidant effects and I the increase of insulin secretion in diabetic mothers causes to reduce blood sugar and prevent the impact of diabetes on the nervous system of the fetus.

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