Effect Of Hordeum vulgar L. on cerebellum cortex development in diabetic Albino mice fetal

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ABSTRACT: Diabetes is one of the chronic diseases which its association with pregnancy can cause several complications for mother and fetus such as abnormal development of embryonic tissues. Common medicine for diabetes are not usable due to many side effects during pregnancy. Considering numerous studies indicating hypoglycemic effects of barley and its treatment role in diabetes. Hence, we examined the effects of barley on cerebral cortex development of diabetic mice embryos. In this study, 60 healthy female rats were used and randomly separated in to four groups. Diabetes was induced in 2 groups, by intraperitoneal injection of streptozotocin. After getting pregnant by natural mating in each 4 group and appointing the zero day of pregnancy, they have been under treatment with barley according to groupings. On the 21th day of pregnancy embryos have been taken out surgically of mother’s body after anesthetizing and by fixing in Formalin sampling was done from their cerebrum for making tissue slides. Microscopic analysis of tissue samples indicated that the rate of differentiation in different parts of embryonic cerebellum cortex was greater in diabetic groups treated with barley than other diabetic groups. Overall results were indicating that intake of barley seeds were effective in promoting the dilatory development process of embryonic cerebellum cortex affected by diabetic mothers.

Keywords: Maternal Diabetes, Hordeum vulgar L, Cerebellum Cortex, Rat

INTRODUCTION

Diabetes is one of the most prevalent endocrine diseases causing high blood sugar and metabolic disorders due to lack or decreased function of insulin(Sivajothi,1997). Considering the shift in culture of using diets and lifestyle from traditional to industrial, the prevalence of diabetes has been increased. In addition to decreasing treatment costs, diet and alternative treatments can also prevent its affection. Maternal diabetes during pregnancy has great importance, because of generating maternal and embryonic complications. And one of the vital issues about embryo and babes of diabetic mothers is the increase in occurrence of congenital disorders. Several synthetic drugs are represented for reducing blood sugar but because of not being fully curable by these available drugs, tendency for using alternative and traditional treatments has been augmented. Meanwhile, the role of herbs in treatment of diabetic patients by reducing blood sugar cannot be ignored (Marles and Fransworth,1995). Treatment of diabetes by traditional medicine and herbs has great background, and several plants in different places of the word have been examined for anti-diabetic effects. Among the, we can refer to anab, olive leaf, distillate of Vinca Rosea and Artemisia Herba Alba, and brewed walnut leaf (Rahman,1989). Barley seeds are of cases which they have been mentioned in Iran's ancient traditional medicine for its role in treatment of diabetes. The reason of using barley is that nourishment specialists suggest using branny grains such as barley for its fibers...
and prevention of cancer, heart disease and specifically diabetes (Sasso, 2006). Barley contains extreme amount of minerals, amino acids, soluble and non-soluble fibers and enzymes. Soluble fiber can reduce blood cholesterol rate and non-soluble is effective in prevention of diabetes. Although fibers of grains are assumed to be effective, there is also ambiguity in this field about what other elements beside fiber contents of barley are effective in reducing the risk of diabetes and stabilization of blood sugar level. It is assumed that the hypoglycemic and hypolipidemic effects of barley may be due to its amino acid or chromium contents (Mahdi and Naismith, 1991). Several physiological researches have shown that most of the congenital abnormalities in infants of diabetic mothers are happened in the beginning of pregnancy because the effects of diabetes on growth and development of the fetus begins in the first 3 months of pregnancy during placenta's expansion and organogenesis. On the other hand, the central nervous system is part of initial systems developed during organogenesis, so by not appropriately controlling mother’s blood sugar levels during first 3 months of pregnancy the chance of expressing developmental defects in different fetal tissues will be elevated (Atasay, 2002; Nold, 2004; Persaud, 2007). On the account of these, we examined barley’s effects as a proper grain on the development of fetal cerebellum cortex of diabetic rats of Wistar strain in the treatment of diabetes by preparing tissue samples.

MATERIALS AND METHODS

Preparation and grouping of studied animals

In this research 60 mature female rats of Wistar strain with mean weight of 150 to 200 grams were prepared from animal section in faculty of pharmacology, Tehran university and were placed in a room with controlled temperature of 21+3°C, light status (12 h light – 12 h night) and proper air conditioning issues. Their nutrition were given with standard plate and appropriate drinkable water. Animals were randomly separated in to 4 groups of 15 rats:

- Group 1: 15 healthy rats treated with regular food (witness group)
- Group 2: 15 healthy rats treated with barley (control group)
- Group 3: 15 diabetic rats treated with regular food (experimental group)
- Group 4: 15 diabetic rats treated with barley (practical group)

Making animals diabetic

After measuring fasting blood sugar of all groups, 45 milligrams of streptozotocin (America, sigma) was injected intraperitoneal y to group 3 and 4. Group 1 and 2 were injected normal saline intraperitoneally with same amount of drug used in 3th and 4h group. For certainly about getting group 3 and 4 diabetic, feeding during 2 days after injection was holded. and on the morning of the next day their blood sugar was measured by glucometer ACCU-CHEK Active after taking blood sample from tail artery. Mice with blood glucose 250 mg/dL was diabetic and lower than the rate excluded category.

Making female rats pregnant and appointing the zero day of pregnancy by observing vaginal plaque

Mouse of each group, was placed in a caged during estrus phase of sexual cycle with a male rat of same strain for mating and fertilization. After seeing vaginal plaque as a proof of mating (Turner and Bagnara, 1971), the zero day of pregnancy was appointed and were immediately taken under mentioned treatments in each group. Un-pregnant rats were also deleted. For the measurement of used barley, weekly use of regular food in each rat was detected first and then on the account of that, mean of daily barley intake was measured 300 grams.

Dissection and taking the fetus out of uterus

Rats were anesthetized with ether on day 21, embryos were taken out by surgery from uterus horns and amnion cavity and then they were kept in 10% formalin for fixation.

Preparation of tissue samples for histopathological studies

5 rats among fixed embryos were chosen randomly and tissue sample were taken from them. After passing tissue preparation phases, paraffin blocks were made from samples by autotechnicon device, then were cut in to 5 micron thick sections by microtome device and were colored by Hematoxylin and eosin stain (H&E), and at the end covered by coverslip. Prepared slides were examined by light microscopes for histological studies. And variable below were observed in embryonic cerebellum cortex of all groups. Qualitative examination was done on
development of molecular, Purkinje and granular layers, white matter and capillaries which make a scaffold for placing cellular layers in cortex part of cerebellum.

RESULTS AND DISCUSSION

After studying microscopic slides taken from cerebellum tissues of day-21 embryos, it was observed that different parts of cerebellum cortex in diabetic mice fetal had obvious and exact changes in comparison to witness group. In a way that, structural order and development in different parts of embryonic cerebellum cortex got lower in diabetic groups with regular food, in comparison to witness group. And congested capillaries and tissue inflammation was detected in structural connective tissue. (Figure 1).

Figure 1. Cross section of cerebellum from 21-day fetuses healthy rats or witness group (A) and diabetic rats or experimental group (B). Power: *40 H&E staining: Meninge(a), Molecular cell layer (b), Purkinje(c) and Granular layer are shown.

Hyperglycemia resulting from mother’s diabetes can cause an increase of glucose in embryo’s brain and damage the nervous system (Wyngarden, 1982). As an example it caused severe inflammation and congested capillaries in cortex part of cerebellum. It seems that presumably elevated blood glucose level, decrease in insulin and increase of blood flow and pressure in microvascular vessels of diabetic mother’s has influenced embryo’s blood circulation, therefore effected development process and levels of embryonic tissues and endangered common growth and development in different parts of nervous system, including the cerebellum (Shooe, 1994). Because, varied levels of mother’s blood glucose is effective on development of embryonic organs as an intimate teratogen (Weintrob, 1996). In this experiment, irregularity in cellular parts of cerebellum cortex and their small differentiation or non-differentiation due to mother’s diabetes was depicted in embryo’s cerebellum tissues of diabetic rats. It was seen in the microscopic section of embryonic cerebellum tissue of diabetic group having barley that the available irregularity and not differentiated in cellular levels of embryonic cerebellum cortex of diabetic group were regulated in some extent and promoted to little regularity and average differentiation; rate of inflammation and congestion of vessels were also decreased noticeably. (Figure 2)

Figure 2. Cross section of cerebellum from 21-day fetuses diabetic rats treated with barley (C). Power: *40 H&E staining: Meninge(a), Molecular cell layer (b), Purkinje(c) and Granular layer are shown.

It is essential to say that, considering microscopic figures, the ratio of cerebellum cortex on center and the process of angiogenesis was lower in diabetic group in comparison to 2 groups of witness and diabetic with barley, according to its little expansion. And suggest the delay development of cerebellum cortex in diabetic rats of fetal.
CONCLUSION

Researchers believe that mother's disease during pregnancy specially diabetes is accounted as one of the teratogens. According to (Wilson and Treize, 1977) the most vulnerable period for teratogens to affect, is the phase of organogenesis and development of generative layers, and one of the effects of teratogens is delaying, slowing down or stopping the differentiation.

Through oxidative pressure resulting from mother's diabetes, Pax3 gene, which has a great role in development of cerebellum system (Engelkamp, 1999), is activated and cause defects in different parts of nervous system, specially the cerebellum (Horals, 2000). Of course it is obvious that severity of defects caused by maternal diabetes is related to the embryonic phase of differentiation and the expansion time of mother's diabetes. Neural growth is happening before the birth even in humans, but it is continued in cerebrum even after the birth. So cerebrum and the brain-spinal axis are more sensitive (Fetita, 2008). According to Pederson hypothesis about fetal diabetic disease (photopathy), most of the embryonic complications caused by diabetes (maternal) during pregnancy are under control of mother's blood sugar. Although we still don't know the mechanism that diabetes cause abnormal development in embryos, but it is shown that hyperglycemia, hyperketonemia and some other metabolic complications caused by lack of insulin have a great role. In a way that, maternal hyperglycemia damage DNA by increasing the metabolic rate of glucose and increases the oxidative pressure by biosynthesizing of hexose amin and maybe lead to elevated glucose levels in the embryo, and hyperketonemia increases the rate of embryonic defects. For reducing severe complications of different synthetic hypoglycemic factors for diabetic mothers, we can utilize the effectiveness of herbs. It is proved that hypoglycemic plants can reduce the harmful effects of cancer inducing materials in the liver of diabetic rats. Because they reduce hepatic cytochrome P450 by which most of drugs used for treatment of diabetes are metabolized (Shewiti et al., 2002). Historical background of barley use in the treatment of diseases such as diabetes demonstrates the non-toxicity trait of this this plant. Further, our histopathological studies expressed for the first time that, barley, as an important grain used in traditional medicine with effects in treatment of diabetes, with reduce maternal hyperglycemic effect as a tratogenic factor leads to reduction complications from it, could cause promotion in the slow and delayed development of cerebellum cortex in diabetic mice fetal and in other words, resulted in improving the outcome of diabetic pregnancies. However, these results represent the positive effects of barley during pregnancy, and must be consulted on human cases.

REFERENCES