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Ameliorative effect of *Morus alba* leaves extract against developmental retinopathy in pups of diabetic and aluminum intoxicated pregnant albino rats

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PEER REVIEW

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Comments
This is an interesting work that showed the ameliorative effect of *M. alba* leaves extract developmental retinotoxicity in pups of diabetic and aluminum intoxicated pregnant albino rats. The scientific part of the manuscript is good and sound and so is the methodology. Results obtained are interesting and encouraging.
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ABSTRACT

Objective: To investigate the possible ameliorative effect of crude water extract of *Morus alba* (*M. alba*) leaves on retinopathy of rat pups maternally subjected to diabetes and/or Al intoxication.

Methods: Both control and experimental groups were subjected to certain integrated approaches, namely, biochemical assessments, light microscopic investigation, transmission electron microscopic investigation, single cell gel electrophoresis (comet assay) and determination of DNA fragmentation.

Results: The retina of pups of diabetic and/or Al-intoxicated mothers exhibited abnormal alterations in retinal cell layers including retinal pigmented epithelium, photoreceptor inner segment and ganglion cells. Increased incidence of DNA fragmentation and apoptosis were evident in pups of diabetic and/or Al-intoxicated mothers. However, retina of pups maternally received *M. alba* extract plus diabetes or Al-intoxicated alone or in combination showed marked amelioration. Less degree of ameliorations was seen in retina of pups maternally subjected to combined treatment. Furthermore, application of crude water extract of *M. alba* resulted in amelioration of the alterations of maternal serum glucose as well as Al concentration.

Conclusions: Based on the results of the present study, *M. alba* extract is effective against experimentally diabetic and Al-induced developmental retinopathy.

KEYWORDS
Morus alba, Retinopathy, Aluminum, Diabetes, TEM, Comet assay

1. Introduction

Diabetes and aluminum (Al) intoxication possess the major health problems. Al intoxication comes from different sources such as cooking utensils, food additives, medicines such as antacids or deodorants, *etc*.[1], drinking water[2], vaccines, inhaled fumes and particles from occupational exposures[3]. Corn, yellow cheese, salt, herbs, spices, tea, cosmetics were found to have increased amounts of Al[4]. Diabetes mellitus is a heterogeneous metabolic disorder characterized by hyperglycemia. The disease is worldwide increasing and affecting children and adolescents in industrialized as well as in developing countries, posing a major challenge to global human health[5] and is now considered as one of the main threats to human health in the 21st century[6].

Several authors who investigated streptozotocin (STZ)-diabetes described segmental demyelination and remyelination as well as abnormalities of the paranodal myelin at a similar rate with the control animals[7], suggesting these alterations to be more related to aging[8,9]. Thomas *et al*.[10] described a reduction in the myelin thickness in chronic STZ-diabetic rats especially if the induction of diabetes was in the early stages of life. More recently, it has been reported that chronic STZ-diabetes was able to cause demyelination,

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especially on the small fibers either on the aortic depressor nerve or the phrenic nerve[11].

The presence of retinopathy, even in its early stages has also been associated with cerebral white matter lesions[12]. In otherwise healthy diabetic adults, a recent exploratory analysis has suggested that increasing severity of retinopathy is related to reductions in cortical grey matter density[13]. Laboratory studies on rats revealed that diabetes of 8 months' duration increased the release of cytochrome c into the cytosol and Bax protein into the mitochondria prepared from the retina, and this phenomenon was not observed in 2 months of diabetes[14]. Diabetes increases oxidative stress, which plays an important role in the development of diabetic complications[15]. Oxidative stress is increased in retina of diabetes and in isolated retinal capillary cells incubated in high-glucose medium. The antioxidant defense system is impaired in the retina of diabetes, glutathione levels are decreased and superoxide production is increased[15,16].

Research works concerning Al retinopathy are evidently scarce[17]. Fry *et al.*[18] intoxicated rabbit with Al and reported neurofibrillary tangles in a subpopulation of retinal ganglion cells (GCs), located primarily in the peripheral retina. The distribution of affected cells suggested a differential susceptibility of GCs to Al intoxication. Following daily injection of 0.3 mL of 4% $AlCl_3$ to 4-week-old Wistar Kyoto rats, Lu *et al.*[19] reported that thin retinal pigmented epithelium (PE) and disappearance of the photoreceptor (Phs) outer and inner segments were the most evident observations.

There is a growing tendency towards using phototherapy owing to the general belief that it has no side effects compared to chemotherapy. Several studies have indicated that hyperglycemia can be controlled via different sorts of medicinal plants[20-24]. Singab *et al.*[25] studied the hypoglycemic activity of the flavonoids rich fraction of 70% alcohol extract of Egyptian *Morus alba* (*M. alba*) root bark in STZ-induced diabetic rats. The authors found that administration of Egyptian *M. alba* root bark for 10 days (600 mg/kg) significantly reduced the amount of glucose from control level to a lower level and significantly increased the insulin level from the control to a high level.

It has been reported that consumption of *M. alba* extract with 75 g of sucrose significantly attenuated the increase in blood glucose concentration in non-diabetic and Type 2 diabetic individuals[26]. *M. alba* leaf contains active compounds that can inhibit galactosidases, such as 1-deoxynojirimycin[27], and this effect may help suppress postprandial hyperglycemia by reducing the rate of digestion and absorption of carbohydrates from the intestine. However, intraperitoneal administration of *M. alba* leaf extract has a hypoglycemic effect in experimentally-induced diabetic mice[28].

The present study dealt with investigating the developmental neurotoxicity of retina of pups of mothers subjected to aluminum chloride ($AlCl_3$) intoxication and/or diabetes during perinatal life. Treatment with *M. alba* leaves extract were carried out to examine its possible ameliorative effect upon the developmental retinopathy. The study involved several integrated parameters conducted on both control and experimental groups: (1) determination of glucose level in the mother's serum; (2) determination of Al concentration in the mother's serum; (3) light and transmission electron microscopic investigation for the development and differentiation of retinal neuronal cells; (4) comet assay; (5) assessment of DNA damage.

2. Materials and methods

2.1. Animals and grouping

Principles of animal care and use were followed during the conducting of the present study. One hundred and forty fertile male and virgin female albino rats (*Rattus norvegius*) weighing (180 ± 20) g were purchased from Hellwan Breeding Farm, Ministry of Health, Cairo, Egypt and used for the experimentation. Rats were housed in individual cages and maintained in a room with good ventilation at 23 ˚C. They were fed on standard diet free from excess fats and free access of food and water was allowed *ad libitum* throughout the experimental period. Females were mated in a special cage (1 male/2 females) during overnight and gestation was determined in the next morning by the presence of sperm in a native vaginal smear. The pregnant rats were arranged into seven groups (15 individuals in each group); control, experimental diabetic, diabetic and *M. alba*, Al-intoxicated, Al intoxicated and *M. alba*, experimental diabetic and Al intoxicated, diabetic and Al intoxicated group plus *M. alba*. The control group was subdivided into two subgroups, the first as control (C) and the second as *M. alba* (M) group. At the end of the experimental period *i.e.* after 7 and 14 days from parturition, mothers and pups of both control and experimental groups were anesthetized by an intraperitoneal injection of sodium pentobarbital solution (50 mg/kg body weight), sacrificed, dissected and eye was separated and processed differently according to the required investigations.

2.2. Water extraction of M. alba leaves

Mulberry leaves were washed and dried in a hot air oven at 50 ˚C for 6-8 h. The dried material was ground to a fine powder and kept in an airtight container at 4 ˚C until further use. Four grams dried *M. alba* leaves were powered and extracted with 50 × (w/v) of hot water (85 ˚C) for 3 h. The extract was filtered with Whatman No.1 filter paper and concentrated to a volume of 1/20 of the initial solution volume by heating at a no boiling temperature near 100 ˚C, and then dried completely under vacuum at 25 ˚C. The dried extract (w/w = 0.5 g, yield = 15%) was used during experimentation. The applied dose of *M. alba* extract was 100 mg/kg body weight[29], and the extract was orally administered after the induction of diabetes every other day till the end of experimentation.

2.3. Induction of diabetes

Diabetes mellitus was induced experimentally in all rat groups except the control and Al intoxicated alone by a single intraperitoneal injection of STZ (60 mg/kg) in citrate buffer (0.05 mol/L, pH 4.5) at 5th day of gestation for two consecutive days and injected within 10 min of dissolution[30]. Control animals were treated with physiological saline as a vehicle. Maternal hyperglycemia was verified by measuring the blood glucose level. Rats with a level of more than 300 mg/dL were selected for this study.