Acta Pharm. Sci. Vol 58 No: 1. 2020
DOI: 10.23893/1307-2080.APS.05807

Effect of Ginger on Hyperglycemia Induced by Streptozotocin in Pregnant Rats and Postnatal Neurodevelopment of their Offspring

Mehouel Raouia[1*], Ferhati Habiba[1], Tahraoui Abdelkrim[1]

1 Badji Mokhtar University, Department of Biology, Laboratory of Neuro-endocrinology Applied, BP 12, 23000, Annaba, Algeria.

ABSTRACT

This study was performed to investigate the consequences of severe maternal hyperglycemia induced by streptozotocin in Wistar rats on postnatal development of offspring. Besides, the ability of a ginger extract to restore glycemic balance in dams and prevent the appearance of disorders in offspring. Diabetes was induced in dams before pregnancy by a single injection of streptozotocin at a dose of 50 mg / kg. Ginger was orally administered from the fourteenth (14th) to the nineteenth (19th) day of pregnancy. Two tests before and after weaning were performed to monitor the offspring development. Our study clearly showed that treatment of diabetic pregnant rats with a ginger extract lowers blood glucose levels and help prevent disorders in offspring.

Keywords: Maternal diabetes, Postnatal neurodevelopment, Streptozotocin, Wistar rats, Ginger

INTRODUCTION

Natural products include a variety of chemical compounds that have been evolutionarily selected for their ability to improve the survival of an organism.[1]

Because of the diversity of their biological activities, they have been widely used in the field of human health as a dietary supplement in traditional medicine for thousands of years.[2]

Ginger (*Zingiber officinale*) is a plant frequently used for thousands of years as a culinary seasoning, in particular in Asian cuisine, and is a common medicinal

*Corresponding Author: Mehouel Raouia, e-mail: mehouel.raouia@gmail.com
Mehouel Raouia ORCID Number: 0000-0001-5749-2091
Ferhati Habiba ORCID Number: 0000-0002-6051-421X
Tahraouia Abdelkrim ORCID Number: 0000-0002-4855-4112
(Received 09 July 2019, accepted 05 November 2019)

agent in the traditional medicine systems of China, India and other Eastern cultures. Ginger has several beneficial pharmacological effects (hypoglycemic, insulinotropic, hypolipidemic) in laboratory animals [3] and in humans.[4]

It has been demonstrated that ginger or its extracts possess certain pharmacological activities, including analgesic effects[5]. Herbal medicines are considered as a fundamental source of new compounds based on pharmacological active principle, where about 11% of basic drugs in the 21st century are derived exclusively from plant origin[6].

In our study, we investigate the effect of maternal diabetes induced by streptozotocin in Wistar rats on postnatal neuro-development of offspring. Moreover, the effect of a ginger extract on maternal hyperglycemia and its impact on the development of offspring.

METHODOLOGY

Experimental Animals

Wistar strain female and male (that will be used for mating) weighing 240 ±10 g were obtained from Pasteur Institute (Algiers, Algeria). The rats were housed in clean polyethylene cages (58 × 38 × 19 cm, 5 rats per cage for each sex) and maintained at standard facility conditions of temperature (25 ± 2 °C), humidity (65 ± 5%) with a photoperiod of 12 h light and 12 h dark cycle. The rats were fed with commercial chow and tap water ad libitum. Procedures for the care and handling of animals were in compliance with current international laws and policies (NIH Publication No. 85–23, 1985, revised 1996).

Study desing

After adaptation period, the female rats were divided into four (4) experimental groups, and treated as follows:

- Group 1: included control dams (C)

- Group 2: included dams treated with ginger extract (G)

- Group 3: included diabetic dams (D),

- Group 4: included diabetic dams treated with ginger extract (D + G)

Animal Treatments

Diabetes Induction

Streptozotocin (STZ) was obtained from Sigma – Aldrich chemicals (USA).

Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (50mg/kg body weight) in 0.1 M citrate buffer (pH 4.5). Animals were considered as diabetic if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The blood glucose levels were mesured from the rat tail vein[7] by using Accucheck verio glucometer (United Kingdom).

Ginger extract administration

The ginger extract used in our experiment is a product of a leading Life Science and High Technology company (Sigma-Aldrich, United States)

(Ginger extract-naturel, Sigma-Aldrich, code: W 252108, CAS Number: 84696-15-1).

Ginger extract was conducted to pregnant rats in groups (G) and (D + G) by oral gavage once daily in 80mg/kg body weight/ day at 08h :30 in 6 consecutive days, from the fourteenth (14th) to the nineteenth (19th) day of pregnancy.

Mating

The female rats of the different groups were individually housed in appropriate polyethylene cages and subjected to the first vaginal smears to determine estrus cycle phases based on a standard cytological analysis.[8] Each pro-estrus female was placed overnight in cage with one male of the same strain. Second vaginal smears were examined the next morning and the presence of spermatozoa was considered as day 1 of pregnancy (gestationel day 1 = GD 1).

Farrowing

Parturition day was designated as PND 0. (PND = Postnatal day).

For the experimental study of offspring, we used 12 male and 12 female rats from each group.

Postnatal development evaluation

In order to monitor the postnatal development, the offspring were weighed in PND 1, PND7, PND 14 and PND 21.

Tests applied in male and female pups (Before and after weaning)

Negative geotaxis (PND8)

The negative geotaxis test is supposed to evaluate motor coordination and the cerebellar integration. The test consists in placing the rattons at PND 8 on an inclined plane at an angle of 25 degrees to the horizontal and their heads pointed down the slope. The measured variable is the latency time for a rat to make a complete half-lap of 180 degrees, ending up at the top of the slope. (measurement of equilibration, maturation of the cerebellum and semicircular canals of the inner ear).[9]

Object recognition test (PND 45)

The test is performed in order to evaluate the recognition memory in rodents because it uses their natural preference for a new object compared to a familiar object. [10]

At postnatal 45, the offspring were placed in an open arena (50 × 50 × 40) and allowed to freely explore two identical objects during a pretest of 5 minutes (exploration phase) and then returned to their cages. After a one-hour retention phase, the pups were resubmitted for 5 minutes at the test session (recognition phase) during which one of the two familiar objects previously explored was replaced by a new object.[11] The recognition memory was evaluated during the test session and expressed by the recognition index which indicates the ratio of the exploration time of the new object on the exploration time of the two objects.

Statistical analysis

All results were expressed as the means ± standard deviation (M±SD).

Statistical calculations were made using Minitab software (version 18.1.0.0, Minitab Inc., USA). The comparison between the different groups was performed using Student's t test.

RESULTS

Maternal glycemia

Table 1. Variation in maternal blood glucose during the experiment (g/l). Results are expressed as the mean ± SD (n = 7).

Groups	Before gestation		During gestation			After gestation	
	Before induction	After induction	GD 1	GD 14	GD 19	PND 1	PND 21
C	**0.94±0.10**	0.89±0.05	0.91±0.04	0.92±0.07	0.90±0.11	0.93±0.03	0.88±0.12
G	**0.90±0.11**	0.92±0.07	0.93±0.09	0.94±0.04	0.89±0.03	0.90±0.08	0.89±0.07
D	**0.92±0.03**	3.04±0.13	3.20±0.12	4.10±0.15	4.32±0.17	4.41±0.10	4.71±0.23
D + G	**0.88±0.05**	3.08±0.09	3.17±0.05	4.13±0.12	2.57±0.21	2.53±0.14	2.52±0.17

On the third day after the induction of diabetes, the blood glucose levels in STZ injected diabetic rats were significantly (P<0.001) increased than of normal control rats. However, we found that the elevated blood glucose levels in diabetic rats (D+G) were significantly (P<0.001) decreased after 6-days ginger administration compared to diabetic rats (D). (Table 1)

Offspring body weight evolution

Pups of diabetic dams (D) gained significantly less weight than those from control dams during the postnatal development ($p < 0.001$). However, pups of diabetic dams treated with ginger (D + G) gained significantly more weight than those from diabetic dams during the postnatal development ($p < 0.001$). The difference at PND 21 is very highly significant (p <0.001), (Fig 1)

Figure 1. Offspring body weight change (g) during the development from PND 1 to 21. Results are expressed as the mean ± SD; n = 24 (12 males, 12 females). (β: comparison vs C), (γ: comparison vs D).

Body weight of male and female pups

Groups	PND 1		PND 7		PND 14		PND 21	
	M	F	M	F	M	F	M	F
C	5.83±0.55	5.66±0.62	13.08±1.11	12.66±1.02	25.50±0.86	24.08±1.32	42.08±1.38	40.58±1.18
G	5.94±0.44	5.76±0.40	14.25±0.59	13.33±1.02	26.75±0.72	24.83±1.14	44.41±0.49	41.50±1.11
D	4.91±0.64	5.16±0.37	8.25±0.92	8.75±1.09	11.25±1.16	11.91±1.03	18.83±0.89	19.66±0.94
D + G	5.33±0.47	5.41±0.49	9.75±0.59	10.16±0.79	15.08±0.64	16.25±0.82	24.50±0.86	26.25±0.92

Table 2. Body weight of male and female offspring (g). Results are expressed as the mean ± SD; n = 24 (12 males, 12 females).

Table 2 shows the body weight (g) of male and female offspring at day PND1, PND7, PND14 and PND21. We found a delay in weight growth in males of diabetic dams (D) compared to females. In PND 1 and PND 7 our results show no significant difference in body weight between males and females from diabetic (D) and diabetic ginger-treated dams (D + G) (P> 0.05). PND 14 and PND 21 showed a very highly significant increase in body weight of females from ginger-treated diabetic dams (D + G) compared with males (p <0.001). In PND 21, our results show a significant increase in body weight of females from diabetic dams (D) compared with males (P <0.05), (Table 2).

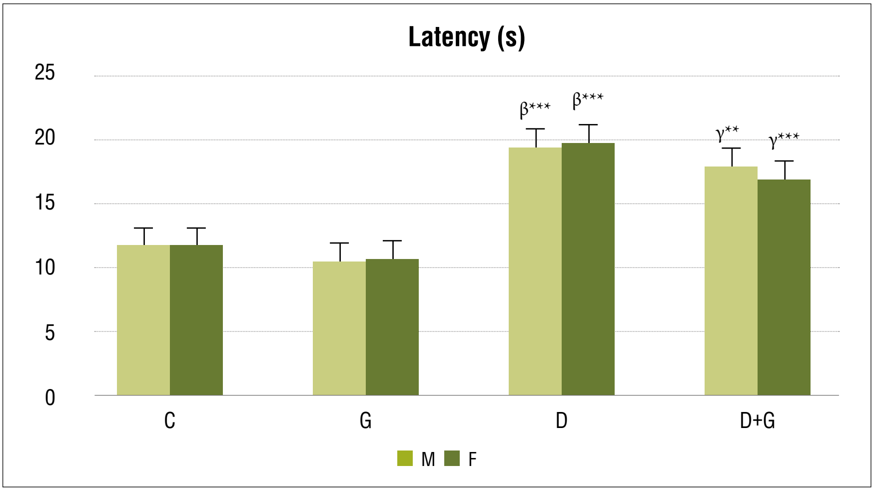

Figure 2. Latency time (s) in the negative geotaxis test at day PND 8. Results are expressed as the mean ± SD ; n = 24 (12 males, 12 females). (β: comparison vs C), (γ: comparison vs D)

Variation in postnatal neurodevelopment parameters of pups:

Negative geotaxis

According to this test, latency significantly increased (p <0.001) in male and female pups of diabetic dams (D) compared to pups of control dams. In contrast, highly significant decrease (p <0.01) in latency in male offspring of diabetic dams treated with ginger (D + G) compared to male offspring from diabetic dams (D), while in female offspring, significant decrease (p <0.001). Latency significantly (P<0.05) decreased in female of diabetic dams treated with ginger (D + G) compared to males (Fig 2).

Object recognition test

As depicted in Fig 3, recognition index significantly ($P<0.001$) decreased in males and females of diabetic dams as compared to pups of control dams. Significantly increase ($P<0.001$) of recognition index in offspring (male and female) of diabetic dams treated with ginger (D + G) compared to offspring of diabetic dams (D), (Fig 3).

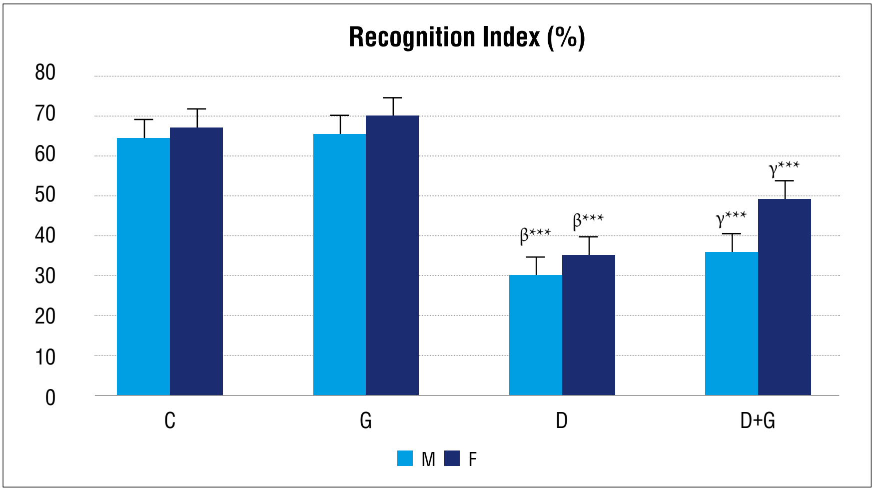

Figure 3. Recognition index in offspring at PND 45. Results are expressed as the mean ± SD; n = 24 (12 males, 12 females). (β: comparison vs C), (β: comparison vs D).

Diabetes mellitus is a group of chronic metabolic disorder characterized by hyperglycemia that causes lesions affecting several devices or systems especially vessels and nerves.[12]

In this study we reported the effects of severe maternal hyperglycemia induced by streptozotocin on development of offspring and the ability of a ginger extract to restore glycemic balance in dams and prevent the onset of disorders in offspring.

Elevated maternal blood glucose in diabetic groups testifies the installation of the diabetic disease.[13] According to the conclusions of several studies, this hyperglycemia is due to the effects of Streptozotocin by its action on pancreatic β cells, leading to a reduction of the cell mass.[14] Streptozotocin activates the expression of protein kinase C, responsible for the dephosphorylation of the insulin receptor.[15]

Ginger has been used traditionally to treat a large number of conditions such as fever and infectious diseases, abdominal pain, abdominal spasms, nausea and vomiting, motion sickness, arthritis, rheumatism, ulcerative colitis, gingivitis hypertension and diabetes.[16] The bioactive components of ginger were characterized by spectroscopic analysis in the form of zingerone, gingerdione, dehydrozingerones which had a potent antioxidant, shogaols, gingerols and a volatile oil.[17]

Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its complications remain a major medical problem in the world's population.

Currently, some medicinal plants are used to treat diabetes, and ginger is one of the most potent herbs traditionally used to treat diabetes mellitus.[18]

Treatment of diabetic pregnant rats (D + G) with ginger extract for six consecutive days reduced glycemia. This is in agreement with previous reports.[3]

According to the literature, two models have been proposed to study diabetes in rodents during pregnancy and its implications for offspring. Moderate diabetes model causes mild hyperglycemia in mothers and macrosomia in the offspring, being similar to repercussions of diabetes type 2 and gestational diabetes mellitus. On the other hand, severe diabetes model causes high hyperglycemia in mothers and microsomia in the offspring.[19,20] Both macrosomic and microsomic infants may have long-term consequences at childhood, adolescence, and adulthood.

Our results showed that the body weight of the offspring of diabetic dams is lower than that observed in offspring of control dams as previously reported by Piazza *et al.*[21].

Compelling evidence have shown that this maternal type 1 diabetes model can induce microsomia in the offspring, possibly as a result of intrauterine growth restriction with placental commitment, [20,22,23] leading to long-term effects, especially in metabolic and neurologic parameters in the infants.[24,25]

The continuation of the hyperglycemic status in diabetic dams (D) after parturition seemed to contribute to the reduced growth during suckle, and pups remained small until weaning age (PND 21) as previously described by Chandna *et al.* [26] The observed effects in offspring may not be only due to the gestational diabetes, but also to the malnutrition at early stages of offspring development, since that maternal type 1 diabetes can delay lactogenesis onset by affecting prolactin secretion and lactose, citrate, and total nitrogen concentrations in the milk.[27]

On the other hand, in the offspring of diabetic dams treated with ginger (D + G), our results showed an increase in body weight, which may be due to the decrease in maternal blood glucose after treatment with ginger extract and stimulation of lactation in these dams.

Developmental milestones play an important role in assessing the maturation of neonatal neurologic reflexes and serve as predictors of behavioral changes in adults.[28]

In our study, the offspring of diabetic dams presented neurodevelopmental delay in the negative geotaxis test according to and complementing the cognitive impairments reported by the clinic.[29,24] Furthermore, a decrease in the object recognition index this is in agreement with the results of Kim *et al.* [30] who have demonstrated, with the same experimental model, impaired memory in step-down avoidance task on PND42.

The administration of ginger in diabetic dams during pregnancy had a beneficial effect on the neurological development of the pups, latency time in the negative geotaxis test was improved especially in females suggesting selective action on one sex. Also, there has been an increase in recognition memory in offspring of diabetic dams treated with ginger.

In addition, this plant extract and its active component, 6-gingerol, also inhibited cholinesterase activity, which increased acetylcholine, a neurotransmitter that plays an important role in learning and memory.[31]

There is considerable recent experimental evidence that ginger treatment has significantly improved word recognition, numerical alertness, choice reaction, digital working memory and spatial working memory scores in middle-aged women.[32]

These studies have shown that ginger can improve cognitive decline in the early stage of dementia in old age. Ginger intake during critical moments of fetal development is associated with an interaction with the neurophysiological processes of postnatal behavior.

Maternal diabetes is an intrauterine environment that is detrimental to the development of offspring, can cause brain damage and increases the risk of neurological disorders.

Our results indicate that severe maternal hyperglycemia during the fetal and perinatal period predisposes offspring to delayed physical development and neurodevelopmental disorders.

Our study clearly showed that treatment of diabetic pregnant rats with a ginger extract lowers blood glucose levels and can help prevent disorders in offspring.

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