

Full Length Research Paper

Hesperidin effects on behavior and locomotor activity of diabetic Wistar rat

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Today, there are sufficient validated scientific data that support the existence of relations between diabetes and certain neuropsychiatric disorders, such as behavioral disorders, anxiety, cognitive decline and depression. The objective of this work was to investigate the effect of a natural bioflavonoid, the antioxidant hesperidin on the neuro behavioral alterations and locomotor function in streptozotocine diabetic wistar rats. Twenty eight male rats were divided equally into four groups; control, and treated series (hesperidin, streptozotocine and hesperidin+ streptozotocine) then exposed to open field test, where animals were individually placed in the center of the compartment for a period of 5 min. Results of the open field test showed high level of anxiety and a slowdown in locomotion and mental flexibility on diabetic rats. Treatment with hesperidin, significantly module these disorders of the animals related to diabetes. Thus, our results confirm the capacity of hesperidin as an antioxidant, to correct neurobehavioral and locomotion disorders related to diabetes and its complications by neutralizing free radicals generated by this metabolic disease.

Key words: Diabetes, oxidative stress, hesperidin, streptozotocine.

INTRODUCTION

Diabetes mellitus is a complex endocrine metabolic disorder and that is multifactorial (Permutt et al., 2005). It causes adverse changes in the central nervous system causing behavioral disorders, depression and cognitive dysfunction (Anderson et al., 2002; Alvarez et al., 2009; Reijmer et al., 2011). Diabetes affect stress response as shown by an increased activity of the hypothalamic-pituitary-adrenal (Saravia et al., 2001; Chan et al., 2003) and also an alterations in vessels, eyes and peripheral

nerves, as evidenced by the frequency of neuropathic pain in diabetics (Schwarz et al., 2009).

The earliest observations have reported the presence of a peripheral neurological deficit, induced by a neuronal degeneration and slowing down the nerve impulses (Gispén and Biessels, 2000). Today it is possible to associate diabetes with psychiatric manifestations; such as depression (Schwarz et al., 2009), behavioral disorders and anxiety (Oldroyd et al., 2005), cognitive

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dysfunction (Adeghate et al., 2006; Sima et al., 2009), a slower speed and mental flexibility (Ramanathan et al., 1998; Brands et al., 2005). Therefore the anxious behavior of high level diabetics was highlighted by an open field tests, as well as in the elevated plus-maze (Adeghate et al., 2006; Miyata et al., 2007).

Although the pathogenesis of defects in learning and memory in diabetics is not well understood, but however it involves several factors, such as metabolic disorders, vascular complications and accumulation of free radicals (Biessels et al., 2007; Kucukatay et al., 2007; Tuma, 2007). It is now accepted that high levels of glucose in the extra and intracellular environments induce stress, which has been defined as an imbalance between pro-oxidant and antioxidant, highlighted in experimental diabetes in animals and patients with type1 (Northam et al., 2006) and type2 (Stewart and Liolitsa, 1999). Oxidative stress has been involved in the past two decades as the main insidious in the genesis of various chronic diseases and degenerative complications (diabetes, atherosclerosis, cancer, rheumatoid arthritis, Alzheimer's disease, and asthma). It is induced either by excessive production of reactive oxygen species, reactive nitrogen species or a depletion of antioxidant defense capabilities (Schaalan et al., 2009), so an antioxidant supplementation has been considered as adjuvant therapy (Favier, 2003). Therefore it is hypothesized that, administration of antioxidants to animals could reduce the risk of developing behavioral and cognitive disorders in diabetic patients.

According to the present literatures, it is hypothesized that the administration of antioxidants reduces the risk of developing behavioral and cognitive disorders in diabetic patients. To confirm this hypothesis it is been assessed that, contribution of an antioxidant in the prevention of abnormal locomotors, behavioral and cognitive functions in diabetic rats has been injected with streptozotocin and later treated with hesperidin.

This is a natural bioflavonoid that possesses a very good antioxidant property which has been proved to be very effective in various neurobehavioral diseases (Maridonneau-Parini et al., 1986). Therefore this present work was conducted by the application of an open field test (Sáenz et al., 2006) that allowed the evaluation of rat basal activity and its behavioral evolution.

MATERIALS AND METHODS

Experimental protocol

Wistar male rats weighing 200 ± 20 g were obtained from Pasteur Institute, Algiers. The rats were reared in the rearing house of the Department of Biology, University of Annaba inside polyethylene cages with a mean temperature of $25 \pm 2^\circ\text{C}$, standardized photoperiod and humidity. Rats were supplied with water and fed on standard diet made up in the form of rods produced by, local food factory (SPA, Bouzareah, Algiers).

Mode of treatment

Twenty eight rats were divided into equal four groups; control vehicle (CV), treated control (rat) with hesperidin (CHS), diabetic induced with streptozotocin considered as diabetic vehicle (DV) and rats treated with hesperidin + streptozotocin represented as (DHS).

Control rat (CV) received daily saline solution of NaCl 0.9% at 1 ml/kg. Hesperidin group (CHS) rats received hesperidin at dose 50 mg/kg daily during 21 days diluted in 1 ml/kg of NaCl 0.9%. Induction of diabetes in the rats (DV) was done by a single intraperitoneal injection of streptozotocin [Sigma ST Louis, Mo] at the dose of 60 mg/kg body weight, with a volume of 1 ml/kg. Streptozotocin was prepared extemporaneously in a citrate buffer 0.1 M (pH 4.5).

Diabetes appears in rats after 48 h. Diabetes was checked by measurement of fasting glucose in blood and confirmed by the presence of glycosuria in urine using dipstick bili labstix®. However, after 72 h of inducing diabetes (DHS), hesperidin (50 mg/kg) was dissolved in NaCl and was given by stomach tube to animals for a period of 21 days.

The open field test

The open field test (OF) was developed in order to measure differences in emotional reactivity of rodents (Sáenz et al., 2006). Therefore the OF evaluates the ambulatory behavior and the environmental neophobia of the tested animals. The structure OF is a Plexiglas unit comprising of (70 X 70 cm) base, surrounded by parapets Plexiglas with a height of 40 cm. The floor of this unit is divided into two areas, central and peripheral area, each measuring 35 cm length divided in squares (1square=1cm²). The central zone is defined as the opened, deems and the peripheral area borders of the walls. This test consists of, introducing the individual rats in the center of the unit and left for five minutes. The test parameters measured were, locomotor activities, standing position, the time of permanence and entry in the center and edge.

All rat movements were recorded by a high resolution camera, thereby recording the number of squares crossed and the time spent in each area, respectively reflecting the locomotor activity and the anxious behavior. The anxiety level is estimated by, the reference to the displacements in two surfaces. It is considered that, when the spent time in the central area is higher the rat's anxiety level will be lower. In general an anxious animal will tend to prefer the peripheral zone, while avoiding entering the central zone. The device is wiped off after each session with an alcoholic solution, to overcome the polarizing effects due to the odors left by the previous rat.

The results were expressed in mean \pm SEM and was compared to control which was analysed using Student's t-test, at $P < 0.05$.

RESULTS

Effect on glycemia

The rats were subjected to treatment in order to induce diabetes. Glycemia increased significantly ($p < 0.001$) for a period of 21 days in comparison to the control. However, this hyperglycemic state decreased significantly after treatment of these animals with hesperidin (Figure 1).

Open field tests

The open field tests were evaluated by the spent time of

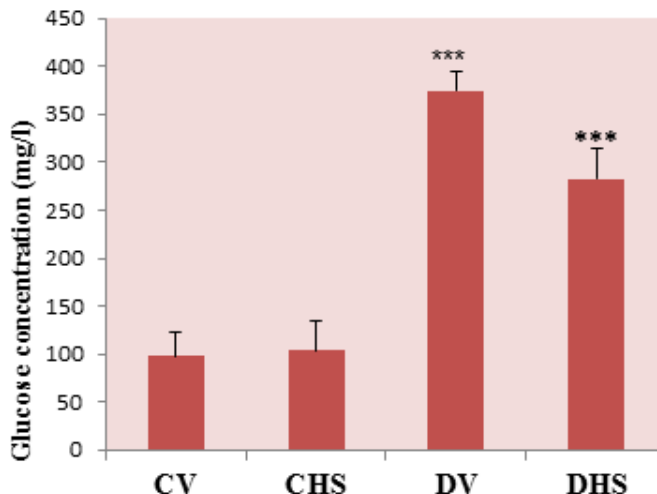


Figure 1. Concentration of blood glucose (mg/l) of the four rat groups; control vehicle (CV), treated control with hesperidin (CHS), diabetic vehicle (DV) and treated with hesperidin + streptozotocine (DHS). (***) = $p < 0.01$.

Table 1. Parameters of the open field test (OF) of the control and treated rat groups.

| Group of rats | The open field test parameters | | |
|--|--------------------------------|---------------------------------|-----------------------------|
| | Spent time (s) in the center | Spent time (s) in the periphery | Traversed distance (cm) |
| Control vehicle (CV) NaCl 0.9% 1 ml/kg | 10.4 ± 0.74 ^a | 289.6 ± 3.14 ^a | 421.3 ± 20.14 ^a |
| Treated control with hesperidin (CHS) 50 mg/kg | 15 ± 3.54 ^b | 285.4 ± 4.07 ^a | 550.6 ± 21.33 ^b |
| Diabetic vehicle (DV) 60 mg/kg | 3.6 ± 0.84 ^c | 297.75 ± 2.83 ^b | 202.3 ± 19.33 ^c |
| Treated streptozotocine and hesperidin (DHS) | 4.3 ± 0.90 ^c | 295.75 ± 3.83 ^b | 380.16 ± 19.67 ^d |

For each group, mean values followed by the same letter are not significantly different ($P < 0.05$).

the rats in the center, periphery and running distance. The results of the different series are represented in Table 1.

Treatment effects on time spent in the center and in the edge

Results show that the group treated rats with hesperidin (CHS) spent less time (second) in the central part and more time in the edge of the device as compared to the control (CV) (15 ± 0.73 against 10.4 ± 0.74) and (285 ± 6.70 against 289.6 ± 3.14) (Table 1). These statistical analyses showed that the differences are significant ($P < 0.05$) (Figure 2A).

These group of diabetic (DV and DHS) spent less time in the central part and more time in the edge of this device than that of the control group (3.6 ± 0.84 and 4.3 ± 0.90 s against 10.4 ± 0.74 s) and (296.4 ± 4.07 s and 295.75 ± 2.83 s against 289.6 ± 3.14 s) (Table 1) with highly significant differences ($P < 0.001$) (Figure 2B). In

the group of treated diabetic rats (DHS), the spent time (second) in the center was slightly higher than the diabetic control (DV) (4.3 ± 0.90 against 3.6 ± 0.84) (Table 1) and consequently, the treated diabetic rats spent less time in the edge than that of diabetic control (295.75 ± 2.83 s against 4.07 ± 296.4 s), but the statistical analysis of these results was not significant (Figure 2A and B).

Treatment effects on the distance traversed

The results of the running distance test by the different groups of rats are shown in Table 1. The assay showed that the number of squares (cm) traversed by rats treated with hesperidin was significantly ($P < 0.01$) (Figure 2C) higher than that traversed by the control (54.75 ± 4.07 cm against 43 ± 5.23 cm). Conversely, diabetic control and treated rats traversed fewer tiles than that of diabetic control (21 ± 3.88 cm and 37.2 ± 3.24 against 43 ± 5.23 cm) (Table 1). The analysis of these results show a highly

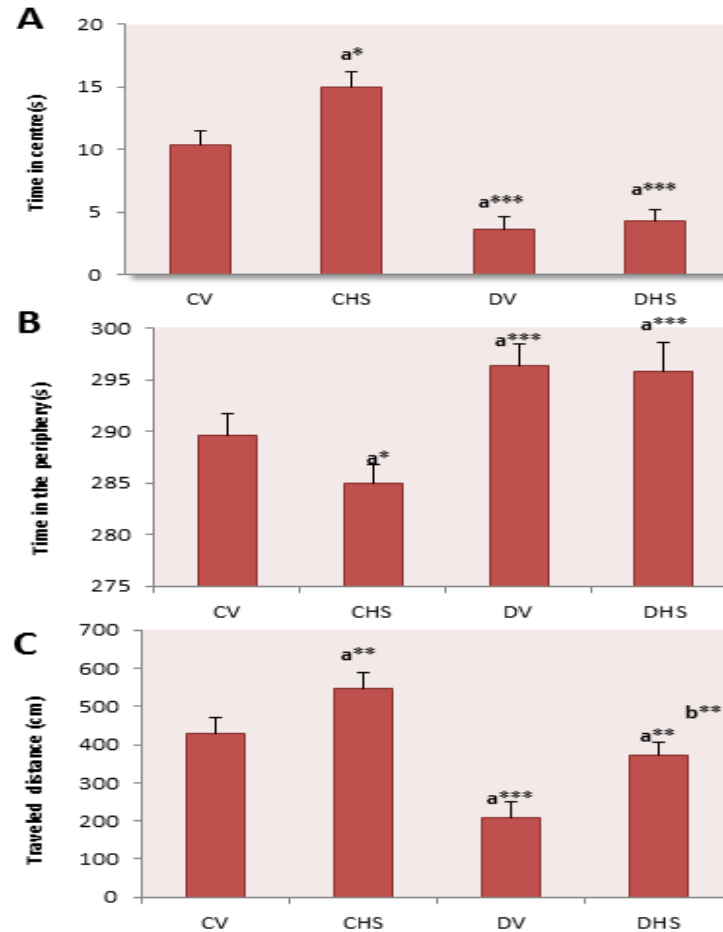


Figure 2. Parameters of the open field test of the four groups. **(A)** The spent time (s) in the center. **(B)** The spent time (s) in the unit edge (periphery). **(C)** Number of squares (cm) traversed. [Treated rats (CV, CHS, DHS) a: compared to the control (CV), b: compared to diabetic rats (DV)]. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

significant difference for diabetic rats ($P < 0.001$) and another significant difference for the diabetic rats treated with hesperidin ($P < 0.01$) (Figure 2C). Indeed, for the treated diabetics, the number of crossed tiles was significant ($P < 0.01$) and higher than that traversed by the diabetic control (37.2 ± 3.24 against 21 ± 3.88 cm).

DISCUSSION

Oxidative stress is considered as the main insidious of various chronic diseases and degenerative complications, like diabetes. (Brands et al., 2005; Alp et al., 2012; Moree et al., 2013). It is induced either by the excessive production of reactive oxygen species or reactive nitrogen species or a depletion of antioxidant defense capabilities. In the present study, diabetes was confirmed by the recorded increase of glucose in blood and urine and this was due to the injected streptozotocin

which has certainly destroyed pancreatic β cells, and led to impaired glucose stimulated insulin release and insulin resistance. Elevation of blood glucose may be attributed to the reduced entry of glucose to peripheral tissues, muscle and adipose tissue which increased glycogen breakdown, gluconeogenesis and hepatic glucose production (Bouhali et al., 2015). Such results agree with the previously published data (Schuster and Duvuri, 2002).

The OF parameters measured in the current investigation were the locomotor activity, standing permanent and entry time in the center and the edge, the time of permanence and entries close to walls. The analysis of these results traduced by the level of anxiety, locomotor function and mental flexibility, showed an increase in time and number of entry in the edge of diabetic and diabetic treated rats. Similar results were obtained when male wistar rats were treated with natural flavonoid like Quercetin (Bouhali et al., 2015). This

demonstrates a high level of anxiety and a slowdown in motor function (Brands et al., 2005). It was reported that music is used as a therapy that modulates a combined predator and noise stress which induced anxiety-like behavior in male wistar rat (Attoui et al., 2015).

In terms of the distance traveled by crossed squares, data analysis showed a clear and significant difference in both diabetic and treated diabetic rats with hesperidin. In diabetic rats injected with hesperidin, the number of crossed squares was significantly ($P < 0.001$) higher than that of the control. Blood glucose in the presence of this antioxidant has returned to normal level as that of the control which confirmed the test results carried out by Ahmed et al. (2010). Similar results have been reported after treatment of *Ruta graveolens* and *rutin* with nicotinamide/streptozotocin diabetic rats (Gispén and Biessels, 2000).

After treating with hesperidin, there was an increase in the anxiety level which improved locomotor activity and recovery time during testing in open field. The same remark was made for treated diabetics (CHS) compared to control.

Hesperidin was reported to ameliorate the behavioral and biochemical indicators of mice thereby modulating the nitrenergic pathway (Viswanatha et al., 2012). These results support the findings of previous studies which showed an improvement in the number of entry and the time spent in the open arms of the treatment after repeated hesperidin. It is concluded that the present study showed a high level of anxiety, and a slowdown in locomotion and mental flexibility. Treatment with an antioxidant remarkably module the disorders related to diabetes (Bouhali et al., 2015). Thus our results confirm the capacity of hesperidin as an antioxidant which corrects neurobehavioral disorders as related to diabetes and its complications by neutralizing free radicals, of cell aging and apoptosis.

Conclusion

The obtained results of the open field tests on diabetic rats showed an increase in anxiety and a decrease in locomotion activity. The supplementation of hesperidin has controlled the neuro-degenerative symptoms. It is therefore suggested that, hesperidin can be used as a therapeutic complement against neuro-behavior disorders of stressed diabetic rats.

Conflict of Interests

The authors have not declared any conflict of interests.

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