Physiology and Pharmacology 26 (2022) 20-29
Original Article

Naringin ameliorates cognitive impairment in streptozotocin/nicotinamide induced type 2 diabetes in Wistar rats

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ABSTRACT

Introduction: Psychomotor slowing and reduced mental flexibility are symptoms of cognitive decline that can occur in type 2 diabetes disease (TD2). Strategies that combine the control of hyperglycaemia with prevention of cognitive decline are desirable. Thus, this study reports the effect of naringin on cognitive deficit in diabetic rats.

Methods: TD2 in Wistar rats was induced with nicotinamide/streptozotocin (NA/STZ). Naringin (50 and 100 mg/kg) or glibenclamide (5mg/kg) was administered for 30 days to diabetic rats. Cognitive performance was investigated using the Morris water maze. Serum glucose, lipid profiles, brain tumour necrosis factor alpha (TNF-α) and acetylcholinesterase (AChE) activity were determined.

Results: Naringin and glibenclamide significantly reduced the escape latency, increased the time spent in the correct quadrant and number of entries in diabetic rats. Also, naringin reduced blood glucose, serum cholesterol, low-density lipoprotein cholesterol levels, triglycerides and prevented a decrease in the level of high-density lipoprotein cholesterol, in diabetic rats. Naringin and glibenclamide treated diabetic rats showed a significant low levels of AChE activity and TNF-α.

Conclusion: Naringin ameliorates diabetes induced cognitive deficit via reduction of inflammation, hyperglycemia, hyperlipidaemia and AChE activity.

Keywords:
Naringin
Diabetes
Cognitive decline
Inflammation
Acetylcholinesterase

Introduction

Diabetes mellitus is a metabolic disorder which is characterized by hyperglycaemia accompanied by impaired metabolism of carbohydrates, lipids and protein (Baynes, 2015). Diabetes can be categorised broadly into insulin dependent diabetes (type 1/TD1), and insulin resistant diabetes (type 2/TD2). In 2019, 463 million cases of diabetes were recorded in the world with 10.2% and 10.9% estimated increase by 2030 and 2045 respectively (Saelens et al., 2019). TD2 is responsible for greater than 90% of the worldwide diabetes cases (Duarte, 2015). Uncontrolled hyperglycemia can results in long-term damage to the kidneys, liver, eyes, nerves, heart, brain and blood vessels.

In this study our interest is focused on the effect of diabetes on the brain which can be structural, neuro-

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Received 12 May 2020; Revised from 2 April 2021; Accepted 6 April 2021

Citation: Oyindamola O. A , Adewale G. B, Christiana T. A, Adebola I. K, Tolulope A. O, Tejumade S. U. Naringin ameliorates cognitive impairment in streptozotocin/nicotinamide induced type 2 diabetes in Wistar rats. Physiology and Pharmacology 2022; 26: 20-29. http://dx.doi.org/10.52547/phypha.26.1.10

physiological and neuropsychological (Brands et al., 2004). Imaging of the various region of the brain of TD2 patients revealed features similar to that of ageing person's brain suffering with cognitive decline (Biessels and Kappelle, 2005). This finding supports the study that reported that TD2 induces advanced brain aging resulting in cognitive performance deficits and increased risk of developing Alzheimer's disease (AD) (Biessels and Kappelle, 2005). AD is a neurodegenerative disorder, a form of dementia that affects memory, thinking and behaviour. AD is responsible for 60-80% of all cases of dementia (Dodel et al., 2013) and about 8% of dementia cases are attributed to TD2 (Kloppenborg et al., 2008). The increased incidence of AD in TD2 patients are linked to insulin resistance, hyperinsulinemia and hyperglycemia, in addition to the normally accompanying hypercholesterolemia, hypertension and obesity (Sima, 2010).

Strategies that combine the control of hyperglycaemia with prevention of cognitive decline is urgently needed to enhance diabetic patient's life quality. Here we evaluated the ability of naringin to protect against diabetes induced cognitive deficit in rats. Naringin, a flavonoid usually seen in some citrus fruits and grapefruit, is hydrolysed upon digestion to its aglycone, naringenin by intestinal bacteria (Owira and Ojewole, 2010; Ribeiro and Ribeiro, 2008). Anti-hyperglycemic effect of naringin has been previously reported (Ahmad et al., 2017; Jung et al., 2004). The potential of its hypoglycaemic and hypolipidemic activities have also been documented in experimental models (Bok et al., 2000; Jung et al., 2006). Naringin has been shown to have antiatherogenic, anti-oxidant, hepatoprotective, neuroprotective, anti-inflammatory, anti-cancer and anti-ulcer properties (Adil et al., 2014; Benavente-Garcia and Castillo, 2008; Choe et al., 2001). Its ability to regulate the transforming growth factor-β and tumour necrosis factor-α (TNF-α) expression which were implicated in the lung injury and pulmonary fibrosis pathogenesis had been documented (Chen et al., 2013). Its *in vivo* and *in vitro* potent cardioprotective and reno-protective activities had been established (Rajadurai and Prince, 2007; Singh and Chopra, 2004). However, information on the effect of naringin to protect against cognitive decline in nicotinamide/streptozotocin (NA/STZ) induced cognitive deficit in diabetic rats is sparse. Thus, the effect of naringin on cognitive deficit in diabetic rat is reported in this study.

Material and methods

Experimental animals

Male Wistar rats (180-200g) were purchased at the Institute of Advanced Medical Research and Training Animal house. They were acclimatized for 1 week and fed daily with regular rat chow from commercial source (ACE feed®, Ibadan) and water *ad libitum*.

The study was approved by both international (Publications of NIH volume 25 no.28. 1996 revised edition) and the Animal Care and Use Research Ethics Committee institutional rules (UI-ACUREC/17/0075).

Chemicals and reagents

Naringin, STZ and NA were procured from Sigma–Aldrich. Glibenclamide, glucose test strips (AccuChek™, Roche, Germany) were purchased from a reputable pharmacy store. Other chemicals used were obtained from local suppliers in Nigeria.

TD2 induction in Wistar rats

The Wistar rats were fasted overnight, subsequently TD2 was induced by administering 110mg/kg NA intraperitoneally in physiological saline 15min prior to intravenous injection of freshly prepared 65mg/kg STZ using 0.1mol/l (pH 4.5) cold citrate buffer. This model is based on the partial protection exerted by NA against the beta-cytotoxic effect of STZ (Masiello et al., 1998). Random blood glucose level (non-fasting) was done 72h after STZ injection. Rats with ≥200 mg/dl blood sugar levels were selected for the experiment. Thirty rats (24 diabetic and 6 non-diabetic) distributed into 5 groups (6 rats per group) received glibenclamide (5mg/kg), naringin (50, 100mg/kg) or 0.9%w/v saline (2ml/kg). Drugs were administered once daily for 30 days orally.

Evaluation of antidiabetic activity

Animals' body weight was measured every week. Glucometer was used to determine the random blood glucose before the administration of the drug and on day 30 post drug administration.

Naringin effect on cognitive function using Morris Water Maze (MWM) in diabetic rats

The MWM assessment on diabetic and non-diabetic rats was performed in accordance with a previously published method (Wang et al., 2015). The rats were trained before the commencement of treatment. Follow-

ing treatment with naringin, MWM assessment was performed on diabetic and non-diabetic rats at day 26 post treatment. Rats were trained from each group to swim freely in a pool of milky water containing a round 14cm diameter Plexiglas platform. The platform was firmly positioned, equidistant from the tank wall centre and immersed 1.5cm beneath the pool. Four training trials per day was done for each of the rat. The rats were placed at one of the four designated start point inside the tank daily, in a pseudorandom order and 4 days training were done to locate the platform. Rats that could not find the platform in 1min, were led manually to the platform and permitted for 15s to stay. On day 30 (24h after the last training), a probe trial was performed after the last training session which consist of a 1min free swim without the platform in the pool. Latency, total time spent in the quadrant containing the platform and numbers of entries into the quadrant were measured. After the experiment, the rats were placed in a warm environment.

Sample collection and preparation

Experimental rats were anaesthetized with ketamine/diazepam 30 days post treatment. Blood samples were gotten through cardiac puncture, allowed to clot and centrifuged (10min at 20,000g). Sera separated from packed blood were stored at –20°C till when needed for analysis. Samples from brain were collected and washed with cold phosphate buffer saline. It was further homogenized in 10% w/v of the buffered saline and centrifuged at 4°C for 10min at 1000g. The supernatant was stored at –20°C.

Determination of serum lipids profile

Cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) were assessed using biochemical kits (Fortress Diagnostic kit®) according to the manufacturer's instruction. Friedewald formula was also used to calculate LDL cholesterol (LDL-C): LDL-C= total cholesterol- (HDL cholesterol+ triglycerides/5) (Friedwald et al., 1972).

Acetylcholinesterase (AChE) activity determination

The brain AChE activity was evaluated based on previously reported method (Ellman et al., 1961). Twenty-five microliter of the rat brain homogenate was mixed with 125µl of 0.1M phosphate buffer (pH 7.4) and 25µl of 5,5′-dithiobis (2-nitrobenzoic acid). Subsequently, 25µl of acetylcholine iodide solution (20mM) was mixed with the reaction. Absorbance at 412nm using spectrophotometer was determined and the absorbance change was measured after two mins. The activity of AChE was presented as mol/min/mg tissue.

Determination of TNF-α in the experimental rats

Brain TNF-α level was assessed with Biolegend® asn say kits as instructed by the manufacturer.

Statistical analysis

Obtained data were presented as the mean±SEM. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Blood glucose of rats in the same group on days 0 and 30 were compared using paired t test. Likewise, the weights of the rats were also compared. All statistical analyses were done using Graph Pad Prism version 5.0 (La Jolla, CA, USA). $P<0.05$ was taken as significant.

Results

Effect on the blood glucose levels

The level of blood glucose in experimental rats is presented in Figure 1. Twenty-four rats were successfully induced with TD2 with evidence of raised blood glucose ranging from 244.67 ± 15.84 to 259.50 ± 14.83 mg/dl while non-diabetic control rats blood glucose was 116.50 ± 4.57 mg/dl ($P<0.001$). Following thirty days treatment with glibenclamide (5mg/kg) and naringin (50 and 100mg/kg), diabetic rats blood glucose was significantly reduced ($P<0.001$).

Effect on the body weight

Figure 2 shows the body weight of the animals. Both diabetic control and treated diabetic rats with selected doses of naringin or glibenclamide on days 0 and 30 have similar weight. In contrast, non-diabetic control rats significantly gained weight.

Cognitive function effect in diabetic rats

Naringin effect on latency to find platform in diabetic rats is presented in Figures 3a and b. Following training for 4 days the escape latency of diabetic rats treated with naringin or glibenclamide decreased compared to diabetic control rats (Figure 3a). By day 5, the probe day, 24h post training, the diabetic control group escape la-

TABLE 1: The effect of treatment with naringin on lipid profile of diabetic rats

Group (mg/kg)	Lipids (mg/dl)			
	Total cholesterol	**Triglyceride**	**HDL-C**	**LDL-C**
Non-diabetic control	47.38± 3.87	38.24± 3.71	39.32± 3.32	0.49± 0.12
Diabetic control	75.77±3.46#	107.0± 6.44#	26.56± 3.05#	26.39± 2.28#
Naringin (50)	55.58± 3.06*	68.13± 11.03*	35.73± 2.56*	6.17± 0.16*
Naringin (100)	60.89±1.11*	76.61± 8.50*	32.60± 4.43	13.11± 1.09*
Glibenclamide (5)	49.69±5.55*	55.70± 2.95*	35.86± 2.69*	2.57± 0.54*

N=6, data are represented as mean±SEM, #Non-diabetic group vs diabetic control group $P<0.05$; *diabetic control group vs all treatment groups $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

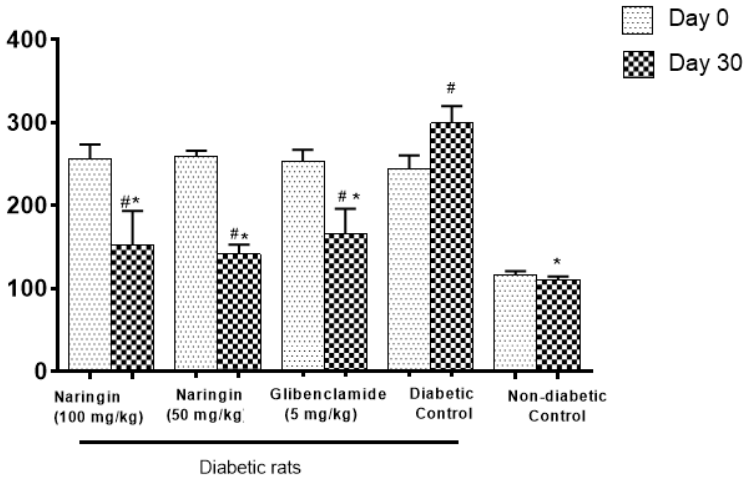

FIGURE 1: The effect of naringin on blood glucose levels in diabetic rats. N=6, data are represented as mean±SEM. *Day 30 diabetic control vs day 30 of all other groups, $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test), #Day 0 vs day 30 of each group $P< 0.05$ (paired t test).

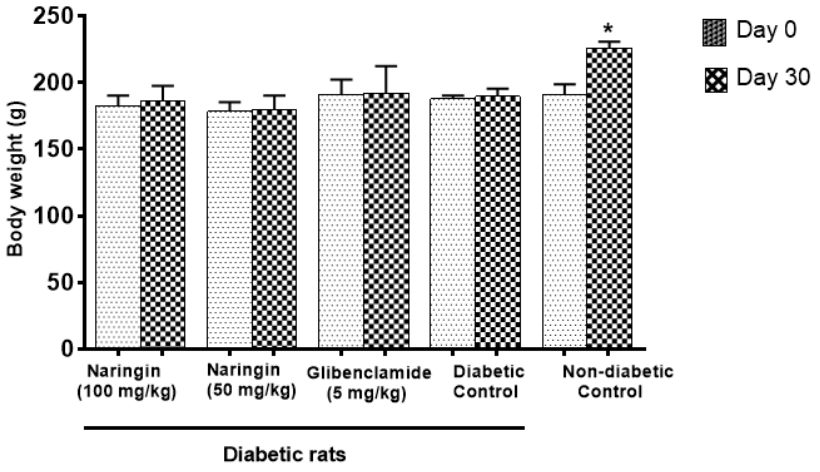

FIGURE 2: Body weight of rats before and after induction of diabetes. N=6, data are represented as mean±SEM. *Day 0 vs day 30 for each group $P<0.05$ (paired t test).

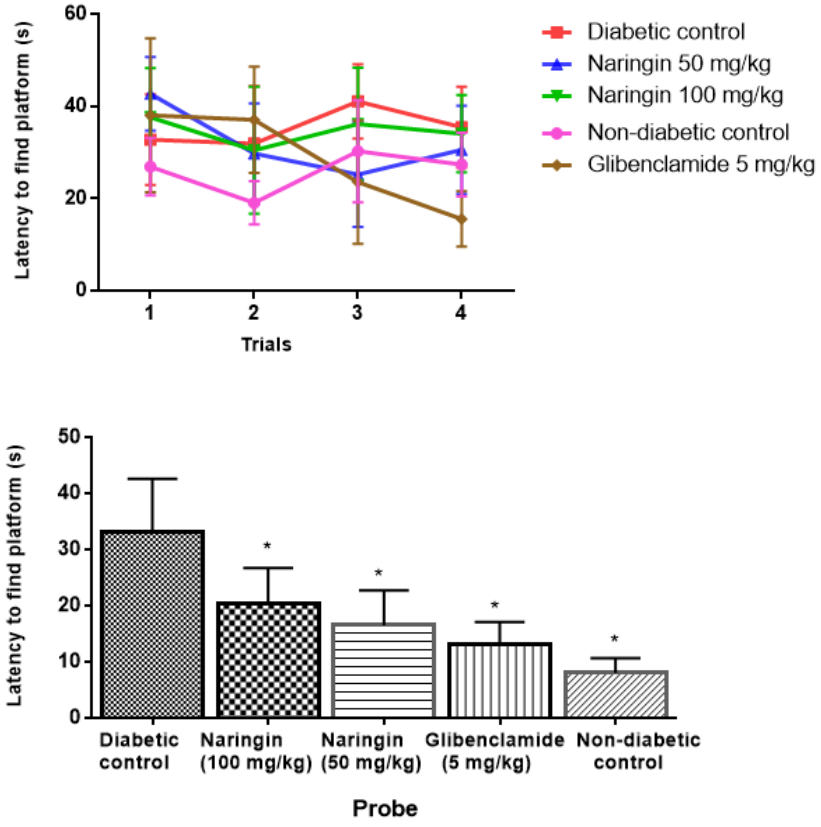

FIGURE 3: (A) Latency to find platform in diabetic rats during four days of training. N=6, data are represented as mean±SEM. (B) Latency to find platform decreases with treatment with naringin. N=6, data are represented as mean±SEM. *diabetic control vs all other groups, $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

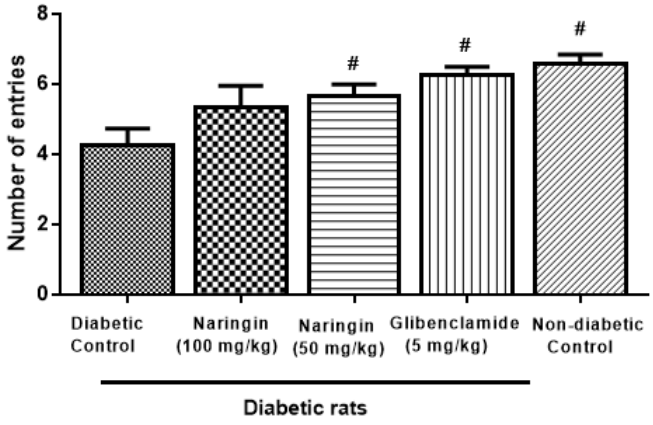

FIGURE 4: Naringin increases the number of entries in target quadrant in diabetic rats. N=6, data are represented as mean±SEM. #Diabetic control vs all other groups $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

tency was markedly increased in comparison to non-diabetic control group ($P=0.0001$). Glibenclamide (5mg/kg) or naringin at 50 and 100mg/kg significantly decreased diabetic rats escape latency (Figure 3b; $P\leq0.02$). Furthermore, the number of entries into the quadrant and the time spent in correct quadrant were significantly decreased in diabetic control rats in contrast to non-diabetic control rats (Figs 4 and 5; $P\leq0.0002$). Treatment of diabetic rats with naringin or glibenclamide increased the entry numbers and the time spent in correct quadrant ($P\leq0.002$).

Effect on serum lipids profile

Serum lipid profile of the experimental rats is present-

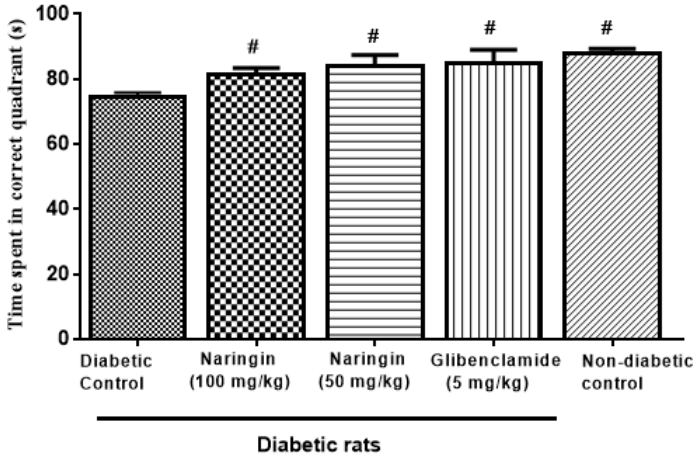

FIGURE 5: Naringin increases time spent in correct quadrant in diabetic rats. N=6, data are represented as mean±SEM. #Diabetic control vs all other groups $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

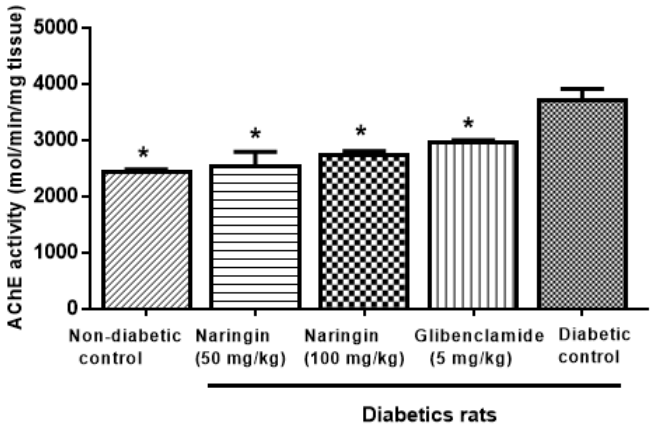

FIGURE 6: Naringin reduces acetylcholinesterase (AChE) activity in the brain of diabetic rats. N=6, data are represented as mean±SEM. #Diabetic control vs all other groups $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

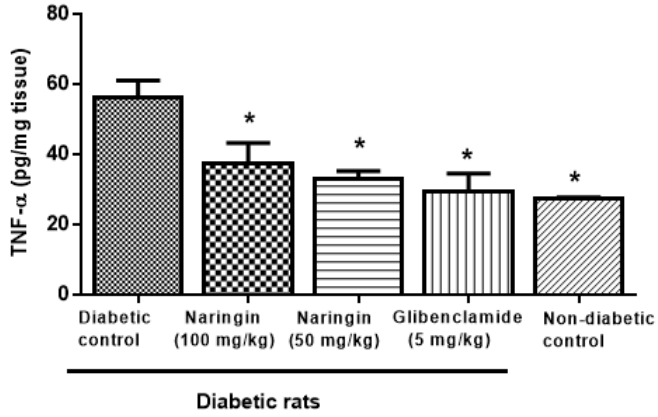

FIGURE 7: Naringin reduces brain TNF- α levels in diabetic rats. N=6, data are represented as mean±SEM. #Diabetic control vs all other groups $P<0.05$ (one-way ANOVA test followed by Tukey post hoc test).

ed in Table 1. Diabetic control rats showed a significant HDL-C reduction, while the triglycerides, cholesterol and LDL-C increased compared to the non-diabetic control rats ($P\leq0.0001$). However, naringin (50 and 100 mg/kg) and glibenclamide (5mg/kg) treatment resulted in a significant low level of cholesterol, triglycerides and LDL-C compared to diabetic control rats ($P\leq0.0082$). Naringin (50mg/kg) and glibenclamide prevented re-

duction in HDL-C level.

Effect on AChE activity in diabetic rats

AChE activity in diabetic control rats was significantly increased (3704.21 ± 210.61 mol/min/mg tissue) in comparison to non-diabetic control rats (2440.4 ± 39.28 mol/min/mg tissue; $P<0.0001$; Figure 6). Following naringin and glibenclamide treatment, AChE activity decreased significantly ($P<0.0001$; Figure 6).

Effect on TNF-α levels in diabetic rats

Brain TNF-α increased significantly in diabetic control rats (56.0±5.0 pg/mg tissue) compared to non-diabetic control rats (27.28±0.42 pg/mg tissue; $P=0.0002$; Figure 7). Treatment with 50 and 100mg/kg naringin (33.07±2.17 and 37.44±5.79 pg/mg tissue) and 5 mg/kg glibenclamide (29.24±5.27 pg/mg tissue) prevented the elevation of brain TNF-α significantly compared to the diabetic control rats ($P\leq0.04$).

Discussion

Diabetes-associated cognitive decline is a neurodegenerative disorder that can lead to dementia and about 8% of dementia cases are attributed to TD2 (Kloppenborg et al., 2008). Strategies that combine the control of hyperglycaemia with prevention of cognitive decline will enhance the quality life of diabetic patients. Thus, the capacity of naringin to protect against diabetic induced cognitive deficit in rats was evaluated in this study.

TD2 induction in experimental rats was achieved by using NA/STZ which resulted in moderate pancreatic β-cells destruction, causing insulin release impairment and subsequent insulin resistance (Masiello et al., 1998). A rise in the level of serum glucose in diabetic rats as opposed to non-diabetic rats, 72h after TD2 induction using NA/STZ observed in this study is similar to previously reported study (Ahmed et al., 2017). Previous studies reported that naringin treatment decreased glucose level in diabetic rats (Ahmed et al., 2017; Jung et al., 2004). Reduced hyperglycaemia observed in rats treated with naringin might prevent the adverse effects of hyperglycaemia on the brain and its vasculature. Hyperglycemia is a central feature of TD2 and may induce changes in cognitive function through a variety of mechanisms including polyol pathway activation, increased formation of advanced glycation end products, diacylglycerol activation of protein kinase C and increased glucose shunting in the hexosamine pathway (Madonna and Caterina, 2011). Glucose, the primary substrate for brain energy metabolism is not stored in the neurons but transported across the blood brain barrier (Jurcovicova, 2014). In insulin resistance diabetes, the brain may not get enough glucose it needs especially for memory because insulin's signal is ignored by cells. Insulin also regulates expression of the neurotransmitters acetylcholine and norepinephrine, both of which are known to influence cognition (Kopf and Baratti, 1999; Rosen et al., 1993). Furthermore, insulin acts to increase cortical cerebral glucose metabolism in brain regions important for learning and memory (Madsen et al., 2002). These many influences of insulin are compromised in insulin resistance state. This is because, there is reduced ability of insulin to exert its action on target tissues which can be associated with neuropathological processes that underlie cognitive aging and dementia (Craft et al., 2013). In this study, cognitive function in diabetic rats treated with naringin was assessed using MWM. The MWM is used to determine learning and spatial memory (Bromley-Brits et al., 2011; Morris, 1981). Naringin reduced the latency time and significantly prolonged the time spent in the correct quadrant containing the platform. There was increase in the number of entries into the correct quadrant in comparison with untreated diabetic control group. Thus, naringin and glibenclamide protects diabetic rats from impaired cognitive performance.

AD and TD2 share the presence of systemic and neuro-inflammation, enhanced production and accumulation of β-amyloid peptide and abnormal levels of the enzymes AChE and butyrylcholinesterase (Mushtaq et al., 2014). Acetylcholine, a neurotransmitter associated with learning and memory, is degraded by the enzyme AChE, terminating the physiological action of the neurotransmitter. Learning disabilities and memory loss have been associated with reduced level of brain acetylcholine (Haam and Yakel, 2017). The inhibition of AChE will enhance cholinergic transmission and lowers the amyloid beta peptide aggregation (Carvajal and Inestrosa, 2011). A reduction in the AChE activity could increase synaptic cleft acetylcholine level and might ameliorate cognitive symptoms partially (Carvajal and Inestrosa, 2011), thus improving the quality life of patients with cognitive impairment

Chronic inflammation is suggested in the pathophysiology of TD2 (Pollack et al., 2016). In addition, astro-

cytes are vulnerable to inflammatory attack and can release inflammatory factors like TNF-α and IL-6 upon activation to trigger neuroinflammation (Namas et al., 2009). The release of TNF-α will potentiate the inflammatory response resulting in pancreatic β-cells destruction (Lin et al., 2012). Thus, leading to insulin resistance (Lin et al., 2012; Stephens et al., 1997) and thereby worsen hyperglycemia-induced brain injury. Agents that target inflammation and reduce hyperglycaemia may improve diabetes by preventing diabetes progression and vascular complications (Pollack et al., 2016). In this study, naringin and glibenclamide markedly decreased the brain TNF-α level in NA/STZ induced diabetic rats. Naringin has been shown by previous reports to inhibit inflammatory process in diabetic rat and mice (Chen et al., 2015; Liu et al., 2016).

Elevation of lipids seen in the serum of untreated diabetic rats might be due to abnormal lipid metabolism. The lipoprotein lipase dysfunction will contribute to hypertriglyceridemia in insulin deficient state due to catabolism of triglyceride rich particles impairment (Dallinga-Thie et al., 2016). Insulin is believed to elevate receptor mediated LDL- cholesterol removal. Thus, the reduced insulin activity in diabetes will lead to high serum LDL-cholesterol level (hypercholesterolemia) (Saravanan and Pari, 2005). Naringin at the doses tested reduced significantly triglycerides, total cholesterol and LDL-cholesterol in diabetic rats. Reduction in HDL-C level was also prevented. It has been shown that naringin and its metabolites naringenin might possess insulinotrophic and insulin sensitizing actions (Ahmad et al., 2017).

Conclusion

The present study showed that naringin significantly reduced hyperglycaemia, hyperlipidaemia and ameliorated diabetes induced cognitive deficits via reduction of AChE activity and inhibition of inflammation in NA/STZ induced diabetes in rats. Naringin might be useful in preventing type 2 diabetes cognitive function impairment.

Acknowledgments

Technical assistance of Ibrahim, of the Department of Physiology and Remi Sosanya and Nora Nwadike of Pharmacology and Therapeutics Department is appreciated.

Conflict of interest

No potential conflict of interest.

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