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Role of aldose reductase and arginase inhibitors in diabetic vascular and behavioral complications.

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ABSTRACT

Diabetes mellitus is an endocrine disorder that is associated with several microvascular and macrovascular complications in addition to complications within the central nervous system (CNS). Diabetic encephalopathy secondary to chronic hyperglycemia is mediated through oxidative stress, increased advanced glycation end products (AGEs), and impairment in cerebral insulin signaling.

The Aim of the work was to investigate whether inhibition of aldose reductase and arginase enzyme can protect against vascular and behavior complications.

Diabetes was induced in male wistar rats by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg). Eight weeks later, diabetic rats were orally treated with ferulic acid (20 mg/kg), cinnamaldehyde (20 mg/kg), norvalline (50 mg/kg), ornithine (200 mg/kg) and citrulline (50 mg/kg) every day for 8 weeks. Body weight, blood glucose, serum AGEs level, blood pressure and behavioral change in memory and cognition were investigated at the end of the study.

Streptozotocin caused a state of hyperglycemia associated with both vascular and behavioral complications as evidenced by the elevation in blood pressure and reduction in the Y-maze score and elevation in the transfer latency in the elevated plus maze. Blockade of aldose reductase and arginase enzyme ameliorated some of these complications without exerting any hypoglycemic effect.

These results suggest the possible effectiveness of aldose reductase and arginase inhibitors in the management of diabetic vascular and behavioral complications together with conventional antidiabetic therapy.

Key words: diabetes, complications, aldose reductase, arginase, inhibitors, protection.

INTRODUCTION

Diabetes mellitus is an endocrine disorder resulting from inadequate insulin release and/or reduced insulin sensitivity. Diabetes-induced microvascular changes can alter the diameter of resistance arteries thereby affecting cardiac output and the distribution of blood flow (Song *et al*., 2008). The complications of diabetes are well characterized in peripheral tissues and there is a growing appreciation that the complications of diabetes extend to the central nervous system (CNS) (Reagan, 2012).

In diabetes, the gradual development of complications in the CNS is termed "diabetic encephalopathy" which is characterized by brain neurophysiological and structural changes, leading to impairment of cognitive function (Taurino *et al*., 2012).

Diabetes-related cognitive dysfunction is a consequence of changes within CNS that are secondary to chronic hyperglycemia (Malone *et al*., 2006). The cerebrovascular changes, oxidative stress, increased advanced glycation end products (AGEs), and impairment in cerebral insulin signaling are thought to be the underlying causes for diabetic dementia (Bhutada *et al*., 2010).

The polyol pathway which is responsible for reducing glucose to sorbitol via aldose reductase enzyme (AR) (He *et al*., 2011) is activated in diabetic conditions resulting in accumulation of sorbitol (SOR) (Nakano *et al*., 2003). Osmotic stress due to accumulation of sorbitol and oxidative stress due to changes in the ratio of NADPH/NADP are major causes of various complications of diabetes (Srivastava *et al*., 2005).

Ferulic acid is a natural polyphenol which is known to inhibit AR and has antioxidant, hypotensive, and anti-inflammatory properties (Badawy *et al*., 2013). Cinnamaldehyde is another AR inhibitor (Lee, 2002) which has antioxidant, anti-inflammatory, immunomodulatory(Chao *et al*., 2008) , and antidiabetic actions (Zhang *et al*., 2008).

Activation of arginase enzyme in diabetes (Jiang *et al*., 2003) leads to competition with endothelial nitric oxide synthase (NOS) for the common substrate arginine (Romero *et al*., 2008), which results in the decrease in NO (Kubes *et al*., 1991) and increase in superoxide production (Caldwell *et al*., 2010), ultimately causing several diabetic complications such as vascular and endothelial dysfunction (Romero *et al*., 2008).

L-norvaline, L-citrulline and L-ornithine are amino acids that work as arginase inhibitors (El-Bassossy *et al*., 2012b).

The present study was designed to investigate the impact of diabetes on animal behavior and also the possible role of aldose reductase and arginase inhibitors in ameliorating these diabetic complications.

MATERIALS and METHODS

Animals

Adult male wistar rats weighing 170±20 g were obtained from the National Research Institute (Cairo, Egypt) and housed in clear polypropylene cages (5 rats per cage) and kept on a 12/12 light –dark cycles under constant environmental conditions. Rats were fed normal pellet diet and water ad libitum. Experimental design and animal handling procedures were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAHZU).

Study protocol

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) in cold normal saline (Ramanathan *et al*., 1998). Diabetes was confirmed by stable hyperglycemia (≥ 300mg/dl) after 3 days of STZ injection. Rats were given 8 weeks after STZ injection to develop vascular complications (Badawy *et al*., 2013). Then rats were randomly distributed among eight experimental groups (n = 6) and were orally treated with ferulic acid (20 mg/kg) (Badawy *et al*., 2013), gliclazide (10 mg/kg), cinnmaldehyde (20 mg/kg) (El-Bassossy *et al*., 2011) daily as a suspension in 0.5% carboxy methyl cellulose (CMC), citrulline (50 mg/kg), norvaline (50 mg/kg) or ornithine (200 mg/kg) daily in distilled water (El-Bassossy *et al*., 2012a) for 8 weeks.

Twelve hrs after the last injection, body weight, blood glucose were measured (Glucometer Bionime GM100 Blood Glucose Meter) and blood pressure was recorded (Power Lab 26T, LTS) in a conscious and slightly restrained rat by tail cuff method as previously described (El-Bassossy *et al*., 2012a). Behavior changes were assessed in Y-maze and elevated plus maze. Performance in Y-maze was recorded as score; 0: no entrance to target arm,1: enter target arm only & stay in it, 2: enter non-target arm first then target arm, 3: enter target arm first and pass three arms in more than four minutes, 4: enter target arm first and pass three arms within four minutes, 5: enter target arm first and pass three arms in less than one minute (Baluchnejadmojarad and Roghani, 2011; Nasri *et al*., 2012). Performance in the elevated plus maze was investigated and transfer latency

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(time taken to move from open arm to closed arm) was recorded in seconds (Abraham *et al*., 2010; Rajashree *et al*., 2011). Then Blood was collected from the retro-orbital plexus under topical ophthalmic anesthetic and centrifuged at 3000 × g, 4 °C, 20 min (HERMLE Z326K®) and serum was analyzed for advanced glycation end products (AGE) fluoremetrically (Munch *et al*., 1997; Sampathkumar *et al*., 2005) at excitation wavelength 370 nm, and emission at 445 nm by LS45 fluorescence spectrophotometer (PerkinElmer®).

Drugs and chemicals

STZ, ferulic acid, cinnamaldehyde, L-norvalline, L-citrulline and L- ornithine were purchased from Sigma-Aldrich (Germany).

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's post Hoc test at $P < 0.05$ using Graphpad Prism software®.

RESULTS

Body weight

Diabetes was associated with a significant decrease in body weight after 4 month in comparison to control rats (204 vs 288.3 gm). Oral administration of ferulic acid, cinnamaldehyde, norvalline, cirtulline, ornithine and gliclazide did not cause any significant change in body weight (fig 1a).

Blood glucose

Figure 1b shows a significant increase in blood glucose in diabetic rats after two and four months of STZ injection in comparison to control rats (440 and 514.7 vs 112.2 mg/dl respectively). Meanwhile, treatment with ferulic acid, cinnamaldehyde, norvalline, cirtulline, ornithine and gliclazide failed to cause any significant change in blood glucose.

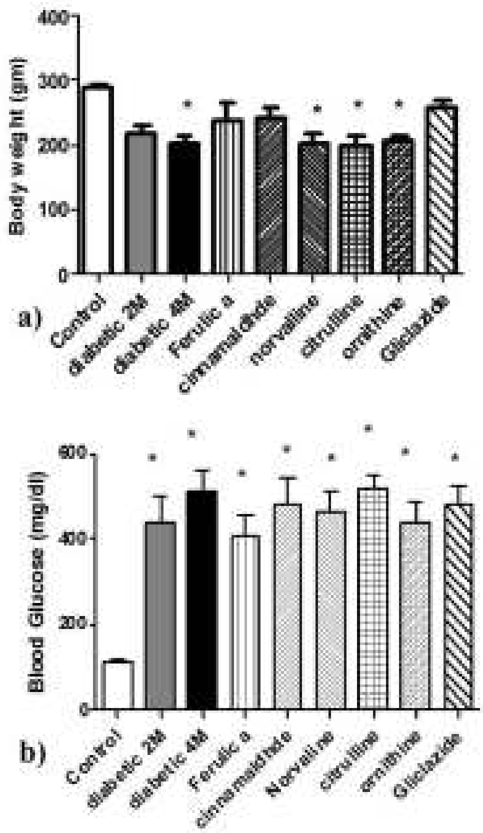

Figure 1. effect of diabetes and treatment with ferulic acid (20mg/kg), cinnamaldehyde (20mg/kg), norvalline (50mg/kg), cirtulline (50mg/kg), ornithine (200mg/kg) and gliclazide (10mg/kg) on: a) body weight and b) blood glucose level.

Data are expressed as mean ± SEM, n = 6.
* significantly different from control group at $P<0.05$ using one way analysis of variance (ANOVA) followed by Tukeys Post Hoc test.

Blood pressure

Figure 2 shows that 4 month of diabetes caused a significant increase in systolic and diastolic blood pressure in comparison to control rats (135.3 vs 114 mmHg and 106.6 vs 73.83 mmHg respectively). Oral administration of gliclazide lead to a significant reduction in systolic and diastolic blood pressure in comparison to diabetic rats (117.7 vs 135.3 mmHg and 77.5 vs 106.6 mmHg respectively) as shown in Figure 2a and b. Oral administration of ferulic acid and

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ornithine lead to a significant reduction in diastolic blood pressure in comparison to 4 month diabetic rats (80.75 and 80 vs 106.6 mmHg respectively) as shown in Figure 2b.

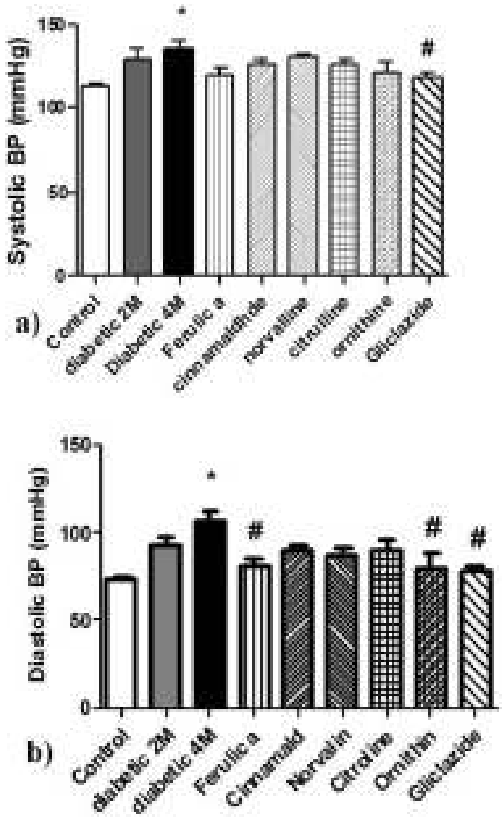

Figure 2. effect of diabetes and treatment with ferulic acid (20mg/kg), cinnamaldehyde (20mg/kg), norvalline (50mg/kg), cirtulline (50mg/kg), ornithine (200mg/kg) and gliclazide (10mg/kg) on: a) systolic blood pressure, b) diastolic blood pressure.
Data are expressed as mean ± SEM, n = 6.
* significantly different from control group, # significantly different from diabetic group at P<0.05 using one way analysis of variance (ANOVA) followed by Tukeys Post Hoc test.

Behavioural changes

Y-maze

Figure 3 shows that 4 months of diabetes induced a significant decrease in Y-maze score in comparison to control rats (1.5 vs 4.7), while treatment with ferulic acid, cinnamaldehyde, citrulline, ornithine and gliclazide lead to a significant increase in Y-maze score in comparison to 4 month diabetic rats (4, 4.7, 4.2, 5 and 4.2 vs 1.5 respectively).

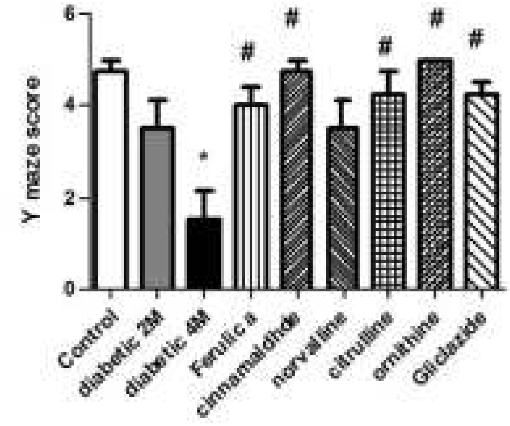

Figure 3. effect of diabetes and treatment with ferulic acid (20mg/kg), cinnamaldehyde (20mg/kg), norvalline (50mg/kg), cirtulline (50mg/kg), ornithine (200mg/kg) and gliclazide (10mg/kg) on score in Y maze.
Data are expressed as mean ± SEM, n = 6.
* significantly different from control group, # significantly different from diabetic group at P<0.05 using one way analysis of variance (ANOVA) followed by Tukeys Post Hoc test.

Elevated plus maze

Figure 4 shows that 4 months of diabetes caused a significant increase in the transfer latency in the elevated plus maze in comparison to control rats (45 vs 3.6 seconds), while treatment with ferulic acid, cinnamaldehyde, norvalline, citrulline, ornithine and gliclazide lead to a significant decrease in the transfer latency in comparison to 4 month diabetic rats (8, 13, 15, 21, 12, 21 vs 45 seconds respectively).

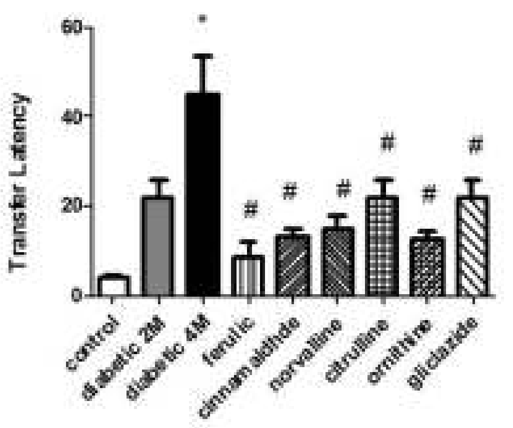

Figure 4. effect of diabetes and

treatment with ferulic acid (20mg/kg), cinnamaldehyde (20mg/kg), norvalline (50mg/kg), cirtulline (50mg/kg), ornithine (200mg/kg) and gliclazide (10mg/kg) on transfer latency of rats.

Data are expressed as mean ± SEM, n = 6. * significantly different from control group, # significantly different from diabetic group at P<0.05 using one way analysis of variance (ANOVA) followed by Tukeys Post Hoc test

Advanced glycation end products (AGEs)

Figure 5 shows that, two and four months diabetic rats had significant increase in serum AGE level in comparison to control rats (276 and 584 vs 161 U/ml respectively). Treatment with ferulic acid, cinnamaldehyde, norvalline, citrulline, ornithine and gliclazide caused a significant decrease in serum AGE level in comparison to 4 month diabetic rats (332, 194, 236, 180, 206 and 219 respectively vs 584 u/ml).

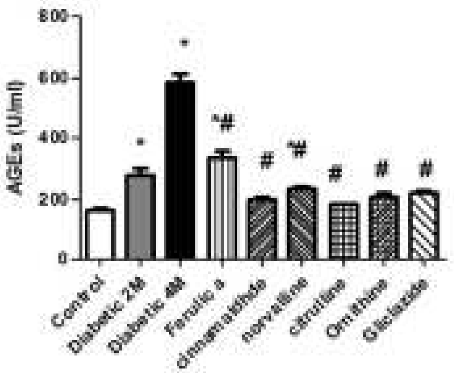

Figure 5. effect of diabetes and treatment with ferulic acid (20mg/kg), cinnamaldehyde (20mg/kg), norvalline (50mg/kg), cirtulline (50mg/kg), ornithine (200mg/kg) and gliclazide (10mg/kg)) on seum level of advanced glycation end products (AGEs).

Data are expressed as mean ± SEM, n = 6.

* significantly different from control group, # significantly different from diabetic group at P<0.05 using one way analysis of variance (ANOVA) followed by Tukeys Post Hoc test

DISCUSSION

Diabetes mellitus (DM) is the most common endocrine disorder and currently affects more than 347 million people worldwide (Huang *et al.*, 2012).It has been well accepted that diabetes results in microvascular and macrovascular complications such as retinopathy, peripheral neuropathy, stroke and coronary heart disease and can also cause complications within the central nervous system (CNS) (Wrighten *et al.*, 2009).

In the current study, diabetes induced in rats by STZ injection caused a significant decrease in body weight after 16 weeks. These findings keep pace with previous studies in diabetic rats (Guglielmotto *et al.*, 2012; Michea *et al.*, 2001). Treatment with aldose reductase inhibitors (ferulic acid, cinnamaldehyde) or arginase inhibitors (citrulline, norvaline, ornithine) for 8 weeks had no effect on body weight.

In the present study, rats subjected to intraperitoneal STZ injection showed increase in blood glucose after 8 and 16 weeks. Similar results were previously reported following STZ injection (Di Filippo *et al.*, 2005; Elsner *et al.*, 2000; Reagan *et al.*, 2000). Treatment with aldose reductase inhibitors (ferulic acid, cinnamaldehyde) or arginase inhibitors (citrulline, norvaline, ornithine) had no action on blood glucose level which indicates that these compounds lack any hypoglycemic action.

Diabetes mellitus is a systemic disease that can cause complications involving both small and large vessels, cranial and peripheral nerves, skin, and eyes. These lesions may lead to hypertension, renal failure, vision loss, neuropathy, myocardial infarction and stroke (J *et al.*, 2009).

The present work demonstrated that diabetes was associated with elevation in blood pressure. Similar results were previously reported following induction of diabetes (Liu *et al*., 1998). Treatment with ferulic acid lead to significant reduction in blood pressure. Similar results were previously reported (Badawy *et al*., 2013; Choi *et al*., 2011). It was shown that plasma angiotensin converting enzyme (ACE) was reduced after administration of ferulic acid, which in turn would reduce the blood pressure (Ardiansyah *et al*., 2008).

Treatment with ornithine lead to significant reduction in blood pressure as shown previously (El-Bassossy *et al*., 2012a). Reducing arginase activity via dietary manganese deficiency was reported to enhance endothelium-dependent vasorelaxation of rat aorta (Ensunsa *et al*., 2004). A previous study has demonstrated enhanced NO generation by arginase inhibition (Santhanam *et al*., 2008). Arginase inhibition restores L-arginine for eNOS and limits NO inactivation by superoxide anion which could explain the protective effect of arginase inhibitors against impairment in endothelium-function and reactive oxygen species (ROS) and AGEs levels were significantly decreased following arginase inhibition (Wautier *et al*., 2001).

The reduction in blood pressure without alteration in blood glucose level implies that these drugs might be useful in the prevention of diabetes vascular complications without alteration in blood glucose level.

Oxidative stress, causes quenching of nitric oxide by free radicals and prevents its vasodilator effect thus promoting endothelial dysfunction (Salman and Inamdar, 2012). Gliclazide is a general free radical scavenger, that was suggested to increase the bioavailability of nitric oxide (Salman and Inamdar, 2012; Sena *et al*., 2009). Treatment with gliclazide lead to significant reduction in blood pressure. Similar results were previously reported (Belcher *et al*.; De Mattia *et al*., 2003).

Diabetes was reported to accelerate the brain aging process, and reduce cognitive reserve and the threshold for the development of Alzheimer's disease symptoms (Gasparini and Xu, 2003). Diabetes and insulin resistance were shown to accelerate biological aging by fostering the formation of AGEs and ROS (J *et al*., 2009). Rats with streptozotocin (STZ)-induced diabetes were previously reported to have reduced nerve fiber diameter and myelin width. These structural abnormalities have been associated with hyperglycemia and increased activity of the polyol pathway as indicated by increased tissue sorbitol levels (Malone *et al*., 1996). Increased nerve sorbitol is associated with reduced concentration of taurine that plays an important role in stimulating neuronal growth during development and regeneration of the central nervous system (Malone *et al*., 2006).

In the present study, diabetic rats showed a decrease in Y-maze score which indicates a deficit in memory and cognition. Similar results were previously reported following induction of diabetes (Kumar *et al*., 2011; Nitta *et al*., 2002). Also the current study showed that diabetic rats exhibited a decrease in memory and cognition as evidenced by the increase in the transfer latency in the elevated plus maze. Similar results were previously reported following induction of diabetes (Xue *et al*., 2012).

Previous studies have shown that, chronic hyperglycemia is associated with the activation of aldose reductase (AR), an increase in cytokines such as TNF-α and IL-8 and oxidative stress which may be responsible for the diabetes-induced cardiovascular diseases such as atherosclerosis and hypertension (Blann and McCollum, 1998; Elkind *et al*., 2002).Treatment with the AR inhibitors as sorbinil or tolrestat, attenuated NF-kB activation and proliferation of cultured vascular smooth muscle cells (VSMC) (Kim *et al*., 2012). Inhibition of AR also prevented protein kinase C (PKC) activation by TNF-α which indicates a pivotal role of AR in the mitogenic signals initiated by cytokines that are elevated in diabetes and its complications (Ramana *et al*., 2003).

Treatment with ferulic acid lead to significant increase in Y-maze score suggesting an improvement in memory and cognition as previously reported (Mohmmad Abdul and Butterfield, 2005). Ferulic acid was shown to provide neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury in rats and attenuated the amyloid-beta-peptide induced memory impairment. Hence, ferulic acid may enhance learning ability and memory function (Cheng *et al*., 2008).

Several studies have demonstrated reduced expression of insulin receptor and related members of the insulin signaling pathway in patients and animals suffering from impaired brain function and Alzheimer's disease (AD) which illustrate that insulin and insulin signaling mechanisms are important for neuronal survival (Anderson *et al*., 2013; McNay and Recknagel, 2011). Treatment with cinnamaldehyde lead to a significant increase in Y-maze score suggesting an improvement in memory and decrease in dementia associated with diabetes. Similar results were previously reported (Frydman-Marom *et al*., 2011; George *et al*., 2013). This might be attributed to the effectiveness of cinnamaldehyde in improving insulin function in addition to its antioxidant and anti-inflammatory actions (Qin *et al*., 2010). In addition, cinnamaldehyde has been shown to alleviate factors associated with Alzheimer's disease and memory loss (Anderson *et al*., 2013).

Treatment with ornithine lead to a significant increase in Y-maze score. Ornithine was shown to enhance rat exploration of a new environment as a result of glutamate/GABA balance restoration (Moinard *et al*., 2004).

Treatment with gliclazide lead to significant increase in Y-maze score. These results are in accordance with previous studies which demonstrated that gliclazide acts by stimulating peroxisome proliferator activated receptor gamma (PPAR-γ) and exerts anti-amyloidogenic, anti-inflammatory, and insulin sensitizing effects, which may play a role in delaying and reducing the risk of neurodegeneration(Alagiakrishnan *et al*., 2013).

Treatment with ferulic acid and cinnamaldehyde lead to significant decrease in transfer latency in the elevated plus maze suggesting an improvement in memory and cognition. Similar results were previously reported following induction of diabetes (Anderson *et al*., 2013; Vijayalakshmi *et al*., 2012). In addition, treatment with norvalline, ornithine and citrulline lead to a significant decrease in transfer latency in the elevated plus maze. Similar results were previously reported (Kurata *et al*., 2011; Li *et al*., 2014; Lopez-Jaramillo and Teran, 1999). A similar improvement in memory and cognition was observed in rats treated with gliclazide as evidenced by the decrease in transfer latency in the elevated plus maze. Similar results were previously reported following induction of diabetes (Strachan, 2005).

Chronic hyperglycemia and hyperinsulinemia stimulate the formation of AGEs, which leads to an overproduction of ROS which are the two main mechanisms involved in biological aging and probably the etiopathogeny of Alzheimer's disease (J *et al*., 2009).

In the current study, diabetic rats showed an elevation in serum AGEs level. Similar results were previously reported following induction of diabetes (Cardoso *et al*., 2012; Guglielmotto *et al*., 2012). Several studies have demonstrated the involvement of AGE in micro- and macrovascular complications of diabetes (Radoi *et al*., 2012).

Treatment with ferulic lead to a significant decrease in serum AGEs. These findings keep pace with previous studies (Castillo and Silván, 2011; Sompong *et al*., 2013). Ferulic acid was shown to reduce the formation of AGEs by acting as an antioxidant, binding amino groups, and inhibiting sugar autoxidation and early Maillard Reaction Products (MRP) degradation (Odjakova *et al*., 2012). Treatment with cinnamaldehyde lead to a decrease in AGEs level as previously described (Kumar *et al*., 2012). Cinnamaldehyde was shown to inhibit the formation of AGEs through its antioxidant and anti-inflammatory actions (Ho and Chang, 2012). Treatment with norvalline, citrulline and ornithine lead to decrease AGEs level as shown previously (El-Bassossy *et al*., 2012a). This might be attributed to the decrease in ROS production. Decrease in AGEs level independent of glucose level has been reported in previous studies (Wautier *et al*., 2001). Treatment with gliclazide caused a significant decrease in AGEs level through its antioxidant action as described previously (Mamputu and Renier, 2002; Mamputu and Renier, 2004).

In conclusion, Long term diabetes is associated with vascular and behavioral complications including hypertension and dementia. Delayed blockade of aldose reductase and arginase enzyme (after 2 month of diabetes in rats) was effective in reducing these complications which suggests the possible use of these inhibitors to manage diabetic complications together with conventional antidiabetic therapies. However, careful analysis of the exact mechanism associated with this action is required.

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دور مثبطات انزيمى الدوزردكتاز والارجيناز فى المضاعفات الدموية والسلوكية لمرض السكرى

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داء السكري هو اضطراب الغدد الصماء مقترن بمضاعفات عدة في الاوعية الدموية الدقيقة و الكبيرة بالإضافةإلى مضاعفات في الجهاز العصبي المركزى.السكري هى اعتلال الدماغ الثانوية لارتفاع السكر المزمن في الدم وزيادة الاكسدة ومنتجات الجليكاشن النهائية المتقدمه وضعف إشارات الأنسولين في الدماغ والهدف من اجراء هذه الدراسة التحقق فيما إذا كان تثبيط انزيمى الدوز ردكتاز والارجيناز يمكن أن يحمي ضد مضاعفات الأوعية الدموية والمضاعفات السلوكية الناتجه عن مرض السكرى.

وقد تمت هذه الدراسة عن طريق حقن الفئران الستربتوزوتوسين بجرعة ٥٠ مجم \ كجم وبعد ثمانية أسابيع، تم علاج الجرذان المصابة بداء السكري عن طريق الفم بحمض الفريوليك او زيت السينمالدهيد بجرعة ٢٠مجم \ كجم او الأورنيثين بجرعة٢٠٠ مجم \ كجم اوسيترولين اونورفالين بجرعة ٥٠ مجم \ كجم يوميا لمدة ثمانية أسابيع اخر ثم حساب الوزن ، ونسبة السكر وضغط الدم ومنتجات الجليكاشن النهائية المتقدمه وتغيير السلوك في الذاكرةوالإدراك.

وقد اثبتت الدراسة ان السكرى تودى الى مضاعفات فى الاوعية الدموية والسلوك للفئران تتضح من ارتفاع في ضغط الدم وانخفاض في درجة الوامز و زيادة الكمون فى المناطق المرتفعة وتشير الى ان استخدام مثبطات انزيمى الدوز ردكتاز والارجيناز يودى الى تحسين هذة النتائج دون التاثير على مستوى السكر فى الدم وهذه النتائج تشير إلى فعالية هذة المثبطات فى تحسين الأوعية الدموية السكري والمضاعفات السلوكية جنبا إلى جنب مع العلاج التقليدي المضادة لمرض السكرى.