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Effect Of Some Leafy Spices (Thymus Vulgaris, Murraya Koenigii And Mentha Spicata) On Some Neuronal Enzymes In The Brain Tissue Of Streptozotocin (STZ)-Induced Diabetic Rats

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Abstract: ***Alzheimer disease is a multi-etiology disorder and the risk factors in non-modifiable factors such as age and genetics, as well as modifiable factors such as dietary and lifestyle choices, co-existing health problems and education. However, the specific mechanistic neuroprotective effects of leafy spices against Streptozotocin on the cholinergic, purinergic and arginase system of neurodegenerative diseases are yet to be fully explored. This study is therefore aimed at determine the effect of Thyme, Curry and Spearmint Leafy Spices on Some Neuronal Enzymes in the Brain Tissue of Streptozotocin (STZ)-Induced Diabetic Rat. Aqueous extracts from the leaves were prepared and were administered at 200 and 400 mg/kg bwt while the extracts effects on cholinesterase, purinergic and arginase enzymes as well as lipid peroxidation were evaluated. Results revealed that induction of STZ at 50 mg/kg bwt caused a significant ($p < 0.01$) increase in the activity of AChE and BChE, Adenosine deaminase and arginase, as well as a significant ($p < 0.01$) increase in malonaldehyde production. Further results showed that the extracts ameliorated the altered activities of AChE and BChE, Adenosine deaminase and arginase and the level of malonaldehyde production in a concentration dependent manner. Findings from the study revealed that the aqueous extracts for 14 days after streptomycin induction in type 2 diabetic rats showed antioxidant property and neuroprotective effect by modulating the arginase, purinergic and cholinergic enzymes activities as well as lowers the levels of aldehydic compounds generated as a product of lipid peroxidation in the brain of diabetic rats thereby contributing to the prevention of neurotransmitter abnormality and consequent vascular complications in diabetic state.***

I. INTRODUCTION

Diabetes mellitus (DM) is a group of non-communicable and metabolic diseases characterized by high blood sugar resulting from defects in insulin secretion and/or increased cellular resistance to insulin with a rising trend in both developed and developing countries (Zimmet *et al.*, 2004). Type 1 diabetes results from the inadequate synthesis of insulin by b-cells of the pancreas, while type 2 diabetes is characterized primarily by insulin resistance and increasing common disorder of carbohydrate and lipid metabolism (Nisoli *et al.*, 2000). Insulin resistance occurs in the early stage of type 2 diabetes, after a long period of insulin resistance, a further decline of beta cells is induced, resulting in hyperglycemia and lipid metabolism confusion. It is also strongly linked with behavioral factors such as dietary habits and physical inactivity (Roberts *et al.*, 2013; Tuomilehto *et al.*, 2001).

Chronic hyperglycemia and other metabolic disturbances of diabetes mellitus leads to longterm tissue and organ damage as well as dysfunction involving the eyes, kidneys, the nervous and vascular system which may be controlled by healthy

eating and being active. Hyperglycemia plays a critical role in the development and progression of diabetic neuropathy. One of the mechanisms by which hyperglycemia causes neural degeneration is through increased oxidative stress that accompanies DM. Metabolic and oxidative insults often cause rapid changes in glial cells (Baydas *et al.*,2003). Diabetes mellitus is known to cause neurodegeneration due to impaired glucose metabolism involving decreased glucose uptake by the brain (Ahmed and Tarannum, 2009).

Since the brain is affected by repeated occurrence of decreased glucose availability and poor diabetic control, protein metabolism undergoes severe changes during this condition
hence, promoting neurological disorders through modification of the cholinergic and purinergic enzymes (Schmatz *et al.*, 2009). The cholinergic and purinergic system plays a very vital role in many functions of both the central and peripheral nervous system (Kahner *et al.*, 2006). Acetylcholinesterase is one of the cholinesterase enzymes that regulates acetylcholine (main neurotransmitter) which plays vital functions such as memory, learning, modulation of cerebral blood flow and movement control while the purinergic system is responsible for neuronal development and repair as well as neurotransmission (Kawashima and Fujii, 2003; Novak, 2008).

These enzymes have been reported to respond to various levels of injury which may alter its activity when subjected to a disease state or oxidative stress (Akinyemi *et al.*, 2015; Mesulam *et al.*, 2002). However, neither insulin nor oral hypoglycemic drugs are certified ideal in the treatment of diabetes due to toxic side effects and sometimes reduction in efficacy after prolonged use (Ndong *et al.*, 2007; Rubin *et al.*, 1994). Hence, the need for alternative/complementary therapy in readily available and relatively cheap functional foods and/or medicinal plants with minimal or no side effects.

The medicinal and aromatic plants contain the chemical constituents which were first used by humans as medicines for various significant treatments, mind relaxing treatments for some patients, as flavoring agents for food and drink, and as mental stimulants for mystic interactions with super natural gods (Inoue and Craker, 2013). Among many aromatic plants, Curry (*Murraya koenigii*) and Thyme (*Thymus vulgaris*, Lamiacea) are aromatic herb that are highly cultivated in many parts of the world, including Africa, and consume as spices and for medicinal/pharmacological purposes (Fachini-Queiroz, 2012; Quiroga *et al.*, 2015). As reported, thyme leaf is rich in many phytochemicals including polyphenols (phenolic acids, flavonoids), biphenyl compounds and, most importantly, terpenes and terpenoids (Fachini-Queiroz, 2012).

Also, spearmint (*Mentha spicata* L.) plays a crucial role, since international demand for spearmint essential oils increased in the past few years. Spearmint produces secondary metabolites applicable in different sectors. Its phenols have also shown strong antioxidant, acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and histone deacetylase inhibitory effects (Arantes *et al.*, 2017; Dwivedi *et al.*, 2004; Hanafy *et al.*, 2017; Mahdavikia *et al.*, 2017). However, there is a dearth of information on the possible mechanisms of action by which these leaves exert their health benefits. Hence, this study, therefore, sought to investigate the effect of *Thymus vulgaris*, *Murraya koenigii*, and *Mentha spicata* leaves on oxidative stress and key enzymes linked to complications of type-2 diabetes.

II. MATERIALS AND CHEMICALS

Gallic acid, streptozotocin, reduced glutathione, adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), 1-chloro-2,4-dinitrobenzene, 2 thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and solvents used were of analytical grade.

A. ANIMALS HANDLINGS

Adult male Wistar rats weighing 200–250 g were used in the experiments. All rats were supplied with food and water ad libitum and were kept on a 12 h light/12 h dark cycle in a room with the temperature regulated to 21–25 C and humidity at roughly 56%. Animals were treated according to standard guidelines of animal care. Inducing Type II Diabetes in Rats. Diabetic induction was carried out as previously described in our group except that 50 mg/kg body weight streptozotocin was used. Briefly, rats were given a single intravenous injection of STZ (50 mg/kg body weight) previously diluted in 0.1 M citrate-buffer (pH 4.5). Control rats received an equivalent amount of buffer. Diabetic state was checked 72 h after induction with STZ. Blood samples taken from the tail vein of rats were used to determine glucose levels using an automatic autoanalyzer (GLUCOTREND, Roche Diagnostics). Animals were considered diabetic when blood glucose level exceeded 250 mg/dL.

B. TREATMENT

The animals were divided randomly into the following groups: Rats in Group 1 received citrate buffer (pH 4.5) and fed with basal diets (Normal control); Group 2: STZ-induced diabetic control rats fed with basal diet (STZ); Group 3: STZ-induced diabetic rats received acarbose orally (25 mg/kg body weight) and were fed with basal diet (STZ + ACA); Group 4: STZ-induced diabetic rats received 200 mg/kg bwt of *Murraya koenigii* (Curry) leaf (STZ + 200 mg/kg bwt CL); Group 5: STZ-induced diabetic rats received 400 mg/kg bwt of *Murraya koenigii* (Curry) leaf (STZ + 400 mg/kg bwt CL); Group 6: STZ-induced diabetic rats received 200 mg/kg bwt of *Mentha spicata* (Spearmint) leaf (STZ + 200 mg/kg bwt ML); Group 7: STZ-induced diabetic rats received 400 mg/kg bwt of *Mentha spicata* (Spearmint) leaf (STZ + 400 mg/kg bwt ML); Group 8: STZ-induced diabetic rats received 200 mg/kg bwt of *Thymus vulgaris* (Thyme) (STZ + 200 mg/kg bwt TL); and Group 9: STZ-induced diabetic rats received 400 mg/kg bwt of *Thymus vulgaris* (Thyme) (STZ + 400 mg/kg bwt TL). Groups 3 to 9 were administered extract orally (once/day) for 14 days after the administration of STZ. At the end of the experimental period, diabetic rats and the corresponding control animals were anesthetized with ether and euthanized by decapitation. Rats were fasted 12 h prior to euthanasia.

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C. PREPARATION OF BRAIN HOMOGENATE

Brain tissues was quickly removed, placed on ice and homogenized in cold 50 mM Tris–HCl pH 7.4. The homogenate was centrifuged at 4,0009g for 10 min to yield the low-speed supernatant (S1) fraction that was used for biochemical assays. For all analyses, protein content was determined by the method of Bradford (1976), using bovine serum albumin as the standard.

D. ACETYLCHOLINESTERASE ACTIVITY ASSAY

The acetylcholinesterase (AChE) inhibition of the plant samples was assessed by colorimetric method (Perry et al., 2000) The AChE activity was determined in a reaction mixture containing 200 µl of a solution of AChE (0.415 U/ml in 0.1 M phosphate buffer, pH 8.0), 100 µl of a solution of 5,5′dithiobis(2nitrobenzoic) acid (3.3mM) in 0.1Mphosphate buffer solution (pH 7.0) containingNaHCO3 (6mM), extract dilutions (0–100 µl), and 500 µl phosphate buffer (pH 8.0). After incubation for 20 min at 25°C, acetylthiocholine iodide (100 µL of 0.05 mM water solution) was added as the substrate, and AChE activities were determined by JENWAY UVVisible spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C. The enzyme inhibitory activities were expressed as percentage inhibition.

E. BUTYRYLCHOLINESTERASE ACTIVITY ASSAY

The butyrylcholinesterase (BChE) inhibition of the plant samples was assessed by colorimetric method (Perry et al, 2000) The BChE activity was determined in a reaction mixture containing 200 µl of a solution of BChE (0.415 U/ml in 0.1 M phosphate buffer, pH 8.0), 100 µl of a solution of 5,5′dithiobis(2nitrobenzoic) acid (3.3mM) in 0.1Mphosphate buffer solution (pH 7.0) containingNaHCO3 (6mM), extract dilutions (0–100 µl), and 500 µl phosphate buffer (pH 8.0). After incubation for 20 min at 25°C, butylrylthiocholine iodide (100 µL of 0.05 mM water solution) was added as the substrate, and AChE activities were determined by JENWAY UV Visible spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C. The enzyme inhibitory activities were expressed as percentage inhibition.

F. ARGINASE ACTIVITY ASSAY

Arginase activity assay was performed according to the method of Kaysen and Strecker (1973). In brief, a solution containing tissue homogenate, 0.01 mM Tris-HCl buffer (pH 7.5) containing 0.05 mM MnCl2, and 50 mM L-arginine was incubated at 37°C for 10 min. The reaction was terminated by adding Ehlrich's reagent (p-dimethylaminobenzaldehyde in 3.6 N HCl). The amount of urea produced was measured spectrophotometrically at 450 nm.

G. ADENOSINE DEAMINASE (ADA) ACTIVITY ASSAY

ADA activity was determined in penile homogenate as previously described (Guisti and Galanti, 1984) with minor modifications. The assay is based on the direct measurements of the formation of ammonia, produced when ADA acts in excess of adenosine. The reaction mixture containing 21 mM of adenosine, penile homogenate, phosphate buffer (pH 6.5), and were incubated at 37^{o}C for 60 min. The reaction was stopped by adding phenol–nitroprusside solution. The reaction mixtures were immediately mixed with 125/11 mM alkaline-hypochlorite (sodium hypochlorite). 75 mM ammonium sulphate was used as ammonium standard. The protein content used for this experiment was adjusted to 0.4–0.6 mg/mL. Results were expressed as unit of specific enzyme activity/mg protein (U/mg of protein for ADA).

H. LIPID PEROXIDATION ASSAY

Lipid peroxidation was determined as the formation of thiobarbituric acid-reactive substances (TBARS) during an acid heating reaction according to the method of Ohkawa *et al.*, (1979) Briefly, the reaction mixture consisting of 200 µL of the penile homogenate or standard (0.03mM malondialdehyde (MDA)), 200 µL of 8.100 mg/kg bwt sodium dodecyl sulfate (SDS), 500 µL of 0.800 mg/kg bwt TBA and 500 µL of acetic acid solution (2.5M HCl, pH 3.4) was heated at 95 °C for 1 h. The absorbance was measured at 532 nm, and the tissue lipid peroxidation levels were expressed as micromoles MDA per milligram of protein.

I. DATA ANALYSIS

All data were expressed as mean ± standard error of mean (S.E.M.). One-way analysis of variance (ANOVA) was used to analyze the differences between the groups with the aid of Graph Pad Prism 5.0 Software.

III. RESULTS AND DISCUSSION

The effect of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves on the activities of acetylcholinesterase (AChE) in the brain of rats is presented in figure 2.1. The result revealed that streptomycin caused a significant increase in the activities of AChE in the brain ($p < 0.01$) of the streptomycin induced group when compared to the normal control. However, treatment with 200 and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves significantly ($p < 0.01$) reduced the activities of AChE in a concentration dependent manner when compared with the streptomycin induced group.

Meanwhile, the effect of Curry, Spearmint and Thyme leaves were also observed on butrylcholinesterase in the brain in figure 2.2. The result indicated that streptomycin caused a significant increase in the activities of BChE in the brain ($p < 0.01$) when compared to the control group. However, treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves significantly reduced the activities of BChE in the brain ($p < 0.01$) in a concentration dependent manner when compared with the streptomycin induced group.

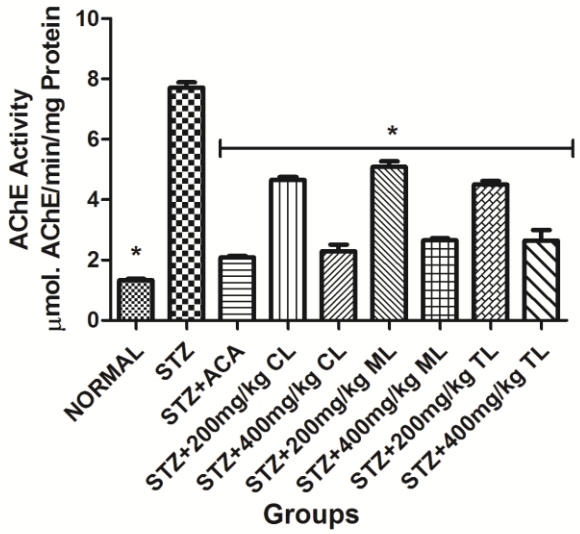

Figure 2.1: Effect of Murraya koenigii (Curry), Mentha spicata (Spearmint) and Thymus vulgaris (Thyme) leaves on acetylcholinesterase activity in the brain of rats

Values represent mean ± standard deviation (n = 5).

*****Values are significantly ($p < 0.01$) different from streptomycin induced group.

KEYS

Group I	Normal control rats
Group II	Streptomycin induced group
Group III	Streptomycin induced + Acarbose
Group IV	Streptomycin induced + 200 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group V	Streptomycin induced + 400 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group VI	Streptomycin induced + 200 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VII	Streptomycin induced + 400 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VIII	Streptomycin induced + 200 mg/kg bwt Thyme (*Thymus vulgaris*) leaves
Group IX	Streptomycin induced + 400 mg/kg bwt Thyme (*Thymus vulgaris*) leaves

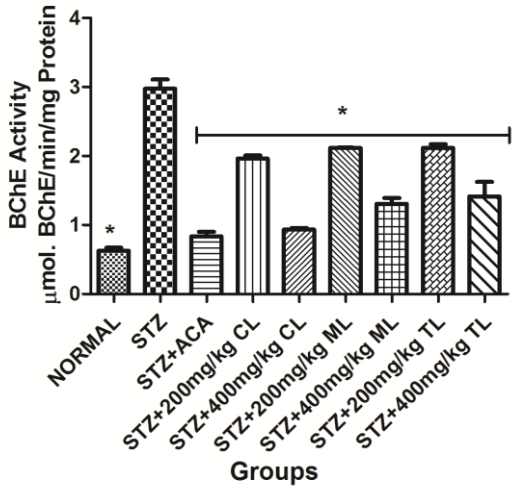

Figure 2.2: Effect of Murraya koenigii (Curry), Mentha spicata (Spearmint) and Thymus vulgaris (Thyme) leaves on butrylcholinesterase activity in the brain of rats

Values represent mean ± standard deviation (n = 5).

*****Values are significantly ($p < 0.01$) different from streptomycin induced group.

KEYS

Group I	Normal control rats
Group II	Streptomycin induced group
Group III	Streptomycin induced + Acarbose
Group IV	Streptomycin induced + 200 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group V	Streptomycin induced + 400 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group VI	Streptomycin induced + 200 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VII	Streptomycin induced + 400 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VIII	Streptomycin induced + 200 mg/kg bwt Thyme (*Thymus vulgaris*) leaves
Group IX	Streptomycin induced + 400 mg/kg bwt Thyme (*Thymus vulgaris*) leaves

Also, the effect of Curry, Spearmint and Thyme leaves on the activities of adenosine deaminase (ADA) in the brain of rats are presented in figure 2.3. The result revealed that streptomycin caused a significant ($p < 0.01$) increase the activities of ADA in the brain of streptomycin induced group when compared with the normal control group. Treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* and *Mentha spicata* leaves significantly ($p < 0.01$) reduced the activities of the enzyme while *Thymus vulgaris* leaves significantly reduced ADA activities at 200 mg/kg bwt ($p < 0.05$) and 400 mg/kg bwt ($p < 0.01$) in concentration dependent manner when compared to streptomycin induced group.

More so, the effect of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves on the activities of arginase in the brain is represented in figure 2.4. The result revealed that streptomycin caused a significant ($p < 0.01$) increase in the activities of arginase in the brain of rats of the streptomycin induced group when compared to the normal control group. Treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* leaves significantly ($p < 0.01$) reduced the activities of the enzyme while *Mentha spicata* and *Thymus vulgaris* leaves significantly reduced arginase activities at 200 mg/kg bwt ($p < 0.05$) and 400 mg/kg bwt ($p < 0.01$) in concentration dependent manner when compared to streptomycin induced group.

Furthermore, there was a significant increase ($p < 0.01$) in the brain level of lipid peroxidation in the streptomycin induced group when compared to the normal control group as represented in figure 2.5. Treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves significantly reduced ($p < 0.01$) the brain lipid peroxidation level in a concentration dependent manner when compared with the streptomycin induced group.

Also, the effect of Curry, Spearmint and Thyme leaves on the activities of adenosine deaminase (ADA) in the brain of rats are presented in figure 2.3. The result revealed that streptomycin caused a significant ($p < 0.01$) increase the activities of ADA in the brain of streptomycin induced group when compared with the normal control group. Treatment

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with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* and *Mentha spicata* leaves significantly ($p < 0.01$) reduced the activities of the enzyme while *Thymus vulgaris* leaves significantly reduced ADA activities at 200 mg/kg bwt ($p < 0.05$) and 400 mg/kg bwt ($p < 0.01$) in concentration dependent manner when compared to streptomycin induced group.

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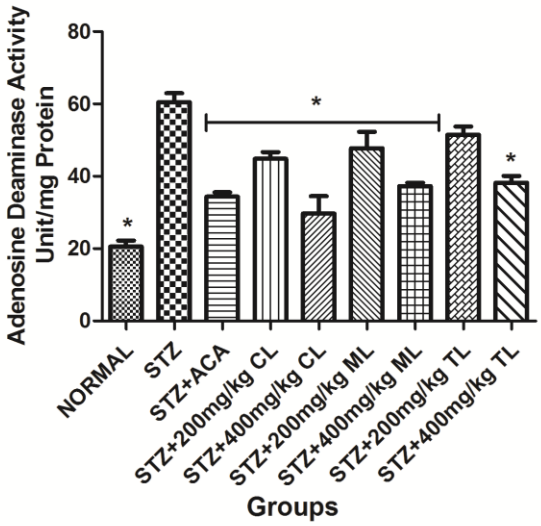

Figure 2.3: Effect of Murraya koenigii (Curry), Mentha spicata (Spearmint) and Thymus vulgaris (Thyme) leaves on adenosine deaminase activity in the brain of rats

Values represent mean ± standard deviation (n = 5).

*****Values are significantly ($p < 0.01$) different from streptomycin induced group.

KEYS

Group I	Normal control rats
Group II	Streptomycin induced group
Group III	Streptomycin induced + Acarbose
Group IV	Streptomycin induced + 200 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group V	Streptomycin induced + 400 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group VI	Streptomycin induced + 200 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VII	Streptomycin induced + 400 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VIII	Streptomycin induced + 200 mg/kg bwt Thyme (*Thymus vulgaris*) leaves
Group IX	Streptomycin induced + 400 mg/kg bwt Thyme (*Thymus vulgaris*) leaves

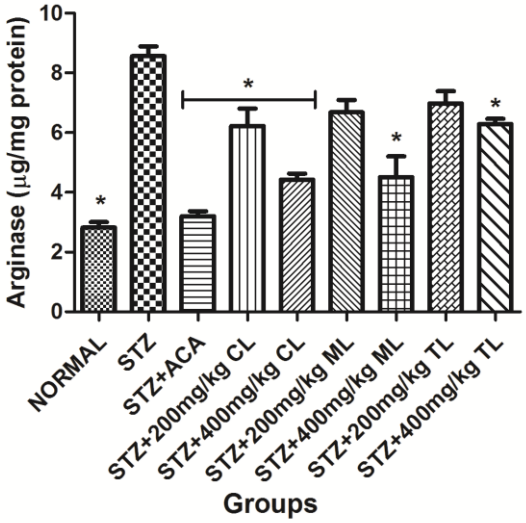

Figure 2.4: Effect of Murraya koenigii (Curry), Mentha spicata (Spearmint) and Thymus vulgaris (Thyme) leaves on arginase activity in the brain of rats

Values represent mean ± standard deviation (n = 5).

*****Values are significantly ($p < 0.01$) different from streptomycin induced group.

KEYS

Group I	Normal control rats
Group II	Streptomycin induced group
Group III	Streptomycin induced + Acarbose
Group IV	Streptomycin induced + 200 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group V	Streptomycin induced + 400 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group VI	Streptomycin induced + 200 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VII	Streptomycin induced + 400 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VIII	Streptomycin induced + 200 mg/kg bwt Thyme (*Thymus vulgaris*) leaves
Group IX	Streptomycin induced + 400 mg/kg bwt Thyme (*Thymus vulgaris*) leaves

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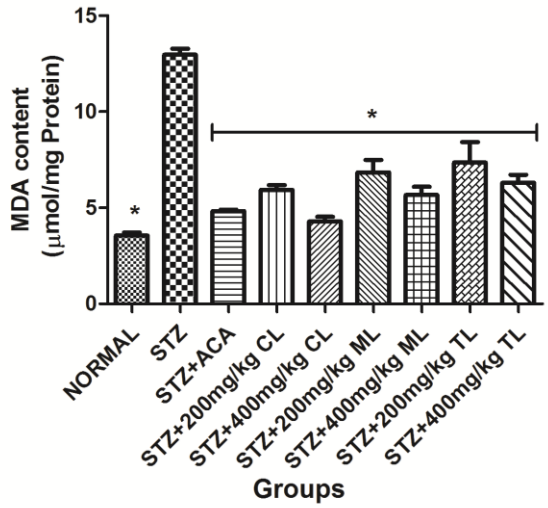

Figure 2.5: Effect of Murraya koenigii (Curry), Mentha spicata (Spearmint) and Thymus vulgaris (Thyme) leaves on lipid peroxidation levels in the brain of rats

Values represent mean ± standard deviation (n = 5).

*****Values are significantly ($p < 0.01$) different from streptomycin induced group.

Keys

Group I	Normal control rats
Group II	Streptomycin induced group
Group III	Streptomycin induced + Acarbose
Group IV	Streptomycin induced + 200 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group V	Streptomycin induced + 400 mg/kg bwt Curry (*Murraya koenigii*) leaves
Group VI	Streptomycin induced + 200 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VII	Streptomycin induced + 400 mg/kg bwt Spearmint (*Mentha spicata*) leaves
Group VIII	Streptomycin induced + 200 mg/kg bwt Thyme (*Thymus vulgaris*) leaves
Group IX	Streptomycin induced + 400 mg/kg bwt Thyme (*Thymus vulgaris*) leaves

IV. DISCUSSION

Findings of the present study showed the neuroprotective effect of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves at 200 mg/kg bwt and 400 mg/kg bwt on streptomycin induced neurotoxicity which involves the Alzheimer disease (AD) related pathological markers. Meanwhile, intravenous streptomycin administered to rats are well-established dementia model (Lannert and Hoyer, 1998). Alzheimer's disease is a progressive disease which destroy memory and cognitive skills and eventually leads to death: it is generally acknowledge as the most prevalent form of dementia (Giannakopoulos and Hof, 2009). AD is a multi-etiology disorder and the risk factors include non-modifiable factors such as age and genetics, as well as modifiable factors such as dietary and lifestyle choices, co-existing health problems and education. However, the specific mechanistic effects of streptomycin on the cholinergic, purinergic and arginase system of neurotransmission in an Alzheimer model are yet to be fully explored.

In this present study, streptomycin was administered intraperitoneally into rats to induce cognitive impairment and memory dysfunction. Available medication or therapies of AD could only provide the symptomatic relief and are not able to prevent the neuronal loss. Presently, reversible acetylcholinesterase (AChE) inhibitor and NMDA receptor antagonist are being used to treat the AD patients (Blennow *et al.*, 2006). Donepezil (Rogers e*t al.*, 1998), rivastigmine (Desai and Grossberg, 2005) and galantamine (Rainer, 1997) are reversible AChE inhibitors which inhibit the AChE enzyme to prevent the breakdown of the acetylcholine (Talesa, 2001). Maintaining Ach level is critical for proper functioning since it is well-known that the cholinergic neurotransmission system in the basal forebrain plays an important role in learning and memory (Blake *et al.*, 2014). There are two types of cholinesterase; the acetylcholinesterase (AChE), which selectively hydrolyses Ach, and the butrylcholinesterase (BChE), which hydrolyses other choline esters in addition to acetylcholine (Lleó, 2007). Acetylcholine is found in the synapses of the cerebral cortex and a depletion of neuronal molecule in the cerebral cortex is one of the major features of impaired cognitive function observed in Alzheimer's disease (Bierer *et al.*, 1995) and this deficiency is due to high activity of the cholinesterase.

The death of cholinergic neurons is a second most acceptable hypothesis for AD pathology. According to the cholinergic hypothesis, the acetylcholine and butyrylcholine levels decrease due to the death of cholinergic neurons. The decreased level of choline caused the declined neurotransmission and contributes in propagation of AD related dementia like symptoms. Therefore, AChE and BChE activities were estimated in streptomycin induced rats. STZ treatment caused increased AChE and BChE activities, thus decreased levels of acetylcholine and butyrylcholine respectively. This observed increase in the cholinesterase activities could be as a result of the oxidative stress induced by the STZ administration. This observation implied that STZ treatment decreased the acetylcholine and butyrylcholine levels in cells causing cholinergic deficiency and mimicking the AD like pathology. The decrease in cholinergic transmission in the brain associated with breaking down of acetylcholine and butyrylcholine is consistent with the findings of McDaniel *et al.*, 2003.

Administration of 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves for 14 days after streptomycin induction led to significant reversal in the effect of streptomycin on the activities of AChE and BChE to a level not significantly different from the normal control group in the brain. The ameliorating effects of the leaves on memory deficit might have resulted from the modulation of acetylcholine levels through an inhibition of AChE and BChE enzymes. The inhibition of AChE and BChE activities prevent these enzymes from degrading acetylcholine and butyrylcholine in the brain and this in turn increased the concentrations of the neurotransmitter at the synaptic cleft which leads to increased communication between the nerve cells that use acetylcholine or butyrylcholine as a chemical

messenger, thus temporarily improve or stabilize the symptoms of Alzheimer's disease (Howes *et al.,* 2003). The resultant increased choline levels have thus been observed to improve cognition in humans and several memories and learning models in rodents (Knoppman, 2001).

More so, the leaves of *Murraya koenigii*, *Mentha spicata* and *Thymus vulgaris* have been observed by Vasudevan and Parle (2009), Marino *et al.,* (2019) and Adefegha *et al.,* (2019) respectively to contain phenolic compounds that can act as neuroprotective agents in the amelioration of neurodegenerative diseases. Furthermore, Akinyemi *et al.,* (2016) also observed that natural products from plant materials rich in antioxidant molecules possess anti-ChEs activities. Summarily, this result may enhance the understanding of the ameliorative role of the phenolic constituents of the leaves in preventing brain disorders and/or managing diabetes complications. Although there was no significant difference in the ability of the plants leaves to ameliorate the cholinesterase disorder, it is noteworthy that phenolic compounds from *Murraya koenigii* had the highest ameliorating potentials, followed by *Mentha spicata* and the least being *Thymus vulgaris.*

Adenosine is a neuromodulator and anti-inflammatory molecules, vital in the purinergic signaling (Marmol *et al.,* 2010). In many pathological conditions, adenosine serves as neuroprotectant via modulation of neurotransmitter release and trophic factors (Akinyemi *et al.,* 2017). In this study, we observed that ADA activity was increased in STZ induced group. An increase of ADA activity increases the hydrolysis of adenosine to inosine. The increase ADA activity excessively reduced adenosine level and its function by irreversibly converting it to inosine (Mahajan *et al.,* 2013). This result compared favorably with Berrie (2004) who reported increased ADA activity in the brain as a result of STZ administration. However, treatment with 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves for 14 days were able to ameliorate the disorder significantly with *Murraya koenigii* leaves possessing the highest inhibitory effect on the adenosine dismutase enzyme activity. The inhibition of ADA has been reported to be a novel therapeutic target in the treatment of various pathologies related to cognitive impairment. This informs possible mechanisms of the plant leaves action on cognitive function. This effect could influence the prevention of adenosine degradation in the CNS, as several antioxidants have been reported to prevent adenosine breakdown in the brain (Akinyemi *et al.* 2016, 2017).

Arginase is a metalloproteinase that catalyzes the metabolism of L-arginine into L-ornithine and urea, and deprives nitric oxide synthase (NOS) of its substrate (L-arginine) for the production of NO, when overexpressed; a condition that could be triggered by oxidative stress (Abdel-Salam *et al.,* 2011, Oyeleye *et al.,* 2019). According to the result of this study, the administration of streptomycin in rats caused significant increase in arginase activity, which could result in consumption of L-arginine and possibly deprived the production of NO. Nevertheless, the treatment of STZ induced rats with *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves for 14 days at varied doses caused significant decrease in arginase activity, which could possibly make L-arginine available to NOS for the production of NO; an active neurotransmitter that mediates memory functions (Pitsikas 2015). According to Paul and Ekambaram (2011) reduced NO level impairs learning and memory function. Therefore, the ability of Curry, Spearmint and Thyme leaves to reverse the elevation in NO content, notably could be another neuroprotective mechanism of *Murraya koenigii*, *Mentha spicata* and *Thymus vulgaris* leaves in the management of Alzheimer's disease.

Furthermore, in the measure of the potential of 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii*, *Mentha spicata* and *Thymus vulgaris* leaves in the reversal of oxidative stress, the increase in lipid peroxidation during diabetes as observed in this study could be attributed to the peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids, which is considered a pointer of cellular deterioration (Mallick *et al.,* 2011). Lipid peroxidation in biological membranes is considered as one of the major mechanisms of cell injury in aerobic organisms subjected to oxidative stress. However, the observed significant ($P < 0.01$) reduction in the lipid peroxidation in *Murraya koenigii*, *Mentha spicata* and *Thymus vulgaris* leaves treated group in descending order when compared with STZ induced group might be due to the high antioxidant potentials of the polyphenolic constituents of leaves as reported in earlier work (Adefegha *et al.* 2019; Marino *et al.,* 2019; Oboh *et al.,* 2013 and Vasudevan and Parle, 2009). The beneficial effects promoted by Padauk leaf could also be attributed to improved antioxidant activity in the brain, which potentially could result in a reduction in membrane lipid peroxidation.

The brain which contains high rate of polyunsaturated fatty acids in membranes as well as being a poor antioxidant defense systems and large consumer of oxygen (Rahman, 2003), is highly susceptible to oxidative stress. Due to the presence of polyphenols which scavenge the very aggressive free radicals (Vaidya *et al.,* 2009), this vegetable may be a good candidate to counteract the oxidative stress induced diabetes-associated brain damages. This beneficial effect can be attributed to free radicals scavenging properties and the presence of phenolic acids and flavonoids in the plants leaves as observed by Vasudevan and Parle (2009), Marino *et al.* (2019) and Adefegha *et al.* (2019). Hence, we could affirm that phenolic acids and flavonoids were able to liberate a hydrogen proton from their hydroxyl group and could scavenge free radicals and prevent the brain from damage induced by hyperglycemia.

V. CONCLUSION

Aqueous extract administration at 200 mg/kg bwt and 400 mg/kg bwt of *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves for 14 days after streptomycin induction in type 2 diabetic rats showed antioxidant property and neuroprotective effect by modulating the arginase, purinergic and cholinergic enzymes activities as well as lowers the levels of aldehydic compounds generated as a product of lipid peroxidation in the brain of diabetic rats thereby contributing to the prevention of neurotransmitter

abnormality and consequent vascular complications in diabetic state. The results also point out that *Murraya koenigii* (Curry) have the highest neuroprotective potential, followed by *Mentha spicata* (Spearmint) and the least potential being *Thymus vulgaris* (Thyme). These results suggest that *Murraya koenigii* (Curry), *Mentha spicata* (Spearmint) and *Thymus vulgaris* (Thyme) leaves for 14 days have therapeutic potentials in preventing and/or treating memory loss in individuals with deficit in memory and neurodegenerative diseases, such as Alzheimer's disease. In addition, this study provides a biochemical rationale for clinical studies.

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