wjpmr, 2020,6(12), 15-18

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

EVALUATION OF SOME TRACE ELEMENTS IN STREPTOZOCIN INDUCED DIABETIC RATS TREATED WITH MORINGA OLEIFERA LEAF POWDER

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Article Received on 01/10/2020 **Article Revised on 22/10/2020** **Article Accepted on 12/11/2020**

ABSTRACT

This work was done to determine the trace elements in strreptozocin-induced diabetic rats treated with *moringa oleifera* leaf. This was conducted using 5 groups of 10 rats each were used. Group1 negative control, group2 positive control (diabetics), groups 3 and 4 treated with 150mg/kg and 300mg/kg of *moringa oleifera* leaf respectively.The parameters analysed includes; zinc, copper, selenium manganese. P. values $p<0.05$ were considered statistically significant. The study showed that there was a significant increase in the magnesium levels of rats in groups 4(0.047±0.19) when compared with groups 1 and 2 (0.19±0.14 and 0.13±0.37), there was a significant increase ($P<0.05$) in the zinc levels of rats in groups 4(0.58± 0.08) when compared with that of groups 1 and 2(0.30±0.15 and 0.39±0.05), there was a significant increase in the selenium levels of rats in group 2 (7.51±1.79) when compared with those in groups 1, 3 and 4(6.43±1.21, 6.30±1.51 and 6.30±1.11). Also there was no significant difference in the Cu levels of rats in all the groups. From this study, it could be inferred that *moringa oleifera* leaf powder used in treatment of streptozocin-induced diabetes in rats reduced the toxic damage to the kidney and liver.

KEYWORDS: Trace elements, streptozocin induced diabetic rats, moringa oleifera leaf powder.

INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia caused by impairment of insulin secretion, transportation, stimulation and insulin action. Extended period of continuous increase in glucose levels may lead to macro/ microvascular complications, such as heart disease, hypertriglyceridemia, nephropathy, and neuropathy. For prevention of the consequences of diabetes mellitus, blood sugar level control through diet is very necessary; this may be achieved using orthodox medication or herbal medication. Many plants are consumed for therapeutic purposes for their nutritional and bioactive compounds constituents. *Moringa oleifera* leaves are one of the plants used for glycermic control due to the nutritional content of its leaves, such as protein, vitamins, and minerals (Misrha *et al.*, 2011).

Moringa oleifera belongs to the family *Moringaceae*, the order *Brassicales*, and the genus *Moringa*, there are 13 species of the plant ranging in height from 5 to 10 m. It has an open crown of drooping, feathery foliage, flowers with distinctive green patches at the tips of the petals and sepals, tripinnate leaves and trunk. It's flowers, pods, and leaves have medicinal benefits owing to various phytochemical constituents. The flower is used to treat inflammation for its stimulant content, the spots and seeds have liver-protective and antihypertensive properties, while the leaves are used to treat microbial infections and to control blood glucose levels. *Moringa oleifera* contains soluble fibers that enhance reduction of glucose levels, proliferation of lymphocytes and induced nitric oxide from macrophages. The leaves contains polyphenols such as quercetin-3-glycoside, rutin, kaempferol and glycosides, and has been found to be useful in diabetes conditions because of their possible capacity to decrease blood glucose concentrations after ingestion (Arora *et al.*, 2013; Al-Malki and El-Rabey, 2015).

Type 2 diabetes is the most common type of diabetes mellitus. It is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion; defective responsiveness of body tissues to insulin is believed to involve the insulin receptor (Shoback and Gardner, 2011). However, the specific defects are not known. Many people with type 2 diabetes usually have initial evidence of "prediabetes" (impaired fasting glucose and/or impaired glucose tolerance) for many years before manifestation of type 2 diabetes.

Type 2 diabetes is primarily due to lifestyle factors and genetics, a number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than 30), stress, and urbanization. Dietary factors also influence the risk of developing type 2 diabetes, lack of physical activity and the type of fats in the diet is also important, saturated fat and trans fats increases the risk and polyunsaturated and monounsaturated fat decreasing the risk, excessive consumption of sugar-sweetened food and drinks is associated with an increased risk (Malik *et al*., 2010).

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveeness. This may be transient, but if untreated can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause infant respiratory distress syndrome.

Complications in diabetes are characterized by inflammation, oxidative stress, and immune failure. These may lead to the loss of intestinal mucosal integrity, and as a result, may decrease the intestinal absorption of essential nutrients and thereby predisposes the individual to increase oxidative stress (Rech *et al.*, 2014). In terminal conditions seen in severely ill patients, the network of antioxidant defense mechanisms (e.g., superoxide dismutase, catalase, and glutathione peroxidase) formed by trace element-dependent enzymes may protect cells from superoxide radicals and nitric oxide (Cander *et al*, 2011). Trace elements such as zinc (Zn), selenium (Se), and copper (Cu) contributes to the protection of cells from oxidative stress (Rech *et al.*, 2014).

Zinc functions in the maintenance of normal growth, immune function, DNA repair, protein synthesis, glucose control, and wound healing. Beseccker *et al*. (2011) reported that zinc deficiency has been seen in critically ill patients with septic shock. Selenium is one of the essential trace elements with antioxidant, immunological, and anti-inflammatory properties. Copper functions as an essential trace element by being an important component of the Cu/Zn superoxide dismutase and serves as a free radical scavenger (Rech *et al.*, 2014).

This work aimed to evaluate study trace elements in strreptozocin-induced diabetic rats treated with pulverised *moringa oleifera* leaf.

MATERIALS AND METHODS

Plant Materials and Preparation

The plant was harvested from garden within Madonna University and was identified in the department of plant science of the University. The leaves were air dried at room temperature for two weeks, after which it was pulverized using electronic blender, the pulverized sample was subjected for extraction using four different solvents namely; ethanol, methanol, ethyl alcohol and water. Each of the extracts was analyzed for bioactive components using Gas Chromatography – Mass Spectrometry (GC-MS).

Animals

Male wistar albino rats (n=60) six weeks old weighing 150-250g was purchased from the animal farm of Madonna University Elele. Each of the animals was housed in animal cage with wire mesh and saw dust lining, and they were kept in a room inside the animal house, with 12 hours light/dark circle, the animals were allowed to acclimatize for 2 weeks, and were given food and water.

Experimental Design

After two weeks, they were numbered and separated into four groups of 10 rats each, group one were fed with animal feed throughout the experimental period, while other groups were fed with high fat diet (HFD) for seven weeks to increase the body mass index. At the end of the 9th week, 0.5ml of of streptozocin 37mg/kg in citrate buffer was administered intraperitoneally to the rats in groups 2, groups 3 and group 4. The rats in groups 3 and 4 in addition to streptozocin were fed with pulverized *Moringa oleifera* leaf daily with the aid of rats cannular, according to the experimental deisgn below. Fasting blood sugar was measured weekly by cutting the tip of the animals tail, using Easy Touch HealthPro glucose monitoring system.

Group 1 (Negative control): The animals in this group were fed with only animal mesh and water throughout the experiment.

Group 2 (Positive control): The animals in this group were given 0.5ml of 37mg/kg of Streptozotocin intraperitoneally in addition to feed and water.

Group 3: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 150mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

Group 4: The animals in this group were given 0.5ml of 37mg/kg of streptozotocin and 300mg/kg of *Moringa oleifera* leave powder daily, in addition to food and water throughout the experiment period.

Determination of Lethal Dose

This involves two steps; in the first step, nine animals were used grouped into three animals, each group was given different doses of the *Moringa oleifera* leaf powder (50, 100, 150mg/kg). The animals were mornitored for 24 hours. Second step three groups of one animal each were given different higher doses of

Moringa oleifera leaf powder (200, 300, and 400mg/kg). The animals were mornitored for 24 hours.

LD50 was determined using the formular;

LD50= $\sqrt{(D_O \text{ X } D_{100})}$

Where Do = the highest dose that gave no mortality

D_{100} the lowest dose that produced mortality

Induction of Diabetes Mellitus in Rats

Diabetes mellitus was induced by intraperitoneally injecting the rats with STZ (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 37 mg/kg body weight (b.w.) after two weeks of adaptation and seven weeks of feeding with high fat diet. STZ was freshly prepared as solution in 10 mM sodium citrate buffer (pH 4.5) and injected to after overnight fasting. Fasting blood glucose was measured before injection. On the third day after the STZ injection, the blood was sample was collected from the tail of STZ-injected animals, and glucose levels were measured using glucometer (Easy Touch HealthPro glucose mornitoring system).

Samples Collection

At the end of the experimental period, the animals were euthanized by exposure to chloroform, blood sample was collected via cardiac puncture. Blood was collected into test tubes labelled accordingly, Serum samples were separated and used for determination of different biochemical parameters. Liver and kidney were surgically removed. Liver and kidney were washed with ice cold (4^0C) phosphate buffer saline (immediately after removal) to remove blood, tissue homogenate was prepared by homogenization of 1g of liver/ kidney using BeadBug 6 position tissue homogenizer, the remaining part of the tissue was preserved using formalin for histological studies.

Laboratory Assays Procedures

All reagents were commercially purchased, / prepared and the manufacturers' SOP was strictly followed.

Determination of Serum Zinc, Copper, Manganese and Selenium By Aas (Agilent 200)

Procedure

The test tubes was labeled and numbered according to the sample label, 1ml of the sample was added to 4mls of distilled water, It was mixed properly and allowed to boil for 20mins at 37°C. The mixture was then measured using FS240AA Agilent atomic absorption spectroscopy at 196nm wavelength with 0.2nm slit and air acetylene flame.

Statistical Analysis

Data obtained from this study were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for windows 7. The results were expressed as mean ± Standard deviation. Independent sample t-test which was used to compare means and values at 95% confidence limit. P values $p<0.05$ were considered statistically significant. Pos-Hoc comparison was carried outusing Turkey LSD. The results are presented in tables, and figures.

RESULTS

Table 1: Shows The Mean ± Values Of The Manganese (Mn), Copper (Cu), Zinc (Zn) And Selenium (Se) Of All The Rats In The Study.

GROUPS	Mn (nmol/l)	Cu (nmol/l)	Zn (nmol/l)	Se(nmol/l)
GROUP 1	0.19±0.14	0.19±0.07	0.30±0.15	6.43±1.21
GROUP 2	0.13±0.37	0.14±0.08	0.39±0.15	7.51±1.79
GROUP 3	0.28±0.24	0.14±0.05	0.37±0.12	6.30±1.51
GROUP 4	0.47±0.19	0.18±0.09	0.58±0.08	6.30±1.10
P VALUES	0.019	0.417	0.000	0.000

The study showed that there is a significant increase ($P<0.05$) in the manganese levels of rats in groups 3 and 4(0.28±0.24 and 0.47±0.19) when compared with groups 1 and 2(0.19±0.14 and 0.13±0.37). This result also shows no significant difference ($P>0.05$) in the copper levels of the rats in all the groups.

The result also showed that there is a significant increase ($P<0.05$) in the zinc level of rats in groups 4 (0.58± 0.08) when compared with that of groups 1, 2 and 3 (0.30±0.15 and 0.39±0.05and 0.37±0.12). The result also shows that there is a significant increase ($P<0.05$) in the selenium levels of rats in group 2 (7.51±1.79) when compared with those in groups 1, 3, and 4 (6.43±1.21, 6.30±1.51, and 6.30±1.11).

DISCUSSION

There was a significant increase ($P<0.05$) in the manganese levels in diabetic rats treated with 150mg of *Moringa oleifera* and in diabetic rats treated with 150mg of *Moringa oleifera* (0.28±0.24 and 0.47±0.19) when compared with non diabetic rats and untreated diabetic rats (0.19±0.14 and 0.13±0.37). Manganese is component of SOD, Manganese superoxide dismutase (MnSOD) is the principal antioxidant enzyme in the mitochondria. Because mitochondria consume over 90% of the oxygen used by cells, they are especially vulnerable to oxidative stress. The superoxide radical is one of the reactive oxygen species produced in mitochondria during ATP synthesis. MnSOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which can be reduced to water by other antioxidant enzymes.

This result also shows no significant difference (P>0.05) in the copper levels of the rats among all the groups.The result also showed that there is a significant increase (P <0.05) in the zinc level in diabetic rats treated with 300mg of *Moringa oleifera* (0.58± 0.08) when compared with that of non diabetic rats, untreated diabetic rats and in diabetic rats treated with 150mg of *Moringa oleifera* (0.30±0.15 and 0.39±0.05and 0.37±0.12). The result also showed that there is a significant increase (P <0.05) in the selenium levels of rats in untreated diabetic group (7.51±1.79) when compared with the non diabetic rats, in diabetic rats treated with 150mg of *Moringa oleifera* and in diabetic rats treated with 300mg of *Moringa oleifera* (6.43±1.21, 6.30±1.51, and 6.30±1.11). This contradicted the report form a study that showed that there is decreased selenium level in type 2 diabetes mellitus due to excessive production of free radicals thereby causing decreased activity of antioxidant enzymes and their corresponding trace elements, leading to complications like metabolic acidosis (Thomas *et al*., 2010). The result also contradicts the report from Basaki *et al.* (2012), who said that trace elements are significantly lowered in diabetic patients.

CONCLUSION

From this study, it could be inferred that *moringa oleifera* leaf powder used in treatment of streptozocin-induced diabetes in rats reduced the toxic damage to the kidney and liver.

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