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Comparative Study Of The Effects Of Vernonia Amygdalina And Moringa Oleifera On The Hippocampal Oxidative Enzymes Of Diabetic Wistar Rats

Acharaike Chidimma A.

Masters Candidate, Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

Ofoego Uzozie C.

Lecturer, Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria

Ezejindu Damian N.

Lecturer, Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

Abstract: ***Diabetes Mellitus is a metabolic disorder that damages virtually most organs including the hippocampus of the brain. The damage to the hippocampus is due to oxidative stress. Vernonia amygdalina (V. amygdalina) and Moringa oleifera (M. oleifera) are plants that are commonly used in Africa and have medicinal values. This research was designed to compare the benefits of V. amygdalina and M. oleifera on the antioxidant enzymes with respect to the hippocampus of diabetic adult wistar rats. The experiment consisted of thirty (30) adult Wistar rats with body weights of 160g – 180g which were divided into six groups of five rats each. The experimental rats except for the normal control group (group A) were intraperitoneally injected with 120mg/kg body weight of prepared alloxan. Group B served as the diabetic control. Group C and B received 250mg/kg and 350mg/kg body weight of aqueous extract of V. amygdalina and M. oleifera for 30 days respectively. Group E received 5mg/kg body weight of glibenclamide for 30 days. Group F received 350mg/kg body weight of V. amygdalina and Group G received 350mg/kg body weight of M. oleifera for 30 days respectively. The findings show that alloxan significantly (P 0.05) decreased catalase (CAT) and superoxide dismutase (SOD), while increasing malondialdehyde levels (MDA) in the hippocampus. The levels of SOD and MDA are markedly elevated by V. amygdalina and M. oleifera, respectively. The CAT levels however, decreased in both plants. According to the research, V. amygdalina has greater antioxidant advantages over M. oleifera.***

Keywords: ***Diabetes Mellitus, antioxidant enzymes, hippocampus, Vernonia amygdalina, Moringa oleifera***

I. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia in the absence of treatment due to defective insulin secretion, insulin action, or both. (WHO, 2019). Globally, this disease is spreading exponentially because of aging, urbanization, changing lifestyles, and population expansion. Rising levels of obesity brought on by bad diets (high calorie intake and rise in consumption of processed foods) and pervasive inactivity or sedentary lifestyles are associated with the emerging global epidemic of DM (Sami et al., 2017). The International Diabetes Federation (IDF) estimates that 463 million people worldwide already have diabetes, and that number will rise to 700 million by the year 2045. (IDF, 2019). In Africa, the prevalence of DM is steadily rising; between 2019 and 2045, the percentage rise will be 143%. According to studies, Nigeria's overall pooled prevalence rate was 5.77%. (Uloko et

al. 2018). According to IDF (2019), 4 million people worldwide died between the ages of 20 and 79 in 2017, while 27 500 people in Nigeria died between the ages of 30 and 69. (WHO, 2016).

Diabetes significantly reduces the brain's ability to use glucose, making it more susceptible to pathological diseases (Pari and Latha, 2004). Cognitive deterioration, vascular anomalies, and behavioral disorders are only a few examples of the pathological changes that can occur in the central nervous system (Hardigan et al., 2016). These modifications are a result of the oxidative damage brought on by hyperglycemia (Pham-Huy et al., 2008). Oxidative stress originates from an excess creation of free radicals that is caused by an obstruction in the antioxidant response system's normal functioning, which lowers the activities of antioxidant enzymes (Salim, 2017). Reactive oxygen species (ROS) are necessary for signaling in the brain, but an excessive buildup causes cellular oxidative damage (Cobley et al., 2018). Cognitively active regions of the brain, such as the hippocampus, are the most vulnerable (Wang and Michealis, 2010). Due to its intricate structure, the hippocampus is very vulnerable, and any harm or atrophy causes cognitive deterioration (Njan et al., 2020).

Herbal medicines have been supported as a management strategy for diabetes and associated complications over the years. *Vernonia amygdalina (V. amygdalina)* and *Moringa oleifera (M. oleifera)* are among the medicinal plants that have been examined and used to treat diabetes as herbs with antidiabetic characteristics. As a result of the numerous health benefits associated with various plant parts, *M. oleifera*, a member of the Moringaceae family, is frequently referred to as the miracle tree (Obembe & Raji, 2018). Numerous phytoconstituents, including tannins, phenolic acids, nitrile glycoside, alkaloids, saponins, glucosinolates, and flavonoids have also been documented to be present in the plant (Mathur et al., 2014; Maldini et al., 2014). According to reports, the plant contains significant antioxidant and anti-diabetic properties (Abdlazeem, 2019; Xu et al., 2019). On the other hand, *V. amygdalina*, also referred to as bitter leaf, is a tropical native plant. The Asteraceae or Compositae family member *V. amygdalina* is distinguished by a bitter taste that results from the anti-nutritional elements of the leaves, including alkaloids, flavonoids, saponins, glycosides, and tannins (Ogunrinola et al., 2019). Locals eat the plant, and it is also used medically for things like antioxidant, antidiabetic, antimalarial, and antihelmintic purposes (Uchendu, 2018; Farombi & Owoeye, 2011; Ijeh & Ejike, 2011), among other things.

This study examined the impact of *V. amygdalina* and *M. oleifera* on the hippocampus oxidant enzymes in diabetes since both plants have been widely utilized to treat a variety of disorders.

II. MATERIALS AND METHOD

A. PLACE OF THE STUDY

This experiment was carried out in the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi, Anambra State.

A. COLLECTION OF PLANT MATERIALS

Moringa leaves (*M. oleifera*) and bitter leaves (*V. amygdalina*) were procured from the Nkwo market in Nnewi, Anambra state.

B. PREPARATION OF MORINGA OLEIFERA AQUEOUS LEAVES EXTRACT

The leaves of *M. oleifera* were prepared separately. They were dried at room temperature after which the leaf powder was prepared using a home blender. Powdered *M. oleifera* leaves weighing 50g were soaked in 500ml of distilled water for 24 hours after which they were sieved and filtered through a Whatman filter paper no. 1 and evaporated to dryness with the aid of a vacuum oven at 40^{o}C. the concentrations 25%, and 35% were prepared, for 25% conc., 25ml of stock was dissolved in 75ml of distilled water and for 35% conc., 35ml of stock was dissolved in 65ml of distilled water. That implies *M. oleifera* leaves extract at doses of 250mg/kg and 350mg/kg. The aqueous extract stock solutions were stored at 4^{o}C for up to 5 days or freshly prepared for each set of experiments.

C. PREPARATION OF VERNONIA AMYGDALINA AQUEOUS LEAVES EXTRACT

After being rinsed, *V. amygdalina* leaves were allowed to air dry for a week at room temperature. They were blended in a household blender until they were pulverized. 50g of powdered leaves were measured out, 500ml of distilled water was added, and the mixture was mixed and allowed to sit for 24 hours before filtering. The mixture was next filtered using Whatman NO.1 filter paper and dried using a vacuum oven set to 40°C. Solutions containing 25% and 35% of the stock of bitter leaf extract were created.

D. EXPERIMENTAL ANIMALS

Thirty (30) adults experimental wistar rats weighing between 160g and 180g were obtained from an animal farm in the College of Health Sciences, Nnamdi Azikiwe University, Nnewi, and housed in the Animal House of the Department of Anatomy, Nnamdi Azikiwe University. The animals were left to acclimatize for two weeks and maintained under the standard environmental conditions and were allowed to have access to food and water ad libitum.

E. INDUCTION OF DIABETES

All rats, excluding those in the Normal Control Group, received an intraperitoneal injection of 120 mg/kg Alloxan. Alloxan monohydrate was dissolved in sterile normal saline solutions to create alloxan. To prevent alloxan-induced hypoglycemia, rats were given 10% glucose solution in their cages for the following 24 hours after receiving alloxan for 6 hours. In addition to a general loss of body weight, the animals were observed for polydipsia, polyuria, and polyphagia. The animals underwent a 72-hour fast, and an Accu-Chek Performa glucometer was used to measure the animals' fasting

blood glucose levels to determine whether the animals had diabetes. Rats were given tail punctures to draw blood samples for the fasting blood glucose measurement. Animals having blood glucose levels greater than 200 mg/dL (more than 11.1 mmol/L) were deemed diabetic, and blood glucose levels were monitored daily for five days.

Treatment commenced on the fifth day; post alloxan injection and was considered the first day of treatment. The treatment lasted for 30 days.

F. EXPERIMENTAL DESIGN

Thirty (30) adult wistar rats were used and randomly assigned into six groups (A-F) with five rats in each group.

GROUP A: Each control rat received 1ml of distilled water daily.

GROUP B: Each diabetic control rat received 1ml of distilled water daily

GROUP C: Alloxan-induced diabetic rats were administered 250mg/kg body weight aqueous extract of *M. oleifera* and *V. amygdalina* leaves once daily.

GROUP D: Alloxan-induced diabetic rats were administered 350mg/kg body weight aqueous extract of *M. oleifera* and *V. amygdalina* leaves once daily.

GROUP E: Alloxan-induced diabetic rats were treated with a standard antidiabetic drug (glibenclamide 5mg/kg body weight) once daily.

GROUP F: Alloxan-induced diabetic rats were administered 350mg/kg body weight aqueous extract of *V. amygdalina* leaves once daily.

GROUP G: Alloxan-induced diabetic rats were administered 350mg/kg body weight aqueous extract of *M. oleifera* leaves once daily.

G. EXPERIMENTAL PROTOCOL

The animals were weighed before and after the experiment. Also, at the end of the experiment, the rats fasted for 24 hours and then sacrificed by decapitation under diethyl ether anaesthesia. The brains of the rats were harvested and weighed before being subjected to homogenization.

H. BRAIN HOMOGENATE PREPARATION

The brain tissue was quickly removed and washed in an ice-cold saline solution. One gram of the brain tissue was homogenized with 10ml of ice-cold 0.05M phosphate buffer pH 7.4. The homogenate was centrifuged at 7,000rpm for 15 min., in the cold medium, and the supernatant obtained was stored at 4°C for further analysis.

I. BIOCHEMICAL PARAMETERS

The oxidative stress marker and antioxidants were assayed using a spectrophotometer. The Malondialdehyde Level (MDA) in the brain tissue was estimated using the technique described by Buege and Aust (1978); Catalase (CAT) activity in brain tissue was determined according to the method of Aebi (1984) and superoxide dismutase (SOD) activity in brain tissue was determined according to the method described by Misra and Fridovich (1972).

J. STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS; Version 20) was used for data analysis, and the results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was applied for determining the significance. The acceptable level of significance was established at $P < 0.05$.

III. RESULTS

	Initial weight (g) MEAN±SEM	Final weight (g) MEAN±SEM	P-value
Group A (Positive control)	144.00±18.79	216.75±7.91	0.02 [a]
Group B (diabetic control)	166.33±18.80	153.00±14.00	0.71 [b]
Group C (DM + 250mg/kg of *M. oleifera* + *V. amygdalina*)	160.00±10.59	180.00±8.50	0.12 [b]
Group D (DM + 350mg/kg of *M. oleifera* + *V. amygdalina*)	130.67±6.76	187.33±13.77	0.06 [b]
Group E (5mg/kg of Glibenclamide)	134.67±11.20	167.33±12.73	0.30 [b]
Group F (350mg/kg of *V. amygdalina*)	171.00±5.00	180.50±6.50	0.56 [b]
Group G (350mg/kg of *M. oleifera*)	162.00±18.78	171.75±23.25	0.82 [b]

Table 1: effect of aqueous extract of M. oleifera and V. amygdalina on bodyweight in diabetic induced toxicity

All data were analysed using a T-test, and were considered significant at $p<0.05$. SEM: Standard error of mean. [a] (significant) [b] (not significant). Table 1 result demonstrated a significant increase in the body weight in-group A, and group B demonstrated a non-significant decrease in the body weight when the initial weight was compared to the final weight. However, groups C, D, E, F, and G had an insignificant increase in bodyweight following comparison between initial and final weight.

	Relative brain weight (g/%) MEAN±SEM	Malondialdehyde (µmol/L) MEAN±SEM
Group A (Positive control)	0.82±0.00 [b]	1.96±0.08 [a]
Group B (diabetic control)	0.92±0.10	2.93±0.08
Group C (DM + 250mg/kg of *M. oleifera* + *V. amygdalina*)	0.80±0.01 [b]	2.46±0.10 [b]
Group D (DM + 350mg/kg of *M. oleifera* + *V. amygdalina*)	0.84±0.06 [b]	1.33±0.11 [a]
Group E (5mg/kg of Glibenclamide)	0.88±0.01 [b]	1.20±0.00 [a]
Group F (350mg/kg of *V. amygdalina*)	0.85±0.02 [b]	2.26±0.46 [a]
Group G (350mg/kg of *M. oleifera*)	0.90±0.01 [b]	2.30±0.05 [a]
F-value	0.89	8.07

Table 2: effect of ethanolic extract of M. oleifera and V. amygdalina on relative brain weight, and Malondialdehyde in diabetic Wistar rats

All data were analysed using ANOVA followed by post HOC LSD multiple comparisons, and values were considered significant at $p<0.05$. SEM: Standard error of mean, [a] (significant) [b] (not significant). Table 2 result demonstrated a

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significant decrease in the MDA level in group A compared to B. Groups D, E, F, and G had a significant decline and group C had an insignificant increase in MDA level compared to group B. The relative brain weight result demonstrated an insignificant rise in group A compared to B. Groups C, D, E, F, and G had an insignificant decline in the relative brain weight compared to group B.

	Catalase (U/L) MEAN±SEM	Superoxide Dismutase (U/L) MEAN±SEM
Group A (Positive control)	41.75±2.91 [a]	24.40±3.46 [a]
Group B (diabetic control)	31.25±5.38	13.50±1.01
Group C (DM + 250mg/kg of *M. oleifera* + *V. amygdalina*)	26.00±2.19 [b]	21.46±1.76 [a]
Group D (DM + 350mg/kg of *M. oleifera* + *V. amygdalina*)	19.85±0.83 [a]	20.93±1.63 [a]
Group E (5mg/kg of Glibenclamide)	16.85±1.76 [a]	19.53±0.67 [a]
Group F (350mg/kg of *V. amygdalina*)	19.45±0.43 [a]	22.60±1.10 [a]
Group G (350mg/kg of *M. oleifera*)	14.35±0.31 [a]	24.93±0.73 [a]
F-value	13.93	4.89

Table 3: effect of ethanolic extract of M. oleifera and V. amygdalina on catalase activity and superoxide dismutase activity in diabetic Wistar rats

Data were analysed using ANOVA followed by post HOC LSD multiple comparisons, and values considered significant at $p<0.05$. SEM: Standard error of mean, [a] (significant) [b] (not significant). Table 3 results demonstrated a significant increase in catalase level in group A compared to B. Groups D, E, F, and G had a significant decline, and group C had an insignificant decrease compared to B. The superoxide dismutase level showed a significant increase in-group A compared to B. Groups C, D, E, F, and G had a significant increase compared to group B.

IV. DISCUSSION

This study was designed to compare the benefits of aqueous extracts of *V. amygdalina* and *M. oleifera* in managing oxidative stress of alloxan-induced diabetes in Wistar rats. First, considering the body weights of the Wistar rats before and after the experiments, there was only a significant increase in the body weight of group A (positive control) while the other groups had an insignificant increase with group B (negative control) having an insignificant decrease. On the relative brain weight, there was no significant difference noted in the treatment groups when compared to the control. This implies that diabetes mellitus causes no significant inflammation or weight loss while on the other hand, *V. amygdalina* and *M. oleifera* extract administration were able to checkmate any inflammation due to lipid peroxidation as a result of their rich antioxidant phytoconstituents such as flavonoids and tannins (Aguwa et al., 2020).

The presence of elevated MDA is one of the most prevalent and dependable indicators of lipid peroxidation and oxidative stress (Debasree et al., 2013). It has been shown to be associated with diabetes mellitus (Sheweita et al., 2016; Calderon et al., 2017). The occurrence of an increase in free radicals causes the overproduction of MDA malondialdehyde (MDA) which is the final product of lipid peroxidation in the cells. The present findings as evident in table 2 of this study demonstrated that the induction of diabetes caused a significant increase in MDA in the diabetic control (group B) when compared to the positive control (group A). This significant increase implies that diabetes mellitus caused an increase in lipid peroxidation in the hippocampus. This is in agreement with Melikiyan et al., (2019) who reported increased levels of MDA in animals induced with diabetes. Low doses of the combined extract (group C) led to an insignificant decrease in MDA level while varying combinations and doses in groups D, E, F, and G are found to reduce lipid peroxidation as evidenced by the significant decrease in MDA level in all the groups when compared to the diabetic control (group B). The combination doses in group D produced a significant increase which is almost equivalent to the standard anti-diabetic drug, glibenclamide (Group E). The decrease in MDA level was however more evident in the group treated with the single dose of *V. amygdalina* than in the group treated with the single dose of *M. oleifera*. This result is in line with the findings of Rahmath et al., (2015) and Kirisattayakul et al., (2013) which showed that *M. oleifera* significantly attenuated MDA levels in the hippocampus when exposed to different stressors or toxicities. It is also in line with the findings of Aguwa et al., (2020) and Nwanjo, (2005) who found that varying doses of *V. amygdalina* extract significantly attenuate lipid peroxidation in the brain and blood serum respectively. All of these are a result of the rich presence of phytochemicals contained in the plant materials such as flavonoids.

Furthermore, oxidative stress could be prevented by the scavenging activity of the antioxidant enzymes (Sharma et al., 2015). And in this study, the activities of the antioxidant enzymes on the hippocampus were observed and it showed that DM significantly reduced SOD and CAT activities as seen in the diabetic control when compared to group A (as presented in table 3). This is because the brain is more vulnerable to oxidative stress due to its high oxygen expenditure and high levels of polyunsaturated fatty acids (Aparecida et al., 2015). However, administration of glibenclamide and varying doses and combinations of *M. oleifera* and *V. amygdalina* as seen in groups C, D, E, F, and G led to a significant increase in SOD level and a significant decrease in CAT level when compared to the diabetic control (group B). The SOD result is in line with the findings of Rahmath et al., (2015) and Aguwa et al., (2020), and while the CAT results, however, disagree with these findings. The antioxidant and scavenging properties were more evident in the group treated with only *V. amygdalina* than in the group treated with *M. oleifera* singly.

Generally, these protective effects against lipid peroxidation and increased activities of free radical scavenging enzymes are a result of the rich bioactive phytoconstituents of *V. amygdalina* and *M. oleifera* as they synergize to combat and attenuate oxidative stress caused by diabetes mellitus. It was reported that the major *M. oleifera* bioactive compounds of phenolics, such as quercetin and kaempferol are responsible for this antioxidant activity and it

also possesses a large number of flavonoids also known to be potent antioxidants (Bajpai et al., 2005; Abd Rani et al., 2018). Many chemical compounds isolated from the leaves of Vernonia amygdalina elicit remarkable antioxidant and chemo-preventive properties in cell cultures and rodent models (Yeap et al., 2014). This is true as the high-performance liquid chromatography (HPLC) fingerprint of *V. amygdalina* elucidated the presence of potent antioxidants and bioactive compounds, such as catechin, chlorogenic acid, quercitrin, quercetin, caffeic acid, and luteolin (Ademosun et al., 2017). However, the aqueous extract of *V. amygdalina* showed better antioxidant properties than M. oleifera. This may be attributed to the high flavonoid and phenolic content in *V. amygdalina* as compared to *M. oleifera* (Olumide, Ajayi & Akinboye, 2019).

Overall, *V. amygdalina* and *M. oleifera* co-administration demonstrated increased antioxidant effects compared to when co-administrered separately. This only indicates that the ameliorative, protective, and preventive benefits of the co-administration of medicinal plants may be enhanced.

V. CONCLUSION

The findings of this study suggest that *V. amygdalina* and *M. oleifera* are very effective in the treatment of diabetes. And it shows that *V. amygdalina* contains more antioxidant benefits than *M. oleifera.*

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