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EVALUATION OF ANTI-DIABETIC AND ANTIOXIDANT EFFECTS OF *CITRULLUS LANATUS* (WATERMELON) SEEDS AND *MORINGA OLEIFERA* LEAVES IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

The use of natural products as a means of treatment for many physiological threats such as diabetes is currently gaining momentum in Nigeria. Diabetes is a metabolic syndrome that afflicts an estimate of 143 million people and that oxidative stress plays a vital role in the development of its related complications. Hence this study was aimed at investigating the hypoglycemic and antioxidant properties of *Citrullus lanatus* (watermelon) seeds and *Moringa oleifera* leaves in Alloxan-induced diabetic Wistar rats. A total of thirty rats were divided into six groups of 5 rats each: Group I: Non Diabetic Control, Group II: Diabetic Control without treatment, Group III and IV represents Diabetic rats administered with ethanolic leaf extract of *Moringa* (200mg/kg and 400mg/kg body weight). Group V and VI represent Diabetic rats treated with watermelon seeds (200mg/kg and 400mg/kg body weight). At the end of the experiment, rats were fasted overnight and blood samples were collected under chloroform anaesthesia for the estimation of fasting blood glucose and antioxidant vitamins A, C, E using standard techniques. The results indicated a significant increase ($p<0.05$) in blood glucose and a significant decrease ($p<0.05$) in antioxidant vitamins A, C and E in diabetic rats compared to non diabetic control. All the rats treated with ethanolic leaf extract of *M. oleifera* and *C. lanatus* seeds recorded a significant decrease in glucose level after 14days. The result indicated a significant increase ($p<0.05$) in antioxidants A, C and E in diabetic Wistar rats supplemented with 200mg and 400mg of *M. oleifera* leaf extract except for vitamin E which was not significantly increased ($p>0.05$) in diabetic rats treated with the low dose of *M. oleifera* leaf (200mg) when compared with the corresponding values in the diabetic control. There is a significant increase ($p<0.05$) in vitamin A and E in diabetic Wistar rats treated with *Citrullus lanatus* seeds with no significant ($p>0.05$) difference in serum level of vitamin C in diabetic rats supplemented with both 200mg and 400mg *C. lanatus* seed extract compared to diabetic control. These findings indicate that *C. lanatus* seed and *M. oleifera* leaves extracts possesses hypoglycaemic and antioxidant properties.

KEYWORDS: Diabetes, *Citrullus lanatus*, *Moringa oleifera*, Antioxidant vitamins, Alloxan.

INTRODUCTION

BACKGROUND

Diabetes mellitus has been described as a metabolic disorder of multiple aetiology characterized by hyperglycemia and alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion by the beta cells of the islets of the pancreas and/or insulin action (Khan *et al.*, 2015).

There are many types of diabetes, however, the two main types of diabetes are Type 1 (insulin-dependent or juvenile onset) caused by lack of insulin secretion by beta cells of the pancreas, accordingly patients must take insulin injections daily to survive. Type 2 (non-insulin dependent or adult onset). It is the most common form of diabetes accounting for about 90% of cases. It is caused by the inability of the body to produce enough insulin or by decreased sensitivity of target tissues to insulin (Hussein *et al.*, 2016). There are also other specific but less common types of diabetes. These include drug-

induced or chemical-induced diabetes, diabetes caused by diseases of the exocrine pancreas (such as cystic fibrosis) or by infections and also gestational diabetes often classified as type 4 (ADA, 2001). Diabetes is characterized by hyperglycemia, glycosuria and several disabling and life threatening complications such as retinopathy, nephropathy, hepatopathy and coronary artery disease (Komolafe *et al.*, 2013).

Several anti diabetic drugs such as biguanid and sulphonyureas along with insulin have been employed for the treatment of this disease. Still none of these drugs were able to cure the disease without adverse reaction (Aja *et al.*, 2015). These associated problems necessitate the search for better drugs with fewer side effects. Many plants are seen to possess hypoglycemic and antioxidant properties (Bnouham, 2006).

The *M. oleifera* tree (*Moringa oleifera*) belonging to family Moringaceae is a fast growing tropical tree. It is called a miracle tree due to its numerous therapeutic benefits, this plant is also prescribed in ancient civilizations, it is well known, cultivated as a crop and consumed as vegetables in many African, Asian, Latin America and Caribbean countries besides its applications in traditional medicine; It is now cultivated as a crop in so many countries in Africa and Asia (Fahey, 2015). Different parts of *M. oleifera* plant contain important minerals as K, Ca, P, Fe, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics as zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol (Anwar *et al.*, 2007; Gowrishankar *et al.*, 2010). It has anti-cancer, anti-inflammatory, and thyroid status regulator efficacies and researchers have reported its hypoglycemic potential (Kar *et al.*, 2003).

Citrullus lanatus is well known as Watermelon plant (Family - Cucurbitaceae). It is grown extensively in south Africa. The seeds contains phytochemical constituent like alkaloids and flavonoids with recognizable hypoglycemic effect (Nasir *et al.*, 2009; Omagie and Agorey, 2014). The leaves of *Citrullus lanatus* is used as anti-inflammatory, analgesic, gonorrhoea, mosquitocidal and has anti microbial property (Ahmed *et al.*, 2011; Rahman *et al.*, 2013). *Citrullus lanatus* possesses numerous bioactivities from natural source which is of better advantage than conventional therapies (Erhirhie and Ekene, 2013).

Multiple studies have shown that the type 2 diabetes is accompanied by increased oxidative damage to all biomolecules in the body including lipids, proteins and nucleic acids and thus leading to cell damage in diabetes (Sinclair, 1993; Birben *et al.*, 2012). Free radicals are either generated by cellular metabolism such as glycolysis, mitochondrial respiration and xenobiotic detoxification or by exogenous factors such as redox reaction. Human beings possesses highly complex and sophisticated antioxidant system (Examples include β-carotene, vitamins A, C, E, and enzymes) that works to protect against free radical damage. Medicinal plants are important source of antioxidants that are active against oxidative damage caused by free radicals (Kasote *et al.*, 2015; Li *et al.*, 2016). Therefore, maintenance of adequate antioxidant levels is essential to prevent or even manage a great number of disease conditions (Carlos and Bucalen, 2008).

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

The seeds of *Citrullus lanatus* (watermelon) and leaves of *M. oleifera* were collected from local market in Birnin-kebbi metropolis of Kebbi state, Nigeria. The plants were identified and authenticated at herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. Voucher number was obtained to be (PCG/UDUS/mori/0001) and (PCG/UDUS/curc/0003) for *M. oleifera* and *Citrullus lanatus* respectively and the specimens were deposited at the herbarium.

PREPARATION AND EXTRACTION

The leaves of *M. oleifera and* watermelon seeds were collected, washed and air-dried at room temperature. The dried leaves and seeds were pulverised to fine powder using laboratory mortar for the leaves and grinder for the seeds. Five hundred (500) g of the grinded seeds was soaked in 1.5 litre of 98% ethanol for 48 hours on a mixer to ensure maximum extraction by percolation using maceration technique under room temperature. This is followed by periodic stirring (Ahmed and Sani, 2013). Two hundred gram (200) g of the powdered leaves was macerated with one litre (1L) of 98% ethanol for 48 hours with occasional shaking. Resulting crude extracts were filtered using what man number 1 filter paper and the filtrates were concentrated in a water bath at 45℃ to obtain 17g of green crude extract of *M. oleifera* leaves and 15g of brownish extract for watermelon seeds. The dried extracts were collected in a sterile storage bottles and kept at 4^0C in a refrigerator until required for use.

CHEMICALS AND REAGENTS

Analytical grade chemicals and reagents were used for this research. Reagent kit for the assay of fasting blood glucose was purchased from Randox Laboratories Limited, United Kingdom.

EXPERIMENTAL ANIMALS

Thirty (30) male Wistar rats (aged 8-12 weeks old), weighing between 190g to 225g were purchased from the animal house, of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The rats were housed in well aerated cages under hygienic conditions in the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were allowed to acclimatise for a period of 2 weeks before the commencement of the experiment. The

animals were maintained as described by Aniagu *et al.*, (2005) in a clean metabolic cage-sand, placed in a well ventilated room conditions with a temperature of 26^0C to 28^0C, photoperiods of 12 hours light and 12 hours darkness; humidity of 40% to 60%.

The animals were fed pelletized feeds (vital®), obtain from Grand Cereals Oil Mills Limited, Jos and were supplied with drinking water *ad labium* throughout the experimental period. Cleaning of the animal cages was carried out daily, and on regular basis. All the experimental protocols were in compliance with the Institutional Animal Ethics Committee guidelines as well as Internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health, 1992), and also in accordance with the recommendation of the International Association for the study of pain (IASP) (Zimmerman, 1983).

RESEARCH DESIGN

Grouping of Animals

The animals were randomly divided into six (6) groups of five rats (5) each. The groups were as follows:

Group I: Control received only rat chow and water

Group II: Diabetic control received only alloxan with no treatment

Group III: Diabetic group and were administered with low dose of *M. oleifera* leaves extract (200mg/kg/day) body weight orally for 14 days

Group IV: Diabetic group and were administered with high dose of *M. oleifera* leaves extract (400mg/kg/day) body weight orally for 14 days.

Group V: Diabetic group and were administered with low dose of watermelon seeds extract (200mg/kg/day) body weight orally for 14 days

Group VI: Diabetic group and were administered with high dose of watermelon seeds extract (400mg/kg/day) body weight orally for 14 days.

Induction of diabetes

To induce experimental diabetes, Alloxan monohydrate was dissolved in saline solution (0.9% sodium chloride, PH 7) and was injected into rats as a single dose of 150 mg/kg intraperitoneally using diabetic syringe as recommended by Ajibola *et al.*, (2014). The rats were placed on 10% glucose for next 24 hours to prevent hypoglycaemia (Misra and Aiman, 2012). After 48 hours, fasting blood glucose (FBG) was determined using On Call Plus one touch glucometer strips (Acon Laboratories) as described by Anees *et al.*, (2007), those with glucose level >180 mg/dl were considered diabetic. The glucose level was assayed weekly to examine the effect of the extracts on the glucose level of the rats.

ANALYTICAL METHODS

Body Weight

The rats in all groups were weighed using a sensitive balance, before commencement of dosing, weekly during the period of dosing and on the day of sacrifice.

Blood Sample Collection and Processing

After 14 days period, the animals were fasted for 12 hours, and were anaesthetized in a glass jar containing wool soaked with chloroform. About five millilitres (5mL) of blood samples were collected from the animals through cardiac puncture, into clean, plain and fluoride oxalate containers. The samples collected in a plain container were allowed to clot at room temperature and later centrifuge at 4000 revolution per minute (4000 rpm) for 10 minutes. The obtained sera were then transferred into labelled sterile cryovials and were tightly caped and stored at -20^OC until the time of assay for the serum levels of antioxidant vitamins A, C and E. The blood sample collected in fluoride oxalate containers was centrifuged as described above and the obtained plasma was used to evaluate the fasting plasma glucose.

Determination of blood glucose

Blood glucose was determined by Glucose oxidase peroxidase method as described by Trinder, (1969).

Determination of antioxidant vitamins

Serum vitamin A concentration was estimated using the method of Rutkowski and Grzegorczyk, (2007).

Serum vitamin C (ascorbic acid) concentration was estimated using the method of Singh and Singh, (2015).

Vitamin E was determined by the method described by Al-Kawaz and Al-Mashhady, (2016).

RESULTS

Change in Body Weight

Table 1 shows the initial and final body weight of diabetic Wistar rats supplemented with both low and high dose of *M. oleifera* leaves extract and *Citrullus lanatus* seeds extract and controls. In this table the final body weight of rats in all the groups are significantly increased ($p < 0.05$) with the exception of diabetic group treated with 200mg *C. lanatus* seed extract when compared with corresponding values in diabetic control which was significantly decreased ($p<0.05$).

Table 1: Changes in Body Weight of Diabetic Wistar Rats supplemented with Ethanolic leaf extract of *M. oleifera* and *C. lanatus* seeds and controls.

Group	(n)	Initial body weight (g)	Final body weight (g)
Group I	5	205.67±1.85	241±4.04
Group II	5	203±2.08	154±5.23
Group III	5	193.67±0.88	227±1.52
Group IV	5	223.33±0.88	248±1.52
Group V	5	207.67±0.88	212±1.45
Group VI	5	213±1.00	222±3.38
P-value		>0.05	<0.001
Post-hoc Analysis			
Group I Vs II		>0.05	<0.05
Group II Vs III		>0.05	<0.05
Group II Vs IV		>0.05	<0.05
Group II Vs V		>0.05	>0.05
Group II vs VI		>0.05	<0.05

Change in blood glucose level

Table 2 and Figure 1 shows the fasting blood glucose of Alloxan-induced diabetic Wistar rats treated with the ethanolic extract of *M. oleifera* leaves and *C. lanatus* seeds and controls. After the first week of treatment there was no significant decrease ($p>0.05$) in glucose level except for diabetic Wistar rats treated with 200mg of *C. lanatus* seeds and *M. oleifera* leaf extract which shows a significant decrease ($p<0.05$) when compared with the diabetic control. The glucose level was significantly reduced ($p<0.05$) in all the groups after 14 days treatment when compared with the diabetic control.

Table 2: Fasting plasma glucose of Alloxan-induced diabetic Wistar rats supplemented with Ethanolic extracts of *M. oleifera* leaves and *C. lanatus* seeds and controls.

GROUP	N	FBG (Day 7)	FBG (Day 14)
Group 1	5	87.67±6.96	81.33±1.85
Group 2	5	335±11.53	512±25.53
Group 3	5	95±4.5	77.67±1.45
Group 4	5	181±39.73	102.33±3.48
Group 5	5	108.67±4.66	96±8.02
Group 6	5	377.33±20.25	103±4.80
P value		<0.001	<0.001
Post hoc analysis			
Group I Vs II		<0.05	<0.05
Group II Vs III		<0.05	<0.05
Group II Vs IV		>0.05	<0.05
Group II Vs V		<0.05	<0.05
Group II Vs VI		>0.05	<0.05

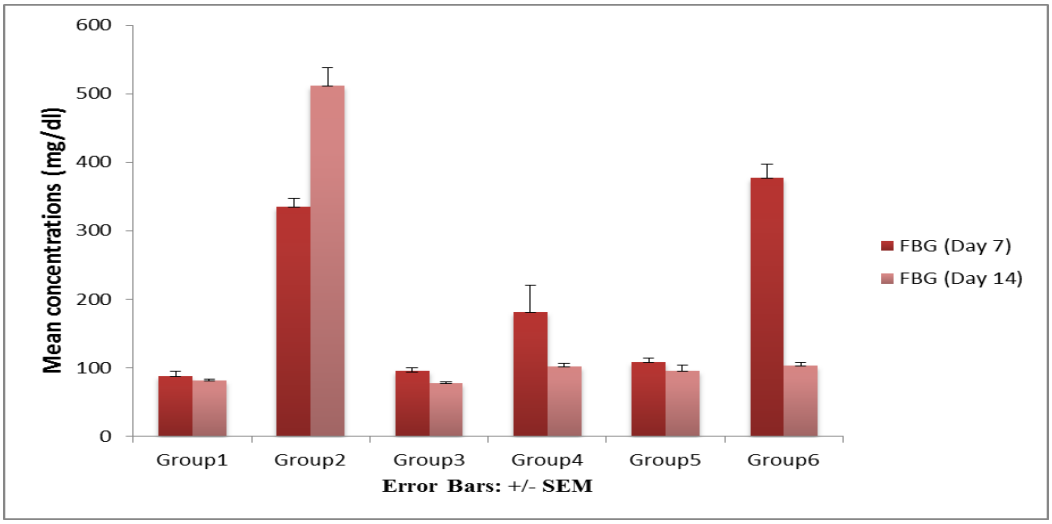

Figure 1: Shows the mean value of week 1 and 2 fasting blood glucose among the six groups.

KEY
Group1= Non Diabetic control
Group2= Diabetic control
Group3=200mg *M. oleifera*
Group4=400mg *M. oleifera*
Group5=200mg *C. lanatus*
Group6=400mg *C. lanatus*
SEM= Standard error of mean

Effect of *M. oleifera* leaves and *C. lanatus* seeds extracts on serum concentrations of antioxidant vitamins

Figure 2 shows the result of the effect of intake of *M. oleifera* leaves and *Citrullus lanatus* seeds on antioxidant vitamins A, C and E. Diabetic Wistar rats showed a significant decrease ($p<0.05$) in the antioxidant vitamins (2.13±0.23, 3.68±0.42, 3.00±0.43) compared with non diabetic control (20.16±0.75, 24.16±1.55, 27.03±2.24) for vitamin A, C and E respectively. The result indicated a significant increase ($p<0.05$) in antioxidants A, C and E in diabetic Wistar rats supplemented with 200mg and 400mg of *M. oleifera* leaf extract except for vitamin E which was not significantly increased ($p>0.05$) in diabetic rats treated with the low dose of *M. oleifera* leaf (200mg) when compared with the corresponding values in the diabetic control. There is a significant increase ($p<0.05$) in vitamin A and E in diabetic Wistar rats treated with *Citrullus lanatus* seeds with no significant ($p>0.05$) difference in serum level of vitamin C in diabetic rats supplemented with both 200mg and 400mg *C. lanatus* seed extract compared to diabetic control.

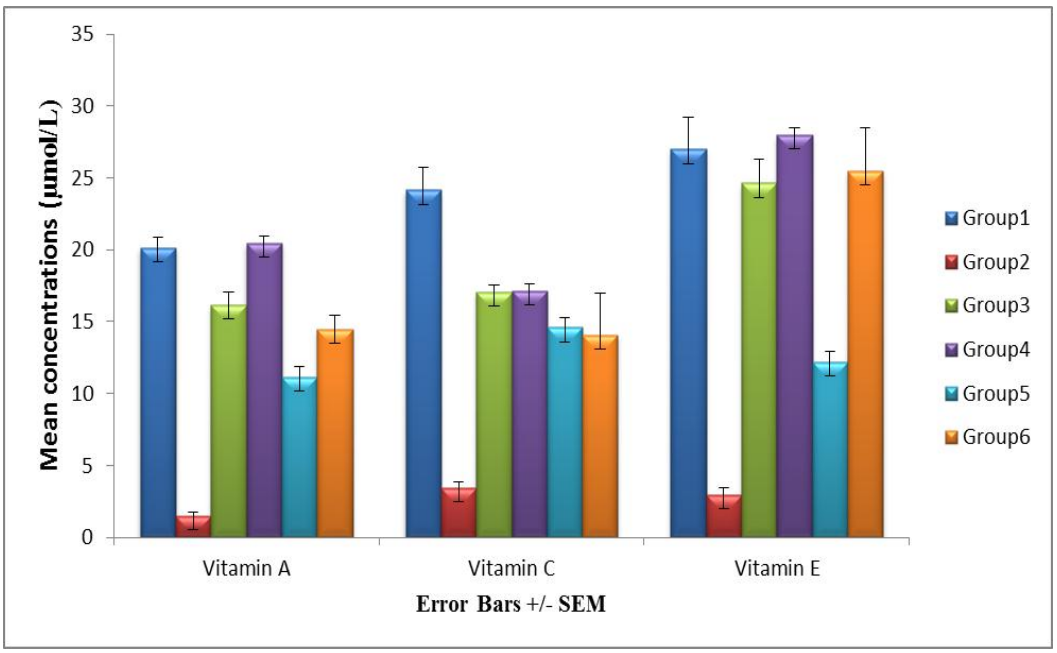

Figure 2: Shows the mean values of antioxidant vitamins among the six groups.

KEY:
Group1= Non Diabetic control
Group2= Diabetic control
Group3=200mg *M. oleifera*
Group4=400mg *M. oleifera*
Group5=200mg *C. lanatus*
Group6=400mg *C. lanatus*
SEM= Standard error of mean

DISCUSSION

Diabetes is a major disease characterized by derangement of carbohydrates, fats and protein metabolism, affecting about 10% of the population. The treatment of DM with oral agents had been being reported to be endowed with characteristic profiles of serious side effects. This leads to increasing demand for herbal products with anti-diabetic factor with little side effects. A large number of plants have been recognized to be effective in the treatment of diabetes mellitus. The present study was carried out to assess the anti-diabetic effect and the antioxidant activity of ethanolic extracts of *M. oleifera* leaves and watermelon seeds.

There was no significant difference in the body weight between all the groups at baseline. However, After 14

days treatment with both the ethanolic extract of *M. oleifera* leaves and *C. lanatus* seeds the final body weight of the rats in all the groups showed a significant increase in body weight ($P<0.05$) when compared with the diabetic group except for the rats treated with 200mg watermelon seeds extract. The result is in agreement with findings of Efiong *et al.,* (2013), Adeeyo *et al.,* (2013) and Aja *et al.,* (2015). The loss of weight seen with diabetic group may be associated with the administration of Alloxan. Wang *et al.,* (2011) reported alloxan to be a toxic substance and loss of weight is a sensitive preliminary index of toxicity (Raza *et al.,* 2002). Another reason for the loss of weight could be due to dehydration and catabolism of fats or breakdown of proteins with consequent muscle wasting (Kimani *et al.,* 2015).

Diabetic Wistar rats treated with ethanolic leaf extract of *M. oleifera* displayed a significant lower glucose level when compared to the diabetic control which is in accordance with the findings of Ajibola *et al.,* (2014), Nabila *et al.,* (2015) and Aja *et al.,* (2015).

The ability of the leaf extract of *M. oleifera* to significantly reduce hyperglycemia induced by alloxan may be as a result of its phytochemical and micronutrient constituents (Nabila *et al.,* 2015). The leaf of *M. oleifera* contains many powerful antioxidant phytochemicals, especially quercetin and kaempferol. Kaempferol has been shown to have hypoglycemic activities (Fuglie 1999; Luangpiom, 2013). Also, the mechanisms of actions could be either by increasing the tissue utilization of glucose (Gray *et al.,* 2000), or inhibiting gluconeogenesis or absorption of glucose into the muscles and adipose tissues (Mbikay, 2012; Soliman, 2013; Ajibola *et al.,* 2014). It could also be by stimulating the *β*-cells of the islets of Langerhans or due to its insulin-like activity (Tende *et al.,* 2011). The *M. oleifera* hypoglycemic activity is reported to be due to the presence of α-glucosidase and pancreatic amylase enzyme inhibitors.

Reduction of serum glucose is the classical and clinical target of any form of diabetes and the results of current study clearly indicate that administration of 200mg and 400mg of *C. lanatus* seed extract to diabetic Wistar rats significantly reduce the plasma glucose ($p<0.05$) when compared with diabetic control. The results are in agreement with Omigie, (2014); Nasir *et al.,* (2009) and Muhammad *et al.,* (2015).

The possible mechanism by which ethanolic fraction of *C. lanatus* seeds extract brings about its hypoglycemic action may be by potentiating the insulin effect by increasing either the pancreatic secretion of insulin from the β-cells of islets of Langerhans or its release from bound insulin. It could also be that the extract caused a hypoglycemic effect by inhibiting the process of glycogenolysis (break down of glycogen to form glucose) or it inhibited the process of glycogenesis by the liver. Omigie, (2014) and Nasir *et al.,* (2009) also suggest that the presence of tannins, Saponins and soluble fibre in watermelon may be the contributing factors to this hypoglycaemic effect.

The current study shows that hyperglycaemia was interrelated to lower total antioxidant capacity, this verify the direct relationship between diabetes and oxidative stress condition. This corroborated with the work of Giridhari *et al.,* (2011) and Rohilla and Ali, (2012) who independently discovered that the total antioxidant status in diabetes was lower and it might be attributed to lower levels of vitamin C, vitamin E in blood or other factors including micronutrients. The result is in consistent with the findings of Merzouk *et al.,* (2003) which observed that the vitamin C levels were not significantly different between control and diabetic subjects. The possible mechanism underlying the decreased serum levels of antioxidant vitamins A, C, and E in the diabetic Wistar rats could be due to to increased generation of reactive oxygen species (ROS) leading to oxidative stress in the experimental rats.

The result of the present study revealed a significant increase in antioxidant vitamins A, C and E in diabetic Wistar rats supplemented with 400mg and 200mg of *M. oleifera* leaf extract with no significant increase in vitamin E concentration seen in the rats supplemented with 200mg *M. oleifera* leaf extract. There was no significant difference observed in the level of vitamin C in the diabetic Wistar rats supplemented with 200mg and 400mg watermelon seeds. However vitamin A and E were shown to be significantly raised when compared with the diabetic control. The result is in aggreement with Aruna *et al.,* (2014) who postulated that the methanolic extract of *Citrullus lanatus* seeds possessed gallic acid, tannic acid, quercetin and vitamins. The significant increase in the antioxidant vitamins in the diabetic Wistar rats treated with ethanolic leaf extract of *M. oleifera* corraborated with several studies (Gowrishankar *et al.,* 2010; El-Dasouki *et al.,* 2015; Hussein *et al.,* 2016).

CONCLUSION

Both the ethanolic extract of *C. lanatus* seeds and *M. oleifera* leaves possesses hypoglycemic effect and are rich source of antioxidant vitamins.

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