International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 9 Issue 2, Mar-Apr 2025 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Comparative Effect of Aqueous Aloe Vera Extracts on Oxidative Stress in Alloxan-Induced Diabetic Wistar Rats

Okonkwo Chukwudi Onyeka John, Ezeokafor Emmanuel Nonso, Nwaefulu Kester Eluemunor

Department of Human Physiology, Faculty of Basic Medical Sciences,
Okofia, Nnamdi Azikiwe University, Nnewi, Nigeria

ABSTRACT

Background: The main pathophysiology of diabetes mellitus (DM) is oxidative stress, which is an imbalance between production of free radicals and antioxidant system in the body. Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), in tissues from glucose auto-oxidation and protein glycosylation. Aim: To investigate the comparative effect of *Aloe vera* gel, rind and a combination of gel and rind on the oxidative stress in alloxan-induced diabetic rats. **Materials and Methods:** 25 male wistar rats weighing between 220–260g were used for this study. They were divided into five groups (A - E) of 5 rats each. Group A served as positive control group and received distilled water only. Group B was the negative diabetic group with no extract administration. Groups C, D and E were diabetic groups that received 300mg/kg of *Aloe vera* rind, 300mg/kg of *Aloe vera* gel and combination of 150mg/kg of *Aloe vera* rind and 150 mg/kg of *Aloe vera* gel respectively. Extract administration was done orally, once daily for 14 days. At the end of administration, oxidative stress biomarkers were assessed. **Results:** There was significant decrease in SOD level in group B (9.87±0.20) when compared with the control (15.88±0.39). Conversely, groups C (17.50±0.35) and D (17.94±0.22) showed significant increase when compared with the control. However, group E showed no significant change when compared with the control (15.88±0.39). On the other hand, there was significant increase in SOD in control group A (15.88±0.39) and all the test groups when compared with group B (9.87±0.20). Similarly, a significant increase in MDA was observed in group B (5.39±0.20) when compared with the control (4.15±0.21). Groups C (3.62±0.20) and D (3.23±0.12) showed significant decrease when compared with control (4.15±0.21). Furthermore, group E (4.14±0.26) showed no significant difference when compared with the control (4.15± 0.2). On the other hand, groups A and all the test groups showed significant decrease in MDA when compared with group B (5.39±0.20).

Conclusion:- *Aloe vera* gel and *Aloe vera* rind extracts singly appeared more potent in reducing oxidative stress in diabetic rats when compared to combined extracts of *Aloe vera* rind + gel at lower doses.

How to cite this paper: Okonkwo Chukwudi Onyeka John | Ezeokafor Emmanuel Nonso | Nwaefulu Kester Eluemunor "Comparative Effect of Aqueous Aloe Vera Extracts on Oxidative Stress in Alloxan-Induced Diabetic Wistar Rats" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-9 | Issue-2, April 2025, pp.1366-1371, URL: www.ijtsrd.com/papers/ijtsrd79829.pdf

IJTSRD79829

KEYWORDS: *Diabetes mellitus, Oxidative stress, Aloe vera, Antioxidant*

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances in carbohydrates, fats, and protein metabolism, resulting from defects in insulin secretion, insulin action or both (Zubin *et al*., 2018). American Diabetes Association (ADA) in 2017 stated that, chronic hyperglycemia is the hallmark of diabetes mellitus, a chronic condition characterized not only by hyperglycemia but also by alterations in protein and lipid metabolism. The main

symptoms of diabetes include increased urination, increased thirst, fatigue, weight loss, blurred vision, increased hunger, and diabetes dermatomes (Hasonadan, 2016).

Whenever a cell's internal environment is perturbed by infections, disease, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption. This "oxidative shielding" acts as a defense mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighboring cells (Naviaux 2012). The main pathophysiology of diabetes mellitus is oxidative stress which is an imbalance between production of free radicals (ROS and RNS) and antioxidant system in the body. Persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), in tissues from glucose auto-oxidation and protein glycosylation (Poitout and Robertson 2002). Reactive oxygen species (ROS) in its excessive level can activate various damaging pathways which have important role in the growth of the diabetes disease (Verma *et al.,* 2018). Some of these important pathways include:- Glucose amine pathway, Sorbitol aldose reductase pathway, Electron transport chain, Protein kinase C stimulation. The stimulation/activation of these pathway and mode of action can lead to artherosclerosis, apoptosis, increased lipid peroxidation, advanced glycation end products (AGEs) formation and failure of pancreatic beta cell function.

Several reports suggested that increased free-radical mediated oxidative stress is involved in diabetic complications such as neuropathy, retinopathy, diabetic nephropathy (Asbun *et al.,* 2006). To cope with the oxidative stress, animal and human cells have developed a ubiquitous antioxidant defense system. Antioxidants are the compounds that can stabilize ROS. These antioxidants can inhibit activity of free radicals through several ways such as; acting as an enzyme, binding metals that stimulate the production of free radicals and act as scavengers of free radicals (Wulan 2019). Antioxidants also help reduce the number of free radicals that form in the body, by lowering the energy levels of existing free radicals, and stopping oxidation chain reactions to lower the amount of damage caused by free radicals. There two major types of antioxidants the enzymatic and non-enzymatic. Enzymatic antioxidants include; super oxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Non-enzymatic antioxidants includes; Vitamins. There are two classes of vitamins, water soluble like vitamin C and fat soluble vitamin A (retinoic acid or retinol) and vitamin E. Therefore, to reduce the risk of complications of diabetes mellitus such as renal failure, blindness and possible limb amputation, the control of both blood glucose levels and antioxidant levels remains paramount.

Throughout history, man has solely relied so much on medicinal plants for health and food needs. *Aloe vera* is xerophytes and as such survives in highly dried and arid conditions mostly found in African countries (Urch1999).It is a species of Aloe that belongs to Liliaceae family. Aloe vera contains different substances and is widely used for a variety of medicinal purposes (Reynolds, 1985). Attention has been focused on herbal medicines such as *Aloe vera* which have antioxidant activities to prevent and protect oxidative damage caused by free radicals (Stavric,1994). Leaf exudates and mucilaginous gel of *Aloe vera* have been reported to possess anti-inflammatory, antifungal, antibacterial, anticancer, antioxidant, cytoprotective, cardiac stimulatory, and immunomodulatory activities (Lanjhiyana2011). Many biological and medicinal properties of *Aloe vera* are associated with inner gel of the leaves.

This study was therefore aimed at investigating the comparative effect of different parts of *Aloe vera* extracts on oxidative stress in alloxan-induced diabetic wistar rats. To our knowledge, no work had been done on this.

2. Materials and Method

Experimental Animals

A total of twenty-five (25) male wistar rats weighing between 220-260g were used for this study. The rats were left to acclimatize for two (2) weeks. All laboratory animals used were kept on a 12 hour light and 12 hour dark cycle in well ventilated room. The rats were fed with vital feed grower mesh manufactured by Grand Cereals Ltd (a subsidiary of UAC Nigeria PLC, Plateau state). The rats had access to feed and distilled water *ad libitum*.

Plant Collection and Extract Preparation

Mature *Aloe vera* leaves were purchased in a local market, Eke Amobi in Nnewi, Anambra state. Plant identification was carried out and the specimen deposited in the Herbarium of Botany department in Nnamdi Azikiwe University, with a voucher number (N.A.U.H-60A). They were thoroughly washed with distilled water to remove dirt and debris, the apex and the base of the leaf was cut off using the surgical blade to prevent the sap/ latex from entering.

Gel extract

The leaves were cut open along its margin thus revealing the transparent mucilage/gel this was scooped into a beaker using a spatula and then homogenized to obtain a finer liquefied form of the gel, then filtered with a standard sieve to obtain, the aloe juice extract (Adesokan *et al.,* 2009). This was stored in a refrigerator till used.

Rind Extract

After removing the gel the rind was obtained. The rind was air dried for two weeks and grounded to powdered form.100 grams of the powdered rind was soaked in 300ml of distilled water for 48hrs and sieved with a standard sieve. The filtrate was oven dried at a temperature of 45^0c for 24hrs to obtain the extract in paste form. The extract was stored in the refrigerator. (Jothi *et al.,*2014). This was reconstituted in distilled water to an appropriate concentration before administration.

Induction of diabetes mellitus

1200 mg of alloxan was measured in a dark environment and dissolved in 16mls of normal saline (0.9%) giving a yield of 75mg/ml (Erhirhie *et al.,* 2014). The colour of the solution obtained was pink to light purple. The experimental group fasted for 24 hours but allowed access to water.

After 24 hours of fasting, alloxan was administered using a single dose of 150mg/kg intraperitonially (Carlvalho 2003).

Formula for determining volume of alloxan solution given: Weight of animal (g) /1000 x 2ml= Xmls ((Erhirhie *et al.,* 2014).

No water or feed was given to the rats until after 30 minutes of administration. In order to prevent alloxan induced fatal hypoglycaemia 10% of glucose was given to the rats in solution bottles by oral gavage for the next 24 hours. After 72 hours of alloxan administration the fasting blood glucose level of the rats was determined from blood samples taken from the tail vein using an Evolve glucometer. Random blood glucose $\geq$ 200mg/dl indicates diabetes mellitus (Lensen, 2008).Three days after induction, surviving and diabetic rats were divided into 5 groups with five rats each.

Experimental design and Extract Administration

After successful diabetes induction, the rats were randomly divided into 5 groups of 5 rats each.

Group I: Positive control non-diabetic group received water and feed *ad libitum.*

Group II: Negative control untreated diabetic group received water and feed *ad libitum*

Group III: Diabetic group treated with 300mg/kg *Aloe vera* Green rind.

Group IV: Diabetic group treated with 300mg/kg *Aloe vera* Gel.

Group V: Diabetic group treated with 150mg/kg *Aloe vera* Rind and 150mg/kg of Aloe vera Gel

Extract administration commenced on confirmation of diabetes. These extracts were administered orally for 14 days by oral gavage.

Determination of oxidative stress biomarkers

12 hours after last administration and feeding, 5 ml of blood sample was drawn via ocular puncture and collected in plain bottles and mixed properly. The coagulated blood was centrifuged for 15 minutes to facilitate separation. Superoxide Dismutase (SOD) and Malondialdehyde (MDA) - a product of lipid peroxidation, were assayed using UV-VIS spectrophotometer (Model 752G, China) according to the standard methods of Rotruk *et al* (1993) and Alam *et al* (2016) respectively.

Statistical Analysis

Results were analyzed and expressed as mean ± SEM using statistical package for social sciences (SPSS version 25). The statistical significance between the means was analyzed using one way analysis of variance (ANOVA) followed by Turkey's multiple range test post-hoc analysis. A p - value of ≤ 0.05 was considered statistically significant.

3. Result:

The Lethal Dose

Acute Toxicity Study

The acute toxicity (LD_{50}) of the extract was estimated in 12 albino rats by oral compulsion as described by Lorke (1983). In brief the method was done in two phases which involved the administration of 3 different doses the first phase and 4 different doses for the second phase. The number of deaths in each group was recorded and LD_{50} was calculated as 2154.07mg/kg/bwt using the formular

$$\sqrt{LD_{50}}=\sqrt{A \times B}$$

A= maximum dose with 0% mortality

B= maximum dose with 100% mortality

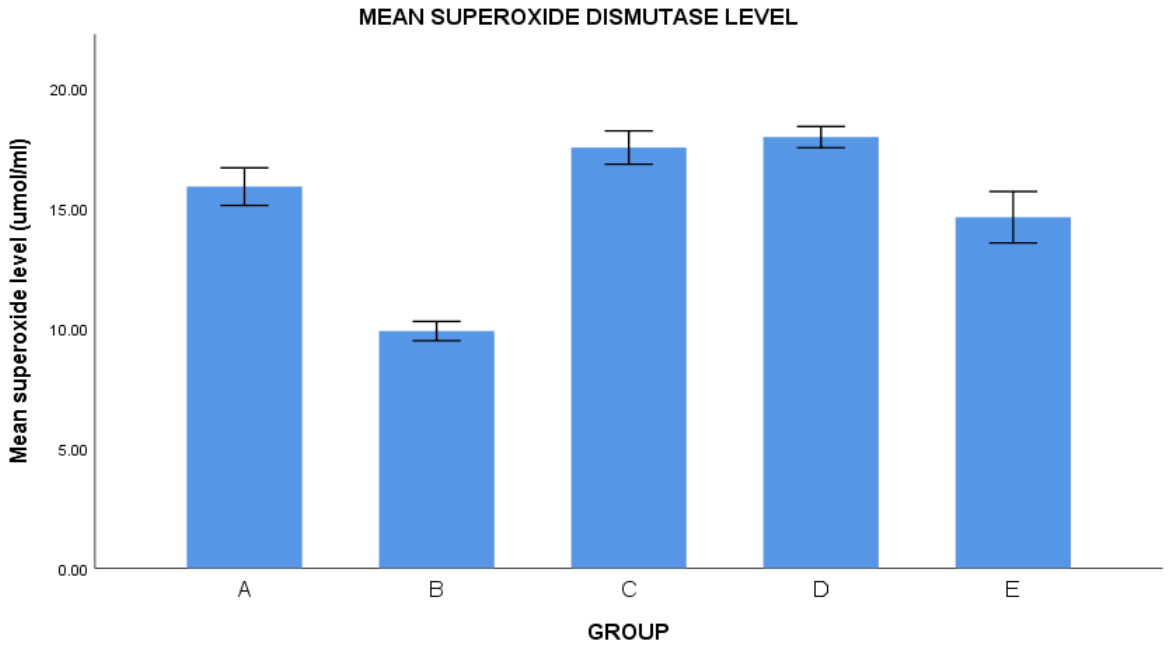

Fig 1: Comparative effect of aqueous extract of Aloe vera on Superoxide dismutase in Alloxan induced diabetic wistar rat.

Result shows significant decrease (P ≤0.05) in SOD level in group B (9.87±0.20) when compared with the control (15.88±0.39). Conversely, groups C (17.50±0.35) and D (17.94±0.22) showed significant increase when compared with the control. However, group E showed no significant change when compared with the control (15.88±0.39).

On the other hand, there was significant increase in SOD in control group A (15.88±0.39) and all the test groups when compared with group B (9.87±0.20).

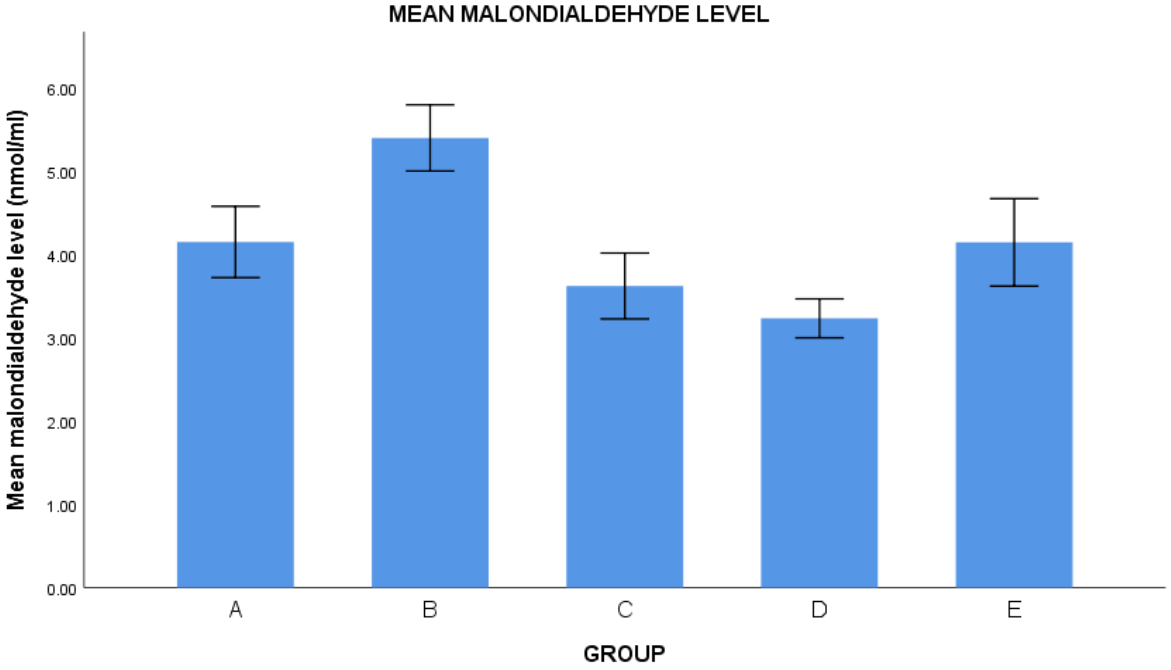

Fig 2: Comparative effect of aqueous extract of Aloe vera on Malondialdehyde in Alloxan induced diabetic wistar rat.

Result showed a significant increase in MDA in group B (5.39±0.20) when compared with the control (4.15±0.21). Groups C (3.62±0.20) and D (3.23±0.12) showed significant decrease when compared with control (4.15±0.21). Furthermore, group E (4.14±0.26) showed no significant difference when compared with the control (4.15± 0.21). On the other hand, groups A and all the test groups showed significant decrease in MDA when compared with group B (5.39±0.20).

4. Discussion

The occurrence of diabetes mellitus has become a serious threat to the health of mankind globally hence the urge to develop solutions with little or no side effects and at a cheaper rate. *Aloe vera* (*Aloe barbadenis mill*) is one of the plants reported to possess antidiabetic /hypoglycemic effect (Lanjihana 2011). This study has evaluated the effects of different *Aloe vera* extracts on oxidative stress in alloxan-induced diabetic wistar rats. So as to ascertain the best part of *Aloe vera* to be consumed amongst individuals with diabetes mellitus.

The untreated diabetic group (B) with highest blood glucose level had the lowest superoxide dismutase level (SOD) when compared to control and other diabetic group treated with *Aloe vera* extracts. This result agrees with the work done by Wulan *et al.,* (2019); Lina *et al.,* 2017 and Abo-Youssef *et al.,* (2013). This decrease in SOD levels in untreated diabetic group indicates oxidative stress caused by an increase in the production of free radicals through glucose autoxidation, protein glycation and lipid peroxidation which leads to the formation of Advanced glycation end products (AGEs). These end products (AGEs) bind to their receptors hence activating it. Its activation leads to the inactivation of antioxidant enzymes (SOD) altering its structure and function hence, decreasing its cellular antioxidant effect. Also in this study, untreated diabetic group showed a significant increase in malondialdehyde (MDA) level when compared with control and diabetic groups treated with *Aloe vera* extracts. This finding is in line with the work done by Betul *et al.,* (2020); Vania *et al.,* (2020) and Lina *et al.,* 2017. Hyperglycemic conditions trigger oxidative stress that can also measured by MDA levels. MDA is a product of lipid peroxidation carried out by the excessive free radical.

On treatment with *Aloe vera gel and Aloe vera rind extracts singly* the level of SOD increased significantly when compared to the normal control but decreased at combined lower doses. Also treatment with *Aloe vera* extracts increased SOD levels significantly when compared to the untreated diabetic group. This could be as a result of the presence of flavonoid and phenolic compounds hence its antioxidant ability according to Christijanti *et al.,(*2019) which coincides with the work done by Wariyah and Riyanto (2015) and Sethi *et al.,*(2012). But amongst the *Aloe vera* treatment groups, Group C (Rind) and GroupD (gel) showed higher SOD level that is they had more scavenging ability. Also on treatment with *Aloe vera* extracts MDA levels in diabetic group decreased greatly when compared to untreated diabetic group B. This finding coincides with the study carried out by Rabab *et al.,*(2019).This is as a result of antioxidant compounds found in *Aloe vera* like vitamins A, E, phenols, flavonoids. Also, Haritha *et al.,*(2014) showed that *Aloe vera* can reduce highly reactive oxygen species that can cause extensive damage to lipid cell membrane thus decreasing MDA levels. Surprisingly, the MDA levels in diabetic group (c) treated with *Aloe vera* rind extract and the combined doses at lower doses showed no significant change when compared to the normal control but showed significant change in the *Aloe vera* gel treated group. The diabetic group treated with only *Aloe vera* gel extract greatly reduced the MDA levels when compared with other *Aloe vera* treatment groups. This could be as a result of higher flavonoid constituent present in the gel Yepbella (2011).

5. Conclusion

Aloe vera extract has a potency of reducing oxidative stress in the diabetic rats by significantly increasing the superoxide (SOD) levels and reducing Malondialdehyde (MDA) levels at the dose of 300mg/kg. But *Aloe vera* gel and *Aloe vera* rind extracts singly appeared more potent in reducing oxidative stress in diabetic rats when compared to combined extracts of *Aloe vera* rind + gel at lower doses.

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