**Manuscript title:** *Kigelia africana* stem bark extract treatment reverses type 1 model of experimental diabetes and associated reproductive impairments in male wistar rats

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**Running title**: Reversal of diabetes-induced reproductive impairments by *Kigelia africana*

ABSTRACT

A key hallmark in diabetes is altered reproductive functions. *Kigelia africana* is reported to offer therapeutic influence in diabetes. However, literature offers nothing concerning its ability to reverse reproductive complications of diabetes. This study investigates the effect of ethanolic extract of *Kigelia africana* (EEKA) on reproductive functions in streptozotocin induced diabetic male rats. Thirty adult male wistar rats (150-200 g) were randomly divided into five groups; Group 1 (control) received distilled water (vehicle). Group 2 was treated with 250 mg/kg EEKA. Groups 3, 4 and 5 were made diabetic using streptozotocin. Thereafter, groups 4 and 5 were treated with 250 mg/kg EEK and glibenclamide respectively. Treatments were by oral gavage for six weeks. Body weight and blood glucose, liver enzymes and proteins, superoxide dismutase (SOD), malondialdehyde activity (MDA), testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), sperm count and motility were assessed. EEKA treatment significantly reduced blood glucose in diabetic rats. Serum testosterone was significantly reduced in diabetic group. However, treatment with EEKA significantly modulated the reduction. EEKA treatment in diabetic rats significantly increased serum LH. Therapeutic EEKA treatment in diabetic rats significantly reversed reduced sperm count and motility. EEKA treatment in diabetic group reduced MDA activity while significantly enhancing SOD activity. The significant increases in serum alanine amino transferase and aspartate amino transferase were significantly reduced with EEKA treatment in diabetic group. Total protein and albumin were significantly reduced in diabetic group but significantly reversed with EEKA treatment. Therapeutic administration of EEKA reduced hyperglyceamia, enhanced sperm count, motility, and serum testosterone levels in diabetic rats. Phytochemical constituents and robust antioxidant activity of EEKA is involved in the modulation and reversal of diabetes and impaired reproductive parameters.

**Key words**: *Kigelia africana*, diabetic male rats, sperm parameters, testosterone, oxidative biomarkers

**Introduction**

There are currently rapidly increasing numbers of men in their reproductive years dealing with the direct consequences of diabetes on their reproductive life (Alves *et al*, 2013, NCD, 2016). Consequently, diabetes is likely to contribute to a decline in global birth rates and sexual functions especially in those societies with a high prevalence (Ding *et al*., 2015). The link between diabetes and male reproductive and sexual impairment is widely reported in literature (Agbaje *et al*.,2007, Calderon *et al*., 2016). Erectile dysfunction secondary to angiopathic, neuropathic, and myopathic damage is a leading complication of diabetes in males. (Alves *et al*., 2013). In addition, hyperglycemia in diabetes can be a stress inducer when excessive glucose reaching the mitochondria lead to an overdrive of the electron transport chain leading to overproduction of superoxide anions (Shrilatha & Muralidhara, 2009). Supersaturation of superoxide anions weakens the mitochondrial SOD and paves way for oxidative stress. This mechanism is fundamental to major pathways preceding vascular (Wiernsperger, 2003), reproductive (La Vignera *et al*., 2012, Ding *et al*., 2015) diabetic complications.

Diabetes is also a risk factor for low testosterone levels, impaired sex steroid status, and hypogonadism in male (Yao *et al*., 2018, Hackett, 2019). Diabetes is also detrimental to sperm quality, motility, count, DNA integrity and constituent of seminal plasma (Ding *et al*., 2015). Epigenetic modification during spermatogenesis causing dysregulation that are inheritable through male germline is also suggested in diabetes complication (Ding *et al*., 2012, Wei *et al*., 2014). Furthermore, patients with diabetes mellitus are reported to experience compromised liver functions liver abscess with higher morbidity and mortality (Islam *et al*., 2020).

Care of type 1 diabetes without undesirable side effects is still a challenge in drug research and development (Chang *et al*., 2013). Plants provides a ready source of natural medicines and a valuable foundation for drug discovery. Report has it that 50% of prescription drugs in the last 25 years have been developed from natural products and their derivatives (Chang *et al*., 2013).

*Kigelia africana* (sausage tree) is a multipurpose herbal plant predominant in Africa. It is composed of alkaloids, sterols, phenols, flavonoids, and tannins (Nabatanz *et al*., 2020). Furthermore, reducing sugars, saponins as well as enzymatic and non- enzymatic antioxidants (catalase, peroxidase, ascorbate oxidase and vitamin C activities) are also reported present depending on the mode of extraction (Nabatanz *et al*., 2020). There are reported medicinal uses of *Kigelia africana* flower, seed, root, leaves and bark. Oral administration of *Kigelia pinnata* flower was reported to reduce blood glucose, serum cholesterol levels and triglycerides levels in diabetic rats (Kumar *et al.*,2012,). In *Clarias gariepinius* (catfish), *Kigelia africana* enriched diet enhanced reproductive functions and better offspring outcome (Adeparusi *et al*., 2010). The fruit extract also facilitates development and maturation of reproductive system in immature rats (Micheli *et al*., 2019). In cisplastin induced testicular toxicity, *Kigelia africana* fruit extract was reported to have cytoprotective effect preventing cell death (Azu *et al.,* 2010).

Despite benefits of *Kigelia africana* in diabetes and reproduction, literature search offers nothing concerning its ability to reverse reproductive complications of diabetes. This study investigates implication of therapeutic EEKA treatment in diabetic male rats on sperm parameters, testosterone, gonadotrophins, liver enzymes and proteins. Estimation of oxidative biomarkers as possible mechanistic activity for *Kigelia africana* functions were also assessed in this study.

**Materials and Methods**

## **Collection and identification of *Kigelia africana* bark**

*Kigelia africana (Lam)Benth* bark was sourced from Mushin Market in Lagos, Nigeria. It was identified and authenticated by Dr. Nodza G.I, of the Department of Botany University of Lagos, Lagos State Nigeria. After due authentication, a sample specimen with reference number 8369 was deposited in the herbarium.

**Preparation of ethanol extract of *Kigelia africana***

The stem barks were cut into pieces, dried under shade and grinded to fine powder using local milling machine. The powdered material (500 g) was soaked in 2 L ethanol for 72 hours (Salami *et al*., 2017). Afterwards, the extract was filtered and evaporated to dryness in a rotary evaporator at 45 °C to produce a semisolid brown mass (89.32 g) and stored in airtight container in a refrigerator below 4 °C.

**Experimental Animals**

Thirty adults male wistar rats weighing about 150-200 g were used in the study. The animals were sourced and housed in the Animal House of the Lagos State University College of Medicine. The animals were maintained under standard laboratory conditions and fed standard pelletized rat feed with water supplied *ad libitum*. Animal use and care was done following standard protocol on animal use and care and in line with the Lagos State University College of Medicine Animal ethical Committee guideline approval.

## **Induction of diabetes in male wistar rats using streptozotocin**

Streptozotocin (STZ) was purchased from AK Scientific, USA and stored in the refrigerator at

-4⁰C to prevent it from being denatured. Dissolution of STZ was done in a dark room due to its light sensitivity. Streptozotocin solution was prepared by dissolving it in freshly prepared citrate buffer (0.01 M, pH 4.5). It was thereafter administered at a dose of 60 mg/kg intraperitoneally (Kumar *et al.,*2012) to fasted wistar rats 10 minutes after preparation. Blood glucose level were checked 72 hours later in blood collected from the tail vein using Accucheck Glucometer (Germany). Animals with serum blood glucose levels >250 mg/dl were considered diabetic, separated, and used for the study.

**Experimental design and treatments**

Thirty adult male wistar rats (150-200 g) were randomly divided into five groups; Group 1 (control) received distilled water (vehicle). Group 2 was treated with 250 mg/kg EEKA. Groups 3, 4 and 5 were made diabetic using streptozotocin. Thereafter, groups 4 and 5 were treated daily with 250 mg/kg EEK and glibenclamide (5 mg/kg) respectively. All treatments were by daily oral gavage for six weeks.

**Collection of blood samples**

Blood samples were rapidly collected across the groups via cardiac puncture under anesthesia into plain sample bottles and cold centrifuged (Uniscope Laboratory Centrifuge, Model SM112, England) for 5 minutes at 3000 rpm to obtain the serum. The serum samples were refrigerated at -40 C and subsequently used for hormonal, oxidative biomarker, liver enzymes, and protein assay.

**Determination of sperm motility and count**.

Sperm count and motility were determined according to the WHO guideline on semen analysis (WHO, 1999). Microscopic evaluation of the sperm displayed both the motile and immotile sperms which were recorded in percentages. For sperm count, diluted semen obtained from the caudal epididymis was placed on the Neubauer counting slide and placed under the microscope. Sperms were counted using the hemocytometer (Deep 1/10; Labart Munich Germany) and results were expressed in million/ml of suspension.

**Assay for serum testosterone, LH and FSH**

Serum concentration of testosterone, LH, and FSH were determined using enzyme-linked immunosorbent (ELISA) test kits (Monobind Inc, USA) for testosterone, LH, and FSH.

### Oxidative biomarkers, liver enzymes and protein assay

## Malondialdehyde activity was assayed by determining thiobarbituric (TBA) reactive products in test serum sample as described by Uchiyama & Mihara, (1978). Aspartate amino transferase (AST) and alanine amino transferase (ALT) levels in the serum were assayed according to the methods of Moss and Henderson, (1999). Estimation of total protein and albumin was done as described in a review by Buzanovskii, (2017)

## **Statistical** **Analysis**

All data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was carried out with Newman keuls post hoc test. Statistical significance was taken at p<0.05 and all analysis and graphical illustrations were done using prism GraphPad (version 5.0) statistical software.

## **Result**

## **Effect of EEKA on weekly body weight and blood glucose in streptozotocin induced diabetic male rats.**

The significant decrease in body weight in diabetic group was significantly attenuated with therapeutic EEKA treatment in diabetic rats (Table 1). Blood glucose levels were significantly increased in diabetic rats. However, therapeutic treatment with EEKA significantly reduced blood glucose in diabetic rats (76.60compared to 411.00 (Table 1)

## **Effect of EEKA treatment on serum Testosterone, LH and FSH in streptozotocin induced diabetic rats**

## EEKA treatment significantly increased testosterone level in non-diabetic group. Serum testosterone level was significantly reduced in diabetic group. However, therapeutic treatment with EEKA significantly modulated the reduction in testosterone level (figure 1). Follicle stimulating hormone levels were not significantly altered across groups (Figure 2). Therapeutic treatment with EEKA in diabetic rats significantly increased serum level of LH (figure 3).

## **Effect of EEKA treatment on sperm count and motility in streptozotocin-induced diabetic rats**

As shown in figure 4 and 5 respectively, sperm count and motility were significantly reduced in diabetic only group. Therapeutic treatment with EEKA in diabetic rats significantly modulated the reduced sperm count and motility.

## **Effect of EEKA treatment on oxidative biomarkers in streptozotocin induced diabetic rats**

MDA activity was significantly enhanced in diabetic group. Therapeutic treatment with EEKA in diabetic group reduced MDA activity (table 2). In addition, SOD activity was significantly reduced in diabetic rats as compared to diabetic EEKA treated group (table 2).

## **Effect of EEKA treatment on liver enzymes and protein in streptozotocin induced diabetic rats**

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were significantly elevated in diabetic only group. Therapeutic treatment with EEKA in diabetic group however significantly reduced ALT and AST (table 3). Total protein and albumin were also significantly reduced in diabetic group. This was significantly reversed in EEKA treated diabetic group (table 3).

## **DISCUSSIONS**

Literature has reported both folkloric and experimental evidence for the use of *Kigelia africana* plant as an antidiabetic agent (Chang *et al*., 2013, Nabatanz *et al*., 2020). Furthermore, studies also affirm the beneficial influence of *Kigelia africana* plant on reproductive function in experimental animals. What is however not clear is if *Kagelia africana* can reverse diabetes and its attendant reproductive impairment in experimentally induced diabetic rats. This study found that EEKA treatment significantly reduced blood glucose level and also improved impaired reproductive endpoints in streptozotocin induced diabetic rats. The presence in *Kigelia africana* stem bark of flavonoids, alkaloids, saponins and tannins we suggest is responsible for its glucose lowering activity as observed in this study. These phytochemicals of EEK may well have protected, repair, or enhanced regeneration of streptozotocin damaged pancreatic β cells. A fact buttressed by progressive reduction in blood glucose over time (table 1) and histological screening of pancreas (data not shown) showing more viable β cells in EEKA treated diabetic rats.Evidences in plants with antidiabetic activity in literature also give credence to our postulation on EEKA. Ginsenosides, a saponin derivatives from *Panax ginseng* (Kim *et al*., 2007), Kinsenoside from *Anoectochilus roxburghii* (Zhang *et al*., 2007) and Berberin an isoquinoline alkaloid from *Berberis vulgaris* (Han *et al*., 2011) protect and repair pancreatic β cells. Conophylline alkaloid from *T divaritica* (Kawakami *et al*., 2010) promote growth, differentiation, and maturation of β cells and reduce blood glucose in streptozotocin-induced diabetic rats (Fuji *et al*., 2009). Silymarin a flavonoid from *Silybum marianum* with potent antioxidant and anti-inflammatory activities protect β cells function. *F japonica* regulate immune cells by reducing leucocytes infiltration (Shen *et al*., 2011), *P peltatum* reduces production of inflammatory cytokines (Alarcon-Aguilar *et al*., 2010) and *Capsicum* that not only regulate immune cells but also protect β cells (Nevius *et al*., 2012).

**Testosterone, LH and FSH.**

Testosterone levels were significantly reduced in diabetic rats. However, therapeutic treatment of diabetic rats with EEKA reversed the reduction in this study. There has been a reported correlation between low testosterone level and incidence of type 2 diabetes (Hackett, 2019). Type 2 diabetes is also reported to be risk factor for testosterone deficiency and impaired sex steroid status (Yao *et al*., 2018). The low testosterone level is linked to metabolic syndrome associated with type 2 diabetes (Corona *et al*., 2011). Particularly, IL-6 and TNF -α are found to be two major inflammatory cytokines that reduce the production of testosterone from Leydig cells (Hong *et al*., 2004) and suppression of hypothalamic gonadotropin -releasing hormone in experimental animals (Watanobe and Hayakawa, 2003). It is noteworthy that this study investigated type 1 diabetes were streptozotocin was used to experimentally cause damage in islet cells. Testosterone levels were however significantly reduced like that reported for type 2 diabetes. Van dam *et al*., (2003) have earlier reported incidences of hypogonadism in men with type 1 diabetes who under treatment with insulin without complications showed a lower free testosterone level as compared to normal men. Consequences of the reduced testosterone led to impairment of some reproductive endpoints observed in diabetic rats in this study.

This study showed that EEKA treatment in type 1 diabetes has the capacity to modulate and reverse reduced testosterone and its likely complications like impaired sexual function and libido. The exact constituent in *Kigelia africana* responsible for this is currently unknown, however, report (Chang *et al*., 2013) have identified the presence of sterols like β sitosterol, stigmasterol, γ sitosterol in stem bark of *Kigelia africana*. These sterols are postulated to bear similarity with synthetic steroids with ability of acting as intermediate in steroid hormone synthesis or in the presence of enzymes in vivo converted to steroid hormones themselves (Salami and Raji, 2015). The presence of these sterols in EEKA we suggest may have enhanced the synthetic activity of testosterone by Leydig cells; particularly the enzyme regulating the rate limiting step in testicular synthesis. Luteinizing hormone that controls amount of testosterone Leydig cells secrete by regulating the expression of 17- β- hydrosteroid dehydrogenase was found to be increased in diabetic rats treated with EEKA in this study.

**Sperm count, motility, and oxidative biomarkers**

Therapeutic treatments with EEKA in this study significantly reversed impaired sperm count and motility in streptozotocin induced diabetic rats. Diabetes disease, and experimentally induced of either type 1 or 2, have shown the ability at having harmful effect on sperm motility by alteration in mitochondrial DNA, DNA integrity and seminal vesicle constituents (Ding *et al*., 2015). Diabetes induced epigenetic modification during spermatogenesis have also been reported inheritable through the male germline (Ding *et al*., 2015). The impairments were attributed to diabetes induced glucose impaired metabolism. Normal glucose metabolism is important in spermatogenesis and maintaining cell activities and functions (Baccetti *et al*., 2002, Agbaje *et al*., 2007). Sperms need energy to acquire and maintain motion competence after epididymal maturation (Yanagimachi ,1994). Much of the adenosine triphosphate present in sperm is used to maintain motility. The reversal of impaired sperm count and motility in EEKA treated diabetic rats in this study can be situated in the ability of EEKA to substantially reduce the hyperglycemia in diabetic rats. Furthermore, another reason from this study showed that testosterone levels were also elevated in diabetic EEKA treated rats as compared to diabetic only rats. Testosterone enhances sexual functions and sperm maturation. Streptozotocin induced diabetes is known to however decrease bioavailability of testosterone and epididymal secretory products like sialic acid, androgen binding proteins and glyceryl phosphorylcholine (Ding *et al*.,2015)

Another plausible explanation for the reversal of impaired sperm count and motility of diabetic rats treated with EEKA in this study is the potent antioxidant activity of EEKA (Nabatanz *et al*., 2020). This study showed that MDA activity were significantly enhanced in diabetic group. SOD activity was also significantly enhanced in diabetic rats therapeutically treated with EEKA as compared to diabetic rats only. Elevated levels of reactive oxygen species (ROS) like nitrate/nitrite in semen of diabetic men have been reported to be cause of ROS induced DNA damage and impaired sperm maturation and function (Karimi *et al*., 2011)

**Liver enzymes and proteins**

Studies have reported that about 30 % of patients with cirrhosis have diabetes (Garcia-Compen *et al*., 2009). The underlying mechanism of diabetes that have been reported to contribute to liver damage is a combination of oxidative stress and aberrant inflammatory response causing damage to hepatocyte (Muhammed *et al*., 2016). Elevated serum aminotransferases are common sign of liver disease and are more frequent in diabetes (Arkkila *et al* 2001) According to Arkkila *et al* (2001) type 1 diabetic complications like neuropathy (that could affect sexual function) and retinopathy are associated with liver enzymes impairments. EEKA treatment to diabetic rats in this study was able to prevent impaired liver enzymes activity. Aspartate amino transferase (AST) and Alanine amino transferase (ALT) levels that were significantly elevated in diabetic rats were significantly reduced in EEKA treated diabetic rats. The potent antioxidant properties of EEKA as previously reported in this study may be responsible for the liver protection against diabetes induced oxidative liver damage and enzymes elevation. This antioxidant approach in patients’ management due to diabetes induced liver damage is currently gaining traction and is subject of intense research by investigators (Mohammed *et al*., 2016).

Conclusively, therapeutic treatment of streptozotocin induced diabetic rats with EEKA significantly modulated diabetes induced complications and secondary damage on some reproductive endpoints in male rats. EEKA robust antioxidant activities and its other phytochemical constituents are implicated in the modulation.

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**Figure 1**: **Serum testosterone level in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, \*=p< 0.05, when compared with control group; abc = p<0.05 when compared with the diabetic only, diabetic+ EEKA treated group, non-diabetic EEKA treated group, **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group



**Figure 2: Serum FSH level in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group

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**Figure 3:** **Serum LH level in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, \*=p< 0.05, when compared with control group; ac = p<0.05 when compared with the diabetic only, non-diabetic EEKA treated group, **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group



**Figure 4: Sperm count in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, \* =p< 0.05, when compared with control group; ac = p<0.05 when compared with the diabetic only, non-diabetic EEKA treated group, **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group



**Figure 5: Sperm motility in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, \* =p< 0.05, when compared with control group; a = p<0.05 when compared with the diabetic only. **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group

**Table 1: Body weight and blood glucose in diabetic rats treated with EEKA**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Week/Grp | CONTROL | KA | DM | DM+KA | DM+GLB |
| Week0  BW(g)  BG(mg/dl) | 239.208.62  86.80 | 240.207.52  86.80±4.55 | 237.8  89.40 | 238.40  94.20 | 222.00  93.80 |
| Week1  BW(g)  BG(mg/dl) | 242.60  90.80 | 233.2021.89  82.80 | 219.80  255.20\*c | 215.60  264.00\*c | 204.80  264.60\*c |
| Week2  BW(g)  BG(mg/dl) | 235.20  88.40 | 238.60  103.80a | 209.60  263.80\* | 203.40  117.40\*a | 180.40\*c  103.60a |
| Week3  BW(g)  BG(mg/dl) | 251.20  89.80 | 232.20  99.40a | 203.206.19\*  280.20\* | 199.80\*  100.40a | 155.00\*\*\*abc  102.00a |
| Week4  BW(g)  BG(mg/dl) | 259.00  91.20 | 225.20  99.60\*ad | 198.20\*  305.80\* | 201.80\*\*  105.60\*a | 153.40\*\*\*abc  111.00\* |
| Week5  BW(g)  BG(mg/dl) | 256.40  96.80 | 230.20  99.20ad | 164.60\*\*\*c  319.60\* | 213..80\*a  100.60ad | 153.60bc  121.80\*a |
| Week6  BW(g)  BG(mg/dl) | 257.00  108.20 | 235.20  97.20a | 158.00\*\*\*  411.00\* | 225.00a  79.60a | 149.0016\*\*\*bc  112.60a |

Value expressed as Mean ± SEM, \*, \*\*, and \*\*\* =p< 0.05, 0.01 and 0.001 respectively when compared with control group; abcd = p<0.05 when compared with the diabetic only, diabetic+ EEKA treated group, non-diabetic EEKA treated group, diabetic+ GLB treated group respectively. BW=body weight, BG= blood glucose, **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group

|  |  |  |
| --- | --- | --- |
| Groups | MDA activity (µmol/L) | SOD (U/ml) |
| Control | 0.91±0.01 | 38.37±0.05 |
| KA | 0.83±0.03 | 39.37±0.28a |
| DM | 1.99±0.10 | 18.77±0.20\* |
| DM+KA | 1.55±0.02 | 47.91±0.66a |
| DM+GLB | 1.61±0.03 | 43.48±0.59a |

Table 2: **Oxidative biomarkers activity in diabetic rats treated with EEKA**

Value expressed as Mean ± SEM, \* =p< 0.05, when compared with control group; a = p<0.05 when compared with the diabetic only group. **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group.

Table 3: **Liver enzymes, protein and albumin level in diabetic rats treated with EEKA**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | ALT  (IU/L) | AST  (IU/L) | ALP  (IU/L) | Total protein  (g/L) | Albumin  (g/L) |
| Control | 121.60±3.33 | 32.40±1.08 | 16.00±0.60 | 86.80±2.63 | 42.20±2.20 |
| KA | 94.29±6.26 | 28.20±2.40 | 17.60±0.75 | 82.40±2.38a | 45.60±3.37a |
| DM | 139.40±3.28\* | 46.20±1.24\* | 17.60±0.93 | 48.60±0.89\* | 27.20±1.16\* |
| DM+KA | 53.80±2.99a | 30.00±2.37a | 16.00±0.89 | 79.60±3.42a | 43.60±2.02a |
| DM+GLB | 46.20±1.77a | 20.20±1.28a | 18.60±0.81 | 66.20±4.18a | 33.60±1.70a |

Value expressed as Mean ± SEM, \* =p< 0.05, when compared with control group; a = p<0.05 when compared with the diabetic only group. **KA** = non-diabetic EEKAtreated group, **DM** = diabetic group, **DM+KA** = diabetic EEKA treated group; **DM+GLB** = diabetic glibenclamide treated group