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Anti-diabetic and toxicological profile of aqueous leaves extract of *Ocimum gratissimum* in alloxan-induced diabetic rats

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ABSTRACT

Background & Aim: *Ocimum gratissimum*is an aromatic plants used among traditional medicine practitioners in the treatment of warts, diarrhoea, headache, diabetes etc. This study aimed at evaluating the anti-diabetic and toxicity profile of aqueous leaf extract of the plant in alloxan-induced diabetic rats.

Experimental: Thirty albino rats (111.33 ±1.50g) were grouped into six (A-F) groups of animals. Group A received 0.5 ml distilled water (*p.o*) for eight days. Diabetes was induced in group B-F animals using 160 mg/kg alloxan (*i.p*) and thereafter administered 2.5 metformin, 125, 250 and 500 mg/kg aqueous leaves extract of *Ocimum gratissimum*, respectively (*p.o*) for 8 consecutive days. Blood sugar level was taken 1 h after drug administration every other day. Body weights of animals were taken before induction, after induction, and on the 8th day. Blood samples and organs (liver, kidney, and pancreas) were collected for biochemical assays and histopathological examinations.

Results: Alloxan significantly ($p<0.05$) increased the glucose, albumin, urea, creatinine, bilirubin, Alkaline phosphatase (ALP), Aspartate transaminase (AST) and Alanine transaminase (ALT) levels of rats compared with the distilled water group. The aqueous leaves extract of *Ocimum gratissimum* significantly ($p<0.05$) reduced the glucose, albumin, urea, creatinine, bilirubin, ALP, AST and ALT levels compared with the diabetic untreated rats. There were no significant histological changes in the liver, pancreas and kidneys of diabetic treated rats compared with diabetic untreated rats which exhibited moderately distorted organ degeneration.

Recommended applications/industries: Aqueous leaves extract of *Ocimum gratissimum* possesses anti-hyperglycemic effects and is relatively safe for use in the treatment of diabetics.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by persistent high blood sugar due to inadequate secretion of insulin by the pancreas or reduced sensitivity to insulin by the tissues (Shoback, 2011). It is associated with the classical symptoms of polyuria, polydipsia, polyphagia and alteration in protein and lipid metabolism which can lead to complications such as neuropathy, nephropathy, atherosclerosis, cardiovascular diseases etc (Koda-Kimble, 2012). Diabetes is among the top 10 causes of death and accounted for about 4 million deaths in 2017 (International Diabetes Federation, 2017). Globally about 500 million people are estimated to be living with diabetes. This is expected to increase by 51 % in

the year 2045 (Saeedi *et al.*, 2019). In Sub-Sahara Africa, 20 million people are living with diabetes is expected to reach 41.4 million by 2035 (World Health Assembly, 2013). The increasing prevalence of diabetes among adolescents and young adults below 20 years of age affect economic productivity due to significant morbidity and mortality (Unnikrishna *et al.*, 2016).

Glycemic control is difficult; comorbidities and risk of complications are high among people living with diabetes (Unnikrishna *et al.*, 2016). However, the use of insulin and oral hypoglycemic agents prevent diabetic-related complications and improve the quality of life (Gaster and Hirsch, 1998; WHO, 2017). Side effects such as hypoglycemia, lactic acidosis and high cost of some of these drugs have posed a great limitation to effectively manage diabetes (Moller, 2001; WHO, 2016). In spite of the availability and intensive use of several antidiabetic drugs, more than 50 % of diabetic patients still suffer poor diabetic control and some develop serious complication within six years of diagnosis (Jarald *et al.*, 2008). Moreover, none of the antidiabetic drugs has antioxidant and lipid lowering effect that would ameliorate the oxidative stress and hyperlipidaemia implicated in the pathogenesis of diabetes (Derek, 2001).

In developing countries with great limitation of resources and inadequate health facilities, there is high dependence on medicinal plants in the management of diabetes (Rao *et al.*, 2010; Ezuruike and Prieto, 2014). *Occimum gratissimum* is a perennial plant, and belongs to the family Labiateae. The plant is found in Asia, and West Africa especially Nigeria (Wagner *et al.*, 1999). It is known as 'Effirin-nla' among the Yorubas, 'Ahuji' among the Igbos and 'Daidoya' among the Hausas (Effraim *et al.*, 2003) and "Asano" among the Warrake people of Edo State, Nigeria. It is used in the treatment of diabetes (Mohammed *et al.*, 2014; Ezuruike and Prieto, 2014); epilepsy, fever, diarrhea (Effraim *et al.*, 2003); mental illness (Akinmoladun *et al.*, 2007); sterilization of wound surfaces, treatment of fungal infections and catarrh (Ijeh and Nwanna, 2005). Previous pharmacological studies have reported hypoglycemic effect of various extract of the plant in type 1 and type 2 animal models of diabetes (Aguiyi *et al.*, 2000; Egusie *et al.*, 2006; Nelson *et al.*, 2012). The plant has also been reported to possess anti-oxidant (Akinmoladun *et al.*, 2007), antidiarrheal (Ilori *et al.*, 1996), antimicrobial (Mann, 2012) and anti-inflammatory activities (Alabi *et al.*, 2018). The 'perceived safety' of *O. gratissimum* and the general acceptability of medicinal plants do not make them free of side effects (Philomena, 2011). In fact, one-third of medicinal plants used in the treatment of diabetes are considered to be toxic (Marles and Farnsworth, 1994). Hence, this work is aimed at evaluating the anti-diabetic activity and toxicity profile of aqueous leaves extract of *Ocimum gratissimum* in alloxan-induced diabetic rats.

2. Materials and Methods

2.1. Drugs/ materials

Alloxan (Sigma Aldrich, Germany), metformin (Bhd Ipoh Malaysia), distilled water (Juhel Pharmaceuticals Nigeria), aqueous leave extract of *Ocimum gratissimum*, Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), urea, creatinine, albumin, bilirubin and total protein assay kits used were products of Randox Laboratories Ltd., Antrim, UK, Accu-check glucometer.

2.2. Plant collection and extraction

Fresh leaves, stems and fruits of the plant were collected from a garden in Oke-Odo Area of Tanke, Ilorin, Kwara State, Nigeria. The plant parts were identified by a Botanist in the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State. Thereafter, the leaves were collected, washed, air-dried under shade for one week and pulverized using an electric blender. About 300g of the powdered plant material was extracted in 2 L of distilled water for 18 h, stirred intermittently and then filtered. The filtrate was concentrated at 45^0C over a water bath to obtain a dried solid mass subsequently referred to as aqueous leave extract of *Ocimum gratissimum* (ALOG) from which freshly prepared solution was made each day of drug administration.

2.3. Ethical approval

Animals were handled according to the guidelines set by the National Research Council of the National Academies for the Care and Use of Laboratory Animals (2011).

2.4. Experimental animals

Thirty (30) Albino rats of average weight of (111.33 ±1.50g) were obtained from the Animal House Facility of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State. They were allowed free access to food and water and acclimatized for one week before experimentation.

2.5. Antidiabetic study

The method described by Yakubu *et al* (2010) was adopted. Animals were fasted for 12 h and the blood glucose levels determined before the administration of alloxan monohydrate at dose of 160 mg/kg intraperitoneally. One hour after the administration of alloxan, animals were allowed their pellet *ad libitum* and 5% dextrose saline in a feeding bottle to overcome the early hypoglycemic phase (Yakubu *et al.*, 2010). On the second day, blood samples were drawn from the tail vein and glucose levels were determined to confirm the induction of diabetes using Accu-Check Active glucometer and test strips. Only animals with blood glucose level higher than 126 mg/dl were used for the study. Preliminary studies (Yakubu *et al.*, 2010) revealed that the untreated diabetic rats could survive up till the 12thday. Thus, the experiment was terminated on the 8th day.

2.6. Animal grouping and extract administration

Thirty male albino rats were randomly divided into 6 groups of 5 animals each. Group 1 (none diabetic) received 10 ml/kg distilled water per oral daily. Group 2, 3, 4, 5 and 6 (all diabetic) received 10 ml/kg distilled water, 2.5 mg/kg metformin, 125, 250 and 500 mg/kg ALOG per oral daily. The body weight of rats was taken before induction, after induction of diabetes and at the end of the experiment. The fasting blood glucose was taken every other day, 1 hour after drug administration.

2.7. Biochemical and histological studies

At the end of 8thday, under diethyl ether anesthesia, blood samples were collected through jugular veins into a clean, dried centrifuge tube for biochemical analysis. The liver, kidney and pancreas were collected, cleansed, weighed and immediately stored in ice cold 0.25 M sucrose solution for histological examination.

2.8. Biochemical assays

Aspartate transaminase (AST) and alanine transaminase (ALT) activities were assayed according to the method described by Reitman and Frankel (1957).

Alkaline phosphatase activity (ALP) and Albumin concentration were assayed according to the method described by Grant (1987). Total protein and serum creatinine were determined according to the method described by Tietz (1995). Bilirubin concentration was determined according to the method described by Evelyn and Malloy (1938). Determination of serum urea was carried out according to the method previously described by Kaplan (1965).

2.9. Histological examination

The liver, kidney and pancreas collected from each experimental group were fixed in 10 % formalin. The specimens were dehydrated in ascending grades of ethanol, cleared in xylene, and processed to paraffin blocks, sectioned (5 µm thick) and stained with Hematoxyline and Eosine stain. They were examined using light microscopy for demonstration of pathological changes including degeneration of β-cells of langerhans, cell destruction, necrosis and the efficiency of *Ocimum gratissimum* extract in alleviating these pathological features (Drury and Wallington, 1980).

2.10. Statistical analysis

All data are expressed as mean ±S.E.M. Statistical analysis was carried out using One-way analysis of variance (ANOVA) followed by Duncan's posthoc test for multiple comparisons using SPSS version 16. Values were considered statistically significant at $p<0.05$.

3. Results and discussion

Despite the availability and intensive use of several antidiabetic drugs, inadequate blood sugar control and development of serious complications in about 50 % of diabetic patients is observed within six years of diagnosis (Jarald *et al.*, 2008). This may stem from inability to tolerate side effects and high cost of these drugs. The wide patronage and general acceptability of medicinal plants is due to their perceived safety.

However, medicinal plants are not free of side effects like other drugs (Philomena, 2011).

The aqueous extract of *O. gratissimum* at all doses and metformin significantly (p<0.05) decreased the fasting blood sugar levels compared with the diabetic untreated group (Figure 1).

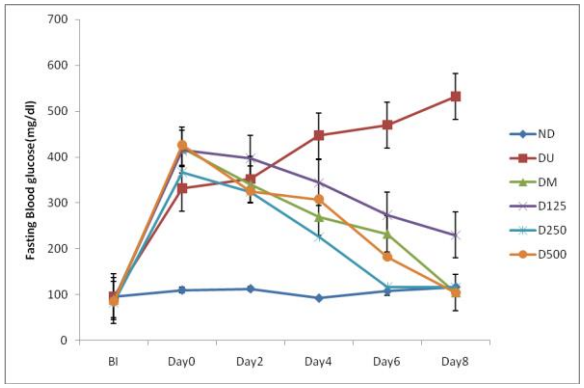

Figure 1. Effect of aqueous leaves extract of *Ocimum gratissimum* on fasting blood glucose level in alloxan-induced diabetic rats. Values are expressed as mean ± S.E.M; n=5; One-way ANOVA; ***p< 0.05* compared to diabetic untreated followed by Ducan post hoc test; ND= non diabetic; DU= diabetic untreated; DM= diabetic treated with metformin; D125= diabetic treated with 125 mg/kg extract; D250= diabetic treated with 250 mg/kg extract; D500= diabetic treated with 500 mg/kg extract; BI= before induction.

Alloxan is a potent urea derivative and well known drug commonly used to induce type 1 diabetes mellitus in laboratory animals by causing selective necrosis of the β-cells of pancreatic islets producing insulin (Maiti *et al.*, 2004; Gupta *et al.*, 2005; Bagri *et al.*, 2009). The necrotic effect of alloxan on pancreatic β-cells involves oxidation of essential sulphydryl (-SH) groups, inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Tabuchi *et al.*, 2003; Ragavan, 2006; Dewanjee *et al.*, 2008). Alloxan is selectively and efficiently taken up by the pancreatic β-cells due to its structural similarity to glucose (Yang *et al.*, 2000; Jalal *et al.*, 2007). The ability of the aqueous leaf extract of *Ocimum gratissimum* to lower blood glucose level in alloxan-induced diabetic rats may be due to direct glucose lowering effect of the extract as seen with the conventional oral antidiabetic drugs or by being able to restore alloxan- induced pancreatic β-cells damage thus retaining the physiological function of the pancreas blood sugar regulation.

Alteration in plasma enzymes activities may sometimes help to detect and localize tissue damage or proliferation and monitoring treatment and disease progression (Philip, 1996). The hepatocytes exhibit broad capacity to metabolize diverse biomolecules, inorganic substances and carry out storage, immunological and detoxification functions. Hepatic enzymes are released into systemic circulation following liver necrosis and are therefore,used as diagnostic indicators for tissue damage (Chikezie *et al;* 2018). Accordingly, elevations in plasma activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), referred to as non-functional plasma enzymes are diagnostic of hepatic dysfunction (Onyema *et al.*, 2006 and Singh *et al.*, 2011). Increased serum transaminases are indications of diabetic complications and such may give rise to hyperglycaemia-induced hepatolysis due to hepatomegaly (Muhammad *et al.*, 2008). The aqueous leaves extract of *Ocimum gratissimum* was able to reduce the elevated serum level of ALP, AST, ALT, Albumin (ALB) and bilirubin (BIL) (Table 1). The ability of aqueous leaves extract of *Ocimum gratissimum* to reduce the serum ALT, AST, ALP, albumin and bilirubin levels may implies prevention of hepatic necrosis thus maintaining the physiological function of the liver.

Table 1. Effect of aqueous leaf extract of *Ocimum gratissimum* on serum biochemical parameters in alloxan-induced diabetic rats.

Treatment /kg	ALP (U/I)	ALT (U/I)	AST (U/I)	ALB (g/l)	BIL (mg/dl)	TP (g/l)
DW 10 ml	1.83±1.73^a	2.50±0.65^a	2.75±0.85^a	48.82±0.36^a	3.11±0.20^a	28.18±1.54^a
Alo+DW 10 ml	5.38±1.49^b	43.50±9.88^b	23.50±2.50^b	55.08±0.72^b	6.26±o.20^b	30.27±1.35^a
Alo+MFM 2.5 mg	2.04±1.53^a	37.50±7.32^a	11.50±1.50^a	48.89±0.33^a	2.82±.014^a	28.71±1.24^a
Alo+ALOG 125 mg	1.97±1.53^a	5.00±0.40^a	8.25±1.11^a	48.21±0.14^a	2.92±0.20^a	28.97±1.56^a
Alo+ALOG 250 mg	1.86±0.91^a	2.75±0.75^a	5.20±0.85^a	47.84±0.19^a	2.92±0.24^a	28.18±0.96^a
Alo+ALOG 500 mg	1.83±0.68^a	1.50±0.50^a	3.00±0.81^a	48.51±0.33^a	2.89±0.24^a	28.44±1.16^a

Values are expressed as mean of four replicates ± S.E.M; One-way ANOVA; $^a p< 0.05$ compared to diabetic untreated followed by Ducan post hoc test; $^b p< 0.05$ compared to DW group; values with the same superscript down the column are statistically not different (P>0.05). ALOG= aqueous leaf extract of *O. gratissimum;* MFM= metformin; Alo= alloxan; DW= distilled water.

The nephron is the functional unit of the kidneys which is physiologically concerned with the removal of wastes, and other substances from the systemic circulation. Elevation in plasma levels of urea and creatinine are indications of compromised renal function (Tawfik *et al.*, 2012; Ezekwe *et al.*, 2017). Creatinine is a bye-product of muscle metabolism in which creatine in the muscle is converted non-enzymatically to creatinine (Joseph, 2017). Alloxan significantly ($p<0.05$) increased the renal creatinine and urea level compared with the control group. The aqueous leaf extract of *Ocimum gratissimum* significantly ($p<0.05$) decreased renal creatinine and urea level compared with the diabetic untreated group (Table 2).

Table 2. Effect of aqueous leaf extract of *Ocimum gratissimum* on renal function indices of alloxan-induced diabetic rats

Treatment /kg	Urea (mg/dl)	CREA (mg/dl)
DW 10 ml	34.61±2.32^a	1.85±0.13^a
Alo+DW 10 ml	40.04±2.32^b	3.69±0.10^c
Alo+MFM 2.5 mg	36.07±3.01^a	1.88±0.18^a
Alo+ALOG 125 mg	35.97±3.01^a	2.37±0.13^b
Alo+ALOG 250 mg	35.29±2.93^a	1.96±0.16^a
Alo+ALOG 500 mg	35.96±2.31^a	1.88±0.10^a

Values are expressed as ± S.E.M; One-way ANOVA; $^a p<0.05$ compared to diabetic untreated followed by Ducan post hoc test; $^b p<0.05$ compared to DW group; $^c p<0.05$ compared to ALOG and MFM groups. Values with different superscript down the column are statistically different ($P<0.05$) ALOG= aqueous leaf extract of *O. gratissimum*; MFM= metformin; Alo= alloxan; DW= distilled water.

Serum creatinine and urea are known to be increased inadequately controlled blood sugar leveland usually correlate with severity renal damage (Zimmet *et al.*, 2001; Shlomo *et al.*, 2011). The ability of aqueous leaves extract of *ocimum gratissimum* to ameliorate the hypercreatininemia and hyperureamia are indicative potential of preventing diabetic complications such as diabetic nephropathy and preserving kidney function.

The alloxan induced diabetic weight loss seen in the diabetic rats probably due to muscle wasting and loss of tissue protein (Shirwaikar *et al.*, 2004) was alleviated in the extract treated groups. This suggests that the extract has protective effect against muscle wasting and loss of tissue protein.

The extract at all doses significantly ($p<0.05$) improved weight loss which was highest at the 500 mg/kg (Table 3).

Table 3. Effects of aqueous leaf extract of *Ocimum gratissimum* on body weight of alloxan-induced diabetic rats.

Treatment /kg	BI (SEM)	A1 (SEM)	D8(SEM)
DW 10 ml	93.80±2.62	102.20±2.06	109.60±3.41
Alo+DW 10 ml	119.20±0.49	114.40±4.15	100.20±3.51
Alo+MFM 2.5 mg	130.00±5.92	128.40±5.46	120.40±5.07
Alo+ALOG 125 mg	103.00±0.71	102.20±5.55	105.00±4.14
Alo+ALOG 250 mg	118.60±1.57	114.80±3.9	120.80±1.85
Alo+ALOG 500 mg	111.00±1.41	122.20±6.70	116.20±7.15

Values are expressed as mean ± S.E.M, n=5; One-way ANOVA; $^{**}p< 0.05$ compared to diabetic untreated followed by Ducan post hoc test; BI= body weight before induction; AI= body weight after induction; D8= body weight on the 8th day; ALOG= aqueous leaf extract of *O. gratissimum*; MFM= metformin; Alo= alloxan; DW= distilled water.

There were no significant pathological changes in the histological examinations of the selected organs and this complements the findings of the biochemical indices thus further suggests the relative safety of the aqueous leaf extract of *O. gratissimum*.

The normal anatomy of the liver at the histologic level was displayed with its divided classic hepatic lobules cords of hepatocytes poked from the central vein by the control group (Figure 2).

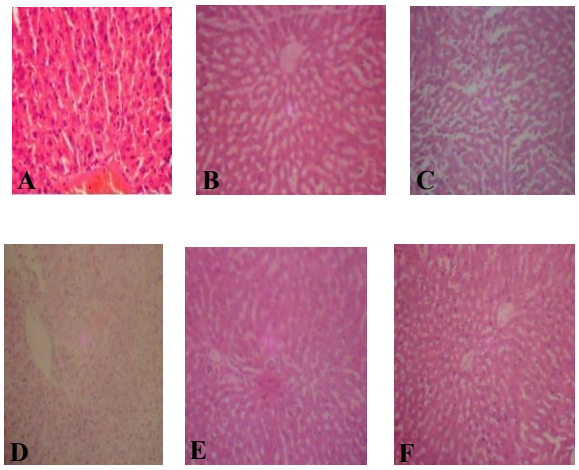

Figure 2: Photomicrograph of the liver. A= control: showing normal liver tissue; B=diabetic untreated: showing moderately degenerated liver tissue; C= diabetic rats treated with metformin: showing mildly degenerated liver tissue; D= diabetic rats treated with 125 mg/kg ALOG: showing mild hepatocellular degeneration and hypochromic liver tissue; E= diabetic rats treated with 250 mg/kg ALOG: showing mild hepatocellular degenerated liver tissue; F= diabetic rats treated with 500 mg/kg ALOG: showing mild hepatocellular degenerated liver tissue.

Photomicrographs of the kidneys of control, diabetic untreated and the diabetic treated groups are shown in Figure 3. No significant pathological changes were observed in the diabetic untreated and the diabetic treated group compared with the control (Figure 3).

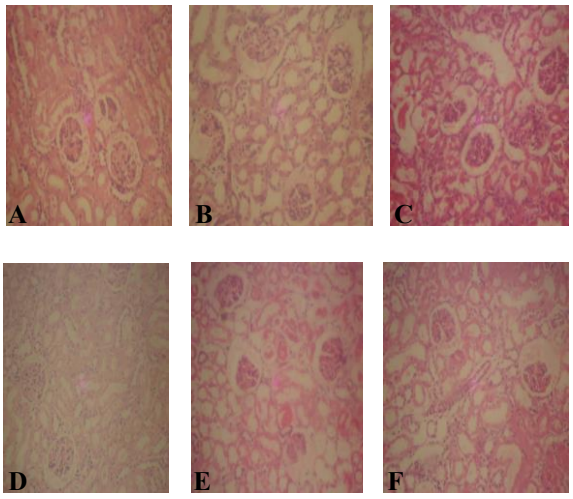

Figure 3. Photomicrograph of the kidney. A= control: showing normal kidney tissue; B= diabetic untreated rats: showing normal but hypochromic kidney tissue; C= diabetic treated with metformin: showing mild glomeruli inflamed kidney tissue; D: diabetic rats treated with 125 mg/kg ALOG: showing normal kidney tissue; E= diabetic rats treated with 250 mg/kg ALOG: showing mild glomeruli inflamed kidney tissue; F= diabetic rats treated with 500 mg/kg ALOG: showing normal kidney tissue with recuperating glomeruli.

Photomicrographs of the pancreas of control, diabetic untreated and the diabetic treated groups are shown in Figure 4. No significant difference was observed when the diabetic untreated, diabetic treated with 250mg/kg, diabetic treated with 500mg/kg extract and metformin treated group were compared. They altogether possess normal pancreatic tissue with no pancreatic cells seen. Whereas there was no difference between diabetic treated with 125mg/kg extract and the control group. They show normal pancreatic tissue with both exocrine and endocrine cells preserved.

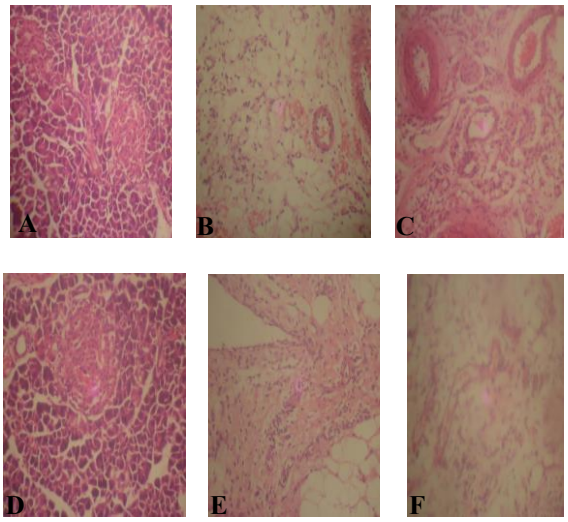

Figure 4. Photomicrograph of the pancreas. A= control: showing normal pancreatic tissue, both exocrine and endocrine parts are preserved; B= diabetic untreated rats: showing normal pancreatic tissue, no pancreatic cells are seen; C= diabetic treated with metformin: showing normal pancreatic tissue, no pancreatic cells are seen; D= diabetic treated with 125mg/kg ALOG: showing normal pancreatic tissue, both exocrine and endocrine parts are preserved; E= diabetic rats treated with 250 mg/kg ALOG: showing normal pancreatic, no pancreatic cells are seen; F= diabetic rats treated with 500 mg/kg ALOG: showing normal pancreatic, no pancreatic cells are seen.

4. Conclusion

The aqueous leaves extract of *Ocimum gratissimum* has anti-hyperglycemic effect and is relatively safe, thus provides the pharmacological rationale for the ethnomedicinal uses of the plant.

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