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Effect of administration of *Ehretia anacua* aqueous extract on blood glucose level in alloxan - induced diabetic rat

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Abstract

The effects of crude aqueous extract of *Ehretia anacua* on alloxan induced diabetic rats was investigated. Male albino rats of weighing between 120 to 150 were used, divided into 6 groups of five animals per group. Group I received distilled water throughout of the experiment and served as the control. Group II received 110 mg/kg of alloxan interperitoneally. Groups III, IV, V and VI received 110 mg/kg of alloxan and in addition administered with aqueous *Ehretia anacua* extract daily for 14 days. Blood glucose level was monitored at five days interval for fourteen days. Target organs (pancrease) was taken from each rat. The histopathological studies of the pancrease were examined. In alloxan - induced diabetic rats, blood glucose level was significantly increased compared with the control rats. Treating diabetic rats with 50, 100 and 200 mg/kg bw *Ehretia anacua* caused a significant decrease in the blood glucose level. The Photomicrograph of the histopathology examination of the pancrease (× 100) of the groups treated with alloxan showed poor architecture was destroyed whereas those treated with *Ehretia nancua* showed normal architecture. This illustrates the amelliorative effects of the extract on the alloxan-induced tocicity. It could be concluded from these results that, *Ehretia nancua* extract should be used in manufacture processes of the natural products as functional foods or as a dietary supplement with anti-diabecretic activity as hypoglycemic effect.

Keywords: *Ehretia anacua;* Diabetes; Alloxan; Blood Glucose; Histopathology

1. Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by defective insulin secretion and / or action, resulting in long term multi-organ complications. Chronic hyperglycemia [1] (Caughron and Smith, 2002) causes damage to the eyes, heart, kidneys, nerves, and blood vessels. The current review focuses on herbal [2] (Lebovitz, 2001) drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses. On the other hand, high glucose level was found to increase the production of free radicals, as determined by cell damage markers. Increased oxidative stress has been implicated in the pathogenesis of diabetic complications and reduced levels of antioxidants are found in blood and tissue in both human and experiments diabetes [3]; [4], [5]. (Cuncio *et al*., 1995; Baynes and Thorpe, 1999; Koleva *et al*., 2002). There are several synthetic drugs that have been used over time for the treatment of diabetes. These include insulin, sulfonylurea, biguanides, α-glucosidase inhibitors, and glinides, which are administered to achieve a better glycemic regulation. Unfortunately, many of these drugs have their limitations and comes with quite a number of adverse effects, such as lactic acidosis, low blood sugar, upset stomach, skin rash or itching, weight gain, kidney complications, upset stomach, tiredness or dizziness, metal taste [6] (Wild *et al*., 2004) etc. Thus managing diabetes using synthetic drugs without side effects remains a challenge [6]; [7]. (Wild *et al*., 2004; Rajagopal*et al.,* 2008). This however has drawn a lot of interest and attention to the curative

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claims and norms of medicinal plants all over the world, especially in under developed countries in Africa and some parts of Asia [8]. (Gagliano *et al*., 2007).

The leaves of *Ehretiaanacua*plant are used as an antidiabetic agent in Nigerian folk medicine. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes there is an increased demand by patients to use the natural products with anti-diabetic activity [9]. (Neuwinger, 2000). One such plant expected to have anti-diabetic activity is *Ehretiaanacua*.

The core aim of the present study was therefore; to examine the effects of *Ehretiaanacua*leaf aqueous extract on lipid profiles and hyperglycaemia in alloxan- induced diabetic rats

2. Justification for the Research

Diabetes mellitus is a complex and multifarious group of disorders characterized by hyperglycaemia that has reached epidemic proportions in the present century. Infection is a leading cause of morbidity and mortality among the diabetic population [10]. (Mottalib *et al.,* 2017). Diabetes is also associated with vascular and renal dysfunctions characterized by hypertension, dyslipidaemia and arteriosclerosis [11]. (Emadian*et al.,* 2015). Numerous studies have provided convincing evidence for the presence of oxidative stress, and its role in the pathogenesis of the complications of diabetes [11]. (Emadian*et al.,* 2015).

The leaves of *Ehretiaanacua*plant are used as an antidiabetic agent in Nigerian folk medicine. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes there is an increased demand by patients to use the natural products with anti-diabetic activity [9]. (Neuwinger, 2000). One such plant expected to have anti-diabetic activity is *Ehretia anacua*

Based on ethnobotanical uses, research on the antidiabetic effects of *Ehretia anacua* leaf and the effects of the extract on the lipid profile via the assessment of the lipid profile seems quite lacking. Therefore, there is a need to investigate the effects of the extract on the blood glucose levels in alloxan-induced diabetic rats.

3. Material and methods

3.1. Plant Materials

Samples of *Ehretia anacua* leaves were collected from a private farm in Ado Ekiti, air dried in the laboratory, pulverized and then stored in an airtight container.

3.2. Reagents and Chemicals

All reagents and chemicals were all of analytical grade.

3.3. Extraction of the extract

Ehretia anacua leaf was air-dried for 30 days at room temperature. The air-dried samples were ground to fine powder using a blender. 500 g of the powdered leaves was soaked in 2000 ml of distilled water for 72 hours. It was then filtered using a cheese cloth, and freeze-dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4^0C for further studies

3.4. Animal's protocol

30 male wistar albino rats weighing 120g – 150g was obtained from the animal house at The Federal Polytechnic, Ado-Ekiti. They were acclimatized in the animal house of the Department of Science Technology, The Federal Polytechnic, AdoEkiti for 2 weeks, housed in clean wire meshed cages under standard conditions temperature (24 ± 1^oC), relative humidity, and 12 / 12-hour light and dark cycle. They were allowed to have free access to food (commercial palletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

3.5. Experimental Design

30 male wistar albino rats were randomly divided into six groups (I-VI) of five animals in each group.

3.6. Animal treatment

The animal treatment is shown in the table below

Table 1 Animal treatment

Groups	Treatment
Group A: Normal control (NC)	Distilled water only for 14 days
Group B: Diabetic control (DC)	110 mg/kg Alloxan alone for a single administration
Group C	110mg/kg Alloxan+100mg/kg *Ehretiaanacua*for 14 days
Group D	110mg/kg Alloxan+200mg/kg *Ehretiaanacua*for 14 days
Group E	110mg/kg Alloxan+ 21.4 mg/kg metformin for 14 days.

3.7. Determination of Blood Glucose Level

The blood glucose level was determined using glucometer

3.8. Dissection of Rats

The rats were dissected and the pancreas were excised using scissors and forceps.

3.9. Histopathological Analysis

Histopathological Analysis was carried out according to the method of [12]. Avwioro (2010).

4. Results

Table 2 Effects of *Ehretiaanacua* leaf extract on the blood glucose level

Group / Treatment	Initial level (before induction)	Induced glucose level	5th day	10th day	14th day
Normal control	98.33 ± 4.13	-	106.23 ±2.10	102.01 ± 5.12	96.14 ± 2.13
Diabetic control	100.23 ± 8.33	307.29±10.5	402.72± 7.21	387.22± 9.20	394.50± 6.72
Alloxan + S. L (150 mg/kg)	107.75 ± 4.5	340.14±2.37	179.12±8.76	102.81±9.00	97.21±2.45
Alloxan + S. L (100 mg/kg)	99.45 ± 3.3	415.09±0.92	203.20±7.22	117.25±7.05	100.43±4.30
Alloxan + S. L (50 mg/kg)	118.12 ± 2.4	478.42 ± 3.61	355.81± 4.50	205.80± 3.05	103.22 ± 5.10
Alloxan + MET (21.4 mg/kg)	105.53 ± 5.5	457.76±5.03	221.43±5.20	106.54±2.70	96.16±4.05

4.1. Histopathological Examination on the Pancreas

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa (red arrow) are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells [Figure1].

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing poor architecture, the parenchyma of the pancreas shows severely thickened vessels and duct (red arrow), and also showing mild peri ductal infiltration (blue arrow). There are diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the borders of the diffuse islets are ill defined (white arrow). There is moderate infiltration of inflammatory cells within the intra acinar space and also seen within the islet (slender arrow), there is mild vascular congestion (black arrow) [Figure1].

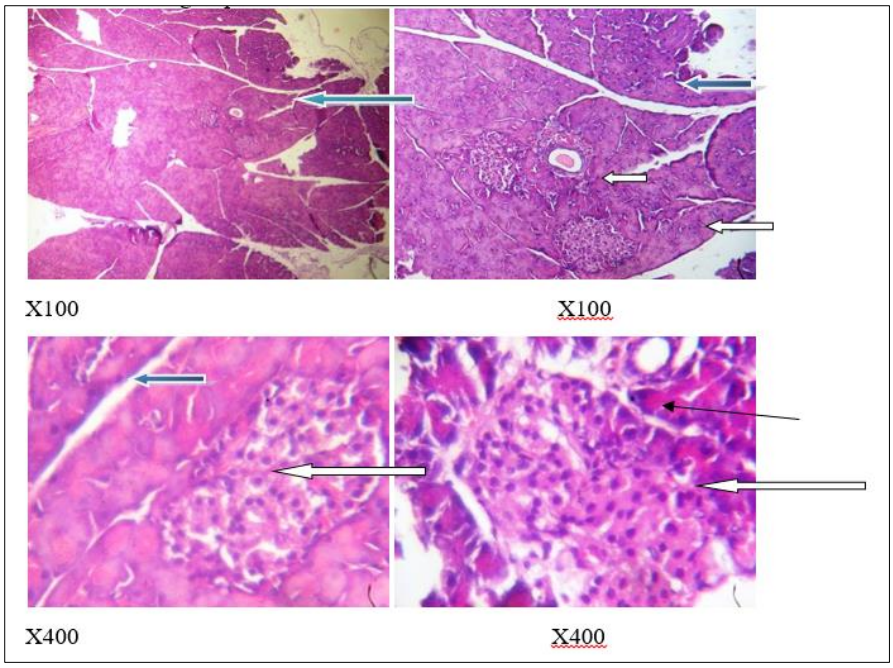

Figure 1 Normal control group

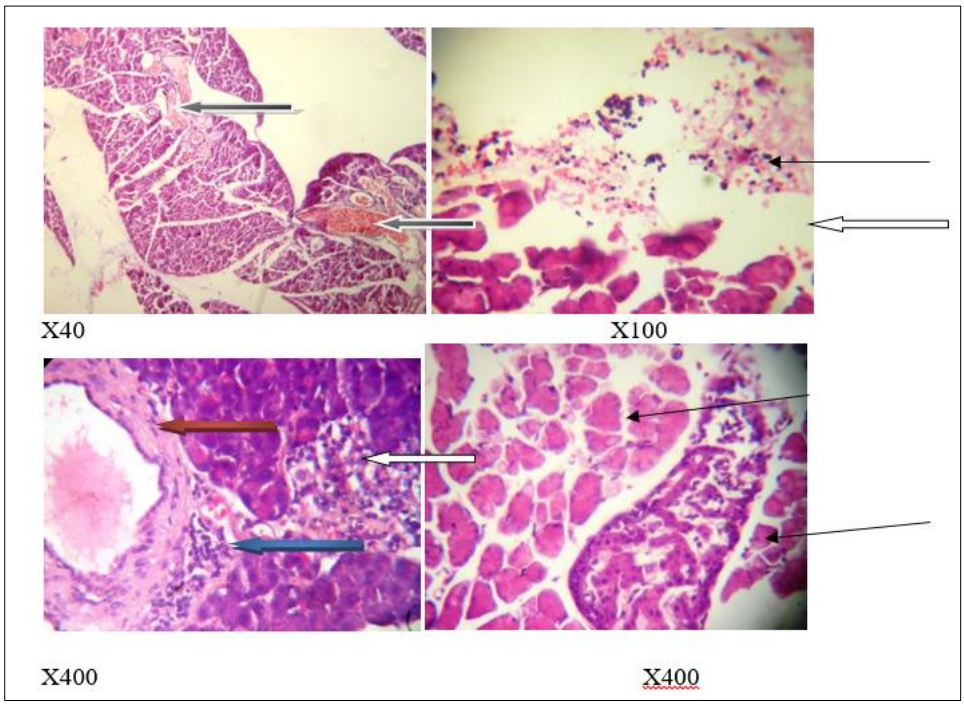

Figure 2 Untreated diabetic group (110mg/kg Alloxan)

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing poor architecture, the parenchyma of the pancreas shows severely thickened vessels and duct (red arrow), and also showing mild peri ductal infiltration (blue arrow). There are diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the borders of the diffuse islets are ill defined (white arrow). There is moderate infiltration of inflammatory cells within the intra acinar space and also seen within the islet (slender arrow), there is mild vascular congestion (black arrow).

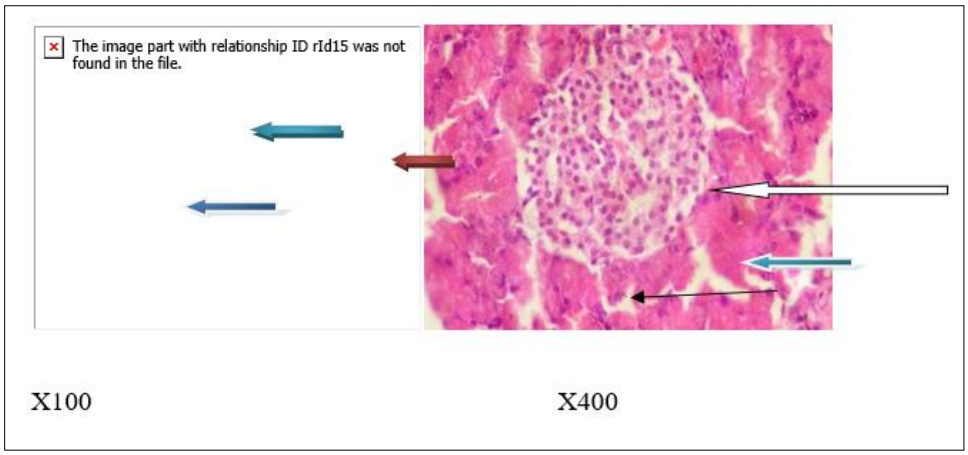

Figure 3 Diabetic group (110mg/kg Alloxan) but treated with *Ehretiaanacua* leaf at 100 mg/kg

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells(slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa(red arrow) are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells.

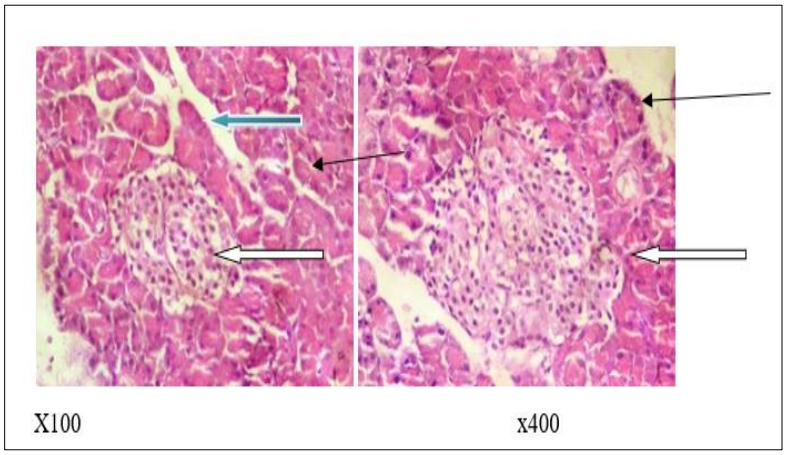

Figure 4 Diabetic group (110mg/kg Alloxan) but treated with *Ehretiaanacua* at 200 mg/kg.

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells.

Photomicrograph of a pancreas section stained by haematoxylin& eosin showing normal architecture, the parenchyma of the pancreas shows normal serous acinar and zymogenic cells (slender arrow) containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues (blue arrow) and septa(red arrow) are seen. There are normal compact islets of langerhans (white arrow) consisting of round to oval collections of endocrine cells (figure5).

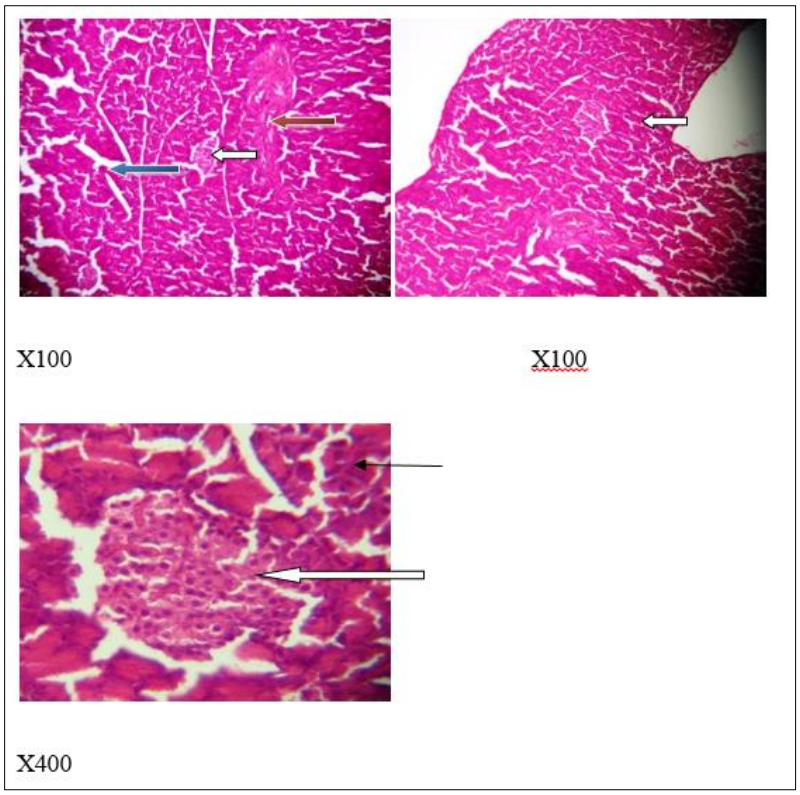

Figure 5 Diabetic group (110mg/kg Alloxan) but treated with synthetic drug (21.4mg/kg metformin)

5. Discussion

The treatment of DM remains a challenging issue. Researchers are exploring safe and effective medications to overcome the detrimental effects of insulin resistance-related metabolic derangement, including hyperglycaemia, hyperinsulinaemia, hyper-lipidaemia, oxidative stress, inflammation, atherosclerosis and other complications [13]. (DeFronzo*et al*., 2013). For patients with DM, no safe treatments yet exist apart from diet and lifestyle modifications [14]. (Elsheikh *et al.,* 2013). However, combined pharmacological therapy is recommended to improve insulin sensitivity in the liver (metformin and pioglitazone) and its periphery (thiazolidinediones), together with other drugs such as betaine, atorvastatin, losartan and orlistate [15]. (Crawford *et al.,* 2009). However, the clinical value of these treatments is very subjective. Patients taking these drugs should be closely monitored due to possible contraindications with DM medications and the vulnerable condition of the liver during the drug detoxification process [16]. (Adams *et al.,* 2006). Antioxidant therapy is a potential future therapeutic strategy; increasing antioxidant levels in patients with DM may hopefully counter the effects of oxidative stress and inflammation, thereby reducing the severity of diabetic complications. A few plant-based products and vitamins have been investigated as ways of protecting against and possibly reversing damage believed to be caused by oxidative stress and inflammation [17]. (Seven *et al*., 2004). Vitamin E and betaine are just a few of the antioxidants which have shown good clinical implications in the reduction of DM severity and the protection of the organs from DM-induced damage [17]. (Seven *et al*., 2004). Therefore, Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [18]. (Okigbo *et al.,*2008). Their properties could be based on the antioxidant, antimicrobial antipyretic, enzyme inhibitory effects of the phytochemicals present in them [19]. (Adesokan *et al.,*2008).

The present study was undertaken to investigate the antihyperglycemic activity of sandpaper leaf *Ehretia anacua* extract in diabetic model rats. Metformin was used as a standard drug for diabetic model rats. It is well established that the only available diabetes medication in the Biguanides class of drug is metformine [20]. (Hundal *et al*., 2000). Biguanides prevent the liver from producing glucose and help to improve the body sensitivity toward insulin. Metformin is commonly used as first line treatment for type 2 diabetes and may occasionally be prescribed in combination with insulin for people with type 1 diabetes. These pills stop the liver from making too much sugar (glucose). They also help the sugar get into the cells [20]. (Hundal *et al*., 2000).

On the other hand, insulin activate glucose uptake in various cells including muscles and adipocytes, stimulates hexose uptake, lipogenesis and inhibit lipolysis and stimulate protein synthesis. Administration of Metformin and extracts into rats almost normalized serum glucose levels. Our results demonstrate that all the extracts of sandpaper leaves and metformin showed significant antihyperglycemic effect in diabetic model rats. Administration of a dose of 150 mg/ kg, 100 mg/ kg and 50 mg/ kg body weight of the extract produced a potent and strong antihyperglycemic effect in diabetic rats. The obtained results are supported by the finding of other investigators [21]; [22]. (Sharma *et al*., 1997; Aderibigbe *et al*., 2001). Antihyperglycemic activity that is found in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the gut by various mechanisms [23]; [24]. (Nahar *et al*.. 2000; Vinik and Wing, 1990). It may be postulated that the extracts of sandpaper leaf might stimulate glycogenesis in the liver, which is enhanced by feeding [25]. (Creutzfeld *et al*., 1979). This effect was confirmed by Perpetusand Salgado, 2003 where they showed that blood glucose level of diabetic rats consuming mango flour for 90 days decreased 66% in comparison to control rats. It was also observed that hepatic glycogen level of those diabetic rats was 64% greater than control. The author claimed that this increase in glycogen level might have contributed to the reduction of blood glucose level in these animals.

According to certain studies, the aberrant signal which promotes glucose production in the liver during DM supposedly also enhances fatty acid oxidation due to a lack of fuel demand [26]. (Reid, 2006). However, other research has found that the liver stops oxidising fatty acids and uses them instead to synthesize triglycerides which then accumulate abnormally in the liver [26]. (Reid, 2006). In type 1 DM, insulin deficiency up regulates hormone-sensitive lipase in the adipose tissues, subsequently leading to increased lipolysis and the circulation of free fatty acids, which subsequently accumulate in the liver. These processes enhance the hepatic uptake of very-low-density lipoproteins and synthesis of triglycerides [26]. (Reid, 2006). Concurrently, elevated glucagon levels inhibit hepatic triglyceride output. Therefore, accumulation of fat in the liver may be due to an imbalance in the uptake, synthesis, export and oxidation of free fatty acids in the liver [27]. (Cohen *et al.,* 2006). Aside from abnormalities in lipoprotein metabolism, an accumulation of hepatic fat in DM may be due to either hyperglycaemia-induced activation of the transcription factor carbohydrate-responsive element-binding protein and sterol regulatory element-binding protein 1c, the up regulation of the glucose transporter 2 protein with subsequent intrahepatic fat synthesis or a combination of these mechanisms [27]. (Cohen *et al.,* 2006).

Poor architecture, mild peri ductal infiltration, mild vascular congestion, moderate infiltration of inflammatory cells within the intra acinar space and within the islet of pancreas, diffuse islets which are composed of trabeculae of endocrine cells interspersed between adjacent acini, the parenchyma of the pancreas shows severely thickened vessels and duct in the pancreas section of Wistar rats treated with alloxan only when compared with the pancreas sections of the control is indicative of alloxan related toxicity.

Administration of the extract *Ehretiaanacua*improved the histo-architecture of the pancreas and by extension restored its functionality.Histoarchitectural distortion, such as, inflammatory cells infiltration observed in the pancreas sections is resultant of alloxanintoxication, while observed histoarchitectural preservation is consequent to treatment with plant extract. This finding is supportive of medicinal plant related studies that have reported hepatoprotective activity of plant extracts; [28]. (Al-Qarawi*et al*., 2012) treatment with plant extract decrease the severity of histopathological changes induced by acetaminophen.Extract of *Ehretiaanacua*presented histoarchitectural preservation of the pancreascells when compared with the control. Treatment with *Ehretiaanacua* demonstrated the plant extract potentials as a free radical scavenger and lipid peroxidation inhibitor, thus helping to maintain the integrity and permeability of cell membranes and protects cells and tissues against oxidative stress induced by free radicals [29]. (Naik and Panda, 2012).

6. Conclusion

Medicinal plants are used in several countries to manage diabetes and diabetes complications and thought to be less toxic than the synthetic drugs. Phytomedicines are also easily available and affordable to many people. In conclusion, the extract of *Ehretiaanacua*reduced the levels of glucose, and preserve the Histoarchitecture of the pancreas in experimentally induced diabetic rats. Therefore, the plant hashypolipidaemic effects on alloxan-induced damage in rats. Based on these findings, the results thereby lend credence to the ethnomedicinal use of the extracts in the management of diabetes at evaluated dosages and their use should be encouraged.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that No conflict of interest in this work.

Statement of ethical approval

All authors hereby declare that the research has been determined exempt from review by the Polytechnic animal research and ethics review committee and that the principles of the laboratory animal care were followed.

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