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Research Article

The Comparative Effects of Alloxan and Streptozotocin in Inducement of Type-1 Diabetes on the Intestinal Microflora of Albino Rats

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ABSTRACT

Alloxan and streptozotocin are the two compounds scientists use for inducing diabetes in experimental animals for diabetes research. Although, both compounds are able to induce diabetes, their effects on the blood, pancreas and microflora of the gastrointestinal tract of animals may not the same. This research therefore, aims to compare the effects of alloxan and streptozotocin used to induce type 1 diabetes on albino rats. 12 young adult male albino rats were divided into 3 groups consisting of 4 rats per group. Group 1 was induced using alloxan, group 2 was induced using streptozotocin and group 3 was control. Both groups showed increased production of urine as compared to the control group and weight loss as well as dull furs few days after induction. The blood sugar level rose from 94 mg/dl to 218 ± 6 mg/dl and 204 ± 5 mg/dl in the alloxan and streptozotocin group respectively. The results of the haematology on the blood of the rats showed that alloxan had more negative effect on the packed cell volume compared to the streptozotocin. Comparatively, while the PCV of the control group was 44.67 ± 0.67%, the group induced with streptozotocin had 41.33 ± 0.67% and the group induced with alloxan had PCV of 38.00 ± 1.15%. Microbiologically, the control had the highest bacterial load of 9.0 ± 1.2 × 103 cfu/ml, while the group treated with alloxan had the least bacterial load of 2.0 ± 0.5 × 103cfu/ml. A total of seven bacteria were isolated from intestine and they are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Clostridium deficile*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* with *E. coli* having the highest frequency of occurrence. Alloxan caused profuse haemorrhage and complete destruction of the acini with prominent destruction of beta cells and washing away of the ductile beta cells of the pancreas, while streptozotocin caused poor formation of the islet of Langerhans and necrotized cells that is profuse as well as haemorrhage of vascularized cells and no visible interlobular duct. The results gathered from this research showed that the two compounds are able to induce type 1 diabetes in the rats. However, the effect of alloxan was more devastating on the pancreas beta cells as well as on the blood parameters of the rats than streptozotocin. It also had the highest reducing effect on the microflora of the GIT of the rats used.

Keywords: *Intestinal microflora; alloxan; streptozotocin; diabetes; Effects.*

INTRODUCTION

Disease conditions such as diabetes mellitus and immune compromised states, have shown to have potentiality in causing species of Candida which are commensal in the oral cavity especially Candida albicans to become pathogenic and causing lesions in the mucosa of the oral cavity[1]. There exist great variability between persons and even in the same person at different occasion in the severity of oral candidiasis is subject to great variability, from one person to another, and in the same person from one occasion to the next [2]. Although topical and systemic treatments offers excellent prognosis, however the disease condition tends to be recurrent and these individuals are always at risk if underlying conditions enhancing pathogenicity of the organism such as reduced salivary flow and immune compromised state continues to be in place [1] . The virulence attributes and Species of Candida isolated from Diabetes mellitus patients requires periodic assessment as these always undergoes changes over time.

Alloxan which is chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog Ref. The compound has the molecular formulae, $C_4H_2N_2O_4$ and a relative molecular mass of 142.06. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes. Among the known diabetogenic agents which include dithizone, monosodium glutamate, gold thioglucose, high fructose load, high glucose load and anti-insulin serum; alloxan and streptozotocin (STZ) are the most widely used in diabetes studies. The current average cost of one gram of alloxan and STZ are respectively 1.5 and 200 US dollars respectively. Due to relative affordability and availability, one will logically expect that alloxan will be more used compared to STZ Ref. However, a literature survey and sub meta-analysis that we

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carried out on the use of both compounds in experimental diabetes studies conducted within the last one and half decade (2000–2016) suggested otherwise. Analysis of the data obtained showed that 30.3% of the studies used alloxan while 57.9% made used of STZ as a diabetogenic agent, and others which used glucose, fructose and genetic diabetic mice constituted the remaining 11.8%.

In 2014, a total of 422 million adults were reported worldwide to have diabetes, the complications of diabetes can lead to heart attack, stroke, blindness, kidney failure and lower limb amputation. Glycemic homeostasis refers to glucose balance or control within circulation in living organisms. It is normally and largely compromised in diabetes. The compromise when exacerbated, leads to several complications including retinopathy, nephropathy and neuropathy which are collectively known as diabetic complications and are the principal actors in co-morbidity and eventual mortality that are often associated with diabetes. Alloxan and streptozotocin are the most popular diabetogenic agents used for assessing the antidiabetic or hypoglycemic capacity of test compounds. Notably, alloxan is far less expensive and more readily available than streptozotocin. On this ground, one will logically expect a preference for use of alloxan in experimental diabetes studies. Surprisingly, a sub meta-analysis of randomly selected studies conducted within the last one and half decade revealed otherwise. This observation necessitated the comparative study of alloxan and streptozotocin as a diabetogenic agent in animal studies.

MATERIALS AND METHODS

Location and environment of study

The study was conducted in Elizade university laboratory, Ilara-Mokin, Ondo State, Nigeria.

MATERIALS

Materials used for this research include Cages, measuring cylinders, conical flask, petri dishes, chloroform, foil paper, cotton wool, paper tape, distilled water, cover slips, slides, specimen bottles, dissecting tools, gloves, Petri dishes, nutrient agar, autoclave.

ANIMALS AND FEEDING

Albino rats (Young adults of four weeks old and all male) were obtained from animal house of Obafemi Awolowo University, Ile-Ife, Nigeria and transported carefully to Elizade University. They were maintained in a customised cage with a mesh top to allow for ventilation at room temperature, the cages were cleaned regularly (twice daily) to prevent infections. Rats' diet consisted of cereals and cereal by-product (rich in starch and proteins) and definitely water ad libitum. They were allowed to acclimatize to the environment for two weeks before the commencement of the research.

Lactose intolerance and potential probiotic treatment

EXPERIMENTAL DESIGN

A total of 12 rats were used for the assay. They were divided into 3 groups consisting of 4 rats per group. The groups and their designations are as follows:

Group 1- Diabetes induced rats using alloxan; Group 2- Diabetes induced rats using streptozotocin; Group 3- Control.

Induction of Diabetes

Experimental diabetes mellitus was induced by a single intra-venous injection of streptozotocin (STZ) dissolved in 0.1M citrate buffer in the adult rats under anaesthetics. After which diabetes was induced and confirmed in four days. Inducement of diabetes using alloxan was done using a single intraperitoneal administration which has been described as the most employed mode that has proved highly effective. The dosage was 150 mg/kg BW as described by[3].

Determination of blood glucose

Blood samples were collected from central auricular artery using insulin syringe and three drops of blood immediately transferred on a clean and dry glass slide for blood glucose estimation using glucometer (Accu-Chek, Roche diagnostics India Pvt. Ltd., Mumbai). Mean of the three readings was recorded as blood glucose level for the sample.

Haematological tests

Haematological tests such as Packed Cell Volume (PCV), Haemoglobin Concentration (HB), Red Blood Cell Count (RBC), Erythrocyte Sedimentation Rate (ESR), White Blood Cell Count (WBC), and White Blood Cell differential count (Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils) were done according to Cheesbrough (2014) using Mindray BC3300 (2016) Modelauto-haematology analyser[4].

Histopathological tests

Histopathological test on the pancreas was performed according to the methods of Baker et al., (2014) and Cheesbrough (2014) in the following process[5]

Fixation

Fixation of the tissue was done to prevent further enzymatic activity that usually leads to post-mortem autolysis. It also hardens tissue as well as kills microbes and keeps the tissue in its original form. The organs of the animals were collected and fixed in 10% buffered formalin.

Trimming

After fixation, the organs were trimmed to about 1-2cm before dehydration.

Dehydration

Dehydration was done by passing the tissue through different concentrations of ethanol. It was done by the use of automatic tissue processor. They were dehydrated in graded percentages (50%, 70%, 80% and 100%) of ethanol for 1 ½ hours each at 30 ± 2°C.

Clearing

Clearing of dehydrated tissue was done using100% xylene. The tissues were left for 2 hours to remove any remnant alcohol completely.

Embedding

This was the placing of the cleared tissue in melted paraffin and allowed to harden. The tissues were left in the molten paraffin wax for 2 hours to embed properly.

Sectioning

This was done using a microtome. The tissues were sectioned to about 3-10µ and floated in water bath at 37°C.

Hydration

This is the process of passing the tissue through water by passing it through different concentrations of alcohol. It was passed through

xylene, 100%, 90%, 80%, 70% and 50% of ethanol for 11/2 hours at each concentration.

Staining

This was done using haematoxylin and eosin (H&E) stains. Haematoxylin stains the nucleus blue while eosin stains the cytoplasm acidophilic.

Dehydration, fixing and microscopy of stained slides

Dehydration was done again by passing the tissue through different concentrations of ethanol. It was done by the use of automatic tissue processor. The tissues were dehydrated in different percentage (50%, 70%, 80% and 100%) of ethanol for 1 ½ hours each and cleared with xylene. The method of Cheesbrough (2014) was used to remove excess stain under tap water[4]. After clearing in xylene, the fixed tissue on a glass slide was fixed with Canada balsam and covered with cover slips [6]. The preparations were left in the oven at 40°C and then placed under the photo-microscope for examination [7].

Determination of bacterial load of the small intestine

The bacterial load of the stomach of the experimental rats was done using the method of Cheesbrough (2014)[3].

Isolation of bacteria from the small intestine

The isolation of bacteria from the small intestine was done using streaking method described by Omoya et al.,[2] (2018).

Purification of bacterial isolates

Different colonies observed on each of the plates used for the isolation of bacteria were picked and streaked on sterile prepared agar plates for recovery of pure culture. These were incubated at 37°C for 24 hrs. Different bacterial pure cultures were then inoculated into Nutrient agar slants, incubated for 24 hrs. in order to ensure proper growth and then kept as stock cultures in the refrigerator at 4°C for identification. Biochemical characterization and identification of the organisms was also carried out using the Bergey's Manual of Determinative Bacteriology, 16th edition by Holt and Krieg (2018)[8].

Characterization of bacterial isolates

The pure culture of each isolate was examined. Microscopic examination, staining techniques and biochemical tests were carried out on the isolates according to the methods described by Olutiola et al. (2000)[9]:

Gram staining

A smear was made on a clean labelled slide using a sterile wire loop and then heat fixed. The smear was then flooded with crystal violet for 1 minute and wash off the crystal violet under running tap water. The slide was flooded with Gram's iodine solution and allowed to react for 1 minute and then was washed off under running tap water. The smear was then decolourized with 95% alcohol for 5 seconds and immediately rinsed off under gently running tap water so as to remove the alcohol effect. The slide was then counterstained with safranin for 1 minute and rinsed under running tap water. The slide was then blotted dry and examined under an oil immersion lens.

Motility test

A little Vaseline drop was placed around the edge of the hollow in a clean cavity slide. A loopful of 24 hrs old culture of the bacteria was aseptically transferred to the centre of a clean cover-slip laid on the bench without spreading it. The cavity slide was then carefully inverted over the cover-slip in such a way that the drop is in the centre of the cavity slide and the slide was gently pressed firmly enough to enable the Vaseline seal the cover-slip in position. The slide was then smoothly inverted in order to allow the drop of culture in hanging position. The preparation was immediately observed under a microscope in a reduced illumination [9].

Catalase test

A drop of 3% (v/v) of hydrogen peroxide was placed on the slide and a loopful of bacterium isolates was added. Effervescence, bubble formation indicates a positive catalase test [8].

Oxidase test

The filter paper spot was first wet with three drops of sterile distilled water after which a large mass of pure 24 h old bacteria culture was aseptically transferred to the wet spot. This was then followed by the addition of one drop of the Kovac's oxidase reagent. The spots were observed for blue colour within 10 seconds and the results compared with the control [9].

Starch hydrolysis test

Starch agar was prepared by fortifying the nutrient agar medium with 1% of soluble starch and autoclaved at 121°C for 15 minutes after which the plates were poured and allowed to gel. The organisms were then streaked once across the surface of the plates and incubated at 37°C for 24 h. The plates were then flooded with some quantity of Gram's iodine. Unhydrolyzed starch will form blue black colour with the iodine while hydrolyzed starch appears as a clear zone which results from α-amylase activity. Reddish brown zones around the colony indicate partial hydrolysis of starch[9].

Citrate utilization test

The test is based on the ability of an organism to use citrate as its only source of carbon. Simmon's citrate agar was prepared in a slope on Bijou bottles as recommended by the manufacturer (it can be stored at 2-8°C). Using a sterile straight wire, first streak the sloop with saline suspension of the test organism and then stab the butt. Incubate at 35°C for 48 hours. Look for a bright blue colour in the medium. Bright blue colour indicates that the citrate is positive, but no change in colour of the medium means the citrate test is negative[9].

Methyl Red Test

Five millilitre (5ml) of glucose phosphate broth (1g glucose, 0.5% KH2PO4, 0.5% peptone and 100ml distilled water) were dispensed in clean test tubes and sterilized at 121°C for 15mins. The tubes were then inoculated with the test organisms and incubated at 37°C for 48hrs. At the end of incubation, five drops of methyl red solution were added to each test tube. The appearance of red colouration indicates positive reaction [9].

Voges Proskauer Test

Nutrient broth 100ml was prepared with (1g) of glucose and sterilized. It was inoculated and incubated at 37°C for 24hrs. 0.5ml of 6% of α–naphthol was added, followed by 0.5ml of 40% potassium hydroxide (KOH). The solution was shaken thoroughly and observed for colour change after 30mins. A strong red colouration formed within 30mins indicates positive result while there were no colour changes in the negative result[9].

Indole test

Three millilitres of Tryptone broth were inoculated with small amounts of a pure culture and incubated at 37°C for up to 48 hrs. Zero-point-five millilitre of kovac reagent was added to the tube as described by Cheesbrough (2006)[8]. A positive indole test is indicated by the formation of pink to red colour ("cherry-red ring") in the reagent layer on the top of the medium within seconds of adding the reagent.

Hydrogen sulphide (H2S) production

An inoculum from a pure culture was transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The inoculated tube is incubated at 37°C for 24 hrs. and the results were determined. A positive hydrogen sulphide (H2S) production test is indicated by the formation of black compound.

Sugar fermentation test

This test shows the ability of bacterial isolates to ferment sugars such as mannitol, glucose, fructose, galactose and lactose. Basal medium of peptone water containing 0.5–1.0% of fermentable substrate was prepared and 1% Andrade's indicator was added. The medium was dispensed into 4 test tubes containing inverted Durham's tubes before autoclaving. The sterile medium was inoculated with broth culture containing the isolates and incubated at 35 oC for 7 days. The cultured tubes were observed for gas production, acid production or both. The presence of space indicates the production of gas as a result of utilization of the sugar by the inoculated organism which brought about colour change [8].

Statistical analysis

Data obtained were subjected to descriptive one way analyses of variance using SPSS version 22 and Duncan New multiple range tests was used as follow up test.

RESULTS

Observation of general condition of the experimental rats

Various observation were made on the activities of both groups (Alloxan and Streptozotocin groups) include increased production of urine as compared to the control group was noticed and weight loss as well as dull furs.

Table 1 shows that streptozotocin and alloxan induced diabetes few days after induction. The initial blood sugar before inducement was 94 mg/dl. An elevation in the blood sugar of the rats can be observed in the first two groups, while the control group maintained blood sugar in the normal range. Table 2 shows the elevation and reduction in weight of various experimental groups. In the first group the weight is seen to have decreased gradually through the week, the second group shows an increase in the weight, in the third group it was observed that the body weight decreased drastically during the first week of induction and gradually increases through the week, the second week showed inconsistency in weight, whereby the weight increased and decreased regularly and finally at the third week there was a drastic reduction in weight is observed compared to the initial weight. The control group however shows increased weight gain from the first week through the third week.

Table 1: Shows the glucose level of experimental rats.

Groups	Week one	Week two	Week 3
Diabetes induced with alloxan	218 ± 6 mg/dl	216 ± 6 mg/dl	212 ± 9 mg/dl
Diabetes induced streptozotocin	204 ± 5 mg/dl	180 ± 4 mg/dl	142 ± 6 mg/dl
Control	90 ± 4 mg/dl	92 ± 5 mg/dl	95 ± 6 mg/dl

Table 2: shows the variation in weights of experimental rats

Groups	Initial weight before induction (g)	Weight in week 1 (g)	Weight in week 2 (g)	Weight in week 3 (g)
Induced diabetes with alloxan	120.40 ± 2.63	115.15 ± 3.81	114.28 ± 2.17	110.23 ± 1.56
Induced diabetes with streptozotocin	130.50 ± 5.02	105.10 ± 3.48	101.91 ± 1.12	107.82 ± 1.05
Control group	120.18 ± 5.42	122.43 ± 4.05	128.12 ± 2.20	130.84 ± 4.54

Results of the haematological analysis

The results of the haematology on the blood of the rats showed that alloxan had more negative effect on the packed cell volume compared to the streptozotocin. Figure 1 shows that while the PCV of the control group was 44. 67 ± 0.67%, the group induced with streptozotocin had 41.33 ± 0.67% and the group induced with alloxan had PCV of 38.00 ± 1.15%. The erythrocyte sedimentation rate (ESR) results showed the same pattern in which the group of rats induced with alloxan had the highest effect on the rats than the ones induced with streptozotocin. The control group had the lowest ESR value. However, the effect of streptozotocin used to induce the diabetes was the same on the red blood cell count with the control group; an indication that the streptozotocin had no negative effect on the red blood cell. The results obtained for the white blood cell count showed a very high value for the two compounds used to induce the diabetes when compared with the value obtained for the control. The same pattern of this result was obtained for the haemoglobin concentration value for the experiment.

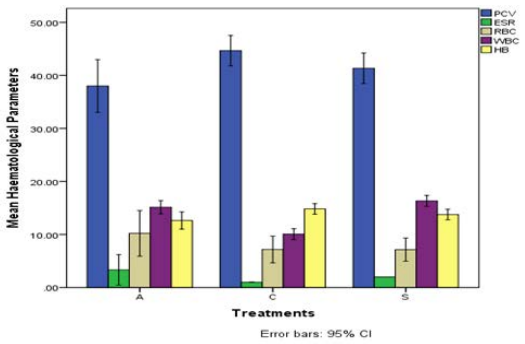
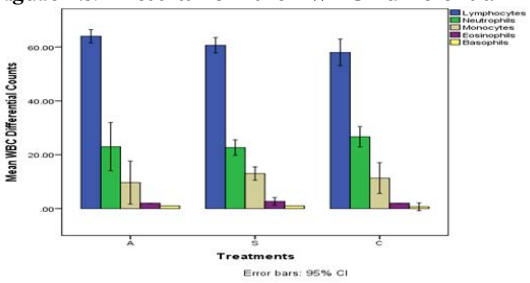


Figure 1: Haematological indices of the experimental rats

The results obtained on the WBC differential counts showed that lymphocyte count was highest on the group induced with alloxan. The same group had the least number on neutrophil count while the group induced with streptozotocin had the highest count of monocytes. The control group on the other hand recorded the least count of eosinophil's and basophil respectively. The result of the white blood cell differential count is represented in

Figure 2.

Figure 2: Results of the WBC differential indices of the



experimental rats

Results of bacterial load and types of bacteria isolated from the small intestine of the experimental rats.

Table 3 shows the bacterial load of the intestine of the experimental rats. The control had the highest bacterial load of 9.0 ± 1.2 x 103 cfu/ml, while the group treated with alloxan had the least bacterial load of 2.0 ± 0.5 x 103cfu/ml. The distribution of bacteria in gastrointestinal tract of the rats specifically the small intestine showed that only four bacteria were identified in the control group which are *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and Proteus vulgaris. However, in the alloxan group, the bacteria isolated include all in the control with the exception of S. pyogenes with Clostridium difficile and *Staphylococcus aureus* which was found in this group. Clostridium difficile was isolated from both experimental group but was not found in the control. Also, S. pyogenes was only isolated from the control group but not found in the two experimental groups.

Table 3: Results of the bacterial load of the rats' intestines

S/N	Sample	Bacterial load (cfu/ml)
1	Alloxan	2.0 ± 0.5× 103
2	Control	9.0 ± 1.2 × 103
3	Streptozotocin	4.7 ± 1.6 × 103

A total of seven bacteria were isolated and identified using morphological, biochemical and fermentation characteristics. The bacteria are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Clostridium deficile*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and Proteus vulgaris. *E. coli* had the highest frequency of occurrence followed by *Klebsiella pneumoniae* while *Staphylococcus aureus* and Streptococcus pneumoniae had the least frequency of occurrence. The result of the isolation and identification process is captured in **Table 4.**

Table 4: Distribution of bacteria

S/N	Sample	Probable bacteria isolated from the group
1	Alloxan	Clostridium difficile, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*
2	Control	*Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and Proteus vulgaris
3	Streptozotocin	Clostridium difficile, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and Proteus vulgaris

HISTOPATHOLOGICAL RESULTS

The results of the histopathology of the pancreas from the 3 groups of rats used for the experiment showed different pathological features based on the treatment each group was subjected to. The group induced with diabetes using alloxan showed features such as profuse haemorrhage and complete destruction of the acini with prominent destruction of beta cells and washing away of the ductile beta cells. The group induced with streptozotocin on the other hand showed a very poor formation of the islet of Langerhans and necrotized cells that is profuse as well as haemorrhage of vascularized cells. There is no visible interlobular duct which means that all formed acini have been destroyed. The control group showed pancreatic lobular cells that are prominent and well-formed and are located with visible connective septae. There is presence of cell infiltrations and very few inflammatory cell infiltrations packed around the islet of Langerhans. Sides' globular pancreatic cells that are well formed with slight anterior washing away of globular cells leading to intercellular drainage of exocrine secretions are pronounced with intercellular deposit of central crystals which are all indications of actively dividing acini. The plates of the histopathology are shown in **Plates 1-3.**

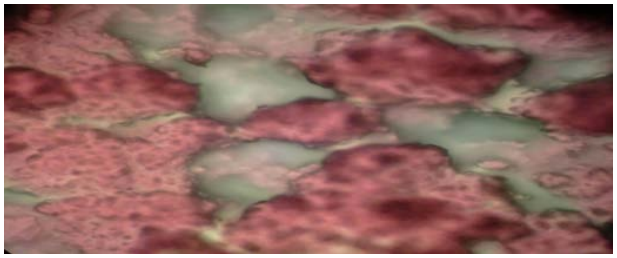
There is complete destruction of the acini of the pancreas and profuse haemorrhage of the ductile cells. This will probably lead to complete destruction of the beta cells of the pancreas. There is also the washing away of the central ductile beta cells of the pancreas in **Plate 1 Table 5.**

Plate 1: depicting the histopathology of rat's pancreas that was induced with alloxan.

Table 5: Probable bacteria isolates obtained from wound.

S/N	Morphological characteristics	Gram reaction	Catalase	Coagulase	Indole	Motility	Spore reaction	Fructose	Sucrose	Galactose	Maltose	Glucose	Lactose	Arabinose	Xylose	Starch hydrolysis	Probable isolate
1	Small, circular, white, translucent, flat, smooth, entire, butyrous.	+	+	+	-	+	-	+	+	+	+	-	-	+	-	-	*Staphylococcus aureus*
2	Medium, circular, milky white, translucent, flat, smooth, entire, butyrous.	+	+	+	-	-	-	+	+	+	-	+	-	-	+	-	*Streptococcus pyogenes*
3	Large. Irregular, white, translucent, flat, smooth, lobate, butyrous.	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	Clostridium difficile
4	Large, irregular, milky white, opaque, raised, rough, lobate, butyrous.	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	*Klebsiella pneumoniae*
5	Large, rhizoid, milky white, opaque, raised, rough, filiform, butyrous.	-	+	-	-	+	-	+	+	+	-	+	+	-	+	+	*Escherichia coli*
6	Small, circular, greenish, translucent, flat, smooth, swarmy, butyrous.	-	+	-	-	+	-	+	+	+	-	+	+	-	+	+	*Escherichia coli*
7	edium, circular, cream, transparent, flat, smooth, entire, mucoid.	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	Proteus vulgaris

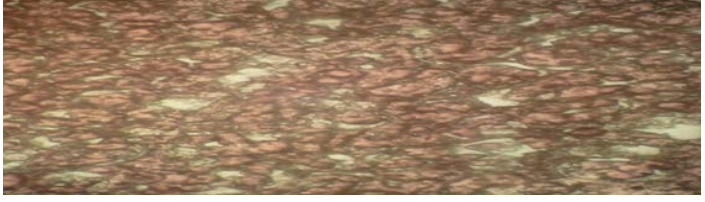
The pancreatic lobular cells are prominent and well-formed which can be located with visible connective septae. There is presence of



cell infiltrations and very few inflammatory cell infiltrations packed around the islet of Langerhans. Sides' globular pancreatic cells that are well formed with slight anterior washing away of globular cells leading to intercellular drainage of exocrine secretions are pronounced with intercellular deposit of central crystals in **Plate 2.**

Plate 2: The histopathology of a normal rat's pancreas.

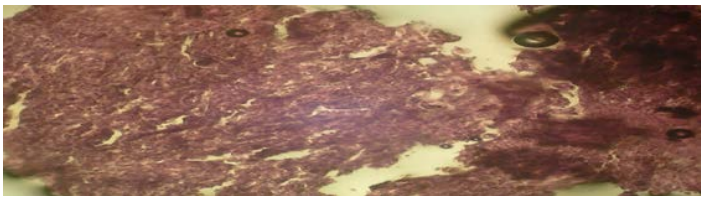
There is poor formation of the islet of Langerhans cells, complete



destruction of beta cells and cracked dot necrotized cells. There is profuse haemorrhage from highly vascularized pancreatic acini

surrounded by parenchyma fat cells. Few intralobular ducts are seen without any visible interlobular duct on the plate as seen in **Plate 3.**

Plate 3: The histopathology of rat's pancreas induced with streptozotocin



DISCUSSION

In **Table 1**, the blood glucose level was elevated to as high as 218mg/dl in the group induced with alloxan compared to the control (90mg/dl) while the group induced with streptozotocin was elevated to 204 mg/dl. Equally, as the induction or diabetes last without treatments in the rats, there was a gradual decrease in the sugar level. Comparatively therefore, the alloxan tend to induce higher sugar level in rats than the streptozotocin. However, the rate of the reduction in sugar level with days even without treatments on the induced diabetes is equally faster in alloxan than in streptozotocin as seen in **Table 1**. Another fact worth noting is that the feed with which the animals were fed also possess the ability to increase the sugar level. By the third week of the experiment, the control group which were fed with basal meal alone had an increase in sugar level of over 3mg/dl.

The mechanism by which streptozotocin (STZ) brings about its diabetic state in animals includes selective destruction of pancreatic beta cells thereby causing the cells to be less active, leading to poor sensitivity of insulin for glucose uptake by tissues according to Elisa et al., (2009)[9]. Diabetes induced by Streptozotocin (STZ) injection provided symptoms like excessive urine production and weight loss, etc. The pancreatic sections stained with Haematoxylin and Eosin in this research showed that streptozotocin caused severe destruction of the pancreas. The diabetogenicity of alloxan is underlined by its selective cellular uptake by beta cells of the pancreas and consequent accumulation in these cells. The chemical properties of alloxan and how they contribute to its toxicity or diabetogenicity is the specific inhibition of a pancreatic glucose sensor enzyme, glucokinase by the compound which is connected with the redox cycling ability of alloxan that results in formation of reactive oxygen species (ROS) generation and this has been traced to both effects of the chemical properties of alloxan as well as its structure.

It was observed that the weight of the rats in the first group decreased from 120.40 ± 2.63 g to 110.23 g ± 1.56g at the third week (**Table 2**). The second group however showed an increase in body weight of 134 ± 2.18 g to 139.23 ± 2.60 g from week one through week three. In the third group, a drastic decrease in weight was observed i.e. from 130.50 ± 5.02 g to 107.82 ± 1.05 g which may be as a result of loss in appetite and poor food intake. The results obtained on the weight analysis of diabetic rats using these compounds as well as the control group is similar to the results obtained by Cani et al., (2017) when they feed diabetic mice with bifidobacteria[2].

The bacteria isolated from the rats are majorly the group of the Enterobacteriacae. According to Prescott et al., (2014), these bacteria should always be present under normal condition in an apparently healthy rats[10]. The distribution of bacteria in gastrointestinal tract of the rats in the small intestine is an indication that the only four bacteria identified in the control group which are *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* are enterobacteriacae. However, in the alloxan group, the bacteria isolated that included all in the control with the exception of S. pyogenes with Clostridium difficile and *Staphylococcus aureus* which was found in this group may be an indication that these bacteria could be contaminants from external sources. Clostridium difficile that was isolated from both experimental group but was not found in the control may be from the compounds. Also, S. pyogenes was only isolated from the control group but not found in the two experimental groups may have been wiped off by these compounds.

According to Omoya et al., (2018), pancreatic cells with infiltrations and diffused globular masses in any animal will definitely make it difficult for such animal to utilize any form of sugar for metabolic activities[10]. Although, there was no necrosis nor haemorrhage in any of the pancreas slide. Alfarag, (2009) stated that the washing away of vacuoles in pancreatic sections shows that the significant content of the pancreas is gone and such pancreas will not be optimal in its metabolic role[10]. The group induced with diabetes using alloxan that showed features such as profuse haemorrhage and complete destruction of the acini with prominent destruction of beta cells and washing away of the ductile beta cells are all negative pathological features that have shown the extent of damage that compound can cause. In a related experiment, Baskaran et al.,(1990) concluded that these are major pathological features of an established diabetes[1]. The group induced with streptozotocin that resulted in a very poor formation of the islet of Langerhans and necrotized cells that was profuse as well as haemorrhage of vascularized cells are indications of the extent of destructions caused on the organ. The fact that there was no visible interlobular duct in this group of rats' pancreas means that all formed acini have been destroyed [10,11]. The interpretations for the histology of the pancreas of the control group (that showed pancreatic lobular cells that are prominent and well-formed and are located with visible connective septae) are indications of positive pathology. According to Graham et al.,(2011), these are signs of active and healthy pancreas in a system where there is no signs and symptoms of diabetes[6]. Also, the presence of cell infiltrations and very few inflammatory cell infiltrations packed around the islet of Langerhans according to Doha et al., (2017) shows actively reproducing cells of the pancreas[4]. Sides' globular pancreatic cells that are well formed with slight anterior washing away of globular cells leading to intercellular drainage of exocrine secretions that were pronounced with intercellular deposit of central crystals are all indications of actively dividing acini [11].

CONCLUSION AND RECOMMENDATIONS

The results gathered from this research showed that the two compounds (alloxan and streptozotocin) are able to induce type 1 diabetes in the rats. However, the effect of alloxan in induction of diabetes was more devastating on the pancreas beta cells as well as on the blood sugar level of the rats than streptozotocin. It also had the highest reducing effect on the microflora of GIT of the rats used in the experiment thereby making streptozotocin safer microbiologically.

I therefore recommend that for experimental induction of diabetes that require urgent acute results using rats, alloxan should be used. However, if the experiment requires microbiological assays and use of bacteria or microorganisms, streptozotocin should be used.

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