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Research Article

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Histopathological Changes Associated with Surgically Created Open Skin Wound Healing and Pancreatic Structure of Alloxan-Induced Diabetic Rabbiits

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Abstract

The study evaluated the histopathological changes associated with the skin wound healing of alloxan-induced diabetic New Zealand White (NZW) rabbits along with the pancreatic cellular responses. Sixteen adult rabbits of either sex, weighing 1.8 - 3.2 kg were used for the study. They were divided into four groups. Group A was designated Non-Diabetic and No Wound and served as control. Group B was Diabetic and No Wound, whereas group C was Non diabetic and Wounded and group D was Wounded and Diabetic. Diabetes induction was accomplished by administration of 100 mg/kg of alloxan monohydrate twice, 72 hours apart in groups B and D only. A 3 cm^2 excisional skin wound was created at the dorsum of each of the rabbits in groups C and D. Skin tissue samples (wounded sites) and pancreas were harvested from twoeuthanized rabbits in each group on day 28 post induction of diabetes for histopathologic examinations. Diabetes was induced in groups B and D on day 3 post treatment. Histopathologic findings in the diabetic rabbits included hyperkeratosis, acanthosis, poor fibrogenesis of the injured skin site, as well as the necrosis and mononuclear cellular infiltration of the islet of Langerhans of the pancreas. In conclusion, alloxan monohydrate administration created a suitable diabetic model rabbit. Diabetes mellitus caused delayed skin wound healing evidenced by hypercellularity and fibrous tissue proliferation, pancreatic necrosis and cellular infiltration of the islets of Langerhans in the diabetic rabbit.

Keywords: Diabetic Wounds, Injured Skin, Pancreas, Rabbits

1. Introduction

Diabetes is a debilitating disease of global concern affecting man and animals [1-3]. It has remained one of the leading causes of death, illness and economic losses in the world [4]. Diabetes is a metabolic and heterogeneous disorder characterized primarily by hyperglycaemia due to insulin deficiency or insensitivity of insulin receptors for normal processes of glucose metabolism in the body [5,6]. It is also a serious endocrine syndrome with poor metabolic control, which is responsible for increased risk of cardiovascular diseases, including atherosclerosis, renal failure, and diabetic cataract [7,8]. Diabetes is characterized by hyperglycaemia, glycosuria, hyperlipidaemia, polyuria, and polydipsia, which causes aberrant expression of various physiologic cells relevant for wound healing to poor response of these wounds to treatment [6, 9-10].

Delayed wound healing has remained one of the major complications of diabetes [11]. This is because of the disruption in the mechanisms of normal progressive phases of wound healing that includes haemostasis, inflammation, proliferation, and remodeling [12,13]. Moreover, wound healing is a coordinated multicellular biologic repair process to restore tissue continuity after disruptive injury. It involves several cell types- including keratinocytes, fibroblasts endothelial cells, macrophages and

platelets-aimed at restoring body tissueesto approximate normal architecture [14,15]. It is essential for preventingpathogenic invasionof damaged tissues and to partially or completely reform the affected tissues [16].

Impairment of wound healing develops due to deleterious factors associated with diabetes. These include slowed metabolic rate, macrovascular, and microvascular dysregulation at the edges of the wounds. Additionally, keratinocyte, fibroblast, and immune cell migration into the wound, and reduced endothelial cell angiogenesis, as well as decreased efferocytosis and peripheral neuropathy occur [17-19]. Furthermore, diabetes decreases biosynthesis of collagen and glucosaminoglycans (GAGs) which in turn result in significant delay in formation of granulation tissue. Moreover, a number of growth factors essential for wound healing including transforming growth factor beta (TGF-β), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) have been found to be reduced in diabetic wound [20]. Consequently, wounds of diabetic patients may defy therapeutic management, degenerate to chronic wounds, and end up in fatality [5].

The geometric prevalence of diabetes is alarming and has an enormous time, energy, and economic implication for research and management of the ailment [10,21]. Nigeria is one of the countries in Africa with the highest number of people living with diabetes (Chinenye and Young, 2011) [22]. Diabetes in Nigeria is estimated at a prevalence of 5.77% [23]. Although Mattin et al. reported a prevalence of 0.34% of diabetes mellitus among dogs attending first opinion practice in the UK Gani and Ihedioha (2016) reported a prevalence of 0.22 % of diabetic mellitus cases among dogs presented for veterinary care in Warri, Delta State, Nigeria [24,25]. These prevalence rates confirm the universality of the diabetes among dogs that require continuous elucidation, especially as it affects wound healing. Besides, the study of pathologic effects of diabetes in tissue repair will be essential in developing valid strategies for management of the ailments [6]. Therefore, the present work evaluated histopathological changes in surgically created skin wound and pancreas of alloxan-induced diabetea in rabbits.

2. Materials and Methods

This study was carried out in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Sixteen adult New Zealand White (NZW) rabbits of either sex, aged 11.6 - 12.4 months and weighing 1.8 - 3.2 kg were used for this study. They were procured from the National Animal Production and Research Institute (NAPRI), Zaria, Kaduna State, Nigeria.

The rabbits were kept in intensive management system in separate cages and fed morning and evening daily with adjusted rabbit-based diet combined (Grand Cereals Limited, Jos, Plateau State) and miller's bran that were mixed in equal proportion in order to regulate the blood glucose level of the hyperglycaemic groups. Daucus carota subsp. sativus leaves was given to the rabbits twice weekly to avoid gastro-intestinal impaction and constipation while water was given ad-libitum throughout the experimental period.

The rabbits were conditioned to the laboratory for two weeks before the commencement of the experiment. During this period, they were administered anthelmintic Ivermectin (Hebei, Kexing pharmaceutical, China) prophylaxis at 0.4mg/kg start, and repeated after two weeks to bolster up protection against internal and external parasites. The rabbits were clinically evaluated using body condition scores, vital and haematological parameters and adjudged apparently healthy for the study before commencement.

2.1 Experimental Protocols

The rabbits were divided into 4 groups (A, B, C, D) of 4 rabbits each; comprising of two males and two females per group. Group A served as control 'No Diabetes induction, No Wound created' (NDNW). Group B had Diabetes induced, but No Wound created (DNW); Group C had No Diabetes induced but Wound created (NDW) and Group D had Diabetes induced and Wound created (DW), respectively.

2.2 Induction and Confirmation of Diabetes in Rabbits

The rabbits were anaesthetized using combination of xylazine hydrochloride (Bioveta a.s., Czech Republic) at 7 mg/kg intramuscular and ketamine hydrochloride (Laborate pharmaceutical, India) at 50 mg/kg intramuscularly. The pinnae were shaved and dabbed with xylene to dilate the marginal ear vein and Alloxan monohydrate at 200 mg/kg (SIGMA-aldrich, UK) was administered intravenously through the marginal ear vein. Blood samples were collected and tested using a glucometer (ACCU-CHEK (R), Roche, Mannheim, Germany) one day prior to alloxan administration (day 0) and subsequently, days 3, 7, 14, 21 and 28 post alloxan administration, for hyperglycaemia and establishment of diabetes in NZW rabbits.

2.3 Skin Wound Creation on the Dorsum of the Rabbits

The dorsum of rabbits in group C (NDW) and group D (DW) were shaved and prepared for aseptical surgery The rabbits were anaesthetized using combination of xylazine hydrochloride (Bioveta a.s., Czech Republic) at 7 mg/kg intramuscular and ketamine hydrochloride (Laborate pharmaceutical, India) at 50 mg/kg intramuscularly [26,27]. A 3 cm2 full-thickness skin incision created on the dorsal portion of the thoraco-lumber region of each of the rabbits, caudal to the cervical region, about 5cm cranial to the sacral region and equidistance from both left and right flanks of the rabbits. The wounds were created using a template designed from x-ray film [28].

The wounds were dressed with sterile gauze and re-dressed only on the days of diabetes/blood glucose evaluations (day 3, 7, 14, 21 and 28 days post alloxan administration.

2.4 Sample Collection and Histopathological Evaluation

Two rabbits from each group were sacrificed on day 28 post alloxan administration The skin wound samples from the back of the uninjured (NDNW and DNW) groups (A, C) and injured (WND and WD) groups (B, D). Pancreas from all the groups were collected and fixed in 10% neutral buffered formalin for histopathologic evaluation according to standard procedures by Drury et al. which involved tissue paraffin embedding, sectioning at 5 µm, and staining with hematoxylin and eosin (H&E) [29].

The stained skin samples were examined by microscopy at various magnifications for evidences of granulation tissue formation, vascularization, epithelialization and fibrous tissue deposition while the pancreas was examined for necrosis and mononuclear infiltration [30]. Subsequently, the other rabbits were nursed back to their normal health using insulin to reverse the diabetes.

2.5 Ethical Approval

Ethical clearance was obtained for this study from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), (Reference number: ABUCAUC/2019/028).

3. Results

3.1 Induction and Confirmation of Diabetes

Diabetes was confirmed by rise in blood glucose level to constant level of 250 - 350 mg/dl between day 3 and 28 of alloxan induction Wang et al. in groups B and D only. The value of blood glucose level remain relatively normoglycaemic in the non-diabetic groups A (100.4 -129.6) and C (103.1- 129.6) throughout the period of the study (Table 1). However, there was exponential rise in blood glucose level of the rabbits in the diabetic group B from induction day 0 (127.8) to post induction day 3 (329.4) and the value remained constantly high throughout the period of the experiment (273.6 - 329.4) as shown in Table 1 [4]. Also, there was exponential rise in blood glucose level of the rabbits in the diabetic and wounded group D from induction day 0 (113.8) to post induction day 3 (344.7) and the value remained constantly higher throughout the period of the experiment (295.2– 358.8) (Table 1). These hyperglycaemic values of rabbits in groups B and D were reversed to normoglycaemic values (less than (150g/dl) within 3 days on administration of insulin at the end of the study.

	Blood Glucose Concentration in g/dl					
Groups	Days					
	0	3	7	14	21	28
A (NDNW);	129.6	126.9	100.4	119.3	109.8	120.6
B (DNW);	127.8	329.4	261.9	302	288	273.6
C (WND).	116.5	119.8	122.9	103.1	106.9	129.6
D (WD).	113.8	344.7	349.7	342	358.8	295.2

Table 1: Blood Glucose Concentration in Rabbits of Experimental Groups A, B, C and D

Key: NDNW = Non-Diabetic and Non-Wounded (NDNW); DNW = Diabetic and Non- Wounded (DNW); WND =Wounded and Non-Diabetic (WND) and WD = Wounded and Diabetic (WD).

3.2 Histopathology of Skin after Wound Creation

The skin of the non-wounded and non-diabetic (NDNW); control rabbits showed the presence of apparently normal epidermis and dermis with hair follicles (Plate 1). The section of the skin of the diabetic and non-wounded (DNW) rabbit revealed the presence of hyperkeratosis and acanthosis with elongated rete pegs (Plate 2). The wound sample (skin) of the wounded groups (WND and WD) of rabbits were characterised by apparently normal epidermis and dermis with hair follicles as well as hypercellularity of the dermis, devoid of hair follicles owing to proliferation of fibroblasts in these groups. However, while there was high density of proliferation of fibroblast and fibrous tissues in the dermis of the group C rabbits (WND) (Plate 3), the dermis of the group D rabbits (WD) was characterised by low density proliferation of fibroblast and fibrous tissues (Plate 4).

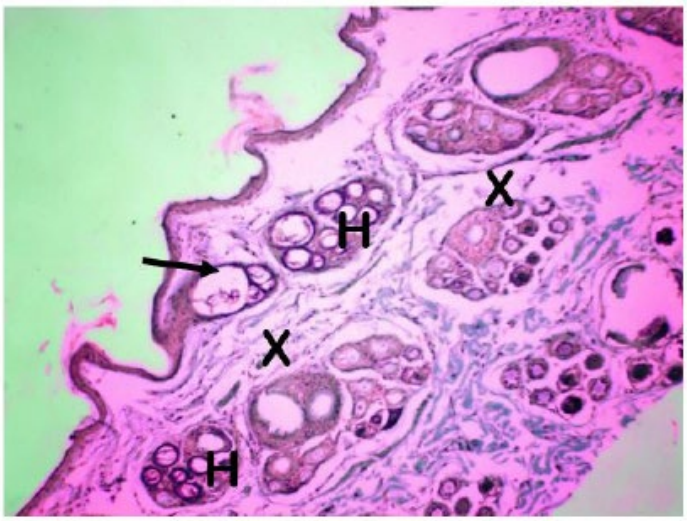

Plate 1: Photomicrograph of the skin of a Non- Diabetic and Non- Wounded (NDNW) Control rabbit. Note the epidermal surface (arrow), dermis (X), and hair follicles (H). H & E x 100

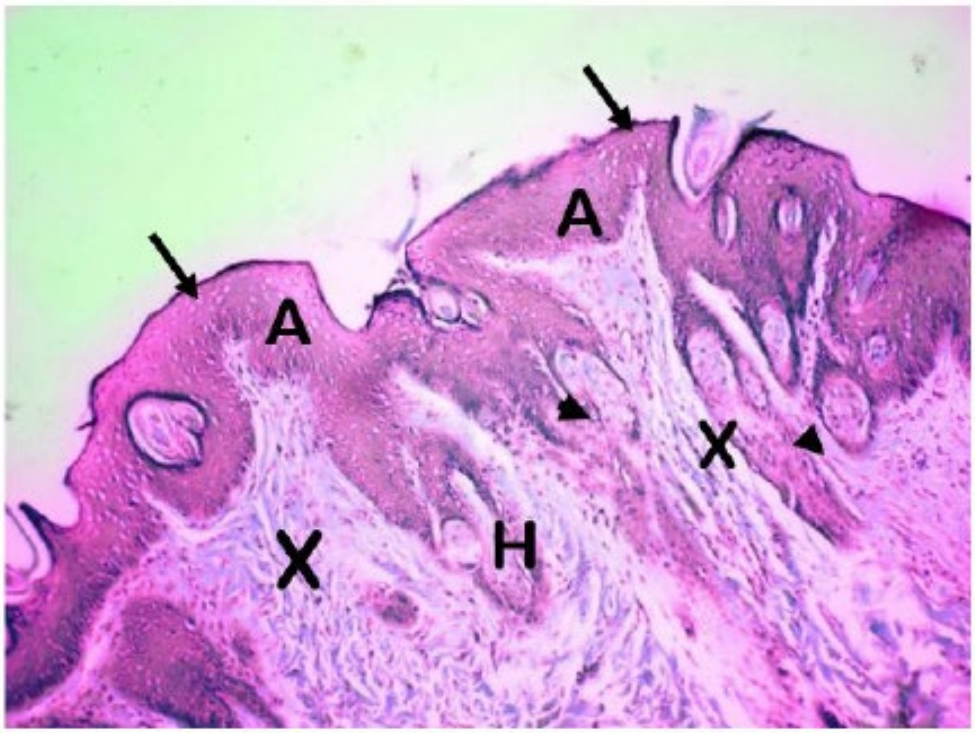

Plate 2: Photomicrograph of the skin of an Alloxan-Induced Diabetic and Non-Wounded (DNW) rabbit on Day 28 Post-Induction. Note the epidermal surface (arrows), acanthosis with elongated rete pegs (arrowheads), dermis (X), and hair follicle (H). H & E x 100

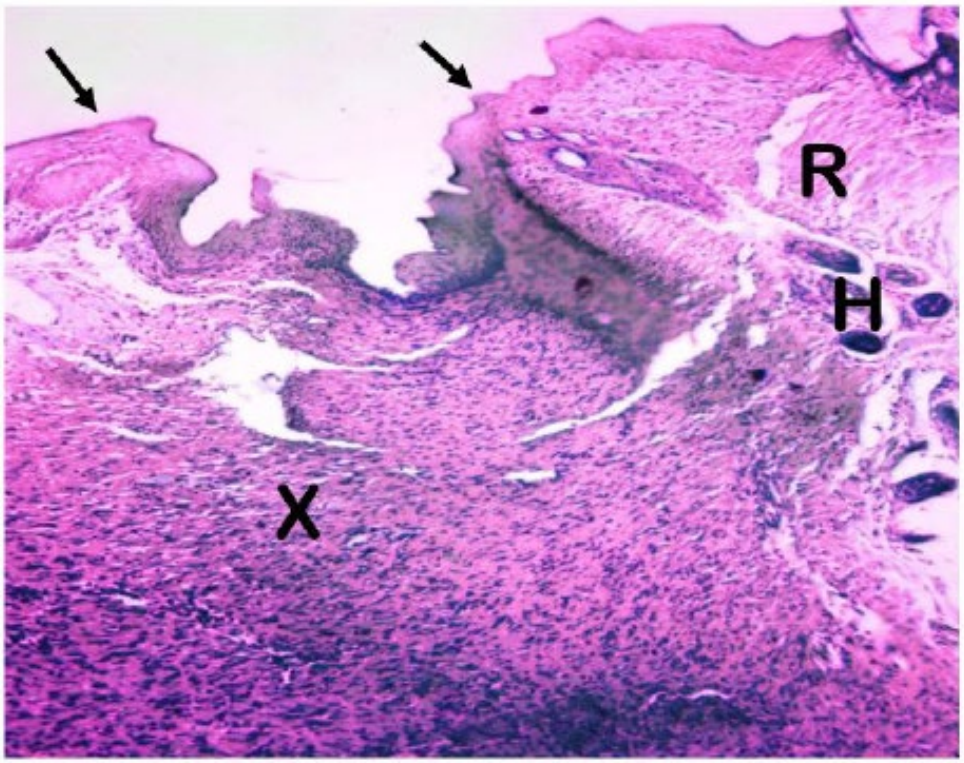

Plate 3: Photomicrograph of the skin of a Wounded and Non-Diabetic (WND) rabbit on day 28 following Wound Creation. Note the epidermal surface (arrow), dermis of the normal part of the skin (R) containing a hair follicle (H), and another part of the skin with proliferated fibrous connective tissues devoid of hair follicles (X). H & E x 100

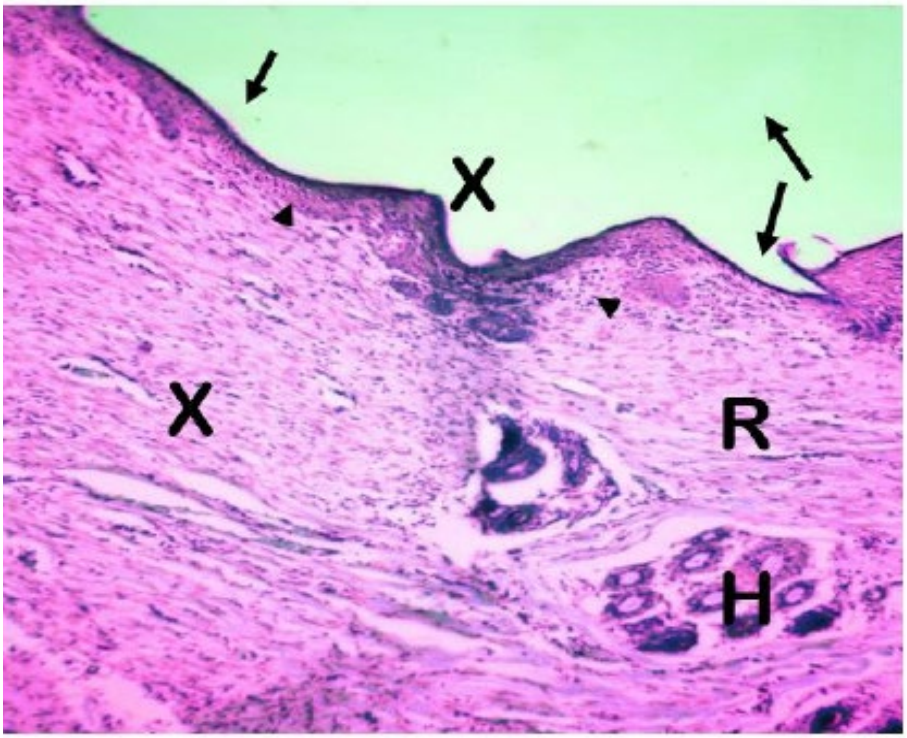

Plate 4: Photomicrograph of the skin of a Wounded and Diabetic (WD) rabbit on Day 28 following Wound Creation. Note the epidermal surface (arrows), dermis of the normal part of the skin (R) containing hair follicles (H) with mononuclear cellular infiltration (arrowheads), and the fibrous part of the skin devoid of hair follicles (X). H & E x 100

3.3 Histopathological Changes in Pancreas

The pancreas of non-wounded and non-diabetic (NDNW) rabbit (control) showed the apparently normal islet of Langerhans and the acinar cells (Plate 5) whereas necrosis with mononuclear cellular infiltration of the acinar cells characterized the section of pancreas of diabetic and non-wounded (DNW) rabbit (Plate 6). The pancreas of wounded and non-diabetic (WND) rabbit showed the apparently normal islet of Langerhans and the acinar cells (Plate 7). However, the section of pancreas of the wounded and diabetic (WD) rabbits was characterised by mononuclear cellular infiltration of necrotic islet of Langerhans, in addition to mononuclear cellular infiltration of the acinar cells (Plate 8)

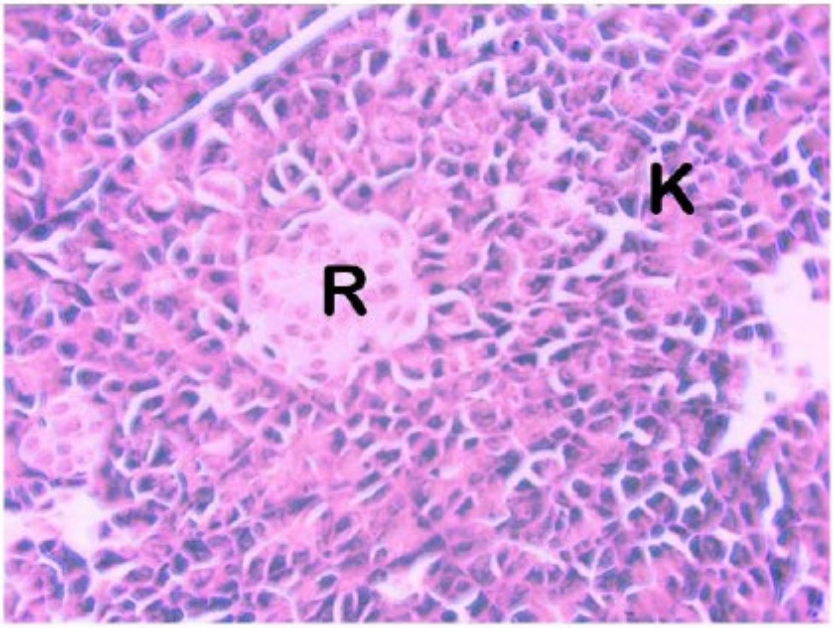

Plate 5: Photomicrograph of the pancreas of a Non-Diabetic and Non-Wounded (NDNW) control rabbit on Day 28. Note the islets of Langerhan (R) and the acinar cells (K). H & E x 400

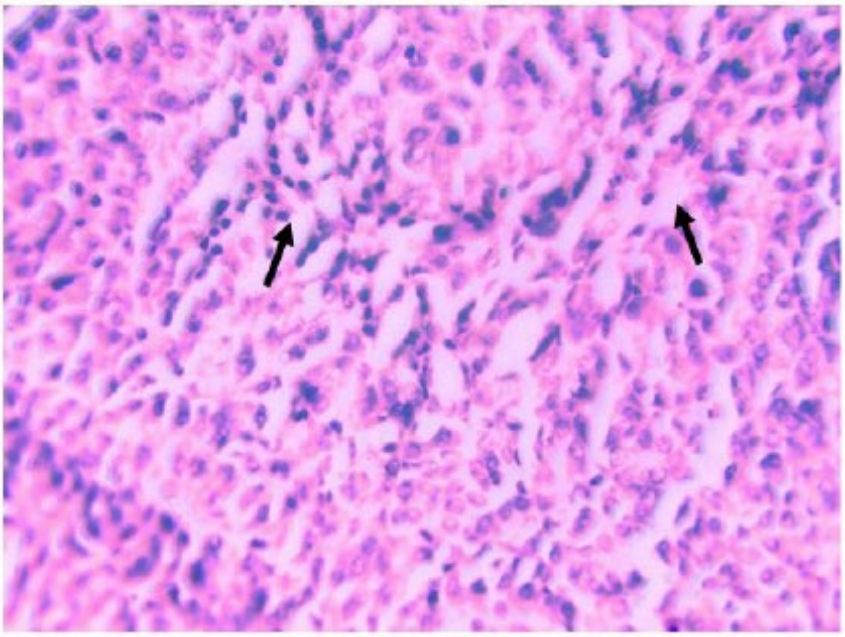

Plate 6: Photomicrograph of the pancreas of an alloxan-induced Diabetic and Non-Wounded (DNW) rabbit on Day 28 Post-Induction. Note the mononuclear cellular infiltration (arrow). H & E x 400

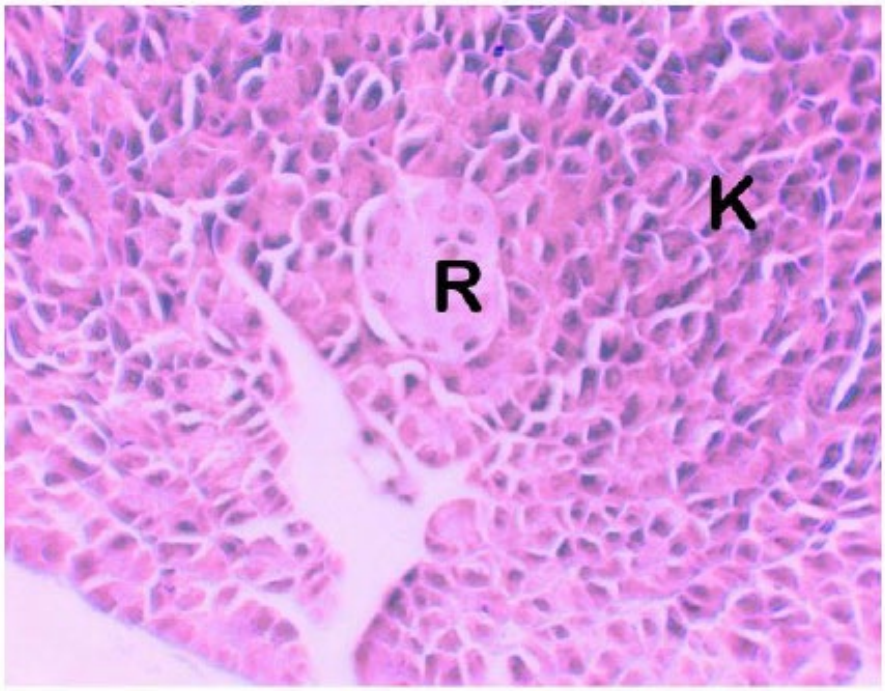

Plate 7: Photomicrograph of the pancreas of a Wounded and Non-Diabetic (WND) rabbit on Day 28 following wound creation. Note the islets of Langerhans (R) and the acinar cells (K). H & E x 400

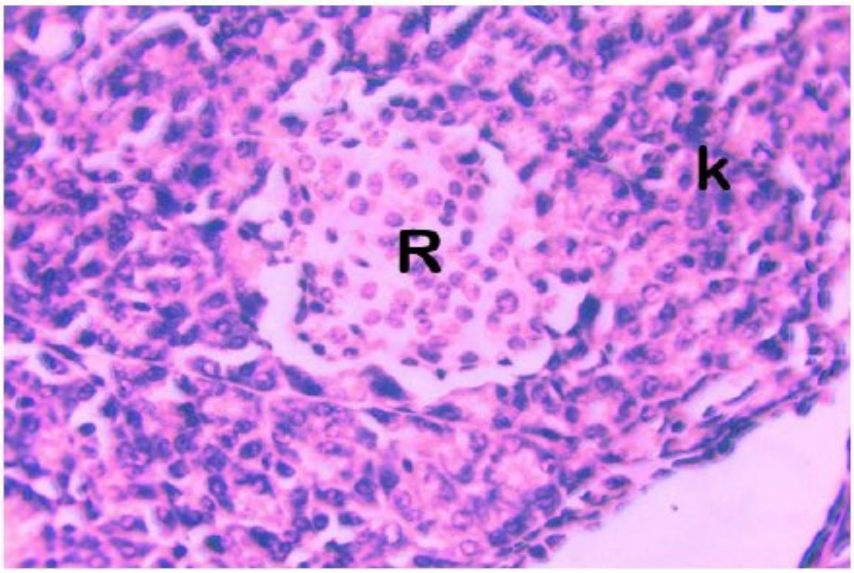

Plate 8: Photomicrograph of the pancreas of a Wounded and Diabetic (WD) rabbit on Day 28. Note the islet of Langerhan (R) with mononuclear cellular infiltration and the acinar cells (K). H & E x 400

4. Discussion

The value of blood glucose level in all the experimental groups remain within normoglycaemic range pre- alloxan induction. These values remain within this range in group A and C where alloxan was not used throughout the study. However there was exponential rise in the blood glucose level of the alloxan groups B and D between the induction day (0) to day 3 post-induction and these values remain hypergrlycaemic throughout the period of the studies and this is indication of alloxan as a reliable diabetogenic drug.

The skin wound of wounded non diabetic group was characterised by mild exudation that disappears after day 3 post-wound creation mild epithelial tissues almost taking over by regenerating skin on day 14 and 21 and moderate rate of contraction on day 7 post-wounding while that of the wounded diabetic group was characterised by prolonged exudation for seven days, moderate epithelial tissues from days 14 to 21 and mild contraction on day 7 post wound creation. Diabetes caused acanthosis with hyperkeratosis observed in the diabetic and non-wounded (DNW) group in the present work. About 30% of patients with diabetes mellitus presents disease-related dermatological problems during the disease, including skin basement membrane thickening and reduction in capillary sizes [31-33]. The hyperkeratosis of the epidermis of cutaneous tissues observed is thought to emanate from an increase in the microcirculatory hydrostatic pressure associated with arteriolar hyalinosis, which have been associated with arteriolar hyalinosis in diabetic patients or as a result of excessive accumulation of abnormal collagen fibres resistant to degradation by collagenase [33,34]. Similarly, it has also been reported that excessive accumulation of abnormal collagen fibres in the skin of a diabetic patient [34]. The hyperkeratosis of the epidermal layers of the cutaneous tissues could also be due to overproduction of collagen and fibroblast synthesis secondary to insulin acting as a growth factor and decreased local oxygen pressure caused by microangiopathy as previously reported [33-35].

The large network/quantity of fibrous tissue deposition observed in the WND group compared to the fewer depositions observed in the WD group might be due to microangiopathy of the cutaneous vessels of the diabetic group caused by diabetes mellitus. The observation is because reduced vasodilation capacity and elasticity of capillaries, delayed rate of exchange of nutrients and migration of necessary cells and growth factors to the wounded sites characterizes microangiopathy of the cutaneous vessels [36]. The compromise in the blood flow predisposed the wounds of these rabbits to delayed marginalization, epiplody, diapedesis, poor granulation tissue formation and its conversion to fibrous tissues, less collagen deposition, aberrant collagen maturation and subsequently ineffective wound maturation and healing as earlier reported by Kraft, in large diabetic excisional wounds that heal by secondary intention [34]. The presence of inflammatory cells in the diabetic wounds on day 28th suggested that diabetes trapped the wounds in a constant inflammatory state, which contributed to the failure of wounds to progress through the normal process of wound healing. Poor circulation culminating in decreased expression of growth factors also impedes the rebuilding process hence lead to prolonged wound healing process in diabetes.

Moreover, growth factors have many activities that make them attractive agents for stimulating tissue repair. Growth factors such as transforming growth factor beta 1, insulin-like growth factor, and vascular endothelial growth factor play critical roles in regulating complex diabetic wound healing. Growth factors also attract cells into the wound, stimulate their proliferation, and have profound influence on extracellular matrix deposition.

The pancreas of the healthy rabbits (control) and the wounded and non-diabetic (WND) rabbit groups on day 28 post-wound creation showed the normal islet of langerhans and the acinar cells, while

the section of pancreas of diabetic and non-wounded (DNW) rabbit group was characterized by mononuclear cell infiltration of the acinar cells. Also, the section of pancreas of wounded and diabetic (WD) rabbits group was characterised by mononuclear cellular infiltration of necrotic islet of langerhans, in adddition to mononuclear cellular infiltration of the acinar cells.

These abberent cellular infiltration in both the islet of Langerhans and acinar cells of the diabetic rabbits are associated with inflammatory reactions that are detrimental to the functional attribute of pancreas; Inflammation of pancreas in diabetic patients is characterized by infiltration of leukocyte and other unchecked inflammatory factors such as B-cells, T-cells, macrophages and Natural Killer (NK) cells in the pancreas, which contribute to β-cell death that characterizes pancreatitis [37]. The autor- reactive effector T-cells abundant in diabetes disease also facilitate the expression of Fas, lytic granules and cytokines such as INF-γ IL-1β, TNFα, INF-γ, IL-6 and IL-8 which are detrimental for β-cell survival [38]. This inflammation and subsequent death of β-cells promote infiltration of immune cells, suppression of β-cell function, reduced insulin exocytosis and increased β-cell apoptosis [39].

The pathologic finding in the pancreatic section was mild when compared with the report of other researchers who noted severe necrotic changes of pancreatic islets, vacuolation, increased eosinophilia, islet congestion and pancreatic congestion in alloxan-induced diabetic rabbits [40- 41]. Although the cause of the variations in these findings is unclear, it might be related to the time when the recording of hyperglycemia started and the dose of administration. In the present study the assessment of diabetes commenced when hyperglcaemia became stable; while other researchers did not consider such a factor [41,42]. Besides, the administration of the alloxan was twice at a dosage of 100 mg/kg IV, 72 hours apart unlike for Sajad who administered the alloxan intraperitoneally at 80 mg/kg every fourth week for five months [41]. The shorter duration of diabetes in this study might also be a contributing factor to the mild pathologic changes in pancreatic structure. Unlike in the present study that lasted 28 days, other researchers reported chronic pancreatitis, haemorrhage, fibroblasts proliferation in some pancreatic lobules, and disorganization of pancreatic acinar in the pancreas of two to five month induced-diabetic rabbits [41-43]. However, Wang et al. reported only marked changes in β-cells and thickened vessel walls in the pancreas of a 12-month alloxan-induced diabetic rabbit.

In conclusion, alloxan monohydrate administration created a suitable diabetic model rabbit for this study. Diabetes mellitus caused delayed wound healing evidenced by hypercellularity and fibrous tissue proliferation in the skin of the diabetic rabbit. Diabetes caused degenerative changes including pancreatic necrosis and cellular infiltration of the islets of Langerhans in the diabetic rabbit.

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Disclosure of Interest

The authors declare that they have no competing interest.

Authors' contributions

JA Onah conceived the idea for manuscript development and wrote the initial draft of the manuscript, ST Fadason, EO Abidoye and KB Kadima appraised the manuscript, SE Abalaka prepared the slides, KB Kadima, JA Onah and SE Abalaka read and interpreted the slides, JA Onah, KB Kadima and ST Fadason did the proof reading and final corrections of the manuscript. All authors approved the final draft.

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