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Histological Effect of Combined Ethanol Extract of *Moringa oleifera* and *Pleurotus ostreatus* on the Pancreas of Alloxan-induced Diabetic Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NAT and CCM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NAT, CCM and LCC managed the literature searches and analyses of the study performed the spectroscopy analysis. Author NAT managed the experimental process. Authors NAT and CCM identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To ascertain the effect of the combined extracts of *Moringa oleifera* and *Pleurotus ostreatus* on the pancreas of diabetic rats through histological studies of the organ.
Study Design: Animal experimental study.
Place of Study: Department of Biochemistry, Faculty of Science University of Port Harcourt P.M.B 5323 Port Harcourt Nigeria.
Methods: Alloxan at concentration of 120 mg/kg body weight was used to induce diabetes mellitus. During the experiment, sixty (60) wistar albino rats with weight range of 160-250 g were used and were grouped into 7 groups. The combined extract was administered to the diabetic wistar albino rats at a different combination percentage of 60% (1,800 mg/kg) *Moringa oleifera* and 40% (600 mg/kg) *Pleurotus ostreatus* and 40% (1,200 mg/kg) *Moringa oleifera* and 60% (900 mg/kg) *Pleurotus ostreatus* respectively. The rest of the diabetic rats were treated with 100% (3,000 mg/kg)

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Moringa oleifera, 100% (1,500 mg/kg) *Pleurotus ostreatus* while 7.1 mg/kg of metformin was used as the standard drug. Histological analysis was carried out on the pancreas sections of the experimental animals used in this study to observe the changes in the organ. This was done immediately after the rats were sacrificed. These organs were cut off and placed in a sample holder containing 10% formal saline. The 10% formal saline kills any bacteria present and ensures that the tissue does not rot or damage. The slides were prepared and stained with haematoxylin and eosin stains. After staining, the sections were prepared for microscopic examination. The slides were then viewed under a microscope.

Results: After 6 weeks of treatment, it was observed that there was obvious regeneration of pancreatic islet with large number of cells and normal acini.

Conclusion: This study concluded that the combined ethanol extracts of *Pleurotus ostreatus* and *Moringa oleifera* produced a significant pancreatic regeneration in diabetic rats.

Keywords: Acini; alloxan; Moringa oleifera; Pleurotus ostreatus.

1. INTRODUCTION

Diabetes mellitus is a chronic disease characterized by disorder in carbohydrate, fats and protein metabolism because of an absolute or relatively deficiency in the action of the insulin hormone produced by the beta cells of the islets of Langerhans of the pancreas [1,2]. The pancreas is an endocrine gland producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide which circulates in blood. The pancreas is also a digestive organ, secreting pancreatic juice containing digestive enzymes that assist digestion and absorption of nutrients in the small intestine. These enzymes help to further break down the carbohydrates, proteins, and lipids in the chyme. Medicinal plants such as *Moringa oleifera* and *Pleurotus ostreatus* which are consumable by man is believed to contribute significantly to the improvement of human health, in terms of prevention and cure of disease because plants have long served as useful and rational source of therapeutic agents e.g. antidiabetic, anti-rheumatic, anti-inflammatory and anti-hypertensive drugs, etc. [3]. Moringa leaves are a potent source of polyphenols, including quercetin-3- glycoside, rutin, kaempferol glycosides, and other polyphenols [4] while mushrooms have higher protein and minerals contents and contain less fat though are rich in B vitamins, vitamin D, vitamin K and sometimes vitamins A and C [5-9]. Since the pancreas houses the β-cells of the islet of Langerhans which produce and secrete the hormone insulin, performs some other vital functions of metabolism (digestive role) in the body, and also knowing fully well that this organ can be possibly be diseased in diabetic condition, the organ (pancreas) is therefore subjected to histological analysis in this present study.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample and Preparation of Leaves Extracts

The leaves of *Moringa oleifera* and *Pleurotus ostreatus* (Mushroom) plant used for extractions were obtained from Dilomat Farms, Rivers State University of Science and Technology, Rivers State [10]. The leaves of *Moringa oleifera* and the *Pleurotus ostreatus* plant were cleaned and shade dried at room temperature, after which they were pounded to yield a powder. Powdered Moringa leaves weighing 1,000 g and powdered mushroom weighing 1,500 g was soaked in 3,000 ml and 4,500 ml of 95% ethanol respectively for 12-48 hours, after which they were sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1. The filtrates were concentrated using a rotary evaporator at 45℃ and 50℃ respectively. The weight of the concentrates were taken and the percentage yield calculated and kept at 4℃ until usage. The extracts were diluted with distilled water at different concentrations for oral administration.

2.2 Reagents

The reagents were sourced commercially and they include: Metformin (Merck Serono Ltd. U.K), chloroform (BDH chemicals Ltd.), alloxan monohydrate (Qualkems Lab. Reagents), formaldehyde, haematoxylin and eosin, ethanol 95% (SIGMA chemicals).

2.3 Experimental Animals

A total of sixty (60) albino rats of both sexes weighing 160-250 g were used and were marked for easy identification. They were housed in the

Pharmacology Department animal house at Ofrima, Abuja park of the University of Port Harcourt, Choba, Rivers State. They were left for 1 week to acclimatize to laboratory conditions during which they had free access to normal feed (Top feeds- grower's mash) and clean water. In the experiment, a total of fifty-seven (57) rats were used, the other three (3) were used for pilot studies to ascertain that the rats could be made diabetic by alloxan treatment at the dose level of 120 mg/kg body weight [11]. The rats were grouped in the following pattern below. Mode of administration of *Moringa oleifera* and *Pleurotus ostreatus* were adopted from Bakre et al. [12] and Imoh and Okon [13] respectively.

- **Group 1:** Non-diabetic animals and were given distilled water and normal feed throughout the course of this study.
- **Group 2:** Diabetic animals but were not treated with any drug or extract.
- **Group 3:** Diabetic animals treated with 7.1 mg/kg metformin
- **Group 4:** Diabetic animals treated with mushroom extract (1,500 mg/kg)
- **Group 5:** Diabetic animals treated with *Moringa oleifera* extract (3,000 mg/kg)
- **Group 6:** Diabetic animals treated with 60% *Moringa oleifera* extract (1,800 mg/kg) and 40% mushroom extract (600 mg/kg)
- **Group 7:** Diabetic animals treated with 40% *Moringa oleifera* extract (1,200 mg/kg) and 60% mushroom extract (900 mg/kg).

2.4 Administration of Alloxan

Alloxan weighing 1.0 g was dissolved in 20 ml of distilled water from which a single dose (120 mg/kg body weight) was slowly administered intraperitoneally to the rats within few minutes of its (alloxan) preparation. Diabetes was confirmed using a drop of blood from the tail artery on a blood glucometer 2-3 days following alloxan injection and was found to have increased by two to three (2-3) times the normal value.

2.5 Histological Analysis

Histological analysis was carried out on the pancreas sections of the experimental animals used in this study to observe the changes in the organ. This was done immediately after the animals were sacrificed. These organs were cut off and placed in a sample holder containing 10% formal saline. The 10% formal saline kills any bacteria present and ensures that the tissue does not rot or damage. Water was removed from the specimen using graded percentage of alcohol in ascending order from a lower concentration to the absolute. Thus, dehydration started with 50% alcohol for two hours, 70% alcohol for two hours, 95% alcohol for twelve hours (overnight) and then absolute (100%) alcohol for two hours. Agitation which is one of the factors for tissue processing was done using Junior Orbit shaker, the alcohol from the blocks or sections of tissue are cleared by immersing them in an ante-medium (Xylene). Other clearing agents include toluene, benzene and chloroform. Thin uniform sections for histological examination were produced using a rotary microtome. A grease-free slide was placed on a warm plate and was flooded with distilled water. A section was laid on the surface of the slide and every major grease was removed by stretching the surrounding wax carefully with mounted needles. As the water warms, the section flattened out. The slide was then removed from the hot plate, labeled and dried. The slides were stained using haematoxylin and eosin stains. They are the most used combination of stains for routine histology. After staining, the sections were prepared as a permanent preparation for microscopic examination. This was accomplished by mounting the section in a suitable medium under a glass cover slip using a mountant. The slides were then viewed under a microscope.

2.6 Ethical Approval

The protocol of this study was approved by the research ethical approval committee of Department of Biochemistry, University of Port Harcourt with authorization number: UPH/BCH/REC/015/028

3. RESULTS AND DISCUSSION

Histological analysis was carried out on the pancreas of the samples. Plates 1- 18 show the findings of the analysis observed in the various groups.

3.1 Architecture of the Pancreas of Normal Rat

From the photomicrograph shown on Plate 1, it was observed that the pancreas of the normal control group has a normal pancreatic islet and acini with numerous cells.

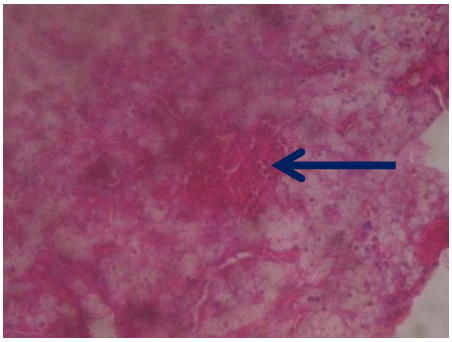

Plate 1. Pancreas photomicrograph of the normal control group showing normal pancreatic islet; magnification x400

3.2 Architecture of the Pancreas of Alloxan-induced Diabetic Rat

From the photomicrograph shown on Plate 2, it was observed that the pancreas of the diabetic control group lacks a normal pancreatic islet and acini rather it had atrophic pancreatic islet with fewer cells and poor pancreatic acini.

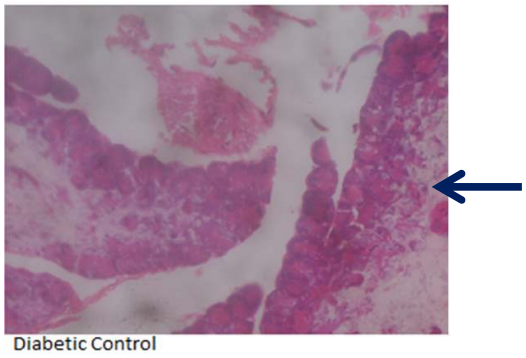

Plate 2. Pancreas photomicrograph of the diabetic control group showing atrophic pancreatic islet with few cells and poor pancreatic acini; magnification x400

3.3 Histological Effect of Metformin on the Pancreas of an Alloxan-induced Diabetic Wistar Albino Rats

From the photomicrograph shown on plate 3-5, it was observed that in week 1 the pancreas had atrophic pancreatic islet with fewer cells and poor pancreatic acini. As treatment progresses to week 3, a recovering effect was seen as the pancreatic acini became normal. In week 5, a better pancreas with normal pancreatic islet cells and acini was seen. The number of cells also increased.

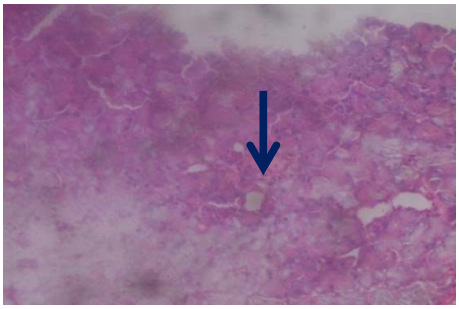

Plate 3. Group MF Week 1; Arrow= Atrophic pancreatic islet

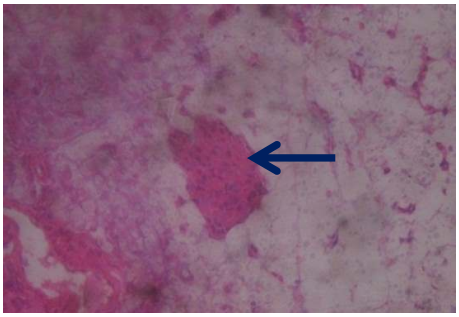

Plate 4. Group MF Week 3; Arrow= Recovering pancreatic islet cells with normal pancreatic acini

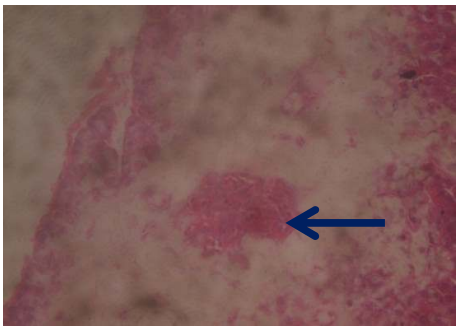

Plate 5. Group MF Week 5; Arrow= Normal pancreatic islet and acini

Plate 3- 5. Pancreas photomicrograph of the group treated with metformin; magnification x400

3.4 Histological Effect of *Pleurotus ostreatus* (Mushroom) Extract on the Pancreas of an Alloxan Induced Diabetic Wistar Albino Rats

From the photomicrograph shown on plate 6- 8; it was observed that in week 1, the pancreas had atrophic pancreatic islet with fewer cells and poor pancreatic acini. As treatment progresses to week 3, a bit atrophic and fewer pancreatic islet

cells were seen. In week 5, a pancreas with smaller holes with an increased pancreatic islet cells was seen.

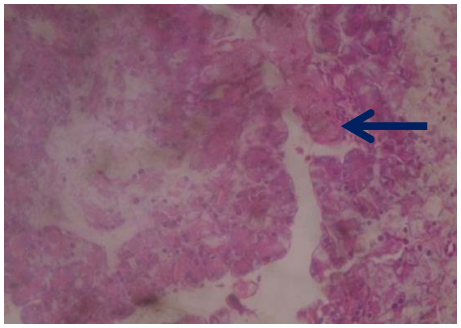

Plate 6. Group MS Week 1; Arrow= Atrophic pancreatic islet with few cells and poor acini

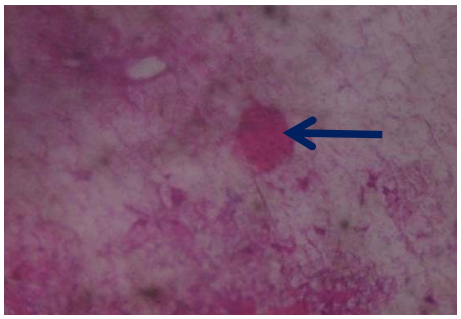

Plate 7. Group MS Week 3; Arrow= A bit atrophic and fewer pancreatic islet cells

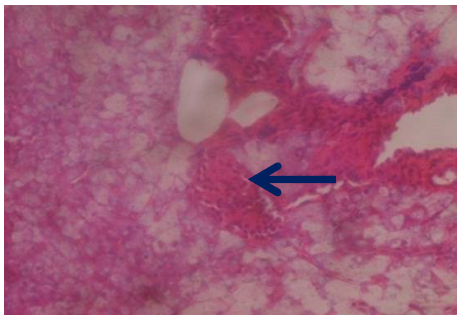

Plate 8. Group MS Week 5; Arrow= Holes getting smaller and increased pancreatic islet cells

Plate 6-8. Pancreas photomicrograph of the group treated with 100% mushroom; magnification x400

3.5 Histological Effect of *Moringa oleifera* on the Pancreas of an Alloxan Induced Diabetic Wistar Albino Rats

From the photomicrograph shown on plate 9-11, it was seen that in week 1 the pancreas had atrophic pancreatic islet with fewer cells and slightly normal pancreatic acini. As treatment progresses to week 3, there was a great recovering effect and also no hole was seen. In week 5, a normal pancreas with numerous pancreatic islet cells and acini was seen.

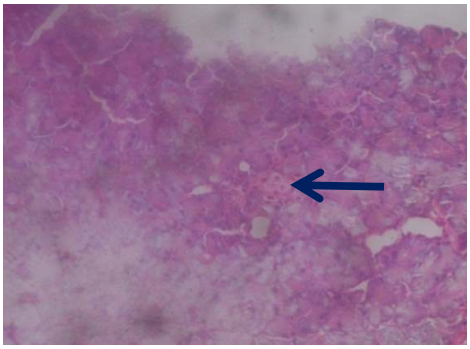

Plate 9. Group MO Week 1; Arrow= Atrophic pancreatic islet with a bit normal acini

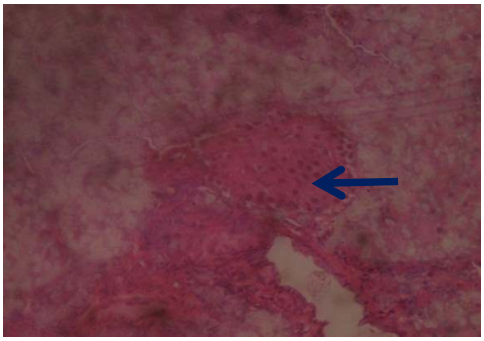

Plate 10. Group MO Week 3; Arrow= Pancreatic cells with holes seen (necrosis)

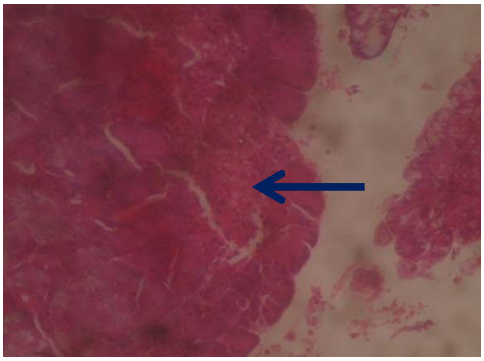

Plate 11. Group MO Week 5; Arrow= Normal pancreatic islet and acini

Plate 9- 11. Pancreas photomicrograph of the group treated with 100% moringa; magnification x400

3.6 Histological Effect of the Combined Extract of 60% *Moringa oleifera* and 40% *Pleurotus ostreatus* (mushroom) on the Pancreas of an Alloxan Induced Diabetic Wistar Albino Rats

From the photomicrograph shown on Plate 12-14, it was observed that in week 1 the pancreas had atrophic pancreatic islet cells with normal pancreatic acini. In week 3, large holes (caused by cell necrosis) were seen in pancreatic islet. In week 5, pancreatic islet was able to regenerate with numerous cells and normal pancreatic acini.

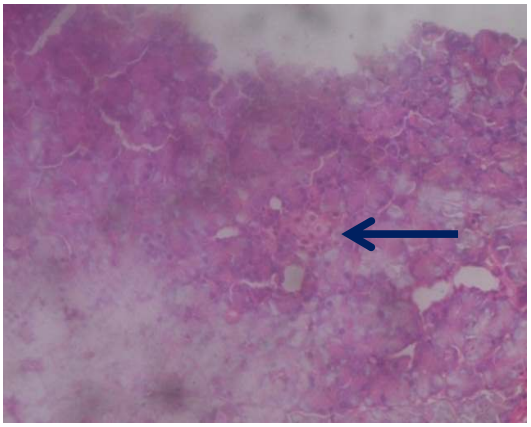

Plate 12. Group MOMS Week 1; Arrow= Atrophic pancreatic islet cells with large holes

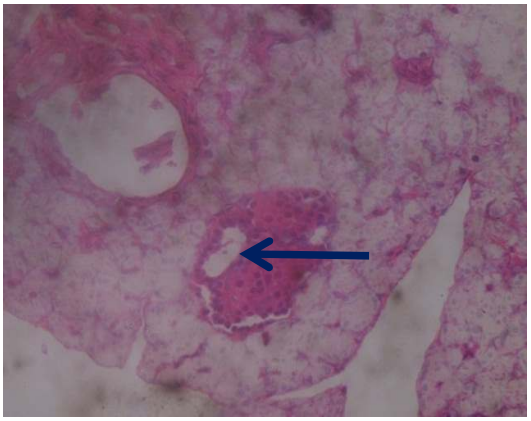

Plate 13. Group MOMS Week 3; Arrow= Large holes seen in pancreatic islet (cell necrosis)

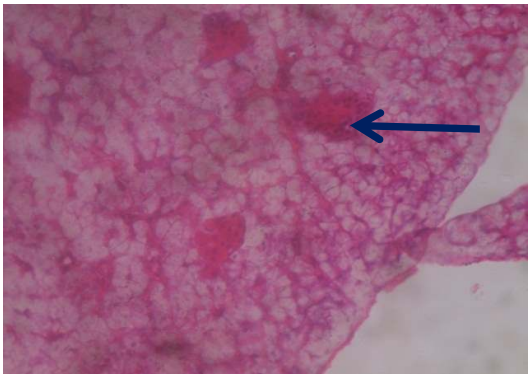

Plate 14. Group MOMS Week 5; Arrow= Pancreatic islet regenerated with fewer cells and normal acini

Plate 12- 14. Pancreas photomicrograph of the group treated with 60% moringa and 40% mushroom at x400

3.7 Histological Effect of the Combined Extract of 60% *Pleurotus ostreatus* (mushroom) and 40% *Moringa oleifera* on the Pancreas of an Alloxan Induced Diabetic Wistar Albino Rats

From the photomicrograph shown on plate 15-17, it was observed that in week 1 the pancreas had atrophic pancreatic islet with few cells, large holes and poor acini. In week 3, the pancreatic islet was able to regenerate with fewer cells and normal pancreatic acini. In week 5, the pancreatic islet regenerated more with large islet, numerous cells and normal pancreatic acini.

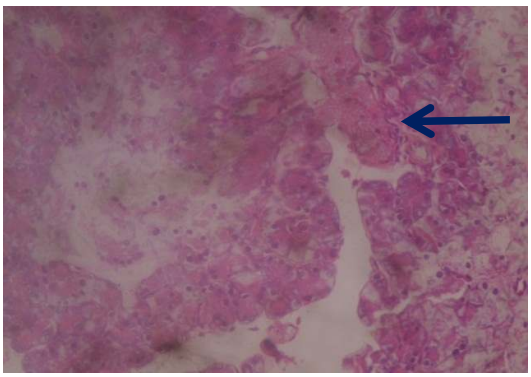

Plate 15. Group MSMO Week 1; Arrow= Atrophic pancreatic islet with few cells, large holes and poor acini

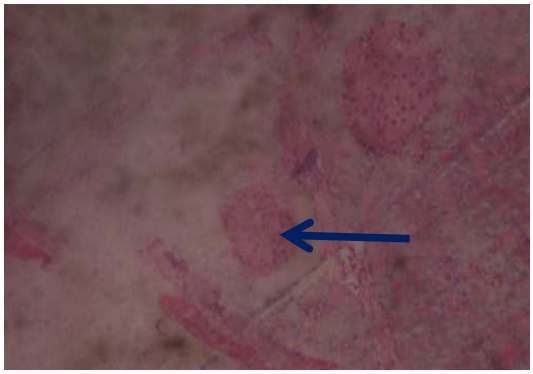

Plate 16. Group MSMO Week 3; Arrow= Regenerating pancreatic islet cells with normal acini

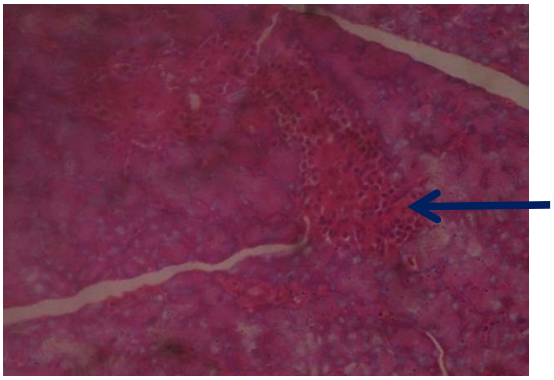

Plate 17. Group MSMO Week 5; Arrow= Larger pancreatic islet cells with and normal acini

Plate 15- 17. Pancreas photomicrograph of the group treated with 60% mushroom and 40%moringa

3.8 Discussion

Histological analysis was carried out on the pancreas of the samples. Pancreatic tissues were collected after animal sacrifice, fixed in 10% formalin, processed routinely, and embedded in paraffin. 5 µm thick sections were prepared and stained with hematoxylin and eosin (H&E) dye for microscopic investigation [14]. The stained sections were examined and photographed under a light microscope at x400. Below are plates (Plates 1-17) showing the photomicrograph of pancreas of the animals in the different groups. From the results, it was observed that the pancreatic photomicrograph of the normal control group at x400 showed normal pancreatic islet (Plate 1). Pancreas photomicrograph of the diabetic control group at x400 (Plate 2) showed atrophic pancreatic islet with few cells and poor pancreatic acini indicating pancreatic damage by diabetes mellitus, thus confirming that alloxan at 120 mg/kg body weight caused pancreatic damage. From the result of the group treated with the reference drug (metformin), there was a great improvement in pancreatic islet cells regeneration as treatment progressed from week 1 to week 5 (Plates 3-5). This shows that metformin is an active antidiabetic drug as it was able to revive a damaged pancreas to normal. Other pancreatic photomicrographs (Plates 6-17) shows that as treatment progressed within the experimental groups been treated, there was a great improvement in the regeneration rate of the pancreas especially those treated with 100% Moringa and the combined treatment of 60% Moringa and 40% mushroom, while other groups treated with 100% mushroom and the combined treatment of 60% mushroom and 40% Moringa were slow in regenerating the pancreas. This shows the ability of *Moringa oleifera* in restoring damaged pancreas to normal. This result is consistent with the findings of Yassa and Tohamy [15] that *Moringa oleifera* aqueous extract restored the pancreas of rats damaged by diabetes.

4. CONCLUSION

This study showed that *Pleurotus ostreatus*, *Moringa oleifera* and the combined treatment of *Moringa oleifera* and *Pleurotus ostreatus* were able to restore and regenerate the pancreatic islet cells of the pancreas of diabetic rats been destroyed by alloxan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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