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VERNONIA AMYGDALINA (BITTER LEAF) SHOWCASES RESTORATIVE POTENTIALS ON THE RENAL HISTOARCHITECTURE OF WISTAR RAT MODELS FOLLOWING ALLOXAN-INDUCED DIABETES MELLITUS

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Abstract: **Background: Diabetic patients' are at risk of developing systemic complications such as nephropathy on the long run. *Vernonia amygdalina* (Bitter leaf) has clearly displayed anti-diabetic and antioxidant properties in several studies. Aim: This study was aimed at investigating effects of the MLEVA; methanol leaf extract of *Vernonia amygdalina* (bitter leaf) on the histology of the kidneys of alloxan-induced diabetes mellitus wistar rats model. Methodology: Thirty (30) adult male wistar rats were grouped into 6 (A-F) (n=5). Group A (control) received normal saline only. Group B (Untreated) received a single intraperitoneal injection of 150mg/kg of Alloxan. Group C, D, E and F received intraperitoneal injections of 150mg/kg of Alloxan, and then treated with 200, 300, 400 mg/kg of the MLEVA and 100mg/kg of Vitamin E respectively daily via oral route. The experiment lasted 16 days. The animals were sacrificed via a median abdominal incision, under ketamine (100mg/ml) as anesthesia 24 hours after their last treatment. The kidneys were carefully harvested, fixed and processed for routine H&E staining. Results: Treatment with MLEVA depicted a clear dose-dependent restoration of the kidney histoarchitecture evident by its tissue stroma appearing normal relative to the control, untreated and Vit E treated animal groups. Conclusion: MLEVA demonstrated a clear restorative potential on the kidney histoarchitecture after injurious changes resulting from alloxan-induced diabetes mellitus in wistar rat model.**

Keywords: Vernonia amygdalina, **Diabetes Mellitus, Alloxan Nephropathy, Author for Correspondence: Christian Chiemeka Ozor.**

1. INTRODUCTION

Diabetes mellitus is evident by the condition of hyperglycemia, and is a common endocrine disease affecting the world population (Teoh *et al*., 2010). It results from a deficiency either in the insulin secretion, insulin action or both (Bastaki, 2005).

Diabetic patients' are at risk of developing long-term systemic complications such as nephropathy, retinopathy and neuropathy (Nathan, 1993). These complications decrease both their quality of life and life expectancy (Ahmed, 2005). Among these complications, diabetic nephropathy seems to be common and results when the function of the kidney is compromised (Pourghasem *et al.*, 2015) leading to tubular proteinuria, sodium and glucose transport disorders (Christiansen *et al.*, 1981; Ziyadeh and Goldfarb, 1991).

Natural remedies have been used in place of allopathic medicine over the years in the management of diabetes mellitus. This is because, it is believed that these natural remedies are safer and have shown beneficial potencies (Joshi and Kaul, 2001). *Vernonia amygdalina* (Bitter leaf) have clearly displayed anti-diabetic properties in several studies (Ijeh and Ejike 2011; Achuba, 2018). It possesses other properties such as being antioxidant, hypolipidemic and anticancerous. Also, its safety through lone consumption as well as in incidences where it is used to treat other toxicants have been established (Lolodi and Eriyamremu, 2013; Abebe and Gebru, 2015; Kadiri, 2017).

2. MATERIALS AND METHODS

Plant Material and Extraction

Fresh matured *Vernonia amygdalina* leaves were procured from a local farm in Enugu South Local Government Area, Enugu State and authenticated at the Faculty of Agriculture, Enugu State University of Science and Technology, Agbani, Enugu.The leaves were washed and air-dried at room temperature for one week. The dried leaves were macerated using a warren blender to a smooth dry powder and was subjected to methanol extraction. The methanol extraction technique used was adopted from Oyedeji *et al*., (2013) and Adefisayo , (2018). The extract was then concentrated using a rotary evaporator at 40–50 °C under reduced pressure was kept in an airtight container afterwards. The extract was stored at −8°*et al.*C until required for use.

Experimental Animals

Thirty adult male wistar rats with average weights of 180-200g were used for this study. This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. There were provided easy access to water and standard livestock pellets (Guinea Feed Nigeria Limited) as food. The animals were kept and maintained under standard laboratory conditions and handling was done following international guidelines on the use of experimental animals. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/FBMS/ETR/23/006.

Experimental Design

The rats were randomly divided into six (6) groups (n=5). Group A (control group) was given normal saline till the end of the experiment. Group B (untreated diabetic group) and received only a single intraperitoneal administration of 150mg/kg/bwt of Alloxan. This dosage of alloxan used was adopted from Cheekati *et al*., (2017). Group C, D and E received a single intraperitoneal administration of 150mg/kg of Alloxan, left for two days (48 hours) to confirm diabetic statuses and then treated with graded doses of 200, 300 and 400 mg/kg of the MLEVA respectively daily for 14 days. The extract dosage was adopted from Adefisayo *et al.*, (2018). Group F received a single intraperitoneal administration of 150mg/kg of Alloxan, left for two days (48 hours) to confirm diabetic status and then treated with 100mg/kg of Vitamin E daily for 14 days. The experiment lasted 16 days.

Confirmation of diabetic status:

All animal were deprived of both food and water for 12 hours. Under aseptic conditions, blood samples were collected on the first day from all groups via tail venipuncture and their blood glucose was determined using a one touch glucometer strip test. Blood glucose levels for all groups ranged from 97mg/dl to 115mg/dl. Group B-F was administered with a single intraperitoneal administration of 150mg/kg of Alloxan immediately after the determination of their blood glucose. To avoid mortalities due to hypoglycemic shock after the induction, their drinking water was replaced with an oral solution of 20% glucose for two days (Bacevic *et al*., 2020).

Blood samples were re-collected from group B-F via tail venipuncture after two days (48 hours) and their blood glucose was determined. Blood glucose levels for these groups after 48 hours ranged from 418mg/dl to 505mg/dl confirming positive hyperglycermia and indicating their diabetic status. Previous studies by Akhtar *et al.*, (2002) and Díez et al., (2013) have reported that animals exhibiting a significant elevation in blood glucose levels above 250 mg/dL should be considered diabetic.

Histological Study

The animals were sacrificed 24 hours after their last administration via a median incision on the abdominal cavity, under ketamine (100mg/ml) as anesthesia. The kidneys were carefully harvested by dissection and immediately fixed with 10% formalin for 72 hours. The fixed tissues were processed using the standard protocols for histological tissue processing and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken using Amscope 14MP USB 3.0 digital microscope camera at X200 magnification.

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3. RESULTS

Histological Analysis

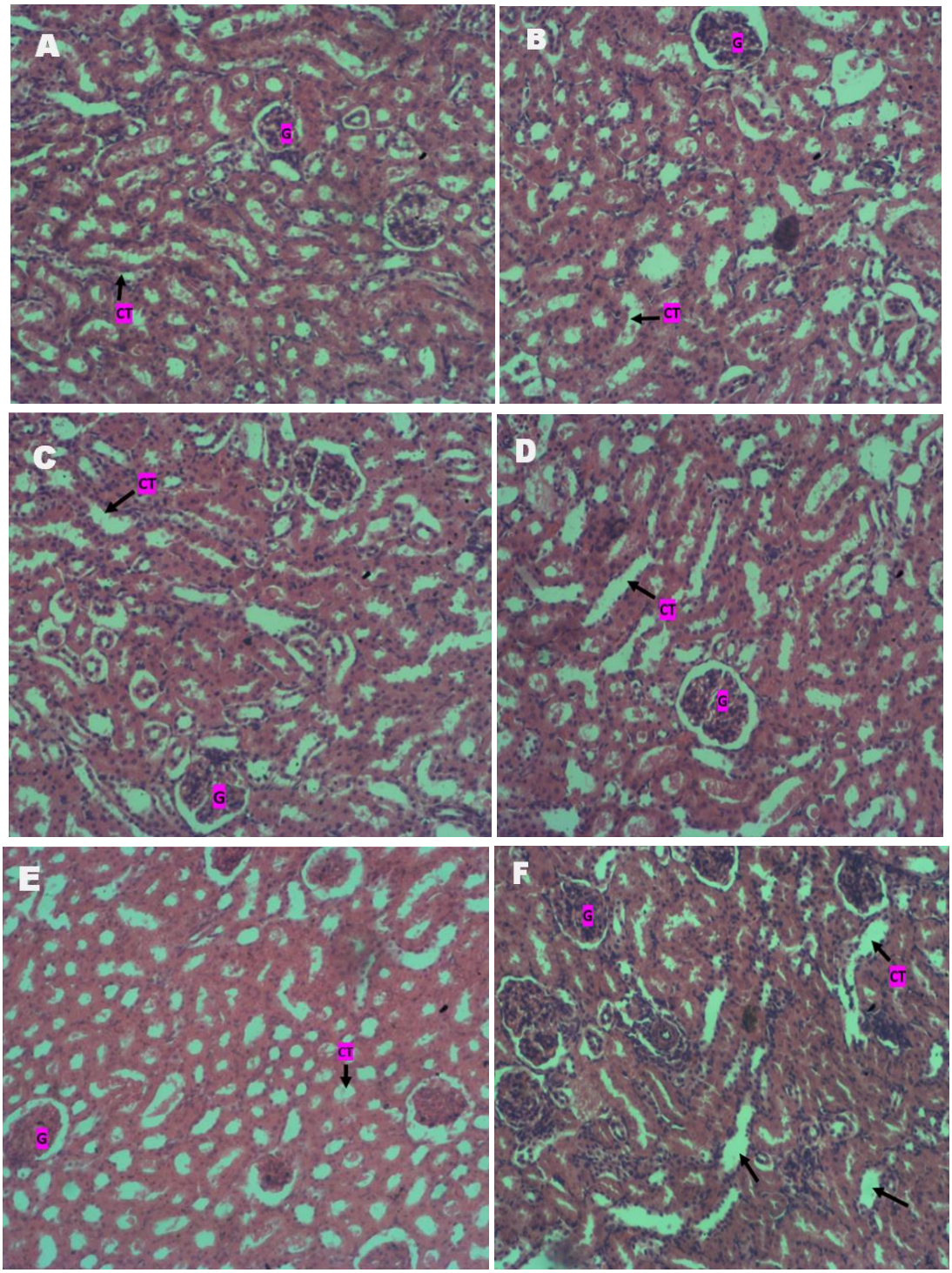

Figure 1(A): Control group. Glomeruli (G) interspersed with several convoluted tubules (CT). General tissue stroma appears normal. H&E. X200. Figure 2(B): Untreated diabetic group. General tissue stroma shows coagulative necrosis of glomeruli and mild capsular hypertrophy. H&E. X200. Figure 3(C): Low dose treatment with MLEVA. General tissue stroma appears normal. H&E. X200. Figure 4(D): Medium dose treatment with MLEVA. General tissue stroma appears normal. H&E. X200. Figure 5(E): High dose treatment with MLEVA. General tissue stroma shows mild capsular enlargement and general loss of tubular nuclei. H&E. X200. Figure 6(F): Treatment with Vit.E. General tissue stroma shows mild tubular loss of brush border (arrow). H&E. X200

4. DISCUSSION

Plant derived remedies for various illnesses have gained popularity principally in the rural areas of most developing countries. These plants contain phytochemicals that have been documented to be medicinal, promoting good health, aiding healing process, while at the same time causing cellular regeneration (Onyeka *et al.*, 2013; Ofoego *et al.*, 2017; Ofoego *et al.*, 2018). The aim of this study was to investigate the effect of the methanol leaf extract of *vernonia amygdalina* (MLEVA) on the microstructure of the kidney in alloxan-induced rat models of diabetes mellitus.

Alloxan-induced rat models of diabetes mellitus caused injurious changes in the kidney histology of the untreated animal groups when compared against the control animal group. These changes were evident as coagulative necrosis of glomeruli and mild capsular hypertrophy. This could without doubt, be as a result of the generation of reactive oxygen species (ROS) that caused damage to the kidney architecture by interfering with various biochemical activities thus impairing the function of the kidney (Donnelly *et al.*, 2000; Lepedda *et al.*, 2016).

Relative to the control, untreated and Vit E treated animal groups, treatment with graded doses of MLEVA depicted a clear dose dependent restoration of the kidney histoarchitecture evident by its tissue stroma appearing normal. The presence of phytonutrients such as flavonoids and tannins found in *Vernonia amygdalina* has the ability to cause regeneration of damaged tissues (Barnes *et al.*, 2020; Oladele *et al.*, 2021). Edet *et al.*, (2020) also demonstrated the protective properties of ethanol leaf extract of *Vernonia amygdalina* against aluminum chloride induced damage to the kidney of male Wistar rats and from his findings histopathological alterations in tissue sections were reversed by the leaf extract.

Notwithstanding, doses of 400 mg/kg of MLEVA still showed mild degenerative changes seen as mild capsular enlargement and a general loss of tubular nuclei suggesting toxicity at high doses. Similar results were also noticed via treatment with Vitamin E. However, Malek *et al.*, (2010) classified vitamin E as a strong antioxidant but its efficacy depends on the used dosage of vitamin E and the time length of administration.

5. CONCLUSION

Treatment with MLEVA demonstrated a clear restorative potential on the kidney histoarchitecture after injurious changes resulting from alloxan-induced rat models of diabetes mellitus. However, large doses may pose extra organ toxicity risks. Caution should be applied while consuming this plant.

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