ejpmr, 2019,6(4), 164-169

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

AMELIORATING EFFECT OF ETHANOLIC LEAF EXTRACTS OF *CARICA PAPAYA* AND *NEWBOULDIA LAEVIS* ON LIVER OF ALLOXAN-INDUCED DIABETIC WISTAR *RATS*

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Article Received on 12/02/2019 **Article Revised on 04/03/2019** **Article Accepted on 25/03/2019**

ABSTRACT

Objective: This study was carried out to investigate the effect of the ethanolic leaf extracts of *Carica papaya (C. papaya)* and *Newbouldia laevis (N. laevis)* on the histology of the liver of alloxan-induced Wistar rats. **Methodology:** Forty male Wistar rats weighing between 160g – 200g were randomly assigned to eight Groups A - H of 5 rats each. Group A served as the control group and was not induced with diabetes, while Groups B – H were induced. Groups A and B received distilled water only, while Groups C - H received 200mg/kg of *C. papaya*, 400mg/kg of *C. papaya*, 200mg/kg of *N. laevis*, 400mg/kg of *N. laevis*, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis* and 400mg/kg of *C. papaya* + 400mg/kg of *N. laevis* respectively for 28 days. On day 29 of the experiment, the animals were sacrificed and liver of each rat was harvested for histological study. **Results**: There were severely damaged hepatic tissue with severe fatty change, cytoplasmic ground glass appearance and clearing of the hepatocytes of the animals in group B when compared with the control group. These effects were ameliorated in Groups C - H which received the variable doses of the ethanolic leaf extracts with more positive effects on the groups that received the combined ethanolic leaf extracts. **Conclusion:** The leaf extracts of *C. papaya* and *N. laevis* have ameliorative effects on the histology of liver of alloxan-induced Wistar rats.

KEYWORDS: *Carica. papaya*, *Newbouldia laevis,* Diabetes Mellitus, liver histology.

1.0 INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic chronic disease that affect the body's utilization of glucose, which is vital to health as it is an important source of energy for the cells that make up the muscles and tissues of the body, and also serve as the brain's source of energy. It results from either the pancreas not producing enough insulin, or the body cells not responding properly to the insulin produced.[1] DM occurs throughout the world but is more common in more developed countries with the greatest increase in rates seen in low- and middle-income countries[2] where more than 80% of diabetic deaths occur.[3] The global number of diabetes cases might increase by 48% between 2017 and 2045.[4] A number of liver abnormalities, such as abnormal glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas (HCCs), abnormal elevated hepatic enzymes, acute liver disease and viral hepatitis are associated with DM.[5,6] Thus there is need to investigate on medicinal plants that will not only treat DM but will also help to ameliorate the complications associated with DM such as fatty liver, liver cirrhosis and kidney diseases.

N. laevis is a medicinal plant belonging to the family of *Bignoniaceae*. *N. laevis* commonly known as Boundary or Border tree is called Ogirisi or Ogilisi in Igbo, Akoko in Youruba and Aduruku in Hausa. It is being used by African traditional healers to treat various ailments like diabetes, rheumatism and toothache.[7] The pharmacological effects of *N. laevis* include antioxidant effect[8], free radical scavengers[9], antimicrobial[10], hepatoprotective[8], anticancer.[11], hypoglycemic[12] and antihypertensive [13]. Studies have shown that *N. laevis* may be used to manage hepatotoxicity[14], hyperglycemia[15] and to protect the liver membrane.[16]

C. papaya is a juicy and tasty fruit medicinal plant belonging to the family *Caricaceace*. *C. papaya* commonly known as Pawpaw is common in tropical and sub-tropical countries.[17] Some of the pharmacological effects of *C. papaya* leaf extract include hypoglycemic[18] and antidiabetic.[19] *C. papaya* leaf according to studies

ameliorates metabolic derangement[20], has antihypoglycemic[21] and antihyperlipidemic[22] effects.

Thus this study was carried out to investigate the combined effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on the histology of the liver in alloxan induced diabetic rats, as no study has been carried out on this.

2.0 MATERIALS AND METHODS

2.1 Animal procurement, care and treatment

Forty (40) wistar rats weighing between 160g to 200g were procured from the animal house of the Department of Anatomy, Nnamdi Azikwe University, Nnewi Campus. They were housed in the Animal house of Anatomy Department, Abia State University, Uturu with wire gauze cages in a well-ventilated area. They were fed with standard commercial pellet diet and water *ad libitum*; and were acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2 Collection and preparation of plant materials

C. papaya and *N. laevis* leaves were harvested from Nkporo in Ohafia L.G.A of Abia State. The leaves were properly washed with water to remove sand and other impurities, and were authenticated at the Herbarium Unit, Botany Department, Abia State University, Uturu. They were air dried and crushed using laboratory blender. Extraction was done using ethanol. The crude ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250mg of these extracts/kg body weight were dissolved in 10mls of distilled water and administered to the animals.

2.3 Induction of diabetes

The rats were divided into non-diabetic control group and experimental group (to be induced with alloxan). Diabetes was induced in the experimental rats by intra-peritoneal administration of 150mg of alloxan per kg body weight of rat (150mg/kg body weight). After the induction, all the rats were allowed free access to the same feed and water. After 72 hours, blood samples obtained through the tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using Glucometer. Diabetes was confirmed at fasting blood glucose levels greater than 200mg/dl.[19]

2.4 Experimental protocol

The animals were grouped into eight (8) groups of five rats each. Different doses of the leaf extracts were administered as shown below:

Group A (The control group) distilled water.
Group B (Diabetic group) distilled water.
Group C Diabetic + 200mg/kg of *C. papaya* leaf extract.
Group D Diabetic + 400mg/kg of *C. papaya* leaf extract.
Group E Diabetic + 200mg/kg of *N. laevis* leaf extract.
Group G Diabetic + 200mg/kg of *C. papaya* and 200mg/kg of *N. laevis* leaf extracts.
Group H Diabetic + 400mg/kg of *Carica papaya* and 400mg/kg of *N. laevis* leaf extracts.

2.5 Sample collection and analysis

The extracts were administered for 28 days. On the 29th day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Liver tissues were harvested from the animals and were fixed in 10% formal saline for four hours, this was followed by histological and histochemical methods of tissue processing.

3.0 RESULTS

3.1 Physical and behavioral changes

During the two weeks of acclimatization, all the animals looked healthy and agile, but on administration of alloxan, they became weak and exhibited labored breathing (dyspnoea), staggering/loss of balance, convulsion, decreased food intake, polydipsia, polyuria, weight loss, hyperglycemic, coma and even death. These signs decreased following administration of ethanolic leaf extract of *C. papaya* and *N. laevis.*

3.2 Histopathological findings

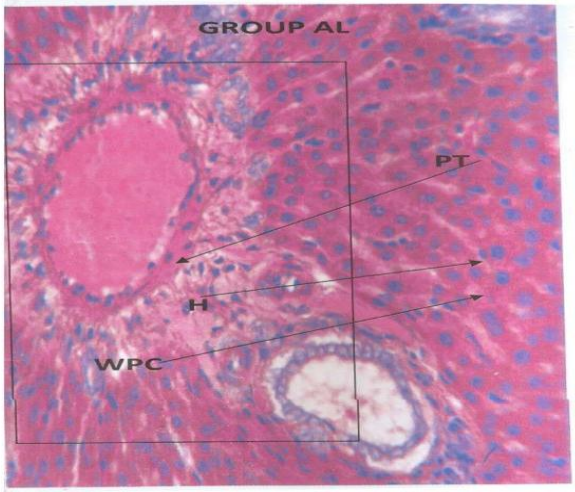

Figure 1: Sectional view of Group A of liver (x400) (H/E) showing normal hepatic architecture with well perfuses cytoplasm (WPC), hepatocyte (H) and portal traid (PT).

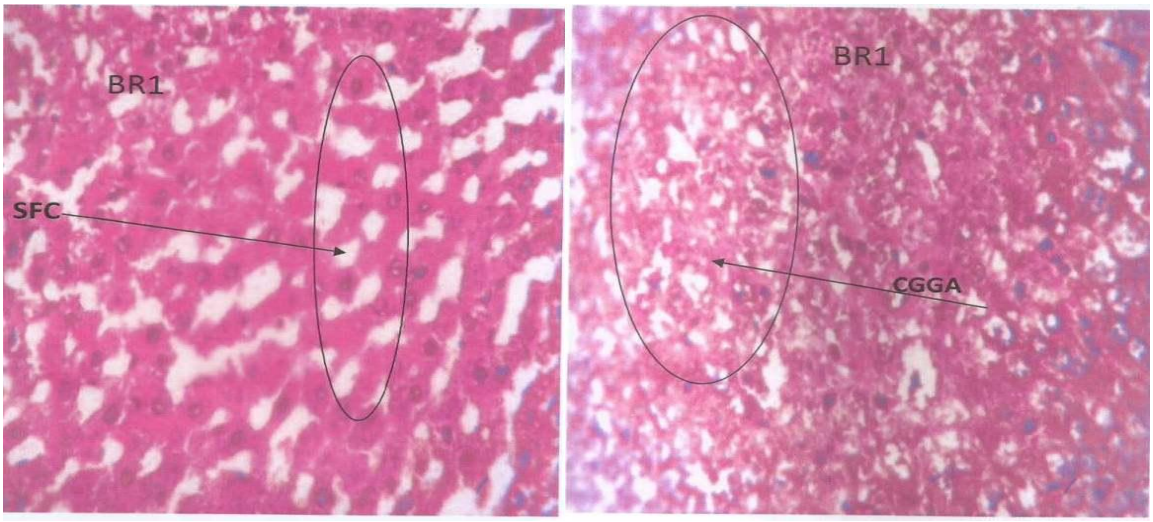

Figure 2: Sectional view of Group B of liver induced with Alloxan (X400) (H/E) showing severely damaged hepatic tissue with severe fatty change (SFC), cytoplasmic ground glass appearance (CGGA) and clearing of the of the hepatocyte.

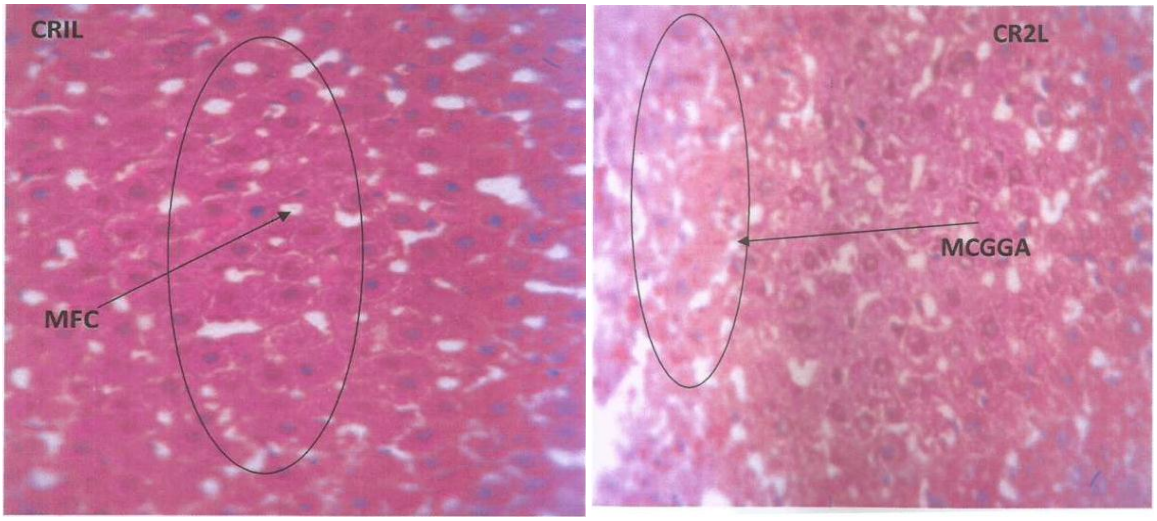

Figure 3: Sectional view of Group C of liver treated with 200mg/kg of *C. papaya* (X400)(H/E) showing mild healing with moderate fatty change (MFC), moderate cytoplasmic ground glass appearance (MCGGA).

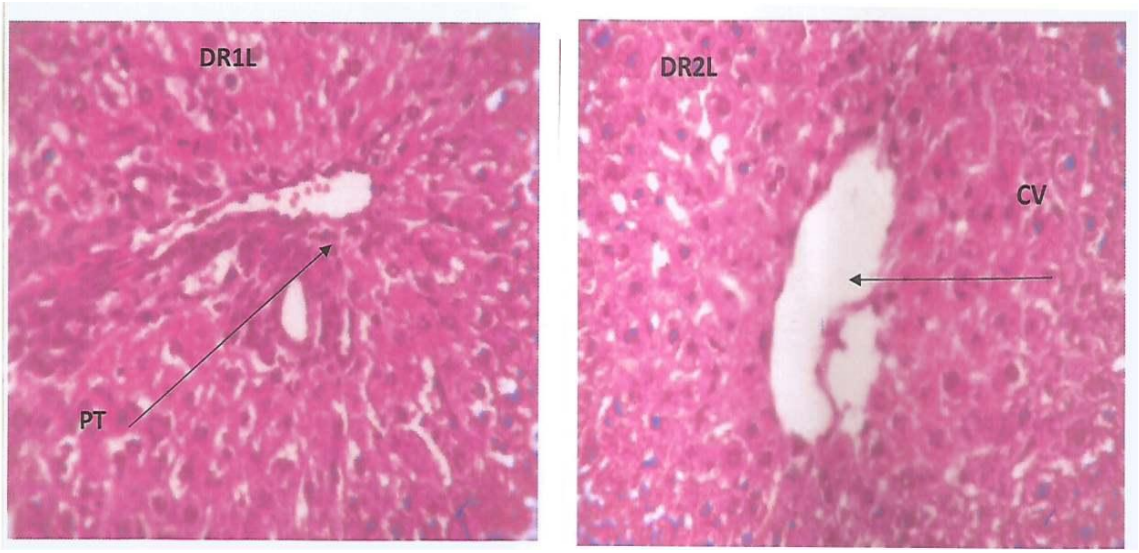

Figure 4: Sectional view of Group D of liver treated with 400mg/kg *C. papaya* X400) (H/E) showing moderate healing with portal inflammation otherwise normal.

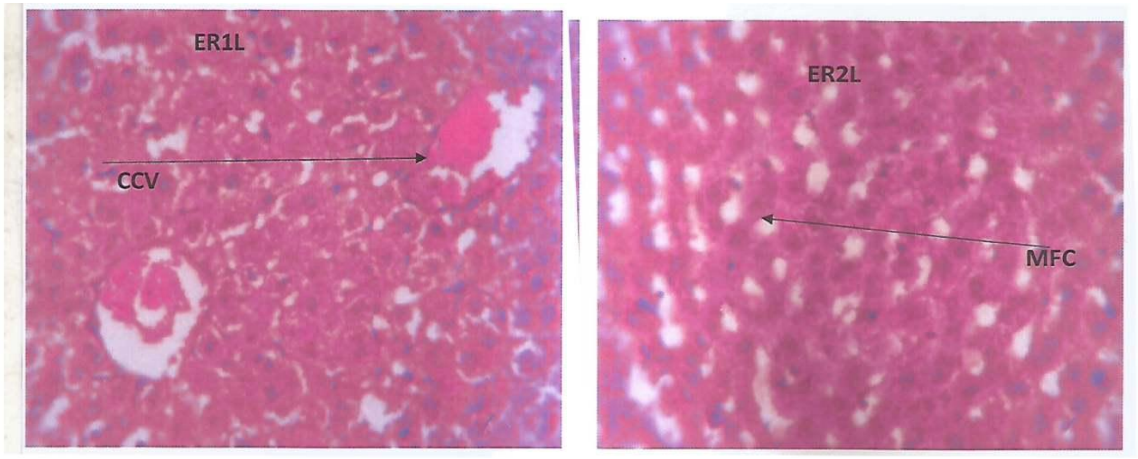

Figure 5: Sectional view of Group E of liver treated with 200mg/kg *N. laevis* (X400) (H/E) showing mild healing with congestion of central vein and moderate fatty change (MFC).

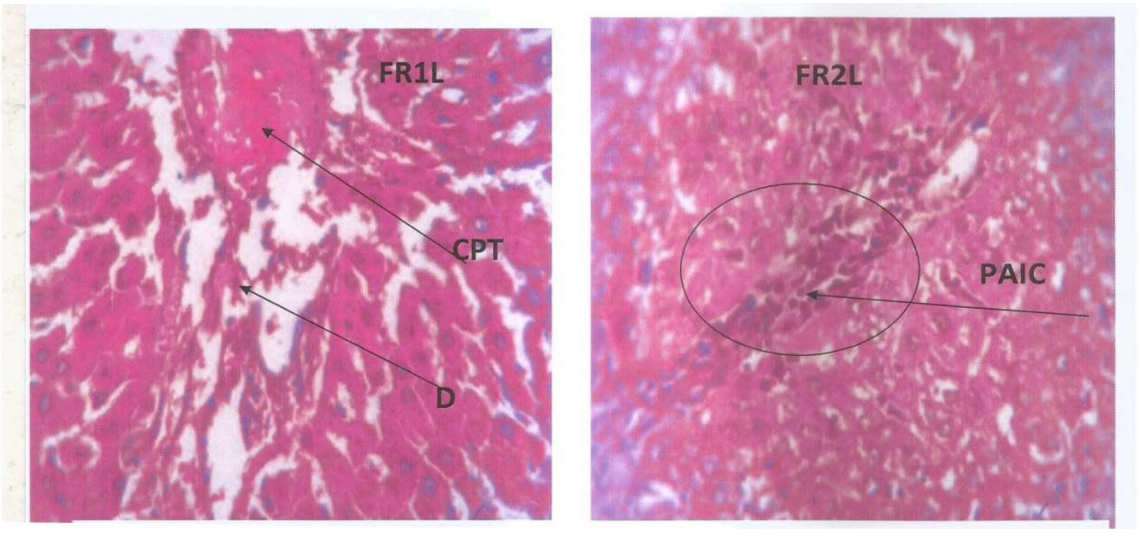

Figure 6: Sectional view of Group F of liver treated with 400mg/kg *N. laevis* (X400) (H/E) showing mild healing with moderate portal aggregate inflammatory cell (MPAIC), dilation (D) and congestion of the portal traid (CPT).

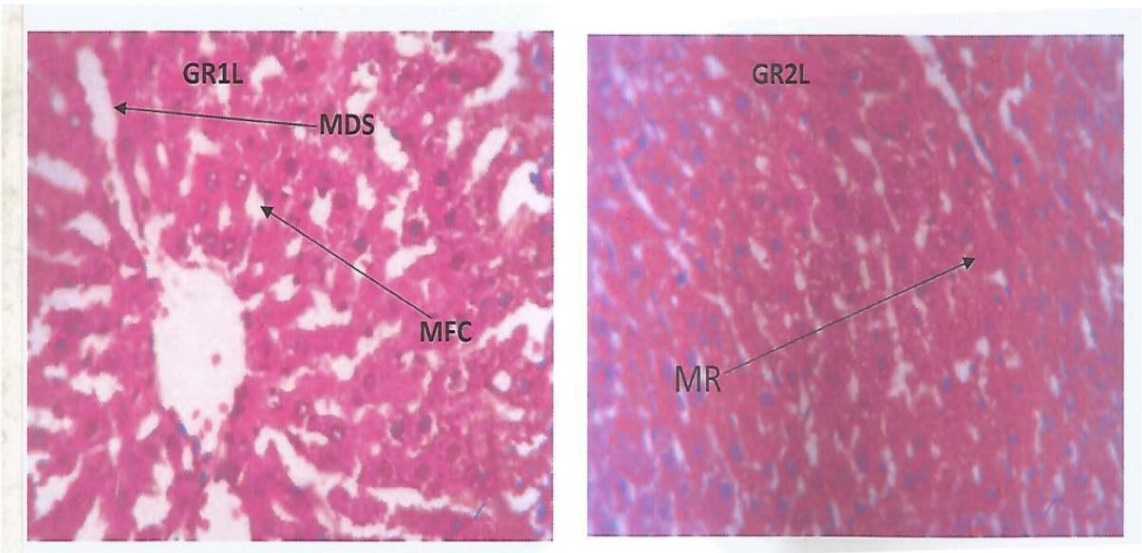

Figure 7: Sectional view of Group G of liver treated with 200mg/kg *N. laevis* and 200mg/kg of *C. papaya* (X400) (H/E) showing mild to moderate regeneration (MR), with dilation of sinusoid (MDS), and mild fatty changes (MFC).

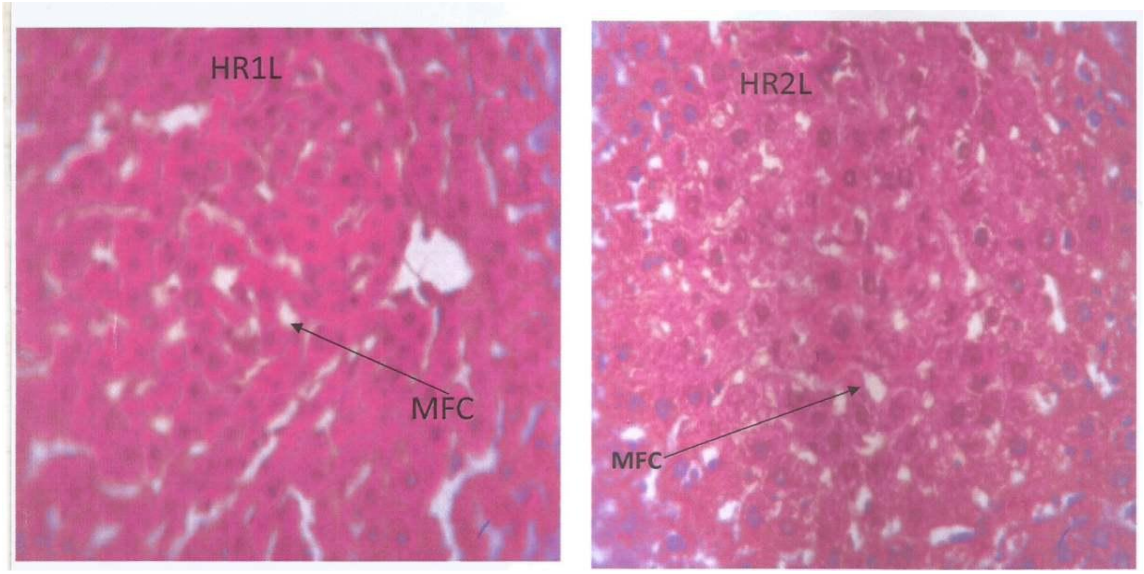

Figure 8: Sectional view of Group H of liver treated with 400mg/kg *N. laevis* and 400mg/kg *C. papaya* (X400) (H/E) showing moderate healing with mild fatty changes (MFC).

4.0 DISCUSSION

In this study, the histopathological findings of group A (Figure 1) indicated normal hepatic architecture with well perfuse cytoplasm (WPC), hepatocyte (H) and portal traid (PT) whereas, group B (Figure 2) indicated severely damaged hepatic tissue with severe fatty change (SFC), cytoplasmic ground glass appearance (CGGA) and clearing of the hepatocyte. This could be attributed to the alloxan monohydrate which damages the pancreatic cells leading to diabetes and its effects on the liver. Alloxan monohydrate induces diabetes in the rats by destroying the insulin producing beta-cells of the pancreas causing cell necrosis.[23,24] However, the results of these researchers suggest that the liver morphological alterations that were observed during the early stages of treatment with alloxan or streptozotocin may be more related to the toxic action of these drugs than to the effects of diabetes mellitus.[24]

Meanwhile the histopathological findings present in ethanolic leaves extract-treated groups (Figures 3 – 8) showed that they could be ameliorating/healing effect of the leaves extract to the liver when compared to the diabetic experimental control group. Adenowo[19] reported that the treatment of the diabetic rats with aqueous extracts of *C. papaya* caused reduction in the activities of liver enzymes when compared to the diabetic group and consequently alleviated liver damage caused by alloxan-induced diabetes[25] which is in line with this study. However, the positive effect was dose dependent and better in the groups treated with the combination of both ethanolic leaves extract of *C. papaya* and *N. laevis* when compared with diabetic non-treated group. Thus combination of both extracts in the management of diabetes could be more acceptable since their ameliorating effects are better than when the extracts are not being combined.

5.0 CONCLUSION

This study confirms that *C. papaya* and *N. laevis* extracts have ameliorating effects on the histology of liver of alloxan-induced diabetic wistar rats. Secondly, the ameliorating effects seen on the groups treated with the combined leaf extracts suggest that the combined doses of ethanolic leaves extracts improve the metabolic disruption of the histology of the liver better than when the leaf extracts of the individual medicinal plants are used in the management of diabetes. Hence, the combination of the two leaf extracts may be more beneficial in the treatment of diabetes mellitus.

Funding: No funding sources.

Conflict of interest: None declared.
Ethical Approval: Approved by Institutional ethical approval.

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