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Ameliorative activity of ethanolic leaf extracts of *carica papaya* and *newbouldia laevis* on kidney of alloxan-induced diabetic *rats*

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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 kidney of alloxan-induced 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 kidney of each rat was harvested for histological study.
Results: There were severely damaged renal tissue with severe tubular necrosis, glomerular atrophy and coagulative necrosis of glomeruli which leads to the closure of the malpighian layer 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 effect on the histology of kidney of alloxan-induced rats.

Keywords: *carica. papaya*, *newbouldia laevis*, diabetes mellitus, kidney histology

1. Introduction
Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized and overproduced causing hyperglycemia [1]. It is a complex chronic illness which requires continuous medical care [1], and is characterized by polyuria, polydipsia, weight loss and increased hunger [2]. If left untreated, DM will lead acute complications such as diabetic ketoacidiosis and nonketotic hyperosmolar coma; and serious long-term complications like heart disease, stroke, kidney failure, foot ulcers and damaged to the eyes [2]. Its primary microvascular complications include damage to the eyes, kidneys and nerves (2). Damage to the kidneys known as diabetic nephropathy can lead to tissue scaring, urine protein loss and eventually chronic kidney disease [2]. Study estimated that 425 million people suffered from DM in 2017; and this number is expected to rise to 629 million by 2045 [3].
Some medicinal plants have shown to have antidiabetic effect over the years (4). Such medicinal plants include *Carica papaya (C. papaya)* and *Newbouldia laevis (N. laevis). C. papaya* belongs to the family *Caricaceae*. It is an herbaceous perennial plant with rapid proliferation rate [3]. It originates from the Southern Mexico, Central America, and Northern South America [5]; and is now cultivated in the Tropics. *C. papaya* is widely cultivated for its edible pleasant fruit which provides good nutritional value and easy digestion [6]. Studies showed that *C. papaya* leaf has antilipidemic [7], hypoglycemic [6], vasodilating and antioxidant effects [8]; and ameliorates metabolic derangement [9].
Newbouldia laevis (*N. laevis*) is a non-leguminous, medium sized angiosperm, commonly known as boundary tree, chieftaincy tree or tree of life [10]. It belongs to the family *Bignoniaceae*. It is called Ogirisi in Igbo, Akoko in Yoruba, and Aduruku in Hausa [11]. It is native to tropical Africa and grows from Guinea Savannah to dense forests. Studies have shown that *N. laevis* leaf is used to manage hyperglycemia, improve haematological and biochemical derangements, control muscle wasting, induce adipogenesis [12]; and has antidiabetic effect [13].
Hence 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 kidney of alloxan-induced diabetic rats, as no study has been carried out on this.

2. 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 [9].

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 F	Diabetic + 400mg/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 *C. 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. Kidney 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.

2.6 Statistical analysis

All data were tabulated and statistically analyzed using SPSS version 20.0. Results were expressed as Mean ± standard error of mean (M ± SEM). One way analysis of variance (ANOVA) followed by Bonferroni's Post-hoc test were used for data comparison. $P < 0.05$ was taken as statistically significant.

Study period: The study commenced from June 2017 and ended on October 2018.

3. 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

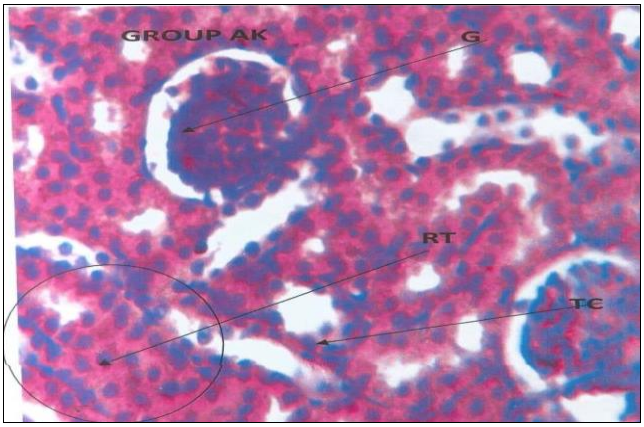

Fig 1: Histopathological analysis of wistar rat kidney: (A) Control.

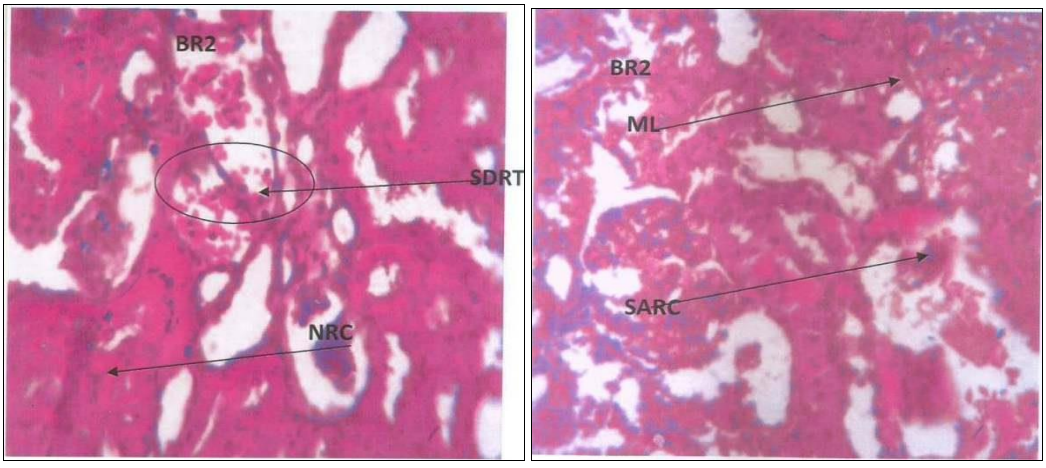

Fig 2: Histopathological analysis of wistar rat kidney: (B) alloxan 150mg/kg of body weight

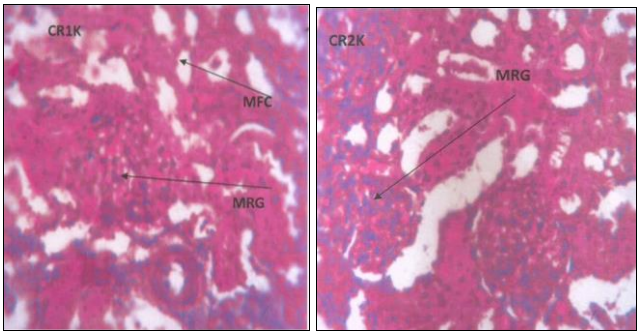

Fig 3: Histopathological analysis of wistar rat kidney: (C) 200mg/kg *C. papaya*.

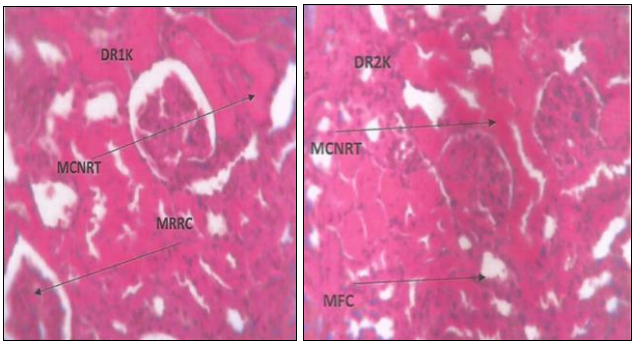

Fig 4: Histopathological analysis of wistar rat kidney: (D) 400mg/kg *C. papaya*.

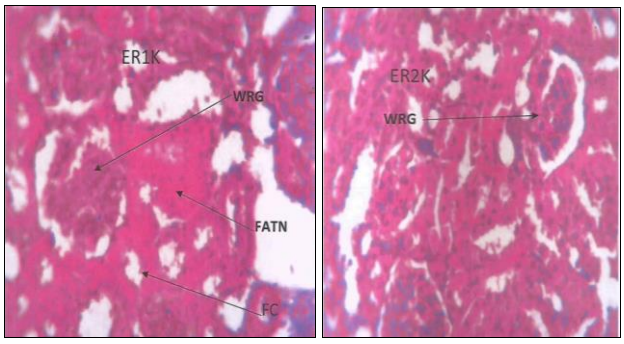

Fig 5: Histopathological analysis of wistar rat kidney: (E) 200mg/kg *N. laevis*.

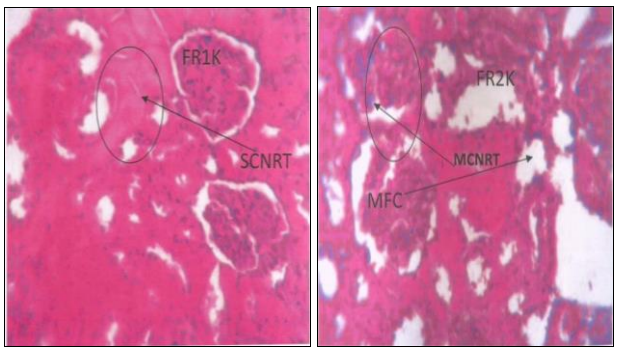

Fig 6: Histopathological analysis of wistar rat kidney: (F) 400mg/kg *N. laevis*.

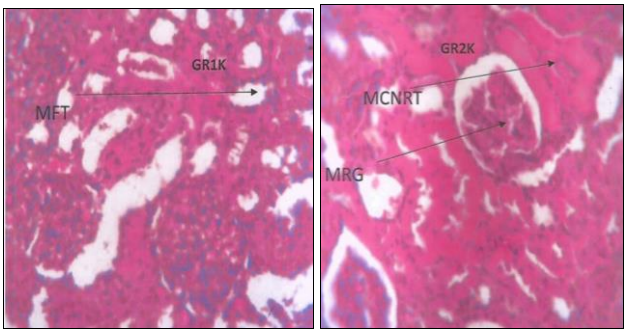

Fig 7: Histopathological analysis of wistar rat kidney: (G) 200mg/kg *N. laevis* and 200mg/kg *C. papaya*.

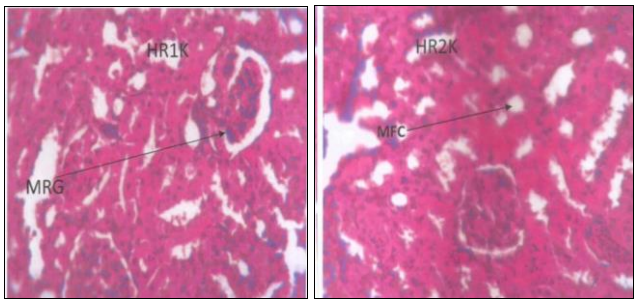

Fig 8: Histopathological analysis of wistar rat kidney: (H) 400mg/kg *N. laevis* and 400mg/kg *C. papaya*.

4. Discussion

In this study, the histopathological finding of kidney cells of the animals in group A (control) showed normal renal architecture with glomeruli, renal tubules and tubular cell that appear normal (figure 1), while that of figure 2 (experimental control B) showed severely damaged renal tissue with severe tubular necrosis, glomerular atrophy and coagulative necrosis of glomeruli that leads to the closure of the malpighian layer. This could be attributed to the alloxan monohydrate which damages the pancreatic cells leading to diabetes and its effects on the kidney. Alloxan monohydrate induces diabetes in the rats by destroying the insulin producing beta-cells of the pancreas causing cell necrosis [14, 15, 16] and kidney toxicity [17]. However, the results of these researchers suggest that the kidney 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 [17].

The histopathological analysis of wistar rat kidney (C) that received 200mg/kg of *C. papaya* showed mild healing with regeneration of glomeruli and moderate fatty change (figure 3). This could be due to stimulatory mechanism on the few surviving β-cells and glomeruli by the leaf extract which may have caused secretion of insulin [18, 19]; and reduction in the levels of creatinine and urea which might have increased due to kidney damage [20, 21] leading to the moderate fatty change observed. The ethanolic leaf extracts *C. papaya* may have ameliorated the damaged kidney by stimulating the

few remaining β-cells and regenerate nephrons back to their normal functions.

Thus C. papaya leaf extract could have ameliorating effect on renal dysfunction resulting from diabetes. however the histopathological analysis of wistar rat kidney of group D that received 400mg/kg *C. papaya* showed mild healing with moderate regeneration of glomeruli, moderate coagulative necrosis of the renal tubules and mild fatty changes (figure 4). This could be due to the fact that C. Papaya leaf extract do not show any toxicity effect with the increasing dosage [12]. Thus, the increased in dosage of the leaf extract stimulated many more β-cells [18, 19] and regeneration of more glomeruli leading to better ameliorating effect on the kidney.

Meanwhile, the histopathological analysis of wistar rat kidney of group E that received 200mg/kg *N. laevis* showed moderate healing with well regenerated glomeruli, mild focal area of tubular necrosis and fatty change. This could be in accordance with the report suggesting that in diabetic rats, the administration of plant extracts can be effective in cell regeneration and restoration of islet size, even producing cell hyperplasia [18, 12] and that the β-cells have shown remarkable potential for regeneration at the pre-clinical stage of diabetes which is a key question when addressing type 1 diabetes [18, 19]. Thus the regenerating effect of the leaf extract brings about the healing/ameliorating effect witness in figure 5. The histopathological analysis of wistar rat kidney of group F that received 400mg/kg *N. laevis* showed mild healing with moderate regeneration of glomeruli, severe coagulative necrosis of the renal tubules and moderate fatty changes. This result observed in group F could be due to the fact that the acute toxicity of *N. laevis* is relatively safe [12]. Thus, better ameliorating effect was observed as 400mg/kg of the leaf extract was given.

Also, the histopathological analysis of wistar rat kidney of group G that received 200mg/kg *N. laevis* and 200mg/kg *C. papaya* shows mild to moderate regeneration of glomeruli with moderate coagulative necrosis of the renal tubules and moderate fatty changes (Figure 7). This could be due to the acute relative safety [12] of the combined leaf extracts which stimulated the regeneration of most of the β-cells and glomeruli, thus ameliorating the kidney better than when the individual leaf extracts were administered. It has been reported that *C. papaya* has protecting effect on the kidney [23] and improves the metabolic disruption produced by diabetes [6], while N. laevis leaf possesses the ability to improve biochemical derangements [13], and also to promote proper kidney functions [24]. However, better ameliorating effect was observed in the Histopathological analysis of wistar rat kidney of group H which received 400mg/kg *N. laevis* and 400mg/kg *C. papaya* (Figure 8). This result shows that the combined leaf extracts of C. papaya and N. laevis have better ameliorating effect on the kidney than when administered single and that the effect is dose-dependent.

5. Conclusion

This study confirms that *Carica papaya* and *Newbouldia laevis* extracts have ameliorating effects on the histology of kidney 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 kidney better than when the leaf extracts of the individual medicinal plants are used in the management of DM. Hence, the combination of the two leaf extracts may be more beneficial in the treatment of DM.

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

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