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Hepatoprotective potential of ethanolic leaf extracts of *Carica papaya* and *Newbouldia laevis* on alloxan-induced diabetic wistar rats

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Abstract
Objective: This study was carried out to investigate the effect of the ethanolic leaf extracts of *C. papaya* and *N. laevis* on the liver enzymes 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 weighed and sacrificed; and blood samples were collected from each of the animals for serum analysis.
Results: There were significant ($P<0.05$) decrease in body weight and significant ($P<0.05$) increase in serum levels of ALT, AST and ALP 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 body weight and serum levels of ALT, AST, and ALP of alloxan-induced Wistar rats.

Keywords: *Carica papaya*, *Newbouldia laevis*, diabetes mellitus, liver enzymes

1. Introduction
Diabetes mellitus has become a growing problem in the world today [1]. According to World Health Organization the diabetic population is likely to increase up to 300 million or more by the year 2025 [2]. At the present rate of its increase, it may turn out to be one of the world's most common diseases and biggest public health burdens, with an estimated minimum of half-a-billion cases over the next decade [3]. Thus there is need to research for Medicinal plants with hypoglycemic agents that will not only ameliorate diabetes mellitus, but will equally help to improve some of the secondary complications of diabetes such as fatty liver, and oxidative stress.
Diabetes mellitus is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced. Development of drugs for the treatment of Diabetes mellitus is one of the major health problems in the world that requires experimental studies using diabetic and anti-diabetic agents [4, 5, 6]. Alloxan is one of such potent diabetic agents that have been used for this purpose in experimental animals [5].
Medicinal plants are plants that have specific bioactive compounds which are responsible for curing or healing certain ailments in human beings or animals. Some of the plants have shown to have some antidiabetic effects over the years [7]. Such medicinal plants include *Carica papaya* and *Newbouldia laevis.*
Carica papaya (*C. papaya*) (*Papaya,* PawPaw, Kates, Papaw,) [8] is a small, sparsely branched tree, usually with a single stem growing from 5 to 10 m (16 to 33 ft) tall. It originates from Southern Mexico, Central America and Northern South America [9] and is now cultivated in all the tropics. It belongs to the genus *Carica* and of the family *Caricaceae.* The phytochemical component of its leaf are carotenoids, polyphenols [10], benzyl isothiocyanates, benzyl glucosinates, [11] and prunasin [12]; while the pharmacological effects *C. papaya* leaf are antidiabetic [13], hypoglycemic [14], antioxidant [15], immunomodulatory [14], hypolipidemic [16] and hepatoprotective [17].
Newbouldia laevis (*N. laevis*) which is commonly known as boundary tree is called *Ogirisi or ogilisi* in Igbo, *Akoko* in Yoruba [18] and Aduruku in Hausa [19]; and it is native to tropical Africa. The phytochemical composition of its leaf are flavonoids, tannins, terpenoids, steroids, cardiac glycosides, alkaloids, and saponins [20]; while its pharmacological effect include antidiabetic [21], hypoglycemic [13], hepatoprotective [22] and antioxidant effect [23].
This study was carried out to investigate the effects of ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on body weight and the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphotase (ALP) of alloxan induced diabetic rats.

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

Carica papaya and *Newbouldia 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 [24].

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 *Carica papaya* leaf extract.
- **Group D:** Diabetic + 400mg/kg of *Carica papaya* leaf extract.
- **Group E:** Diabetic + 200mg/kg of *Newbouldia laevis* leaf extract.
- **Group G:** Diabetic + 200mg/kg of *Carica papaya* and 200mg/kg of *Newbouldia laevis* leaf extracts.
- **Group H:** Diabetic + 400mg/kg of *Carica papaya* and 400mg/kg of *Newbouldia 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. Blood samples were assayed for levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphotase (ALP) using randox kit method.

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.

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 *Carica papaya* and *Newbouldia laevis.*

Table 1: The effects of ethanolic leaf extracts of *Carica papaya* and *Newbouldia laevis* on the body weight of alloxan induced diabetic Wistar rats

Groups	No. of Rats (n)	Treatment received	Body weight (g)
A	5	Control (Distilled water only)	190.0 ± 10.0
B	5	Positive control (Diabetes + no treatment)	160.0 ± 7.07*
C	5	Diabetes + 200mg/kg *Carica papaya*	182.0 ± 10.95
D	5	Diabetes + 400mg/kg *Carica papaya*	220.0 ± 20.0*
E	5	Diabetes + 200mg/kg *Newbouldia laevis*	202.0 ± 10.95
F	5	Diabetes + 400mg/kg *Newbouldia laevis*	222.0 ± 20.49*
G	5	Diabetes + 200mg/kg *Carica papaya* + 200mg/kg *Newbouldia laevis*	244.0 ± 16.73*
H	5	Diabetes + 400mg/kg *Carica papaya* + 400mg/kg *Newbouldia laevis*	258.00 ± 13.04*

*P>0.05: significant when compared with the control group

Table 1 shows significant decrease (P<0.05) in body weight of animals in group B (160.0 ± 7.07) when compared with the control group A (190.0 ± 10.0). There was no significant difference (P>0.05) in body weight of animals in groups C (182.0 ± 10.95) and E (202.0 ± 10.95) when compared with the control group A (190.0 ± 10.0). However, groups D (220.0 ± 20.0), F (222.0 ± 20.49), G (244.0 ± 16.73) and H (258.00 ± 13.04) show significant increase in body weight when compared with the control group A (190.0 ± 10.0).

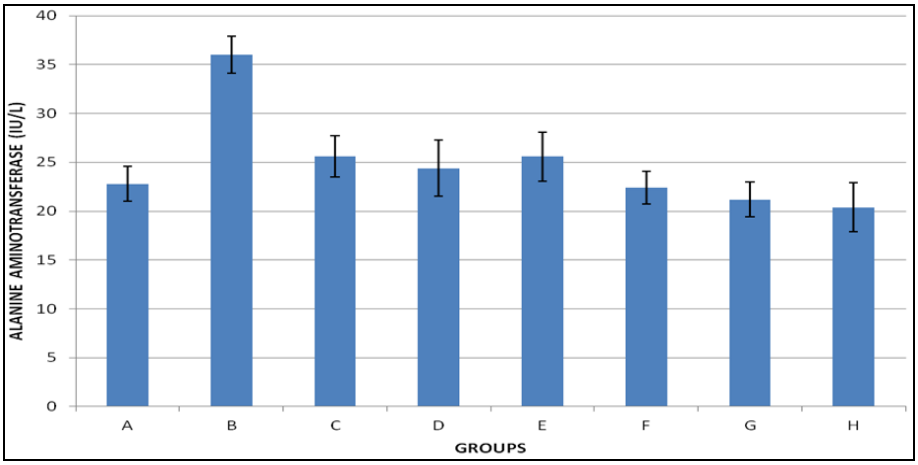

Fig 1: Effects of Ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on serum Alanine Aminotransferase (ALT) of Alloxan induced diabetic Wistar rats.

Figure 1 shows significant increase (P<0.05) of serum alanine aminotransferase in group B (36.0 ± 1.87) when compared with control group A (22.8 ± 1.79). Conversely, no significant difference was seen in serum alanine aminotransferase of groups C (25.6 ± 1.34), D (24.4 ± 2.88), E (25.6 ± 2.51), F (22.4 ± 1.67), G (21.0 ± 1.79) and H (20.4 ± 2.61) when compared with control group A (22.8 ± 1.79).

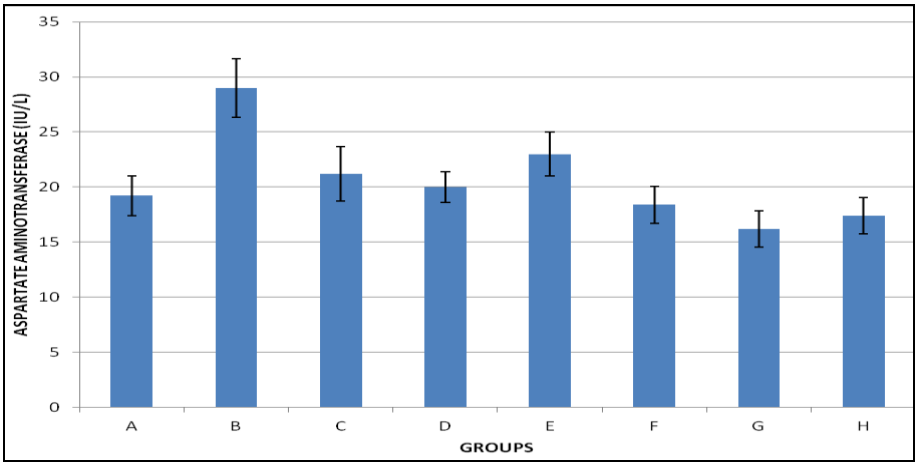

Fig 2: Effects of ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on serum aspartate aminotransferase (AST) of alloxan induced diabetic wistar rats.

Figure 2 shows the significant increase (P<0.05) in serum aspartate aminotransferase in group B (29.0 ± 2.65) when compared with the control group A (19.2 ± 1.79). But no significant change (P>0.05) was observed in serum aspartate aminotransferase in groups C (21.2 ± 1.67), D (20.0 ± 1.41), E (23.0 ± 2.0), F (18.4 ± 1.68), G (16.2 ± 1.64) and H (17.4 ± 1.34) when compared with the control group A (19.2 ± 1.79).

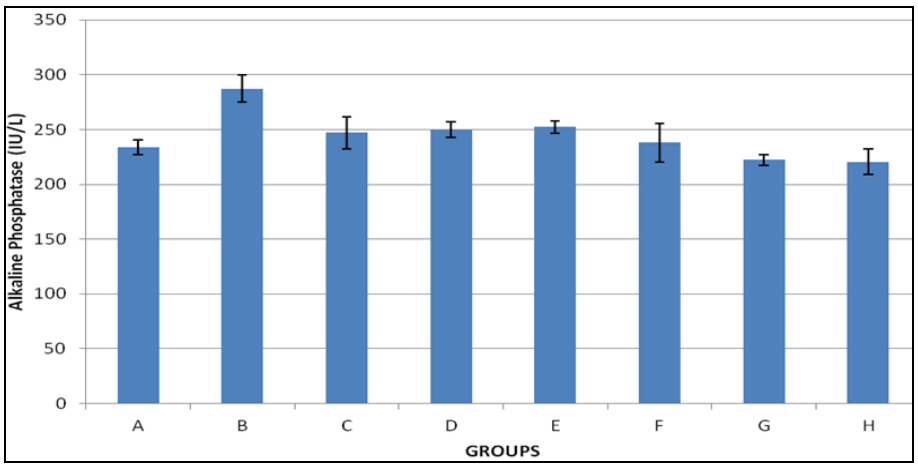

Fig 3: Effects of ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on serum alkaline phosphatase (ALP) of alloxan induced diabetic wistar rats.

Figure 4.3 shows significant increase (P<0.05) in serum alkaline phosphatase in group B (287.4 ± 12.4), when compared with the control group A (233.8 ± 6.87). However, no significant difference (P>0.05) was observed in serum alkaline phosphatase in groups C (247.0 ± 14.5), D (249.6 ± 7.13), E (252.4 ± 5.6), F (238.0 ± 17.7), G (222.4 ± 4.77) and H (242.0 ± 11.65) when compared with the control group A (233.8 ± 6.87).

4. Discussion

In this study, the significant decrease (P > 0.05) in weights observed in non-extract treated diabetic group (Table 1) could be due to insufficient insulin which prevents the body from getting glucose from the blood into the body's cell. When this occurs, the body starts burning fat and muscle for energy causing a reduction in overall weight. This result is in agreement with the result of research work carried out by Ewenighi [25] which reported decreased in body weight in alloxan induced diabetic rats. Junod [26] and Montano [27] also reported significant weight loss after inducing diabetes with streptozotocin on Wistar rats in their respective studies. The extract-treated groups however showed significant (P < 0.05) increase in weight gain following extract administration which was comparable with that of the normal control. This could be due to improve glycaemic control and the drugs' protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis [28].

The significant increase in serum enzymes - ALT, AST and ALP in group B (Figures 1, 2 and 3) when compared with control group A could be due to liver damage. Arkkila [29] reported that elevated activities of serum enzymes are common signs of liver disease and are observed more frequently among people with diabetes than in the general population. Anaduaka [22] reported that diabetes is known to involve oxidative stress, and that high levels of ALT, ALP and AST are typical of oxidative stress condition. The non-significant difference in liver enzymes observed in groups C - H (figures 1, 2 and 3) showed that the extracts may possess an ameliorative effect. The ethanolic leaf extracts of *C. papaya* and *N. laevis* have been reported to have antioxidant effect on the liver [22] thus, decreases the liver enzymes. The decrease in activity of ALT, AST, and ALP by the leaf extracts suggests hepatoprotective potential of the extracts. This may be due to the reported antioxidant property of the extracts [15]. Antioxidants scavenge free radicals and prevent lipid peroxidation of biomembranes thereby reducing the leakage of intracellular enzymes [30].

5. Conclusion

This study confirms that *Carica papaya* and *Newbouldia laevis* extracts have ameliorating effects on liver enzymes and body weight 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 liver better; and also control muscle wasting, reduce levels of liver enzymes 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.

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Conflict of interest: None declared.

Ethical Approval: Approved by Institutional ethical approval.

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