**EFFECT OF ETHANOLIC LEAF EXTRACTS OF CARICA PAPAYA AND NEWBOULDIA LAEVIS ON KIDNEY ENZYMES OF 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 kidney 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 final weights of the animals were determined and they were sacrificed; blood samples were collected from each of the animals for serum analysis. **Results:** There were significant (P˂0.05) increase in serum levels of urea and creatinine 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:** This study has revealed that ethanolic leaf extracts of *C. papaya* and *N. laevis* have ameliorative effects on the body weight and serum levels of urea and creatinine on alloxan-induced Wistar rats.

**KEYWORDS:** *Carica papaya, Newbouldia laevis, Diabetes Mellitus, kidney enzymes.*

1.0 **INTRODUCTION**

Diabetes mellitus (DM) is a typical metabolic disease usually characterized by hyperglycemia, engendered by defects in insulin secretion (Insulin-Dependent Diabetes Mellitus, IDDM or Type 1 DM) and peripheral tissue resistance to insulin (Non-Insulin-Dependent Diabetes Mellitus; NIDDM or Type II DM) or both.[1,2] Chronic hyperglycemia in concert with dyslipidemia elicits hyperoxidative stress, which in turn, have been implicated in the pathogenesis of micro- and macrovascular complications such as retinopathy, nephropathy, neuropathy, myocardial infarction and atherosclerosis in DM state.[3,4,5] Furthermore, DM pathophysiology manifest in form of tissue/organ dysfunctions with multidimensional alterations in cellular metabolism.[6,7] Epidemiological survey showed that DM, among other dilapidating pathologic conditions, is the leading cause of morbidity and mortality of all age groups worldwide.[8] According to the World Health Organization report of the year 2000, 2.8% of global population was afflicted with DM and epidemiological projections showed that the number is expected to rise to 366 million (4.4% of global population) by 2030.[9] These statistics obviously underscore the enormous public health concerns of DM pathology, which calls for the development of new anti-diabetic remedies and improvement in already established anti-diabetic therapies for attainment of global health and wellness. Studies have shown that patients suffering from chronic diseases such as DM are turning away from the use of synthetic drugs to herbal remedies as alternative therapeutic strategy.[10,11]

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.[12, 13, 14] Apart from being the primary source of food for animals including humans, a great proportion of plant species have shown over times to have wonderful medicinal values.[15, 16] Some of the plants have shown to have some antidiabetic effects over the years.[15, 17] Such medicinal plants include *Carica papaya* (*C. papaya*) and *Newbouldia laevis* (*N. laevis*).

*C. papaya* is an herbaceous plant with prominent leaves (20-60 cm long), a member of the *Caricaceae* family and is widely cultivated for its edible pleasant fruit, which provides good nutritional value and easy digestion. Its leaf has been proven to have antidiabetic effect[18], hypoglycemic properties[19], antioxidant, immunomodulatory, and hypolipidemic effects[20], and is
also helpful in preventing diabetic complications by dyslipidemia improvement.\textsuperscript{[21]}

\textit{N. laevis} as one of such plants is a tree of the family Bignoniaceae in the order Bignoneae and is a genus of one species. It is used in folkloric medicine to treat a number of diseases. Some of which include treatment of ear aches, sore foot, chest pain, fever, convulsion and epilepsy in children\textsuperscript{[22, 23]}, diarrhea,\textsuperscript{[24]} Studies have shown that \textit{N. laevis} leaf extracts is used to treat diabetes mellitus\textsuperscript{[25, 26]}, have hyperglycemic effect\textsuperscript{[27]} and anti-diabetic properties\textsuperscript{[28]}, and lower blood glucose level in diabetic rats.\textsuperscript{[29]}

Thus this study was carried out to investigate the combined effect of ethanolic leaf extracts of \textit{C. papaya} and \textit{N. laevis} on the serum levels of urea and creatinine in alloxan induced diabetic rats, as no study has been carried out on this.

\section{MATERIALS AND METHODS}

\subsection{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 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.

\subsection{Collection and preparation of plant materials}

\textit{Carica papaya} and \textit{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.

\subsection{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 intraperitoneal 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 food 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.\textsuperscript{[29]}

\subsection{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.

\begin{itemize}
  \item \textbf{Group A} \hspace{1cm} (The control group) distilled water.
  \item \textbf{Group B} \hspace{1cm} (Diabetic group) distilled water.
  \item \textbf{Group C} \hspace{1cm} Diabetic + 200mg/kg of \textit{Carica papaya} leaf extract.
  \item \textbf{Group D} \hspace{1cm} Diabetic + 400mg/kg of \textit{Carica papaya} leaf extract.
  \item \textbf{Group E} \hspace{1cm} Diabetic + 200mg/kg of \textit{Newbouldia laevis} leaf extract.
  \item \textbf{Group F} \hspace{1cm} Diabetic + 400mg/kg of \textit{Newbouldia laevis} leaf extract.
  \item \textbf{Group G} \hspace{1cm} Diabetic + 200mg/kg of \textit{Carica papaya} and 200mg/kg of \textit{Newbouldia laevis} leaf extracts.
  \item \textbf{Group H} \hspace{1cm} Diabetic + 400mg/kg of \textit{Carica papaya} and 400mg/kg of \textit{Newbouldia laevis} leaf extracts.
\end{itemize}

\subsection{Sample collection and analysis}

The extracts were administered for 28 days. On the 29th day, the animals were sacrificed by anaesthetizing under chloroform vapour and dissected. Blood samples were assayed for levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using randox kit method.

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

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO. OF RATS (n)</th>
<th>TREATMENT RECEIVED</th>
<th>BODY WEIGHT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>Control (Distilled water only)</td>
<td>190.0 ± 10.0</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>Positive control (Diabetes + no treatment)</td>
<td>160.0 ± 7.07*</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>Diabetes + 200mg/kg <em>Carica papaya</em></td>
<td>182.0 ± 10.95</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>Diabetes + 400mg/kg <em>Carica papaya</em></td>
<td>220.0 ± 20.0*</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>Diabetes + 200mg/kg <em>Newbouldia laevis</em></td>
<td>202.0 ± 10.95</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>Diabetes + 400mg/kg <em>Newbouldia laevis</em></td>
<td>222.0 ± 20.49*</td>
</tr>
<tr>
<td>G</td>
<td>5</td>
<td>Diabetes + 200mg/kg <em>Carica papaya + 200mg/kg Newbouldia laevis</em></td>
<td>244.0 ± 16.73*</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>Diabetes + 400mg/kg <em>Carica papaya + 400mg/kg Newbouldia laevis</em></td>
<td>258.0 ± 13.04*</td>
</tr>
</tbody>
</table>

*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 (258.0 ± 13.04) show significant increase in body weight when compared with the control group A (190.0 ± 10.0).

Figure 1: Effects of ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on serum urea level of alloxan induced diabetic wistar rats.

Figure 4.4 shows significant increase (P<0.05) in serum urea level in group B (15.6 ± 0.59) when compared with the control group A (10.7 ± 0.76). Conversely, no significant difference (P>0.05) in serum urea level was observed in groups C (10.7 ± 0.42), D (10.4 ± 1.34), E (10.9 ± 0.63), F (10.1 ± 0.71), G (9.7 ± 0.82) and H (9.84 ± 0.32) when compared with the control group A (10.7 ± 0.76).
Figure 2: Effects of ethanolic leaf extract of *Carica papaya* and *Newbouldia laevis* on serum creatinine level of alloxan induced diabetic wistar rats.

Figure 4.5 shows no significant increase (P<0.05) in serum creatinine level in group B (1.19 ± 0.04) when compared with the control group A (0.93 ± 0.07) in the bar chart above. However, groups C (1.01 ± 0.07), D (1.02 ± 0.06), E (0.99 ± 0.04), F (1.04 ± 0.05), G (0.95 ± 0.07) and H (1.20 ± 0.08) showed no significant difference (P>0.05) in serum creatinine level when compared with the control group A (0.99 ± 0.04).

4.0 DISCUSSION

In this study, the significant decrease 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 carried out by Ewenighi[30] which reported decreased in body weight in alloxan induced diabetic rats. Also, significant weight loss reported after inducing diabetes with streptozotocin on Wistar rats is in agreement with the result. [31, 32] The extract-treated groups however showed significant 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. [33]

The significant increase in serum urea and creatinine levels in group B (Figures 1 and 2) when compared with the Control group A could be due to renal damage. Anjaneyulu[34], Ezeigbo[35] and Yakubu[36] reported of elevated levels of serum urea and creatinine due to progressive renal damage, metabolic disorders in diabetes and continuous catabolism of amino acids leading to a higher production of urea by the urea cycle respectively. However, groups C - H (Figures 1 and 2) which showed no significant difference in serum urea and creatinine levels when compared with the control group A could have been positively ameliorated by the ethanolic leaf extracts of *N. laevis* and *C. papaya*.

5.0 CONCLUSION

This study confirms that *C. papaya* and *N. laevis* extracts have ameliorating effects on kidney 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 kidney better; and also control muscle wasting, reduce levels of kidney enzymes better than when the leaf extracts of the individual medicinal plants are used in the management of diabetes. Thus, the combined 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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