

Effect of *Gongronema latifolium* Leaf Extract on some Liver Enzymes and Protein Levels in Diabetic and non Diabetic Rats

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Abstract: The effect of leaf extract of *Gongronema latifolium* (GL) on some tissue enzymes and protein level of alloxan-induced diabetic rats was studied. Graded doses of the extract (200, 300 and 400 mg/kg b. w.) were administered to the diabetic and corresponding non-diabetic rats for 14 days. At the end of which total protein, aminotransferases (ALT and AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) were assayed in the liver whole homogenate (LWH). Total liver protein concentration which was significantly increased ($p < 0.05$) in untreated diabetic rats when compared to non-diabetic control rats was non significantly ($p > 0.05$) changed in all test groups after treatment, compared to the control. Whereas in the non diabetic counterparts, protein level decreased in all treatment groups relative to the non diabetic control. However, this decrease was only significant ($p < 0.05$) in the group administered 200mg/kg b. w. The decrease was therefore independent of the dose of extract. There was observed general significant decrease ($p < 0.001$) in AST, ALT, ALP and GGT activities of diabetic test groups when compared to the corresponding non-diabetic groups. Compared to the diabetic control there was significant decrease in AST, ALT, ALP and GGT activities ($p < 0.001$ for AST; $p < 0.05$ for ALT, ALP and GGT) of diabetic rats administered 300mg/kg b. w. However at a test dose of 400mg/kg body weight, ALP and GGT activities rather increased significantly ($p < 0.05$ for ALP; $p < 0.001$ for GGT) relative to diabetic control. Hence the water soluble fraction of ethanol extract of *Gongronema latifolium* leaf is not likely to cause liver pathology, and can provide alleviation and protection to the animal liver in chemical diabetes with a dose up to 300 mg/kg b. w.

Key Words: Alloxan, diabetes, *Gongronema latifolium*, liver enzymes, liver proteins, liver function.

INTRODUCTION

The liver, like the salivary gland and pancreas, is an outgrowth of the digestive tract, a very large and versatile organ endowed with metabolically active cells which independently coordinate virtually all biochemical transformations. Accordingly, the regenerating power of these cells is tremendous and involved metabolic, secretory, excretory, storage, detoxification, protective and synthetic functions, among others. Many plasma proteins are synthesized in the liver and their plasma levels therefore depend on the balance between synthesis and catabolism and/or loss from the body^[1]. These liver enzymes and proteins generally are very important biomarkers in the body utilized in the diagnosis and assessment of normal function or otherwise of the tissues, organs and the body as a whole. Major or minor changes in the integrity of cellular membranes in tissues or organs have culminated in changes in enzyme activities. For instance alanine amino transferase (ALT) and Aspartate aminotransferase (AST) are useful in detecting alterations in liver disease - hepatocellular damage or increased permeability of liver cells while ALT and

gamma glutamyl transferase (GGT) are implicated in extra-hepatic or intra-hepatic obstruction^[1]. This knowledge is also usually applied in monitoring the safety of xenobiotics such as trial drugs (including medicinal plant extracts), since the liver is preoccupied with the function of biotransformation of xenobiotics^[2]. The purpose of this study was therefore to investigate the impact of *Gongronema latifolium* leaf extract, an antidiabetic medicinal plant, on liver enzyme activities of diabetic rats with a view to ascertaining the toxicity implications of the plant when used traditionally in the management of diabetes.

MATERIALS AND METHODS

All reagents and chemicals used in this work were of analytical grade.

Collection and preliminary preparation of plant materials

Fresh leaves of *Gongronema latifolium* were obtained from Akpabuyo, Cross River State, Nigeria in the month of June, 2005. The leaves were identified and authenticated by a botanist in Department of Botany, University of Calabar,

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Nigeria. The leaves were oven-dried and crushed using laboratory KENWOOD blender (Kenwood Electric, KENWOOD LTD, England). The ground leaf powder was stored in a glass bottle with a plastic screw cap and kept in a refrigerator (4°C) prior to extraction.

Preparation of plant extract

The grounded leaves of *Gongronema latifolium* was extracted in ethanol by regular shaking in bottles for 12 hours. The mixture was then filtered and the filtrates concentrated *in vacuo* using a rotary evaporator (Labo rota 3000 Resona, Edwards model). Thereafter the extract was transferred into a round bottom flask and allowed to evaporate completely under pressure at 50°C. The extract was stored in a refrigerator until required for use. The concentration of the extract was gravimetrically determined.

Animal handling and treatment protocol

Fifty six (56) male albino rats of Wistar strain were used in this study. The animals were fasted for 24 hrs [3] and were made diabetic by intraperitoneal injection of 150 mg/kg body weight of alloxan monophosphate (purchased from Alpha Aesar, Johnson Matthey Shore Road, Heysham, Lance, Canada) reconstituted in a buffer. Diabetes was confirmed after 7 days in rats with fasting blood glucose (FBG) >300mg/dl. Other overt features of the diabetic state were loss of body weight, polyuria and polyphagia. The 56 male albino rats were divided into 2 major sets of 28 rats (diabetic and non-diabetic) each. A set comprised of 8 groups (4 diabetic and 4non-diabetic sub-groups) of 7 each, consisting of one control and 3 test groups. The control groups received placebo treatment (distilled water), while the test groups 2, 3 and 4 in each subgroup administered by oral intubation 200, 300 and 400mg/kg b.w. of the extract. Both feed and water were given *ad libitum* and treatment lasted for 14 days.

Preparation of sample (liver whole homogenate)

The experimental animals were after the last administration fasted for about 10-12hours and thereafter anaesthetized under chloroform fumes and sacrificed. Whole liver tissues were surgically removed weighed and stored frozen until use. About 1g of each liver tissue was chopped and thoroughly homogenized using ceramic/porcelain mortar and pestle. The homogenate was made up to a total volume of 5ml with Tris-buffer. Whole liver tissue protein level was measured for each sample using Biuret method. The whole homogenate was diluted 1ml in 10ml with the buffer and used for analysis of enzyme activities.

Biochemical assays

The analysis for ALP activity in the diluted sample was determined by an optimized and standardized colorimetric (Radox kit) method according to the recommendation of the German Society of Clinical Chemists (GSCC) [4]. Gamma glutamyl transferase (GGT) activity in the sample was determined using Biolabo S. A. kinetic method of Szasz [5]. The absorbances were measured using Optima Spectrophotometer SP-300 (Optima Inc. Chicago, USA). Alanine and aspartate aminotransferases' activities were also determined using analytical kits obtained from Radox with the method of Reitman and Frankel [6].

Statistical analysis

Data was expressed as mean ± standard deviation (SD) and analysed with the use of ANOVA, student's t-test at p < 0.05 confidence.

RESULTS

The results of liver protein levels and enzyme activities of diabetic rats are shown in table 2 while that of the non-diabetic rats in table 1. Total liver protein concentration was significantly increased (p<0.05) in untreated diabetic rats when compared to non-diabetic control rats (Table 1).

Table 1: Effect of *Gongronema latifolium* leaf extract on activities (u/g tissue) of selected enzymes and protein level in liver whole homogenate of non- diabetic rats.

	Group I	Group II	Group III	Group IV
		(control)		
Liver protein (g/100g tissue)	17.56±3.86	17.07±2.12 b ¹	17.40±2.37	15.29±2.99
Aspartate amino-transferase	1824.06±128.21	1753.83±53.56	2118.32±83.97	1504.41±84.14 b ¹
Alanine amino-transferase	1952.42±130.36	1915.71±108.04 b ¹¹	1992.35±69.41 b ¹	1739.17±57.67 b ¹
Alkaline Phosphatase	1035.63±44.65	1020.92±42.16	935.35±37.00	1352.73±38.17 b ¹¹
γ – glutamyl transferase	2199.25±86.86	2071.72±113.44	2299.73±78.14	2611.15±74.64

*Mean± SD of 7 determinations, b = significant vs control : I = p<0.05, II = p<0.001

Within the diabetic rats, liver protein levels were not significantly (p>0.05) changed in all test groups when

compared to the control. In the non diabetic counterparts, protein level decreased in all treatment groups relative to the non diabetic control. However, this decrease was only

significant ($p < 0.05$) in the animal group administered 200mg/kg b. w. and not in the 300 and 400mg treated groups. The decrease was therefore independent of the dose of extract used. There was observed general significant decrease ($p < 0.001$) in AST, ALT, ALP and GGT activities of diabetic test groups (table 2) when compared to the corresponding non-diabetic groups (table 1). Compared to the diabetic control there was significant decrease in AST,

ALT, ALP and GGT activities ($p < 0.001$ for AST; $p < 0.05$ for ALT, ALP and GGT) of diabetic rats administered 300mg/kg b. w. However at a test dose of 400mg/kg body weight, ALP and GGT activities rather increased significantly ($p < 0.05$ for ALP; $p < 0.001$ for GGT) relative to diabetic control. Also, these changes were not dose dependent.

Table 2: Effect of *Gongronema latifolium* leaf extract on activities (u/g tissue) of selected enzymes and protein level in liver whole homogenate of diabetic rats.

	Group I (control)	Group II	Group III	Group IV
Liver protein (g/100g tissue)	19.21±3.73 a ¹	21.10±2.98 a ¹	15.77±1.57 a ¹	18.06±1.57 a ¹
Aspartate amino- transferase	933.64±40.42 a ¹¹	1013.13±100.48 a ¹¹	804.02±61.79 a ¹¹ , c ¹	893.20±39.83 a ¹¹
Alanine amino- transferase	1242.63±169.04 a ¹¹	1197.57±54.21 a ¹¹	889.33±60.45 a ¹¹ , c ¹	1097.09±85.03 a ¹¹
Alkaline Phosphatase	305.83±68.77 a ¹¹	266.71±22.13 a ¹¹	244.18±3.91 a ¹¹ , c ¹	423.27±51.55 a ¹¹ , c ¹
γ - glutamyl transferase	1183.21±148.29 a ¹¹	1170.16±129.55 a ¹¹	952.87±18.28 a ¹¹ , c ¹	2189.60±129.94 a ¹¹ , c ¹¹

*Mean± SD of 7 determinations, a = significant vs corresponding non-diabetic group, c = significant vs diabetic control I= $p < 0.05$, II= $p < 0.001$.

DISCUSSION

The changes in the liver enzyme system have been used clinically in evaluating the toxicity of any extraneous substance to the living system. This is so, because, any derangement of biochemical processes in experimental animals due to presence of a xenobiotic would reflect as increase or decrease in the activity of such enzymes including AST, ALT and GGT used as indicators of liver injury^[7,8].

The pattern of change in AST and ALT activities in diabetic rats were not the same as in non-diabetic rats. In this study, the test and control diabetic groups showed decreases in AST activity relative to ALT, whereas in non-diabetic rats test group which received 300mg/kg body weight showed an increase in AST activity relative to ALT. This and the decrease in AST/ALT ratio in diabetic rats may be indicative of the pathology of the liver in diabetic condition. Increases in both aminotransferases (AST and ALT) are found in liver diseases with ALT activity being much higher than AST activity^[9,8]. Atangwho et al^[10] had earlier reported significantly high liver enzyme activity in diabetic rats compared to the non diabetic. From the present results the increase in enzyme activities were not dose dependent as was the case also with whole liver protein level. For instance at a dose level of 300mg/kg body weight, in diabetic rats, the AST, ALT, ALP and GGT activities were decreased significantly when compared to the control, whereas a test dose of 400mg/kg body weight, increased significantly ALP and GGT activities when compared to the control. Although this appears difficult to explain, but it clearly emphasizes the sensitivity and relevance of dose margins and precision in toxicity studies.

Gamma glutamyl transferase (GGT) is essential to the metabolism of antioxidant glutathione and as such is

believed to be important in providing protective properties against oxidative stress^[11]. High levels of GGT activity have been implicated in either primary or secondary liver cancer. Gamma glutamyl transferase (GGT) is also reported to be more sensitive than alkaline phosphatase (ALP) in detecting all forms of liver disease. The result of diabetic rats at a dose level of 400mg/kg body weight showed that ALP and GGT activities increased significantly when compared to the control. This observation may indicate induction by the leaf extract at that dose level. It is also increased by enzyme-inducing drugs. The results in Tables 1 and 2 showed that at a dose level of 400 mg/kg body weight, the leaf extract increased GGT activity considerably; indicating that at that dose the extract may be acting as an enzyme inducing drug, similar to some anticoagulants^[9]. Alkaline phosphatase has been reported to be involved in protein synthesis, glycogen metabolism and syntheses of certain enzymes and the transport of metabolites across the cell membranes^[12]. The results of alkaline phosphatase (ALP) at the dose level of 400mg/kg body weight, where its activity increased significantly, may have been due to disturbances in the secretory activity, or in the transport of metabolites or other hepatotoxic conditions^[12]. More importantly, at the dose level of 400mg/kg body weight, an increase in ALP activity could be drug induced as observed with over-dose of paracetamol, methyl dopa, isoniazid and certain steroids^[9]. The observed increase in liver total protein level of diabetic rats when compared to non-diabetic rats could possibly be due to liver tissue protein inductive response to the destruction of β-cells of the pancreas by alloxan. This response may be responsible for increasing the rate of metabolism required to eliminate foreign compounds^[13,14]. This observation may also indicate the frequent

susceptibility of diabetic animals including humans to infections^[15].

Conclusion

In general, these results suggest further and strongly too that water soluble portion of ethanol extract of *Gongronema latifolium* leaves is not likely to cause liver pathology in experimental animals particularly at doses below 400mg/kg b.w. Where such liver pathology had been developed as in the case of alloxan diabetogenesis in experimental rats, treatment with graded doses of the leaf extract up to 300 mg/kg body weight provided alleviation and protection to the liver of such animals. Generally, alloxan diabetogenesis was shown to suppress liver enzyme and protein expression in the liver.

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