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EVALUATION OF THE EFFECTS OF GONGRONEMA LATIFOLIUM ETHANOL LEAF EXTRACT ON THE LIVER ENZYMES OF IN THE ALLOXAN- INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Gongrenema latifolium is used by traditional healers in different localities for the treatment of various ailments including Diabetes mellitus without any scientific proof or validation. This study is aimed at investigating the effects of ethanol leaf extracts of *Gongrenema latifolium* on liver enzymes in adult albino rats. Statistical analysis was carried out using ANOVA. The different doses of the extracts also showed a significant decrease ($p<0.05$) in the value of Alkaline phosphatase (ALP), Alanin aminotransferase (ALT) and Aspartate aminotransferase (ASP). The extracts did not show any adverse effect in the liver based on the liver function test function test. This study has therefore provided some vital pieces of scientific information about the hepatoprotective effects of the extracts of *Gongronema latifolium* which can possibly contribute to its use as a herbal therapy in the management of diabetic patients

Keywords: *Gongronema latifolium, liver enzymes, Alloxan- induced diabetic albino rats*

INTRODUCTION

The use of plants for healing purposes predates human Herbal medicine, also called botanical medicine or phytomedicine refers to the use of parts of plants such as seeds, berries, roots, leaves, bark or flowers for medicinal purposes (Ehrlich, 2019). It may also be defined as popular stock or knowledge about medicinal properties of herbs and roots as treatment for common remedies and other diseases in the society, which had been handed down from generation to generation (Ayodhya and Baba, 2017). Studies have confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus (Bnouham *et al,* 2016). Numerous mechanisms of actions have been proposed for these plant extracts. Some hypotheses relate to their effects on the activity of pancreatic B-cells (synthesis, release, and cell regeneration) or the increase in the protective/inhibitory effect against insulinase and the increase in insulin sensitivity or the insulin-like activity of the plant extracts. Other mechanisms may involve improved glucose homeostasis (increase in peripheral utilization of glucose, increase in synthesis of hepatic glycogen and/or inhibition of intestinal glucose absorption), reduction of glycaemic index of carbohydrates, reduction of the effect of glutathione. All of these actions may be responsible for the reduction and or abolition of diabetes complication (Bnouham *et al,* 2016). Herbalism has a long tradition of use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing of disease (Ehrlich, 2019).

Blood glucose levels are controlled by a complex interaction of multiple chemicals and hormones in the body including the hormone insulin made in the beta cells of the pancreas. Diabetes mellitus consists of a group of syndromes characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease. Criteria for the diagnosis of diabetes mellitus have been proposed by several medical organizations (WHO, 2919). In recent years, developed and developing nations have witnessed an explosive increase in the prevalence of diabetes mellitus predominately related to life style changes and the resulting surge in obesity (King et al, 2018). The metabolic consequences of prolonged hyperglycemia and dyslipidemia, including accelerated atherosclerosis, chronic kidney disease and blindness pose an enormous burden on patients with DM and on the public health system (Goodman and Gilman, 2016).

Diabetes mellitus occurs throughout the world but is more common (especially type 2) in the more developed countries (Rother, 2017). The greatest increase on

prevalence is, however, expected to occur in Asia and Africa, where most patients will likely be found by 2030 (Roglic et al, 2014). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "western-style" diet. For past 20 years, diabetes rates in North America have been increasing substantially.

The study was done to determine the effect of the Gongronema latifolium ethanol leaf extract on the liver enzymes such as aspartate amino-transferase (AST), Alanine amino transferase (ALT) and alkaline phosephatase (ALP) in the Alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

STUDY AREA

This study was carried out in the department of Pharmacology, faculty of Pharmacy,Madonna University, Elele Rivers State, Nigeria.

Collection and identification of plant

The leaves of *Gongonema latifolium* plant were collected from a farmland in Umunze in Anambra State, South East, Nigeria and were identified by Mrs.Victoria Alozie, a taxonomist in Botany department of University of Nigeria Nsukka where a voucher specimen was deposited in the herbarium

EXPERIMENTAL ANIMALS

One hundred and thirteen (113) adult albino rats of both sexes weighing (150-180)g were obtained from the animal house of the department of Pharmacology and toxicology Madonna University, Elele, Rivers State Nigeria. The animals were housed in cages in a well-ventilated area in the animal house under room temperature and were allowed to acclimatize for two weeks before the experiment. The animals were fed on commercial pellet feeds and were given potable water ad libitum. They were fasted for 12hours before the commencement of the experiment, but were allowed water ad libitum.

Animal Ethics Approval from University senate Research committee

The study was conducted after obtaining full approval (reference number: MAU/SREC/A/17) from University senate Research and ethics committee of Madonna university Nigeria

EXTRACTION OF PLANT EXTRACT MATERIALS

The leaves of *Gongronema latiflium* were thoroughly washed with clean tap water and air-dried in the laboratory for 14 days. The leaves were pulverized into fine powder using electric grinding machine. Three vehicles were used for the extraction. For ethanol extraction, 500g of the powdered material was soaked in 1000ml of 80% ethanol, extract was concentrated using a rotary evaporator. The same process was repeated using distilled water and methanol respectively. The solid ethanol extract of the ethanol was kept in air-tight container and stored in a refrigerator and was used throughout the study of 40%.

DETERMINATION OF YIELD OF PLANT EXTRACT

This was carried out according to method proposed to Okoli et al, (2010). The plant extract was evaporated to dryness at a temperature of 40• in a hot oven using a previously weighed empty beaker.

Yield in percent was calculated using the formula;

Yield weight (g) of residue x 100

Weight of coarse powder

Induction of hyperglycaemia (experimental diabetes)

Alloxan (Sigma St. Louis U. S. A.) was dissolved in normal saline and was given to the animals at a dose of 150mg/kg intraperitoneally (LP) for 7days. Rats with blood glucose levels equal to or greater than 200mg/dl (11.lmmol/ were considered diabetic and used for this study.

Anti-diabetic treatment (Experimental Design)

Alloxan-induced diabetic albino rats which were fasted for 12 hrs were placed in five (5) groups (1-5) of 20 rats each and treated as follows; Group 1: Received normal saline intraperitoneally (ip) (negative).

Group 2: Received 5mg/kg body weight of glibenelamide intraperitoneally (i.p.) (positive control)

Group 3: Received 250mg/kg body weight of the ethanol leaf extract ip. Group 4: Received 500mg/kg body weight of the ethanol leaf extract ip. Group 5: Received l000mg/kg body weight of the ethanol leaf extract ip. All these groups received these treatments for 14 days.

Determination of Blood glucose level

All blood samples were collected by cutting the tail-tips of the overnight fasted rats. Blood samples for blood glucose estimation were collected at intervals 0, 2,4,8 and 24hrs following treatment.

These tests were carried out using a glucose enzymatic-colorimetric test kit-Glucose oxidase-peroxidase (GOD-POD) method, produced by Cyprus diagnostics (Belgium). The test principle is based on the oxidation of glucose by glucose oxidase (GOD) to gluconic acid and hydrogen peroxide. The hydrogen peroxide(H202)forms a red violet colour with a chromogenic oxygen acceptor, phenolaminophenazone in the presence of peroxidase (POD). The colour intensity is proportional to glucose concentration in the sample and the results were recorded as mMol/1.

Estimation of Liver Enzymes in the alloxan-induced Albino Rats after treatment withEthanol extract of Gongronema latifolium (Tietz, 2018)

After the alloxan-induced diabetic albino rats were treated for 14 days using the Ethanol leaf extract of Gongronema latifolium, the treated albino rats were placed into three (3) groups of twenty rats per group. Group 1 that received 250mg/kg body weight, group 2 that received 500mg/kg body weight of the Ethanol extract and group 3 that received l000mg/kg body weight of the Ethanol extract. Blood samples for Liver Enzyme assay were collected in tubes by cutting the tail-tips of the treated albino Rats. The Liver enzyme assayed to know if the extract had toxic effect on the liver cells were Alanine aminotransferase, (ALT), Aspatate aminetransferase (AST) and Alkaline phosphatase (ALT). The assays were carried out using ultra violet (UV) kinetic kits produced by Cypress diagnostics. The test is based on photometric determination of rate of

nicotinamide adenine' dinucleotide (NADH) consuption by pyruvate and oxaloacetate which is directly related to ALT and AST activities. At alkaline PH alkaline phosphatase catalyses the hydrolysis of p-nitrophenyl phosphate to yellow coloured p-nitro- phenolate and phosphate; the change in absorbance measured at 415nm is directly proportional to the enzyme activity (table 4.3). The normal ranges for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphate (ALP) were 3-15lU/L, 5-18l u/L and 25-92lU/L respectively.

STATISTICAL ANALYSIS

Statistical analysis was carried out using statistical package for social sciences (SPSS) version 19. One way analysis of variance (ANOVA) was adopted for comparison and the results were subjected to post hoc test using least square deviation (LSD). The data were expressed as ± standard error of mean (SEM).

RESULTS

Determination of liver enzymes activities (IU/L)

Class ±	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal Control	13.5±4.0	15.0±1.1	20.2±2.0
Diabetic control	16.0±2,0	20.5±1.0	100.2±.2.0
Diabetic+250mgGL	10.5±3.0	11.4±2.0	40.6±4.0
Diabetic+5mgGL	9.0±1.0	10.0±1.0	38.1±1.0
Diabetic+500mgGL	8.4±10	7.0±3.0	34.10±1.0
Diabetic+l000mgGL	6.2±10	5.1±1.0	30.20±1.0

ALT= Alanine amino transferase, AST = Aspartate aminotransferase, ALP= Alkaline Phosphatase

Assay for liver enzymes namely ALT,AST and ALP is important in assessing optimal liver function in diabetics treated with the extracts of Gongronema latifolium. The increase in ALT, AST and ALP were not statistically different from the normal control group, indicating possible hepato-protective effect of the plant extracts. The group that was administered the extract of Gongronema latifolium showed a significant reduction in ALT, AST and ALP levels compared to the diabetic control.

DISCUSSION

The ASP, ALT and ALP levels support the fact that the extract played a protective role since their levels were significantly reduced (P<0.05) in the groups of diabetic rats were administered with the extract compared to the diabetic control.

Reduction in the activity of these enzymes compromises their function and might account in part for the increased oxidative stress in the diabetic control rats in this study. Diabetes-induced alterations in glutathione peroxidase activity were reversed by treatment with the ethanol leaf extract of *Gongronema latifolium*. The ethanol leaf extracts of Gongronema latifolium in this study were effective in restoring the activity of glutathione peroxidase, superoxide dismutase and catalase.

The liver enzymes Alanin aminotransferase, Asparate aminotransferase and alkaline phosphatase assayed in this study showed that the ethanol leaf extract of Gongronema latifolium caused no hepatotoxic effect on the liver because there was no increase in the activities of these enzymes. All the liver enzymes of the treated rats were within normal ranges showing that the ethanol leaf extract of *Gongronema latifolium* did not cause any adverse effect on the liver.

CONCLUSION

In conclusion, the results of this study were able to establish that the ethanol extract of Gongronema latifolium possessed hypoglycaemic effect and was relatively safe, because it was non-toxic to the liver which might contribute to its use as a herbal therapy in the management of diabetes.

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