



SERUM TRANSAMINASE ACTIVITIES, ALBUMIN AND BILIRUBIN LEVELS IN SALT LOADED EXPERIMENTAL RATS TREATED WITH ETHANOL EXTRACT OF *GONGRONEMA LATIFOLIUM*.

*¹Ohadoma S.C. and ²Nnatuanya I.N.

¹Department of Pharmacology, College of Medicine, Imo State University, Owerri, Nigeria.

²Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Madonna University, Elele, Nigeria.

*Author for Corresponding: Dr. Ohadoma S.C.

Department of Pharmacology, College of Medicine, Imo State University, Owerri, Nigeria.

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ABSTRACT

Objective: To evaluate the effect of ethanol leaf extract of *Gongronema latifolium* on activities of serum transaminase, albumin, and bilirubin levels, which are indicators of liver function, in salt loaded rabbits. **Material and Methods:** Four groups comprising five rats each were used. Group I served as control (non-loaded with salt and non-treated with extract); Group IV received continuous salt loading; while Groups II and III were administered 100 and 300 mg/kg of extracts respectively, once daily for 14 days. The alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB) and bilirubin (BIL) levels were evaluated using Assay Kits (Randox Laboratories Ltd, United Kingdom BT 294 QY). Students t-test, ANOVA and Turkey-Kramer test were used to assess significance of difference due to administration of extract and the control. **Results:** Continuous salt loading showed significantly ($p < 0.05$) elevated serum ALT, AST, ALB and total BIL when compared with the control. Treatment with *G. latifolium* ethanol leaf extract, in the salt loaded rats, exhibited dose-dependent non-significantly ($p > 0.05$) compared with the control group. **Conclusion:** The leaf extract of *G. latifolium* was seen to be safe considering its effects on serum ALT, AST, albumin and bilirubin of the experimental rats; and may be useful in the management of possible deleterious effects of salt load to the liver.

KEYWORDS: Serum transaminase, albumin, bilirubin, salt loaded, *Gongronema latifolium*.

1. INTRODUCTION

Excessive dietary salt intake has been implicated in cardiovascular diseases especially hypertension.^[1] Elevated blood pressure leads to damages in various organs of the body including heart, kidneys, blood vessels, eyes, and the brain. As evidence on the possible relationship between excessive salt ingestion and hypertension in human abound^[2,3], excess salt also presented deleterious renal effects in spontaneously hypertensive rat (SHR). This is reflected by a massive proteinuria after 8 weeks of 8 percent salt loading.^[4] There are evidences that diet with high-salt was an independent determinant of renal injury^[5,6], and in normotensive and SHR rats, salt loading enhanced ventricular and renal fibrosis.^[7,4] The deleterious effect of salt load to the liver poses a lot of challenges in view of its metabolic function. In traditional settings, these deleterious effects can be managed with the use of medicinal plants such *Acalypha wilkesiana*.^[8] In this work, *G. latifolium* extract was treated in salt loaded rats to assess the liver function.

G. latifolium belongs to the family Asclepiadaceae, and referred to as bush buck.^[9] In South-eastern and South-western Nigeria, *G. latifolium* is known as “utazi” and “arokeke” respectively.^[10] The versatility in the use of *G. latifolium* have been reported for: maintenance of blood glucose level^[11], anthelmintic effect^[12], anti-inflammatory effect^[9], antioxidative effect^[10]; stimulation of appetite, reduction of post-partum contraction as well as enhance the resumption of the menstrual cycle in women that have recently put to bed.^[11] *G. latifolium* has been reported to contain a plethora of phytochemicals such as saponins, flavonoids, terpenoids, steroids, glycosides, alkaloids, tannins, and ascorbates.^[13,14]

2. MATERIALS AND METHODS

2.1. Plant material

Fresh *G. latifolium* leaves were obtained from Owerri, Imo State, Nigeria; and authenticated at the Department of Pharmacognosy, Madonna University, Elele, Nigeria. The leaves were air-dried at room temperature for 4 weeks and ground into fine powder.

Portion (200 g) of the powdered leaves was soaked in 800 ml of ethanol (95%) for 72 h. The mixture was then filtered using a sintered funnel (equivalent to sheet of cheese cloth or four-fold bandage). The filtered was then concentrated using rotary evaporator.

2.2. Preparation of salt-loaded Diet

Sodium Chloride (8%) was prepared by mixing 8 g of analytical NaCl (BDH chemicals, England) with 92 g of the feed. The mixture was then fed to the experimental rodents as shown in the experimental design.

2.3. Animals

Twenty (20) adult Wister rats (150-220 g) kept in the Laboratory Animals facility of Department of Pharmacology and Toxicology, Madonna University, Elele, Nigeria, were used in this study. The animals were maintained under standard laboratory situations and had free access to standard pellets (Vital Feeds Plc, Nigeria) and clean water. The animals were transferred to work area prior to experimental use and allowed two weeks of acclimatization.

2.4. Experimental Design

The animals were randomly selected into four groups (I-IV) comprising five rats each.

Group I: Non-loaded (with salt) and non-treated (with extract) and served as control.

Group II: Salt-loaded and treated with 100 mg/kg of extract

Group III: Salt-loaded and treated with 300 mg/kg of extract

Group IV: Continuous salt-loading.

The animals in Groups II-IV were fed with the salt-loaded diet for a period of 60 days, after which Group II and III were treated with 100 and 300 mg/kg of ethanol extract respectively for 14 days. Group IV animals were administered salt-loaded diet continuously until the 74th day; while Group I animals were neither administered salt-loaded diets nor treated with the extract hence, served as control.

2.5. Collection of blood

After 60 days of salt-loaded diets and prior to treatment (day 61) with the extract, blood samples were collected from the vein located on the dorsal side of the ear lobes of the experimental rats, using sterilized hypodermic

needles. At day 75 after treatment (day 74) with the extract, blood samples were also collected. Samples were collected into fluoride oxalate and plane universal bottles immersed in ice. Immediately after collection of blood, the tubes were centrifuged at 3,500 rpm for 10 min to obtain clear plasma (fluoride oxalate) and serum (plane bottles) for subsequent analysis.

2.6. Biochemical Assay

Alanine transaminase (ALT), Aspartate transaminase (AST), Albumin (ALB) and Bilirubin (BIL) levels were evaluated using Assay kits (Randox Laboratories Ltd, United Kingdom BT 294 QY). The principle was based on colorimetric measurement.

2.7. Statistical Analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM) (n=5). Significance of difference was tested by Student's t-test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San Diego, California USA). Statistical significance was set at $p < 0.05$.

3. RESULT

The outcome of oral administration of ethanol extract of *G. latifolium* leaves on some serum parameters in salt-loaded experimental rats, are as stated below. After 60 days of salt-loading, serum ALT and AST activities were shown to be significantly ($p < 0.05$) higher in all the salt loaded groups, as compared with the non-loaded (control) group (Table I). After administration of the extract, only Group IV (administered continuous salt load) at day 74 maintained the increase ($p < 0.05$) as compared with the control (Table II). Other groups (II and III) showed dose dependent non-significantly ($p < 0.05$) higher values for ALT and non-significantly ($p > 0.05$) lower AST activities as compared with the untreated group. There were significant reduction of ALB serum level in rats administered with the extract which were concentration-dependent. The lower concentration 100 mg/kg showed greater effectiveness than 300 mg/kg. The ethanol of *G. latifolium* leaves and/or treatment with salt loading showed non-significant ($p > 0.05$) effects in serum bilirubin of the experimental rats.

Table I: Day 61 effect of the serum ALT, AST, ALB and BIL of salt-loaded rats treated with ethanol extract of *G. latifolium*.

Group	ALT(U/L)	AST (U/L)	ALB (mg/dL)	BIL(mg/dL)
I Control (No salt, no extract)	24.30 $\pm$ 1.40	29.65 $\pm$ 6.10	2.6 $\pm$ 0.24	1.10 $\pm$ 0.04
II Salt loaded + extract (100 mg/kg)	33.67 $\pm$ 7.00	71.82 $\pm$ 8.90	2.5 $\pm$ 0.54	1.08 $\pm$ 0.03
III Salt loaded + extract (300 mg/kg)	37.17 $\pm$ 2.50	64.00 $\pm$ 4.50	2.8 $\pm$ 0.90	1.16 $\pm$ 0.31
IV Continuous Salt loading	45.33 $\pm$ 2.70*	73.50 $\pm$ 13.50*	2.6 $\pm$ 0.16*	1.22 $\pm$ 0.28*

Where ALT = alanine transaminase, AST = aspartate transaminase

ALB = albumin, BIL = bilirubin. Data represent means $\pm$ SEM

(n=5), * $p \leq 0.05$, SEM = Standard error of the mean.

Table II: Day 75 effect of the serum ALT, AST, ALB and BIL of salt-loaded rats treated with ethanol extract of *G. latifolium*.

Group	ALT (U/L)	AST (U/L)	ALB (mg/dL)	BIL (mg/dL)
I Control (No salt, no extract)	27.60 $\pm$ 4.25	34.73 $\pm$ 6.08	1.60 $\pm$ 9.40	0.82 $\pm$ 0.04
II Salt loaded + extract (100 mg/kg)	32.17 $\pm$ 4.30	34.67 $\pm$ 2.70	1.50 $\pm$ 3.15	0.79 $\pm$ 0.09
III Salt loaded + extract (300 mg/kg)	39.00 $\pm$ 1.05	33.07 $\pm$ 3.71	1.80 $\pm$ 6.15	0.63 $\pm$ 0.12
IV Continuous Salt loading	53.17 $\pm$ 3.28*	66.00 $\pm$ 10.70*	3.20 $\pm$ 0.16*	0.85 $\pm$ 0.09*

Where ALT = alanine transaminase, AST = aspartate transaminase

ALB = albumin, BIL = bilirubin. Data represent means $\pm$ SEM

(n=5), * $p \leq 0.05$, SEM = Standard error of the mean.

4. DISCUSSION

Serum ALT and AST activities were significantly ($p < 0.05$) higher in all the salt-loaded groups, as compared with the control group. Only Group IV which was continuously administered salt-loaded, maintained the increase ($p < 0.05$) at day 74; while other Groups (II and III) exhibited dose-dependent non-significantly ($p > 0.05$) values as compared with the untreated (control) group. This may indicate the protective tendency of the plant against the possible hepatocellular harm emanating from the salt load. The *G. latifolium* leaf extract may have acted on glutathione peroxidase which later reduced oxidative stress that normally would damage the hepatocytes.^[15] The persistent rise in ALT and AST activities of the group administered continuous salt load as against decrease activities of the treated groups is indicative of the possible adverse effect of the salt-load on assessment of the liver functional status. The decrease in ALT and AST activities in the treated groups also indicated the possible protective effect of the plant against the adverse effects on the liver occasioned by the salt load. High levels of ascorbates found in *G. latifolium* leaves^[14] which are antioxidants may be attributed to the hepatoprotection. Ethanol extract of *G. latifolium* leaves and/or treatment with total bilirubin (conjugated and unconjugated) levels of the experimental rats. This is in agreement as elevated total bilirubin levels are observed in cases of intravascular haemolysis.^[16]

One of the common tools for the assessment of the usefulness of herbs and likelihood to reveal their potential toxicity or safety is biochemical evaluation of medicinal plants. Alanine transaminase (ALT) also called alanine aminotransferase or serum glutamate pyruvate transaminase is an enzyme predominant in hepatocytes. Upon a cell damage, ALT leaks into the blood together with other cellular contents where they can be measured in acute liver damage, ingestion of xenobiotics, acetaminophen overdose or viral hepatitis, ALT rises dramatically.^[17] Aspartate transaminase (AST)

also called aspartate aminotransferase or serum glutamate oxalate transaminase (SGOT) is similar to ALT in that it is another enzyme associated with parenchymal cells of the liver. AST is elevated in acute liver damage, but not specific to liver because it is also present in red blood cells, skeletal and cardiac muscles. Sometimes, the ratio of AST to ALT is useful in differentiating between causes of liver damage.^[18] AST can also be used as a cardiac marker and not specific for liver damage.

CONCLUSION

G. latifolium leaf may be useful in the management of any possible deleterious effects of salt-load to the liver, considering its effects on serum ALT, AST, Albumin and bilirubin of the experimental rats.

CONFLICT OF INTEREST: No conflict of interest declared.

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