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EFFECT OF AQUEOUS EXTRACT OF VERNONIA AMYGDALINA AND ZINGBER OFFICINALE ON BIOCHEMICAL PARAMETER IN ALLOXAN INDUCED DIABETIC WISTER RATS

H. O. Asuzu-Samuel*

Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt.

***Corresponding Author: H. O. Asuzu-Samuel**
Biomedical Technology, School of Science Laboratory Technology, University of Port Harcourt.

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ABSTRACT
Focus on plant research has increased in recent times all over the world and results have shown an immense potential of some plants in various traditional use as effective in medicinal values as well as food supplements. Following the rise in people suffering from one or more kidney diseases, this study is aimed at investigating the effects of the combination of extracts from both bitter leaf and ginger on the biochemical indices of alloxan induced diabetic rats. A total of 25 malewister rats weighing 136-200g/wt were purchased from the animal house at Choba, Port Harcourt and were transported to the Department of Biomedical Technology Laboratory, University of Port Harcourt. At the end of 7 days of acclimatization, the animals were randomly selected into 5groups (GROUP A- E) (n=5) 10% of alloxan solution was made (1g Alloxan/ 100ml of distilled water). 2ml of the drug (alloxan solution)was administered to the experimental group B-E for a day. This enabled us to determine the diabetic status of the experimental animals. After this process, the rats were observed for 24 hours, at the end of which the proper treatment (administration) of plant extracts started. They were allowed for three days to confirm if the rats were diabetic or not. After the confirmation, 2 ml of bitter leaf extract was given to the bitter leaf group (Group C) and 2 ml of ginger extract was given to the ginger group (Group D), then 2 ml of both extracts (bitter leaf and ginger extracts) was given to the ginger and bitter leaf group(Group E) for fourteen days. The results of the study showed that oral administration of aqueous bitter leaf and ginger extracts, administered at 2ml had little or no significant effect on the biochemical parameters except on bicarbonate which was significant at ($P<0.05$.) as shown in tables. Also, ginger and bitter leaf extracts decrease blood glucose and can possibly aid diabetic healing with increase time and possibly dose. From the results obtained, it shows that the administration of bitter leaf and ginger has the ability to relives diabetics and has no harmful effect on the kidney of Wister rats.

KEYWORDS: Aqueous Extract, *Vernonia Amygdalina, Zingber Officinale and* Biochemical.

INTRODUCTION
The term disease broadly refers to any condition that impairs normal function, and is therefore associated with dysfunction of normal homeostasis. Normal renal function is very important for homeostasis, so much, that situations in which renal functions are impaired can be life threatening. The basic functional unit of the liver is the liver lobule which is a cylindrical structure several millimeters in length and 0.8 to 2 millimeters in diameter. The human liver contains 50,000 to 100,000 individual lobules (Guyton & Hall, 2006). Its anatomical position is placed it in a greater part of the right hypochondriac region, part of the epigastric region and extending into the left hypochondriac region. Its upper and anterior surfaces are smooth and curved to fit under surface of the diaphragm (Guyton & Hall, 2006). Hence, the main function of the liver is filtration and storage of blood, metabolism of carbohydrate, proteins, fats, hormones, and foreign chemicals, as well as formation of bile, vitamins, irons and coagulation factors, making the liver is an extremely active organ. The kidney is another interesting organ that plays the important role of ridden the body of its waste materials that are either ingested or produced by metabolism. It also plays the vital role of body fluid balancing in the body. For water and virtually all electrolytes in the body, balance between intake (due to ingestion or metabolic production) and output due to excretion or metabolic consumption) is maintained in large part by the kidneys (Guyton & Hall, 2006). The human body has two kidneys which lie on the posterior wall of the abdomen, outside the peritoneal cavity. Each kidney of the adult human weight about 150 grams and is about the size of a clenched fist. The medial side of each kidney contains indented region called the hilum though

which pass the renal artery and vein, lympatics, nerve supply, ureter, which carries the final urine from the kidney to the bladder, where it is stored until emptied. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in International trade (Ahmad *et al.*, 2008). In certain African countries for instance, up to 90% of the population still relies exclusively on plants as a source of medicines (Ahmad *et al.*, 2008). The aim of this study is to ascertain the effects of aqueous combined bitter leaf and gingers extracts on liver and kidney of alloxan induced written rate. A complex disease like diabetes mellitus, where little is talked about in aspects of prevention and curation, but rather management, there is an increased focus on plants in the search for appropriate hypoglycaemic / antihyperglycaemic agents (Ahmad *et al.*, 2008). Ginger (*Zingiber officinale*) is also one of the most widely consumed spices for the flavoring of food worldwide (Li *et al*., 2012), It has been that, ginger had reported several beneficial pharmacological effects such as hypoglycemic, insulinotropic and hypolipidemic activities in humans (Andallu *et al*., 2003 and Bhandari *et al*., 2005), and in experimental animals (Ojewole, 2006, Madkor *et al*., 2010 and Abdulrazaq *et al*., 2011). The major chemical constituents of ginger rhizome are essential volatile oil and non-volatile pungent compounds, such as gingerols, shogaols, paradols and zingerone (Masood and Tauseef, 2011). *Vernonia amygdalina*, commonly called bitter leaf, is a small shrub that is native to tropical Africa. It is a member of the Asteraceae family whose leaves have been consumed for many years, either as a vegetable (macerated leaves in soup) or aqueous extracts for the maintenance of good health. One family of plant compounds, phytosterols, found naturally, mainly as β-sitosterol, campesterol and stigmasterol in *Vernonia amygdalina* and many food substances of plant origin with vegetable oils (especially unrefined oils), nuts, seeds and grains as the major dietary sources (Ahmad *et al.*, 2008) have been investigated for their biochemical roles in chronic diseases. β-sitosterol had earlier been reported in the literature to be effective as nutraceuticals and in combination therapy for the treatment of hypercholesterolemia, benign prostatic hyperplasia and breast cancer. It is also suggested to be immunomodulatory (Ahmad *et al.*, 2008), anti-artherogenic and antioxidative (Ahmad *et al.*, 2008).

MATERIALS AND METHODS

Sample Collection

A total of 25 female Wister albino rats weighing 136-200g/wt were purchased from the animal house at Choba, Port Harcourt and were transported to the Laboratory of the Department of Biomedical Technology, University of Port Harcourt. Fresh Ginger *(Zingiber officinale)* rhizomes were purchased from Choba Community Market in Obio-Akpor Local Government Area of Rivers State, Nigeria and Bitter leave *(Vernonia amygdalina)* were also collected from a garden in the same community. The plant materials were taken to the Department of Plant Science and Biotechnology, University of Port Harcourt, where they were identified correctly by Dr. B. C. Ndukwu of same department, with a voucher sample deposit at the Department of Plant Science and Biotechnology. Plant materials were returned to Laboratory of the Department of Biomedical Technology, where they were washed with sodium chloride solution to remove contaminants, they were dried at room temperature of (273^0C), they were further grinded to powder form, using the electric blender.

Acclimatization of the Animals

The animals were kept in the laboratory, where they were fed with animal feeds (finisher) and tap water for 7 days acclimatization.

Extraction of Bitter Leave and Ginger

200g of the powdered plants of ginger and bitter leave were separately soaked in 200ml of distilled water at ambient temperature for 48hours and filtered. The filtrates were stored in a refrigerator until when needed.

Administration of Alloxan

At the end of the 7 days acclimatization, the animals were weighed after which randomly selected into 5groups (GROUP A- E) (n=5)10% of alloxan solution was made (1g Alloxan/ 100ml of distilled water). 2ml of a drug (alloxan solution) was administered to the experimental group B-E for a day (24 hours), while group A (normal control) is administered with distilled water.

At the end of the 24hours, the diabetic status of each rat, were tested, using a glucometer and collecting blood from the tail of the rats. After this process confirms the diabetic status of the rats to be positive, proper administration of plant extracts begins.

Administration of Plant Extracts

At the end of the one day administration of alloxan solution, group C-E of the rats were administered with 2ml of plant extracts, group C with bitter leave extract, group D with ginger extract and group E with both ginger and bitter leave extracts. Group A is normal control and group B as diabetics control. Both group A and B were administered with only distilled water and their normal feeds, the period of administration was 14 days.

At the end of the administration, the rats were anaesthetized with diethylether in a desiccator before sacrifice. The rats were held in position on a dissecting board with dissecting pins and sacrificed by decapitation. Blood samples were collected and put into heparinized bottles that were properly labeled from which the renal biochemical parameters were determined.

Analysis of Renal Biochemical Parameters
Sodium
Specimen: plasma
Principle: the principle is based on the modification by Maruna and Trinder, in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate with the excess uranium then being reacted ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Assay Procedure
1. Labelled test tubes as blank, standard, control, etc.
2. Pipetted 1.0ml of filtrate reagent to all tubes
3. Added 50ul of sample to all tubes and distilled water to the blank
4. Shook all tubes vigorously and mixed continuously for 3 minutes.
5. Centrifuged tubes at high speed (1,500rpm) for 10 minutes and test the supernatant fluids as follows;

- Labelled test tubes corresponding to the above filtrate tubes
- Pipetted 1.0ml acid reagent to all tubes
- Added 50ul of supernatant to respective tubes and mixed.
- Added 50ul of colour reagent to all tubes and mixed.
- Zero spectrophotometer with distilled water at 550nm
- Read and recorded absorbance of all tubes.

Potassium
Specimen: plasma
Principle: the principle is determined using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7mEq/L.

Assay Procedure
1. Labelled test tubes as blank, standard, control, etc.
2. Pipetted 1.0ml of potassium reagent to all tubes
3. Added 0.01ml (10ul) of sample to respective tubes mixed and sat at room temperature for 3 minutes
4. After 3 minutes, set the wavelength of the spectrophotometer to 500nm, zero spectrophotometer with reagent blank. Read and recorded the absorbance of all tubes.

Chloride
Specimen: plasma
Principle: $Hg(SCN)_2 + 2Cl^- - HgCl_2 + 2SCN^-$

$3SCN^- + Fe^{3+}$ - $Fe(SCN)_3$ red complex
Chloride ion from a soluble non-ionized compound with mercuric ions will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480nm. The intensity of the colour complex produced is directly proportional to the chloride concentration.

Assay Procedure
1. Labelled test tubes as blank, calibrator, control.
2. Pipetted 1.5ml of chloride reagent to each tubes
3. Added 0.01ml (10ul) of calibrator or sample to respective tubes and mixed
4. Incubate at room temperature for at least five minutes
5. Set spectrophotometer to 480nm-520nm and zero with reagent blank.
6. Read and record the absorbance reading of all tubes.

Urea
Specimen: plasma
Principle: urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelots reaction.
$Urea + H_2O - \overset{Urease}{} 2NH_3\, CO_2$
NH_3 + Hydrochlorite + Phenol – Indophenol (blue compound)

Assay Procedure
1. Selected URMD in the run test screen and carry out a water blank
2. Pipetted 50ul of reagent to all tubes
3. Mixed and incubated at 37^0C for 15 minutes
4. Insert into the Monza flowcell folder and press read. The colour of the reaction of the reaction is stable for at least 8 hours.
5. Read and recorded the samples.

Creatinine
Specimen: plasma
Principle: Creatininein alkaline solution, react with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Assay procedure
Using fresh ddH_2O perform a new gain calibration in cuvette mode. Select CREA in the run test screen and carry out water blank as instructed.

RESULTS AND DISCUSSION
Table 1: Body Weight.

	Dose (mg/kg)	Initial	3 days after induction	One week of treatment	2 weeks of treatment
Normal control		160±0.00	168.40±2.41	169.2±9.4	184.20±7.43
Diabetic control	75±0.00	228±0.95	221.80±11.90	221.6±11.9	187±6.1
Bitter leaf	200±0.00	160±0.00	157±4.0	152±2.70	129±3.2
Ginger	200±0.00	160±0.00	154±2.5	174±25.6	121±5.0
Bitter leaf & Ginger	200±0.00	200±0.00	193±2.9	162.6±11.1	163±9.2

Data are expressed as mean ±SEM n=5

Table 2: Mean blood Glucose (Mmol/dl) of the experimental animal treated with combined extract of *Vernonia amygdalina* and *Zingiber officinale.*

	Dose (mg/kg)	Initial	3days after induction	One week of treatment	2 weeks of treatment
Normal control		2.7±0.8	8.7±1.9	7.6±1.4	4.2±0.1
Diabetic control	75	5.0±0.7	15.92±0.89	10±0.75	9.81±0.4
Bitter leaf	200	3.76±0.84	13±0.9	6.9±1.0	6.7±1.5
Ginger	200	3.48±0.42	10.2±0.8	6.32±0.9	7.08±1.3
Bitter leaf & Ginger	200	3.94±0.2	8.2±0.8	6.3±0.87	6.28±0.89

Data are expressed as mean ±SEM n=5

Table 3: Effects of aqueous extract of bitter leave and ginger on plasma level of renal biochemical parameters.

Paramters	Sodium (meq/l)	Potasium (meq/l)	Bicarbonate	Chloride (meq/l)	Urea (mmol/l)	Creatinine
Normal control	52.42±4.3	13.8±1.10	26.46±1.6	8.9±1.5	58.0±1.5	28.0±0.29
Diabetic control 75mg/kg	54.34±3.2	14.0±1.05	25.84±3.76	7.55±2.5	51.0±3.2	31.6±2.3
Bitter leaf 200mg/kg	48.8±8.9	12.5±1.3	48.5±36.9	4.908±0.91	51.8±21.3	0.62±1.9
Ginger 200mg/kg	40.4±17.7	10.6±2.0	26.92±1.43*	5.418±1.5	53.0±19.0	21.4±37.2
Bitter leaf and ginger 200mg/kg	59.3±9.6	13.3±1.1	25.5±1.66	7.05±2.7	45.3±26.9	0.0±0.00

Values are significant at (p<0.05), significance = *, N = 5

DISCUSSION

The plasma sodium, potassium, bicarbonate, chloride, urea and creatinine are a very important biochemical markers employed in renal dysfunction, impairment or in diabetics.

In the present study, the fasting blood glucose level was higher in groups B, C, D and E than in group A rats, before the administration of plant extracts. Study according to table 2, also reveals a decrease in blood glucose, which indicates improvement on the diabetic status of rats from an initial 3.76±0.84 to 13±0.9 three days after induction of alloxan, to 6.9±1.0 in a week of treatment and 6.7±1.5 in two weeks of treatment with bitter leave extracts only in group C. In group D, there was a decrease plasma glucose from an initial 3.48±0.42 to 10.2±0.8 three days after induction of alloxan, to 6.32±0.9 in a week of treatment and 7.08 ±1.3 in two weeks of treatment with only ginger extracts in group D. similar simultaneous decrease of plasma glucose occurred from an initial 3.94±0.2 to 8.2±0.8, three days after induction of alloxan, to 6.3±0.87 in a week of treatment and 6.28 ±0.89 in two weeks of treatment with combination of bitter leaf and ginger extracts in group E, though, no significant difference was observed across all groups, possibly an extended week of continuous treatment would have resulted in a significance change. The results as shown in table 4.1, also reveal a continuous decrease in body weight of rats in the first and second weeks of treatment with plant extractsacross groups (C, and E) from an initial 160±0.00 to 157±4.0 three days after induction of alloxan, to 152±1.0 in a week of treatment and 129±3.2 in two weeks of treatment with only bitter leaf extracts in groupC. In the case of groupD, there was an almost significant increase in body weight of rats at one week of treatment but with a decrease body weight of rats in the second week of ginger extract administration, from an initial 160±0.00 to 154±2.5 three days after induction of alloxan, to 174±25.6 in a week of treatment and 121±5.0 in two weeks of treatment with only ginger extracts in group D. However, the case was different in group E as body weight of rats slightly increased in the second week of administration of both bitter leaf and ginger extracts, from an initial 200±0.00 to 193±2.9 three days after induction of alloxan, to 162±11.1 in a week of treatment and 163±9.2 in two weeks of treatment with both bitter leaf and ginger extracts in group E. At the end of the first and second weeks of administration of bitter leaf, ginger and both ginger and bitter leaf extracts to group C, D and E respectively and only distilled water to group B, plasma sodium was decreased to 48.8±8.9 the treatment group C, 40.4±17.7 in group D, while it increase to 59.3±9.6 in group E from the control 52.42±4.3, however, no significant changes were obtained. Plasma potassium recorded a decrease in across group C, D and E to 12.5±1.3, 10.6±2.0 and 13.3±1.1 respectively from the normal control13.8±1.10 with no significance. This may be due to the dose response or short period of extract administration. Plasma bicarbonate recorded an increase value in group C (48.5±36.9) and a significant decrease ($p<0.05$) in group D (26.92±1.43). This indicates an improvement in renal function with the administration of only ginger extracts. Further decrease in bicarbonate occurred in group E (25.5±1.66) when compared to the normal controlof 26.46±1.6 with no significant change. Plasma chloride recorded a continuous decrease in group C, D and E to 4.908±0.9, 5.418±1.5 and 7.05±2.7 respectively from the normal control of 8.9±1.5. However, there was no significance

but result shows a possible improvement in plasma chloride in rats that receives bitter leave and ginger extract. Plasma urea also recoded also recorded a decrease across all groups from the normal control (58.0±1.5), to 51.8±213 in group C, 53.0±19.0 in group D and 45.3±26.9 in group E. The results show that there are not different in plasma creatinine with a 0.62±1.9 in group C, 21.4±37.2 and 0.0±0.00 in group E, from the normal control 0f 28.0±0.29. This indicates that ginger and bitter leaf extracts administered together an completely clear from the blood. Possible reason for this result may be that the beta cells have already been completely destroyed by alloxan or is no longer sufficient for its activities and the possible improvement by the function of the remaining beta cells was not sufficient to return the plasma sodium, potassium, bicarbonate, chloride, urea, creatinine and blood glucose level to normal or significant state in spite of the effect of ginger and bitter leave extracts. The decline in the effects of ginger and bitter leaf extracts on the body weight of rats could possibly be that the administered dose of alloxan was excess and has totally destroyed all the beta cells which would have ease healing.

CONCLUSION

Oral administration of aqueous bitter leaf and ginger extracts, shows that Ginger causes significant increase ($P<0.05$.) in bicarbonate, with no significance in sodium, potassium, chloride, urea and creatinine. Also, ginger and bitter leaf extracts decrease blood glucose and can possibly aid diabetic healing with increase time and possibly dose. From the results obtained, it shows that the administration of bitter leaf and ginger has the ability to relives diabetics and has no harmful effect on the kidney of Wister rats.

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