African Journal of Pharmacology and Therapeutics Vol. 5 No. 4 Pages 235-240, 2016
Open Access to full text available at http://journals.uonbi.ac.ke/ajpt

Research Article

Biochemical parameters in alloxan induced diabetic rats treated with glibenclamide, metformin and two polyherbal bitters

Emmanuel I. Etim [a,*], Awanabasi E. Udoh [b], Idara N. Udodok [a], and Nsikan M. Udo [b]

[a] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria*
[b] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria*

* **Corresponding author:** Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, P.M.B 1017, Uyo, Nigeria. **Tel:**+234-80-23792373, **Email:** iwetim@gmail.com

Background: Manufacturers and promoters of various polyherbal bitters claim that, being of natural origin, they could be co-administered with therapeutic drugs with no adverse effects, and that it could be used to treat a wide array of ailments including diabetics. Most Nigerians use the bitters and their conventional drugs concurrently.

Objectives: To assess the effects(s) or otherwise of the co-administration of two popular bitters in Nigeria market S-bitter and Y-bitter with two therapeutic antidiabetic drugs glibenclamide and metformin on some liver and kidney biochemicals and lipid profile.

Methodology: Therapeutic doses of glibenclamide, metformin and the bitters alone and a combination of the drugs and the bitters corresponding to the body weight of the rats were administered orally to different groups daily for fourteen days. On the 15th day, the rats were sacrificed and plasma collected was analyzed for the hepatic biochemicals (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total and direct bilirubin), lipid biochemical (high density lipid cholesterol, low density lipid cholesterol, total cholesterol and triglycerides) and renal biochemicals (creatinine and urea).

Results: When metformin, glibenclamide and the bitters were administered alone, there was a marginal decrees in the levels of alanine aminotransferase, and a significant increase ($p<0.05$) in the plasma levels of creatinine and blood urea nitrogen. A combination of the bitters with metformin and glibenclamide caused a significant decrease ($P<0.05$) in the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin, but there was an increase in the levels of total cholesterol, high density lipid cholesterol, low density lipid cholesterol, triglycerides and albumin.

Conclusion: From the results, we conclude that the co-administration of the bitters with therapeutic drugs is hepatoprotective by reducing the levels of liver enzymes and bilirubin, and by increasing levels of lipid biochemicals, it could lead to the development of heart disease. We therefore advise users of the bitters to do so separately and not in combination with conventional drugs.

Key words: Polyherbal bitters, biochemicals, glibenclamide, metformin.

Received: November, 2016
Published: December, 2016

1. Introduction

Diabetes mellitus is a complex chronic illness requiring continuous medical care (WHO, 2014). As of 2013, 382 million people had diabetes worldwide (IDF, 2016). The pancreas is a storage deport for digestive enzymes and hormone, among which is insulin which promotes uptake of glucose by most cells especially those of the liver, skeletal muscle and adipose tissues thus lowering plasma glucose concentration, (Rang et al, 2007). The complications of diabetes mellitus are morphological consequences of many altered metabolic pathways which may be associated with increased free radical, hepatic enzymes and plasma lipid activity, (Etim et al,

 ISSN 2303-9841

2011; Oberley, 1988). Alloxan, a β-cytotoxin produces massive destruction of the β-cells of the islet of Langerhans, resulting in decreased endogenous insulin release which leads to decrease glucose utilization by tissues and a resultant diabetic (hyperglycemia) condition (Rang et al, 2007). An abnormality in glucose metabolism influences liver function enzymes, lipid metabolism and other endogenous biochemicals (Etim et al, 2011).

The bitters are galenical oral preparations made from a blend of various parts and fruits of plants. Manufacturers of the various bitters claim that, they cure a wide variety of ailments among which are kidney and bladder infections, normalize intestinal motility, lower blood sugar, act as digestive aid and detoxify the body, (Etim et al, 2016). Some researchers agree that some bitters have potent antidiabetic efficiency, (Jimmy and Udofia, 2014). The bitters are widely used for diabetic treatment in Nigeria (Jimmy and Udofia, 2014). Components of the bitters are known to interact with and influence the pharmacokinetics and blood glucose levels of therapeutic drugs, (Jimmy and Udofia, 2014; Etim et al, 2016).

Some enzymes act as indicators of disease states. Enzymes levels in the serum or plasma form an integral part of diagnosis. Increase in the level of enzyme in plasma is indicative of cell damage. Liver disease is the most important cause of increased alanine aminotransferase (ALT) and aspartate aminotrasferase (AST) activity. In hepatocellular injury or necrosis, there is leakage of cytoplasmic enzymes into the systemic circulation and as such the level of the enzymes in the plasma in higher (Hasper and Jorres, 2011; Etim et al, 2013).

Lipid profile which involve total cholesterols (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and triglycerides (TG), serve as diagnostic indices in conditions such as chronic obstructive jaundice, hepatitis, coronary heart disease, while cholesterol is the major lipid constituent of arteriosclerotic plague, (Rang et al, 2007; Ekpo et al, 2007).

Creatinine wich is derived from creatine and phosphocreatine is a major constituent of muscles. When creatinine is released from the muscle into plasma, it is excreted almost exclusively by the kidney through glomerular filtration process. It is neither re-absorbed, secreted, synthesized nor metabolized by the kidney hence the clearance of creatinine is equal to the glomerular filtration rate (GFR). A decrease in the GFR would result in an increase in the plasma creatinine concentration, thus the determination of the plasma creatinine concentration is used in the clinical evaluation of patients with suspected renal disease, (Onyeneke et al, 2000; Marshal and Bangert, 2009).

The end product of protein metabolism is urea and it is produced solely by the liver. After production, it travels through the blood and is excreted by the kidneys. Because the blood urea nitrogen (BUN) is completely filtered at the glomerulus of the kidneys, then reabsorbed and tubularly secreted within the nephrons, the concentrations of BUN reflect renal function. Hydration status, protein intake and some drugs which affect renal blood flow will affect the BUN of individuals (Etim et al, 2012).

Since consumers of these bitters may do so concurrently with some therapeutic drugs, this study sought to assess the effect or otherwise on liver enzymes and other biochemicals, lipid profile, creatinine and urea levels in alloxan induced diabetic rats treated separately and concurrently with two brands of widely used bitters in Nigeria SB and YB and two popular therapeutic antidiabetics GLI and MET. The results obtained would enable us to ascertain the safety or otherwise of these act, and to advise consumers accordingly.

2. Methodology

2.1 Animals

A total of sixty healthy albino rats of both sexes (Wister strain) weighing between 160 – 230 ± 21g were used in the study. They were maintained under standard environmental condition and had free access to food and water at the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo.

Permission and approval for animal studies were obtained from College of Health Science, Animal Ethics Committee, University of Uyo.

2.2 Induction of diabetes

The animals were fasted overnight, and diabetes was induced by a single intraperitoneal injection of freshly prepared solution of alloxan (120 mg/kg body weight) in ice-cold 0.9% saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia (Okokon et al, 2009). Control groups both positive and negative were injected with normal saline alone.

Seventy two hours after the administration of alloxan, the blood glucose levels of the alloxan treated rats was checked for the development of diabetes, and those with moderate diabetes having hyperglycemia (blood glucose level range above 200mg/dl) were considered as diabetic and used for the various drug combination treatment (Okokon et al, 2009).

2.3 Experimental design

The diabetic rats were divided into nine groups A to I, with six animals in each group. Eight groups were for the various drug combination treatment while the ninth group was the negative control and was treated with distilled water.

A positive control group comprising six non-diabetic rats were treated with distilled water only and was used for comparison of the normal variation of the various biochemicals in rats (Okokon et al, 2009).

2.4 Administration of test materials

The various drugs and combinations were administrated daily for 14 days as shown in **Table 1**. At the end of 14 days, the animals were sacrificed under chloroform anaesthesia and blood was collected by cardiac puncture, into EDTA tubes. The whole blood collected was allowed

to equilibrate with the anticoagulant. The blood was then centrifuged for 20 minutes at 5,000 rpm. The plasma was aspirated into sterile sample tubes and stored at -4^{o}C for the various analysis.

Table 1: Administration of test substances

Group	Test substance administered
A	Glibenclamide alone 5mg/kg body weight
B	Glibenclamide 5mg/kg + S-bitters 15ml/kg
C	Glibenclamide 5mg/kg + Y-bitters 20ml/kg.
D	Metformin alone 15mg/kg
E	Metformin 15mg/kg + S-bitters 15ml/kg
F	Metformin 15mg/kg + Y-bitters 20ml/kg
G	S-bitter alone 15ml/kg
H	Y-bitter alone 20ml/kg
I. (diabetic rats)	(Negative control) distilled water 3ml/kg
J. (non-diabetic rats)	(Positive control) distilled water 3ml/kg

2.5 Analysis of plasma:

The prepared plasma was analyzed using BS – 120 Chemistry Analyzer after mixing the plasma with different diagnostic kits and incubating at 37^{o}C for time depending on the compound analyzed, following the methods as outlined in Clinical Chemistry Solutions Product List Mindray Brochure. The compounds were measured as follows;

Hepatic Biochemicals:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using ALT and AST diagnostic kit according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) without pyridoxal phosphate activation (Marshall and Bangert, 2009 ; Chemistry Solutions Brochure, 2012). The absorbance was read at 340nm against sample blank which automatically recorded the concentration of ALT and AST in international unit (U/L)

Alkaline phosphatase (ALP) was measured using ALP diagnostic kit with the modified IFCC method. The absorbance was read at 405nm against sample blank and the concentration of ALP was recorded in international unit (U/L).

Total and direct bilirubin (T-BIL. And D-BIL) were analyzed with the various diagnostic kit using the Diazotized Sulfonic Acid (DSA) method. The absorbance was read at 546nm against sample blank and the concentration of T-BIL and D-BIL recorded in µmol/L (Whitby et al, 1984).

Albumin (ALB) was measured using Bromocresol Green Method (BCG). The absorbance was read at 578nm and the ALB concentration was recorded in g/L (Marshall and Bangert, 2009; Chemistry Solutions Brochure, 2012).

Lipids:

High density lipid cholesterol (HDL-C) and low density cholesterol (LDL-C) were measured with the respective kits using the direct method by incubating for 5 minutes at 37^{0}C and measuring the absorbance at 600nm. HDL-C and LDL-C were given in mmol/L.

Total Cholesterol (TC) was measured with TC diagnostic kit using Cholesterol oxidase-Peroxidase (CHOD-POD) method. The absorbance was read at 510nm against sample blank and the concentration of TC was recorded in mmol/L.

Triglycerides (TG) was measured with TG diagnostic Kit using Glycerokinase Peroxidase –Peroxidase method (GPO-POD). The reagents and plasma were thoroughly mixed and incubated at 37^{0}C for 10 minutes. The absorbance was read at 510nm against sample blank, and the concentration of TG was recorded in mmol/L (Marshall and Bangert, 2009).

Renal Biochemicals:

Creatinine was measured with creatinine diagnostic kit using modified Jaffe method. The absorbance was measured at 510nm and creatinine concentration was given in µmol/L, while Urea kit using Urease-glutamate Dehydrogenase UV method at 340nm was used to measure urea (Marshall and Bangert, 2009; Onyeneke et al, 2003)

2.6 Statistical analysis

Data were expressed as mean ±SEM. Statistical comparisons between groups were performed using analysis of variance (ANOVA). Difference between mean were determined by Turky – Kramer pair-wise comparison test at a level of $p<0.05$

3. Results and Discussion

The results for the administration of MET, GLI, the bitters and the various combination, for liver enzymes ALT, ALP and AST as well as bilirubin are given in **Table 2**. The values are compared to the positive control which were given neither the drug, the bitters nor their combination. When MET and GLI and the bitters were administered alone, there was a marginal decrees in the levels of ALT, but a combination of the bitters and the conventional drugs MET and GLI caused significant decrease ($P<0.05$) in the levels of ALT. The same trend was observed for ALP and AST (**Table 2**).

These results suggest a hepatoprotective effect by components of the bitters and synergism in restoring liver function when used with therapeutic drugs (Kazim et al, 2015). MET, GLI, SB and YB when administered alone caused a significant decrease ($P<0.05$) in the levels of TBIL and CBIL (**Table 2**). Low levels of CBIL indicates that bile is properly excreted and these is no obstruction in the bile duct or gall bladder. Low levels of unconjugated bilirubin indicates that less haemoglobin is being destroyed or that the liver is actively treating the

haemoglobin it is receiving. (Grazisdei 2011). There was no effect on the levels of plasma albumin when the drugs and bitters were administered as mono-therapy and in combination with each other as shown in **Table 2**.

There was an increase in the levels of total cholesterol, HDL, LDL and triglycerides when MET, GLI, SY, YB and their various combinations were administered to the diabetic rats compared to negative controls (**Table 3**). Clinical knowledge of the levels of plasma lipids is an important biochemical tool in the toxicity or beneficial effects of foreign compounds. Plasma lipids are predominantly resident in body tissues. The physiological and pathological state of body tissues is highly associated with metabolism, level of plasma lipids and lipid peroxidation (Etim et al, 2011). In situation where there is high activity of these lipids in the body tissues due to oxidative damage, associated with lipid metabolism, the administration of antioxidants ameliorates tissue dysfunction (Robert, 2012).

Hyperlipidemia is one of the risk factors for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plague (Etim et al, 2011). The gradual increase in the lipid profile of diabetic rats treated with GLI, MET, SB, YB, and their combination and negative control indicates that there might be a gradual buildup of lipid which may result in coronary heart disease (Ekpo et al, 2007). The increase in the level of triglyceride in diabetic rats is because of the destruction of the acinar cells in pancreas responsible for the secretion of pancreatic lipase essential for lipid digestion. The high level of the various lipids indicates that the antidiabetic drugs and the bitters alone and in combination do not have the ability of rapid regeneration of the acinar cells (Hensley et al, 2000).

Table 2: Plasma level of liver enzymes and biochemicals in rats treated with therapeutic drug (GLI and MET), the bitters (SB and YB) and a combination of therapeutic drugs with the bitters.

	ALT (U/L)	**TBIL**(µmol/L)	**DBIL**(µmol/L)	**ALP** (U/L)	**AST** (U/L)	**ALB** (g/L)
MET	107.2±6.11	8.78±2.87	4.65±1.77	612.5±16.71	322.4±11.37	38.40 ±2.11
MET+SB	96.3±20.91	10.44±1.63	9.31±2.8	491.9±18.4	186.85±16.5	37.80±1.20
MET+YB	129.1±1.56	10.53±1.01	5.67±1.10	460.5±11.32	239.9±3.91	38.00±1.00
GLI	111.45±2.41	7.62±0.05	5.79±0.35	998.6±13.51	334.65±0.15	38.70±0.51
GLI+SB	66.5±6.31	10.82±2.15	8.06±2.65	433.3±20.52	250.65±23.65	38.70 ± 0.90
GLI+YB	67.2±1.90	10.36±1.12	8.66±0.46	402.8±9.801	226.05±11.45	38.40±1.00
SB	101.3±8.55	6.71±0.41	4.85±0.21	582±16.13	297.15±18.5	38.25±.25
YB	111.75±2.75	7.46±1.42	4.81±0.49	699.4±18.11	355.7±9.1	37.95±1.33
CON NC	96.4±6.71	12.94±1.33	9.06±2.13	579.2±7.63	203.30±6.11	33.00±3.12
CON PC	121.16±2.11	12.70±1.91	9.93±2.00	736.7±8.71	168.7±3.7	35.80±1.20

Values are expressed as mean ± SEM significance relative to positive control P<0.05, (n=6)

Table 3: Lipid profile of rats treated with therapeutic drug (GLI and MET), the bitters (SB and YB) and a combination of therapeutic drugs with the bitters.

Material administered	**Lipid profile (mmol/L)**			
	TC	**HDL –C**	**LDL – C**	**TG**
GLI alone	2.78± 0.45	1.02 ±0.07	1.14±0.10	1.27±0.14
GLI + SB	2.93 ± 0.91	1.51 ±0.17	0.93±0.30	1.30±0.17
GLI + YB	2.32 ± 0.42	1.40±0.07	1.05± 0.15	1.30±0.14
MEt alone	2.11 ±0.63	1.32±0.17	1.22± 0.15	1.21±0.11
MET + SB	2.42±0.63	1.11±0.21	0.96± 0.27	1.05±0.09
MET + YB	3.21±0.93	1.15±0.15	1.04±0.11	1.51±0.04
SB alone	2.17±0.40	1.19±0.18	0.86±0.43	1.45±0.49
YB alone	2.29± 0.10	1.11±0.21	1.18±0.11	1.25±0.14
Distilled water(NC)	2.67 ±0.43	1.48±0.23	1.61±0.06	1.77±0.12
Distilled water (PC)	1.31 ± 0.32	0.90± 0.01	0.56±0.01	0.75±0.31

Values are expressed as mean ± SEM significance relative to positive control p<0.05,(n=6)

 ISSN 2303-9841

Table 4: Plasma levels of creatinine and urea in rats treated with therapeutic drug (GLI and MET), the bitters (SB and YB) and a combination of therapeutic drugs with the bitters.

	CREA – J	UREA
MET	77.90±2.18	25.40±1.33
MET+SB	65.25±3.75	12.36±2.45
MET+YB	83.70±2.11	29.87±3.13
GLI	81.20±3.10	10.35±1.50
GLI+SB	59.45±2.95	10.35±2.20
GLI+YB	73.20±6.52	12.25±3.21
SB	76.15±3.65	16.36±3.41
YB	63.30±3.40	22.94±3.54
CON NC	51.40±2.11	8.41±1.36
CON PC	63.30±3.40	10.32±1.10

Values are expressed as mean ± SEM significance relative to positive control p<0.05, (n=6)

There was a significant increase in the plasma levels of creatinine and BUN when GLI, MET, SB and YB were administered as monotherapy (**Table 4**). This suggest a decrease in glomerula filtration and an adverse effect on the kidney (Etim et al, 2013). When GLI and MET were respectively administered with SB, the levels of both creatinine and BUN returned to normal. This was not the case when they were administered with YB. This suggest that there may be a component of SB which reacts with MET and GLI respectively to enhance kidney functions.

4. Conclusion

The results vary with the parameter studied. Combination of the bitters with therapeutic drugs cause synergism in liver protection, while there was increase in lipid profile which is a pointer to cardiovascular diseases. With renal efficacy, one bitter enhances renal function while the other had no effect. With the high number of bitters flooding our markets, and the high level of uncertainty of actions in various organs, we conclude that it is best to administer the bitters separate from therapeutic drugs.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Mr. Aniefiok Ukpong, laboratory technician, of the animal house of the department of Pharmacology and Toxicology, University of Uyo and Mrs. Ekaete Umoh, the Chief Technologist of the Department of Pharmaceutical and Medicinal Chemistry, University of Uyo for their technical assistance.

References

Clinical Chemistry Solutions Product List Brochure. Mindray Healthcare 3rd. version 2012. Hong Kong. Pp 6-63.

Ekpo A. Eseyin OA, Ikpeme AO, Edoho EJ. (2007). Studies on some biochemical effects of *Vernonia amygdaline* in rats. *Asian J. Biochem*. **2**:193-97

Etim OE, Bassey EI, Ita SO, Udonkang IC, Etim E. I and Jackson IL (2011). Modulatory effect of sweet almond extract on some serum lipid, lipid peroxidation and histology of pancreas in alloxen-induced diabetic rats. *Int. Res. J. Pharm. Pharmacol.* **1**:155-61.

Etim EI, Udoh IE, Udoh AE. (2012). Evaluation of plasma creatinine and urea levels after oral administration of artemisinin and some artemisinin combination therapy regimens to healthy Nigerian males. *World J. Appl. Sci. Technol.* **4**:1 - 6

Etim El, Essien EE, Eseyin OA, Udoh IE (2013). Effect of some artemisinin combination therapy regimens with send without concomitant administration of phospholipids on the levels of plasma aminotransferases and bilirubin in Nigerian male subjects. *Afr. J. Pharmacol. Ther.* **2**:17-25.

Etim El, Silas LM, Umoh UF, Akwaowo AE and Udodok IN. (2016). Effect of two polyherbal bitters on the pharmacokinetics of glibenclamide and metformin in rats. *World J. Pharm. Pharm. Sci.* **5:** 1259- 66

Graziadei IW (2011). The clinical challenges of acute or chronic liver failure. *Liver Int.* **31**:24-6.

Hasper D and Jorres A (2011). New insights into the management of hepato-renal syndrome. *Liver Int*. **31**:27-30.

Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA (2000). Reactive oxygen species cell signaling and cell injury. *Free Radic. Biol. Med*. **28**: 1456-61.

International Diabetes Federation (IDF) 2014 www.idf.org. (Accessed 10th.June, 2016)

Jimmy EO and Udofia AJ (2014). Yoyo bitters a potent alternative drug in the treatment of diabetes. *Int. J. Innov. Med. Health Sci.* **2**:1-5.

Kasim LS, Ewuoso OV, Etim EI, Daodu JO, Aderinola AA (2015). Screening of hepatoprotective effects of four different brands of polyherbal bitter formulations against tetrachloromethane

(CCl_4) induced hepatotoxicity in Wister rats. *Nig. J. Pharm. Appl. Sci. Res.* **4**:26 32.

Marshall, WJ and Bangert SK (2009). Text book of clinical chemistry 6th ed. Edinburg London. Oxford Philadelphia. pp 69-116.

Oberley IW (1988). Free radicals and diabetics. *J. Free Radic. Biomed*. **5**:113-24.

Onyeneke EC, Oghenejode EO, Okonkwo CJ and Okpagba NA (2003). Serum urea and creatinine levels in Nigeria human malaria patients. *Glob. J. Med. Sci.* **2**:103- 6

Okokon JE, Umoh EE, Etim EI, Jackson CL. (2009). Antiplasmodial and antidiabetic activities of ethanolic leaf extract of *Heinsia crinata*. *J. Med. Food.* **12**: 131-36.

Rang HP, Dale MM, Ritter, Flower RJ. Rang and Dale's Pharmacology. 6th.ed. Churchill Elsevier, Livingstone (2007). pp 397-409.

Robert KI (2012). Diabetes treatment bridging the divide. *New Engl. J. Med.* **356**: 1499-1501.

Whitby LG, Percy-robb IW and Smith AF. Enzyme test in diagnosis. In: lecture notes on Clinical Chemistry. 3rd.ed. Boackwall Scientific Publication, London (1984) pp138 -68.

World Health Organization. Diabetes facts sheet, 2014. www.who.int/diabetes/facts/en. (Accessed 13th. June, 2016).

 ISSN 2303-9841