ORIGINAL ARTICLE

Momordica charantia mitigates hepatic injury following adjuvant treatment with antiretroviral drugs in diabetic animal models

Ugochukwu Offor[1] · Edwin C. S. Naidu[1] · Oluwatosin O. Ogedengbe[1,2] · Peter I. Aniekan[1,3] · Onyemaechi O. Azu[1,4]

Abstract

Momordica charantia (*M. charantia*) is a medicinal plant, used in traditional practice for treating diseases like hypertension and diabetes mellitus. This study investigated the possible hepato-protective effect of *M. charantia* following treatment with highly active antiretroviral therapy (HAART) in diabetic rats. 48 adult male Sprague Dawley rats were divided into seven groups (A–G) of 7 animals per group and treated according to protocols. Diabetes was induced with streptozotocin (STZ) by intraperitoneal injection (45 mg/kg body weight). The animals were euthanized on the 10th week with liver removed for examination and blood obtained via cardiac puncture and centrifuged to collect the sera. Blood glucose levels (BGL) were consistently and significantly raised ($p < 0.05$) in all groups not receiving the adjuvant *M. charantia*. Treatment with *M. charantia* reverses the increase in BGL to near normal. Markers of liver injury assayed showed significant increase ($p < 0.05$) in AST, ALP and ALT levels in groups not receiving *M. charantia*. Adjuvant HAART and *M. charantia* caused significant declines in the liver enzymes ($p < 0.05$). Serum GGT was not markedly altered. Treatment with *M. charantia* significantly restored liver enzymes elevations to near normal comparable to control. Histopathological observations ranged from severe hepatocellular distortions, necrosis and massive fibrosis following treatment of HAART in diabetic groups not receiving *M. charantia*. Treatment with *M. charantia* did not show any sign of hepatotoxicity as judged from the histological and biochemical observations.

Keywords Highly active antiretroviral therapy · Hepatotoxicity · Liver · Diabetes mellitus · *Momordica charantia* · Sprague-dawley rats

Introduction

Antiretroviral (ARV) drugs have been shown to suppress HIV replication while reducing morbidity and mortality. However, as a counterweight to this positive impact, ARV drugs carries along deleterious effects, which challenge the management of HIV-infected patients to a great extent [1]. Some of these effects are nephrotoxicity, hepatotoxicity and other metabolic disorders such as hypertension and diabetes mellitus. These effects have been linked to the continuous use of ARV drugs [2].

ARV drugs were shown to inhibit insulin-stimulated glucose disposal by blocking the glucose uptake through glucose transporter isoform 4 (GLUT 4) [3]. Disturbances in glucose-insulin homeostasis through insulin resistance and impaired beta cell function predisposes to hyperglycemia and eventually diabetes mellitus and further complications of diabetes such as diabetic liver disease often called hepatogenous diabetes [4]. It is not clear how diabetic liver disease develop but however scientist have reported Porto systemic shunts and decreased overall liver mass through down-regulation of insulin receptors as a possible mechanism [3].

There are compelling evidences that increased consumption of fruits and vegetables reduces the risk of

✉ Ugochukwu Offor
ugochukwuoffor@yahoo.com

1 Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

2 Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Nigeria

3 Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo-Nigeria, Uyo, Nigeria

4 Department of Anatomy, School of Medicine, University of Namibia, Windhoek, Namibia

various pathological events, hence *Momordica charantia* L. (Cucurbitaceae) known as bitter melon, have been traditionally used worldwide, as an adjuvant in the therapeutic strategy for glycemic control aimed at preventing the development and progression of chronic liver failure associated with uncontrolled diabetes mellitus [5]. Studies have documented the hypoglycemic effects of *M. charantia* through physiological, pharmacological and biochemical mechanisms. It is believed to act via stimulation of peripheral skeletal muscle glucose utilization, inhibition of intestinal glucose uptake, [6], suppression of key gluconeogenic enzymes, stimulation of key enzyme of hexose mono phosphate (HMP) pathway, and preservation of β cell islet and its functions [5].

However, there is paucity of literature explaining the attenuating influence of *M. charantia* on the liver injury following treatment with ARV drugs and diabetic condition. Our study therefore sought to investigate the therapeutic potential of *M. charantia* in treating diabetic liver disease following antiretroviral treatment.

Materials and methods

Preparation of *M. charantia* fruit ethanolic extract

Fifty kilogram of fresh unripe green fruits of *M. charantia* was purchased from a local market in Durban South Africa and were authenticated in the Department of Life Science, Westville Campus, University of KwaZulu-Natal, with voucher no: 4617. The fruits of *M. charantia* was cleaned and sliced into small pieces and the seeds separated from it. The sliced green fruit was weighed and dried in shade and weighed again after drying to obtain the actual weight before pulverizing into fine powered in a grinder and stored at 5 °C until ready for extraction. Fruit powder was extracted with solvent (ethanol) by soxhlet extraction. Solvent was evaporated in a rotary evaporator at 40–50 °C with the percentage yield of 85.25%. The extract was filtered through whatman filter. The concentrated extract was stored at 4 °C until ready for use.

Drug and chemical

Highly active antiretroviral (HAART) regimen (Triplavar) (*Cipla-Medpro*) containing Lamivudine 150 mg, Nevirapine 400 mg and Zidovudine 300 mg, was used for this study. The drug was obtained from Pharmed pharmaceuticals, Pty (Ltd) Durban, South Africa. Streptozotocin (Sigma-Aldrich, St.

Louis, MO, USA) which was of analytical grade quality was purchased from Capital Lab Supplies, Durban, South Africa.

Animal management and experimental design

The University of KwaZulu Natal Animal Research Ethics Committee gave full approval of the project and assigned Reference Number: **AREC/033/016D**. A total of forty-eight (n = 48) adult male Sprague-Dawley rats weighing 178–232 grams (219.31 ± 36.17) were used for the study. Animals were housed in well ventilated plastic cages (4 rats per cage having dimensions of 52 cm long × 36 cm wide and 24 cm high with bedding of soft wood shavings. They were maintained under standardized animal house conditions (temperature: 23–25 °C; light: approximately 12 h natural light per day) and were fed with standard rat pellets from Meadow feeds (Division of Astral Operations Limited, Durban, South Africa) and given tap water ad libitum. The initial body weights of the animals were recorded before treatment. The animals were randomly assigned to the 7 treatment groups, (B–G) with 7 animals per group and 6 animals in the (−ve) control group A.

Group A served as −ve control
Group B served as +ve control (Diabetic)
Group C received Triplavar (Diabetic)
Group D received *M. charantia* (200 mg/kgbw) (Diabetic)
Group E received *M. charantia* (400 mg/kgbw) (Diabetic)
Group F received Triplavar + *M. charantia* (200 mg/kgbw) (Diabetic)
Group G received Triplavar + *M. charantia* (400 mg/kgbw) (Diabetic)

The therapeutic dose of triplavar was adjusted for animal weight using the human therapeutic dose equivalent for the rat model [7]. These substances were administered daily via oral route with orogastric tube. The study lasted for 10 weeks. Monitoring of the animals was carried out by the research team. Thereafter, the animals were euthanized at the 10th week and the tissues were harvested and prepared for observation.

Induction of diabetes mellitus

All rats were placed on a 12 h fast to obtain baseline fasting blood glucose levels (FBG). The diabetic groups were given intra-peritoneal streptozotocin (STZ) (Sigma-Aldrich Chemical Company, Missouri, St Louis, USA) at 45 mg/kg body weight dissolved in a citrate buffer (pH 4.5) [8]. Successful induction of diabetes was determined

by observation of polyuria and polydipsia and confirmed by a 72 h post STZ FBG level ≥ 11 mmol/L.

Measurement of blood glucose

Blood samples were obtained from the tail using sterile needle prick. Glucose levels were measured once a week during the 10 weeks treatment using the one touch ultra-glucometer (Boehringer-Mannheim, Germany).

Body and liver weight

Body weights of animals were recorded on the first day before treatment (initial), thereafter weekly during the 10 weeks treatment till the day of sacrifice (final). Liver weight (LW) was measured by an electronic balance (Mettler Toledo; Microsep (Pty) Ltd, Greifensee, Switzerland).

Assessment of liver function

Blood samples were collected through cardiac puncture and allowed to clot for 30 min and centrifuged for 15 min at 3000 revolutions per minute. The serum was decanted into Eppendorf tubes and prepared for biochemical analyses. Biochemical analyses of the serum enzymes for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were spectrophotometrically determined by the method of Reitman and Frankel [9].

Histopathological examination of liver tissues

Liver were weighed and examined for gross pathology. A phosphate buffer solution (PBS) was used to wash out blood before preparation for tissue fixation. They were sectioned at 5 μm thickness using Leica RM 2255 microtome. Tissues were stained with Haematoxylin and Eosin (H and E), for general assessment of liver structure. For histochemical studies, the tissues were stained with Masson's trichrome (MT) for the assessment of possible liver fibrous architecture [10]. The stained slides were scanned using a Leica SCN 400 (Leica Microsystems GmbH, Wetzlar, Germany) and measured at 200 magnification using image analyser Leica (DMLB) and Leica QWIN software.

Statistical analysis

Continuous variables (liver and body weights and liver function test), were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison posttest using Graph pad prism® statistical software 6.02. The results are expressed as mean ± SD (standard deviation). Values were considered significant at $p < 0.05$.

Results

Blood glucose levels

The blood glucose concentration for diabetic +ve control (group B) and diabetic treated with HAART-regimen (triplavar) alone was significantly high ($p < 0.05$) (Fig. 1). This level was sustained throughout and was peaked between 6th and 10th weeks. Treatment with *M. charantia* alone at both low and high dose as well as co-administration of *M. charantia* (low and high dose) and HAART showed significant mild hyperglycemia ($p < 0.05$) compared to normal control. From the 4th to 6th week of treatment, adjuvants treatment with *M. charantia* in all groups receiving *M. charantia* extract showed a significant lowering of blood glucose ($p < 0.05$). Between weeks 8 and 10, groups receiving *M. charantia* (low and high dose) together with HAART showed significant ($p < 0.05$) reduction in blood glucose to near normal levels (Fig. 1). This reduction of blood glucose level shows in addition, a dose dependent mitigation of hyperglycemia (Fig. 1).

Body weight and organ (liver) weight changes

While there was an overall increase in the final body weight (BW) in all groups, Groups, E and G recorded the highest increase in BW significant at $p < 0.05$ with the percentage BW gain recording 34.9% and 20.9% respectively, but however, lower when compared to control animals. Although there was a lower rate of body weight gain in all groups when compared to control, this was particularly so in Groups (B, C and F). Diabetes and HAART were related to lower rate of body weight gain as observed in Groups (B and C) with the percentage body weight
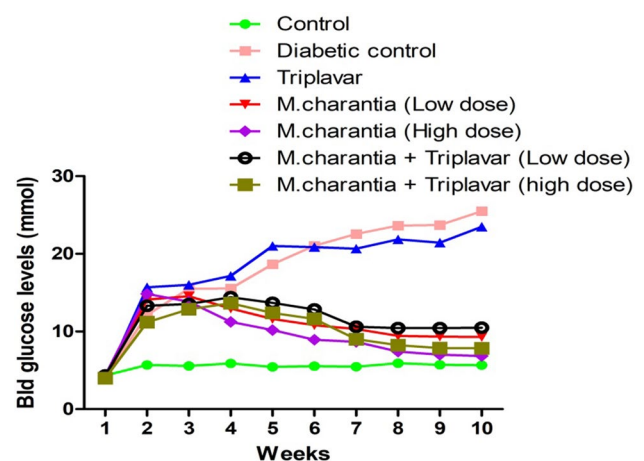

Fig. 1 Graphical representation of blood glucose levels

recording 11.74%, 12.77%. Groups D (administered with low dose (200 mg/kgbw) of *M. charantia* extract alone), and Group F (administered with low dose of *M. charantia* extract + HAART) showed a narrower range of weight gain when compared with Groups E (administered with high dose (400 mg/kgbw) and Group G (administered with high dose of *M. charantia* extract + HAART). Mean liver weight corresponds with the percentage BW gained across groups with the control group having an optimal mean liver weight (Table 1).

Liver function test

As shown in Table 2, the levels of AST, ALT and ALP were significantly decreased ($p < 0.05$) in animals treated with *M. charantia* alone as well as *M. charantia* + HAART (low and high dose) as seen in groups (D, E, F, G) when compared to groups (B and C). Treatment with HAART (group C) as observed showed significant increase in liver function parameters ($p < 0.05$). Similarly, the liver functional indices were elevated in the diabetic +ve control animals (group B).

Histopathological examination (H and E and MT)

The H&E stained sections of the liver tissues in control animals and animals treated with *M. charantia* alone and its co-administration with HAART revealed adequate preservation of hepatocellular architecture with the normal hepatocytes and no cytoplasmic vacuolation of liver cells and fine sinusoidal spaces (Fig. 2). The outline of hepatocytes and sinusoidal spaces were seen with no pathologies. Histopathological evaluation of groups treated with HAART alone as well as untreated diabetic group (B and C) showed various degrees of distortions and cytoarchitectural patterns ranging from extensive necrosis of hepatocytes with occlusion of sinusoidal spaces and cytoplasmic vacuolation.

Masson's trichrome stains fibrous tissue blue while the cytoplasm of hepatocytes are stained red and nuclei could be dark or red as well. Sections of liver in control group and groups treated with *M. charantia* alone and its co-administration with HAART showed normal red-stained hepatocytes. Photomicrographs from liver tissues of groups treated with HAART alone as well as untreated diabetic group showed extensive network of blue-stained fibrous components in a necrotized, disorganized hepatic tissue cord (Fig. 3).

Table 1 Body weight and liver weight of experimental and control groups

Groups	Initial BW (g)	Final BW (g)	Mean BW	BW difference	BW Diff in (%)	Mean LW	LWBR
A	178.1	351.5	261.7 ± 127.0	173.4	66.26	12.69 ± 1.41	4.85
B	227.1	255.3	240.1 ± 18.28	28.2	11.74	10.30 ± 1.79	4.29
C	227.1	258.1	242.6 ± 21.92	31.0	12.77	10.55 ± 1.22	4.35
D	212.6	258.0	235.3 ± 32.12	45.4	19.29*	10.07 ± 1.56	4.28
E	240.3	342.1	291.2 ± 72.02	101.8	34.9*	12.43 ± 1.89	4.27
F	217.9	249.9	233.9 ± 22.63	32.0	13.68	10.19 ± 1.58	4.36
G	232.1	286.3	259.2 ± 38.32	54.2	20.9*	11.34 ± 0.75	4.37

LWBR = (Mean LW/Mean BW) × 100. BW diff in% = (BW diff/Mean BW) × 100

BW body weight of rats, *LW* liver weight of rats, *LWBR* liver weight body ratio

*Values are expressed as mean ± SD for each group and considered statistically significant at $p < 0.05$

Table 2 Effect on serum AST, ALT ALP and GGT following treatment with HAART and *M. charantia*

Groups	AST (IU/L)	ALT (IU/L)	AST/ALT ratio	ALP (IU/L)	GGT (IU/L)
A	24.67 ± 3.05	27.67 ± 3.22	0.89	104.7 ± 29.37	4.33 ± 4.04
B	134.7 ± 31.50	104.0 ± 22.87*	1.29	558.7 ± 140.6*	19.00 ± 3.00
C	113.0 ± 44.54*	87.00 ± 22.27	1.29	225.3 ± 40.10	11.67 ± 2.52
D	32.33 ± 6.51*	33.67 ± 8.51	0.96	126.7 ± 14.19*	15.33 ± 6.81
E	31.67 ± 4.04	40.67 ± 6.03*	0.78	119.0 ± 26.63*	11.67 ± 6.51
F	29.33 ± 6.81	36.00 ± 12.53*	0.81	108.7 ± 27.79	9.00 ± 5.00
G	30.33 ± 5.13*	37.00 ± 4.58*	0.82	117.7 ± 14.57	4.33 ± 2.52

AST alanine amino aspartate, *ALT* alanine amino transferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transferase

*Values are expressed as mean ± SD for each group and considered statistically significant at $p < 0.05$
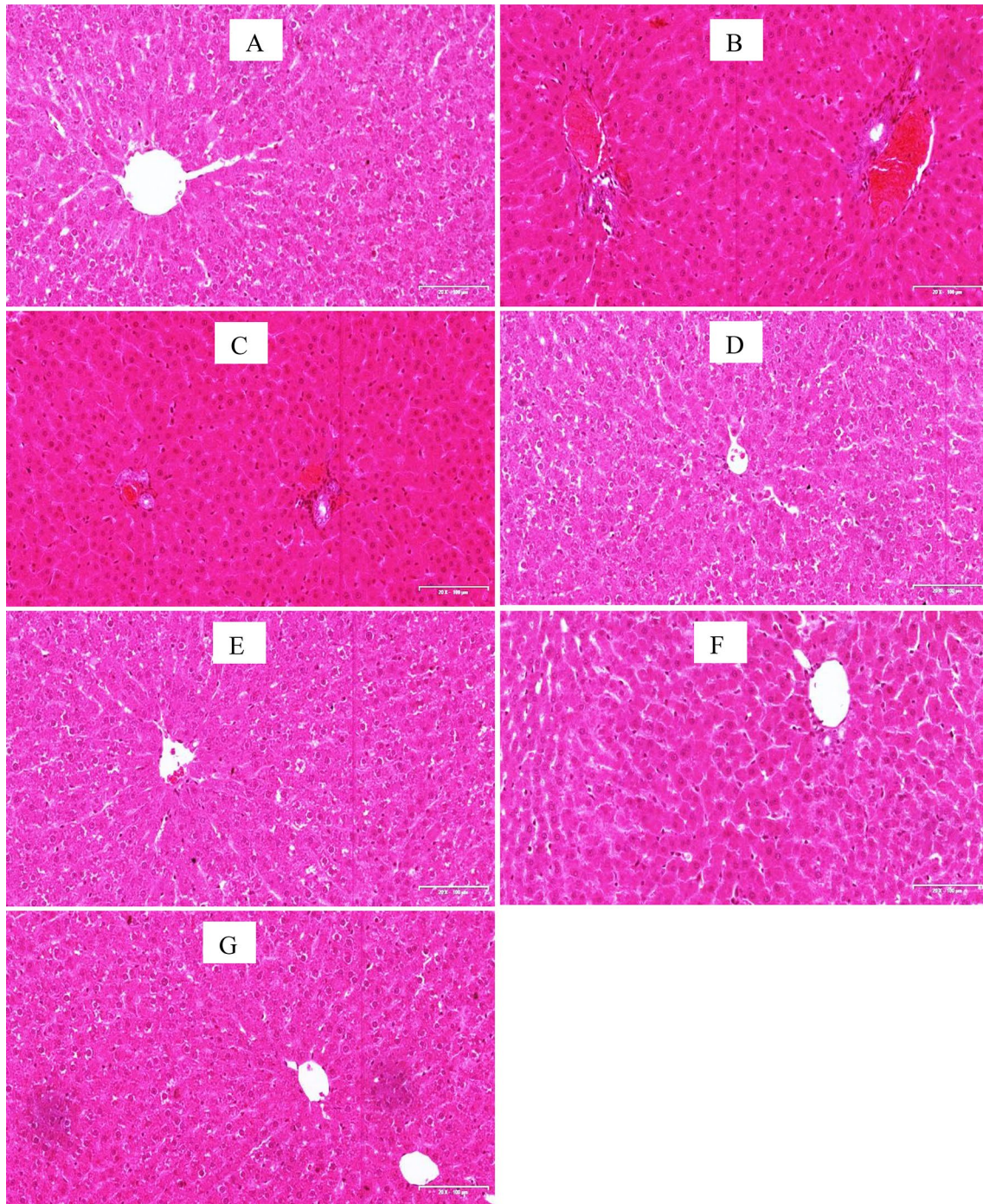
Fig. 2 Photomicrographs of liver sections (H &E stains). Scale bar 20×200 µm. Note the normal architecture of hepatocellular cords sinusoidal spaces and central vein in groups A, D, E, F and G. There are various degrees of distortion (mild to severe) in the radial arrangement of hepatic cords seen in groups B, extensive necrosis are observed in groups C. **a** −ve Control, **b** Diabetic +ve Control **c** Diabetic + HAART **d** Diabetic + *M. charantia* 200 mg/kg **e** Diabetic + *M. charantia* 400 mg/kg, **f** Diabetic + *M. charantia* 200 mg/kg + HAART **g** Diabetic + *M. charantia* 400 mg/kg + HAART

Discussion

Despite the substantial benefit in the reduction of morbidity and mortality by HAART, organ toxicities (especially liver) are frequently becoming a major concern for scientist and researchers ranging from mild side effects (elevated liver enzymes) to fatal complications (chronic liver failure, diabetic liver disease etc.) [11]. The liver performs numerous
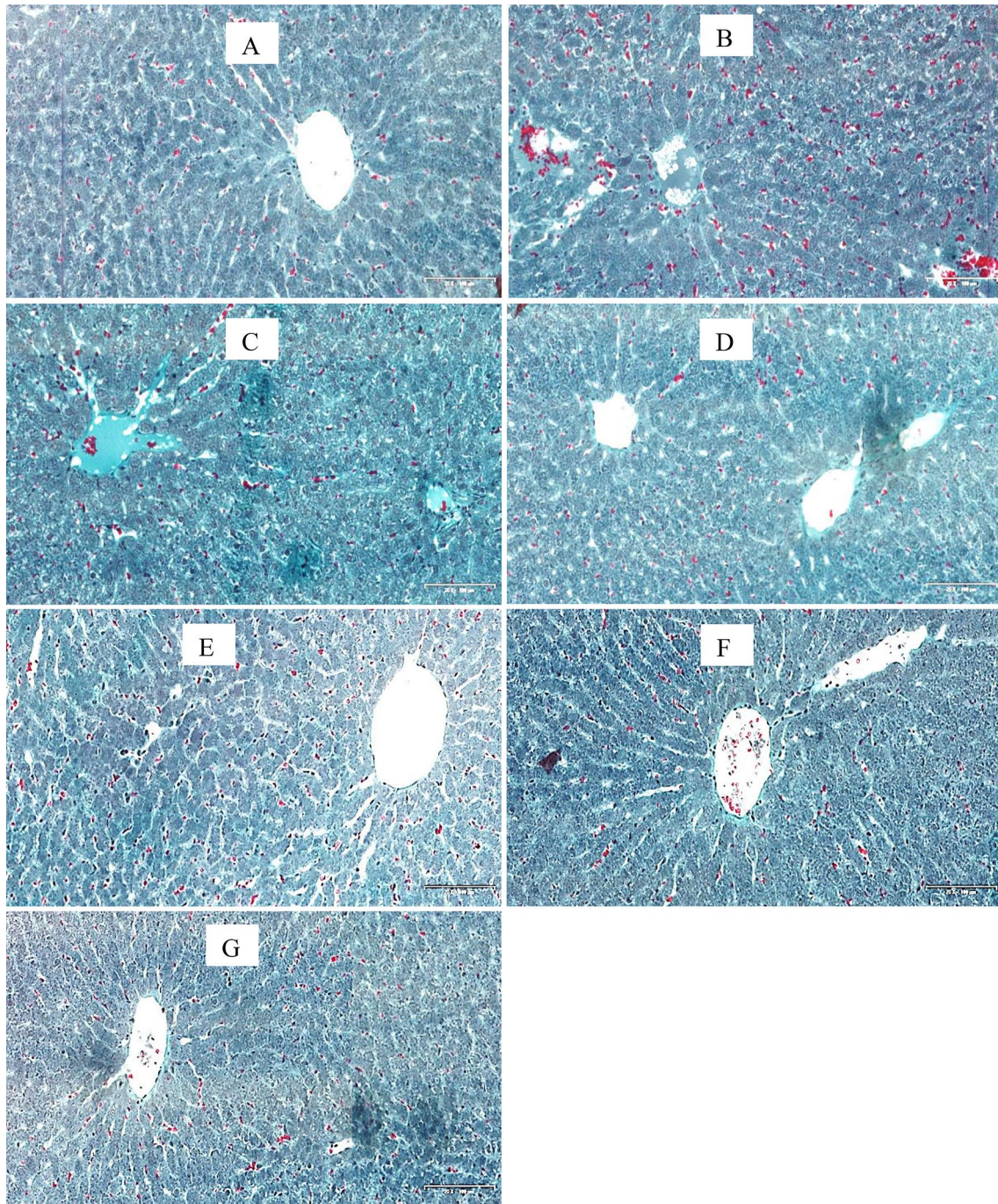
Fig. 3 Photomicrographs of liver sections (MT stains). Scale bar 20×200 μm. Note the normal architecture of the liver with intact hepatocytes stained red in groups A, D, E, F and G. Nuclei can be seen as dark red to black structures within cells and fibrous elements stained light blue. There is extensive necrosis of central veins, hepatic cords and massive fibrosis in groups B, and C. **a** –ve Control, **b** Diabetic +ve Control **c** Diabetic + HAART **d** Diabetic + *M. charantia* 200 mg/kg **e** Diabetic + *M. charantia* 400 mg/kg, **f** Diabetic + *M. charantia* 200 mg/kg + HAART **g** Diabetic + *M. charantia* 400 mg/kg + HAART

vital metabolic activities, including synthetic and excretory functions of drugs/chemicals. Due to this central role, it is thus exposed to toxic injuries. In other to mitigate some of these toxicities, people living with HIV/AIDS (PLWHAs) in sub-Saharan Africa rely on medicinal plants rich in phytochemical components as a complementary or alternative therapy for the alleviation of some of the toxicities of HAART [12].

In our study, we evaluated alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline

phosphatase (ALP) and gamma-glutamyl transferase (GGT) on the serum samples. These liver enzymes are helpful in understanding inflammatory and necrotic changes in the liver [13]. It has been reported that elevated levels of these liver enzymes are associated with complications of diabetes mellitus [14], and commencement of antiretroviral therapy [15]. Although this can be exacerbated by other co-infections like hepatitis B or C virus [16]. Our study revealed an elevation of these liver enzymes (ALT, AST and ALP) above normal levels in the diabetic +ve control animals and the diabetic animals treated with HAART. This elevations perhaps could reflect a major permeability problem or cell disruptions in the liver [17]. Elevations of these enzymes is a sensitive signal for liver injury and may worsen into severe complications of the liver with the continuation of therapy [15].

In the quest for better outcome and well-being of PLWHAs, there is increased utilization of alternative and complementary medicines in association with HAART, although many of these adjuvants have been implicated in drug-induced liver/other organ's injury [7]. From our study we observed lower levels of ALT, AST and ALP in diabetic rats treated with *M. charantia* alone and its concomitant treatment with HAART, indicating that consumption of *M. charantia* had a beneficial effect in suppressing elevation of liver enzymes levels. The decreased levels of these liver enzymes (especially following adjuvant HAART with *M. charantia*) in our study supports previous reports by [18] on effects of *M. charantia* on the serum and liver triglyceride levels in rats. While the levels of ALT, AST and ALP were reduced in our study, GGT showed normal levels in all groups. The tendency of *M. charantia* to suppress the rise in values of the liver enzymes provides substantial evidence that treatment with *M. charantia* extract could mitigate the progression of liver dysfunction.

Liver dysfunction is known to be associated with complications of diabetes and assessment of blood glucose level plays a critical role in evaluating the functional capacity of the liver following antiretroviral treatment in STZ induced diabetic rats. Elevated levels of blood glucose are associated with complications arising from occlusion of the vascular wall [19]. This pathogenesis is clearly complex and occurs as a result of cytokines secretion increase which induces dysregulation of endothelial and vascular smooth muscle cell growth [20, 21]. In the present study, elevated level of blood glucose level was observed when rats were treated with HAART regimen alone indicating that HAART regimen suppresses glucose uptake by the tissues. While blood glucose levels were elevated in animals treated with HAART alone, it was lowered when *M. charantia* was used as adjuvant to HAART. It is believed that *M. charantia* could exert their hypoglycemic properties via stimulation of peripheral skeletal muscle glucose utilization [22, 23] inhibition of adipocyte differentiation [24], stimulation of key enzyme of hexose mono phosphate (HMP) pathway, and preservation of β cell islet and its functions [25].

We correlated our findings with the histopathological assessment of the liver tissues. HAART-treated groups revealed disorganized architecture and extensive network of fibrotic strands and hepatic structural injury. The pathogenic pathway for HAART-mediated hepatic injury has been associated with mitochondrial toxicity especially with NRTIs [26, 27]. HAART inhibit the mitochondrial DNA resulting in inhibition of normal mitochondrial replication which in turn decrease cellular respiratory chain and inhibits fatty acid β-oxidation pathway [26]. Studies have also concretely proved that interruption in electron transport chain results in the release of increased intracellular reactive oxygen species (ROS) leading to oxidative stress [28, 29]. These free radicals can cause lipid peroxidation of fatty acid present in the hepatocytes membrane consequently to release profibrogenic cytokines, and nuclear factor κB to counteract the effect [30]. It is reasonable to believe that a perturbed antioxidant defense system could perhaps be a contributory arm towards the histopathological observations in our study.

Our observations in the H and E stains corresponded with the MT staining intensities of liver sections and there is a supporting link. Photomicrographs from liver tissues of groups B and C showed extensive network of blue-stained fibrous components in a necrotized, disorganized hepatic tissue cord possibly indicating low deposits of collagen fibres. These derangements could be as a result of low glucose metabolism and is supported by [31] involving PIs regimens. It is interesting to note that while all these deleterious effects were seen in HAART treated groups, *M. charantia* reversed the pathologies and showed good cyto-protective effects and maintained hepatic structural integrity. Although [7] reported that the pharmacokinetic interactions between antiretroviral therapy and plant based adjuvants can be unfavourable due to drug- drug interactions, the exact mechanistic pathway for *M. charantia* boosting in hepatic tissues in our study still remain a subject for further investigation.

Body and organ weight analysis offers insight on inflammatory changes and thus important in the identification of target organ by toxicants. In this study, HAART with *M. charantia* induced observational changes in the body weight of experimental animals. While loss of weight may be associated with induction of diabetes, initiation of HAART may as well contribute to the low pace of weight gain due to other metabolic activities relative to lipid and sugar [7]. Our study agrees with [7] that long term liver enzyme elevation in addition to other positive indices tilts the balance towards liver fibrosis. These perturbations were mitigated by *M. charantia* and its concomitant treatment with HAART.

Compliance with ethical standards

Conflict of interest The authors declared that there is no conflict of interest.

References

1. Havlir DV, Currier JS (2015) CROI 2015: complications of HIV infection and antiretroviral therapy. Top Antivir Med 23:56–65
2. Bonacci M, Lens S, Mariño Z, Forns X (2016) Challenges in special populations: HIV/HCV coinfection, liver transplantation and patients with end-stage renal disease. Dig Dis 34:317–326
3. Goswami A, Bhargava N, Dadhich S, Kulamarva G (2014) Insulin resistance in euglycemic cirrhosis. Ann Gastroenterol Q Publ Hell Soc Gastroenterol 27:237
4. Takahashi H, Eguchi Y, Anzai K (2016) Pathogenesis of hepatogenous diabetes. Nihon Rinsho Jpn J Clin Med 74:587
5. Kwatra D, Dandawate P, Padhye S, Anant S (2016) Bitter melon as a therapy for diabetes, inflammation, and cancer: a panacea? Curr Pharmacol Rep 2:34–44
6. Perumal V, Murugesu S, Lajis N, Khatib A, Saari K, Abdul-Hamid A et al (2015) Evaluation of antidiabetic properties of *Momordica charantia* in streptozotocin induced diabetic rats using metabolomics approach. Int Food Res J 22:1298–1306
7. Azu OO, Jegede AI, Ugochukwu O, Onanuga IO, Kharwa S, Naidu EC (2016) Hepatic histomorphological and biochemical changes following highly active antiretroviral therapy in an experimental animal model: does *Hypoxis hemerocallidea* exacerbate hepatic injury? Toxicol Rep 3:114–122
8. Etuk E (2010) Animals models for studying diabetes mellitus. Agric Biol J N Am 1:130–134
9. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63
10. Naruse K, Tang W, Makuuchi M (2007) Artificial and bioartificial liver support: a review of perfusion treatment for hepatic failure patients. World J Gastroenterol 13:1516
11. Bissell DM, Gores GJ, Laskin DL, Hoofnagle JH (2001) Drug-induced liver injury: mechanisms and test systems. Hepatology 33:1009–1013
12. Offor U, Jegede A, Onanuga I, Naidu E, Azu O (2016) Does Hypoxis hemerocallidea mitigate renal histopathological injuries following highly active antiretroviral therapy? An experimental animal study. Minerva Urol Nefrol 69:391–399
13. Ramaiah SK (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 45:1551–1557
14. Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G et al (2006) Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 44:865–873
15. Carr A, Cooper DA (2000) Adverse effects of antiretroviral therapy. Lancet 356:1423–1430
16. Weber R, Sabin CA, Friis-Moller N, Reiss P, El-Sadr WM, Kirk O et al (2006) Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. Arch Intern Med 166:1632–1641
17. Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R et al (2000) Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory beta-cell hyperplasia. Diabetes 49:1880–1889
18. Senanayake GV, Maruyama M, Shibuya K, Sakono M, Fukuda N, Morishita T et al (2004) The effects of bitter melon (*Momordica charantia*) on serum and liver triglyceride levels in rats. J Ethnopharmacol 91:257–262
19. Kohner EM, Patel V, Rassam SM (1995) Role of blood flow and impaired autoregulation in the pathogenesis of diabetic retinopathy. Diabetes 44:603–607
20. Vlassara H, Palace M (2002) Diabetes and advanced glycation endproducts. J Intern Med 251:87–101
21. Ahmed N (2005) Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes Res Clin Pract 67:3–21
22. Abas R, Othman F, Thent ZC (2015) Effect of *Momordica charantia* fruit extract on vascular complication in type 1 diabetic rats. EXCLI J 14:179
23. Kang J, Zeng B, Tang S, Wang M, Han X, Zhou C et al (2016) Effects of *Momordica charantia* Saponins on in vitro ruminal fermentation and microbial population. Asian-Australas J Anim Sci 29:500–508
24. Perumal V, Khoo W, Abdul Hamid A, Ismail A, Shaari K, Murugesu S et al (2015) Evaluation of antidiabetic properties of *Momordica charantia* in streptozotocin induced diabetic rats using metabolomics approach. Int Food Res J 22:1298–1306
25. Aljohi A, Matou-Nasri S, Al-Khafaji N, Slevin M, Ahmed N (2016) *Momordica charantia* (bitter melon) extracts promote angiogenesis in vitro via the receptor for advanced glycation endproducts (RAGE). J Int Soc Antioxid Nutr Health 3:514–515
26. Barve S, Kapoor R, Moghe A, Ramirez JA, Eaton JW, Gobejishvili L et al (2010) Focus on the liver: alcohol use, highly active antiretroviral therapy, and liver disease in HIV-infected patients. Alcohol Res Health 33:229
27. McInnes J (2013) Mitochondrial-associated metabolic disorders: foundations, pathologies and recent progress. Nutr Metab 10:63
28. Zhang DX, Gutterman DD (2007) Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. Am J Physiol Heart Circ Physiol 292:H2023–H2031
29. Forbes JM, Coughlan MT, Cooper ME (2008) Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 57:1446–1454
30. Browning JD, Horton JD (2004) Molecular mediators of hepatic steatosis and liver injury. J Clin Investig 114:147–152
31. Herman J, Easterbrook P (2001) The metabolic toxicities of antiretroviral therapy. Int J STD AIDS 12:555–564