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CONSUMPTION OF RIDA BITTERS ATTENUATES HYPERGLYCEMIA, INSULIN RESISTANCE AND OXIDATIVE STRESS, AND SUPPRESS INFLAMMATION IN A RAT MODEL OF TYPE 2 DIABETES

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ABSTRACT

Background: Diabetes mellitus has been identified as a chronic syndrome that has become wide-ranging and yet without type distinction is a leading cause of manifold complications. The disease is associated with glucose metabolism resulting from defects in insulin secretion and action**. Aim:** This study aimed to explore the effect of RIDA herbal bitters on diabetic insulin-resistant rat model. **Materials and Methods:** 32 Adult male rats of Wistar strain, weighing 160g ±20g were randomized into 4 groups of n=8. Diabetes was induced by High-fat fed diet and 25 mg/kg *b.w*intraperitoneal Streptozotocin. Treated animals were administered 200 mg/kg *p.o* of the reference drug and 0.3ml p.o RIDA bitters orally. Samples were collected by cardiac puncture after 28 days of administration. **Results:** There was an increase in body weight ($p<0.05$), hyperglycemia ($p<0.05$), insulin resistance and HOMA-IR ($p<0.05$) in high-fat fed and STZ induced diabetic group when compared with control. IL-6, TNF-α, Adiponectin and Insulin concentration were significantly reduced in RIDA/Metformin treated groups.The administration of RIDA bitters significantly decrease ($p<0.05$) total cholesterol, low density lipoprotein, triglyceride, SOD, CAT, GPx, Urea, Uric acid, Creatinine and significantly increase ($p<0.05$)High Density Lipoprotein-Cholesterol and MDA activities in the RIDA/ Metformin treated groups compared to STZ group. In addition, GGT, Aspartate aminotrasferase, Alkaline Phosphatase and Alanine Transaminase activities were significantly reduced ($p<0.05$) in the RIDA group compared to STZ. **Conclusion:** The results provide evidence that RIDA is a possible beneficial product for type II diabetes and its associated metabolic disorders.

KEYWORDS: Diabetes mellitus, RIDA bitters, Streptozotocin, High-fat diet, Inflammation, oxidative stress.

1. INTRODUCTION

Diabetes mellitus is a chronic syndrome that has become wide-ranging.[1] It is a condition with abnormally high level of sugar in the blood.[2] Diabetes mellitus is characterized by quantification of disturbances in glucose-insulin homeostasis as a result of defects in insulin secretion and functionality.[3] Sellamuthu*et al.*, defined it as a disease that is characterized by elevated glucose level and either by not producing insulin and/or defects of insulin action.[4] Interestingly, several reports have shown concern of a combination of hereditary and environmental factors (which include physical inactivity, high intake of processed foods and obesity etc) to be problems related to diabetes mellitus.[5] Globally, the occurrence of diabetes without type distinction was approximately 415 million in 2015 and is estimated to reach 642 million by 2040.[6] According to WHO, it is projected that 3% of the world's populace have diabetes and the prevalence is probable to double to 6.3% by the year 2025.[7][8] In Nigeria, the current incidence of diabetes among adults aged 20-69 years is recounted to be 1.7%.[9]

Type II diabetes mellitus which is independent insulin type of diabetes, is characterized primarily by chronic unusually high concentration of sugar in the blood and insulin resistance in peripheral tissues.[10] Type II diabetes has been shown with manifold complications including diabetic nephropathy (DN), which is a leading cause of chronic kidney diseases in human.[10][11] In T2DM, pancreatic beta cells produce deficient considerable measure of insulin to maintain normoglycemia or produce exceeding amounts due to failure in the peripheral tissues insulin action, which roots insulin resistance.[11][12] Evidence from several reports has shown that oxidative stress has great significance among the complications of type II diabetes.[10] Crook[13] revealed a robust interplay between hyperglycemia, hyperglycemic-induced oxidative stress, inflammation and the development and progression of

type II DM.[13] This has been reported as a known alleyway in the origin and development of diabetic complications.[13] It is believed to upsurge the levels of pro-inflammatory proteins with permeated macrophages secreting inflammatory cytokines, thus leading to local and systemic inflammation.[14]

In the demand of precluding and treatment of diabetes, several synthetic drugs including Sulphonylureas, thiazolidinediones, Glinide, and Metformin etc, have been investigated and industrialized. These owns to the perception that they are not optimum solution, thus, they do retain many side effects and are moderately expensive. However, the uses of medicinal plants for managements and treatment of certain conditions have become common medications and remedies in many Nigerian homes.[15] Whilst in the main, evidence from Ajao *et al.*,[2] reported that RIDA bitters helps to increase body's levels of antioxidants and decrease establishment of free radicals.[2] Thus, there is incentive to use Herbal medicinal compounds, which has available resources, economical and less side effects.[16]

RIDA herbal bitter is a polyherbal formulated blendmade from more potent ethnomedicinal plants.[2] It's a 100% natural formulation consisting of several herbs with synergistic and complimentary properties (Marvel Natural Products Plc). According to RIDA bitters leaflet, the natural herbal bitters is prepared from many plant species as the *Hunteria umbellate* (9.18%), *Colocynthiscitrullus* (24%), *Uvariachamae* (9.41%), *Curgulicopilosa* (20.48%) and *Senna alata* (11.4%).[2] RIDA herbal mixture is acclaimed by its producer to have numerous medicinal benefits. The herbal remedy is specified n previous studies for several ailments including obesity, diabetes, impotence, arthritis, gastrointestinal and heart disease amongst others.[2] Phytochemical investigations of RIDA show that the many plant species of the product have antidiabetic activity in a genetic model of diabetes mellitus with hyperinsulinemia.[2] RIDA has also been reported to possess antitumor, and immunomodulatory activities.[2] Therefore, this present study is designed to examine effects of RIDA bitters in diabetic insulin-resistant induced by streptozotocin and high-fat fed diet, through wide-ranging physiological depiction, blood biochemical analysis and insulin production evaluations.

2. MATERIAL AND METHODS

Drugs, Chemicals and Herbal bitters

All chemical, drug and herbal materials that were used in this study including Streptozotocin, Metformin, and RIDA herbal bitters were produced by Sigma-Aldrich, Germany; HOVID Bhd, Ipoh, Malaysia; and Marvel Natural Products Plc rspectively.

High-fat Fed Diet Formulation

The High fat feed was fortified and formulated at the Department of Animal production and Health, LAUTECH, Ogbomoso, Oyo State, Nigeria. The diet was supplemented by quantifying a percentage amount of feed ingredients using computerized linear program (CPL) as described by Black and Hlubik.[17] The theory was based on solving complex models of optimal feed mix. All the ingredients used in formulation were purchased from Wholesales store (GloryVet Nig. Enterprises, General, Ogbomoso, Oyo State, Nigeria).

Study designs

Thirty-two (32) male rats *(Rattusnorvergicus)* of Wistar strain, weighing between 160g ±20g were obtained and kept in the animal house of the Department of Physiology, Ladoke Akintola University of Technology, Ogbomoso, having standard conditions, at a temperature of 18-24ºC and 12 hours light and darkness. Animals that fulfilled obesity (body weight 250 ± 20 gm) with persistent blood glucose level exceeding >200 mg/dl, hyperinsulinemia, and dyslipidemia criteria after 48 hours intraperitoneal injection of 25 mg/kg Streptozotocin were used in the study. The animals were randomized into 4 groups of n=8 in metabolic cages under standard laboratory conditions.Animals in control groups were fed normal pellet and other experimental rat in other groups were fed high-fat fed diet and water *ad-libitum* until the last day of the experiment.

The animals were grouped as follows

CONTROL: Normal control rats on normal feed only.
HFD + STZ: Diabetic rats induced with Streptozotocin (25mg/kg i.p) after feeding with high fat diet for a period of six weeks.
HFD + STZ + RIDA: Diabetic rats treated with 0.3ml p.o. of *Rida bitters.*
HFD + STZ + MET: Diabetic rats treated with Metformin (200mg/kg p.o).

Sampling and Measurements

After the last dose of treatment, the rats were deprived of food overnight and were sacrificed by cervical dislocation. Blood samples was collected by cardiac puncture into heparinised tubes and centrifuged at 3,500 rpm for 15min. The supernatant and plasma was separated and stored at a temperature of -4^{o}c until it is required for assaying.

Determination of Blood Glucose Level

Fasting blood glucose levels were determined by glucose oxidase method[18] using a digital glucometer (Accu-Chek, Roche Diagnostic, Germany). At 72 hrs of STZ induction, the blood samples were collected from the tail vein of the rats and during last day of treatment.

Blood analysis

Estimation of Serum Insulin Concentration

Plasma insulin concentration was estimated by radioimmunoassay (RIA) using coat -A- count insulin kit.[19]

Estimation of Homa-IR

Homeostasis model assessment was evaluated according to the method of Mathew *et al.*[20] The insulin resistance was calculated by the model and was described by means of a set of simple, mathematically-derived nonlinear equations as below.

HOMA-IR = (serum/plasma glucose, mmol/L x serum/plasma insulin, µIU/ml) / 22.5

Determination of Superoxide dismutase (SOD), Malondialdehyde (MDA), GGT, Glutathione peroxidase (GPx) and Catalase (CAT).

GPx activity was determined in accordance with the principle of Paglia and Valentine[21], Thomas *et al.*,[22], and Pippenger*et al.*,[23] to catalyze the oxidation of Glutathione (GSH) by cumenehydroperoxide. The level of plasma MDA was measured using Thiobarbituric Acid Reactive Substances (TBARS) as described by the principles of Angulo *et al.*,[24] and Botsogolou *et al.*,[25] The spectrophotometric procedures were used in GGT Assay and the procedure were based on the method of Ellman[26] GPx activity was determined spectrophotometrically in absorbance of 340 nm according to Paglia and Valentine[21] and the modification described by Chen *et al.*,[27] Catalase activity was determined by the method of Johannson and Borg.[28]

Determination of Urea, Uric acid, and Creatinine

Plasma urea was determined by colorimetric test in accordance with the principle of Fawcett and Scott[29] using Fortress reagent kits. Plasma uric acid was measured by direct enzymatic assay according to Fossati*et al.*,[30] principles using Fortress reagent kits. Bartels *et al.*,[31] principles was adopted in Plasma creatinine estimation. Plasma creatinine was determined by kinetic test without deproteinization using Fortress reagent kits.

Determination of ALT, ALP, and AST

ALT, ALP, and AST was estimated in accordance with the Method recommended by the International Federation of Clinical Chemistry, Scientific committee (IFCC).[32][33]

Statistical Analysis

Results obtained were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Bonferoni post hoc statistical test was used to separate and compare means. Data were considered statistically significant at $p < 0.05$. Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Inc.) was used for analysis.

4. RESULTS

Effect of RIDA Bitters in high fat diet and STZ-induced type 2 diabetic rat model

Food Intake and Body Weight

Figure 1A illustrates the effect of RIDA bitters on food intake in HFD+ STZ diabetic rats. There was no significant increase in food intake of HFD+ STZ diabetic rats compared to control. Treatment with RIDA bitters resulted in a significant reduction ($p<0.05$) in food intake level compared to HFD+ STZ diabetic rats. At the end of high-fat dietary manipulation and streptozotocin injection, there was significant decrease ($P<0.05$) in the body weight of diabetic rats when compared with control. Administration of RIDA bitters however significantly increase ($P<0.05$) the body weight of RIDA bitters treated rats compared with HFD+ STZ diabetic rats. The effect was comparable to the standard drug metformin group (figure 1B).

Blood Glucose, plasma insulin concentration and homeostasis model assessment for insulin resistance (HOMA-IR)

The fasting blood glucose of the control and experimental animals were shown in figure 3. There was a significant increase ($p<0.05$) in blood glucose level of HFD+ STZ diabetic rats compared to control. Treatment with RIDA bitters resulted in a significant reduction ($p<0.05$) in the blood glucose level compared to HFD+ STZ diabetic rats (figure 2A). The changes in plasma glucose level (figure 1B) were accompanied by significant decreased ($p<0.05$) plasma insulin level in HFD+ STZ diabetic rats compared to control rats. Treatment with RIDA bitters resulted in significant increase ($p<0.05$) in plasma insulin levels compared to HFD + STZ diabetic rats (figure 2B). The degree of insulin resistance as calculated by HOMA was higher in HFD + STZ diabetic rats. Oral administration of RIDA bitters to HFD + STZ diabetic rats for four weeks significantly ($p <0.05$) reduced HOMA values (Figure 2C). These effects were comparable to the reference drug, Metformin.

Lipid profiles

The lipid profiles assessed in RIDA bitters and metformin treated HFD + STZ diabetic rats were presented in Table 1. HFD + STZ diabetic rats exhibited significant increases ($p<0.05$) in total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and atherogenic Index (LDL-C/HDL-C) but lower levels of high density lipoprotein cholesterol (HDL-C) compared to control rats. However, treatment with RIDA bitters showed significant reductions ($p<0.05$) in TC, TG, LDL-C, VLDL-C levels and Atherogenic Index and increase in HDL-C levels compared to HFD + STZ diabetic rats.

Kidney and Liver Functions

Markers of kidney and liver functions were assessed in high-fat fed and streptozotocin-induced diabetic treated rats (Table 2). There were significant increases ($p<0.01$) in urea, uric acid and creatinine levels in the HFD + STZ group compared to control. Treatments with RIDA caused significant reductions ($p<0.05$) in levels of urea, uric acid, and creatinine in the RIDA group compared to HFD + STZ group. Significant increases ($p<0.05$) in

Aspartate Aminotransferase (AST), gamma-glutamyl transferase (GGT), Alkaline Phosphatase (ALP) and Alanine Transaminase (ALT) activties was observed in the high-fat fed and streptozotocin-induced diabetic rats compared to control. Treatment with RIDA resulted in significant reductions ($p<0.05$) in markers of liver function assessed in the RIDA group when compared with HFD + STZ group. The effects observed in the RIDA treatred group were comparable to metformin group.

Oxidative Stress Indices

Oxidative stress indices were assessed in high-fat fed and streptozotocin-induced diabetic treated rats (Table 3). The malondialdehyde level was increased significantly ($p<0.05$) while levels of superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx), decreased significantly ($p<0.05$) in HFD + STZ group compared to control. However, treatment with RIDA bitters resulted in significant increases ($p<0.05$) in SOD, CAT and GPx enzyme activities in RIDA group compared to HFD + STZ group. Also, the lipid peroxidation decreased significantly in the RIDA group. These results were comparable to the metformin-treated group.

Inflammatory markers and Adiponectin

Levels of IL-6 and TNF-alpha were significantly increased ($p<0.05$) in HFD + STZ rats when compared to control. Treatment with RIDA bitters resulted in significant reductions ($p<0.05$) in IL-6 and TNF-alpha levels in comparison to HFD + STZ rats (Figure 3a and b).

Adiponectin level in HFD + STZ group reduced significant ($p<0.05$) when compared with control. However, treatment with RIDA bitters caused significant increase in RIDA group compared to HFD + STZ group. These results were comparable to the metformin-treated group.

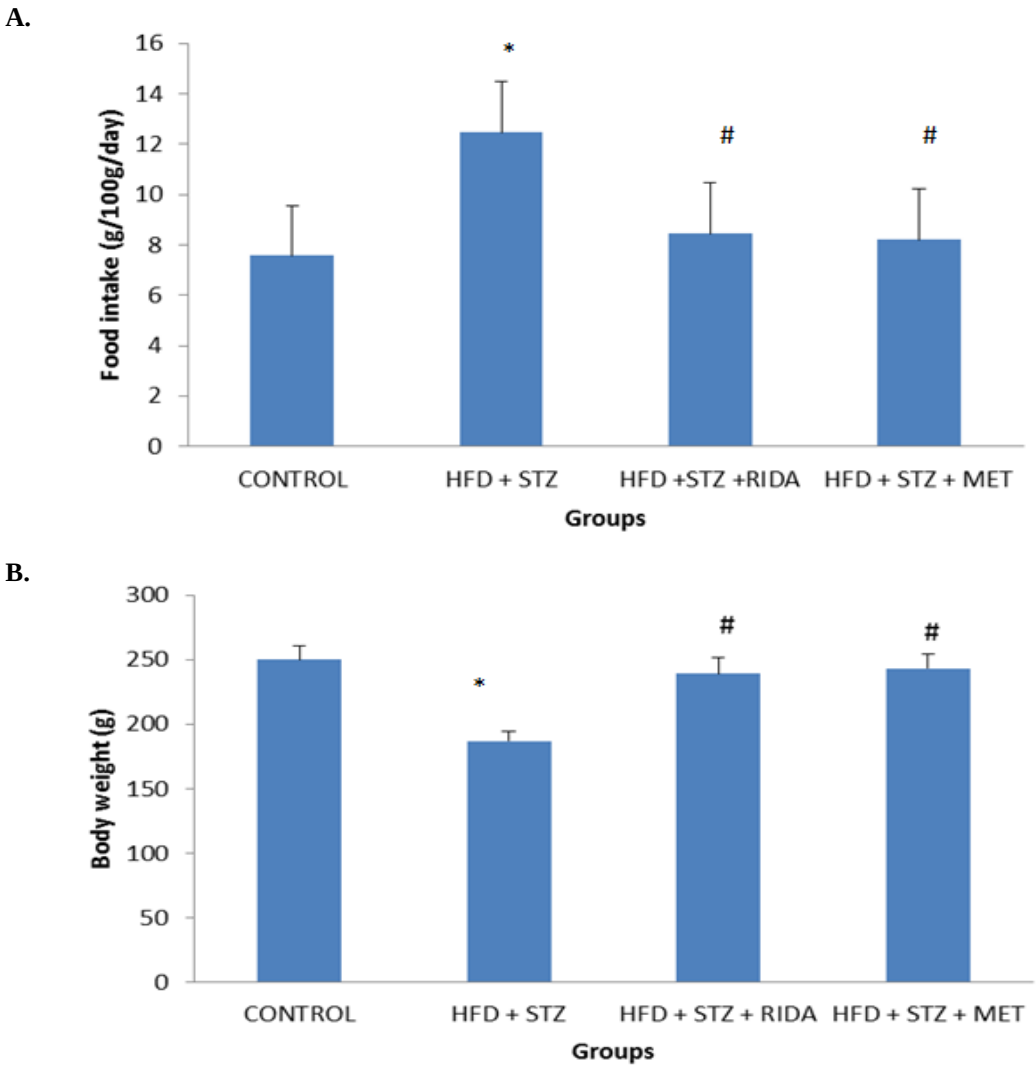

Figure 1: Effect of RIDA bitters on (A) food intake and (B) body weight in high fat-diet and streptozotocin-induced diabetic rats. ***Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, [#]- significant at p<0.05 compared with HFD + STZ group.*** **CON- Control, STZ Streptozotocin, RIDA- RIDA bitters, MET- Metformin HFD- High Fat Diet.**

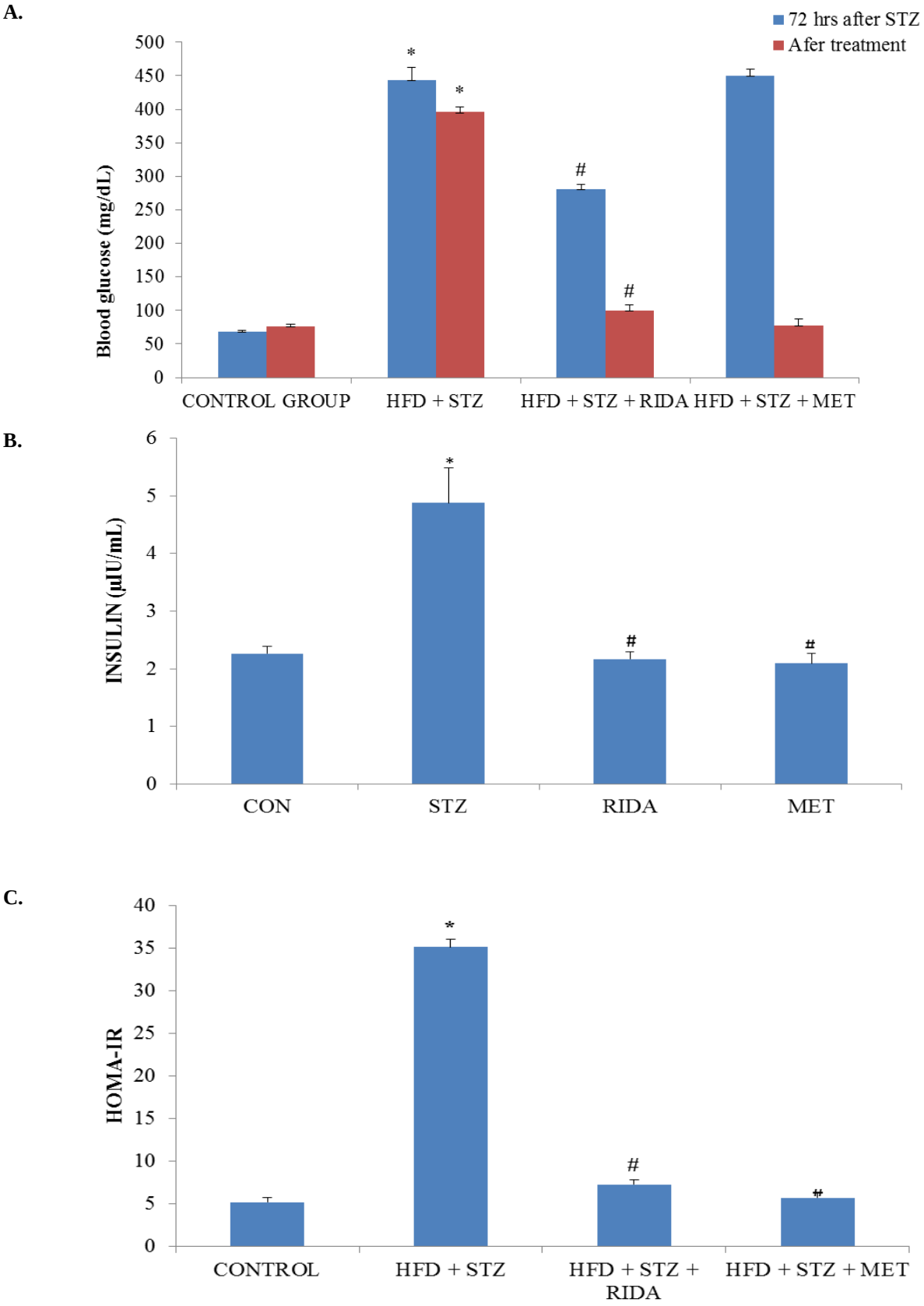

Figure 2: Effect of RIDA bitters on (A) blood glucose, (B) plasma insulin concentration and (C) HOMA-IR in high fat-diet and streptozotocin-induced diabetic rats. ***Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, #- significant at p<0.05 compared with STZ group.*** **CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, MET- metformin, HFD- High Fat Diet.**

Table 1: Effects of RIDA bitters on lipid profile in high fat-diet and streptozotocin-induced diabetic rats.

GROUPS	LIPID PROFILE					
	TC (mg/dl)	TG(mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	Atherogenic Index (LDL/HDL)
CON	168.67±7.76	50.86±4.49	91.11±9.38	24.03±0.74	10.17±0.90	3.80±0.38
HFD + STZ	280.33±8.57*	93.14±5.08*	196.76±18.78*	14.35±1.07*	18.63±1.02*	6.47±0.81*
HFD + STZ + RIDA	167.5±9.14$^{\#}$	53.93±9.94$^{\#}$	96.72±4.18$^{\#}$	25.49±0.36$^{\#}$	10.79±1.99$^{\#}$	3.95±0.20$^{\#}$
HFD + STZ + MET	174.75±6.36$^{\#}$	50.00±8.85$^{\#}$	99.01±5.28$^{\#}$	26.08±1.99$^{\#}$	10.00±1.77$^{\#}$	3.79±0.36$^{\#}$

Values are expressed as mean ± SEM (n=8). *******- *significant at p<0.05 compared with control,* $^{\#}$*- significant at p<0.05 compared with STZ group.* **CON**- Control, **STZ**- Streptozotocin, **RIDA**- Rida bitters, **MET**- Metformin, **TC**- Total Cholesterol, **TG**- Triglyceride, **LDL-C**- Low Density Lipoprotein Cholesterol, **VLDL-C**- Very Low Density Lipoprotein Cholesterol.

Table 2: Effects of RIDA bitters on some markers of kidney and liver functions in high fat-diet and streptozotocin-induced diabetic rats.

PARAMETERS /GROUPS	CONTROL	HFD + STZ	HFD + STZ + RIDA	HFD + STZ + MET
UREA (mg/dl)	37.17±1.58	85.46±2.55*	42.52±1.79$^{\#}$	41.61±2.33$^{\#}$
URIC ACID (mg/dl)	2.94±0.45	11.63±0.49*	2.73±0.77$^{\#}$	3.15±0.73$^{\#}$
CREATININE (mg/dl)	94.57±4.04	213.86±15.93*	97.14±5.53$^{\#}$	102.22±4.53$^{\#}$
AST (U/L)	30.61±3.41	75.74±5.05*	45.18±37.71$^{\#}$	42.69±33.76$^{\#}$
ALT (U/L)	77.37±5.90	224.01±14.69*	82.90±7.47$^{\#}$	76.45±8.93$^{\#}$
ALP (mol/ml/min)	48.39±1.37	76.65±7.19*	45.37±2.60$^{\#}$	46.15±4.46$^{\#}$
GGT (U/L)	25.76±1.80	63.58±4.98*	23.00±2.86$^{\#}$	20.86±1.16$^{\#}$

Values are expressed as mean ± SEM (n=8). *******- *significant at p<0.05 compared with control,* $^{\#}$*- significant at p<0.05 compared with STZ group.* **CON**- Control, **STZ**- Streptozotocin, **RIDA**- Rida bitters, **MET**- Metformin, **HFD**- High-fat diet **AST**-Aspartate aminotransferase, **ALT**-Alanine Transaminase, **ALP**-Alkaline phosphatase, **GGT**- gamma-glutamyl transferase.

Table 3: Effects of RIDA bitters on antioxidant enzymes and lipid peroxidation in high fat-diet and streptozotocin-induced diabetic rats.

PARAMETERS /GROUPS	CONTROL	HFD + STZ	HFD + STZ + RIDA	HFD + STZ + MET
CAT (umol/ml/mins)	26.49±1.38	14.58±0.35*	28.45±0.84$^{\#}$	26.72±1.05$^{\#}$
GPx (U/L)	695.5±29.18	392.1±13.18*	779.85±23.34$^{\#}$	741.3±26.72$^{\#}$
SOD (µ/ml)	1.70±0.04	0.71±0.08*	1.76±0.04$^{\#}$	1.66±0.05$^{\#}$
MDA (µM)	8.60±1.04	30.53±1.29*	7.50±0.55$^{\#}$	7.90±1.01$^{\#}$

Values are expressed as mean ± SEM (n=8). *******- *significant at p<0.05 compared with control,* $^{\#}$*- significant at p<0.05 compared with STZ group.* **CON** - Control, **STZ** - Streptozotocin, **RIDA**- Rida bitters, **MET** - Metformin, **HFD** - High-fat diet, CAT - Catalase, **GPx** - Glutathione peroxidase, **SOD** - Superoxide dismutase, **MDA** – Malondialdehyde..

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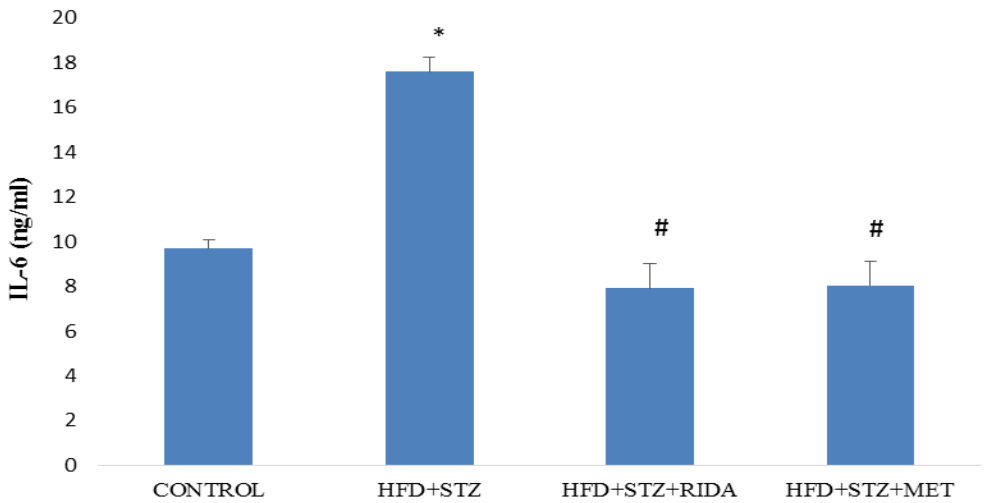

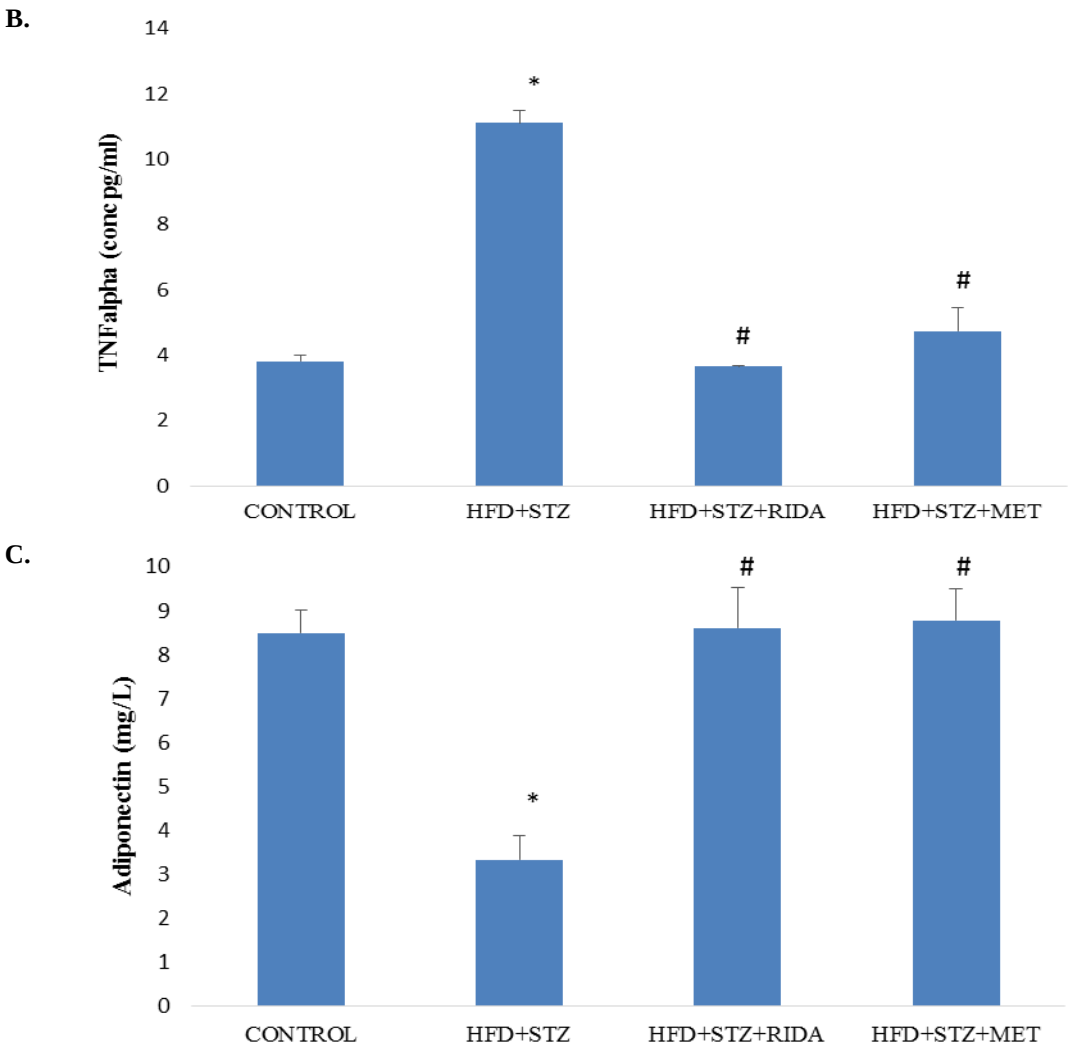

Figure 3: Effect of RIDA bitters on (A) Interleukin 6, (B) Tumor Necrotic factor alpha and C) Adiponectin in high fat-diet and streptozotocin-induced diabetic rats. Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, #- significant at p<0.05 compared with STZ group. CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, MET- metformin, HFD- High Fat Diet.

5. DISCUSSION

Diabetes mellitus is chronic condition characterized by quantification of disturbances in glucose-insulin homeostasis as a result of deficiencies in insulin secretion and functionality.[11] It has been identified as one of the four major syndromes that demands crucial consideration globally in an effort to lessen its prevalence and associated complications.[34] Evidence from several studies have shown that it is one of the highest ten causes of death globally, killing about 1.7 million people worldwide and estimated to reach 642 million by 2040.[6] Diabetes is seen as the third top risk factor for wide-reaching early mortality due to hyperglycaemia and hyperglycaemic-induced oxidative stress and inflammation. It has been shown that a strong link occurs between hyperglycaemia, hyperglycaemic-induced oxidative stress, inflammation and the growth and progression of type 2 diabetes mellitus.[35] Various reports have shown that chronic substandard inflammation is associated with the possibility of developing type 2 diabetes and that insulin resistance is attributed from sub-clinical inflammation and is linked to the characteristics of metabolic syndrome such as hyperglycaemia.[36] A combination of low dose STZ and high fat diet has been shown to closely resemble the natural process of the type 2 diabetes occurrence and its metabolic disturbances.[37]

In this study, the HFD/STZ-induced diabetic rats showed signs of polyphagia, polyuria and polydipsia associated with reduction of body weight. The observed decrease in body weight in HFD/STZ-induced diabetic rats was as a result of marked muscle destruction or degradation of structural proteins.[38, 39] Treatment with Rida bitters and metformin increased body weight, decreased food intake, and water intake in HFD/STZ-induced diabetic rats. The body weight increase observed in rida bitters treated group could be as a result of increased synthesis of structural proteins and improvement in glycaemic control.

Low dose streptozotocin is known to induce rapid destruction of pancreatic β-cells leading to impaired glucose-stimulated insulin release and insulin resistance, both of which are marked features of type 2 diabetes.[40] In this study, the levels of blood glucose and homeostatic model assessment of insulin resistance (HOMA-IR) were significantly increased whereas the levels of plasma insulin were significantly decreased. However, treatment with rida bitters and metformin brought the levels of blood glucose, plasma insulin and HOMA-IR back to near normal. These results indicate that rida bitters exhibited its glucose lowering action both by stimulating the surviving β-cells of Islets of Langerhans to release more insulin and also by lowering HOMA-IR indices thus restoring of insulin sensitivity.[41][42]

Type-2 diabetes is often associated with dyslipidemia.[43] Sout, (2005) reflected that diabetic dyslipidaemia and hyperglycaemia can be a predictors of cardiovascular complications.[51][44] Chronic dyslipidemia leads to lipid deposition and pancreatic lipotoxicity, thus impairing insulin secretion and promoting the progression of type-2 diabetes.[45] In the present study, increases in plasma triacylglycerol, total cholesterol, low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol, and decrease in high density lipoprotein (HDL) indicated significant imbalance of lipids (dyslipidemia) in diabetic rats (Table1). Treatment with rida bitters significantly attenuate dyslipidemia suggesting inhibition of pancreatic lipase and fat absorption.[46]

Evaluation of kidney functional markers gives vital information about the functionality of kidneys. In this present study, levels of the urea, creatinine levels and uric acid were significantly elevated in HFD/STZ-induced diabetic rats indicating deteriorated alteration in normal kidney functions to eliminate waste products from the body (Table 2). Findings of Giribabu et al.[47] who reported deteriorated alterations in kidney markers and histopathology in diabetic rats supported the result of this study. Treatment of HFD/STZ-induced diabetic rats with rida bitters however, improved kidney functions by reversing the altered biomarkers, demonstrating the renoprotective of rida bitters. The hepatic damage in the diabetic rats was manifested by increased levels of liver enzymes, such as ALT, AST, ALP, and GGT. The increased levels of these enzymes are markers of hepatocellular damage, which allows these liver functional enzymes to escape from cytosol into the bloodstream.[48] The present findings corroborate with the results from previous studies showing increased serum levels of ALT, AST, ALP, and GGT in diabetic rats.[49] While, diabetic rats, treated with rida bitters, significantly prevented the histopathological changes and attenuated the altered enzymatic markers functions, suggesting the role of rida bitters in restoring liver damage caused by diabetes.

The oxidative stress induced by reactive oxygen species is a pathophysiology in diabetes mediated complications.[50] Oxidative stress occurs in cells when there is an imbalance between oxidants and antioxidant defenses.[51] In this study, the markers of oxidative stress, such as MDA levels (lipid peroxidation) and NO levels, were significantly increased with significant decrease in plasma activities of SOD, CAT, and GPx of diabetic rats. Previous studies supported the findings from this study.[52] Administration rida bitters to diabetic rats attenuate the altered markers of oxidative stress, suggesting the anti-oxidant effect of rida bitters on HFD/STZ diabetes-induced oxidative stress.

Data from this study revealed elevated levels of TNF-α and IL-6 in HFD/STZ-induced diabetic rats. Excess ROS can also stimulate NF-$_k$B, a transcription factor that elicits the expression of TNF-α, IL-6, and inducible NO synthase (iNOS). These ROS-mediated effects elucidate the elevated hepatic MDA and NO levels as well as the pro-inflammatory cytokines observed in diabetic rats. Previous studies have reported increased circulating levels of TNF- α and IL-6, and liver NF-$_k$B expression in HFD/STZ-induced diabetic rats.[53][54] T2DM patients have also been shown to exhibit increased circulating levels of TNF- α and IL-6.[55] Increased IL-6 is related to impaired glucose tolerance[56] and insulin resistance in hepatocytes.[57] IL-6 reduces insulin receptor substrate (IRS)-1 tyrosine phosphorylation and inhibits insulin-dependent activation of protein kinase B/Akt in hepatocytes.[57] TNF- α impairs the ability of insulin to suppress hepatic glucose production and stimulate peripheral glucose uptake.[58] It also increases lipolysis in adipocytes[59], and impairs insulin signaling in muscle cells.[60] Therefore, ameliorating oxidative stress and inflammation will improve insulin sensitivity.

The effect of rida bitters on plasma adiponectin of HFD/STZ-induced diabetic rats was also evaluated. Diabetic rats showed a significant decrease in plasma adiponectin as earlier reported.[61][62] Lowered serum level of adiponectin is associated with insulin resistance and the etiology of obesity and type 2 DM.[63] Treatment of HFD/STZ- induced diabetic rats with rida bitters resulted in increased plasma level of adiponectin. These results were correlated with an improved glucose tolerance, insulin sensitivity, hepatic glucose output, and peripheral glucose uptake. Adiponectin has been reported to stimulate AMP-activated protein kinase resulting in an increased insulin sensitivity and regulation of glucose metabolism.[63] In addition, adiponectin decreases the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxylase, leading to a decreased hepatic glucose output via inhibition of hepatic gluconeogenesis.[63] Furthermore, adiponectin can activate peroxisome proliferator activated receptor-(PPAR-) α, decrease the hepatic and skeletal muscle triglyceride content[64], and enhance the oxidation of muscle fat through inhibition of acetyl-CoA carboxylase inhibition.[65]

CONCLUSION

This study showed for the first time that rida bitters improves insulin sensitivity and glucose tolerance in HFD/STZ type 2 diabetic rats. Rida bitters increased peripheral glucose uptake, improved lipid profile, suppressed hepatic glucose output, and prevented oxidative stress and inflammation in diabetic rats. In addition, rida bitters increased the plasma adiponectin levels of the diabetic rats. These findings suggests the role of adiponectin in mediating the antidiabetic effect of rida bitters; however, further studies to determine its exact mechanism of action are recommended.

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Conflict of interest

The authors have no conflict of interest to declare.

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