

Liver Function Parameters of Diabetic Rats Co-administered with Metformin and Aqueous Leaf Extract of *Bryophyllum pinnatum*

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(Received January 29, 2021; Accepted May 17, 2021)

ABSTRACT

The co-administration of antidiabetic herbs with conventional medicines raises safety concerns due to the complex interactions of multiple bioactive components. This study aimed to assess the impact of co-administering metformin and the aqueous leaf extract of *Bryophyllum pinnatum* (BPP) on liver function indices of diabetic rats. Forty Wistar rats were divided into five groups of 8 animals each namely; C (Control, distilled water only), DC (Diabetic untreated), Met (120 mg/kg body weight Metformin only), BPP (400 mg/kg b.wt. BPP only), BPP + Met (400 mg/kg b. wt. BPP and 120 mg/kg b.wt. Metformin). Rats were treated for 28 days, sacrificed 24 hours after and their liver and blood collected for the analysis of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, globulin, and total bilirubin. The results revealed a significant increase in total protein and globulin concentrations in the BPP and BPP + Met groups compared to the control (C) group. Conversely, albumin concentration decreased significantly in the diabetic untreated (DC), Metformin (Met), BPP, and BPP + Met groups. Total bilirubin concentration significantly reduced in the BPP + Met group compared to C, DC, and BPP. ALP activity increased significantly in Met, BPP, and BPP + Met groups compared to C, while liver ALP activity showed no significant change. Both serum and liver ALT activities decreased significantly in the experimental groups compared to C. Serum AST activity decreased significantly in Met and BPP + Met groups, while liver AST activity showed significant alterations only in the BPP group. In conclusion, the coadministration of metformin and BPP in diabetic rats did not reverse or improve diabetic-induced liver function indices alterations, suggesting caution in their concurrent use in diabetic conditions.

Keywords: Aminotransferase; Albumin/globulin ratio; Herb; *Bryophyllum pinnatum*; Liver

INTRODUCTION

Diabetes mellitus, a chronic metabolic disease, characterized by chronic hyperglycemia, remains a global health concern with significant implications for various organ systems, including the liver (Mohamed *et al.*, 2016; Yazdi *et al.*, 2019). Its global prevalence, mortality and that of chronic liver disease had increased conceivably due to changes in diet and lifestyle. According to the Institute for Health Metrics and Evaluation, approximately 1,690 million patients had chronic liver disease including cirrhosis in 2019 (prevalence rate 22.7%), and about 1.47 million people globally died of chronic liver disease (2.6% of global all-cause mortality) (IHME, 2019). The ninth edition of the International Diabetes Federation Diabetes Atlas in 2019 reported that among the global adult population in the age range of 20–79 years, approximately 463 million patients had DM (prevalence rate 9.3%), and approximately 4.2 million people have died of DM (11.3% of global all-cause mortality) (IDF, 2019).

The liver plays a crucial role in glucose homeostasis, and its function can be profoundly influenced by diabetes and antidiabetic medications (Crawford and Iacobuzio-Donahue, 2009). Diabetes-induced alterations in liver function are multifaceted, involving disruptions in glucose metabolism, lipid homeostasis, and oxidative stress (Rehman *et al.*, 2017; El-Akabawy and El-Kholy, 2018). Insulin resistance, a hallmark of type 2 *diabetes mellitus*, causes peripheral adipocytes to undergo lipolysis. Free fatty acids are then released to the bloodstream and eventually accumulate in the liver. At the same time, adipocytokines release tumour necrosis factor- α and leptin, worsening the hepatocyte damage by increasing oxidative stress in the mitochondria. The combined action of mitochondrial oxidative stress, hyperinsulinaemia and hyperglycaemia produce free radicals which in turn induce inflammation and cellular necrosis. Tissue inflammation stimulates the hepatic stellate cells to produce collagen, leading to fibrosis, cirrhosis and, finally, hepatocellular carcinomas (Mohamed *et al.*, 2016).

Metformin, a widely prescribed oral hypoglycemic agent, is known for its effectiveness in improving glycemic control and reducing hepatic glucose production. It is a first-line therapeutic option for type 2 diabetes, is recognized for its ability to enhance insulin sensitivity and suppress hepatic gluconeogenesis (Viollet *et al.*, 2012). According to the existing studies, metformin therapy was reported safe in T2DM patients with cirrhosis, and further prolong patient's survival time (Ampuero *et al.*, 2012). Metformin had also been shown to be related with reduced incidence of Hepatocellular carcinoma and liver-related death/transplantation in T2DM patients with cirrhosis due to hepatitis C virus (Nkontchou *et al.*, 2011). It was also reported to reduce the risk of death by 57% in T2DM patients with cirrhosis (Zhang *et al.*, 2014).

Bryophyllum pinnatum is a succulent plant with a history of traditional medicinal use, has been investigated for its potential antidiabetic properties. It is commonly known as "life plant" or "miracle leaf," has been traditionally used for its purported antidiabetic and hepatoprotective properties (Asare *et al.*, 2012; Asuzu, 2015).

The concurrent use of antidiabetic herbs and pharmaceutical medicines has raised safety concerns. Unlike pharmaceutical medicines, where the ingredients are well defined and characterized, herbal medicine contains multiple bioactive components for which there is a lack of understanding of how these components interact with each other and with pharmaceutical medicines when taken in combination. Although many studies regarding herb–drug interactions emphasize the potential harmful effects of such interactions, positive interactions between herbs and drugs may lead to enhanced effectiveness of the antidiabetic agents through additive or synergistic actions (Gupta and Tyagi, 2017). Interaction between herbal and orthodox drugs is a double-edged sword and is of concern to both patients and health care practitioners. It is necessary to continue research on potential risks and benefits associated with these interactions

Co-administration of metformin with plant extracts has been explored as a strategy to enhance therapeutic efficacy while minimizing adverse effects (Erukainure *et al.*, 2018). Despite the individual attention given to metformin and *Bryophyllum pinnatum* in the context of diabetes management, there is scarcity of research investigating the combined effects of these agents on liver function parameters. The study aimed to contribute to the understanding of the interplay between metformin and *Bryophyllum pinnatum* in the context of liver function, providing insights that could inform more comprehensive and synergistic approaches to diabetes management. The assessment of liver function parameters in diabetic rats receiving combined treatment with metformin and *Bryophyllum pinnatum*, will offer valuable information on the potential hepatoprotective effects of this co-administration. The findings may have implications for the development of novel therapeutic strategies that address not only glycemic control but also mitigate the hepatic complications associated with diabetes.

MATERIALS AND METHODS

Plant Collection and identification

Fresh leaves of *Bryophyllum pinattum* plant were harvested from a garden within Ilorin, Kwara state (8°31'37.12116"N 4°35'19.21848"E), Nigeria, at 7am in the morning, February, 2023. It was authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. A voucher specimen was deposited under a voucher number; UILH/001/909/2023, for reference purpose in the department.

Experimental Animals

Forty male Wistar rats of average weight 100 ± 20 g were obtained from and kept at the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in well-ventilated cages of dimensions 9.06 x 6.89 x 6.3 inch and allowed to acclimatize to animal house condition of; temperature 28 – 31°C, 12 hours of natural light and 12 hours darkness and humidity 50 – 51% for 14 days. The rats were fed with growers feed and they were given water. The cages were contained in a well-ventilated standard housing condition.

Reagents

Streptozotocin (Sigma-Aldrich, St. Louis, Mo, USA) and D-Fructose (MOLYCHEM, Mumbai, India) were purchased from a local research laboratory in Ilorin, Nigeria. Metformin (Eden U.K. Pharmaceutical Ltd.) was purchased from a local pharmacy store in Ilorin, Nigeria. Assay kits for total bilirubin, albumin, ALT, AST, ALP were products of RayBiotech, 3607, Parkway Lane, Suite 100, Nor cross, GA 30092, USA. Other reagents used were of analytical grade, prepared in glass apparatus with distilled water.

Aqueous Plant Extract Preparation

The *Bryophyllum pinnatum* leaves collected were rinsed with water, air-dried to a constant weight and ground into fine powder using a hammer mill (Model PC 200 x 300, DEWO Machinery Company Ltd, Zhengzhou, Henan, China). A known weight of 426.73 g of the pulverized *Bryophyllum pinnatum* leaves were percolated in 6000 ml (6L) of distilled water for 72 hours with intermittent stirring using a glass rod. It was filtered using cotton wool and whatman (no. 1) filter paper. The resulting filtrate was concentrated on water bath at 40°C.

Induction and Confirmation of Diabetes

All experimental rats except those in group A (control group) were administered orally with 10% fructose solution for 14 days and group A were given distilled water.

At the end of the 14 days administration of fructose solution, streptozotocin (STZ) was freshly dissolved in ice-cold 0.1 M citrate buffer (pH 4.5) and was administered to rats in groups B to E by a single intra-peritoneal injection at a dose of 40 mg/kg body weight (Wilson and Islam, 2012) after fasting them for 8 hours to induce *diabetes mellitus*. Seventy-two (72) hours after STZ was administered, the Fasting Blood Glucose (FBG) level of the rats was determined using Accu-chek active glucometer and compatible strips. Rats showing FBG level above 200 mg/dl were considered diabetic.

Experimental Design

The male rats obtained were randomly divided into 5 groups consisting of 8 rats each. The grouping of the rats is shown below;

C (Control) - Non diabetic healthy rats administered orally with 1 ml of distilled water daily

DC (Diabetic untreated) - Diabetic rats administered orally with 1 ml of distilled water daily

Met - Diabetic rats treated with the metformin only (120 mg/kg body weight daily)

BPP - Diabetic rats treated with aqueous leaf extract of *Bryophyllum pinnatum* only (400 mg/kg body weight daily)

BPP + Met - Diabetic rats treated with aqueous leaf extract of *Bryophyllum pinnatum* (400 mg/kg body weight) and metformin (120 mg/kg body weight) daily

Each group received the various treatments described above once daily orally for 28 days.

Animal Sacrifice and Tissue Isolation

The rats were sacrificed 24 hours after 28 days of treatment. They were anaesthetized with dichloromethane and sacrificed by simply incising the jugular vein, the organ of interest (liver) was harvested and preserved in 10% formalin. Blood samples were collected into clean sample bottles and was allowed to clot at room temperature and the serum was collected using Pasteur pipette.

Biochemical Assays

Fasting blood glucose (FBG) Determination

Before induction of *diabetes mellitus* in experimental rats (day 0), Fasting blood glucose (FBG) concentration of all experimental groups was determined using a glucose oxidase-based glucometer (AccuChek active, Roche Diagnostic). Blood was withdrawn from the caudal vein of the rat tail using insulin syringe and it was dropped on the center of the green field on the strip. The test strip was gently inserted into the glucometer and the FBG concentration in mg/dl was displayed on the glucometer. After induction of diabetes have been confirmed, FBG concentration was checked every other day throughout the experimental period.

Liver function parameters assay

Serum albumin, total bilirubin, total protein, serum and liver ALP, ALT and AST were determined by following the manufacturer's instruction in assay kits (RayBiotech, 3607, Parkway Lane, Suite 100, Nor cross, GA 30092, USA.). Serum globulin was determined from the difference between total protein and albumin.

Statistical analysis

Data were expressed as mean of 8 determinations $\pm$ SEM. The data were subjected to statistical analysis using the IBM[®] statistical package for social sciences (SPSS) software version 20. All significant differences were determined by one way analysis of variance (ANOVA). Post hoc multiple comparisons were done using Duncan's multiple range test. The level of significance was set at $p < 0.05$ (confidence level = 95 %).

RESULTS

Induction and Confirmation of diabetes in experimental rats

Table 1 showed the result of the induction and confirmation of *diabetes mellitus* in the experimental rats. There was a significantly increase ($p < 0.05$) in the blood glucose concentration of all the groups administered with fructose and STZ (DC, Met, BPP, BPP +Met) compared to the control group (C).

Table 1: Fasting blood glucose of rats before and after diabetes induction

Group	Fasting blood glucose (mg/dl)	
	Before induction	After induction
C	68.50 ± 1.39 ^a	90.00 ± 4.40 ^a
DC	69.13 ± 4.00 ^a	275.63 ± 6.75 ^b
Met	74.63 ± 1.74 ^a	270.88 ± 6.06 ^b
BPP	65.88 ± 3.36 ^a	271.50 ± 6.86 ^b
BPP + Met	62.50 ± 1.86 ^b	291.88 ± 4.88 ^b

Values are expressed as mean of 8 determinations ± SEM. and those with different superscripts down the column are statistically different ($p < 0.05$); C - Control; DC – diabetic control; Met – Metformin (120 mg/kg b.wt.); BPP – *Bryophyllum piannatum* (400 mg/kg b.wt.); BPP + Met - *Bryophyllum piannatum* (400 mg/kg b.wt) + Metformin (120 mg/kg b.wt.).

Selected serum liver function indices of rats co-administered with metformin and aqueous leaf extract of *Bryophyllum pinnatum*

Table 2 showed the result of selected non-enzymic liver function indices of rats co-administered with metformin and aqueous leaf extract of *Bryophyllum pinnatum*. Total protein and globulin concentrations of BPP and BPP + Met increased significantly ($p < 0.05$) compared to C. While albumin concentration decreased significantly for rats in DC, Met, BPP and BPP + Met groups compared to C. Total bilirubin concentration for those in BPP + Met reduced significantly compared to C, DC, and BPP.

Table 2: Selected serum liver function indices concentration of rats co-administered with metformin and aqueous leaf extract of *Bryophyllum pinnatum*

Group	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G Ratio	Total bilirubin (umol/L)
C	7.60 ± 0.56 ^a	3.95 ± 0.23 ^a	3.65 ± 0.33 ^a	1.08 ± 0.01 ^a	60.37 ± 1.49 ^a
DC	7.87 ± 0.24 ^a	3.23 ± 0.21 ^b	4.64 ± 0.03 ^b	0.70 ± 0.00 ^b	53.69 ± 1.48 ^b
Met	7.44 ± 0.25 ^a	2.72 ± 0.07 ^c	4.74 ± 0.18 ^c	0.57 ± 0.01 ^c	53.20 ± 1.89 ^b
BPP	9.25 ± 0.35 ^b	1.90 ± 0.12 ^d	7.35 ± 0.23 ^d	0.26 ± 0.00 ^d	53.37 ± 1.00 ^b
BPP + Met	8.11 ± 0.26 ^{ab}	1.90 ± 0.08 ^d	6.21 ± 0.18 ^e	0.31 ± 0.00 ^e	52.17 ± 1.81 ^{bc}

Values are expressed as mean of 8 determinations ± SEM. and those with different superscripts down the column are statistically different ($p < 0.05$); C - Control; DC – diabetic control; Met – Metformin (120 mg/kg b.wt.); BPP – *Bryophyllum piannatum* (400 mg/kg b.wt.); BPP + Met -

Bryophyllum piannatum (400 mg/kg b.wt) + Metformin (120 mg/kg b.wt.); A/G –Albumin to globulin ratio

Selected enzymic liver function indices of rats co-administered with metformin and aqueous leaf extract of *Bryophyllum pinnatum*

There was significant increase ($p < 0.05$) in the serum ALP activity of MET, BPP, and BPP + Met compared to C, while there was no significant change ($p > 0.05$) in the liver ALP activity of the experimental groups compared to C (Table 3). The activity of both serum and liver ALT decreased significantly ($p < 0.05$) compared to C, while for AST activity, serum AST decreased significantly ($p < 0.05$) for Met and BPP + Met compared to C. In the liver, there was no significant alteration ($p < 0.05$) for AST activity of the treatment groups except BPP (Table 3).

Table 3: Selected enzymic liver function parameters activities of rats co-administered with metformin and aqueous leaf extract of *Bryophyllum pinnatum*

Groups	ALP (U/L)		ALT (U/L)		AST (U/L)	
	Serum	Liver	Serum	Liver	Serum	Liver
C	55.96 ± 1.27 ^a	60.81 ± 1.87 ^a	58.58 ± 1.87 ^a	110.40 ± 1.50 ^a	43.07 ± 1.15 ^a	32.40 ± 2.65 ^a
DC	62.45 ± 0.98 ^b	61.49 ± 1.04 ^a	63.22 ± 0.25 ^b	107.06 ± 3.31 ^a	52.36 ± 1.94 ^b	28.96 ± 3.37 ^a
Met	58.86 ± 0.94 ^c	58.90 ± 1.55 ^a	48.83 ± 3.28 ^c	89.28 ± 4.31 ^b	35.48 ± 1.24 ^c	30.13 ± 2.33 ^a
BPP	57.54 ± 1.69 ^{ac}	59.11 ± 3.28 ^a	51.25 ± 2.23 ^c	76.66 ± 2.35 ^{bc}	42.82 ± 1.21 ^a	28.67 ± 1.79 ^a
BPP + Met	57.50 ± 4.33 ^{ac}	57.51 ± 4.05 ^a	57.06 ± 2.96 ^a	63.83 ± 2.56 ^c	32.18 ± 1.24 ^c	29.18 ± 0.85 ^a

Values are expressed as mean of 8 determinations ± SEM and those with different superscripts down the column are statistically different ($p < 0.05$); C - Control; DC – diabetic control; Met – Metformin (120 mg/kg b.wt.); BPP – *Bryophyllum piannatum* (400 mg/kg b.wt.); BPP + Met - *Bryophyllum piannatum* (400 mg/kg b.wt) + Metformin (120 mg/kg b.wt.); ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase

DISCUSSION

The use of animal models by researchers have enabled the successful study of pathophysiology of all types of diabetic conditions and discovered suitable methods for therapy. Based on the result of our study (Table 1), *diabetes mellitus* was successfully induced in Wistar rats using fructose and STZ. This finding is in agreement with previous study that utilized fructose solution and STZ to induce type 2 diabetes in Wistar rats (Wilson and Islam, 2012; Soji-Omoniwa and Oloyede, 2021). Diets high in fructose cause hyperlipidemia and hypertension in

various animal models (Hwang *et al.*, 1987) and have been linked over the years to insulin resistance (IR), a hallmark of DM (Tappy and Le, 2010). High circulating lipid concentrations in the blood secrete humoral factors such as resistin and adiponectin that alter insulin sensitivity, leading to IR (Trujillo and Scherer, 2006; Barnes and Miner, 2009). Fructose has been shown to be more hyperlipidemic than both glucose and starch with increased levels of low-density lipoprotein (LDL), very low density lipoprotein (VLDL) and total cholesterol in rats (Benado *et al.*, 2004). Other studies have also reported that higher serum triglycerides alone or accompanied with low serum high density lipoprotein (HDL-cholesterol) is a risk factor for hepatic IR as well as type 2 *diabetes mellitus* (Elliot *et al.*, 2002; Singla *et al.*, 2009).

The result of the liver function indices evaluated in this study showed a significant decrease in the albumin concentration of the diabetic untreated group (DC) (Table 2). This decline might be associated with the diabetic condition itself. Literature had established a relationship between liver function and *diabetes mellitus* where the combined action of mitochondrial oxidative stress, hyperinsulinaemia and hyperglycaemia resulting in generation of free radicals, in turn induce inflammation and cellular necrosis. Likewise, tissue inflammation stimulates the hepatic stellate cells to produce collagen, leading to fibrosis, cirrhosis and, finally, hepatocellular carcinomas (Mohamed *et al.*, 2016). Albumin, a plasma protein, is synthesized in the liver and as such, its decrease in the serum might suggest that the synthetic function of the liver might be impaired. Similarly, globulin, another class of plasma protein was elevated significantly in the diabetic untreated group (DC). Globulin is a marker of inflammation and immune function. Immune system activation is highly related to T2D incidence and progression and adaptive and innate immunity are involved in adipose tissue inflammation (Tsalamandris *et al.*, 2019). Treatment of the diabetic rats with Met, BPP, BPP + Met did not reverse the albumin and globulin concentrations of the diabetic untreated group (DC). It rather worsens the condition as shown by the significant decrease in the albumin concentration of MET, BPP and BPP + Met groups for albumin and significant increase of the globulin concentration of MET, BPP and BPP + Met groups. This finding is contrary to earlier reports that metformin is related with reduced incidence of hepatocellular carcinoma and liver-related death/transplantation in T2DM patients (Nkontchou *et al.*, 2011; Zhang *et al.*, 2014). Also, *Bryophyllum pinnatum* was earlier reported to possess hepatocellular properties (Asuzu, 2015). The albumin/globulin ratio of less than one for the treatment groups recorded in this study, further confirmed derangement in the liver function. Further study is however needed to elucidate the agents and probable mechanism responsible for the alternation in the studied parameters.

Serum total bilirubin concentration often give information on the excretory function of the liver. Bilirubin in plasma is mostly produced by the destruction of erythrocytes where heme is metabolized into biliverdin via heme oxygenase and then into bilirubin via biliverdin reductase inside the macrophages. Bilirubin is then released into the plasma and transported to the liver bound by albumin, since it is insoluble in water in this state. In this state, bilirubin is called unconjugated. In the liver, unconjugated bilirubin is up-taken by the hepatocytes and subsequently conjugated with glucuronic acid via the enzyme uridine diphosphate-glucuronyl

transferase. In this state, bilirubin is soluble in water and it is called conjugated bilirubin. Conjugated bilirubin is excreted into the bile ducts and enters the duodenum. During its transport to the colon, it is converted into urobilinogen by bacterial enzymes. Most of the urobilinogen is further reduced into stercobilinogen and is excreted through faeces. In our study, the concentration of bilirubin for the diabetic rats (DC) and treatment groups (Met, BPP and BPP + Met) were significantly low compared to Control. This alteration might be caused by diabetes in the experimental rats since low bilirubin is associated with kidney disease, a major complication of *diabetes mellitus*. Low bilirubin concentration has also been linked to increased risk of cardiovascular disease. Overall, administration of metformin and BPP did not improve the alteration in bilirubin concentration of the diabetic rats.

A remarkable alteration was recorded in the liver enzymes studied. Aminotransferase (AST and ALT) are markers of hepatocellular injury. They participate in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively. AST is present as cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It is not as sensitive or specific for the liver as ALT and elevation in AST may be seen as secondary to non-hepatic causes as well (Oh *et al.*, 2017).

ALT, a cytosolic enzyme is found in high concentrations in the liver. ALT is usually higher than AST in most types of liver disease in which the activity of both enzymes is predominantly from the hepatocyte cytosol. Hepatocellular injury and not necessarily cell death triggers the release of these enzymes into circulation. Alkaline phosphatase on the other hand, is part of a family of zinc metalloenzymes that are highly concentrated in the microvilli of the bile canaliculus as well as several other tissues (e.g., bone, intestines, and placenta) (Iluz-Freundlich *et al.*, 2020). The non-significant change in the activity of ALP for all the treatment groups compared to Control suggested there was no damage to the hepatocellular membrane. However, there was a significant decrease in ALT activity for the treatment groups (Table 3) compared to Control. This decrease did not result in significant increase in the serum ALT activity which suggested there might be down-regulation or repression in the synthesis of ALT. For AST, there was no significant alteration in the liver AST activity but there was a significant increase in the serum AST for BPP group, which suggested that the source of AST in the serum might be from other tissues aside the liver.

CONCLUSION

In conclusion, co-administration of 120 mg/kg body weight metformin and 400 mg/kg body weight *Bryophyllum pinnatum* aqueous leaf extract for 28 days in Wistar rats did not reverse neither improved the diabetic-induced liver function indices alterations but rather worsen the condition. Therefore, their concurrent use in diabetic condition is not recommended.

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