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Comparative Study on the Antidiabetic Activity of Fresh and Fermented Fruit Juice of *Morinda citrifolia* in Alloxan-Induced Diabetic Rats

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ABSTRACT

Background and Objective: Diabetes mellitus is a disorder of glucose metabolism that causes mortality and morbidity. Both fresh and fermented fruit juice of *Morinda citrifolia* have antidiabetic activity. This study compared the antidiabetic activity of the fresh and fermented fruit juice of *Morinda citrifolia*. **Materials and Methods:** The 45 Wistar rats were randomly divided into 9 groups. Group 1 was the normal control. Groups 2-9 were induced with diabetes using alloxan. Group 3 was administered a standard drug, glibenclamide. Groups 4, 5, 6 were administered 20, 40 and 80 mL/kg b.wt., fresh fruit juice of *Morinda citrifolia*, respectively, while groups 7, 8 and 9 were administered 20, 40 and 80 mL/kg b.wt., fermented fruit juice of *Morinda citrifolia*, respectively. The fasting blood glucose level of the rats was taken at an interval of 2 days using a glucometer. The initial and final weights of the rats were taken. After sacrificing the animals on the 15th day, the concentration of albumin, alanine transaminase, aspartate transaminase and alkaline phosphatase was determined using standard methods. **Results:** The fasting blood glucose level of the groups administered 80 mL/kg b.wt., fresh fruit juice of *Morinda citrifolia* and 80 mL/kg b.wt., fermented fruit juice of *Morinda citrifolia* was comparable with the control by day 7. The weight gains of the diabetic rats that received 80 mL/kg b.wt., fresh *Morinda citrifolia* and fermented fruit juice of *Morinda citrifolia* were not significantly different from each other. The level of the serum liver indices of rats administered 80 mL/kg b.wt., fermented fruit juice of *Morinda citrifolia* was not significantly different from the control. **Conclusion:** High dose of fermented fruit juice of *Morinda citrifolia* attenuated liver damage caused by diabetes mellitus. Fermented fruit juice of *Morinda citrifolia* is more effective in treating liver damage caused by diabetes mellitus.

KEYWORDS

Diabetes, *Morinda citrifolia*, liver damage, hyperglycemia, fruit juice

INTRODUCTION

Diabetes mellitus is a metabolic disorder of carbohydrates due to insulin deficiency resulting from dysfunction of pancreatic β-cells[1]. It results from defects in insulin secretion, action or both[2]. Diabetes

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mellitus is characterized by hyperglycemia. It is estimated that more than half a billion people are currently living with diabetes worldwide and this figure will project to 1.31 billion by 2050[3].

There are 2 types of diabetes mellitus, type 1 and type 2. Type 1 (T1DM) which is also called insulin-dependent diabetes mellitus occurs when there is a complete lack of insulin due to autoimmune destruction of the pancreatic β-cells while type 2 diabetes mellitus (T2DM) also called non-insulin dependent diabetes mellitus occurs due to impaired secretion and action of insulin[4]. Symptoms of T1DM includes weight loss, polydipsia, polyuria, ketoacidosis and polyphagia[5].

Diabetes mellitus can be managed by changing diet and by the use of synthetic drugs (insulin and/or hypoglycemic drugs such as biguanides and sulphonylurea[6]. These synthetic drugs have so many side effects which include hypoglycemia by sulphonylureas, gastrointestinal symptoms by acarbose, B_{12} and folate malabsorption by metformin and weight gain by sulphonyureas and thiazolidinediones[7]. There is therefore need to search for newer antidiabetic agents with no or lesser side effects. Medicinal plants are one such agent. A lot of plants have been reported to have antidiabetic activity. Amongst such plants is *Morinda citrifolia*.

Morinda citrifolia L (Rubiaceae) also known as noni is used traditionally for the treatment of many ailments which include diabetes, cancer, skin inflammation, wounds, arthritis and circulatory weakness[8-11]. Studies have shown that *M. citrifolia* fruit juice has hypoglycemic activity and can thus be used to treat diabetes[12]. Several mechanisms of antidiabetic activity of *M. citrifolia* fruit juice have been proposed. Studies have shown that *M. citrifolia* fruit juice exerts an insulin-mimetic activity[13-15]. Also, *M. citrifolia* fruit juice has been reported to contain iridoids (deacetylasperulosidic acid, asperulosidic acid and asperuloside) which have several biological activities[16]. Iridoid glycosides are bitter in taste) and recognized as potentially toxic via bitter taste receptors (T2R's)[17]. The T2R's present in some gastrointestinal cells secrete the peptide hormone; glucagon-like-peptide-1 (GLP-1) which stimulates the secretion of insulin in response to an increase in the blood glucose level[18,19]. This has been proposed as another mechanism by which *M. citrifolia* fruit juice exerts its hypoglycemic activity.

Fermented *M. citrifolia* fruit juice has also been shown to have antidiabetic activity[13]. Fermentation is advantageous because it enhances the digestibility of foods and helps to reduce the antinutrients in foods. This study therefore compared the antidiabetic activity of fermented *M. citrifolia* fruit juice and fresh *M. citrifolia* fruit juice.

MATERIALS AND METHODS

Study area: The study was conducted in the Biochemistry Laboratory of Adamawa State University, Mubi, Adamawa State, Nigeria between July, 2023 and September, 2023.

Methods: The 500 g of fresh fruits of *M. citrifolia* were collected from Yola, Adamawa state. It was identified by a botanist. The fresh fruits were washed with lukewarm water. The fruit was chopped into small pieces and the seeds were removed. The juice was extracted using a fruit juice extractor. This constitutes the fresh fruit juice and was prepared daily. It was then used for the analysis. For the fermented fruit juice, the fresh fruit juice was left for seven days to ferment.

Phytochemical analysis was done using the standard procedure described by Hussain *et al.*[20]. The 45 healthy male Wistar rats were obtained from the animal house, Department of Biochemistry, Adamawa State University, Mubi. They were housed in well ventilated cages. The rats were given standard feed pellets and water *ad libitum*. They were handled according to the guidelines for the protection and

handling of laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the Ethical Committee of Adamawa State University, Mubi and was given an approval number: ADSU/IACEC/ANP-A046/2023.

The Wistar rats were randomly divided into nine groups. Groups 1 was the normal control and was given normal saline. Diabetes was induced to groups 2-9. After confirmation of diabetes, group 3 received 2.0 mg/kg b.wt., glibenclamide, groups 4, 5 and 6 received 20, 40 and 80 mL/kg b.wt., fresh fruit juice of *M. citrifolia*, respectively, while groups 7, 8 and 9 received 20, 40 and 80 mL/kg b.wt., fermented fruit juice of *M. citrifolia*, respectively for 14 days. The initial and final body weight of the rats were taken.

To induce diabetes, 40 mg/kg b.wt., alloxan was injected intravenously. After five days, rats with fasting blood glucose ≥7.0 mmol/L were considered diabetic.

Fasting blood sugar levels was done using the glucometer (glucose-oxidase principle). Blood samples were collected from the tail veins of the rats.

The rats were sacrificed on the 15th day and serum collected was used for the determination of albumin, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Albumin was determined using the method described by Doumas *et al.*[21], ALT and AST were determined using the method described by Cabaud *et al.*[22], while ALP was determined by the method described by Sherphard *et al.*[23].

RESULTS

Table 1 shows the result of the phytochemical screening. Phenols, saponins, flavonoids, alkaloids and tannins were found present in both the fresh and the fermented fruit juice of *M. citrifolia* while steroids were absent in both.

The fasting blood glucose level of alloxan-induced diabetic rats administered fresh and fermented fruit juice of *M. citrifolia* is shown in Fig. 1. The fasting blood glucose of all the groups except the control increased on day 5 (≥7.0 mmol/L) indicating that diabetes was induced in the rats. The fasting blood

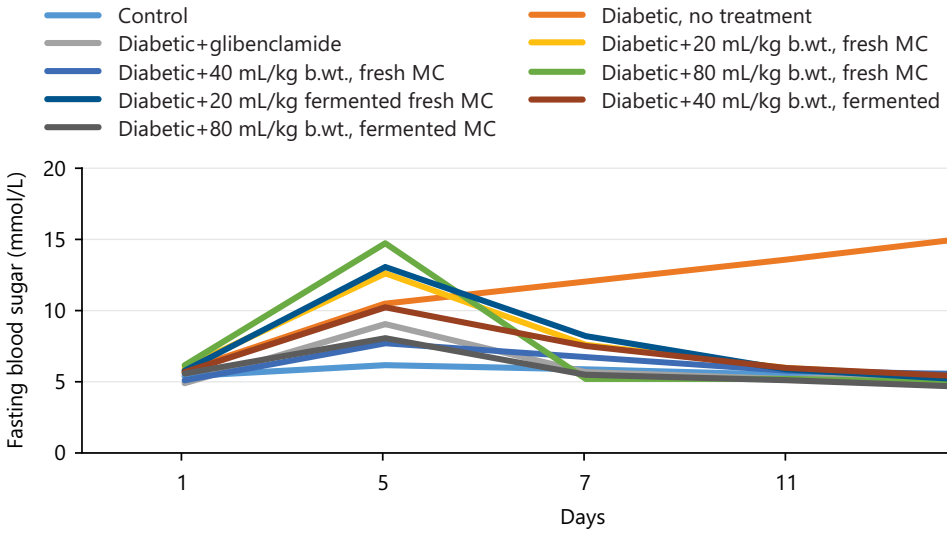

Fig. 1: Fasting blood glucose level of alloxan-induced diabetic rats administered fresh and fermented fruit juice of *Morinda citrifolia*

Table 1: Qualitative phytochemical screening of fresh and fermented fruit juice of *Morinda citrifolia*

Phytochemical	Fresh *Morinda citrifolia* fruit juice	Fermented *Morinda citrifolia* fruit juice
Steroid	-	-
Phenols	+	+
Saponins	+	+
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+

+: Present and -: Absent

Table 2: Body weight gain/loss of alloxan-induced diabetic rats treated with fresh and fermented fruit juice of *Morinda citrifolia*

Group	Weight gain/loss (g)
Control	70.00±7.23^{e}
Diabetic untreated	-30.00±3.7^{a}
Diabetic+glibenclamide	54.67±0.88^{d}
Diabetic+20 mL/Kg b.wt., fresh MC	45.33±2.72^{c}
Diabetic+40 mL/Kg b.wt., fresh MC	50.33±0.88^{c}
Diabetic+80 mL/Kg b.wt., fresh MC	57.33±3.28^{d}
Diabetic+20 mL/Kg b.wt., fermented MC	41.67±3.67^{b}
Diabetic+40 mL/Kg b.wt., fermented MC	47.33±2.97^{c}
Diabetic+80 mL/Kg b.wt., fermented MC	54.00±4.00^{d}

Values are Mean±SEM. Mean values with different superscript letters down the column are significantly different from each other

Table 3: Serum liver function indices of alloxan-induced diabetic rats administered fresh and fermented fruit juice of *Morinda citrifolia*

Group	Albumin (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
1	3.50±0.06^{a}	13.33±0.88^{a}	57.67±1.45^{a}	91.33±1.20^{a}
2	4.70±0.06^{e}	34.67±0.33^{g}	143.33±0.33^{i}	128.67±0.88^{h}
3	4.27±0.19^{d}	25.67±0.88^{e}	87.67±1.76^{e}	113.33±1.20^{g}
4	4.30±0.06^{d}	20.33±0.67^{c}	82.33±1.45^{d}	102.33±1.45^{f}
5	4.13±0.07^{c}	19.00±0.56^{b}	75.00±2.08^{c}	96.67±1.20^{d}
6	3.67±0.09^{a}	14.67±0.33^{a}	64.00±2.08^{b}	94.33±0.33^{c}
7	4.23±0.03^{d}	28.33±0.88^{f}	127.33±0.88^{h}	96.00±0.58^{e}
8	3.97±0.03^{b}	22.00±1.73^{d}	122.00±1.15^{g}	94.33±0.67^{c}
9	3.67±0.03^{a}	15.33±0.33^{a}	63.00±2.18^{a}	90.00±0.58^{a}

ALT: Alanin transaminase, AST: Aspartate transaminase and ALP: Alkaline phosphatase, values are Mean±SEM. Mean values with different superscript letters down the column are significantly different from each other

glucose level of the group that received no treatment continued increasing on day 7, 11 and 14, while by day 14, the fasting blood glucose of the treatment groups was comparable to the normal control. The fasting blood glucose of the groups that received 80 mL/kg b.wt., fresh *M. citrifolia* juice and 80 mL/kg b.wt., fermented *M. citrifolia* juice was comparable with the control right from day 7.

Table 2 shows the body weight gain or loss of alloxan-induced diabetic rats treated with fresh and fermented fruit juice of *M. citrifolia*. The rats that were diabetic and received no treatment significantly lose weight (-30.00±3.70). The weight gained by the treated groups was not comparable with the control. The weight gain by diabetic rats that received 80 mL/kg b.wt., fresh and fermented *M. citrifolia* was not significantly different from each other.

The serum liver function indices of alloxan-induced diabetic rats administered fresh and fermented fruit juice of *M. citrifolia* are shown in Table 3. The serum liver function indices of rats in group 2 (diabetic rats that received no treatment) significantly increased when compared to that of the rats in group 1 (control). There was a significant decrease in the serum liver indices of rats in groups 3-9 (treatment groups) when compared to group 2 (the untreated diabetic group). All the tested serum liver function indices of rats in group 9 (diabetic rats that received 80 mL/kg b.wt., fermented *M. citrifolia*) were not significantly different from group 1 (control rats).

DISCUSSION

The phytochemicals present in both fermented and fresh *M. citrifolia* fruit juice are similar. These similar phytochemicals present may be responsible for the non-significant difference in the hypoglycemic activity of both fruit juices. Saponin have been reported to inhibit gastric emptying either by inhibiting the degradation of glucagon-like peptides 1 (GLP-1) or by promoting its secretion. This slows down the process of nutrient absorption into the bloodstream and thus, is effective in managing hyperglycemia[13]. The decreased degradation of GLP-1 may also stimulate insulin release from the pancreas[24]. Also, saponin may have a glucagon decreasing effect which may enhance glucose utilization and thus lower blood glucose[13].

The continuous increase in the fasting blood glucose of rats that received no treatment indicates untreated diabetes while the observed comparable decrease in fasting blood glucose of rats administered a high dose (80 mL/kg b.wt.,) of fresh and fermented fruit juice of *M. citrifolia* indicates that their hypoglycemic activity is comparable. This may be due to the similar phytochemicals present in both the fresh and fermented fruit juice.

The observed decrease in the body weight of diabetic rats that received no treatment indicates weight loss. Insufficient insulin in people with diabetes prevents the body from moving glucose from the blood into the cells for energy, the cells therefore result in burning fat and muscle for energy leading to overall weight loss. The no significant difference in the body weight of rats that received high doses (80 mL/kg b.wt.,) of fresh and fermented *M. citrifolia* fruit juice indicates that fermentation did not have any effect on the ability of the fruit juice to restore weight gain in diabetic condition.

Guven *et al*.[25] described diabetes mellitus is associated with a number of liver abnormalities which include abnormally elevated liver enzymes and acute liver disease. Studies have shown that there is a decrease in catalase and superoxide dismutase activities in hyperglycemia state which leads to an increase in reactive oxygen species (ROS). This increase in ROS leads to oxidation induced liver damage[26,27]. The observed significant increase in the liver serum indices of diabetic rats that did not receive any treatment indicates liver damage. This liver damage was attenuated by both fresh and fermented fruit juice of *M. citrifolia*. However, the fermented *M. citrifolia* at 80 mL/kg b.wt., effectively attenuated the liver damage when compared to fresh fruit juice. Reports have shown that fermented fruit juice has hepatoprotective properties[13].

Flavonoids have antioxidant properties. The flavonoid present in *M. citrifolia* may be responsible for the attenuation of oxidation-induced liver damage. Fermentation has been shown to markedly increase the total flavonoid content of food[28]. This increase in flavonoids maybe responsible for the effective liver damage attenuation by high dose of fermented fruit juice of *M. citrifolia*. Zhao *et al*.[29] reported that fermentation can improve the antioxidant activity of plant-based food materials by increasing phytochemicals, antioxidant polysaccharides and antioxidant peptides produced by microbial hydrolysis or biotransformation. This may contribute to the effective liver damage attenuation by 80 mL/kg b.wt., fermented fruit juice of *M. citrifolia*.

CONCLUSION

The fresh and fermented fruit juice of *Morinda citrifolia* are comparable in their hypoglycemic activity and its weight gain ability. Fermented fruit juice efficiently attenuated liver damage better than fresh fruit juice. Fermented fruit juice is better than fresh fruit juice in the treatment of liver damage arising from diabetes mellitus.

SIGNIFICANCE STATEMENT

The use of both the fresh and fermented fruit juice of *Morinda citrifolia* has been scientifically validated but there is no information on which of the two is better. This study therefore compared the antidiabetic

activity of the fresh and fermented fruit juice of the plant. The study found that fermentation does not have any effect on the ability of the fruit juice to lower fasting blood glucose but it increases the hepatoprotective potential of the fruit juice. Therefore, fermentated *Morinda citrifolia* fruit juice is recommended because it is more effective in the treatment of liver damage caused by diabetes.

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