Asian Journal of
Science, Technology, Engineering, and Art

e-ISSN : 3025-4507
p-ISSN : 3025-5287
Index : Harvard, Boston, Sydney University, Dimensions, Lens, Scilit, Semantic, Google, etc

https://doi.org/10.58578/AJSTEA.v2i2.2775

ANTIDIABETIC AND HEPATOPROTECTIVE EFFECTS OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF Mitracarpus hirtus ON ALLOXAN-INDUCED DIABETIC RATS

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Feb 10, 2024	Feb 20, 2024	Feb 25, 2024	Feb 29, 2024

Abstract

Diabetes mellitus (DM) is a metabolic disorder that remains a major health concern worldwide. It is associated with derangement of carbohydrate, protein, and lipid metabolism. This study evaluates the antidiabetic and hepatoprotective effects of aqueous and ethanolic leaf extracts of Mitracarpus hirtus on alloxan-induced diabetic rats. Fifty-five male Wistar rats were divided into 11 groups, with diabetes induced by alloxan. Treatment with aqueous and ethanolic extracts, along with Glibenclamide, lasted three weeks. At the end of the treatment period, the rats were sacrificed and blood samples were collected via cardiac puncture for biochemical analysis. Findings revealed a significant decrease ($p<0.05$) in fasting blood sugar (FBS) in group 10 (99.8 ± 15.74 mg/dL) when compared with group 2 (274.2 ± 7.95 mg/dL) in week 1. Week 2 also revealed a significant decrease ($p<0.05$) in group 11 (84.6 ± 14.98 mg/dL) when compared with group 2 (262.8 ± 15.94 mg/dL). Week 3, further revealed a significant decrease ($p<0.05$) in group 9 (79.4 ± 25.13 mg/dL) and group 11 (83.6 ± 8.35 mg/dL) when compared with group 2 (289.6 ± 20.89 mg/dL). Aspartate Aminotransferase (AST) revealed a significant decrease ($p<0.05$) when group 11 (54.38 ± 15.74 U/L) was compared with group 2

(146.66 ± 11.35 U/L). Similarly, Alanine Aminotransferase (ALT) significantly decreased ($p<0.05$) when group 11 (53.46 ± 4.6 U/L) was compared with group 2 (101.42 ± 16.01 U/L), while total protein and albumin increased in all treated groups. Group 5 (0.64 ± 0.11 mg/dL) in Total bilirubin (TB) had the lowest mean when compared with group 2 (2.69 ± 0.52 mg/dL). All treatment groups significantly decreased ($p<0.05$) Triglyceride (TG) and Cholesterol (CHOL). From this study, it has been shown that the aqueous and ethanolic leaves extracts of Mitracarpus hirtus may have the potential to ameliorate the complications due to diabetes in a dose dependent manner.

Keywords: Antidiabetic, Hepatoprotective, Alloxan, Mitracarpus Hirtus, Glibenclamide

INTRODUCTION

Diabetes mellitus is a prevalent metabolic disorder affecting glucose, lipid, and protein balance in the endocrine system (Effiong *et al.*, 2013). It stems from insufficient insulin secretion by pancreatic β-cells or reduced tissue cell sensitivity to insulin (Effiong *et al.*, 2013; Nirmala *et al.*, 2009). This lifelong condition requires constant monitoring and control, significantly impacting overall well-being (Okoronkwo *et al.*, 2015). The global rise in diabetes mellitus and its complications is a growing concern (IDF, 2019), contributing to premature deaths, including those of distinguished leaders and renowned scholars (Oputa and Chinenye 2015).

Complications of diabetes, such as foot infections, ulcsers, and the subsequent need for lower extremity amputation, along with kidney, liver, and heart diseases, have been documented (Lipsky *et al.*, 2012). Despite the significant economic impact, diabetes management using conventional drugs like insulin, biguanides, and sulfonylureas raises concerns due to serious side effects such as hypoglycemic coma, hematological and gastrointestinal reactions, and disturbances in kidney, liver, and heart functions. The pursuit of more effective and safer therapeutic agents for diabetes mellitus and its complications remains a crucial area of research (Akomas *et al.*, 2014; Ayinla *et al.*, 2014).

Diabetes mellitus-induced damage to liver cells results in the release of liver function marker enzymes like alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase into the plasma (Mankhatithan *et al.*, 2011). Monitoring the activities of these enzymes in the plasma provides an indirect assessment of liver function. Elevated

concentrations beyond homeostatic limits indicate potential liver tissue disorder (Friday *et al.*, 2010).

Medicinal plants have been documented as having beneficial properties used for the management of various ailments (Ochalefu *et al* 2023). *Mitracarpus hirtus* (L.) DC., also known as 'Harwatsi' in Hausa is a weed plant wildly grown everywhere in Nigeria. The juice obtained from the plant is quite common for the treatment of eczema (Malami *et al.*, 2023). *Mitracarpus hirtus* L. has been widely used to treat ringworm, rashes, toothache, itch, eczema, venereal diseases (B/kudu *et al.*, 2018), boils, measles (Idris *et al.*, 2015), skin diseases (Suleiman and Suleiman, 2015), and as an antidote for bites and stings (Pansuksan *et al.*, 2014). Therefore, this study was aimed at investigating the antidiabetic and hepatoprotective effects of aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* on alloxan-induced diabetic rats.

METHODS

Sample Collection and Preparation

Fresh leaves of *Mitracarpus hirtus* were collected from its natural habitat in Sondi, Wukari L.G.A, Taraba State, Nigeria. Identification and authentication were done at the herbarium in the Department of Plant Sciences of Modibbo Adama University Yola, Nigeria. It was air dried at room temperature and was ground using electric blender.

Experimental Animals

Fifty-five (55) wistar rats were purchased, maintained at room temperature, fed with broilers starter and water *ad-libitum* and allowed to acclimatize for a week before commencement of the experiment.

Ethanolic Extraction of *Mitracarpus hirtus* leaves

The extraction process followed the method outlined by Yakubu *et al.* (2013) and Ezeonwu and Dahiru (2013). Initially, 400 grams of pulverized sample were soaked in absolute ethanol at a ratio of 1:4 w/v (400g: 1600 mL) for precisely 24 hours. After the soaking period, the extract was filtered using a clean white sieving mesh followed by Whatman No. 1 filter paper to remove any solid particles. Subsequently, the filtrate was concentrated

using a rotary evaporator at a temperature of 68°C. Once concentrated, the extract was transferred into air-tight containers, corked, and preserved in a refrigerator at 4°C until further use.

Aqueous Extraction of *Mitracarpus hirtus* leaves

The extraction process followed the method outlined by Yakubu *et al.* (2013) and Ezeonwu and Dahiru (2013). Initially, 400 grams of pulverized sample were soaked in absolute distilled water, totaling 1600 mL, for precisely 24 hours. After the soaking period, the extract was filtered using a clean white sieving mesh followed by Whatman No. 1 filter paper to remove any solid particles. Subsequently, the filtrate was concentrated using a rotary evaporator at a temperature of 68°C. Once concentrated, the extract was transferred into air-tight containers, corked, and preserved in a refrigerator at 4°C until further use.

Induction of Diabetes

Diabetes was induced in the experimental rats by administering a single intra-peritoneal injection of alloxan monohydrate at a dosage of 150 mg/kg, dissolved in distilled water as a vehicle, following the method described by Carvalho *et al.* (2003). Prior to the injection, the rats were fasted for 12 hours. Immediately after the injection, the animals were provided with a glucose solution to drink to mitigate the drug-induced hypoglycemia.

Forty-eight hours post-alloxan administration, blood samples were collected from the tail of the rats, and their glucose levels were measured using a One Touch Glucometer and test strips. Rats exhibiting a fasting blood glucose concentration greater than 196 mg/dl were considered to have developed diabetes and were selected for inclusion in the experiment, as per the criteria outlined by Carvalho *et al.* (2003) and Yakubu *et al.* (2020).

Experimental Design

Fifty-five (55) male Wistar rats were used and distributed randomly into eleven groups consisting of five animals each, as described below.

Group 1: Normal control (Non diabetic, no treatment).

Group 2: Negative control (Diabetic, no treatment).

Group 3: Diabetic treated with Glibenclamide 5 mg/kg (positive control).

Group 4: Diabetic treated with 200 mg/kg of aqueous leaves extract of *Mitracarpus hirtus*.

Group 5: Diabetic treated with 400 mg/kg of aqueous leaves extract of *Mitracarpus hirtus.*

Group 6: Diabetic treated with 200 mg/kg of ethanolic leaves extract of *Mitracarpus hirtus.*

Group 7: Diabetic treated with 400 mg/kg of ethanolic leaves extract of *Mitracarpus hirtus***.**

Group 8: Administered 200 mg/kg of aqueous leaves extract of *Mitracarpus hirtus* before administration of alloxan.

Group 9: Administered 400 mg/kg of aqueous leaves extract of *Mitracarpus hirtus* before administration of alloxan

Group 10: Administered 200 mg/kg of ethanolic leaves extract of *Mitracarpus hirtus* before administration of alloxan

Group 11: Administered 400 mg/kg of ethanolic leaves extract of *Mitracarpus hirtus* before administration of alloxan.

Treatments were given to the rats orally for 21 days. The fasting blood glucose determinations were determined weekly using glucometer.

Fasting Blood Glucose Determination

This was carried out as described by Yakubu *et al.* (2013) and Uhuo *et al*., (2022). A drop of blood was collected through the tail of the over-night fasted rats to test for glucose on an assay strip and read using Accu-Check glucometer. This was carried out on a weekly basis for 21 days.

Animal Sacrifice and Collection of Samples

This was carried out as described by Imo *et al.* (2013). The animals were anaesthetized with chloroform; incisions were made into their thoracic cavity and blood samples were collected by cardiac puncture using a 10ml syringe and dispensed into dry tubes and allowed to clot for fifteen minutes after which it was centrifuged for 10 minutes at 4000 rpm. Serum was separated from the clot using Pasteur pipette for the serum biochemical analysis.

The serum activities of selected liver marker enzymes (ALT and AST) and serum levels of Total protein (TP), Total Bilirubin (TB), Direct Bilirubin (DB), albumin (ALB),

Triglycerides and Cholesterol were assayed using an autoanalyzer (Cobas C111 Chemistry Analyzer).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 23. One-way analysis of variance (ANOVA) was conducted to assess the differences between groups. Subsequently, Duncan multiple comparisons were applied to further analyzes the data. The significance level was set at $p < 0.05$. The results are presented as mean ± standard deviation, with a sample size of $n = 5$ for each group.

RESULTS

The administration of alloxan resulted in a significant increase ($p < 0.05$) in blood glucose levels in experimental rats compared to control rats. However, treatment with both aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* significantly decreased blood glucose levels ($p < 0.05$) in alloxan-induced diabetic fasted rats. The hypoglycemic activity observed was comparable to that of Glibenclamide (5mg/kg bsody weight/day) treated positive control rats.

Table 1 Fasting blood sugar concentration in alloxan-induced diabetes rats treated with aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* in week 1 revealed a significant decrease in group 10 (99.8 ± 15.74 mg/dL) when compared with group 2 (274.2 ± 7.95 mg/dL). In Week 2, group 11 (84.6 ± 14.98 mg/dL) significantly decreased when compared with group 2 (262.8 ± 15.94 mg/dL). Week 3, revealed a significant decrease in group 9 (79.4 ± 25.13 mg/dL) and group 11 (83.6 ± 8.35 mg/dL) when compared with group 2 (289.6 ± 20.89 mg/dL).

Table 1: Fasting Blood Sugar (FBS) levels in normal and diabetic rats treated with aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* and Glibenclamide (mg/dL)

GRPS	Treatment	DAY 1 mg/dL	WK1 mg/Dl	WK2 mg/dL	WK3 mg/dL
1	Normal Control	88.60 ± 09.18^{a}	83.20 ± 9.01^{a}	93.20±10.52^{a,b}	89.40 ±8.62^{ab}
2	Negative Control	291.20 ±10.28^{de}	274.20±7.95^{e}	262.8±15.94^{e}	289.6±20.89^{e}
3	Diabetic+ Glibenclamide	264.40±12.18^{cde}	162.2±126.3^{bcd}	126.80±13.03^{cd}	110.2±8.41^{bc}
4	Rats treated with 200mg/kg Aq (Mh)(Post alloxan induced Diabetes)	283.20±58.26^{de}	167.80±25.47^{cd}	138.40±22.86^{d}	117.40±25.36^{d}
5	Rats treated with 400mg/kg Aq (Mh)(Post alloxan induced Diabetes)	298.20±24.98^{e}	146.20±66.27^{abcd}	94.40±18.80^{ab}	100.40±5.50^{abc}
6	Rats treated with 200mg/kg Et (Mh)(Post alloxan induced Diabetes)	259.40±54.39^{cde}	171.20±8.26^{cd}	128.6±40.56^{d}	100.20±7.60^{abc}
7	Rats treated with 400mg/kg Et (Mh)(Post alloxan induced Diabetes)	241.20±44.38^{cd}	130.80±15.77^{abcd}	115.40±9.50^{bcc}	95.60±17.54^{abc}
8	Rats administered with 200mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	273.60±22.12^{cde}	178.40±26.75^{d}	122.20±15.37^{cd}	99.60±16.98^{abc}
9	Rats administered with 400mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	189.80±61.82^{b}	105.80±33.36^{abc}	100.60±14.15^{abc}	79.40±25.13^{a}
10	Rats administered with 200mg/kg Et (Mh) (Pre alloxan induced Diabetes)	223.60±21.89^{bc}	99.80±15.74^{ab}	90.40±14.36^{ab}	87.40±13.28^{ab}
11	Rats administered with 400mg/kg Et (Pre alloxan induced Diabetes)	190.20±13.30^{b}	110.60±18.32^{abcd}	84.60±14.98^{a}	83.60±8.35^{a}

Values are expressed as mean ± Standard deviation; N=5.Values with different superscript down the column are considered statistically significant at p≤0.05.

The table 2. below shows results of liver function tests – Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Protein (TP), Albumin (ALB).

The result of AST revealed a significant decrease when group 11 (54.38 ± 15.74 U/I) was compared with group 2 (146.66 ± 11.35 U/I). Similarly, ALT significantly decreased when group 11 (53.46 ± 4.6 U/I) was compared with group 2 (101.42 ± 16.01 U/I). TP revealed a significant increase when group 11 (7.45 ± 0.5 gm/dL) was compared with group 2 (4.23 ± 0.47 gm/dL). Albumin also significantly increased in group 11 (5.02 ± 0.75 gm/dL) when compared with group 2 (0.24 ± 0.08 gm/dL).

Table 2: Concentration of selected liver function parameters of rats treated with aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* and Glibenclamide

GRPS	TREATMENTS	AST (U/L)	ALT (U/L)	TP (gm/dL)	ALB (gm/dL)
1	Normal Control	42.18 ± 2.7^{a}	47.59 ± 7.14^{a}	6.39 ± 0.94^{cd}	3.89 ± 0.2^{bc}
2	Negative Control	146.66 ± 11.35^{h}	101.42 ± 16.01^{e}	4.23 ± 0.47^{a}	1.24 ± 0.08^{a}
3	Diabetic+ Glibenclamide	68.14 ± 7.96^{cd}	51.28 ± 4.28^{ab}	5.03 ± 0.90^{ab}	3.98 ± 0.40^{bc}
4	Rats treated with 200mg/kg Aq (Mh)(Post alloxan induced Diabetes)	105.46 ± 6.66^{g}	78.21 ± 8.81^{d}	4.29 ± 0.88^{a}	3.14 ± 2.51^{b}
5	Rats treated with 400mg/kg Aq (Mh)(Post alloxan induced Diabetes)	56.35 ± 6.32^{bc}	74.54 ± 4.56^{cd}	6.33 ± 0.75^{cd}	3.03 ± 0.26^{b}
6	Rats treated with 200mg/kg Et (Mh)(Post alloxan induced Diabetes)	84.59 ± 3.56^{ef}	91.26 ± 9.77^{e}	5.65 ± 0.65^{bc}	4.19 ± 0.14^{b}
7	Rats treated with 400mg/kg Et (Mh)(Post alloxan induced Diabetes)	92.15 ± 15.35^{f}	72.22 ± 6.85^{cd}	$6.52\pm0.93^{c,d,e}$	3.09 ± 0.77^{bc}
8	Rats administered with 200mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	78.41 ± 8.43^{de}	71.51 ± 11.39^{cd}	6.61 ± 0.06^{cde}	3.28 ± 0.20^{b}
9	Rats administered with 400mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	73.62 ± 7.09^{de}	67.88 ± 7.28^{cd}	6.97 ± 0.42^{de}	3.11 ± 0.13^{b}

10	Rats administered with 200mg/kg Et (Mh) (Pre alloxan induced Diabetes)	82.15±7.96^{ef}	63.46±12.48^{bc}	5.77±0.42^{bc}	3.09±0.70^{b}
11	Rats administered with 400mg/kg Et (Pre alloxan induced Diabetes)	54.38±15.74^{a}	53.46±4.60^{ab}	7.45±0.50^{e}	5.02±0.75^{c}

Values are expressed as Mean ± Standard deviation; N=5.Values with different superscript down the column are considered statistically different at $p \leq 0.05$.

The table 3 shows the result of Total bilirubin, Direct Bilirubin (DB), and Indirect Bilirubin (I.D BIL) with a significant ($p>0.05$) increase TB in the diabetic control compared to the normal control. All treatment groups revealed a significant decrease in all parameters. However, group 5 (0.64 ± 0.11 mg/dL) in TB had the lowest mean when compared with group 2 (2.69 ± 0.52 mg/dL). Similarly, DB revealed a significant decrease in group 5 (0.11 ± 0.09 mg/dL) when compared with group 2 (0.81± 0.09 mg/dL).IDB revealed a significant decrease in group 9 (0.31 ± 0.14 mg/dL) and 11 (0.34 ± 0.19 mg/dL) when compared with group 2 (0.99 ± 0.91 mg/dL).

Table 3: Serum bilirubin concentration of rats treated with aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* and Glibenclamide

GRPS	TREATMENT	TB (mg/dL)	DB (mg/dL)	IDB (mg/dL)
1	Normal Control	0.54±0.06^{a}	0.11±0.07^{a}	0.37±0.16^{a}
2	Negative Control	2.69±0.52^{ab}	0.81±0.09^{c}	0.99±0.91^{b}
3	Diabetic+ Glibenclamide	0.59±0.04^{g}	0.3±0.33^{ab}	0.44±0.08^{a,b}
4	Rats treated with 200mg/kg Aq (Mh)(Post alloxan induced Diabetes)	0.71±0.04^{ab}	0.26±0.18^{ab}	0.80±0.12^{ab}
5	Rats treated with 400mg/kg Aq (Mh)(Post alloxan induced Diabetes)	0.64±0.11^{abc}	0.11±0.09^{a}	0.64±0.66^{a,b}
6	Rats treated with 200mg/kg Et (Mh)(Post alloxan induced Diabetes)	1±0.44^{de}	0.34±0.31^{ab}	0.55±0.23^{ab}
7	Rats treated with 400mg/kg Et (Mh)(Post alloxan induced Diabetes)	0.89±0.12^{bcde}	0.62±0.19^{bc}	0.53±0.26^{ab}
8	Rats administered with 200mg/kg Aq (Mh) (Pre	1.14±0.24^{ef}	0.64±0.10^{bc}	0.81±0.19^{ab}

	alloxan induced Diabetes)			
9	Rats administered with 400mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	0.94 ± 0.09^{def}	0.59 ± 0.03^{bc}	0.31 ± 0.14^{a}
10	Rats administered with 200mg/kg Et (Mh) (Pre alloxan induced Diabetes)	1.38 ± 0.27^{f}	0.86 ± 0.79^{c}	$0.76\pm0.59^{a,b}$
11	Rats administered with 400mg/kg Et (Pre alloxan induced Diabetes)	1.04 ± 0.05^{de}	0.50 ± 0.33^{abc}	0.34 ± 0.19^{a}

Values are expressed as mean ± Standard deviation; N=5.Values with different superscript down the column are considered statistically significant at $p\leq0.05$.

All treatment groups significantly decreased in triglycerides (TG) when compared with group 2 (2.60 ± 2.13 mmol/L). Cholesterol (CHOL) significantly decreased in all treatment groups when compared with group 2 (3.82 ± 0.82 mmol/L). However, group 7 (1.49 ± 0.70 mmol/L) revealed a significant increase when compared with other treatment groups

Table 4: serum Triglycerides (TG) and Cholesterol (CHOL) levels in normal and diabetic rats treated with aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* Glibenclamide.

GRPS	TREATMENT	TG(mmol/L)	CHOL(mmol/L)
1	Normal Control	0.47 ± 0.37^{a}	0.43 ± 0.48^{a}
2	Negative Control	2.60 ± 2.13^{b}	3.82 ± 0.82^{c}
3	Diabetic+ Glibenclamide	0.47 ± 0.27^{a}	0.32 ± 0.80^{a}
4	Rats treated with 200mg/kg Aq (Mh)(Post alloxan induced Diabetes)	0.71 ± 0.33^{a}	0.93 ± 0.33^{a}
5	Rats treated with 400mg/kg Aq (Mh)(Post alloxan induced Diabetes)	0.54 ± 0.53^{a}	0.76 ± 0.58^{a}
6	Rats treated with 200mg/kg Et (Mh)(Post alloxan induced Diabetes)	0.65 ± 0.39^{a}	0.77 ± 1.16^{a}
7	Rats treated with 400mg/kg Et (Mh)(Post alloxan induced Diabetes)	0.51 ± 0.24^{a}	1.49 ± 0.70^{b}
8	Rats administered with 200mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	0.44 ± 0.16^{a}	0.90 ± 0.44^{a}
9	Rats administered with 400mg/kg Aq (Mh) (Pre alloxan induced Diabetes)	0.17 ± 0.12^{a}	0.69 ± 0.11^{a}

10	Rats administered with 200mg/kg Et (Mh) (Pre alloxan induced Diabetes)	0.71±0.22[a]	0.39±0.30[a]
11	Rats administered with 400mg/kg Et (Pre alloxan induced Diabetes)	0.29±0.12[a]	0.28±0.25[a]

Values are expressed as mean ± Standard deviation; N=5.Values with different superscript down the column are considered statistically significant $p \leq 0.05$.

DISCUSSION

The present study focused on evaluating the antidiabetic and hepatoprotective effects of aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* in alloxan-induced diabetic rats. The overall effects of aqueous and ethanolic extract of *Mitracarpus hirtus* were carried out on the antidiabetic and hepatoprotective activity. Research indicates that inducing diabetes chemically through the intraperitoneal administration of alloxan monohydrate leads to Type I diabetes in laboratory animals (Viana *et al.,* 2004; Etuk, 2010). Alloxan monohydrate, derived from urea, causes diabetes by selectively damaging the pancreatic beta cells of Langerhans (Iranloye *et al*., 2011). This impairment affects the synthesis and release of endogenous insulin, resulting in insufficient availability and leading to hyperglycemia (Nastaran *et al*., 2011). Alloxan's toxic effects on pancreatic beta cells involve inhibiting the glucokinase enzyme, generating free radicals, disrupting intracellular calcium balance, and oxidizing essential sulphydryl (-SH) groups (Dunn *et al*., 1983; Szkudelski, 2001; Dhanesha *et al*., 2012).

The preliminary phytochemical screening of *Mitracarpus hirtus* by Binoodha *et al.* (2022) revealed the presence of alkaloids, flavonoids, steroids, tannins, phenolics, glycosides, carbohydrates, proteins and amino acids, which explains that the plant have valuable medicinal properties.

Elevated levels of glucose in the environment can contribute to apoptosis (Allen *et al*., 2003), potentially damaging cells due to hyperglycemia in diabetes. Reactive oxygen species (ROS) play a crucial role in inducing β-cell death during the progression of diabetes mellitus (DM). It's been suggested that high glucose levels can lead to the generation of ROS and nitrogen species in various cell types. The production of superoxide due to high glucose is well-documented and primarily occurs through the mitochondrial electron

transport chain (Swagat *et al*., 2016). Another source of glucose-induced oxidative stress is the polyol pathway, where glucose is converted to sorbitol by aldose reductase, a process that consumes NADPH. This impedes the NADPH-dependent synthesis of glutathione, a vital cellular antioxidant (Weiss and Sumpio, 2006). In this study significant hyperglycemia was achieved after alloxan injection. However, the aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* administration in day 1 revealed a significant decrease fasting blood sugar (FBS) in group 11(Rats administered with 400 mg/kg Et (Mh) (pre-alloxan-induced diabetes) (190.2 ± 13.31 mg/dl). In week 1, group 10 (Rats administered with 200 mg/kg Et (Mh) (pre-alloxan-induced diabetes) (99.8 ± 15.74 mg/dL) significantly decreased FBS when compared with group 2 (274.2 ± 7.95 mg/dl). Week 2 also revealed a significant decrease of FBS in group 11 (84.6 ± 14.98 mg/dl) when compared with group 2 (262.8 ± 15.94 mg/dL). Week 3, revealed a significant decrease in group 9 (Rats administered with 400mg/kg Aq (Mh) Mh) (pre-alloxan-induced diabetes) (79.4 ± 25.13 mg/dL) and group 11 (83.6 ± 8.35 mg/dL) when compared with group 2 (289.6 ± 20.89 mg/dL) in a dose dependent manner. These findings align with those of previous studies (Chaturvedi *et al*., 2004; Abubakar *et al*., 2016). This could be due to the presence of phenolic compounds, which are known to interact with proteins and hinder enzymatic activity (Suryanarayana *et al*., 2004). Phenols derived from plant extracts used in food, which inhibit α-amylase activity, are considered potentially safe and may be a preferable option for regulating carbohydrate digestion and managing the glycemic index of food products. Additionally, alkaloids found in *Mitracarpus hirtus* leaves have been reported to inhibit α-glucosidase activity. This suggests that both aqueous and ethanolic leaf extracts of the plants may have insulin-like effects on peripheral tissues, either by enhancing glucose uptake or metabolism, inhibiting hepatic gluconeogenesis (Ali *et al*., 1993), or limiting glucose absorption into muscles and adipose tissues (Kamanyi *et al*., 1994). This could stimulate a regeneration process and revitalize remaining beta cells (Shanmugasundaram *et al*., 1990).

Enzyme activities in tissues are frequently utilized as indicators to detect early toxic effects of introduced foreign substances in experimental animals (Akanji and Ngaha, 1989; Adesokan and Akanji, 2004). This is because any changes in the biochemical processes in these animals resulting from the presence of a foreign compound would manifest as either increased or decreased activity of such enzymes (Kandem *et al*., 1982; Zilva and Pannal, 1984). Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) serve as marker enzymes, indicating liver injury or impairment in liver functions, and are crucial in

metabolism (Reitman and Frankel, 1957). AST and ALT are both cytosolic enzymes. AST facilitates the transfer of an amino group from aspartate to α-ketoglutarate, yielding oxaloacetate and glutamate. ALT, on the other hand, is involved in gluconeogenesis and amino acid metabolism, catalyzing the transfer of an amino group from alanine to α-ketoglutarate, resulting in glutamate and pyruvate. In damaged livers with hepatocellular lesions, these marker enzymes are released from damaged tissues, leading to increased levels in the bloodstream (Nkosi *et al*., 2005).

ALT and AST serve as prominent diagnostic indicators of liver damage. The ratio of these two enzymes in the serum is employed to distinguish liver damage from damage to other organs (Nathwani *et al*., 2005). ALT activity, in particular, is the most commonly used biomarker for hepatotoxicity. Hepatotoxicity causes an increase in the normal values of these enzymes because the body may struggle to excrete them through bile due to congestion or obstruction of the biliary tract within the liver. Elevated serum activity of these enzymes is typically observed during liver damage. In this study, diabetes induced damages to the liver. Table 2 revealed the significant ($p<0.05$) increase in the level of AST and ALT and significant ($p<0.05$) decrease in the level of Total protein (TP) in diabetics control (group 2) when compared with the normal control (group 1). Also there was a significant ($p<0.05$) decrease in Albumin (ALB) in diabetics control (group 2) when compared with the normal control (group 1). The significant elevation in the marker enzymes in diabetic control (group 2) in this current study as shown in the table 2 above is a confirmation of previous research work on alloxan-induced diabetes mellitus (Atmaca *et al.,* 2019). The increase in the activities of serum AST and ALT and decrease in total protein and albumin indicated that diabetes may result due to liver dysfunction and acute hepatocytes affection (Alshawsh *et al*., 2011). Thereafter, increase in the enzyme activities may be mainly due to leakage of these enzymes from the liver cells into the blood stream which gives an indication on the hepatotoxic effect of alloxan induction. However, the extracts administration caused a significant decrease in AST and ALT in all treatments groups; however the best result was obtained when group 11 (54.38 ± 15.74 U/L) was compared with group 2 (146.66 ± 11.35 U/L). Similarly, ALT also significantly decreased when group 11 (53.46 ± 4.6 U/L) was compared with group 2 (101.42 ± 16.01 U/L). This report agreed with Ugwu *et al.* (2013); Yakubu *et al.,* (2020) and Abu *et al*., (2022). The decrease in AST and ALT in the treatments groups may be attributed to the presence of flavonoids which possess a wide range of biological benefits, including organ protection,

hypoglycemic, lipid-lowering, anti-oxidative, and anti-inflammatory properties (López-Lázaro, 2002). Therefore, it is possible to suggest that these extracts are safe and might confer protection against diabetes-induced hepatocellular damage as evidenced by reduction in the serum levels of AST and ALT in the treatments groups (Yakubu *et al.,* 2020).

There was a notable ($p<0.05$) reduction in the average total protein (TP) in the diabetic control group (group 2) compared to the normal control group (group 1). This decrease in protein levels could be attributed to microproteinuria, a crucial clinical indicator of diabetic nephropathy (Tuvemo *et al*., 1997; Makare *et al*., 2001), and possibly increased protein breakdown (Almdal and Vilstrup, 1988). Additionally, insulin deficiency can lead to decreased RNA and mRNA levels, contributing to the decline in total protein (Fu *et al*., 2013). This study's findings align with previous research, particularly that of Kemasari *et al.* (2011). However, treatment with both aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* resulted in a significant ($p<0.05$) increase in TP across all tested groups. Notably, group 10 (7.45 ± 0.5 gm/dL) in the treatment groups exhibited a remarkable increase compared to group 2 (4.23 ± 0.47 gm/dL). These findings are consistent with the work of Reda (2006) and Najafi (2011), who demonstrated that *date seed* extract has the potential to restore normal function and protect the liver in rats against alloxan-induced toxicity.

Albumin also significantly decreased in diabetic control (group 2) when compared with the normal control (group 1). This could be as a result of damaged caused by alloxan in the liver as a result of oxidative stress (Watkins and Seef, 2006). Administration of aqueous and ethanolic leaf extracts of *Mitracarpus hirtus*, caused a significant increase in albumin when group 9 (5.02 ± 0.75 gm/dL) was compared with group 2 (1.24 ± 0.08 gm/dL), and consequently may improve liver damage caused by alloxan-induced diabetes (EL-Demerdash *et al*., 2005). This agreed with the findings of (Ugwu *et al*., 2013). This increased in albumin may be attributed to the presence of free radical scavenging antioxidants in the extracts (Li *et al.,* 2015). The results also obtained correlate with the above findings.

Table 3 revealed the concentrations of total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IDB). All treatment groups revealed a significant decrease in all parameters when compared with diabetic control. However, group 5 (0.64 ± 0.11 mg/dL) in TB had the lowest mean when compared with group 2 (2.69 ± 0.52 mg/dL). Similarly, DB revealed a significant decrease in group 5 (0.11 ± 0.09 mg/dL) when compared with

group 2 (0.81± 0.09 mg/dL). IDB revealed a significant decrease in group 9 (0.31 ± 0.14 mg/dL) and 11 (0.34 ± 0.19 mg/dL) when compared with group 2 (0.99 ± 0.91 mg/dL). This result agrees with various research involving alloxan as toxicity inducing agent by Shah and Kahn, (2014); Yakubu *et al.* (2020) and many other researchers reported the high levels of these marker enzymes in rats treated with alloxan. The total bilirubin level serves as a crucial indicator of liver function as it undergoes conjugation in the liver for potential excretion via the kidneys or bile. Elevated bilirubin levels were observed in diabetic rats, indicating compromised liver function, which aligns with findings from Shah and Kahn (2014). This increase in plasma bilirubin may result from reduced liver uptake, conjugation, or increased bilirubin formation following liver damage as noted by Rana *et al.* (1996). Treatment with *Mitracarpus hirtus* extracts for 21 days significantly reduced bilirubin levels in diabetic rats, indicating improved liver function, consistent with Shah and Khan's (2014) findings using Sida cordata extract in alloxan-induced diabetic rats for 15 days. Similarly, Sunmonu and Afolayan (2013) reported a notable decrease in bilirubin levels in STZ-induced diabetic rats treated with *Artemisia afra* aqueous extract for 15 days.

Hypertriglyceridemia stands as the prevalent lipid anomaly in diabetic populations (Al-Shamaony *et al.*, 1994). According to the findings presented in table 4, alloxanization induces a notable elevation in serum triglyceride (TG) and cholesterol (CHOL) levels among diabetic subjects in comparison to their non-diabetic counterparts (Al-Shamaony *et al.*, 1994; Bako *et al.*, 2014). This increase is likely attributable to the disruption in the regulation of lipase activity by insulin owing to its deficiency stemming from the alloxan-triggered destruction of beta islet cells (Al-Shamaony *et al.*, 1994). Lipase, responsible for converting triglycerides into free fatty acids and glycerol, is normally inhibited by insulin in adipose tissue (Al-Shamaony *et al.*, 1994). In the absence of insulin, plasma free fatty acid levels surge. Subsequently, in the liver, free fatty acids undergo catabolism into acetyl CoA, with surplus acetyl CoA being transformed into cholesterol, triglycerides, and ketone bodies, thereby inducing ketosis (Al-Shamaony *et al.*, 1994). Alloxan-induced diabetes fosters an excess of plasma fatty acids, prompting their conversion into triglycerides and cholesterol in the liver, which are then released into the bloodstream as lipoproteins (Bopanna *et al.*, 1997). In table 4, all treatment groups significantly decreased in TG when compared with group 2 (2.60 ± 2.13 mmol/L), CHOL also significantly decreased in all treatment groups when compared with group 2 (3.82 ± 0.82 mmol/L), this may be attributed to the presence of phytochemicals such as alkaloids which have been reported to

exhibit lipid lowering potential activity in rats (Bako *et al*., 2014). Glycocides also present in the leaves of *Mitracarpus hirtus* have been proven to decrease Hb1Ac level, oral glucose tolerance test, triglyceride level and fatty acid synthase activity (Bako *et al*., 2014). According to Claudia *et al*. (2006) and Bopanna *et al*. (1997), various plant extracts possess therapeutic potential in mitigating atherosclerosis, a significant complication of diabetes, by reducing serum lipid levels, particularly total cholesterol, triglycerides, and low-density lipoprotein levels (Luka and Tijjani, 2013).

CONCLUSION

The results from this study indicated that the aqueous and ethanolic leaf extracts of *Mitracarpus hirtus* possess hypoglycemic, hypolipidemia, and hepatoprotective effects in alloxan induced diabetic rats in a dose-dependent manner, this it does by reversing alterations and stabilizing the biochemical parameters which are indicators of organ failure and malfunctions in diabetes, thus scientifically validating the possible use of both extracts of the plants in folkloric medicine in the management of diabetes mellitus and hepatic diseases. The results therefore suggest that the extracts (especially group 9 and 11) which were pre-administered and at higher doses (400 mg/kg) showed great antidiabetic properties, thus potential sources of natural products that may be of great importance for the treatment of diabetes and diabetes related diseases.

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