ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

ANTIDIABETIC AND HEPATOPROTECTIVE EFFECTS OF COMBINED AQUEOUS LEAF EXTRACT OF *Azadirachta indica* AND *Mangifera indica* IN ALLOXAN MONOHYDRATE-INDUCED DIABETIC WISTAR RATS

Omeodu SI[a], Uahomo PO[b] , Porobe VL[a]

[a]*Department of Biochemistry, Faculty of Sciences, University of Port-Harcourt, Rivers State, Nigeria.*
[b]*Department of Biomedical Technology, School of Science Laboratory Technology, University of Port-Harcourt, Rivers State, Nigeria.*

Corresponding Author: Omeodu S.I., Department of Biochemistry, Faculty of Sciences, University of Port Harcourt, Rivers State, Nigeria.

Article DOI: https://doi.org/10.36713/epra11592
DOI No: 10.36713/epra11592

ABSTRACT

This study was aimed at assessing the antidiabetic and hepatoprotective effects of combined aqueous leaf extract of Azadirachta indica and Mangifera indica in alloxan monohydrate-induced diabetic Wistar rats. Fresh leaves extract of Azadirachta indica and Mangifera indica were prepared using distilled water in appropriate stock concentrations and used in a 14-day treatment. Acute toxicity studies of the combined extract conducted in rats showed no toxicity up to 5000mg/kg. Experimental animals were acclimatized for 14 days while diabetes was induced using alloxan monohydrate (150mg/kg) intraperitoneally. After 14 days of treatment of diabetic rats with 300mg/kg, 500mg/kg 700mg/kg respectively as well as with 150mg/kg metformin, there was increase in weight, the blood glucose levels decreased significantly ($p<0.05$) whenc compared to untreated diabetic rats (diabetic control). Also, there was a significant $p<0.05$) reduction in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels of rats treated with different doses of the extract compared to the diabetic control. A dose dependent antidiabetic and hepatoprotecitve effect was observed and the combined extract had a close similar effect as the standard drug metformin. Therefore, combined aqueous leaf extract of Azadirachta indica and Mangifera indica exhibit antidiabetic and hepatoprotective and this is due to the presence of phytochemical constituents in the leaf part of the plants.

KEY WORDS: ***Azadirachta indica, Mangifera indica combined aqueous leaf extract, diabetes, hepatotoxicity, Bioactive constituents***

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that affects more than 100 million people worldwide. It is a chronic metabolic disease caused by an absolute or relative lack of insulin and or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism. Over time, uncontrolled diabetes can lead to serious damage to the various body systems (Bajaj and Madan, 1995; Rambhade *et al*., 2010). Diabetes was described more than 2000 years ago. For the past 200 years, it has featured in the history of modern medicine. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. Type 2 DM is the prominent form of diabetes worldwide, accounting for 90% of cases worldwide. An epidemic of type 2 DM is under way in both developed and developing countries. According to World Health Organization (WHO) the prevalence of diabetes worldwide is 180 million and will reach the 300 million in 2025 (Bennet, 1998). In Nigeria, the current prevalence of DM among adults aged 20–69 years is reported to be 1.7% (International Diabetes Federation, 2017). The prevalence of diabetes is rising because of increase in the life expectancy as well as a substantial increase in obesity and sedentary life style. The factors for this steep rise include genetic predisposition, urbanization, ethnicity, insulin resistance and central obesity (Zargar, 2002).

Antidiabetic medicines lack rigorous control on DM and exhibit different troublesome adverse effects. Therefore, medicinal plants and food wastes are explored for pronounced antidiabetic activity and less severe adverse effects (Akhtar *et al*., 2016; Fatima *et al*., 2019). The attention to the use of plants is due to the presence of bioactive constituents present in them capable of antidiabetic effects. Medicinal plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world. Search for antidiabetic molecules from natural sources are going on. Hence, this research work was aimed at assessing the synergistic potentials of aqueous leaf extract of *Azadirachta indica* and *Mangifera indica* in lowering blood sugar as well as liver protective effect in alloxan-induced diabetic rats.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

MATERIALS AND METHOD

Collection of Plant Materials

The leaves of the plants *Azadirachta indica* and *Mangifera indica* were collected from Abuja Campus of the University of Port Harcourt in Obio/Akpor Local Government Area of Rivers State and identified in the herbarium of the Department of Plant Science and Biotechnology in the University of Port Harcourt by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology.

Preparation of Aqueous Extract

The leaves of the plants *Azadirachta indica* and *Mangifera indica* were pulverized and air tried for three weeks after which the leaves were ground into fine powder using an Electric Blender. 25g of *Azadirachta indica* and 25g of *Mangifera indica* powder were combined and soaked in 500ml of distilled water and stirred intermittently and was left over night. The macerated pulp was dried at reduced temperature. The dry mass served as combined aqueous extract of leaves of *Azadirachta indica* and *Mangifera indica* for experimentation.

Toxicity studies of Combined Aqueous leaf extract of *A. indica* and *M. indica*

Acute toxicity method by Lorke (1983) as described by Okoli *et al.* (2010) was used.

Phytochemical Screening of Combined Aqueous leaf extract of *A. indica* and *M. indica*

Alkaloids, tannins, saponnins, flavonoids, phenol, Terpenes, steroid and anthraquinones were quantitatively determined using standard methods (Harborne, 1973; Van-Burden and Robinson, 1981; Obadoni and Ochuko, 2001, and Uahomo *et al.*, 2022).

Procurement of Animal

For this investigation, adult Wistar rats of either sex weighing 140–210g were used. They were acquired from the Animal House of the Department of Pharmacology at the University of Port Harcourt in River State, Nigeria, and were acclimatized for two weeks. They were kept in a conventional laboratory environment with 28°C temperature (28±2ºC), relative humidity (46±6%), a 12-hour light/dark cycle, and adequate ventilation. The animals were given access to water and a commercial feed (Vital Feed Nig. Ltd.) *ad libitum*. Twelve hours prior to the experiments, food was withheld, although water was always available for free.

Drug Purchase and Preparation

Metformin hydrochloride (MET) by Pfizer was obtained from Alpha Pharmacy and Stores, a licensed pharmacy in Port Harcourt, Rivers State, Nigeria. In order to prepare the powder for administration to the test animals, the tablets were crushed into a fine powder and the proper concentrations produced in distilled water. Alloxan monohydrate, another substance utilized, was also bought from the same pharmacy to cause diabetes in rats. All chemicals and solvents used were of analytical grade.

Induction of Diabetes

Alloxan monohydrate, freshly made with distilled water as the vehicle, was diluted to a concentration of 150mg/kg body weight and administered intraperitoneally to rats to cause diabetes. Three days later, diabetes was identified in alloxan-induced rats with Random Blood Glucose (RBG) levels ≥200mg/dL. Glucose levels were monitored using a hand held glucometer (Accu-CHEK) to test blood samples taken from the tail vein.

Experimental Design

Thirty (30) male rats were randomly selected into six experimental groups of 5 animals each.

Group	Treatment
Group I	Non-diabetic rats. They received only water and normal rat chow.
Group II	Untreated diabetes rats.
Group III	Diabetic rats treated with 150mg/kg body weight of Metformin
Group IV	Diabetic rats treated with 300mg/kg body weight of combined aqueous leaf extract of *Azadirachta indica* and *Mangifera indica*
Group V	Diabetic rats treated with 500mg/kg body weight of combined aqueous leaf extract of *Azadirachta indica* and *Mangifera indica*
Group VI	Diabetic rats treated with 700mg/kg body weight of combined aqueous leaf extract of *Azadirachta indica* and *Mangifera indica*

The rats in group III were given oral dose of 150mg/kg daily for 14 days. A safe oral dose of metformin HCl for rats is 100-200mg/kg (Quaile *et al*., 2010). Rats in group IV to VI were given an oral dose of 300mg/kg, 500mg/kg and 700mg/kg combined aqueous leaf extract of *Azadirachta indica* and *Mangifera indica* respectively daily for 14 days. All 6 groups of rats were sacrificed on last day (14th day) of treatment after 12 hours of fasting and given a chloroform anesthetic. Blood was collected by cardiac puncture into heparinized sample bottles for biochemical estimations. Body weights of all the animals were recorded prior to the treatment and sacrifice. Blood glucose level was determined using a hand held glucometer (Accu-CHEK) before and 48 hours after alloxan monohydrate administration, for the confirmation of the diabetic state of animals.

Biochemical Assay

Glucose was determined using methods of Malloy and Evelyn (1937) as modified by Tietz (1996), while serum lipids including total cholesterol, total protein and triacylglycerol were determined using the method of Tietz (1990). The method of

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

Reitman and Frankel (1957) was adopted for the ALT and AST assay. ALP was estimated using the method of King and King (1954) as adapted by Cheesbrough (2000).

Ethical Clearance

All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt and NIH guidelines for care and use of laboratory animals.

Method of Data Analysis

Data were analyzed using SPSS version 23.0. All data obtained were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) was used to compare the means between and within the groups and a *p*-value <0.05 was considered significant. A *Tuckey's* post-hoc test was also applied to assess significant differences between groups.

RESULTS

Acute toxicity test on combined aqueous leaf extract of *A. indica* and *M. indica*

Even at the greatest dose tested (5000mg/kg body weight), no mortality was seen over a period of 24 hours using Lorke's (1983) methodology. However, at dosages of 3000 and 5000mg/kg body weight, certain symptoms of listlessness, shivering, and mouth scratching were seen in experimental rats. As a result, it was evident that the extracts were acutely non-toxic to experimental animals.

Quantitative phytochemical analysis of aqueous leaf extracts of *A. indica* and *M. indica*

Phytochemical analysis of both plants showed the presence of contained alkaloids, tannins, saponins, flavonoids, Flavonoids, terpenoids, and phytosterol.

Table 1: Phytochemical (quantitative) analysis of the plants

Metabolites	Test	*A. indica*	*M. indica*
Alkaloids	a. Mayer's test	+	+
	b. Dragendorf's test	+	+
	c. Wagners reagent	+	+
Tannins	a. Lead acetate	+	+
	b. Ferric chloride test	+	+
Saponins	a. Frothing test	+	+
Flavonoids	a. Ferric chloride test	+	-
	b. NaOH test	+	-
	c. Shinoda test	+	+
Phenols	a. Ferric chloride test	+	-
Terpenoids	a. Lieberman-Buchard	+	+
	b. Salkowski test	+	+
Steroids	a. Lieberman-Buchard	-	-
Phytosterol	a. Salkowski test	+	+
Glycosides	a. Keller-Killians test	-	+

Key: + presence of constituent, - absence of constituent

Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on the body weight of diabetic rats

Body weight loss was observed in untreated diabetic rats (Table 2) which is one of the threats associated with DM. Treatment with combined aqueous leaf extract of *A. indica* and *M. indica* extract (300mg/kg, 500mg/kg and 700mg/kg) showed signs of recovery as comparable with the standard drug metformin .

Table 2: Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on the body weight of diabetic rats

Group	Pre-Treatment	Day 7	Day 14
Normal Control	160.05±5.11	164.55±4.62	171.18±5.22
Diabetes Control	153.62±6.25	138.90±7.42*	127.82±5.89*
150mg/kg Metformin	154.62±5.23	166.50±6.10*#	172.80±5.86*#
300mg/kg Extract	152.48±4.94	162.55±7.10*#	170.20±7.65#
500mg/kg Extract	155.26±5.18	164.33±5.52#	169.78±6.12*#
700mg/kg Extract	152.47±5.29	167.15±6.85*#	171.66±6.13#

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on blood glucose levels in alloxan induced diabetic rats

In untreated diabetic rats, there was a significant (p<0.05) rise in blood glucose level when compared with untreated non-diabetic rats. Results of this study showed that 14 days treatment with combined aqueous leaf extract of *A. indica* and *M. indica* extract (300mg/kg, 500mg/kg and 700mg/kg) produced a significant (p<0.05) decrease in blood glucose level in experimental rats. A dose-dependent reduction in blood glucose level was observed with the extract dose of 700mg/kg as the most effective dose. Hence, the combined aqueous leaf extract of *A. indica* and *M. indica* extract showed dose-dependent hypoglycemic effect by lowering the blood glucose (Table 3).

Effect of Metformin on blood glucose level in alloxan–induced diabetic rats

Metformin produced significant (p<0.05) decrease in blood glucose levels in alloxan induced diabetic rats. With 150mg/kg the blood glucose levels was 4.92±0.61mmol/L compared to blood glucose levels of 13.88±3.16mmol/L in diabetic control animals (Table 3).

Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on lipid parameters in alloxan induced diabetic rats

There was a decrease in total protein and total cholesterol values and an increase in triglyceride value in untreated diabetic rats. The treatment with combined aqueous leaf extract of *A. indica* and *M. indica* extract (300mg/kg, 500mg/kg and 700mg/kg) caused a significant (p<0.05) increase in total protein value and a significant (p<0.05) decrease in total cholesterol and triglyceride values when compared with untreated diabetic rats. The effect of the combined aqueous leaf extract of *A. indica* and *M. indica* extract was dose-dependent and had a close similar effect as the standard drug, metformin (Table 3).

Table 3: Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on some lipid profile parameters of alloxan-induced Wistar rats

Groups	Glucose (mmol/L)	Total Protein (g/L)	T. Cholesterol (mmol/L)	Triglyceride (mmol/L)
Normal Control	4.43±0.48	61.50±3.42	4.15±0.26	1.55±0.39
Diabetes Control	13.88±3.16*	48.00±3.16*	6.80±0.85*	2.58±0.28*
150mg/kg Metformin	4.92±0.61*#	61.88±2.84#	4.12±0.18#	1.48±0.72#
300mg/kg Extract	9.08±2.26*#	52.02±3.46*#	5.45±0.66*#	1.90±0.18*#
500mg/kg Extract	7.10±1.86*#	55.25±2.28*#	4.46±0.49*#	1.88±0.39*#
700mg/kg Extract	3.96±0.32*#	63.60±2.97*#	3.64±0.55*#	1.34±0.38*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on Liver enzyme parameters of alloxan-induced Wistar rats

There was an increase in liver enzyme markers such as AST, ALT and ALP in untreated diabetic rats. The administration of the combined aqueous leaf extract of *A. indica* and *M. indica* extract (300mg/kg, 500mg/kg and 700mg/kg) significantly (p<0.05) brought back almost to normal the levels of AST, ALT and ALP when compared with the untreated diabetic rats. The effect of the combined aqueous leaf extract of *A. indica* and *M. indica* extract on liver enzyme markers was dose-dependent and had a close similar effect as the standard drug, metformin (Table 4).

Table 4: Effect of combined aqueous leaf extract of *A. indica* and *M. indica* extract on some Liver enzyme parameters of alloxan-induced Wistar rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal Control	8.75±1.50	9.00±2.58	45.52±3.68
Diabetes Control	23.20±3.15*	21.20±2.28*	91.16±6.23*
150mg/kg Metformin	8.82±2.23*#	10.06±1.50*#	46.08±4.15*#
300mg/kg Extract	19.50±3.42*#	15.08±2.58*#	68.44±5.02*#
500mg/kg Extract	13.41±2.33*#	12.40±2.60*#	56.11±4.56*#
700mg/kg Extract	8.20±1.09*#	10.40±1.67*#	46.90±3.87*#

Each value represents mean ± SEM, values marked with (*) differ significantly from normal control group (*p<0.05) while those marked with (#) differ significantly from diabetes control group (#p<0.05).

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

DISCUSSION

A. indica and *M. indica* are two plants that have been researched for their hypoglycemic activities (Kemasari *et al*., 2011; Basha *et al*., 2011; Anggit *et al*., 2014; Arika *et al*., 2016). *A. indica*, also known as neem tree, was reported to have various biological and pharmacological activities, including antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, antiulcer, spermicidal, anthelminthic, larvicidal and fungicidal activities (Atowadi and Atowadi, 2009). Previous researches have reported that most of the active compounds of *A. indica* include flavonoids, phenolic compounds, tannins, saponins, alkaloid, glycoside, reducing sugars (Ujah *et al*., 2021, Hikaambo *et al*., 2022). *Mangifera indica* which is native to Asians and it is a member of the Anacardiaceae family. It is widely grown in different parts of Africa, especially in the southern part of Nigeria. *Mangifera indica* is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains, malaria (Madunagu *et al*., 1990) and diabetes (Muruganandan *et al*., 2005). Phytochemical study on different parts of *M. indica* has revealed the presence of phenolic constituents, triterpenes, flavonoids, phytosterol, and polyphenols (Saleh and Ei-Ansari, 1975; Kharn *et al*., 1994; Anjaneyulu *et al*., 1994; Selles *et al*., 2002; Singh *et al*., 2004). This species is purposed to possess numerous therapeutic uses including analgesic, anti inflammatory (Garrido *et al*., 2001), immunostimulant (Makare *et al*., 2001), antioxidant (Martinez *et al*., 2000; Sanchez *et al*., 2000), spasmolytic, anti diarrhea (Sairam *et al*., 2003), antilipidemic (Anila and Vijayalakshmi, 2002), antidiabetic (Aderibigbe *et al*., 1999), antiamebic (Tona *et al*., 1998), anthelminthic, antiallergic (Garcia *et al*., 2003) and anti bacterial applications.

Phytochemical analysis of both plants, showed the presence of contained alkaloids, tannins, saponins, flavonoids, Flavonoids, terpenoids, and phytosterol. These phytochemicals help to explain some of the effects of using these plants as herbal remedies.

The antiglycemic effect of plants is believed to be through different mechanisms (Chakravarthy *et al*., 1980). Plants exhibiting antidiabetic effects stimulate beta-cell in the pancreas by activating regeneration of pancreatic cells (Boppanna *et al*., 1997; Chorvathova *et al*., 2000). Fiber of plant also interferes with carbohydrate absorption, affecting blood glucose level. Studies show that chemical induction of diabetes by intraperitoneal administration of a diabetogenic agent, alloxan monohydrate induces Type I diabetes in experimental animals (Viana *et al*., 2004; Etuk, 2010). Alloxan monohydrate is derived from urea and induces diabetes by selective necrosis of pancreatic beta-cells of Langerhans (Iranloye *et al*., 2011). This therefore, affects endogenous insulin synthesis and release making it biologically unavailable or insufficient and thus results in hyperglycemia (Nastaran *et al*., 2011). The toxic alloxan confers its toxicological effect on pancreatic beta cells through inhibition of glucokinase enzyme, generation of free radicals, disturbances in intracellular calcium homeostasis and oxidation of essential sulphydryl (-SH groups) (Dunn *et al*., 1983; Szkudelski, 2001; Dhanesha *et al*., 2012). The underlying mechanism of action involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells (Lenzen, 2008).

Body weight loss was observed in untreated diabetic rat which is one of the threats associated with DM. Treatment with combined aqueous leaf extract of *A. indica* and *M. indica* extract showed signs of recovery as comparable with the standard drug metformin. This is agreement with the study by Basha *et al*. (2011) and Kemasari *et al*. (2011).

Administration of alloxan significantly increased the level of glucose when compared to normal control rats, which might account for the cytotoxic effect of alloxan on beta-cells. Alloxan is relatively toxic to insulin producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the transmembrane carrier protein glucose transporter 2 (Vuksan and Sievenpiper, 2005). This cytotoxic action is mediated by reactive oxygen species (ROS) source of generation of ROS is dialuric acid, a reduction product of alloxan. These radicals undergo dismutation to hydrogen peroxide (H_2O_2). The action of ROS with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells (Szkudelski, 2001; Omeodu *et al*., 2022) thereby decreasing the secretion of insulin, which in turn increase the blood glucose level.

In the present study there is increase in blood glucose in untreated diabetic rats (alloxan induced) when compared to normal control group, which account for the cytotoxic action of alloxan. Administration of combined aqueous leaf extract of *A. indica* and *M. indica* extract remarkably reduced the altered sugar level. It has been reported that in diabetic subjects the levels of HbA1C increases (Paulsen, 1973; Sokiprim *et al*., 2022). This is as a result of reaction of excess of blood glucose with haemoglobin leading to the formation of HbA1C (Koening *et al*., 1976). Administration of combined aqueous leaf extract of *A. indica* and *M. indica* extract for 2 weeks reduced the blood glucose at a dose-dependent level by the normalization of fasting blood glucose levels. This suggests that the combined aqueous leaf extract of *A. indica* and *M. indica* may possess insulin-like effect on peripheral tissues by either promoting glucose uptake or metabolism, by inhibiting hepatic gluconeogenesis (Ali *et al*., 1993) or absorption of glucose into the muscles and adipose tissues (Kamanyi *et al*., 1994), by the stimulation of a regeneration process and revitalization of the remaining beta cells (Shanmugasundaram *et al*., 1990). The combined leaf extract at a dose of 500mg/kg and 700mg/kg appeared to have similar potency as the reference drug (metformin) in reducing the blood glucose level.

Reduction in plasma total protein and increase in total cholesterol and triglyceride levels were observed in alloxan induced rats. The decrease in protein may be due to microproteinuria which is an important clinical marker of diabetic nephropathy (Tuvemo *et al*., 1997; Makare *et al*., 2001) and may be due to increased protein catabolism (Almdal and Vilstrup, 1988). Lack of insulin also reduces RNA and mRNA, which is another factor for the reduction of total protein (Fu *et al*., 2013). The finding of this study correlates with the above findings and

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

hence agrees with the study by Kemasari *et al*. (2011). Treatment with combined aqueous leaf extract of *A. indica* and *M. indica* extract for 2 weeks reduced was able to cause an increase in total protein and a decrease in total cholesterol and triglyceride values and this can be attributed to the presence of phytochemical constituents in the plants. Hence, the potentials of the combined extract of *A. indica* and *M. indica* to exhibit antidiabetic effects bringing about normalization of body weight, blood glucose and serum lipid markers such as total protein, total cholesterol and triglgceride can be attributed the presence of phytochemicals.

AST, ALT and ALP are the specific markers to assess hepato-cellular damage leading to liver cell necrosis (Amacher, 1998). In present study, the activities of AST, ALT and ALP were assessed as they are the specific index of liver cell damage in experimental animals (Mitchell *et al*., 1974). High level of these liver enzyme markers indicates hepato-cellular damage and lowering of these enzymes content in serum is an indication of hepatoprotective action of a drug. Activities of AST, ALT and ALP in serum were increased in untreated diabetic rats but the the treatment with combined extract of *A. indica* and *M. indica* afforded a significant protection against alloxan induced increase in the serum enzyme level. The combined extract of *A. indica* and *M. indica* may induce accelerated regeneration of liver cells by reducing the leakage of AST in to blood there by lowering its value to normal levels. ALT is more specific to the liver and a better parameter for detecting liver damage (Chandiran *et al*., 2011). In the present study, increased ALT level was brought back to normal by the administration of combined extract of *A. indica* and *M. indica*. This agrees with the study of Kemasari *et al*. (2011). The ability of the combine extract to bring to normal liver enzyme markers can be attributed to the presence of phytochemicals such as terpenoids, flavonoids and phytosterols which have hepatoprotective effects. The finding of this studies suggest that the combined extract of *A. indica* and *M. indica* can be very beneficial in diabetes management and its associated complications, holding hope of the new generation antihyperglycemic drug.

CONCLUSION

The results from this study indicated that combined extract of *A. indica* and *M. indica* possess hypoglycemic and hepatoprotective effects in alloxan induced diabetic rats, thus scientifically validating the possible use of the leaves of both plants in folkloric medicine in the management of diabetes mellitus and hepatic diseases. These actions were exhibited due to cumulative effect of phytoconstituents present in the extract including free and bound alkaloids, tannins, saponins, flavonoids, Flavonoids, terpenoids, and phytosterol. However, further investigation should be done in order to isolate the constituents responsible for the antidiabetic effect of this plant through bioassay guided fractionation so as to corroborate the findings of this study. Moreover, the organic solvent extraction for this plant should also be done to compare the antidiabetic and hepatoprotective activities of the combined aqueous and organic fractions.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. *Bajaj, J.S and Madan, R. (1995). Diabetes in tropics and developing countries. Bulletin of the International Dairy Federation, 38, 5-6.*
2. *Rambhade S., Chakraborty A.K., Patil U.K., Rambhade A. (2010). Diabetes Mellitus-Its complications, factors influencing complications and prevention-An Overview. J. Chem. Pharm. Res., 2:7–25*
3. *Bennet P.H. (1998). Joslin's Diabetes Mellitus (1), 13th Edition, BI Waverly Pvt. Ltd, New Delhi; 379-390.*
4. *International Diabetes Federation (2017). Diabetes atlas. 8th ed. Brussels: International Diabetes Federation; 2017.*
5. *Zargar AH. (2002). Epidemic of diabetes mellitus in the subcontinent. Now is the time to declare war on this major public health problem. The Journal of Medical Sceince, 5(1): 46-49.*
6. *Akhtar, M.F.; Ashraf, M.; Javeed, A.; Anjum, A.A.; Sharif, A.; Saleem, A.; Akhtar, B. (2016). Textile industrial effluent induces mutagenicity and oxidative DNA damage and exploits oxidative stress biomarkers in rats. Environ. Toxicol. Pharmacol., 41, 180–186.*
7. *Fatima S., Akhtar M.F., Ashraf K.M., Sharif A., Saleem A., Akhtar B., Peerzada S., Shabbir M., Ali S., Ashraf W. (2019). Antioxidant and alpha amylase inhibitory activities of Fumaria officinalis and its antidiabetic potential against alloxan induced diabetes. Cell. Mol. Biol.; 65:50–57.*
8. *Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. Archives of Toxicology, 53, 275-287.*
9. *Okoli CO, Ibiam AF, Ezike AC, Akah PA, Okoye TC. (2010). Evaluation of antidiabetic potentials of Phyllanthusniruri in alloxan diabetic rats. African Journal of Biotechnology, 9(2):248-259.*
10. *Harborne, J.B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London. Pp. 279.*
11. *Van-Burden TP, Robinson WC. (1981). Formation of complexes between protein and tannin acid. Journal of Agriculture and Food Chemistry, 1:77–82*
12. *Obadoni, B.O. and Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homeostatic Plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science, 8, 203-208.*
13. *Uahomo PO, Isirima JC, Akoko S. (2022). Evaluation of Phytochemicals and Bioactive Properties in Leaf and Root Parts of Cyathula prostrata (Pasture Weed) – A Qualitative and Quantitative Analysis. Asian Plant Research Journal, 9(3): 8-16.*
14. *Quaile, M.P., Melich, D.H., Jordan, H.L., Nold, J.B., Chism, J.P., Polli, J.W., Smith, G.A., & Rhodes, M.C. (2010). Toxicity and toxicokinetics of metformin in rats. Toxicology and Applied Pharmacology, 243(3), 340–347.*
15. *Malloy HJ, Evelyn KA (1937). The determination of bilirubin with the photometric colorimeter. J Biol Chem., 119: 481-490.*
16. *Tietz NW (1996). Lever Function Tests, Nitrogen Metabolites and Renal Function In: Fundamentals of Clinical Chemistry. 3rd ed. W.B. Saunders, Philadelphia pp. 476 – 576.*

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

17. *Tietz NW (1990) Clinical Guide to Laboratory Tests; 2nd Edition (Philadelphia: W B Saunders Company) pp: 554-556*
18. *Reitman, S. and Frankel, S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. American Journal of Clinical Pathology, 28, 56-63.*
19. *King EJ, King PR (1954). Estimation of serum phosphatase by determination of hydrolysed phenol with amino – antipyrene. J. Clin. Path., 7: 322 – 327.*
20. *Cheesbrough M (2000). Clinical chemistry in: District and Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, Cambridge p. 318*
21. *Kemasari P., Sangeetha S. and Venkatalakshmi P. (2011). Antihyperglycemic activity of Mangifera indica Linn. in alloxan induced diabetic rats. J. Chem. Pharm. Res., 3(5):653-659*
22. *Basha DP, Katikala PK, Bulusu BT and Mannam S. (2011). Antidiabetic activity on extracts of Mangifera indica in Alloxan monohydrate induced diabetic rats. Drug Invention Today, 3(7), 165-168*
23. *Anggit Listyacahyani Sunarwidhi, Sudarsono Sudarsono, Agung Endro Nugroho (2014). Hypoglycemic Effect of Combination of Azadirachta indica A. Juss. and Gynura procumbens (Lour.) Merr. Ethanolic Extracts Standardized by Rutin and Quercetin in Alloxan-induced Hyperglycemic Rats. Adv Pharm Bull., 4(2), 613-618*
24. *Arika WM, Nyamai DW, Agyirifo DS, Ngugi MP, Njagi ENM (2016). In Vivo Antidiabetic Effect of Aqueous Leaf Extract of Azardirachta indica, A. juss in Alloxan Induced Diabetic Mice. J Diabetic Complications Med., 1: 106.*
25. *Atowadi SE, Atowadi JC. (2009). Azadirachta indica (neem): A Plant of Multiple Biological and Pharmacological Activities. Phytochem Rev., 8:601-20.*
26. *Ujah I.I, Nsude C.A, Ani O.N, Alozieuwa U.B, Okpako I.O and Okwor A.E. (2021). Phytochemicals of neem plant (Azadirachta indica) explains its use in traditional medicine and pest control. GSC Biological and Pharmaceutical Sciences, 14(02), 165–171*
27. *Hikaambo, C., Kaacha, L. , Mudenda, S. , Nyambe, M. , Chabalenge, B. , Phiri, M. , Biete, L. , Akapelwa, T. , Mufwambi, W. , Chulu, M. and Kampamba, M. (2022) Phytochemical Analysis and Antibacterial Activity of Azadirachta indica Leaf Extracts against Escherichia coli. Pharmacology & Pharmacy, 13, 1-10.*
28. *Madunagu, B.E., Ebana, R.U.B., & Ekpe, E.D. (1990). Antibacterial and antifungal activity of some medicinal plants of Akwa Ibom State. West African Journal of Biological and Applied Chemistry, 35, 25–30*
29. *Muruganandan S, Scrinivasan K, Gupta S, Gupta PK, Lal J. (2005). Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J Ethnopharmacol.; 97:497–501.*
30. *Saleh NA, Ei- Ansari M. (1975). Polyphenolics of twenty local varieties of Planta Med., 28(2):124-130.*
31. *Kharn MA, Nizami SS, et al. (1994). New triterpenes from MangiferaIndica. J.Nat Prod.,, 28: 124 – 130.*
32. *Anjaneyulu V, Babu JS, Connolly JD (1994). 29-hydroxymangiferonic acid from Mangifera indica. Phytochemistry, 35(5): 1301-1303.*
33. *Selles NAJ, Castro HTV et al. (2002). Isolation and quantitative analysis of phenolic antioxidants free sugar and polyols from mango(MangiferaIndica Linn) stem bark aqueous decoction used in cuba as a nutritional supplement. J. Agri Food Chem., 50:762-766.*
34. *Singh UP, Singh DP, Singh M , Maurya S, Srinivatava JS, Singh RB & Singh SP (2004). Characterization of phenolic compound in some Indian medicinal mango cultivars, Int J Food Science Nutrition, 55:163-169*
35. *Garrido G, González D, Delporte C, Backhouse N, Quintero G, Núñez-Sellés AJ, Morales MA (2001). Analgesic and anti-inflammatory effects of Mangifera indica L. extract (Vimang) Phytother Res., 15:18–21.*
36. *Makare, N., Bodhankar, S., & Rangari, V. (2001). Immunomodulatory activity of alcoholic extract of Mangifera indica L in mice. Journal of Ethnopharmacology, 78(2–3), 133–137.*
37. *Martinez G, Delgado R, Perez G, Garrido G, Nunez Selles AJ, Leon OS (2000). Evaluation of the in-vitro antioxidant activity of Mangifera indica L: Extract (Vimang) Phytother Res., 14:424–7*
38. *Sanchez GM, Re L, Giuliani A, Nuñez-Selles AJ, Davison GP, Leon-Fernandez OS (2000). Protective effects of Mangifera indica L.extract, mangiferin and selected antioxydants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmacol Res., 42:565–73.*
39. *Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Sarkar M, et al. (2003). Evaluation of anti-diarrhoeal activity in seed extracts of Mangifera indica. J Ethnopharmacol., 84:11–5.*
40. *Anila, L. and Vijayalakshmi, N.R. (2002) Flavonoids from Emblica officinalis and Mangifera indica—Effectiveness for Dyslipidemia. Journal of Ethnopharmacology, 79, 81-87.*
41. *Aderibigbe AO, Emudianughe TS, Lawal BA. (1999). Antihyperglycaemic effect of Mangifera indica in rat. Phytother Res., 13:504–7*
42. *Tona, L., Kambu, K., Ngimbi, N., Cimanga, K., & Vlietinck, A. J. (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of ethnopharmacology, 61(1), 57–65.*
43. *García, D., Escalante, M., Delgado, R., Ubeira, F. M., & Leiro, J. (2003). Anthelminthic and antiallergic activities of Mangifera indica L. stem bark components Vimang and mangiferin. Phytotherapy research : PTR, 17(10), 1203–1208*
44. *Chakravarthy B.K., Gupta S., Gambhir S.S., Gode K.D. (1980). Pancreatic beta cell regeneration: a novel antidiabetic mechanism of Petercarpus marsupium. Indian J Pharmacol, 12, 123-128*
45. *Bopanna KN, Kannan J, Gadgil S, Balaraman R, Rathod SP. (1997). Antidiabetic and antihyperlipaemic effects of neem seed Kernel powder on alloxan diabetic rabbits. Indian J Pharmacol., 29:162–7.*
46. *Chorvathova, V., P. Bobek, E. Ginter and J. Klvanova (1993). Effect of the oyster fungus on glycaemia and cholesterolaemia in rats with insulin-dependent diabetes. Physiol. Res., 42: 175-179.*
47. *Viana GS, Medeiros AC, Lacerda AM, Leal LK, Vale TG, et al. (2004) Hypoglycemic and anti-lipemic effects of the aqueous extract from Cissus sicyoides. BMC Pharmacol 8: 4-9.*
48. *Etuk EU (2010). Animals models for studying diabetes mellitus. Agric Biol J N Am., 1: 130-134.*
49. *Iranloye BO, Arikawe AP, Rotimi G, Sogbade AO (2011). Anti-diabetic and antioxidant effects of Zingiber Officinale on alloxan-induced and insulin-resistant diabetic male rats. Niger J Physiol Sci 26: 89-96.*

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 8| Issue: 10| October 2022|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2022: 8.205 || ISI Value: 1.188

50. *Nastaran JS (2011). Antihyperglycaemia and antilipidaemic effect of Ziziphus vulgaris L on streptozotocin induced diabetic adult male Wistar rats. Physiol Pharmacol 47: 219-223.*
51. *Dunn JS, Sheehan HL, Mclethie NGB (1983). Necrosis of islets of Langerhans produced experimentally. Lancet 1: 484-487.*
52. *Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in β- cells of the rat pancreas. Physiology Research 50: 536-546*
53. *Dhanesha N, Joharapurkar A, Shah G, Dhote V, Kshirsagar S, Bahekar R, and Jain M (2012). Exendin-4 ameliorates diabetic symptoms through activation of glucokinase. J. Diabetes 4, 369–377.*
54. *Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia, 51: 216-226.*
55. *Vuksan V, Sievenpiper JL. (2005). Herbal remedies in the management of diabetes: lessons learned from the study of ginseng. Nutr Metab Cardiovasc Dis., 15:149–160.*
56. *Paulsen, E.P. (1973). Heamoglobin A1C in childhood of diabetes. Metabolism, 22: 269- 271.*
57. *Sokiprim A., Dagogo M.O., and Roseline M.A. (2022). Medication Adherence and its Determinants among Patients with Type-2 Diabetes Attending University of Port Harcourt Teaching Hospital. World Journal of Advance Healthcare Research, 6 (6), 62-71*
58. *Koenig, R. J., Peterson, C. M., Jones, R. L., Saudek, C., Lehrman, M., & Cerami, A. (1976). Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. The New England journal of medicine, 295(8), 417–420.*
59. *Ali, L., Azad Khan, A.K., Mamun, M.I.R., Mosihuzzaman, M., Nahar, N., Nur-E-Alan, M. and Rokeya, B. (1993). Studies on the hypoglycaemic effects of fruits pulp, seed and whole plant of Momordica charantia on normal and diabetic model rats. Planta Medica 59: 408–412.*
60. *Kamanyi, A., Djamen, D. and Nkeh, B. (1994). Hypoglycemic properties of the aqueous roots extract of Morinda lucida (Rubiacea) study in the mouse. Phytother. Res. 8: 369–371.*
61. *Shanmugasundaram, E.R.B., Gopinath, K.L., Shanmugasundaram, K.R. and Rajendran, V.M. (1990). Possible regeneration of the islets of Langerhans in streptozotocin-diabetes rats given Gymnema sylvestre leaf extracts. J. Ethnopharmacol. 30: 265–279.*
62. *Tuvemo, T., U. Ewald, M. Kobbah and L.A. Proos (1997). Serum magnesium and protein concentrations during the first five years of insulin-dependent diabetes in children. Acta Paediatrica, 86: 7-10.*
63. *Almdal JP and Vilstrup H. (1988). Strict insulin therapy normalizes organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia, 31: 114-118*
64. *Fu Z, Gilbert ER, Liu D. (2013). Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev., 9(1):25-53.*
65. *Amacher, D.E. (1998). Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. Regul. Toxicol. Pharmacol., 27: 119-130.*
66. *Mitchell, J. R., Thorgeirsson, S. S., Potter, W. Z., Jollow, D. J., & Keiser, H. (1974). Acetaminophen-induced hepatic injury: protective role of glutathione in man and rationale for therapy. Clinical pharmacology and therapeutics, 16(4), 676–684.*
67. *Chandiran, I. S., Jayaveera, K. N., & Reddy, R. (2011). S., High-Throughput Liquid Chromatography–Tandem Mass Spectrometric Method for Simultaneous Quantification of Carvedilol and Its Metabolite 4-Hydroxyphenyl Carvedilol in Human Plasma and Its Application to Bioequivalence Study. Journal of Chemical and Pharmaceutical Research, 3(2), 341-353.*