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Research Article

HYPOGLYCEMIC AND HEPATOCURATIVE ACTIVITIES OF AJU-MBAISE ON ALLOXAN-DIABETIC MODEL IN MALE WISTAR RAT

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Abstract

Diabetes mellitus is a disorder that results from impairment of insulin action or secretion seen in chronic hyperglycemia and long-term severe vascular complications. The study aimed to determine the hypoglycemic and hepatocurative activities of Aju-mbaise on the alloxan-diabetic model in male Wistar Rat. Thirty male Wistar rats weighing 140-190g were employed for the study and grouped into six groups of five animals each. Group A received feed and distilled water only, group B diabetic control, group C received alloxan and treated with 150mg/kg of Aju-mbaise. Group D received alloxan and treated with 600mg/kg of Aju-mbaise. Aju-mbaiseextract was administered through oral gavage, and lasted for 21. Data obtained for blood glucose, Alkaline Phosphatase, Aspartate aminotransferase, Alanine aminotransferase, liver weight, and body weight were subjected to SPSS version 25 (IBM, USA, 2018). ANOVA was used to analyzed the data followed by post HOC Turkey HSD, and values were considered significant at $p<0.05$. The study findings revealed a non-significant ($p>0.05$) increase in the bodyweight in-group C and D compared to group B. Also, relative liver weight had a non-significant ($p>0.05$) difference in-group C, E, and F, with a significant decrease ($p<0.05$) in group D compared to group B. Also, aspartate aminotransferase and alkaline phosphatase showed a significant ($p<0.05$) difference in the treated group D, E, and F compared to group B. further, alanine aminotransferase showed a significant ($p<0.05$) difference in the treated group C, D, E, and F compared to group B. The blood glucose level at day 0 showed a significant ($p<0.05$) increase in group B, C, D, E, F when compared to group A. Day 7, 14, and 21 results revealed a significant ($p<0.05$) decrease in the blood glucose level in group C, D, E, and F as compared to group B. In conclusion, Aju-mbaise possesses anti-diabetic and hepatocurative activities on alloxan diabetic model in Wistar rats based on dose-dependent.

Keywords: Aju-mbaise, Oxidative stress, Diabetes mellitus, Blood glucose, Hepatocurative.

INTRODUCTION

Diabetes mellitus (DM) is a prevalent disease affecting both developed and developing countries, and affects 25% of the world population (1). DM is a disease of the endocrine gland system caused by the abnormality of carbohydrate metabolism, linked to low blood insulin level or insensitivity of target organs to insulin (1,2). Majorly it is classified into; Type I diabetes caused by insulin secretion deficit and type II diabetes, accompanied by a progressive rate of insulin resistance in liver and peripheral tissues, reducing β-cells mass, and deficient insulin secretion (3). Diabetes mellitus has several acute metabolic side effects, including ketoacidosis, hyperosmolar coma accompanied with chronic disorders. It has long-term adverse side effects such as retinopathy, renal failure, neuropathy, skin complications, and increasing cardiovascular complication risks (4–6). It is linked with a spectrum of liver diseases like non-alcoholic liver disease (NALD), steatohepatitis, and liver cirrhosis with their increased complications and mortality(7). The liver plays a significant role in regulating carbohydrate metabolism, as it uses glucose as a fuel; it can store glucose as glycogen and synthesize glucose from non-carbohydrate sources (8). The liver is more susceptible to diseases in subjects having a metabolic disorder, especially for DM. In Type II diabetes mellitus (T2DM), the loss of a direct effect of insulin to suppress hepatic glucose production and glycogenolysis in the liver causes increased hepatic glucose production (9,10).

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In Type II diabetes mellitus, hyperinsulinemia combined with a high free fatty acid flux and hyperglycemia is known to up-regulate lipogenic transcription factors. The increased availability of free fatty acid, glucose, and insulin lead to the accumulation of fatty acids in the liver, finally leading to non-alcoholic fatty liver disease (NAFLD) in T2DM patients (7,11,12). The NAFLD causes an asymptomatic abnormality of liver enzyme levels (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Among these liver enzymes, ALT is most closely related to liver fat accumulation and consequently a biomarker of NAFLD. Serum aminotransferase such as ALT and AST indicate the concentration of hepatic intracellular enzymes leaked into the circulation, which are indicators for hepatocellular injury and are used as primary markers (13,14). The liver enzymes ALT, AST, ALP, and γ-glutamyl-transferase (GGT), increases during liver diseases and type II diabetes-related cases (15) are used in the assessment of liver toxicity in DM. The ALP is also used to evaluate liver function and reaches incredibly high levels in biliary obstruction. The altered ALP activity may reflect an increased hepatic insulin resistance or oxidative stress (16). Medicinal plants have been the primary source of medicines since ancient times; as treatments and palliatives, all human societies have practically utilized plants as sources of nutrition and therapy against certain diseases and ailments (17,18). Plants of medicinal value have become of great interest to society due to their applications in modern medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (17). Herbal medicines encompass the combination of indigenous systems of medicine and several

therapeutic experiences of many previous generations (19), which delivers valuable guidelines to the selection, preparation, and application of herbal formulation for the treatment, control, and management of a range of illnesses. Plant-based drugs are reported to be successfully used to cure skin diseases, tuberculosis, jaundice, hypertension, mental disorders, cancer, AIDS, diabetes, and many other infectious diseases (20). Aju Mbaise is a combination of plant leaves decocted and administered to women after childbirth in treatment or diabetes mellitus; it is also believed to have antimicrobial activity(21). The decoction is composed of a combination of different roots, leaves, and trunk of medicinal plant wrapped together; it was reported that this plant decoction contains a reasonable amount of essential proteins, minerals and vitamins, and antibacterial activity (21,22). There is limited literature on the hypoglycemic and hepatocurative activities of Aju-mbaise on the alloxan-diabetic model; the study tends to investigate in male Wistar Rat.

MATERIALS AND METHODS

Location of the Study: This study was carried out in the Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Ethical approval consent was obtained for the progress of this study, from the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. Rats handling and treatments conform to guidelines of the National Institute of Health (NIH publication 85-23, (1985) for laboratory animal care and use.

Materials: Alloxan Monohydrate (Sigma Aldrich, USA), Aju-Mbaise, Normal saline, Distilled Water, oral cannula, Automatic Water distiller (SZ-1 Search Tech Instrument), Glucometer (Accu-check active, Mannheim, Germany), Glucose strips (Accu-check strips), chloroform (JHD Chemicals, Guangdong China), Randox Kit, heparinized capillary tube, Electronic weighing balance (M-Metallar M311), 2ml hypodermic sterile syringe, animal weighing balance (Camry LB11), Normal laboratory chow (Standard Pellet), and standard cage. Centrifuge 90(1) (Alpin Medical, England), UV-VIS 752N Spectrophotometer (Shanghai, Yoke Instrument Co., Ltd. China), S. Pyrex Beakers (Techmel, USA), and Measuring cylinder (MINGHE).Latex Medical Hand gloves (Supermax Gloves, Selangor, Malaysia), and Cotton wool (KENS LINT, Benin City, Nigeria).

Experimental Animals

A total of 30 male Wistar rats weighing of 140g-190g were used in this study. The animals were obtained from the Animal house, Faculty of Basic medical Sciences, Nnamdi azikiwe University, Nnewi Campus. The rats were housed, five animals in each standard laboratory cage. During the experiment the rats had access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for two weeks before commencement of the experiment.

Experimental Groupings

The animals were divided into six (6) groups each comprising five (5) animals; Group Areceived food and water only; Group B received 120mg/kg of Alloxan Monohydrate; Group C received Ajumbaise150mg/kg/body weight; Group Dreceived Ajumbaise 600mg/kg/body weight. Group E received 120mg/kg of Alloxan Monohydrate and treated with 150mg/kg of Ajumbaise, and Group F received 120mg/kg of Alloxan Monohydrate and treated with 600mg/kg of Ajumbaise. The administration of the decoction (AjuMbaise) was done for 21 days through oral garvage.

Induction of Diabetes Mellitus: Diabetes Mellitus (Hyperglycemia) was induced in the experimental rats by a single dose of intraperitoneally injection of 120mg/kg of Alloxan Monohydrate. The animals were fasted 12-hours prior to the induction of diabetes and was administered the Alloxan Monohydrate. Hyperglycemia was confirmed when fasting blood glucose concentration was greater than 200mg/dL for three consecutive days. This confirmed diabetes has taken place, and was done using an acute check. 1.5gram of Alloxan monohydrate was dissolved in 15mls of distilled water to obtain a stock solution of 100mg/ml.

Sample collection: Animals were anaesthetized with chloroform in an enclosed container 24 hours after the last administered dose of the Aju-mbaise, and blood were collected using heparinized capillary tube and put in a plain container (23), and centrifuge using centrifuge (England) and serum were retrieved and used for serum liver enzyme (AST, ALT, and ALP). Blood glucose level wereestimated using Accu-checker by puncturing the tail of the animals with a lancelet and placed it on Accu-check strip and blood glucose level was documented.

Liver enzyme estimation

Aspartate Aminotransferase (AST) Estimation: This test was carried out according to the method described by (24).

Principle: The substrate in the reaction are alpha ketoglutaric acid and L- Aspartate. The products formed by enzyme action are glutamate and oxaloacetate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using Spectrophotometer.

Alanine Aminotransferase (ALT) Estimation: This test was carried out according to the method described by (24).

Principle: The substrate in the reaction are alpha ketoglutaric acid and L- Aspartate. The products formed by enzyme action are Glutamate and Pyruvate. Addition of 2, 4 dinitrophenyl hydrazine results in the formation of hydrazine complex with ketoacids. A red colour is produced on the addition of sodium hydroxide. The intensity of colour is related to the enzymatic activity and this can be measured at 550 nm wavelength using Spectrophotometer.

Alkaline Phosphatase (ALP) Estimation: This test was carried out according to the method described by(25).

Principle: Alkaline Phosphatase in Alkaline medium hydrolyses Phenyl phosphatase in 15minutes at 37°C and pH of 10 to release phenol which in the presence of potassium ferricyanide reacts with 4-aminophenazone to give a red-pink colour which is measured Spectrophotometrically at 510nm wavelength. The intensity of the colour indicates ALP activity in the sample.

Data analysis: Data obtained were subjected to SPSS version 25 (IBM, USA, 2018). ANOVA was used to analyzed the blood glucose level, Body weight, liver function test (AST, ALT, and ALP), and relative liver weight followed by multiple comparism using post HOC Turkey HSD. Values were presented as MEAN±STD, and data were considered significant at $p<0.05$.

RESULTS

Table 1 results revealed a significant decrease ($p<0.05$) in the relative liver weight in-group D, group C and F had a non-significant ($p>0.05$) decrease, and group E had a non-significant ($p>0.05$) increase as compared to group B. Group A when compared to group B showed a non-significant ($p>0.05$) increase in the relative liver weight. The bodyweight result revealed a non-significant ($p>0.05$) increase in group C, D, E, and F as compared to group B. Group A when compared to group B revealed a non-significant ($p>0.05$) increase in the bodyweight. Table 2 result revealed a significant ($p<0.05$) decrease in AST level in-group E and F, group D had a significant ($p<0.05$) increase, and group C had a non-significant ($p>0.05$) increase as compared to group B. Group A when compared to group B revealed a significant ($p<0.05$) increase in AST level. The result of ALT level showed significant ($p<0.05$) decrease in-group E and F; group C and D had a significant ($p<0.05$) increase as compared to group B. Group A when compared to group B revealed a significant ($p<0.05$) increase in ALT level. The ALP result showed a significant ($p<0.05$) decrease in-group E and F, group D had a significant ($p<0.05$) increase, and group C had a non-significant ($p>0.05$) increase as compared to group B. Group A when compared to group B revealed a significant ($p<0.05$) increase in ALP level. Table 3 result revealed a significant ($p<0.05$) increase in the blood glucose level in-group B, D, E, and F, and group C had a non-significant ($p>0.05$) increase as compared to group A at day 0. The results for Day 7, 14, and 21 revealed a significant decrease in the blood glucose level in group C, D, E, and F as compared to group B, while group A had a significant increase ($p<0.05$) compared against group B.

DISCUSSION

DM is a metabolic disorder with multiple etiologies characterized by chronic hyperglycemia with impaired protein and lipids metabolism (26). Oxidative stress, resulting from the pathogenesis and complications of diabetes mellitus, has resulted in the increased need for natural ways to boost the antioxidant capacity (27). Recent exploration of Medicinal plants has shown to reduce diabetic complications through biological methods with fewer adverse effect (28). Phytochemical screening of Aju-mbaise shows a high concentration of phytochemical compounds such as; Alkaloids, Flavonoids, Glycosides, Hydrogen Cyanide, Phenols, Saponins, Steroids, Tannins, Terpenoids. These phytochemical compounds were involved in many therapeutic processes at all levels, including diabetes and its pathogenesis. These phytochemicals are potential antioxidants possessing antidiabetic, cardioprotective, anti-inflammatory, anti-carcinogenic and anti-mutagenic effects (21). Tannins reduce diabetic complications by inhibiting adipogenesis and increasing tissue sensitivity, thereby enhancing glucose uptake; thus, herbs containing tannin in high concentration like

Table 1. Effect of Ajumbaise on Liver weight and Body weight on alloxan induced diabetic rats' model

Groups	Relative Liver weight (g)	Body weight (g)
	MEAN±STD	MEAN±STD
Group A (Control)	$3.40\pm1.03_{NS}$	$173.33\pm15.28_{NS}$
Group B (Diabetes Only)	4.11±0.02	140.00±0.00
Group C (150mg/kg AjuMbaise)	$3.56\pm0.05_{NS}$	$160.00\pm20.00_{NS}$
Group D (600mg/kg AjuMbaise)	$2.56\pm0.06_{**}$	$143.33\pm5.00_{NS}$
Group E (Alloxan + 150mg/kg AjuMbaise)	$4.25\pm0.05_{NS}$	$186.66\pm73.71_{NS}$
Group F (Alloxan + 600mg/kg AjuMbaise)	$3.85\pm0.05_{NS}$	$146.66\pm5.77_{NS}$

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$*

Table 2. Effect of Ajumbaise on AST, ALT, and ALP activity on alloxan induced diabetic rats' model

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
	MEAN±STD	MEAN±STD	MEAN±STD
Group A (Control)	36.50±0.50***	14.50±0.50***	125.69±0.44***
Group B (Diabetes Only)	75.00±4.00	60.83±0.76	286.22±1.05
Group C (150mg/kg AjuMbaise)	$76.00\pm1.00_{NS}$	67.00±1.00**	$299.31\pm1.17_{NS}$
Group D (600mg/kg AjuMbaise)	89.00±1.00***	97.50±2.50***	375.83±25.48***
Group E (Alloxan + 150mg/kg AjuMbaise)	67.50±0.50**	51.00±1.00***	213.08±3.02***
Group F (Alloxan + 600mg/kg AjuMbaise)	53.50±0.50***	46.50±1.50***	207.73±1.51***

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$*

Table 3. Effect of Ajumbaise on blood glucose level on alloxan induced diabetic rats' model

Groups	Day 0	Day 7	Day 14	Day 21
	MEAN±STD	MEAN±STD	MEAN±STD	MEAN±STD
Group A (Control)	78.33±1.50	79.33±4.84**	82±2.04***	74.00±1.02
Group B (Diabetes Only)	533.33±20.00***	536.33±17.16	498.22±11.05	400.67±9.05
Group C (150mg/kg AjuMbaise)	$88.12\pm1.12_{NS}$	80.57±4.20**	$75.31\pm1.17_{**}$	75.33±2.42**
Group D (600mg/kg AjuMbaise)	90.21±1.02**	86.50±2.50**	80.13±2.18***	78.30±0.12**
Group E (Alloxan + 150mg/kg AjuMbaise)	450.14±5.56**	360.25±3.14**	210.08±4.02***	105.33±5.25**
Group F (Alloxan + 600mg/kg AjuMbaise)	470.22±8.96***	349.35±5.50**	185.73±5.51***	99.33±4.33**

Data was analyzed using ANOVA followed by Post-Hoc Turkey HSD and values were considered significant at $p<0.05$. $p<0.001$***, $p<0.01$**, and $p<0.05$*

Aju-mbaise can be a potential treatment for type 2 diabetes mellitus (29,30). Flavonoids have multiple positive health effects on diabetes. Vast enquiries have stressed the significance of flavonoids, phenols, alkaloids and some glycosides in preventing the pathogenesis of certain metabolic disorders, including diabetes (31,32). Sequel to the antidiabetic effect of Aju-mbaise through its numerous secondary metabolites or phytochemicals, this phytochemical compound also modulates oxidative stress through their impact on neutralizing free radicals; these, in turn, reduce diabetic complications. These phytochemicals combine to improve insulin signalling and secretion regulation, carbohydrate digestion, glucose uptake, and fatty deposition. The result for liver weight revealed a significant decrease in-group D and non-significant differences in the relative liver weight compared to diabetic control. The bodyweight result showed a non-significant increase in all Aju-mbaise treated groups compared to group to diabetic group. Normal control, when compared to the diabetic group, revealed a non-significant increase in the bodyweight. The physiology behind the non-significant difference seen in the bodyweight is not well elucidated. The study of Ijioma *et al.,* (33) reported a significant ($p<0.05$) difference in body weight, which contradicts the report of this study.

The study result revealed a significant ($p<0.05$) decrease in AST level in-group E and F, group D had a significant ($p<0.05$) increase and group C had a non-significant ($p>0.05$) increase as compared to group B. Group A when compared to group B revealed a significant ($p<0.05$) increase in AST level. The substantial reduction seen in treated diabetic groups with Aju-mbaise results from the potency of the phytochemicals, such as flavonoids and saponin, which reduces AST activities (22). The report of this study has similarities with the findings' of Ijioma et al., (33), who revealed a significant increase in AST level, which was seen in group D, revealing that Aju-mbaise could show signs of toxicity for a prolonged time if consummate only. The study results showed similarities with the Iweala and Oludare(34) report, revealing that AST had no significant change than control. The study result agrees with the findings of Islam et al., (16), indicating a significant rise in AST activities in diabetic control compared to the standard control. The result of ALT level showed a significant ($p<0.05$) decrease in-group E and F; group C and D had a significant ($p<0.05$) increase as compared to group B. Group A when compared to group B, revealed a significant ($p<0.05$) increase in ALT level. The significant rise in ALT activities shown in diabetic control revealed the damage of hepatic membrane following oxidative stress from Alloxan toxicity on the liver, which indicates liver injury.

The study has similarities with the reports following alloxan toxicity in the diabetic model(16,35,36). Further, the significant reduction in ALT level revealed the decoction (Aju-mbaise) has high flavonoid content, which lowered ALT activities in the diabetic treated groups. Ijioma et al., (33) reported a significant increase ($p<0.05$) in ALT level following Aju-mbaise administration, which has a similar report with this study's report. The ALP activity revealed a significant ($p<0.05$) decrease in-group E and F, group D had a significant ($p<0.05$) increase, and group C had a non-significant ($p>0.05$) increase as compared to group B. Group A when compared to group B revealed a significant ($p<0.05$) increase in ALP level. The significant higher ALP activities observed in the diabetic treated groups showed liver injury resulting from altered AST and ALT in the hepatic membrane, which have similarities with this study finding. Ijioma et al., (33) reported a significant increase ($p<0.05$) in ALT level following Aju-mbaise administration, which has a similar report with this study's finding. The study's findings revealed a significant ($p<0.05$) increase in blood glucose level in all groups compared to the normal control group except for the group that received 150mg/kg, the increase in non-significant at day 0. The persistent rise in blood glucose level is attributed to oxidative stress from accumulated alloxan monohydrate, which brings about auto-oxidation of glucose (37). The study has similarities with (38), who revealed a significant change in glucose level following damages from the pancreas resulting from alloxan toxicity.

On Day 7, 14 and 21, the blood glucose level revealed a significant ($p<0.05$) decrease in all groups C, D, E, and F compared to the diabetic group. The hypoglycemic effect of Aju-mbaise is attributed to the Flavonoids and polyphenols present (39), which has the potency of reducing the high blood glucose level, as well as regeneration of insulin sensitivity to the pancreas and muscle cells. The precise mechanisms for these actions are not fully elucidated. The report of (40) showed a significant decrease ($p<0.05$) in blood glucose level in diabetic model following S. mombin. Iweala and Oludare(34)reported a significant reduction ($p<0.05$) in blood glucose level in diabetic model treated with S. mombin. The findings of (37) reported a poly-herbal mixture constituent having hypoglycemic activities following alloxan monohydrate activities on the diabetic model. The study of (41) had a similar report with this study finding, following administration of S. mobin on the diabetic model.

Conclusion

The study showed that Alloxan monohydrate had a significant increase in liver enzymes activities (AST, ALT, and ALP), revealing hepatic damage. Also, there were higher blood glucose activities caused by Alloxan monohydrate, indicating pancreatic tissue damage; however, the extract (Aju-mbaise), when administered, showed hepato-curative and hypoglycemic activities on the diabetic model of Wistar rats. In addition, the extract had no effect on the body weight as well as liver weight.

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Statement of competing interests

The authors have no competing interest.

List of Abbreviations

DM: Diabetes mellitus
NALD: Non-alcoholic liver disease
T2DM: Type II diabetes mellitus
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase

REFERENCES

1. Arumugam G, Manjula P, Paari N. A review: Anti diabetic medicinal plants used for diabetes mellitus. J Acute Dis. 2013;2(3):196–200.
2. Bahmani M, Zargaran A, Rafieian-Kopaei M, Saki K. Ethnobotanical study of medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest Iran. Asian Pac J Trop Med [Internet]. 2014 Sep 1 [cited 2021 May 10];7(S1):S348–54. Available from: https://pubmed.ncbi.nlm.nih.gov/25312149/
3. Kazemi S, Asgary S, Moshtaghian J, Rafieian M, Adelnia A, Shamsi F. Liver-protective effects of hydroalcoholic extract of allium hirtifolium boiss. In rats with alloxan-induced diabetes mellitus. ARYA Atheroscler [Internet]. 2010 [cited 2021 May 10];6(1):11–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22577407
4. Daryabor G, Atashzar MR, Kabelitz D, Meri S, Kalantar K. The Effects of Type 2 Diabetes Mellitus on Organ Metabolism and the Immune System [Internet]. Vol. 11, Frontiers in Immunology. Frontiers Media S.A.; 2020 [cited 2021 May 10]. p. 1582. Available from: /pmc/articles/PMC7387426/
5. Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, et al. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. Lancet Glob Heal [Internet]. 2018 Dec 1 [cited 2021 May 10];6(12):e1352–62. Available from: www.thelancet.com/lancetghVolhttp://dx.doi.org/10.1016/S0140-6736
6. Mathur S, Kumar Mehta D, Kapoor S, Yadav S. Liver Function in Type-2 Diabetes Mellitus Patients. Int J Sci Study [Internet]. 2016;(10). Available from: www.ijss-sn.com
7. Dharmalingam M, Yamasandhi Pg. Nonalcoholic fatty liver disease and Type 2 diabetes mellitus. Indian J Endocrinol Metab [Internet]. 2018 [cited 2021 May 11];22(3):421. Available from: /pmc/articles/PMC6063173/
8. Han HS, Kang G, Kim JS, Choi BH, Koo SH. Regulation of glucose metabolism from a liver-centric perspective [Internet]. Vol. 48, Experimental and Molecular Medicine. Nature Publishing Group; 2016 [cited 2021 May 11]. p. 48. Available from: www.nature.com/emm
9. Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis [Internet]. Vol. 1411, Annals of the New York Academy of Sciences. Blackwell Publishing Inc.; 2018 [cited 2021 May 11]. p. 21–35. Available from: /pmc/articles/PMC5927596/
10. Galicia-garcia U, Benito-vicente A, Jebari S, Larrea-sebal A. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(6275):1–34.
11. Sheng X, Che H, Ji Q, Yang F, Lv J, Wang Y, et al. The Relationship between Liver Enzymes and Insulin Resistance in Type 2 Diabetes Patients with Nonalcoholic Fatty Liver Disease. Horm Metab Res [Internet]. 2018 May 1 [cited 2021 May 11];50(5):397–402. Available from: https://pubmed.ncbi.nlm.nih.gov/29723898/
12. Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016 May 1;31(5):936–44.
13. Shibabaw T, Dessie G, Molla MD, Zerihun MF, Ayelign B. Assessment of liver marker enzymes and its association with type 2 diabetes mellitus in Northwest Ethiopia. BMC Res Notes [Internet]. 2019 Oct 29 [cited 2021 May 11];12(1):1–5. Available from: https://doi.org/10.1186/s13104-019-4742-x
14. Mathur S, Mehta DK, Kapoor S, Yadav S. Liver Function in Type-2 Diabetes Mellitus Patients. Int J Sci Study. 2016;3(10):43–7.
15. Kobayashi A, Suzuki Y, Sugai S. Specificity of transaminase activities in the prediction of drug-induced hepatotoxicity. Vol. 45, Journal of Toxicological Sciences. Japanese Society of Toxicology; 2020. p. 515–37.
16. Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N. Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinol Diabetes Metab [Internet]. 2020 Apr 1 [cited 2021 May 10];3(2). Available from: /pmc/articles/PMC7170449/
17. Aidi Wannes W, Marzouk B. Research progress of Tunisian medicinal plants used for acute diabetes. J Acute Dis. 2016 Sep 1;5(5):357–63.
18. Chaachouay N, Benkhnigue O, Fadli M, El Ibaoui H, Zidane L. Ethnobotanical and ethnopharmacological studies of medicinal and aromatic plants used in the treatment of metabolic diseases in the Moroccan Rif. Heliyon [Internet]. 2019 Oct 1 [cited 2021 May 10];5(10). Available from: /pmc/articles/PMC6838988/
19. Skalli S, Hassikou R, Arahou M. An ethnobotanical survey of medicinal plants used for diabetes treatment in Rabat, Morocco. Heliyon. 2019 Mar 1;5(3):e01421.
20. Giovannini P, Howes MR, Edward SE. Data on medicinal plants used in Central America to manage diabetes and its sequelae (skin conditions, cardiovascular disease, kidney disease, urinary problems and vision loss. Data Br. 2016;(7):1217–1220.
21. Nnadiukwu AT, Monago-Ighorodje CC, Chuku LC. Phytochemical Composition of Ethanol Extract of a Cocktail Herbal Mixture (Aju Mbaise). J Adv Biol Biotechnol [Internet]. 2019 Jul 16 [cited 2021 May 10];22(1):1–11. Available from: https://journaljabb.com/index.php/JABB/article/view/30103
22. Nnadiukwu TA, Ighorodje CCM-, Chuku LC. Nutritional Composition of 'Aju Mbaise' Herbal Cocktail. Asian J Res Biochem. 2020 Aug 31;30–7.
23. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother [Internet]. 2010 Jul [cited 2021 Apr 10];1(2):87–93. Available from: /pmc/articles/PMC3043327/
24. REITMAN S, FRANKEL S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol [Internet]. 1957 [cited 2021 May 11];28(1):56–63. Available from: https://pubmed.ncbi.nlm.nih.gov/13458125/
25. KING EJ, JEGATHEESAN KA. A method for the determination of tartratelabile, prostatic acid phosphatase in serum. J Clin Pathol [Internet]. 1959 [cited 2021 May 11];12(1):85–9. Available from: https://pubmed.ncbi.nlm.nih.gov/13631088/
26. Erion DM, Park HJ, Lee HY. The role of lipids in the pathogenesis and treatment of type 2 diabetes and associated co-morbidities. BMB Rep [Internet]. 2016 Mar 1 [cited 2021 May 11];49(3):139–48. Available from: /pmc/articles/PMC4915228/

27. Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong XB, et al. The Pathogenesis of Diabetes Mellitus by Oxidative Stress and Inflammation: Its Inhibition by Berberine [Internet]. Vol. 9, Frontiers in Pharmacology. Frontiers Media S.A.; 2018 [cited 2021 May 10]. p. 782. Available from: www.frontiersin.org
28. Unuofin JO, Lebelo SL. Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review [Internet]. Vol. 2020, Oxidative Medicine and Cellular Longevity. Hindawi Limited; 2020 [cited 2021 May 10]. Available from: /pmc/articles/PMC7042557/
29. Laddha AP, Kulkarni YA. Tannins and vascular complications of Diabetes: An update [Internet]. Vol. 56, Phytomedicine. Elsevier GmbH; 2019 [cited 2021 May 10]. p. 229–45. Available from: https://pubmed.ncbi.nlm.nih.gov/30668344/
30. Fraga-Corral M, Otero P, Cassani L, Echave J, Garcia-Oliveira P, Carpena M, et al. Traditional Applications of Tannin Rich Extracts Supported by Scientific Data: Chemical Composition, Bioavailability and Bioaccessibility. Foods (Basel, Switzerland) [Internet]. 2021 Jan 26 [cited 2021 May 10];10(2). Available from: http://www.ncbi.nlm.nih.gov/pubmed/33530516
31. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules [Internet]. 2019 Sep 1 [cited 2021 May 10];9(9). Available from: /pmc/articles/PMC6769509/
32. Meng Q, Qi X, Fu Y, Chen Q, Cheng P, Yu X, et al. Flavonoids extracted from mulberry (Morus alba L.) leaf improve skeletal muscle mitochondrial function by activating AMPK in type 2 diabetes. J Ethnopharmacol. 2020 Feb 10;248.
33. Ijioma SN, Emmanuel O, Nosiri CI, Ugbogu EA. Evaluation of toxicity profile and pharmacological potentials of Aju Mbaise polyherbal extract in rats. Sci African. 2021 Mar 1;11:e00681.
34. Iweala EEJ, Oludare FD. Hypoglycemic effect, biochemical and histological changes of Spondias mombin linn, and Parinari polyandra benth. seeds ethanolic extracts in alloxan-induced diabetic rats. J Pharmacol Toxicol. 2010;6(2):101–12.
35. Aprioku JS, Amah-Tariah FS. Garlic (Allium sativum L .) Protects Hepatic and Renal Toxicity of Alloxan in Rats. J Pharm Res Int. 2017;17(6):1–7.
36. Mandal A, Bhattarai B, Kafle P, Khalid M, Jonnadula SK, Lamicchane J, et al. Elevated Liver Enzymes in Patients with Type 2 Diabetes Mellitus and Non-alcoholic Fatty Liver Disease. Cureus [Internet]. 2018 Nov 23 [cited 2021 May 10];10(11). Available from: /pmc/articles/PMC6347442/
37. Ojiako OA, Chikezie PC, Ogbuji AC. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. J Tradit Complement Med [Internet]. 2016 Apr 1 [cited 2021 May 10];6(2):184–92. Available from: /pmc/articles/ PMC4833464/
38. Yarmolenko O, Bumeister V, Demikhova N, Prykhodko O, Gordienko O, Khotyeev Y. The Effect of Alloxan-Induced Hyperglycemia on the Myocardium of Experimental Animals. Rom J Diabetes Nutr Metab Dis [Internet]. 2020 Aug 13 [cited 2021 May 10];27(2):80–4. Available from: http://rjdnmd.org/index.php/RJDNMD/article/view/626
39. Hussain T, Tan B, Murtaza G, Liu G, Rahu N, Saleem Kalhoro M, et al. Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy [Internet]. Vol. 152, Pharmacological Research. Academic Press; 2020 [cited 2021 May 10]. Available from: https://pubmed.ncbi.nlm.nih.gov/31918019/
40. Ojo OA, Afon AA, Ojo AB, Ajiboye BO, Oyinloye BE, Kappo AP. Inhibitory effects of solvent-partitioned fractions of two nigerian herbs (Spondias mombin Linn. and mangifera indica L.) on α-amylase and α-glucosidase. Antioxidants [Internet]. 2018 Jun 1 [cited 2021 May 10];7(6):73. Available from: www.mdpi.com/journal/ antioxidants
41. Saronee F, Bekinbo M, Ojeka S, Dapper D. Full-text Available Online at Comparative Assessment of Methanolic Extracts of Hog Plum (Spondias mombin linn) Leaves and Turmeric (Curcuma longa L .) Rhizomes on Blood Glucose and Glycosylated Haemoglobin in Male Wistar Rats SARONEE, F ; BEKINBO, M. 2019;23(9):1631–6.

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