GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Effect of ethanolic leaf extracts of *Medicago sativa, Gongronema latifolium* and *Pterocarpus sanalinoides* on the liver integrity of alloxan-induced diabetic albino rats

Obijuru N.T *, Igwe, C.U, Ene A. C and Nwaoguikpe R. N

Department of Biochemistry, Medical Unit, Federal University of Technology, Owerri, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2023, 25(01), 178–185

Publication history: Received on 08 September 2023; revised on 16 October 2023; accepted on 19 October 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.1.0427

Abstract

The study investigated the effects of ethanolic leaf extracts of *Medicago sativa (*MS), *Pterocarpus santalinoides* (PS) and *Gongronema latifolium* (GL) on the liver function profile and organ histology integrity of alloxan-induced diabetic albino rats. Sixty male rats were divided into 10 groups, made up of 1 group each of normal, diabetic, and positive controls, 3 treatment groups each of the single and pairwise extract combinations, and 1 group of the ternary extracts combination. Hyperglycaemia was induced in the treatment groups by a single intraperitoneal injection of alloxan at 150 mg/kg body weight. The positive control was treated with metformin (100 mg/kg), while the extracts were administered via oral intubation to the single (200 mg/kg of each extract), double (combination of 2 extracts at 100 mg/kg each) and ternary (combination of the 3 extracts at 66.7 mg/kg each) treatment groups for a total period of 4 weeks. Induction of diabetes significantly ($p<0.05$) increased AST, ALT and ALP activities and total bilirubin concentration, but treatment with metformin and the plants' extracts significantly ($p<0.05$) reduced the enzymes activities and total bilirubin concentration to levels comparable to those of the normal control. Generally, the combination therapies had non-significantly stronger effects than the single extract treatments. Histological evaluation confirmed that combinatorial compared to individual extracts treatment reverted more the damage to the liver organ caused by alloxan induction. Thus, *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* are possible lead plants for potential production of hepatoprotective agents.

Keywords: *Medicago sativa*; *Pterocarpus santalinoides*; *Gongronema latifolium*; Hepatoprotective; Hyperglycaemia; combination therapies

1. Introduction

Diabetes mellitus is a common health problem with multiple etiology mainly characterized by hyperglycemia resulting from insulin secretion and/or function abnormalities[1]. Alterations in hepatic cell growth and cell number alter the liver size during diabetes. It has been proposed that the most important cause of liver damage in diabetic patients is hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolisms[2]. It has been reported that hyperglycemia-induced oxidative stress and subsequent disturbance in carbohydrate, protein and lipid metabolisms are the most important causes of liver damage in diabetic patients[3]. Chronic hyperglycaemia of diabetes mellitus has been strongly associated with damage to several organs including the liver[4]. Hence, the need to assess the hepato-protective effects of plant extracts in alloxan induced diabetic rats. Diabetes mellitus (type 2) has been treated using anti-diabetic medications. The majority of currently used hypoglycemic medications, such as thiazolidinediones, biguanides, meglitinides, and sulfonylureas, as well as benzoic acid derivatives (repaglinide), are taken orally as monotherapy or in conjunction with other drugs to manage blood sugar levels[5]. However, gradual resistance to these agents, in addition to their various adverse effects, has increased the need for finding alternative therapies with less or even no side effects for diabetic patients. In recent years, the use of herbal medications has developed to minimize hyperglycemia and other diabetes associated complications. Practitioners of traditional

* Corresponding author: Obijuru N.T

medicine in South East Nigeria use *Medicago sativa, Gongronema latifoliun and Pterocarpus santalinoides*, as a folk remedy for treating diabetes mellitus. Hence we made an attempt to study the validity of this folk remedy by investigating the effects of ethanol extracts of these leaves and effect of their combination in alloxan induced diabetic rats .

2. Material and methods

The research was carried out in the department of Biochemistry, Federal University Of Technology Owerri.

2.1. Collection and preparation of extract

Medicago sativa, Pterocarpus santalinoides and *Gongronema latifolium* were purchased from market and was identified and authenticated by taxonomist at Department of Botany, Imo State University. The collected plant material was washed with tap water carefully and was completely dried under the shade. The shade-dried plant material was finely powdered. The aqueous extracts were prepared by soaking about 100 g of powdered plant material in 1000 ml ethanol for 24 hours. The extract was filtered and the solvents removed with rotary evaporations to obtain crude active ingredient.

2.2. Experimental animal

60 Male Wistar albino rats (180–200 g) were used for the antidiabetic evaluation. The rats were housed in laboratory conditions in stainless steel cages at standard conditions (temperature 24 ± 2 ℃, relative humidity 45–55% and 12 h light/- dark cycle). They were provided with commercial rat feed and water *ad libitum*.

2.3. Induction of diabetes mellitus

In overnight fasted rats, diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150 mg/kg) dissolved in normal saline. Diabetes was confirmed in rats by determining glucose level with a glucometer after 72 hrs of alloxan monohydrate injection. Experimental rats having blood glucose level above 120 mg/dl were believed to be diabetic and included for further studies.

2.4. Experimental design

The study was carried out for 31 days. The rats were acclimatized for one week and maintained standard laboratory conditions. Diabetes was induced in rats a week before the start of the experiment. After the induction of diabetes, they were divide into different groups. Varying concentrations of the crude extracts of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their mixture were administered via oral intubation to the animals Blood samples were collected and serum was separated for glucose, kidney and liver enzymes estimation. kidney and liver organs were harvested for histological studies.

Table 1 Grouping of albino rats

No	Groups	Treatment
Group 1	Normal control	Feed and water only
Group2	Diabetic control	Feed and water only
Group 3	Positive control	Metformin at 100 mg/kg b.w.
Group 4	Diabetic + *P.santalinoides,*	200 mg/kg b.w.
Group 5	Diabetic + *M.sativa*	200 mg/kg b.w.
Group 6	Diabetic + *G.latifolium*	200 mg/kg b.w.
Group7	Diabetic + *P.santalinoides and G.latifolium*	100 mg/kg.b.w each
Group 8	Diabetic + *P.santalinoides and M.sativa*	100 mg/kg.b.w each
Group 9	Diabetic + *G. latifolium and M.sativa*	100 mg/kg.b.w each
Group 10	Diabetic + *P. santalinoides, G. latifolium and M.sativa*	66.7 mg/kg.b.w each

2.5. Assays

The Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method[6]. Bilirubin by the method[7]. Histology of the liver is by the method[8].

2.6. Statistical analysis

All the results obtained were expressed as mean ± S.E. Data will be analyzed statistically by analysis of variance, for statistical significance using DMR test, one way ANOVA. Differences between means were regarded significant at $P < 0.05$

3. Results

Fig. 1 showed the effect of ethanolic extract of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their combination on AST level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in AST level in negative control group when compared to normal control group indicating that alloxan causes elevation of AST level in rats but the administration of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium*, and their combinations non- significantly ($P < 0.05$) lowered the AST level. There was no significant difference between different treated groups as compared to standard drug (positive control).

Fig. 2 showed the effect of ethanolic extract of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their combinations on ALT level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in ALT level in negative control group when compared to normal control group indicating that alloxan causes elevation of ALT activity in rats but the administration of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium*, and their combinations significantly ($P < 0.05$) lowered the ALT level. There was no significant difference between different treated groups as compared to standard drug (positive control). A higher percentage decreases were observed in ternary treated diabetic rats when compared to other extract treated rats.

Fig. 3 showed the effect of ethanolic extract of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their combinations on ALP level of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in ALP level in negative control group when compared to normal control group indicating that alloxan causes elevation of ALP activity in rats but the administration of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium*, and their mixture significantly ($P < 0.05$) decreased the ALP level. There is no significant difference between different treated groups as compared to standard drug (positive control).

Fig. 4 showed the effect of ethanolic extract of *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their combinations on total bilirubin concentration of rats after treatment of diabetes. There was a significant ($P < 0.05$) increase in Total bilirubin concentration in negative control group when compared to normal control group indicating that alloxan causes raise in total bilirubin concentration in rats but the administration of *Medicago sativa, Petrocarpus santalinoides* and *Grongonema latifolium*, and their combinations significantly ($P < 0.05$) decreased the total bilirubin concentration. There was no significant difference between different treated groups as compared to standard drug (positive control).

4. Discussion

The present study demonstrates that liver function is significantly altered in patients with diabetes mellitus. Alloxan induced diabetic rats are one of the animal model for diabetes. In diabetic animal's alteration in the activity of serum enzymes directly related to changed metabolism in which enzymes are involved. Increased activity of liver enzymes in diabetic animals are reported by many researchers. Increased aminotransferase level in absence of insulin because of increased amino acid activity in hyperglycemic condition are responsible for ketogenesis and gluconeogenesis[9,10]. The present study represents increased activities of serum AST, ALT and ALP level indicated that hepatic dysfunction may be induced due to hyperglycemia in diabetic rats. Along with that reported in [11] their study that individuals with diabetes are at a high risk of liver function abnormalities as compare to normal. Slight chronic elevation in aminotransferase activity most often reflect the insulin resistance. Single, binary and tenary combinations significantly improved the alterations in serum liver enzymes ALP, ALT and AST (Fig 1,2,3). AST is a nonspecific marker for hepatic injury while ALT is a specific marker for hepatic parenchymal injury. They are both used in the evaluation of Liver disorders [12,13,14]. Alkaline phosphatase is a membrane bound glycoprotein enzyme. High amount of this enzyme is present in the sinusoids and in the endothelium of the central and periportal veins.

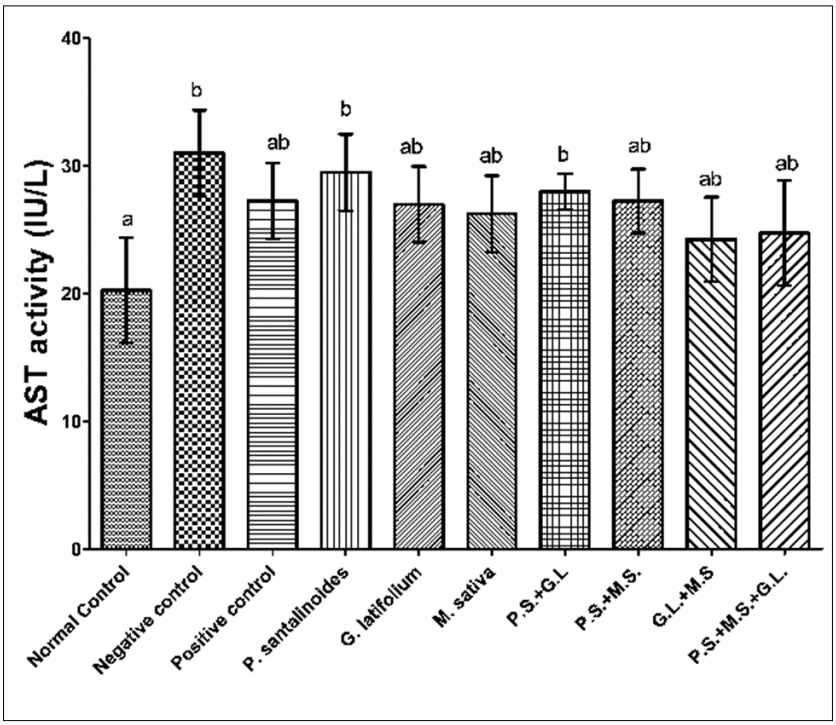

Figure 1 Aspartate aminotransferase activity (IU/L) of alloxan-induced diabetic albino rats treated with single, binary and ternary combinations of leaf extracts of *Medicago sativa* (M.S), *Pterocarpus santalinoides* (P.S.) and *Gongronema latifolium* (G.L)

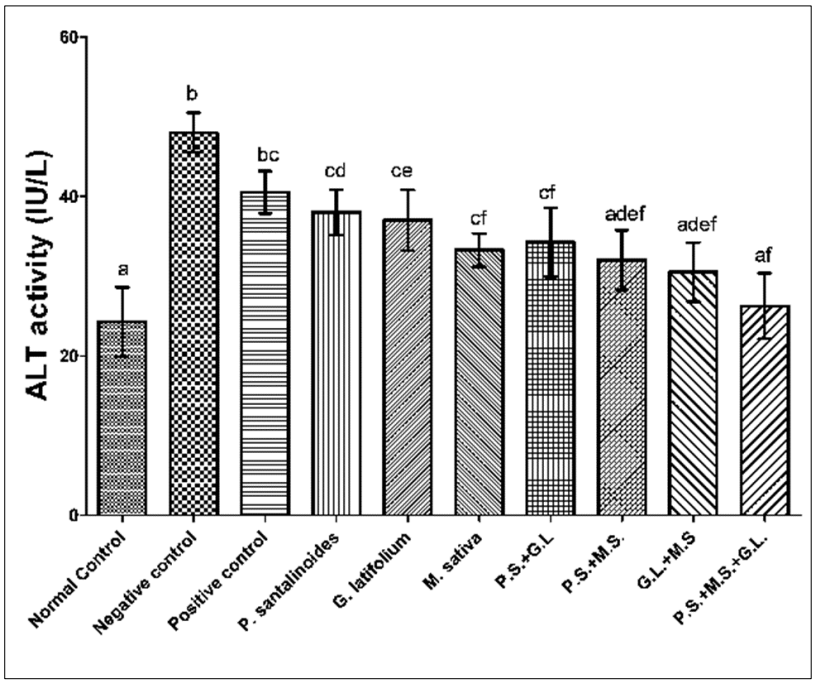

Figure 2 Alanine aminotransferase activity (IU/L) of alloxan-induced diabetic albino rats treated with single, binary and ternary combinations of leaf extracts of *Medicago sativa* (M.S), *Pterocarpus santalinoides* (P.S.) and *Gongronema latifolium* (G.L)

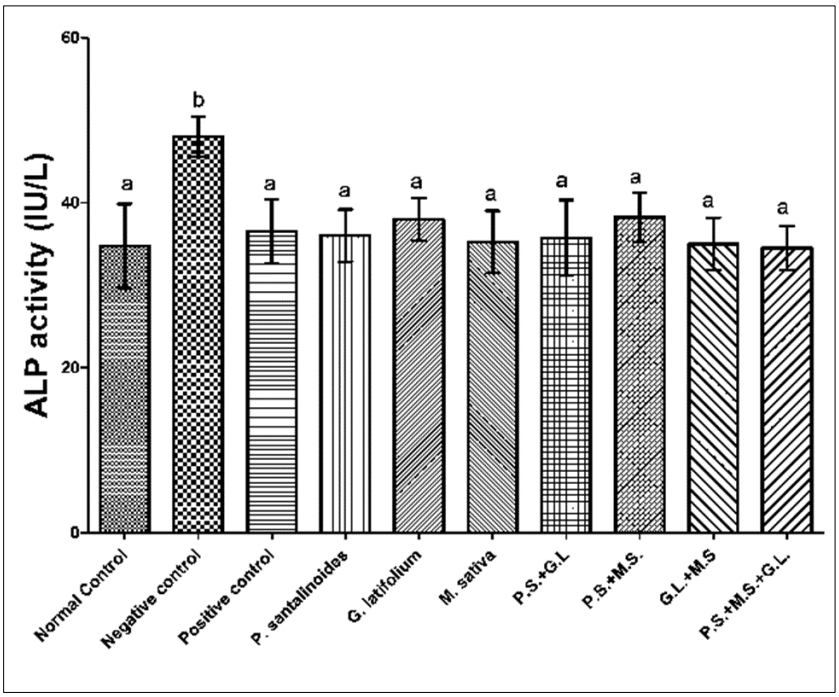

Figure 3 Alkaline phosphatase activity (IU/L) of alloxan-induced diabetic albino rats treated with single, binary and ternary combinations of leaf extracts of *Medicago sativa* (M.S), *Pterocarpus santalinoides* (P.S.) and *Gongronema latifolium* (G.L)

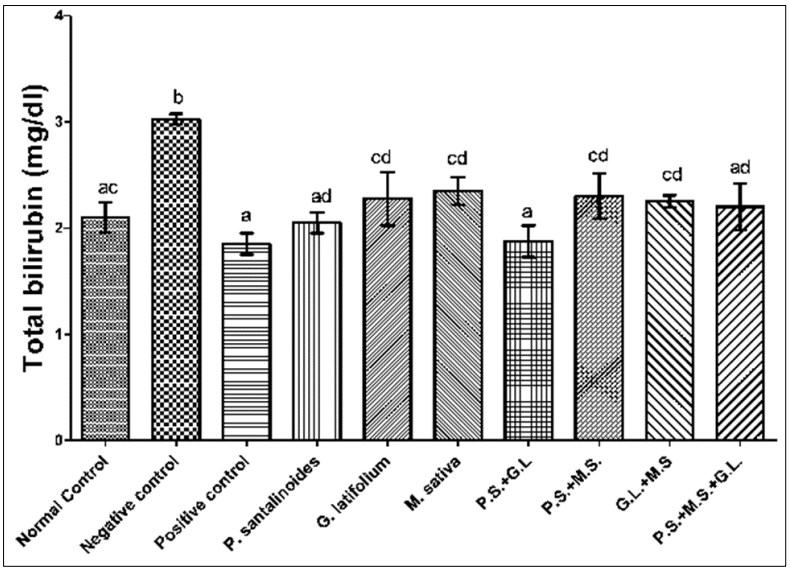

Figure 4 Total bilirubin concentration (mg/dl)of alloxan-induced diabetic albino rats treated with single, binary and ternary combinations of leaf extracts of *Medicago sativa* (M.S), *Pterocarpus santalinoides* (P.S.) and *Gongronema latifolium* (G.L)

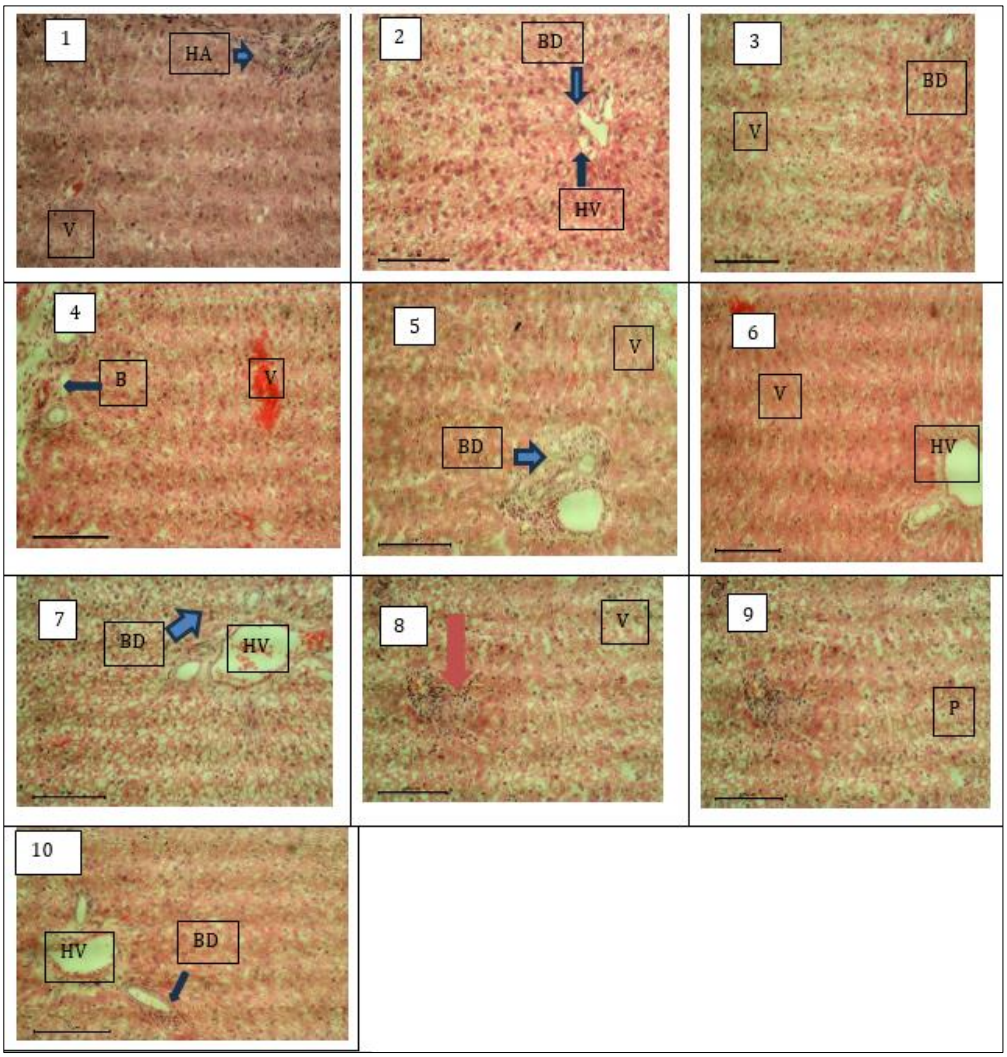

Figure 5 : Photomicrograph of section of the Liver of diabetic rats (X400). Stain: H&E. HA-hepatic artery, V-central vein, BD-bile duct, HA-hepatic artery, P-portal area. The sections of the liver presented in group 1 showed the normal hepatic histo-architecture. The sections of the liver presented in group 2 showed a widespread vacuolar degeneration of the hepatocytes caused by alloxan induction. The sections of the liver presented in group 3 showed a moderate widespread vacuolar degeneration of the hepatocytes. The sections of the liver presented in group 4 showed a mild widespread micro-vesicular lipidosis of the hepatocytes. The sections of the liver presented in group 5 showed mild sprinkling of inflammatory leukocytes around the portal areas. The sections of the liver presented in group 6 showed mild vacuolation of the hepatocytes in the mid-zonal areas of the hepatic lobules. The sections of the liver presented in group 7 showed mild widespread vacuolation of the hepatocytes. The sections of the liver presented in group 8 showed mild widespread vacuolation of the hepatocytes and random multiple areas of inflammatory cellular infiltration (red arrow). The sections of the liver presented in group 9 showed mild sprinkling of inflammatory leukocytes around the portal areas. The sections of the liver presented in group 10 showed moderate multifocal vacuolation of the hepatocytes (arrow), hence the ternary reduced the damage to the liver more than other treatments.

An increase in these enzyme activities is indicative of liver damage[15]. Cell damage to the liver causes these cytosolic enzymes to spill into the sinusoids and finally into the blood stream. In this study we reported a significant increase in the levels of AST, ALT and ALP in the diabetic control (untreated) rats when compared with the normal control. Bilirubin is a product of heme metabolism and it is lipid-soluble. The significant increase in total and conjugated bilirubin levels observed in the diabetic control groups in this study corroborates with the studies done by[16]. The significant decrease in bilirubin levels in the extracts treated groups (fig.4), therefore suggests that the three plant extract possess bilirubin lowering effects. [17]reported that induction of diabetes with monohydrate alloxan resulted in necrosis of the liver of rats. Hence, the increases observed in the activities of AST and ALT may result from the leakage of these aminotransferase enzymes from the cytosol of the liver into the blood[18], which therefore indicates the hepatotoxic impact of alloxan. Our result is in agreement with the reports of other researchers who observed similar elevations in activities of liver

enzymes following alloxan induction[19,20,21]. Chronic and untreated diabetes tends to induce liver injury and damage, since this organ is the central processing unit for fuels whose metabolism have been drastically altered in diabetes. Histology of the liver of rats treated with the various plant extracts: Histological examination of the liver revealed that induction of the rats with alloxan resulted in a characteristic periportal infiltrates of inflammatory cells, portal vascular congestion and oedema as well as portal vascular ulceration of the liver cells. Treatment of the diabetic rats with extracts showed a characteristic normal hepatocytes as those recorded in the control groups.

5. Conclusion

Diabetes mellitus has been a serious disease all over the world. Plants play an important role for the treatment of this disease due to their active constituents. The present study indicates that *Medicago sativa, Pterocarpus santalinoides* and *Gongronema latifolium* and their combinations normalized liver enzyme activities in alloxanized diabetic rats and it could be used as an adjunct in the management of hyperglycemic conditions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present method on wistar albino rats was submitted to the Medical and Health Research Ethics Committee.

References

[1] Ruch, W. and Zumsteg, U. (1988). Diabetes or hyperglycemia? *Schweizerische medizinische Wochenschrift*. 118(8):264-266.

[2] Levinthal, G.N. and Tavill, A.S. (1999). Liver disease and diabetes mellitus. *Clinical diabetes*. 17(2):73.

[3] Mohamed, J., Nafizah, A.N., Zariyantey, A. and Budin, S.B.(2016). Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. *Sultan Qaboos University Medical Journal*. 16(2):e132.

[4] Lyra, R., Oliveiria, M., Lins, D. and Cavalcanti, N. (2006). Prevention of type 2 diabetes mellitus. *Arq. Bras. Endocronol. Metab*, 50: 239 – 249.

[5] Shams, G.A., Kamel, M.A. and Ezzat, R.M.(2018). Adverse Effects of Repaglinide Alone and in Combination of Vitamin E. Article 36, Volume 35, Issue 2, , Page 413-421.

[6] Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28, 56–66.

[7] Jendrassik, L. & Grof, P. (1938). Simplified Photometric Methods for the Determination of Bilirubin. *Biochemical Journal*, 297, 81-89.

[8] Akparie, S.O. (2004). General veterinary pathology. *Stirling-Horden Publishers* (Nig.) Ltd, Oyo, Nigeria, 136pp.

[9] Baldi, A. and Goyal, S. (2011). Hypoglycemic effect of polyherbal formulation in alloxan induced diabetic rats. *Pharmacologyonline* 3, 764–773.

[10] Atta, A., Mustafa, G., Sheikh, M.A., Shahid, M. and Xiao, H. (2017). The biochemical significances of the proximate, mineral and phytochemical composition of selected vegetables from Pakistan. *Mat. Sci. Pharma* 1 (1), 06–09.

[11] Salih, D.H. (2013). Study of liver function tests and renal function Tests in diabetic type II patients. *IOSR J. Appl. Chem*. 3, 42–44.

[12] Jus´kiewicz, J., Zdun´czyk, Z., Jurgon´ski, A., Brzuzan, Ł., Godycka- Kłos, I., Ewa, Z. and Ary-Sikorska, A. (2008). Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutr. Res.* 28:343–349.

[13] Sepodes, B., Maio, R., Pinto, R., Marques, C., Mendes-do-Vale, J., McDonald, M.C., Thiemermann, C. and Mota-Filipe, H.(2004). Tempol, an intracelullar free radical scavenger, reduces liver injury in hepatic ischemia-reperfusion in the rat. *Transplant Proc*. 36:849–853.

[14] Bi, W., Cai, J.,Xue, P., Zhang, Y., Liu, S., Gao, X., Li, M., Wang, Z., Baudy-Floc'h , M., Green, S.A. and Bi, L. (2008). Protective effect of nitronyl nitroxide-amino acid conjugates on liver ischemia-reperfusion induced injury in rats. *Bioorg. Med. Chem. Lett*. 18:1788–1794.

[15] Elisa, J., Daisy, P., Ignacimuthu, S. and Duraipandiyan, V. (2009). Antidiabetic and antilipidemic effect of eremanthin from Costus speciosus (Koen.) Sm., in STZ-induced diabetic rats. *Chem. Biol. Interact.* 182:67–72.

[16] Omonkhua, A.A., Adebayo, E.A., Saliu, J.A.,Ogunwa, T.H. and Adeyelu, T.T. (2014). Liver function of Streptozotocin-Induced Diabetic Rats Orally Administered Aqueous Root-Bark Extracts of *Tetrapleura tetraptera* (Taub). *Nigerian J. Basic and Appl Sci*. 22(3&4): 99-106.

[17] Ohaeri, O.C. (2001). Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. *Biosci. Rep.* 21:19–24.

[18] Navarro, C.M., Montilla, P.M., Martin, A., Jimenez, J. and Utrilla, P.M. (1993). Free radicals scavenger and antihepatotoxic activity of Rosmarinus tomentosus. *Planta Med.* 59:312–314 .

[19] Zafar, M., Naqvi, S.N., Ahmed, M. and Kaimkhani, Z.A. (2009). Altered Liver Morphology and Enzymes in Streptozotocin Induced Diabetic Rats. *Int. J. Morphol.* 27(3): 719- 725.

[20] Najla, O.A., Olfat, A.K., Kholoud, S.R., Enas, N.D. and Hanan, S.A.(2012). Hypoglycemic and Biochemical Effects of *Matricaria chamomilla* Leave Extract in Streptozotocin-Induced Diabetic Rats. *J. Health Sci*. 2(5): 43-48.

[21] Soliman, G.Z.A. (2013). Effect of Vitamin C and/or Vitamin E on Kidney, Liver and Brain Functions of Streptozotocin-Induced Diabetic Rats. *The Egypt. J. Hosp. Med*. 53: 799– 808.