RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2014.177.183

Ameliorative Effects of the Ethanolic Seed Extract of *Mucuna pruriens* in Alloxan-induced Biochemical Alteration in Male Wistar Rats

[1]Stephen O. Majekodunmi, [2]Ademola A. Oyagbemi, [3]Oluwatoyin A. Odeku

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria

[2]Department of Veterinary Physiology, Biochemistry and Pharmacology,

[3]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Backgournd: To evaluate the ameliorative effect of ethanolic seed extract of *Mucuna pruriens* in alloxan-induced biochemical alteration in male Wistar rats. **Materials and Methods:** Ethanolic seed extract of *Mucuna pruriens* was administered orally in doses of 5, 10, 20, 30, 40, 50 and 100 mg kg^{-1} body weight (b.wt.) to alloxan-induced rats. The plasma biochemical and lipid profiles and the histopathological evaluation of the liver of the normal and diabetic rats were carried out and the results compared with those diabetic rats administered with *M. pruriens* extract and glibenclamide (5 mg kg^{-1} body weight). **Results:** The alloxan treated rats showed significantly ($p<0.05$) elevated levels of plasma cholesterol, triglycerides, urea, creatinine, serum aspartate aminotransferase and serum alanine aminotransferase with concomitant decreased in total protein level when compared to the control rats. Administration of *M. pruriens* seed extract and glibenclamide significantly ($p<0.05$) decreased the plasma cholesterol, triglycerides, low density lipoprotein-cholesterol, creatinine, alkaline phosphatase levels while high density lipoprotein-cholesterol levels significantly ($p<0.05$) increased after 12 weeks of treatment of the diabetic rats. The effect of *M. pruriens* seed extract was dose dependent and the plasma and lipid profiles and the electrolytes levels were restored to near normal levels. There were no significant ($p>0.05$) difference in the ameliorative effect of *M. pruriens* extract and glibenclamide. **Conclusion:** *Mucuna pruriens* ethanolic seed extract has both hepatoprotective and lypogenic activities in alloxan induced diabetic rats and could be substituted for standard oral hypoglycaemic agent. *Mucuna pruriens* extract in addition to possessing hypoglycaemic properties could be used to ameliorate the biochemical alterations induced by diabetes.

Key words: Diabetes, *Mucuna pruriens*, glibenclamide, ameliorative effect, hepatoprotection

Pharmacologia 5 (5): 177-183, 2014

INTRODUCTION

Diabetic mellitus is a disorder characterized by hyperglycemia (Panunti *et al*., 2004; Davi *et al*., 2005), altered metabolism of lipids, carbohydrates and proteins (Davis, 2006) which results from either the relative impairment in pancreatic insulin secretion or varying degrees of peripheral resistance to the action of insulin, or both (Obaid and Turtle, 2004). It is accompanied by many serious and long-term complications including hypertension, hyperlipidaemia, nephropathy and retinopathy (Jarrett *et al*., 1982; Wingard *et al*., 1983). Cirrhosis of the liver can develop in diabetics as a result of progressive fatty steatosis, pericentral hepatic fibrosis and at times, central hyaline sclerosis (Falchuk and Conlin, 1993). High blood pressure due to high cholesterol level with the attendant risk of coronary artery disease and dyslipidemia (increased serum triglyceride and decreased high-density lipoprotein cholesterol concentrations) is common in diabetic patients (Gordon *et al*., 1977; Nathan *et al*., 2005) and treatments include cholesterol lowering. Cholesterol and other fatty blood components often referred to as 'blood lipids' are divided into different fractions or components: Low Density Lipoprotein (LDL) and High density lipoprotein (HDL), cholesterol and triglycerides. High levels of LDL and low levels of HDL are associated with increased risk of coronary heart disease (CHD).

Mucuna pruriens L. (Family Leguminoseae) also known as velvet beans or Cowitch in English, Werepe in Yoruba; Karara in Hausa and Agbara in Ibo, is an annual climbing plant indigenous to tropical regions, especially Africa, India and the West Indies. It is one of the plants that have been shown to demonstrate hypoglycaemic activity in normal and diabetic rats (Dhawan *et al*., 1980; Akhtar *et al*., 1990; Rathi *et al*., 2002; Bhaskar *et al*., 2008;

Corresponding Author: Oluwatoyin A. Odeku, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria Tel: +2348057320466

Majekodunmi *et al*., 2011). In a recent study, the acute and chronic oral administration of the ethanolic seed extract of *M. pruriens* has been shown to result in a dose dependent reduction in the blood glucose level in alloxan-induced diabetic rats comparable with the effects of glibenclamide (5 mg kg^{-1} body weight, b.wt.) (Majekodunmi *et al*., 2011). Administration of the extracts to diabetic rats also led to a significant ($p<0.001$) reduction in the weight loss often associated with diabetes indicating its usefulness as an antidiabetic agent comparable with standard drugs. However, the ameliorative effects of *M. pruriens* extract in alloxan-induced biochemical alteration has not be investigated. Thus in the present study, the ameliorative effects of *M. pruriens* seed extract in alloxan-induced biochemical alterations in rats have been investigated and compared with the effect of standard hypoglycaemic agent, glibenclamide. The effects of the extract on the liver of the diabetic rats were also investigated.

MATERIALS AND METHODS

Materials: Alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, M.O., USA) and glibenclamide tablet was obtained from the Nigerian-German Chemicals Plc (Ota, Ogun State, Nigeria). All reagents were of analytical grade. The collection and authentication of *Mucuna pruriens* seed and the extraction procedures have been given elsewhere (Majekodunmi *et al*., 2011).

Induction of diabetes: Male Wistar rats (180-240 g) obtained from the animal house of the Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Ibadan (Ibadan, Nigeria) were acclimatized for a period of 2-3 days before the start of the experiments. Diabetes was induced by the administration of single intraperitoneal injection of 100 mg kg^{-1} b.wt. of alloxan monohydrate (dissolved in 0.9% normal saline) after an overnight fast (Silva *et al*., 2002). Hyperglycaemia (blood glucose level of 450-500 mol dL^{-1}) was confirmed 72 h after alloxan injection and the animals were randomly distributed into groups of six rats each.

Administration of the doses: Group A served as control and received 10 mL kg^{-1} b.wt. of normal saline, Group B served as the diabetic control while Groups C, D, E, F, G, H and I were given 5, 10, 20, 30, 40, 50 and 100 mg kg^{-1} b.wt. respectively, of the ethanolic seed extract, administered orally for 12 weeks and group J received glibenclamide (5 mg kg^{-1} b.wt.) as standard reference drug.

Plasma biochemical parameters: After 12 weeks of daily oral administration of the extract or glibenclamide, the animals were fasted overnight and 3-5 mL of blood was collected through the retro-orbital venous plexus under light anaesthesia (ether). Blood was collected with the aid of heparinised capillary tube into lithium heparinised sample bottle and centrifuged at 3000 revolution min^{-1} (rpm) for 10 min. Clear plasma obtained was used for the analysis of plasma biochemical and lipid profiles. All the rats were thereafter sacrificed by cervical dislocation.

The following biochemical assays were carried out as described; serum alkaline phosphatase, ALP (Silva *et al*., 2002), serum aspartate aminotransferase, AST (Tietz and Shuey, 1986), serum alanine aminotransferase, ALT (Bergmeyer *et al*., 1985), albumin (Klauke *et al*., 1988), total bilirubin and direct bilirubin, D. Bil (Schlebusch *et al*., 1995). Gamma-glutamyl transferase (γ-GT) in the plasma was estimated using L-γ-glutamyl-3-carboxyl-4-nitroanilide and glycylglycine as substrates (Szasz, 1974). Total protein was determined in the plasma by the method of Lowry *et al*. (1951). Total Plasma triglycerides were assayed by the peroxidase-coupled method (Bucolo and David, 1973), total plasma cholesterol was measured by the enzymatic method (Allain *et al*., 1974). High Density Lipoproteins (HDL) cholesterol was determined after precipitation of very Low-Density Lipoproteins (LDL), Very Low-Density Lipoproteins (VLDL) and chylomicrons using magnesium chloride and dextran sulphate while low density lipoprotein-cholesterol concentration was calculated from the above data using the Friedewald formula (Friedewald *et al*., 1972). Plasma Sodium (N^+), Potassium (K^+) and Phosphate (PO_4^{2-}) ions were determined by flame photometry and the concentration of K^+ was calculated using the standard calibration method (Kolthoff and Elving, 1976). Bicarbonate, HCO_3^- and Chloride, Cl^- anions were measured as described by Van Slyke and Aulle (1971) and Schales and Schales (1971), respectively. The Blood Urea Nitrogen (BUN) and creatinine was estimated as described by Harrison (1947).

Histopathological evaluation: Small pieces of liver tissues were collected in 10% formal saline buffer for proper fixation. These tissues were processed and embedded in paraffin wax block and sections of 5-6 μm in thickness were cut and stained with haematoxylin and eosin for histopathological examination (Luna, 1968). The slides were examined microscopically for morphological changes.

Statistical analysis: All values were expressed as Mean±S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. Differences of means were considered significant at $p<0.05$.

RESULTS

Liver function has been assessed by biochemical parameters such as Alkaline Phosphatase (ALP), release

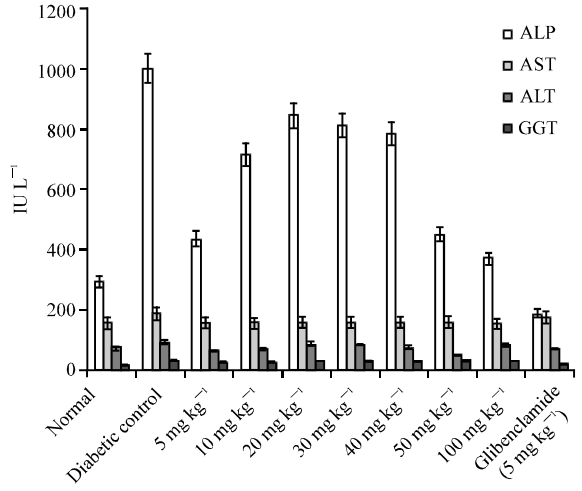

Fig. 1: Effect of ethanolic seed extract of *Mucuna pruriens* (MP) on the liver function tests Alkaling phosphatase (ALP), Aspartame aminotransferse (ALT), Alanine aminotransferse (AST) and Gamma glutt amyltransferse (GGT) (Mean±SEM, n = 6)

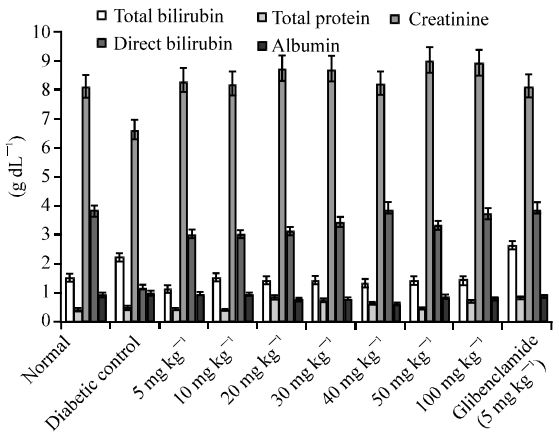

Fig. 2: Effect of ethanolic seed extract of *Mucuna pruriens* on the bilirubin, protein, albumin and creatinine levels (Mean±SEM, n = 6)

of aspertate aminotranferase (AST), alanine aminotranferase (ALT) and gamma-glutamyltransferase (GGT) (Sherlock and Dooley, 1993). The result of the liver function test shown in Fig. 1 indicates that ASL, AST, ALT and GGT were significantly ($p<0.001$) higher in the diabetic rats compared with the control rats. Administration of *M. pruriens* extract or glibenclamide led to a dose dependent reduction in the parameters to near normal levels. The result of the bilirubin, protein, albumin and creatinine tests shown in Fig. 2 indicates that diabetes induced significant ($p<0.05$) decrease in the total protein and albumin levels and significant ($p<0.05$)

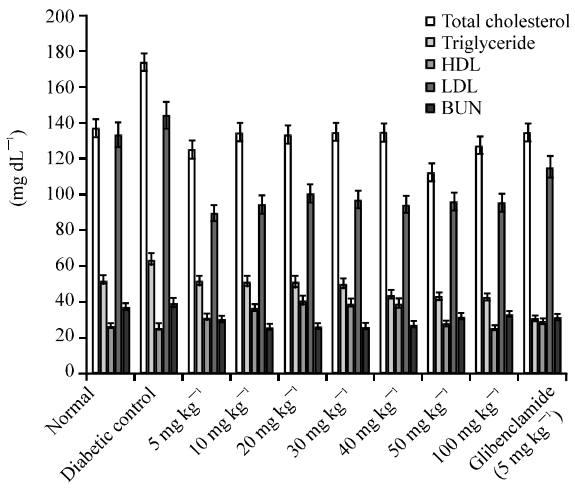

Fig. 3: Effect of ethanolic seed extract of *Mucuna pruriens* on the plasma total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) (Mean±SEM, n = 6)

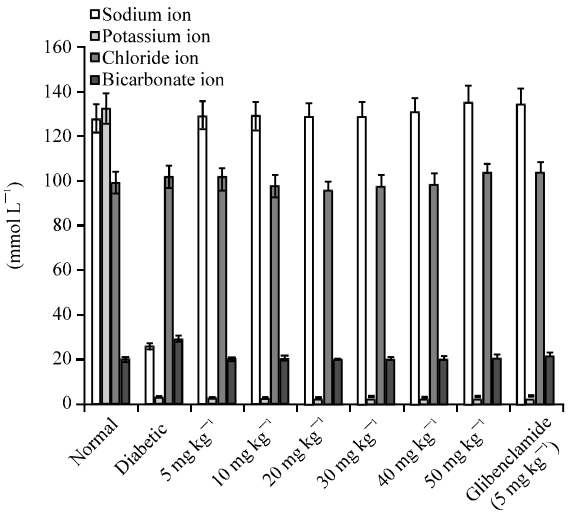

Fig. 4: Effect of ethanolic seed extract of *Mucuna pruriens* on the plasma electrolytes of sodium ion (N^+), potasium ion (K^+), chloride ion and bicarbonate (Mean±SEM, n = 6)

increase in the total bilirubin and creatinine levels. Administration of *M. pruriens* extracts restored the parameters to normal level in a similar manner to glibenclamide The results of the plasma lipid and metabolites profiles are presented in Fig. 3. The results showed that alloxan induced diabetic rats showed significantly ($p<0.05$) high levels of plasma cholesterol, triglycerides, low density lipoprotein, LDL and blood urea nitrogen (BUN), with concomitant decrease in high density lipoprotein (HDL) when compared to the

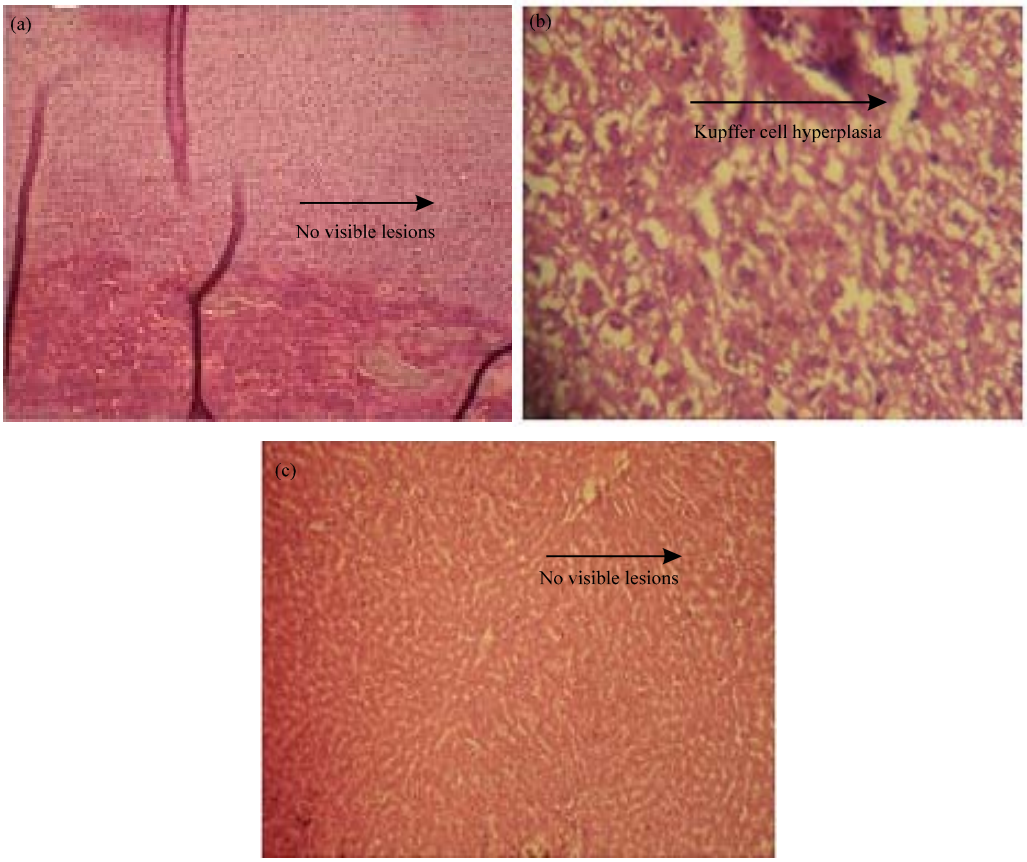

Fig. 5(a-c): Histopathology of the section of the liver of rats, (a) Norml control, (b) Diabetic control and (c) Treted with *M. pruriens* extract

control animals. Oral administration of *M. pruriens* extract or glibenclamide for 12 weeks significantly ($p<0.05$) decreased the plasma cholesterol, triglycerides, LDL and urea levels compared to the diabetic rats while the HDL increased significantly ($p<0.05$). The effect of *M. pruriens* extract was found to be dose dependent. There were generally no significantly ($p>0.05$) difference in the effects of the extracts compared with glibenclamide especially at high doses (1g kg^{-1} b.wt.).

The result of the plasma electrolytes levels shown in Fig. 4 also showed significant ($p<0.001$) decrease in plasma Na^+ level and significant ($p<0.05$) increase in HCO_3^- level in the diabetic rats while the difference was not significant for K^+ and Cl^- ions. Administration of *M. pruriens* seeds extracts showed a dose dependent amelioration of the depletion of the electrolytes in a similar manner to glibenclamide.

The histopathological changes that occurred in the liver of the control (A), diabetic (B) and treated (C) rats are presented in Fig. 5. The results showed that after 12 weeks of treatment, no changes were detected in the liver of the normal rats (A) compared with rats on the diabetic rats (B) where hyperplasia of the kupffer and central venous congestion were observed. However, there were no visible lesions for the extract and glibenclamide treated animal. This result confirmed the result of the biochemical analysis.

DISCUSSION

The major functions of the liver are detoxification of bilirubin, epimerization of galactose to glucose as uridine-5-phosphate derivatives, synthesis of protein (albumin) and prothrombin, handling of enzymes, such as alkaline phosphatase (ALP), release of aspertate aminotranferase (AST), alanine aminotranferase (ALT) and gamma-glutamyltransferase (GGT) (Sherlock and Dooley, 1993). Hence, liver damage has been assessed by significant increase in the serum levels of ALT, AST, ALP and total bilirubin levels (Sallie *et al*., 1991). Elevated levels of these enzyme in the blood has been ascribed to

damaged structural integrity of the liver and are indicative of cellular leakage and loss of functional integrity of the cell membrane (Rajesh and Latha, 2004). Their estimations are useful quantitative marker for the extent of hepatocellular damage (Kumar *et al*., 2004). Alkaline phosphatase mainly arises from the lining of canaliculi in hepatocytes and also brush border of the renal tubule. It is excreted normally via bile through liver and involves an active transport across the capillary wall. Increased activity of alkaline phosphatasewhich occurs due to de novo synthesis by liver cells, is a reliable marker of hepatobiliary dysfunction due to damage in liver and kidney. The determination of serum GGT activity is a well-established diagnostic test for hepatobiliary disease and is used as a sensitive marker of alcohol consumption and abuse (Rollason *et al*., 1972).

The results showed that the diabetic rats exhibited significantly high levels of ALT, AST ALP and GGT. This is indicative of liver damage induced by diabetes. However, administration of *M. pruriens* or glibenclamide induced a significant reduction in these parameter after 14 weeks of daily administration. The administration of the extract was found to be dose dependent, with the values obtained for the administration of 100 mg kg^{-1} b.wt. of *M. pruriens* extract not being significantly ($p>0.05$) different from the values obtained for glibenclamide. The decrease in the plasma enzymes suggests the ameliorative effects of *M. pruriens* extract on the liver against alloxan-induced hepatotoxicity in a similar manner to glibenclamide.

Bilirubin has been shown to increase in the blood because of regurgitation of bile due to obstruction within the liver by the damage or inflammation caused by diabetes (Schaefer *et al*., 2002) and the regurgitation of bile has been shown to result in the increase of ALP activity (Stocker *et al*., 1987). Increase in the levels of plasma bilirubin is usually reflected by the depth of jaundice (Saraswat *et al*., 1993). The results showed that bilirubin which was significantly high in the diabetic rats was restored to normal levels by the administration of the extracts. Yamini and Anil (2001) reported that *M. pruriens* possessed antioxidant property. The effects of *M. pruriens* extract on diabetic rats could be due to the antioxidant property of *M. pruriens* in trapping the superoxide radical. Although, free radicals are considered to be important for normal physiology, when produced in excess, they cause cellular damage. Hence, it is possible that the mechanism of hepato-protection of *M. pruriens* may be due to its antioxidant effect.

The protein and albumin levels which were significantly reduced by the induction of diabetes was restored to normal values by the administration of *M. pruriens* which suggests the stabilization of the endoplasmic reticulum required for protein synthesis (Patel *et al*., 2011). The effects of *M. pruriens* was found to be dose dependent and comparable with those of glibenclamide. The synthesis of total protein and albumin has been shown to accelerate the regeneration process and the protection of liver cells.

The BUN and creatinine levels increase significantly ($p<0.05$) with the induction of diabetes using alloxan. High levels of these parameters have been said to be indicative of injury to the nephrons (kidney cells) (Oyagbemi and Odetola, 2010). These parameters decrease in a dose dependent manner with the administration of *M. pruriens* comparable with the effects of glibenclamide. The observable decrease in plasma urea and creatinine following administration of *M. pruriens* extract confirmed that *M. pruriens* could ameliorate kidney damage which is one of the complications of diabetic mellitus indicating the nephroprotective effect of the plant extract.

Diabetes mellitus is characterized by a marked increased risk of cardiovascular disease due to the often elevated plasma triglycerides, cholesterol and LDL, as well as decreased HDL compared to the control subjects (Nicholls *et al*., 2005; Hardman and Limbard, 2001). Furthermore, insulin deficiency has been known to stimulate lipolysis in the adipose tissue and give rise to hyperlipidemia and fatty liver. Thus, in diabetes, hypercholesterolemia and hypertriglyceridaemia often occur (Barter *et al*., 2004). The results showed that the *M. pruriens* seed extract produced significant reduction in LDL-cholesterol, total cholesterol and triglyceride with appreciable increase in HDL-cholesterol. Studies have shown that the plasma concentration of High-Density Lipoproteins (HDL) has been reported to be inversely related to the risk of cardiovascular disease (Hardman and Limbard, 2001) although the mechanisms of the protective actions of HDL are not fully understood. However, there is mounting evidence that HDL has other effects that are independent of the cholesterol transporting functions, including antioxidant, antiinflammatory and antithrombotic properties (Hardman and Limbard, 2001; Negre-Salvayre *et al*., 2006; Matz, 1994). *Mucuna pruriens* extract promote appreciable increase in HDL cholesterol and could therefore, ameliorate cardiovascular complications associated with diabetes.

Diabetic ketoacidosis (DKA) and hyperosmolar hyperglycemia (HHS) are the two most serious acute metabolic complications occurring in both type I and type II diabetes, responsible for 5-15% mortality (Wingard *et al*., 1983). Both DKA and HHS are associated with glycosuria, leading to osmotic diuresis, with loss of water, sodium, potassium and other electrolytes (Carroll and Matz, 1983). The result showed the significant loss in plasma sodium in the dibaetic rats

compared to the normal rats. The plasma electrolytes levels were restored to normal levels by the administration of *M. pruriens* seeds extracts in dose dependent manner similar to the effects of glibenclamide. Thus, *M. pruriens* seeds extracts could be useful in the melioration of the depletion in electrolyte levels in diabetic rats and restored it to normal levels and could therefore be used to ameliorate complications associated with diabetes mellitus.

The results also showed that histological changes occurred in the liver of the diabetic rats where hyperplasia of the kupffer and central venous congestion were observed whereas no changes were detected in the liver of the normal rats and the extract or glibenclamide treated animal. These results confirm the result of the biochemical analysis which indicates tht diabetes induced liver damage while the damage ws ameliorated by the administration of the extracts to the diabetic animals.

CONCLUSION

The results showed that ethanolic seed extract of *Mucuna pruriens* has both hepato-protective and lypogenic effects in alloxan induced diabetic rats and could be substituted for standard oral hypoglycaemic agent (glibenclamide). *Mucuna pruriens* extract in addition to possessing hypoglycemic properties could be used to ameliorte the biochemical alterations induced by diabetes.

REFERENCES

Akhtar, M.S., A.Q. Qureshi and J. Iqbal, 1990. Antidiabetic evaluation of *Mucuna pruriens*, Linn. seeds. J. Pak. Med. Assoc., 40: 147-150.

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Barter, P.J., S. Nicholls, K.A. Rye, G.M. Anantharamaiah, M. Navab and A.M. Fogelman, 2004. Antiinflammatory properties of HDL. Circ. Res., 95: 764-772.

Bergmeyer, H.U., M.R. Horder and R. Rej, 1985. Approved recommendation of IFCC methods for the measurement of catalytic concentration of enzymes part 3. IFCC method for alanine aminotransferase. J. Clin. Chem. Clin. Biochem., 24: 418-489.

Bhaskar, A., V.G. Vidhya and M. Ramya, 2008. Hypoglycemic effect of *Mucuna pruriens* seed extract on normal and streptozotocin-diabetic rats. Fitoterapia, 79: 539-543.

Bucolo, G. and H. David, 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., 19: 476-482.

Carroll, P. and R. Matz, 1983. Uncontrolled diabetes mellitus in adults: Experience in treating diabetic ketoacidosis and hyperosmolar nonketotic coma with low-dose insulin and a uniform treatment regimen. Diabetes Care, 6: 579-585.

Davi, G., A. Falco and C. Patrono, 2005. Lipid peroxidation in diabetes mellitus. Antioxid. Redox Signal, 7: 256-268.

Davis, S.N., 2006. Insulin, Oral Hypoglycemic Agents and the Pharmacology of the Endocrine Pancreas. In: Godman and Gilman's. The Pharmacological Basis of the Therapeutics, Brunton, L.L., J.S. Lazo and K.L. Parker (Eds.). McGraw-Hill, New York, pp: 1613-1645.

Dhawan, B.N., M.P. Dubey, B.N. Mehrotra, R.P. Rastogi and J.S. Tandon, 1980. Screening of Indian plants for biological activity: Part IX. Indian J. Exp. Biol., 18: 594-606.

Falchuk, K.R. and D. Conlin, 1993. The intestinal and liver complications of diabetes mellitus. Adv. Int. Med., 38: 269-286.

Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Gordon, T., W.P. Castelli, M.C. Hjortland, W.B. Kannel and T.R. Dawber, 1977. High density lipoprotein as a protective factor against coronary heart disease: The framingham study. Am. J. Med., 62: 707-714.

Hardman, J.G. and L.E. Limbard, 2001. Insulin, Oral Hypoglycemic Agents and the Pharmacology of the Endocrine Pancreas. In: Goodman and Oilman's the Pharmacological Basis of Therapeutics, Hardman, J.G. and L.E. Limbird (Eds.). 10th Edn., McGraw-Hill, New York, pp: 1383-1399.

Harrison, G.A., 1947. Chemical Methods in Clinical Medicine. In: Hypochlorite-Induced Oxidation of Amino Acids, Peptides and Proteins, Hawkins, C.L., D.I. Pattison and M.J. Davies (Eds.). 3rd Edn. Churchill, London, pp: 259-274.

Jarrett, R.J., H. Keen and R. Chakrabarti, 1982. Diabetes, Hyperglycaemia and Arterial Disease. In: Complications of Diabetes, Keen, H. and R.J. Jarrett (Eds.). 2nd Edn., Arnold, London.

Klauke, R., E. Schmidt and K. Lorentz, 1988. Recommendations for carrying out standard ECCLS procedures for the catalytic concentrations of creakinase kinase. aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase at 37°C. Eur. J. Clin. Chem. Clin. Brochem., 31: 901-909.

Kolthoff, I.M. and P.J. Elving, 1976. Treatise on Analytical Chemistry. Part 1 and 2. Vol. 15, John Wiley and Sons, USA., pp: 15-100.

Kumar, G., G.S. Banu, P.V. Pappa, M. Sundararajan and M.R. Pandian, 2004. Hepatoprotective activity of *Trianthema portulacastrum* L. against paracetamol and thioacetamide intoxication in albino rats. J. Ethnopharmacol., 92: 37-40.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Luna, L.G., 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Edn., McGraw-Hill Book Company, Toronto and London.

Majekodunmi, S.O., A.A. Oyagbemi, S. Umukoro and O.A. Odeku, 2011. Evaluation of the antidiabetic properties of *Mucuna pruriens* seed extract. Asian Pacific. J. Trop. Med., 4: 632-636.

Matz, R., 1994. Hyperosmolar Nonacidotic Diabetes (HNAD). In: Diabetes Mellitus: Theory and Practice, Porte, D. Jr. and R.S. Sherwin (Eds.). 5th Edn., Elsevier, New York, pp: 845-860.

Nathan, D.M., P.A. Cleary, J.Y. Backlund, S.M. Genuth and J.M. Lachin *et al*., 2005. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N. Eng. J. Med., 353: 2643-2653.

Negre-Salvayre, A., N. Dousset, G. Ferretti, T. Bacchetti, G. Curatola and R. Salvayre, 2006. Antioxidant and cytoprotective properties of high-density lipoproteins in vascular cells. Free Radical Biol. Med., 41: 1031-1040.

Nicholls, S.J., K.A. Rye and P.J. Barter, 2005. High-density lipoproteins as therapeutic targets. Curr. Opin. Lipidol., 16: 345-349.

Obaid, M. and J.R. Turtle, 2004. Type 2 diabetes: An epidemic in the making. Am. Diab. Found, 5: 29-34.

Oyagbemi, A.A. and A.A. Odetola, 2010. Hepatoprotective effects of ethanolic extract of *Cnidoscolus aconitifolius* on paracetamol-induced hepatic damage in rats. Pak. J. Biol. Sci., 13: 164-169.

Panunti, B., A.A. Jawa and V.A. Fonseca, 2004. Mechanisms and therapeutic targets in type II diabetes mellitus. Drug Discovery Today: Dis. Mech., 1: 151-157.

Patel, K.N., G. Gupta, M. Goyal and B.P. Nagori, 2011. Assessment of hepatoprotective effect of *Tecomella undulate* on paracetamol-induced hepatotoxicity in rats. Braz. J. Pharm., 21: 133-138.

Rajesh, M.G. and M.S. Latha, 2004. Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. J. Ethnopharmacol., 91: 99-104.

Rathi, S.S., J.K. Grover and V. Vats, 2002. Anti hyperglycemic effects of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. Phytother. Res., 16: 236-243.

Rollason, J.G., G. Pincherle and D. Robinson, 1972. Serum gammaglutamyltranspeptidase in relation to alcohol consumption. Clin. Chim. Acta, 39: 75-80.

Sallie, R., J.M. Tredger and R. Williams, 1991. Drugs and the liver Part 1: Testing liver function. Biopharm. Drug Dispos., 12: 251-259.

Saraswat, B., P.K. Visen G.K. Patnaik and B.N. Dhawan, 1993. Anticholestic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurrooa*, against carbon tetrachloride induced cholestasis. Ind. J. Exp. Biol., 31: 316-318.

Schaefer, E.J., J.R. McNamara, P.K. Shah, K. Nakajima and L.A. Cupples *et al*., 2002. Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the framingham offspring study. Diabetes Care, 25: 989-994.

Schales, O. and S.S. Schales, 1971. Determination of chloride in Laboratory. J. Biol. Chem., 140: 879-879.

Schlebusch, H., C. Schneider and N. Liappis, 1995. Bilirubin determination in neonatal sera: Precision accuracy and sensitivity to hemoglobin interferences of six runtime methods. Clin. Chem., 6: 95-95.

Sherlock, S. and J. Dooley, 1993. Diseases of the Liver and Biliary System. 9th Edn., Blackwell Scientific Publication, USA.

Silva, F.R.M.B., B. Szpoganicz, M.G. Pizzolatti, M.A. Willrich and E. de Sousa, 2002. Acute effect of *Bauhinia forficata* on serum glucose levels in normal and alloxan-induced diabetic rats. J. Ethnopharmacol., 83: 33-37.

Stocker, R., Y. Yamamoto, A.F. McDonagh, A.N. Glazer and B.N. Ames, 1987. Bilirubin is an antioxidant of possible physiological importance. Science, 235: 1043-1046.

Szasz, K., 1974. Gamma Glutamyl Transpeptidase. In: Methods of Enzymatic Analysis, Bergmeye, H. (Eds.). 2nd Edn., Academic Press, New York, USA., Pages: 715.

Tietz, N.W. and D.F. Shuey, 1986. Reference intervals for alkaline phosphatase activity determined by the IFCC and AACC reference methods. Clin. Chem., 32: 1593-1594.

Van Slyke, W. and H.S. Aulle, 1971. Text Book of Clinical Chemistry. W.B. Saunders Co., Philadelphia, pp: 112-197.

Wingard, D.L., E. Barrdt-Conner, M.H. Criqui and L. Suarez, 1983. Clustering of heart disease risk factors in diabetic compared to nondiabetic adults. Am. J. Epidemiol., 117: 19-26.

Yamini, B.T. and K.U. Anil, 2001. Antioxidant property of *Mucuna pruriens* Linn. Curr. Sci., 80: 1377-1378.