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RESEARCH ARTICLE

BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES ON ALLOXAN INDUCED DIABETIC ALBINO RATS TREATED WITH LEAVES OF MORINDA TINCTORIA ROXB

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ABSTRACT

The aim of study is to analyze the biochemical and histopathological changes of oral administration of various formulations of *Morinda tinctoria* leaves on alloxan induced diabetic rats. Ethanol and Ethyl acetate extracts of leaves of *M.tinctoria*. on serum glucose, haemoglobin, glycosylated haemoglobin (HbA1c), plasma insulin, C-peptide and urea levels were assessed and the histological changes in the liver, pancreas and kidney tissues of control and experimental groups were examined. Oral administration of ethanol and ethyl acetate extracts of Mt. for 35 days significantly reduced the levels of serum glucose, HbA1c and urea while it increases the levels of plasma insulin, haemoglobin and C-peptides. Supplementation of ethyl acetate extract of *M.tinctoria* extract improves histopathological alterations in the liver, pancreas and kidney. Therefore, it could be used as a natural source for prevention or early treatment of diabetes mellitus.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia associated with impairment in insulin secretion and/or insulin action as well as aberrations in intermediary metabolism of carbohydrates, proteins and lipids. The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rise to around 439 million (7.7%) by 2030 (Wild *et al.,* 2004). Diabetes mellitus may present with classical characteristic features such as blurring of vision, excessive thirst (polydypsia), excessive feeding (polyphagia), excessive urination (polyuria), and weight loss. In its most severe forms, ketoacidosis may develop leading to stupor, coma and, in absence of effective treatment death ensues. There are two main types of diabetes mellitus: i. Type 1 diabetes, also called Insulin Dependent Diabetes Mellitus (IDDM), is caused by lack of insulin secretion by beta cells of the pancreas. ii. Type 2 diabetes, also called Non-Insulin Dependent Diabetes Mellitus (NIDDM) is caused by decreased sensitivity of target tissues to insulin. In both types of diabetes mellitus, metabolism of all the main foodstuffs is altered. The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body (**ADA., 2001**).

Type 1 diabetes (β -cell destruction, usually leading to absolute insulin deficiency) or Immune-mediated diabetes which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At the stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac spruce, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

Type 2 diabetes (ranging from predominantly insulin resistance with relative insulin deficiency or secretary defect) which accounts for 90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes or adult diabetes, encompasses individuals who have insulin resistance and

usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional may have an increased percentage of body fat distributed predominantly in the abdominal region. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal (**Blood, 1975**).

Generally two groups of drugs are commonly used in the treatment of diabetes: insulin and its preparation and Oral Hypoglycemic Drugs (OHD). These OHD drugs are used to treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, liraglutide and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors. Currently, six classes of oral antidiabetic drugs are available, such as

1. Sulphonylureas – first generation (tolbutamide, chlorpropamide); second generation – (glibenclamide, glipizide, gliclazide, glimiperide)
2. Biguanides – metformin
3. Meglitinides – repaglinide, nateglinide
4. Thiazolidinediones – rosiglitazone, pioglitazone
5. Alpha glucosidase inhibitors – acarbose, miglitol
6. Dipeptidyl peptidase-4 (DPP-4) inhibitors – sitagliptin

Even there are lot of synthetic drugs available that have been used to control and treat diabetic patients only partial recovery results from this dreaded disease. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Several medicinal plants have been investigated for their beneficial use in different types of diabetes. Several phytonutrients have been identified from medicinal plants and this presents an exciting opportunity for the development of new types of therapeutics for diabetes mellitus. Most abundant phytonutrients present in medicinal plants are the alkaloids, terpenes, and phenolics. Phytomedicine has been used since ancient time in many parts of the world where access to modern medicine is limited. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. The treatment of diabetes with synthetic drugs in the developing countries is expensive due to poverty and lack of access to Medicare. Hence, phytotherapy has significant role to play in the developing countries compared to synthetic drugs because it is safe, less expensive and available as a gift of nature.

The study material *M. tinctoria* belongs to the Rubiaceae family that grows wild and is distributed throughout Southeast Asia. It is commercially known as Nunaa, indigenous to tropical countries and is considered as an important folklore medicine. The tribes of Australia have used the ripe fruits of *M. tinctoria* for the treatment of respiratory infections (**Wang *et al.*, 2002**). There is a great demand for its fruit juice in treatment of arthritis, cancer, gastric ulcer and other heart diseases (**Farine *et.al*., 1996**). Fruit extracts of *M.tinctoria* also inhibit glucose diffusion using an in vitro model of glucose absorption (Thirupathy kumaresan *et al*., 2014). Octoamic acid, Vitamin C, terpenoids such as scopoletin, flavones, glycosides, linoleic acid, anthraquinones, morindone, rubiadin and alizarin are the major components identified in the Nunaa plant (Harbone, 1998). Since there were no documented reports available on the hypoglycemic activity of *M. tinctoria* leaves either in experimental or clinical studies. So the present investigation was envisaged to evaluate its anti diabetic potential in alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Preparation and fractionation of plant extracts

The leaves of *M. tinctoria* were collected from the banks of Cauvery river at Government Arts College, Kumbakonam, Tamil Nadu, India. The plant was identified and authenticated by Dr. N. Ramakrishnan, Head, Department of Botany, Government Arts College, Kumbakonam, Tamil Nadu, India. Fresh leaves from the plant were washed and dried in air at room temperature. The shade dried leaves (1 kg) were ground in to a powder form, macerated and soaked with 95% ethanol for three days at room temperature. The extracts were filtered and the filtrates are subjected to evaporation under reduced pressure to remove the excess solvents using a rotary evaporator. The crude filtrate was then extracted successively with ethyl acetate; then the extracts were condensed separately to obtain powder to use further for biological activities. The yields obtained for each fraction with respect to the initial dry material were ethanol 0.36% and ethylacetate 0.30%. The available standard procedures were followed to carry out preliminary phytochemical screening.

Experimental Animal

Adult male albino rats weighing of 175 to 200 gm were procured from Haja Nursery Garden, Mayiladudhurai, Tamil Nadu. They were housed in standard cages and maintained on standard rat chow with water *ad libitum*. The rats were acclimatized for two weeks before the commencement of the experimentation in order to adapt the rats with the environmental conditions. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Induction of Diabetes

The animals were fasted over night and diabetes was induced by a single intra peritoneal injection of freshly prepared solution of alloxan monohydrate (120 mg/kg body weight dissolved in 0.9% saline). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Two days after alloxan injection, blood glucose content was measured by using glucose test meter one touch horizon (China make) using a blood sample obtained from the tail vein. Those animals, whose blood glucose level above

200mg was considered as diabetic for the present study. Treatment with *M. tinctoria* extract was started 48 hours after alloxan injection. Retro orbital venous blood samples were collected in the fasting state at specific intervals during 0, 7, 14, 21, 28 and 35 days were tested for presence of glucose concentration.

Experimental Design

The animals were randomly divided into five groups and each group consists of six animals and experiment was made for 35 days.

Group 1: Normal rats (Control)
Group 2: Diabetic Control (DC)
Group 3: Diabetic rats treated with 250mg/kg body weight of Ethanol fractions of *M. tinctoria* leaves (MtLEEt)
Group 4: Diabetic rats treated with 200 mg /kg body weight of Ethyl acetate fractions of *M. tinctoria* leaves (MtLEaEt)
Group 5: Diabetic rats administered orally with the reference drug Glibenclamide 5mg/kg body weight per day (DG)

Determination of biochemical assays

At the end of the experimental period, the rats were anaesthetized and sacrificed. Blood samples were collected through retro – orbital plexus puncture and stored in with or without disodium ethylene diamine tetra acetate depending on the respective biochemical parameter estimation. The levels of serum glucose, serum urea, haemoglobin, glycosylated haemoglobin, insulin and C-Peptide were estimated using the methods in the available literature (Sunil *et al*., 2011).

Histopathology analysis

The kidney, liver and pancreas were preserved in 10 % formalin immediately after removal from the animal.

Tissue processing

Liver, kidney and pancreatic tissues were placed in 10% formalin (diluted to 10% with normal saline) for 1 hr to rectify shrinkage due to high concentration of formalin. The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 hour. The dehydrated tissues were cleared in two changes of xylene, 1 hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were mounted and cut with rotary microtome at 5 µ thickness.

Tissue staining

The sections were deparaffinised by immersing in xylene for 10 min in horizontal staining jar. The deparaffinised sections were washed in 100% isopropyl alcohol and stained in Eosin and Hematoxylin for 8 min in horizontal staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (70% alcohol). The sections were then placed in running tap water for 10 min for bluing (slow alkalization). The sections were counter stained in 1% aqueous eosin (1 gm in 100 ml tap water) for 1 min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was ensured by placing the sections in the incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mount having the optical index of glass. The architecture was observed low power objective under microscope. The cell injury and other aspects were observed under high power dry objective (Dunn, 1974).

Statistical analysis

All the data expressed as Mean ± SEM of six animals in each group were evaluated by one-way analysis of variance (ANOVA), followed by least significant differences test. P-values less than 0.05 were considered as statistically significant.

RESULTS

Effect of M. tinctoria leaves on Serum glucose and Serum urea levels

In the present study, alloxan induced diabetic uncontrolled rats showed elevated levels of serum glucose (255.4 ± 1.35) and serum urea (58.2 ± 1.19) compared to serum glucose (92.0±4.3) and serum urea (35± 0.65) of normal rats at the end of 35 days. Oral administration of ethyl acetate extract leaves of *M. tinctoria* leaves and glibenclamide significantly decreased the serum glucose as 140.8 ± 1.38 and 134.2 ± 1.294. respectively while the serum urea was reduced as 38.8 ± 1.29 and 30 ± 0.79 respectively at the end of the 35th day (Table-1).

Table 1 Effect of *M. tinctoria* leaves on serum glucose and serum urea levels of control and experimental animals

Group	Serum glucose (mg/dL)		Serum urea (mg/dL)	
	0th day	35th day	0th day	35th day
Normal	88.0 ± 5.2	92.0 ± 4.3	30 ± 0.79	35± 0.65
Diabetic control	247 ± 1.03	255.4 ± 1.35*	55.4 ± 1.03	58.2 ± 1.19*
MtLEEt	245.4 ± 0.57	161.8 ± 1.09*	51.6 ± 1.09	56.6 ± 2.01*
MtLEaEt	236.4 ± 0.90	140.8 ± 1.38*	46 ± 0.79	38.8 ± 1.29*
DG	241.4 ± 1.15	134.2 ± 1.294*	40.6 ± 0.57	30 ± 0.79*

Each value is mean ± S.E for six rats in each group. Values are statistically significant at *p<0.05
Diabetic rats were compared with Control rats, while other treatment rats were compared with diabetic control.

Effect of M. tinctoria leaves on haemoglobin and glycosylated haemoglobin level

The decreased level of haemoglobin (6.6±0.63) and increased level of glycosylated haemoglobin (13.52±0.2) noticed in alloxan induced diabetic control rats (Table-2). Administration of both ethyl acetate and glibenclamide to diabetic rats restored the total haemoglobin and HbA1c to almost near normal control levels.

Table 2 Effect of *M. tinctoria* leaves on haemoglobin and glycosylated haemoglobin level of control and experimental animals

Group	Haemoglobin (g/dl)		HbA1c (%)	
	0th day	35th day	0th day	35th day
Normal	14.3 ± 1.72	14.4±1.74	5.94±0.14	6.08±0.13
Diabetic control	7.5 ± 0.87	6.6±0.63*	6.01±0.12	13.52±0.2*
MtLEEt	10.1 ± 0.41	10.56±0.5*	5.85±0.43	11.94±0.5*
MtLEaEt	11.2 ± 0.81	11.8±0.88*	5.53±0.27	7.08±0.28*
DG	11.8 ± 0.69	11.9±0.7*	6.02±0.13	7.96±0.22*

Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05
Diabetic rats were compared with control rats, while other treatment rats were compared with diabetic Control

Effect of M. tinctoria leaves on Insulin and C-peptide levels

Diabetic controlled rats shown very low level of Insulin and C-Peptide compared to normal control. Administration of Ethyl

acetate extracts of *M. tinctoria* for a period of 35 days significantly increased the level of insulin (12.8±0.19) and C-peptide (244.9±9.4) which was nearing that of the normal control group and glibenclamide treatment (Table-3).

Table 3 Effect of *M. tinctoria* leaves on Insulin and C-peptide levels of control and experimental animals

Group	Insulin (µU/mL)		C-Peptide	
	0th day	35 th day	0 th day	35 th day
Normal	15.97±0.24	15.99±0.25	263.07 ±10.4	265.17 ± 6.8
Diabetic control	9.72±0.13*	6.68±0.16*	65.8± 9.6*	44.6±10.8
MtLEEt	9.34±0.03*	11.97±0.70*	63.5± 7.4*	219.4±7.5*
MtLEaEt	9.57±0.21*	12.8±0.19*	58.8± 9.6*	244.9±9.4*
DG	9.14±0.28*	13.20±0.25*	66.9± 7.4*	237. 4±7.6*

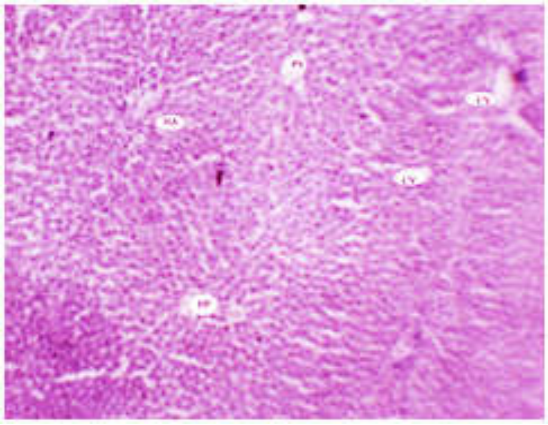
Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05
Diabetic rats were compared with control rats, while other treatment rats were compared with diabetic control rats.

Histopathological studies

Histopathology of kidney, liver and pancreas of normal, diabetic untreated and diabetic treated groups was assessed after 35 days of treatment.

Liver

Normal liver tissue section shows sinusoidal cards of hepatocytes with central vein and portal tracts. The portal tracts show the lympho plasmacytic infiltration of some, portal triad with portal vein, hepatic artery (**FIG-1a**). In diabetic uncontrolled rats, the hepatocytes were enlarged with vacuolar cytoplasm and hypertrophic nuclei. The hepatocyte form branching and anastomosing cards radiating from the central vein. The vesicular nuclei and some of them appeared binucleated. The cells appeared to separate by the blood sinusoids that were seen. The stroma displayed discrete disorganization with enlargement of the space between the hepatocyte plaques, sinusoidal dilation, and an infiltrative inflammatory process in the periportal region (**FIG-1b**). The diabetic rats treated with the ethyl acetate (200 mg/kg body weight) of *Morinda tinctoria* showed hepatocyte brought back the cellular vein and reduced necrosis. The portal tract, central vein and hepatic artery are back into normal structure, which are correlate with the reference drug glibenclamide treated group (**FIG-1c,d).**



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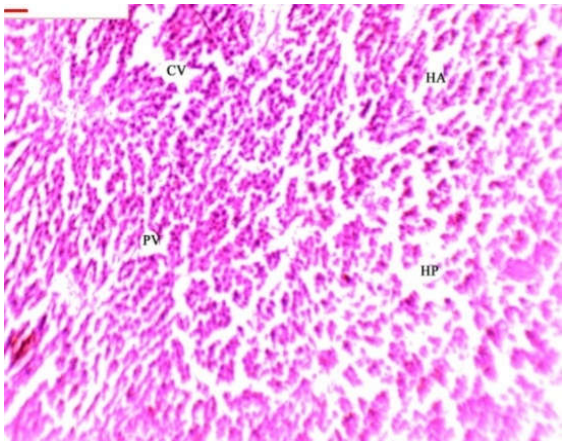

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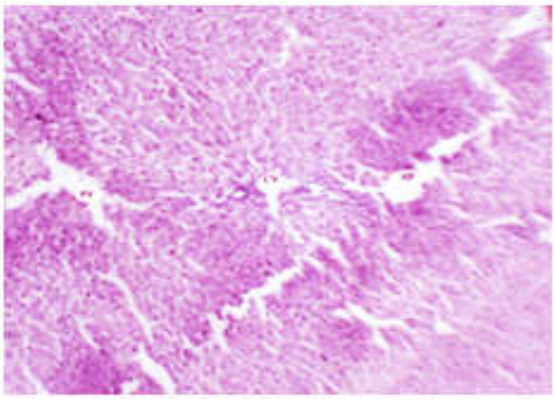

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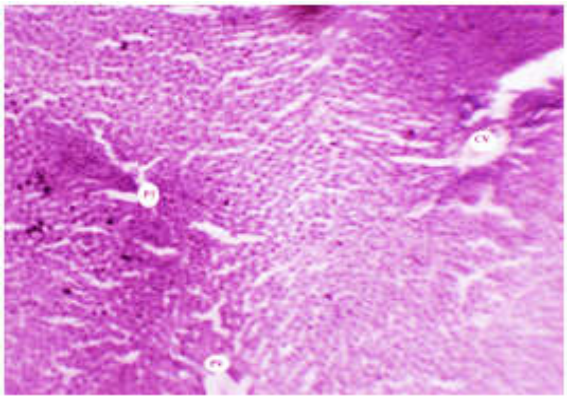

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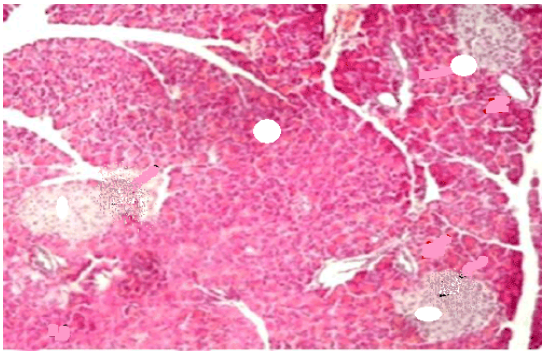
Figure 1 Effects of the *M. tinctoria* treatments on histopathological parameters of liver : **A**: The photomicrograph shows the normal architecture of liver, HE-stained, showing PT, PV, HP, CV 100X. **B**: The photomicrograph shows diabetic rat liver inflammatory process in the portal and vein and portal tract is enlarged stroma 100X. **C**: The diabetic kidney treated with ethyl acetate extract of *M. tinctoria* displaying improvement in the morphology of PT, PV. 100X. **D:** The photomicrograph shows diabetic liver treated with the glibenclamide. Both **C** and **D** indicated the better improvement like a normal architecture. PT-portal tract, PV-portal vein, HP-hepatocytes , CV- central vein.



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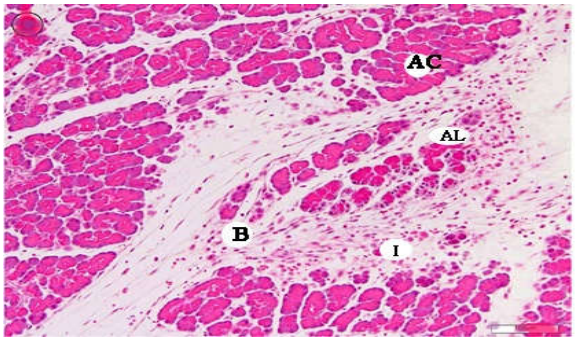

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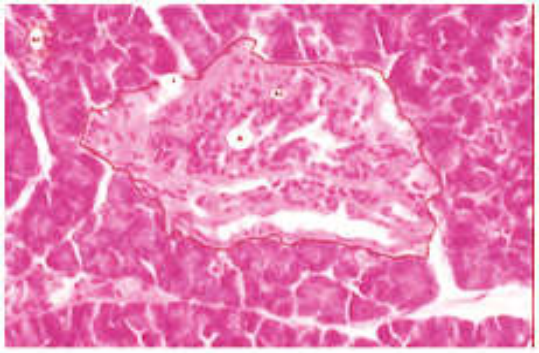

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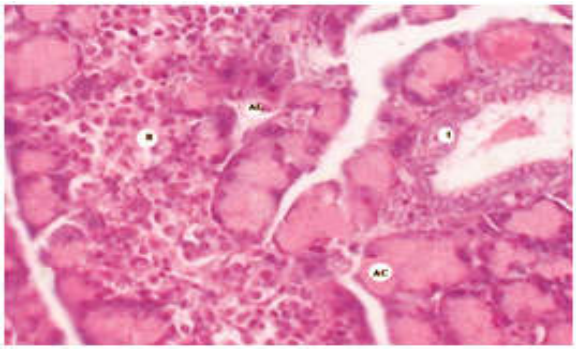


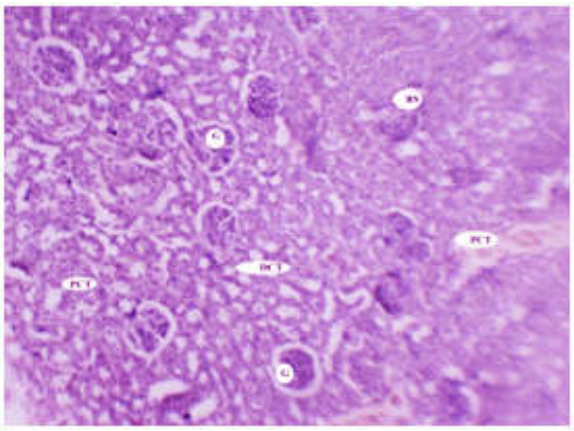
(D)

Figure 2 Photomicrographs showing effects of *M. tinctoria* treatments on histopathological parameters of pancreatic islets(100X). **A**: Photomicrograph of normal architecture pancreatic islet control group, islets cells (I), Acinar cells (AC), (Al) alpha cells, beta cells (B) are stained with HE. **B**: Photomicrograph of diabetic control group of pancreatic islets is contain low number of alpha cells (AL) and show the acinar cells are damaged. **C:** Diabetic group treated with ethyl acetate extract of *M. tinctoria* displaying increase the number of alpha and beta cells in the islets and the acinar cells are arranged in the normal architecture. **D**: Diabetic group treated with glibenclamide shows the islets back to normal architecture (400X).

The damage or necrosis of β cells was caused by the alloxan used to induce diabetes were more prominent (**FIG-2b**). Administration of ethyl acetate extract (200 mg/kg body weight) of *M. tinctoria* increases the recovery of necrotic β cells (**FIG-2c)**. The damaged β cells and acinis are seen after the initial induction of diabetes were no longer observed after treatment with *M. tinctoria* extract. These actions are correlate with the reference drug glibenclamide treated group (**FIG-2d).**

Kidney

Histological study of the normal kidney of the non diabetic rats revealed normal glomerulus surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes (**FIG-3a**). The kidneys of untreated diabetic rats showed degenerated glomeruli infiltrated by the inflammatory cells and thickening of the basement membrane. The proximal convoluted tubule exhibited edematous changes with deposition of mucopolysaccharide and hyaline substances. All the necrotic changes observed in the proximal and distal convoluted tubules along with the deposits were found (**FIG-3b**). The diabetic rats treated with the ethyl acetate extract (200 mg/kg body weight) of *M. tinctoria* showed salient features of healing i.e. normal glomerulus, absence of inflammatory cells, normal basement membrane and the capillary loops with increase in the thickness of the wall, decrease in the mucopolysaccharide and hyaline deposit, respectively. The grade of tissue necrosis was also observed very low degree in the group treated with ethyl acetate extracts of *Morinda tinctoria*. These results are correlate with the reference drug glibenclamide treated group organisms (**FIG-3c,d).**



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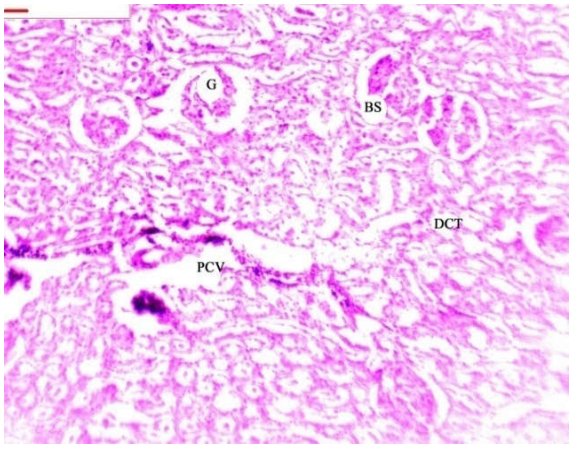

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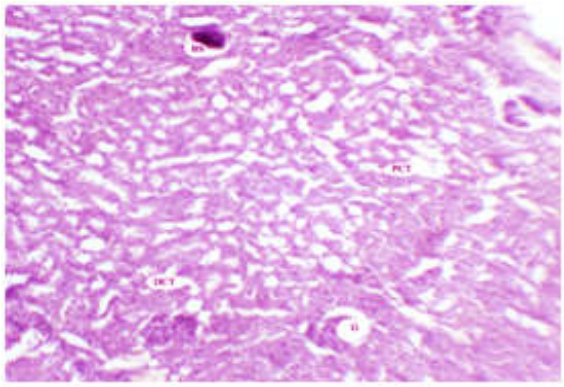

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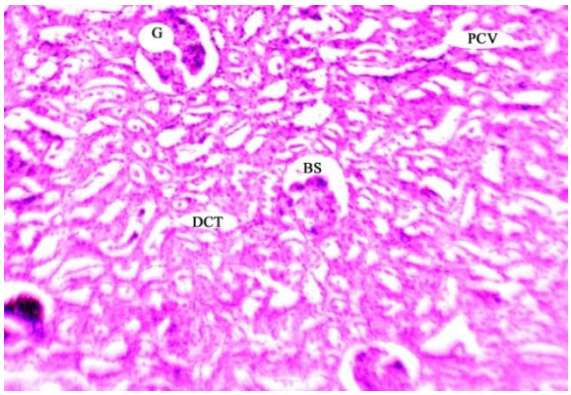

(D)

Figure 3 - Effects of the *M. tinctoria* on histopathological parameters kidneys **A**: the photomicrograph shows the normal architecture of kidney stained with the HE-stain (100 X). **B**: The kidney from diabetic animal with inflammatory process in the gomerulus membrane was denature and enlarged the (PCT and DCT) 100X. **C**: Kidney section indicate the untreated diabetic control group treated with ethyl acetate extract of *M. tinctoria* displaying normalization of altered glomerular morphology, and dilatation of tubules (PCT, DCT), 100X. **D**:The photomicrograph shows the kidney treated with glibenclamide which was similar to normal kidney (100 X).**PCT-** Proximal convoluted tubule, **DCT-** distal convoluted tubule, **G-** glomerulus, **BS-** bowman's capsule space.

DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. The alloxan induced hyperglycemia as an experimental model because it is one of the best model to study the effect of the antidiabetiogenic agent. Excessive usage of chemicals, such as derivatives, meglitinides and alpha glucoside inhibitors causes unwanted side effect. The efficacies of these compounds are debatable and there is a demand for new compounds with highest potential is in need for an hour. Hence plants have been suggested as a rich, as yet, unexplored source of potentially useful antidiabetic drugs. However only a few have been subjected to detailed scientific investigation due to lack of mechanism based available *in vitro* assays. Optimal pancreatic β-cell function is essential for the regulation of glucose homeostasis in both humans and animals and its impairment leads to the development of diabetes. Hence the present study was undertaken to investigate the biochemical and histopathological effect of ethanol and ethyl acetate fractions of *M. tinctoria* leaves on alloxan induced diabetic rats. Oral administration of ethanol and ethyl acetate extract of *M. tinctoria* leaf decreased serum glucose and serum urea level in diabetic rats. This hypoglycemic effect may be due to depression of key gluconeogenic enzymes or the increase in the levels of glucose transports and stimulation of uptake in peripheral tissues (Ji su kim *et al.,* 2008). Another effect of these plants extract may be that it preserve the cells of islets of langerhans of β-cells functions, which results in a significant increase in insulin activity (Palayan Muralidharan *et al.,* 2009). The possible mechanism of hypoglycemic action may be through potentiation of pancreatic secretion of insulin or due to enhanced transport of blood glucose to the peripheral tissues (Doda *et al.,* 1996).

The reduced Hb and elevated HbA1c levels were observed in diabetic rats than normal control rats which may be due to hyperglcemic condition. The decreased level of haemoglobin in diabetic rats is mainly due to the increased formation of HbA1C. During diabetes, the excess glucose present in the blood binds with haemoglobin to form HbA1C (Koening *et al*., 1976). The normalization of glycosylated haemoglobin was observed on ethyl acetate extract of *M. tinctoria* leaf treated rats. It indicates that there is decreased glycation of proteins which is similar to glibenclamide. The present study revealed that 200 mg/kg body weight of ethylacetate extract of *M. tinctoria* is more potent than 250 mg/kg body weight of ethanol to control hyperglycemia in diabetic rats. This points out that ethyl acetate fraction of *Morinda tinctoria* has got more soluble compounds which show remarkable improvement in maintaining serum glucose levels same like glibenclamide treatment.

Insulin and C-peptide are the products of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of both C-peptide and insulin levels have been reported to be a valuable indices of insulin secretion than insulin alone. In the present study, treatment with *M. tinctoria* extract showed significant increase in plasma insulin and C-peptide levels in diabetic rats. These results indirectly indicate that part of the antihyperglycaemic activity of this plant is through release of insulin from the pancreas. Perhaps the *M. tinctoria* extract treatment could play a critical role in repairing the damage of the pancreatic β-cells and promoting insulin synthesis. Diabetics rats administrated with ethanol and ethyl acetate extract, also improved the biochemical parameters, due to its non toxic nature and multivalent potentiality. The ethyl acetate extract exhibit significant antihyperglycemic activities in alloxan induced hyperglycemic rats without significant change in body weight. It also improved conditions of diabetic mellitus by lowering serum blood glucose level. The results are similar with preliminary studies on antihyperglycemic effect of ethyl acetate extract of *M. tinctoria* (Ramasubramanian *et al.,* 2015).

The administration of *M. tinctoria* ethyl acetate extract was more effective than ethanol to manage diabetes mellitus. The active ingredients present in the extract may helpful to cure the diabetic state by stimulating the existing β cells or by modulating intracellular glucose utilization. The results cited here are similar with *Eugunia jambolana* root of *Musa paradisiac* extract against STZ induced diabetic male albino rat carried out by Chanda *et al.,* 2006. These damage of pancreas has normalized in those animals treated ethyl acetate extract. Diabetes mellitus is the only disease in which both the afferent and efferent glomerular artery show hyaline thickening the deposition of hyaline material in the mesangium of the lobules of the glomerulus. It may be deposited or diffused and more or less evenly throughout the glomerulus or unevenly as one or more nodules. The two type of lesion are

often present together, as in this case there is diffused infiltration of the glomerular tuft with eosnophilic material and also heavy focal deposition. The diffused infiltrate appears to be in the basement membrane of proximal and distal convoluted tubules. It is well established that in severe diabetes, a catabolic response develops in tissues, such as the liver muscle and adipose tissue, with the prevalence of more catabolic over anabolic processes. However, in other tissue such as kidney the reverse may be true. The diabetic kidney is characterized by some metabolic alteration that entail enhanced protein synthesis. In the kidneys of animals with the experimental diabetes, the protein synthesis has been reported to be significantly elevated. The results are similar with fenugreek treatment against albino rat, were carried out by Naveen helmyabou *et al.,* 2007.

The availability of the HbA1c test has been a major advantage in diabetic care and its measurement has become an integral part for the management of diabetes. The histological examination of liver of diabetes rats showed periportal necrosis of the hepatocyte near the portal vessels as well as areas of inflammatory cell infiltration and pericentral glycogen depletion. Such kinds of deformities are rapidly cured up by the ethyl acetate extract of *M. tinctoria* than the ethanol extract.

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