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Melatonin and Magnesium Suppresses Diabetic Kidney Disease in Streptozotocin-induced Diabetic rats.

[1]Godam ET, [2]Hamman WO, [3]Oladele S, [4]Samaila MO, [1]Ordu KS and [1]Jacks TW.

[1]Neuroscience and Molecular and cell Biology Unit, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria.

[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University Zaria.

[3]Department of veterinary Pathology, Faculty of Veterinary Medicine Ahmadu Bello University, Zaria

[4]Department of Pathology, Faculty of Basic Clinical Sciences, Ahmadu Bello University Zaria

Corresponding Author. Godam ET
Email: elvis.godam@ust.edu.ng; +2348063339602

ABSTRACT

Diabetic kidney disease accounts for more than 40% of all end stage kidney disease (ESKD) in the United States and the world at large. The aim of this study was to evaluate the histological and biochemical effects of magnesium and melatonin on the kidney of streptozotocin induced diabetic rats. To achieve this aim fifty-four Wistar rats were used in the study. Streptozotocin (STZ) was used to induce chemical type 1 diabetes mellitus (T1DM) after two weeks acclimatization period. Forty-eight diabetic rats were randomly distributed in eight groups and six normal normoglycaemic rats were used as control. The animals were assigned into nine groups as follows, Normal control group (NC), Diabetic control (DC) group, Melatonin Low dose group of 10 mg/kgbw (MLD), magnesium low dose group of 240 mg/kgbw (MgLD), melatonin and magnesium combined low dose group of 10mg/kgbw+240mg/kgbw (MMgLD), melatonin high dose group of 20mg/kgbw (MHD), magnesium high dose group of 480mg/kgbw (MgHD), melatonin and magnesium high dose combined group of 20mg/kgbw+480mg/kgbw (MMgHD) and insulin at 500mg/kgbw group (IN). Melatonin and insulin were administered through intraperitoneal injections (IP) while magnesium was by oral administration. The control groups were given placebo and all group treatment was for twenty-one days. The kidney tissues were evaluated for nephropathy using H&E and Massons trichrome stain. Histopathological results showed that melatonin given at low dose, high dose and when combined with low dose magnesium suppresses histopathological features of Diabetic kidney disease in streptozotocin-induced diabetic rats.

KEY WORDS. Diabetes, Streptozotocin, Melatonin. Magnesium, Nephropathy, Glomerulopathy, Kidney.

INTRODUCTION

Diabetes is the second most common cause of kidney failure in the United States. Diabetic Kidney Disease (DKD) embraces structural and functional abnormalities involving the kidneys. Clinically, these changes result in hypertension, proteinuria, and progressive decline in kidney function, ultimately leading to end stage kidney disease (ESKD). Diabetic kidney disease accounts for more than 40% of all ESKD in the United States, [1,2].

Kidney disease has been described as the most neglected chronic disease in the world, [3]. Diabetic glomerular hypertrophy constitutes an early event in the progression of glomerular pathology which occurs in the absence of mesangial expansion, [4]. Although, the mechanism of renal hypertrophy is unknown, evidence suggest that local alterations in the production of one or more growth factors and or their receptors are crucial to this process. [5,] proposed that development of renal hypertrophy in insulin dependent diabetes mellitus (IDDM) was associated with over expression of transforming growth factor (TGF) – beta 1 in the kidney especially in proximal convoluted tubules (PCT) cells and glomerular mesengial cells. [6] reported that renal hypertrophy, increased glomerular volume, mesangial proliferation and accumulation of glomerular extracellular matrix were due to growth hormone (GH) And insulin like growth factors (IGFs) through a complex System consisting of growth hormone binding proteins (GHBP), insulin like growth factors (IGFS), insulin like Growth factors receptors (IGF-receptors) and insulin like Growth factor binding proteins (IGFBP). [7] attributed the renal hypertrophy and hyperplasia to increased epidermal growth factor (EGF). [8] reported that diabetic renal hypertrophy might be due to HGF (hepatocyte growth factor) and c-met proto-oncogene product, a tyrosine kinase receptor for HGF. Possible Mechanism for renal enlargement could be the direct effect of growth hormone (GF) and insulin like growth

factor (IGF)-1, [9]. [10] investigated the mechanism of renal hypertrophy.
They proposed that renal hypertrophy might be due to an increase in the rate of protein synthesis and decrease in the degradation of renal extracellular matrix components which occur early after induction of experimental diabetes before the onset of typical structural changes in the kidneys. Accelerated renal protein turnover and hypertrophy are early manifestations and perhaps harbingers of more severe renal changes in diabetes. All cell types in the glomerulus as well as proximal and distal tubule cells appear to be involved. [11] proposed that during diabetic renal hypertrophy, the cellular autophagy is inhibited in distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) cells, suggesting that both type of cells contributed in a balanced manner to the hypertrophic growth of kidney cortex. [12] proposed that increase in kidney weight is associated with the increase in renal expression of angiogenic factors such as vascular endothelial growth factor. Prevention of diabetic complications, particularly DKD, by long-term intensive glycemic control from early in the course of diabetes is well established for type 1 and type 2 diabetes mellitus, [13], However, intensive glucose control after onset of complications or in longstanding diabetes has not been shown to reduce risk of DKD progression or improve overall clinical outcomes, [14]. Despite current approaches to management of diabetes and hypertension and the use of ACE inhibitors and ARB, there is still large residual risk in DKD. Novel agents targeting mechanisms, such as glomerular hyper filtration, inflammation, and fibrosis, have been a major focus for development of new treatments, [15]. Hence our present experimental study uses melatonin a potent antioxidant and magnesium a bioactive element essential to all cellular life to ameliorate toxicological changes to the kidney caused by streptozotocin-induced diabetes mellitus in rats.

Melatonin is a circulating neuro-hormone secreted predominantly at night. It is important in conveying the daily cycle of light and darkness to the body, thus regulating circadian rhythms. In addition to its' regulatory role, melatonin has antioxidative capacity, immunomodulatory potency, and also appears to be protective against certain types of cancers, [16]. Type 1 diabetes mellitus is a T cell-mediated autoimmune disease characterized by excess inflammation, independent of adiposity and glycemic control. Melatonin has also been demonstrated to have hypoglycaemic effect, pancreatic Beta cells regeneration and up regulation of intracellular antioxidant (GPx, SOD and CAT) and reduction in intracellular free radicals generated Malondialdehyde (MDA) in Streptozotocin (STZ) induced diabetes in Wistar rats, [17].

Magnesium (Mg) is an electrolyte of chief physiological importance in the body, being the most abundant divalent intracellular cation in the cells, the second most abundant cellular ion next to potassium and the fourth cation in general in the human body, [18]. Type 2 diabetes mellitus (T2DM) is often accompanied by alteration of Mg status. An increased prevalence of Mg deficits has been identified in diabetes mellitus patients, especially in those with poorly controlled glycemic profiles, with longer duration of the disease and with the presence of micro and macrovascular chronic complication, [19].

MATERIALS AND METHODS

The following materials were used in the study, Plastic Cages, Spectrophotometer auto analyzer, blood sample containers, organ sample containers, Centrifuge, Temperature controlled refrigerator, Microwave oven, water bath, humidity chamber, Leica Auto processor, Leica Auto stainer, Leica DM750, Camera ICC50 E, AmScope D200 digital camera, MRC, Microwave oven, spectrophotometer.

Bioactive compounds and drugs used in the study were: Melatonin M5250-1G (Sigma Aldrich, USA), Magnesium (Randox, USA) Streptozotocin SP0130 (Sigma Aldrich, USA), Haematoxylin and Eosin Stain (H&E), Masson trichrome stains, insulin (Novo Nordisk, Switzerland) were used in the study.

Source of animals and management: Sixty-four Male Wistar rats weighing of 120–150 g, were purchased from the Faculty of Pharmaceutical Sciences Animal House of the Ahmadu Bello University, Zaria and selected for the study. The rats were maintained on a day and night cycle at room temperatures and have *ad libitum* access to food (Standard feeds, standard rat pellets) and water. All experiments were performed between 08:00 and 12 hours.

Induction of Type 1 Diabetes Mellitus: Type 1 diabetes mellitus (T1DM) was induced after 2 weeks acclimatization period, a baseline blood glucose levels and behavioral and cognitive assessment were performed for all test animals. This was done to ensure that the animals were all normoglycaemic and that they all exhibit normal cognitive function to remove bias using the elevated plus maze (EPM). Fifty-Eight male Wistar rats were randomly selected and given a single dose of intra peritoneal injection of streptozotocin, (STZ) (Sigma, Aldrich, USA), at 55mg/kg body weight in citrate buffer (0.1M, pH 4.5). The solution (STZ in citrate buffer) was used within 5 minutes to induce chemical diabetes in the Wistar rats after an overnight fast of twelve hours, [20]. The Wistar rats were randomly divided into the following groups of 6 rats each in the table below.

Hyper glycaemia screening and confirmation of T1DM: Four days after streptozotocin was used to induce diabetes mellitus, blood was collected from the tail vein following an overnight fast [20, 21]. Fasting blood sugar (FBS) was measured with a standard glucometer

(Optimum, Germany). The day that hyperglycaemia above 200mg/dl (11 mmol/l) was confirmed was considered to be diabetic day 1. Rats with fasting blood glucose levels lower than 200 mg/dL (11mmol/L) were excluded from the study.

Animal grouping and treatment procedure: Forty eight diabetic rats and 6 normoglycaemic rats were randomly divided into nine groups are shown in the table 1.0 below.

Table 1: Animal grouping and treatment protocol

GROUPS	Treatment
NC	Normal control + Normal saline (orally)
DC	Diabetic control + Normal saline (orally)
M	Diabetic + Melatonin (10mg/kgbw), (IP)
Mg	Diabetic + Magnesium (240mg/kgbw), (Orally)
MMgLD	Diabetic+Melatonin (10mg/kgbw) (IP) + Magnesium (240mg/kgbw), (orally)
MHD	Diabetic+Melatonin(20mg/kgbw) (IP)
MgHD	Diabetic+ Magnesium (480mg/kgbw) (orally))
MMgHD	Diabetic + Melatonin(20mg/kg bw) (IP) + Magnesium (480mg/kgbw), (orally)
IN	Diabetic + Insulin (500mg/kgbw), (IP)

NC (control), DC (Diabetic control), M (Melatonin), MG (Magnesium), MMGLD (Melatonin and magnesium low dose), MGHD (Magnesium high dose), MHD (Melatonin high dose), MGHD (Magnesium high dose), MMGHD (Melatonin and Magnesium high dose), IN (Insulin).
n=6 rats per group, Melatonin route of administration = intraperitoneal, Magnesium = oral, Insulin = intraperitoneal
Treatment duration is once daily for 21 days

Histochemical Studies: Tissue samples were harvested and fixed in 10% normal buffered formalin for 72 hours. The samples were grossed and labelled in tissues cassettes and processed histological by different concentration of alcohols (70, 80, 90 and 100%) for dehydration, cleared through three changes of Toluene, infiltrated, embedded in molten paraffin wax and blocked on cold ice packs. The tissues were sectioned using a Rotary Microtome (Leica, Germany) and the ribbons obtained were picked on clean grease free Leica charged slides for general histological and histochemical studies. The slides were drained and heat fixed on a hot plate at 2 degrees above wax melting point. The tissues were further dewaxed in Toluene, cleared in decreasing reverse order above for alcohol and taken to water before proceeding to all staining procedures. Portions of the kidney, were stained using Haematoxylin and Eosin (H&E) methods for general histological studies and Masson Trichrome stains method was used to study kidney nephropathy and glomerulopathy, [22]. The slides obtained were screened and photomicrographs were taken using a Leica DM 750 ICCE 50 Microscope and digital camera and AmScope DM 200 digital microscope mounted on an Olympus Light microscope.

RESULTS

Haematoxylin and Eosin study of the kidney: Figure 1A Photomicrograph section of kidney tissues of control rats (NC) showing normal kidney glomerulus, proximal and distal tubules. Figure 1B Photomicrograph section of STZ induced diabetic rats of Diabetic control group (DC) given normal saline showing glomerunephritis with cellular and tissue damage with hazy, swollen proximal and distal tubules while Figure 1C melatonin treated group (MLD) with 10mg/kgbw showed recovered glomerulus and proximal and distal tubular cellular properties. Figure 1D photomicrograph section of kidney tissues (MgLD group) magnesium treated at 240 mg/kgbw showing glomerunephritis, proximal and distal tubular cellular degeneration. Figure 2E (MMgLD group) photomicrograph of a section of STZ induced diabetes and treated with melatonin and magnesium treated at 10 mg/kgbw) + (240 mg/kgbw), showed glomerular tissue regeneration and reduced swelling in proximal and distal tubular tissues. Figure1F is a photomicrograph section of kidney tissue of STZ induced diabetic rats treated with melatonin at 20mg/kgbw showing mild regeneration of glomerular tissues with tubular hydropic change. Figure 1G Photomicrograph of a section of kidney tissue from magnesium treated diabetic rats at 480mg/kg (MgHD) showed glomerulopathy and distal tubules necrosis and proximal tubular recovery. Figure 1H Photomicrograph of section of kidney tissue from STZ induced diabetic Wistar rats treated with 20mg/kgbw and 480mg/kgbw of magnesium (MMgHD), showed glomerulopathy, tubular swelling and necrosis. Figure 1I Photomicrograph of a section of kidney in STZ induced diabetes and treated with 2IU insulin (IN group) showed glomerulonephritis with proximal and distal tubular necrosis and hydropic change.

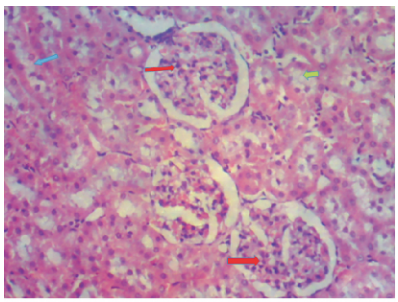

Figure 1A: Photomicrograph section of a normal kidney tissue (NC) showing glomerulus (red arrows), proximal tubules (green arrow) and distal tubules (blue arrow), (H&E x100).

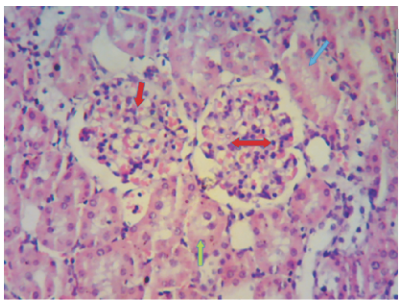

Figure 1B: Photomicrograph section of a kidney tissue of STZ induced diabetes control (DC group), showing glomerunephritis (red arrow) with cellular and tissue damage and hazy swollen proximal tubules (green arrow) and distal tubules (blue arrow), (H&E x100).

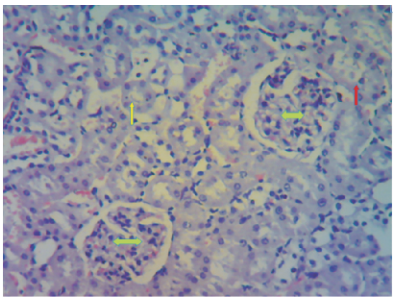

Figure 1C: Photomicrograph of a section of kidney tissue from STZ induced diabetic rat's treated with Melatonin (10mg/kgbw) (MLD) showing very mild glomerunephritis (green arrows) and improved proximal (yellow arrows) and distal tubules (red arrow) cellular properties, (H&E x100).

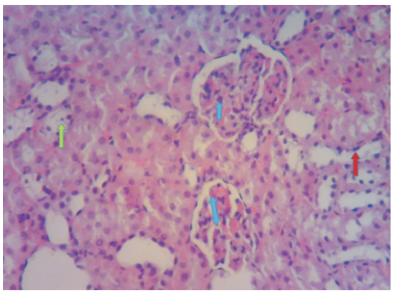

Figure1D: Photomicrograph section of liver tissue of STZ induced diabetes in Wistar rats treated with magnesium (240 mg/kgbw) (MgLD group), showing glomerunephritis (blue arrow) and proximal (green arrow) and distal (red arrow) tubular degeneration and necrosis. There was no improvement of the cellular profile of the kidney tissues, (H&E x100).

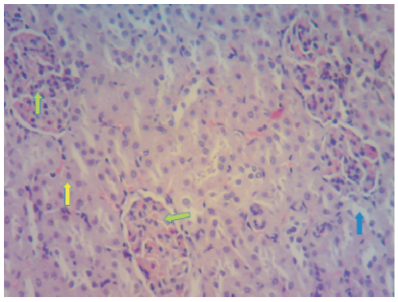

Figure 1E: Photomicrograph of a section of kidney tissue of STZ induced diabetes in melatonin treated (10 mg/kgbw) and magnesium treated (240 mg/kgbw) (MMgLD group). Kidney tissue shows glomerular tissue regeneration (green arrow) and reduced swelling in distal (blue arrow) and proximal (yellow arrow) tubular tissues, (H&E x100).

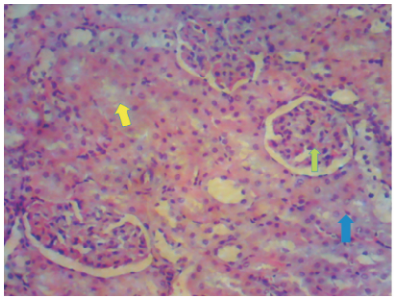

Figure1F: Photomicrograph of a section of kidney tissue of STZ induced diabetic wistar rats treated with melatonin (20 mg/kgbw) (MHD group), showing mild regeneration of glomerular tissues with tubular swelling (yellow arrow) and hydropic change (blue arrow), (H&E x100).

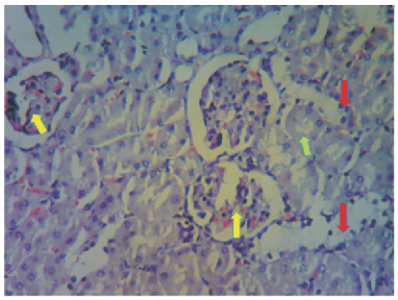

Figure1G: Photomicrograph of a section of a kidney of STZ induced diabetes treated with Magnesium (480 mg/kgbw) (MgHD), showing glomerulopathy (yellow arrow) and distal tubular swelling and necrosis (red arrow), while proximal tubules are preserved (green arrow), (H&E x100).

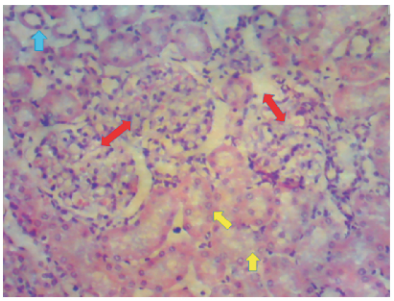

Figure 1H: Photomicrograph of a section of kidney tissue of STZ induced diabetic Wistar rats treated with melatonin (20mg/kgbw) and magnesium (480 mg/kgbw) (MMgHD group), showing glomerulopathy (red arrows) and distal tubular swelling and hydropic change (yellow arrows) and proximal tubules necrosis (blue arrow), (H&E x100).

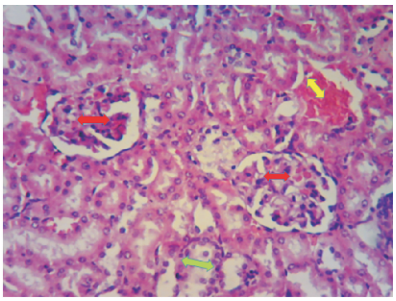

Figure 1I: Photomicrograph of a section of kidney of STZ induced diabetes in Wistar rats administered Insulin (2IU), (IN group), showing glomerunephritis (red arrows), proximal and distal tubular necrosis (yellow arrow) and hydropic change (green yellow), (H&E x100).

Masson Trichrome Stain for Glomerular and Tubular Pathology: Figure 2A (NC group) Photomicrograph of a section of kidney tissue of control group showing normal glomerulus, proximal and distal tubules with normal staining reaction in glomerulus and basement membrane. Figure 2B Photomicrograph section of kidney tissue (DC group) showing glomerular nephropathy. There is thickening of the glomerular basement membrane with mesangial nodular lesion of the glomerulus and thickening of the tubular basement membrane with atrophy. These results were similar to histological features in Figure 2D (MgLD group) magnesium treated (240 mg/kgbw) diabetic rats, Figure 2G (MgHD group) magnesium treated (480 mg/kgbw) diabetic rats. Figure 2H (MgHD Group) melatonin and magnesium treatment at 20 mg/kgbw+480 mg/kgbw (MMgHD Group) and Figure 2I insulin treated diabetic rats (IN group).
Figure 2C Photomicrograph of kidney section from melatonin treated (10 mg/kgbw) MLD group of STZ induced diabetic rats showing glomerulus membrane recovery with less tubular basement membranes connective tissues staining reactions indicating recovery of normal histoarchitechture, while Figure 2E (MMgLD Group) melatonin and magnesium treated (10 mg/kgbw+240 mg/kgbw) diabetic rats and Figure 2F (MHD group) shows similar results when compared with Figure 2A (NC group).

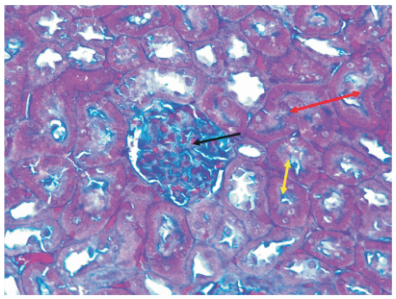

Figure 2AI: Photomicrograph section of kidney tissue of normal control Wistar rats (NC group), showing preservation of the glomerulus (black arrow) and proximal tubules (yellow double arrow) and distal tubule (red double arrow). (Masson Trichrome. Leica DM 750, Camera ICC50 E x400).

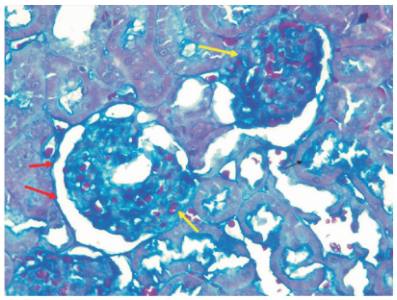

Figure 2B: Photomicrograph of section of the kidney of STZ induced diabetes (DC group) showing glomerular nephropathy confirmed by thickening of glomerular basement membrane (red arrow) and mesangial nodular lesion of the glomerulus (yellow arrow), with thickening of the tubular basement membrane and atrophy (black arrow), (Masson Trichrome Stain. Leica DM 750, Camera ICC50 E x400).

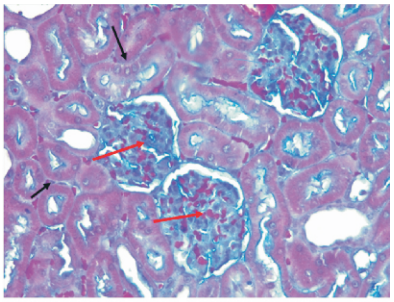

Figure 2C: Photomicrograph section of Kidney tissue from STZ induced melatonin treated (10mg/kgbw) diabetic rats (MLD group). There is recovery of the glomerulus and membranes (red arrows) and tubules basement membranes less reaction (black arrows) signifying recovery of normal histoarchitechture of the kidney. (Leica DM 750, Camera ICC50 E, (Masson Trichrome stain, x400).

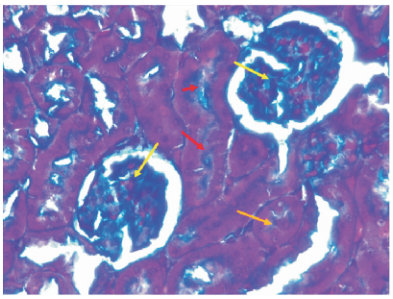

Figure 2D: Photomicrograph of kidney section of STZ induced diabetes treated with Magnesium (240 mg/kgbw) in Wistar rats. Showing diffuse glomerular lesions (yellow arrows) and tubular interstitial basement membrane thickening (red arrows) and atrophy (orange arrow), (Leica DM 750, ICC50 E, x400).

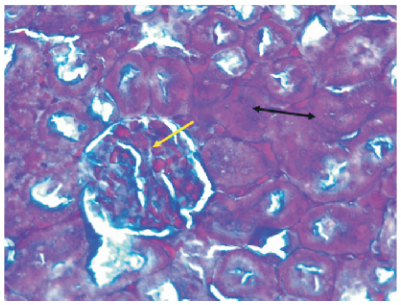

Figure 2E: Photomicrograph of a section of kidney tissue from STZ induced Diabetic rats (DMMgLD group) treated with Melatonin (10 mg/kgbw) and Magnesium (240 mg/kgbw) showing mild glomerular recovery (yellow arrow) and atrophic necrotic tubules (black double arrow), (Leica DM750, ICC50 E, x400).

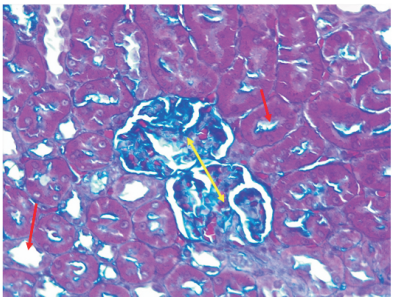

Figure 2F: Photomicrograph of a section of Kidney of STZ induced diabetic rats (DMHD group) treated with Melatonin (20 mg/kgbw). Section shows mild glomerular recovery (yellow double arrow) and tubules (red arrows), (Leica DM 750 ICC50 E. Masson Trichrome Stain. X400)

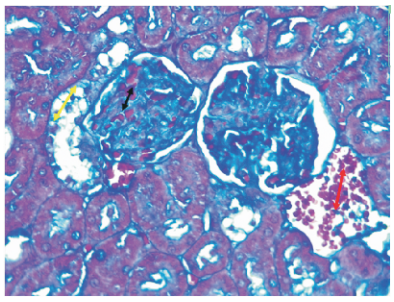

Figure 2G: Photomicrograph of a section of kidney tissue of STZ induced diabetic rats treated with 480 mg/kgbw magnesium (MgHD group). Section showed necrotic changes in the glomerulus (red arrow) and in distal and proximal tubules (yellow arrow). There is hydropic change, swelling and condensation of basement membranes tissues, (Leica DM 750 ICC50 E, (Masson trichrome, x400.)

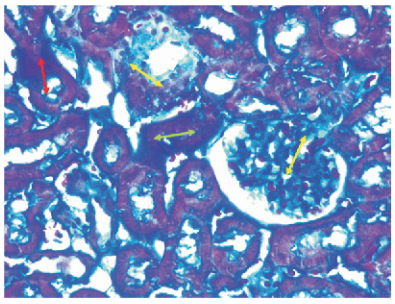

Figure 2H: Photomicrograph of a section of kidney tissue from STZ induced diabetic rats treated with Melatonin 20mg/kg and Magnesium (480 mg/kgbw), (DMMgMD). Shows glomerular (yellow arrow) and tubular (red arrow) necrosis and atrophy (green arrow). (Leica DM 750, Camera ICC50 E, x400).

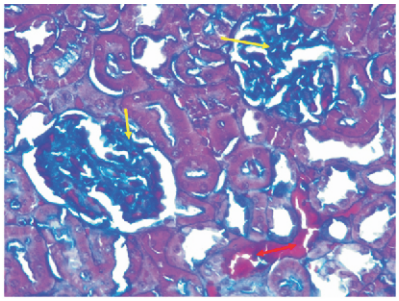

Figure 2I: Photomicrograph of a section of Kidney of STZ induced diabetes and Insulin treated (2IU) IN Group. Section shows tubular focal necrotic changes (red arrow) and glomerular degeneration (yellow arrow), Leica DM 750, ICC50 E, (Masson trichrome, x400).

DISCUSSION

Kidney tissue section of control rats showed normal kidney glomerulus, proximal and distal tubules while STZ induced diabetic rats (Diabetic control) given normal saline had glomerunephritis with cellular and tissue damage with hazy, swollen proximal and distal tubules while magnesium treated (240 mg/kgbw), magnesium treated (480 mg/kgbw), melatonin and magnesium treated (10 mg/kgbw)+(480 mg/kgbw) and insulin treated STZ induced diabetic groups showed similar results when compared to diabetic control group. Melatonin treatment at low and high doses and when combine with low dose magnesium showed similar results compared with normal control group expressing kidney glomerulus, proximal and distal tubules recovery. Glomerulopathy was further studied using Masson Trichrome stain which showed that the normal control group with normal glomerulus, proximal and distal tubules with no normal staining reaction in glomerulus and basement membrane. Section of kidney tissues in Diabetic control group showed glomerular nephropathy. There is thickening of the glomerular basement membrane with mesangial nodular lesion of the glomerulus with atrophy.

This result was similar to histological features in magnesium treated (240 mg/kgbw) diabetic rats, magnesium treated (480 mg/kgbw) diabetic rats, melatonin and magnesium treatment at 20 mg/kgbw+480 mg/kgbw and insulin treated diabetic rats. Kidney tissues from melatonin treated (10 mg/kgbw) shows glomerulus and membrane recovery with less tubular basement membranes connective tissues staining reactions indicating recovery of normal histoarchitechture while melatonin and magnesium treated (10 mg/kgbw+240 mg/kgbw) diabetic rats' shows similar results when compared. Melatonin and magnesium treatment at 10 mg /kgbw, 20 mg/kgbw and with magnesium (240 mg/kgbw) showed reversal of kidney nephropathy changes and recovery and regeneration of tissues and cells of the glomerulus, proximal and distal tubules. These results supports reports by [23 ,24] stated that when the blood glucose rises beyond the kidneys capacity to reabsorb glucose from renal ultra-filtrate, glucose remains diluted in the fluid, raising its osmotic pressure, and causing more water to be carried out, thus, increasing the excreted urine volume (polyuria). The increased volume dilutes the sodium chloride in the urine, signaling the macula Densa to release more renin, causing vasoconstriction, a survival mechanism to retain water by passing less blood through the kidneys. These changes develop over a period of time and results in features of clinical renal disease including proteinuria, hypertension, and declining glomerular filtration rate, [25, 26]. This nephropathological changes leads to expansion of glomerular mesangial regions at the expense of filtration surface area and subsequent thickening of the glomerular and tubular basement membranes, [27, 28] and [29, 30, 31]. Melatonin and magnesium in our study reversed these nephropathological changes associated with Diabetic Kidney Disease in STZ induced diabetes rats.

CONCLUSION

Melatonin administration to STZ induced diabetic rats at 10mg/kgbw, 20mg/kgbw and when 10mg/kgbw of melatonin was co-administered with 240 mg/kgbw magnesium was able to ameliorate nephropathy and glumerulopathy associated histopathological changes in experimental model of diabetic kidney disease.

ACKNOWLEDGEMENTS

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