Histology is the study of micro anatomy of specific tissues and organs through the examination of the microscopical architecture of tissues and the relationship between the different types of cells and tissue types found within tissues and organs. The study of abnormal cells and tissues is histopathology. Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level (Aughey & Frye, 2001). It is commonly performed by examining cells and tissues under a light microscope or electron microscope, which have been sectioned, stained and mounted on a microscope slide. Histological studies may be conducted using tissue culture, where live human or animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine. The architectural dynamics of a tissue are very essential for maintaining the tissue integrity and for effective physiological, biochemical functions. The cellular and sub-cellular constituents of tissue in terms of size, shape and number play an important role in the physiological and metabolic functions. Under physiological conditions the diversified structural and biophysicochemical components of the cell act and maintain a dynamic synergistic equilibrium (anatomophysiological synergism) determined and regulated in an orderly manner by intrinsic mechanism in conjunction with the environmental condition of the cell. In abnormal condition of a structural, metabolic or functional character, reversible changes (histometabolic dysergia) take place. However, if such endogenous or exogenous phenomena are repeated or perpetuated without control and exceed the physiologic endurance, then eventually disorganization or dissolution of the integrated anatomophysiological synergistic equilibrium follows with consequent irreversible changes of structural (degeneration), metabolic (histometabolic pathergia) and functional (dysfunction or paralysis) character takes place in cell. Therefore, the histological structure of tissue in an animal has a profound influence on its function (Savithri et al., 2014).

Kidney histopathology under Diabetic conditions:

It is essential to evaluate renal tissue using appropriate standards for renal biopsy. These include hematoxylin and eosin, periodic acid–Schiff (PAS), Masson trichrome, and periodic acid methenamine silver stains for light microscopy. Biopsies
should contain at least 10 glomeruli, 14 excluding incomplete glomeruli along the biopsy edge. Immunofluorescence requires the use of antibodies against IgA, IgG, IgM, C3, C1q, and kappa and lambda light chains to rule out other renal diseases. Virtually any glomerular disease can accompany diabetic nephropathy (DN), postinfectious glomerulonephritis (GN) (Mazzucco et al., 2002; Haas, 2003) and membranous glomerulopathy being the most common (Agati et al., 2005).

**Classes of Glomerular Lesions**

**Class I: Glomerular Basement Membrane Thickening.**

If the biopsy specimen shows no or only mild, nonspecific changes by light microscopy that do not meet the criteria of classes II through IV [in effect, in the absence of mesangial expansion, nodular increases in mesangial matrix (Kimmelstiel–Wilson lesions), and global glomerulosclerosis of more than 50% of glomeruli] the biopsy is assigned to class I, in which by direct measurements with electron microscope the glomerular basement membrane (GBM) on average is thicker than 430 nm in males 9 years and older and thicker than 395 nm in females. These cutoff levels are based on a deviation from normal GBM thickness plus 2 standard deviations as recently determined by Haas (Haas, 2009). Light microscopic changes in the GBM and epithelial foot process effacement by EM have no influence on the classification. Class I incorporates cases that have been called “normal or near normal DN”, but in human system, a certain degree of chronic and other reactive changes (e.g., changes of arterionephrosclerosis, ischemic type changes, or interstitial fibrosis) are accepted as part of this category (Fioretto et al., 1996). Diagnosing DN in cases without characteristic light microscopic glomerular lesions may be difficult, especially when a thicker GBM is also seen with aging or hypertension. The presence of arteriolar hyalinosis may be helpful in these cases, although it is not a prerequisite. GBM thickening is a characteristic early change in type 1(Drummond et al., 2002) and type 2 DN (White and Bilous, 2000) and increases with duration of disease (Perrin et al., 2006). GBM thickening is a consequence of extracellular matrix accumulation, with increased deposition of normal extracellular matrix components such as collagen types IV and VI, laminin, and fibronectin (Kim et al., 1991). Such accumulations result from increased production of these proteins, their decreased degradation, or a combination of the two. GBM thickening may already be present in
type 1 diabetes patients who are normoalbuminuric (Drummond et al., 2002). GBM thickening has even been described as a “prediabetic” lesion: In patients with proteinuria and isolated GBM thickening but without overt diabetes, 20% were positive on a blood test for diabetes at the time of biopsy, whereas 44% were diagnosed with diabetes at 6 months, and 70% at 2 years after the biopsy was taken (Mac-Moune et al., 2004). Long-term glucose control and urinary albumin excretion (UAE) correlate strongly with basement membrane thickness (Bangstad et al., 1994). Jensen et al. (1979) were among the first to measure GBM thickness using the orthogonal intercept method. In brief, a grid with eight evenly spaced intersecting lines (four horizontal and four vertical) is placed over a photomicrograph, and GBM measurements are made at each point that a line on the grid intersects an endothelial-GBM interface. Currently, some laboratories use computer-assisted measurements by which the mean width is calculated from approximately 50 measurements of the GBM at five different locations. The GBM width is then compared with GBM width from normal subjects, as determined previously by Steffes et al. (1978) and recently updated by Haas, (2009). Ideally, glutaraldehyde- fixed, plastic resin-embedded tissue should be used for EM, keeping in mind that other methods, particularly the reprocessing of paraffin tissue for EM, may cause artifactual GBM thinning as recently reported by Nasr et al. (2007).

Class II: Mesangial Expansion, Mild (IIa) or Severe (IIb)

Class II encompasses on classification of mild or severe mesangial expansion but not meeting inclusion criteria for class III or IV and is analogous to the previously used term “diffuse diabetic glomerulosclerosis.” Mesangial expansion is defined as an increase in extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules. The difference between mild and severe mesangial expansion is based on whether the expanded mesangial area is smaller or larger than the mean area of a capillary lumen. If severe mesangial expansion is seen in more than 25% of the total mesangium observed throughout the biopsy, the biopsy is classified as IIb. If this is not the case, but at least mild mesangial expansion is seen in more than 25% of the total mesangium, the biopsy is classified as IIa. Expansion of cellular and matrix components in the mesangium is a hallmark of type 1 and type 2 DN (White et al., 2000). It can be detected in some patients within a few years after the onset of type 1
diabetes (Drummond et al., 2002). When the mesangium expands, it restricts and distorts glomerular capillaries and diminishes the capillary filtration surface. Various indices have been proposed to describe the amount of mesangial expansion in DN. Mauer et al. (1984) define mesangial expansion by mesangial fractional volume or volume density (Vv), defined as the fraction or percentage of the cross-sectional area of the glomerular tuft made up by mesangium, expressed in the formula: Vv(mes/glom). Using this formula, many correlations have been made between mesangial expansion and clinical parameters of DN, particularly showing highly inverse correlations exist between Vv(mes/glom) and GFR (Caramori et al., 2002; Najafian et al., 2003). There is also a relationship between Vv (mes/glom) and UAE (Caramori et al., 2002) and blood pressure (Mauer et al., 1992). Another index to express mesangial expansion is the so-called “index of mesangial expansion” (IME) for DN. The IME is determined by a semiquantitative estimate of the width of mesangial zones in each glomerulus: grade 0 is normal, 1 is twice normal thickness, 2 is three times normal thickness, and so forth; half grades can also be assigned. The mean of the grades for each glomerulus for IME can thus be determined from a single biopsy. The IME closely correlates with the Vv (mes/glom). The new classification for IgA nephropathy in which it is defined as an increase in the extracellular material in the mesangium such that the width of the interspace exceeds two mesangial cell nuclei in at least two glomerular lobules (Cattran et al., 2009).

**Class III: Nodular Sclerosis (Kimmelstiel–Wilson lesions)**

If at least one convincing Kimmelstiel–Wilson lesion is found and the biopsy specimen does not have more than 50% global glomerulosclerosis it is classified as class III. Kimmelstiel–Wilson lesions appear in type 1 and type 2 diabetes as focal, lobular, round to oval mesangial lesions with an acellular, hyaline/matrix core, rounded peripherally by sparse, crescent-shaped mesangial nuclei (Stout et al., 1993). Paul Kimmelstiel and Clifford Wilson, a German and an Englishman who met at Harvard, first described nodular lesions in glomeruli from eight maturity onset diabetes patients in 1936 (Kimmelstiel et al., 1936). According to Cameron (2006), they barely noted the association with diabetes, and it was Arthur Allen who clarified the association in 105 patients with diabetes in 1941. Nodular sclerotic lesions may also occur in the absence of DN that are clinically related to hypertension, smoking, hypercholesterolemia, and extrarenal vascular disease (Markowitz et al., 2002). It is
claimed that in the initial stage of developing nodular sclerotic lesions in DN, two important processes take place: lytic changes in the mesangial area called mesangiolysis and detachment of endothelial cells from the GBM (Nishi et al., 2000). Exactly how these two processes relate remains uncertain. Paueksakon et al., detected fragmented red blood cells in Kimmelstiel–Wilson lesions, which supports the theory that microvascular injury contributes to the pathogenesis of these lesions (Paueksakon et al., 2002). Dissociation of endothelial cells may disrupt the connections between the mesangial area and the GBM. This process precedes expansion of the Kimmelstiel–Wilson lesion (Nishi et al., 2000). These lesions consist of an accumulation of mesangial matrix with collagen fibrils, small lipid particles, and cellular debris (Glick et al., 1992). A completely developed Kimmelstiel–Wilson lesion destroys the normal structure of glomerular tuft with a decrease in mesangial cells, especially in the central area. In 1992, a graphic method of analysis of the position of Kimmelstiel–Wilson lesions demonstrated the nodules were distributed in a horseshoe-shaped area corresponding to the peripheral or intralobular mesangium (Sandison et al., 1992), excluding the possibility of hyperfiltration as being their main cause of development. The presence of at least one Kimmelstiel–Wilson lesion associates with longer duration of diabetes and less favorable clinical parameters (Hong et al., 2007). Kimmelstiel–Wilson lesions are often found in combination with mesangial expansion. The occurrence of Kimmelstiel–Wilson lesions is widely considered transitional from an early or moderately advanced stage to a progressively more advanced stage of disease (Mason et al., 2003).

Class IV: Advanced Diabetic Glomerulosclerosis

Class IV implies advanced DN and designates those biopsies with more than 50% global glomerulosclerosis in which there is clinical or pathologic evidence that the sclerosis is attributable to DN. Glomerulosclerosis in DN is the end point of multifactorial mechanisms that lead to excessive accumulation of extracellular matrix proteins such as collagen types I, III, and IV and fibronectin in the mesangial space, which through stages of mesangial expansion and development of Kimmelstiel–Wilson lesions finally result in glomerulosclerosis (Qian et al., 2008). The clustering of sclerotic lesions in columns perpendicular to the kidney surface suggests that vascular factors relating to the interlobular arteries also contribute (Horlyck et al., 1986). Designation of class IV lesions in our classification system is restricted to
those cases in which there is evidence for DN. This evidence can come from other lesions in the biopsy as described for classes I through III. The occurrence of hyalinosis of the glomerular vascular pole or a capsular drop may also be taken as evidence for the presence of DN. Alternatively, if DN is the likely clinical diagnosis (e.g., by the presence of retinopathy) a biopsy with extensive glomerulosclerosis can also be classified as class IV. Glomerulosclerosis without evidence of DN should be mentioned as such in the conclusion of the pathology report but should not be assigned class IV.

Results and discussion:

The kidney tissue of the Normal control (NC), *Xanthium indicum* treated (Xi), α-tocopherol treated (Tpt), Diabetic control (DC), Glibenclamaide treated diabetic (Di + Glbt), α-tocopherol treated diabetic (Di + Tpt) and *Xanthium indicum* treated diabetic (Di + Xi) rats were examined for structural changes under the light microscope using hemotoxylin and eosin staining. Histological examinations of the kidney by light microscope are figured in Plates 1, 2, 3, 4, 5, 6 and 7.

The diabetic nephropathy has been considered an important cause of mortality and morbidity and many of the end stage renal failure results due to diabetic nephropathy (Boonna *et al.*, 2006). In the diabetic animals, a significant increase in the kidney weight was observed. The result of this study is in accordance with the findings of earlier research studies (Yadav *et al.*, 2005). It has been described that the kidney enlargement in DM is attributed to certain factors like glucose over-utilization and subsequent enhancement in increase uptake, glycogen accumulation, lipogenesis and protein synthesis in the kidney tissue (Sun *et al.*, 2002). Glycation is a nonenzymatic reaction between sugars and the free amino groups of materials in a hyperglycemic situation such as diabetes mellitus. The glycation of materials induces a wide range of chemical, cellular and tissue effects and leads to nephropathy development. Sabbatini *et al.* (1992), showed that the early glycation products (EGPs) induce glomerular hyperfiltration even in normal rats. Thickening of tubular basement membrane (TBM) closely parallels that of glomerular basement membrane (GBM) thickening, implying that glomerular hemodynamic perturbations are not required for these changes to occur (Brito *et al.*, 1998). Mesangial expansion, predominantly due to an increase in mesangial matrix, develops later although an increase in the matrix
component of the mesangium. Both mesangial expansion and GBM and TBM thickening are a consequence of extracellular matrix (ECM) accumulation, with increased deposition of the normal ECM local components of types IV and VI collagen, laminin and fibronectin (Kim et al., 1991) due to their increased production, decreased degradation or both. In contrast to the mesangium, initial interstitial expansion is primarily due to an increase in the cellular component of this renal compartment; increase in fibrillar collagen is, in fact, a relatively late finding in this disease, measurable only in patients with an already established decline in glomerular filtration rate (Katz et al., 2002).

The glycation process is reversible but over time, it becomes irreversible and EGPs develop into advanced glycation end products (AGEs). AGE influences charge, solubility and conformation of ECM. Therefore, the early diagnosis and treatment of hyperglycemia prevents AGE production. Renal investigations have demonstrated that hyperfiltration is associated to vasodilatation and the consequent increase in blood flow and glomerular capillary pressure. The blood flow increase is the primary and main cause of structural and functional disorders in the kidney and blood flow correction in the early stage of diabetes inhibits further complications in the kidney (Pourghasem et al., 2014). The kidney weight increased when compared to control animals. This result indicates that the onset of renal enlargement can be a characteristic feature of diabetic kidney. It has manifested that the kidney enlargement is caused by certain factors like glucose over-administration, glycogen accumulation, lipogenesis and protein synthesis in the diabetic kidney (Sun et al., 2002). Actually, it is due to glomerular hypertrophy and nephromegaly.

The present study has been conducted to find earlier possible histological changes in the kidney of diabetic rats. 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 (Plate-1). Four weeks after the initiation of diabetes mellitus, the histological study of kidney of STZ induced diabetic rats demonstrated the presence of abnormal cells in the wall of renal tubules which could lead to cell damages and tubular damage, haemorrhage in the Bowman’s capsule due to glomerular damage. The cytoplasm resolution of abnormal cells changed and vacuolar modifications occurred. According to the association between cell shape and cell function, these changes may correspond to an
adaptation of cells to a new situation such as increased load due to congestion (Plate - 4). The results indicate a primary and a secondary effect of the diabetic state on the kidney of the rat. The primary effect, the diabetes factor was associated with hyperglycaemia and was responsible for dilatation of proximal and distal tubules in the cortex. The secondary effect, named the individual response factor, was associated with inflammatory processes (Leegwates et al., 1984). Diuresis is a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus (Das et al., 1996). Lipid peroxides, hydroperoxides and protein carbonyls are the secondary products of oxidative stress and are unleashed as a result of the toxic effect of reactive oxygen species produced during lipid peroxidation in diabetes. Lipid peroxidation may also play a crucial role in diabetic glomerulosclerosis. The initial hyperfiltering phase of diabetic kidney disease, heralded by the presence of microalbuminuria, is followed by gross proteinuria, progressive diminution in glomerular filtration rate and excessive amassing of extracellular matrix proteins in the glomerular capillaries and mesangium. The latter process leads the way to eventual glomerulosclerosis and renal failure (Salahudeen et al., 1997).

All the necrotic changes observed in the proximal and distal convoluted tubules along with the deposits were found to be absent in the diabetic rats treated with the hydro methanolic extract of Xanthium indicum and its bioactive compound α-Tocopherol. The two groups (group-VI: α-tocopherol treated diabetic rats and and group-VII: Xanthium indicum treated diabetic rats) showed features of healing i.e. normal glomerulus, normal basement membrane and renal tubules, absence of congestion, and degenerative changes, decrease in hyaline deposit. The tissue necrosis was also observed to decrease in these groups (Plates -6 and 7). α-Tocopherol, on the other hand, acts as a non-enzymatic antioxidant and reduces lipid peroxidation and glutathione (Punithavathi et al., 2008; Minamiyama et al., 2008). α-tocopherol is very effective in glycemic control, lowering the HbA1c levels (Ihara et al., 2000) and preventing the hypertrophic effects of hyperglycemia (Nascimento et al., 2005). Diffuse thickening of the glomerular basement membrane depends on the severity of the disease. α-tocopherol reduce the thickness of the basement membrane (DavilaEsqueda et al., 2005). This normalization may be accomplished by the antioxidant and free-radical quenching nature of α-tocopherol and plant extract (Kumkum & Ranjana, 2014: Anjoo et al., 2015).
Plate-1:

- Photomicrograph of normal control (Group-I) rat kidney renal parenchyma showing normal architecture with normal Bowman’s capsule (BC) and renal tubule (RT).

Plate-2:

- Photomicrograph of Xanthium indicum treated (Xit) (Group-II) rat kidney renal parenchyma showing normal architecture with normal Bowman’s capsule (BC) and renal tubule (RT).
Plate-3:

- Photomicrograph of α-tocopherol treated (Tpt) (Group-III) rat kidney renal parenchyma showing normal architecture with normal Bowman’s capsule (BC) and renal tubule (RT).

Plate-4:

- Photomicrograph of diabetic (DC) (Group-IV) rat kidney renal parenchyma showing degenerative changes in glomeruli/ Bowman’s capsules (BC) and renal tubules along with necrotic changes (NC) and congestion (C).
Plate-5:
- Photomicrograph of Glibenclamaide treated diabetic (Di + Glbt) (Group-V) rat kidney renal parenchyma showing Regeneration changes in glomeruli (G) and Bowman’s capsule (BC) where necrosis takes place.

Plate-6:
- Photomicrograph of α-tocopherol treated diabetic (Di+Tpt) rat (Group- VI) kidney renal parenchyma showing regeneration changes in distal tubules (D) and Bowman’s capsule (BC) where necrosis takes place.
Plate-7:

- Photomicrograph of *Xanthium indicum* treated diabetic (Di + Xit) rat (Group-VII) kidney renal parenchyma showing Regenerative changes in Glomeruli (G), distal tubules (D) and Bowman’s capsule (BC) where necrosis takes place.