ALTERATIONS IN BODY WEIGHT, PROTEIN PROFILE, NON-PROTEIN NITROGEN CONSTITUENTS AND KIDNEY STRUCTURE IN DIABETIC RATS UNDER GLIBENCLAMIDE TREATMENT

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ABSTRACT The effect of glibenclamide administration to alloxan diabetic rats on body weight, protein profile and non-protein nitrogen constituents has been assessed. Histological changes of kidney were also studied. Diabetic and glibenclamide-treated animals showed significant decrease in body weight gain from controls recording percentage values of 36.37% and 21.14%, respectively throughout the study. Highly significant decrease in serum total protein and albumin levels was recorded in diabetic rats throughout the study with percentage decrease of 21.74% and 20.19%, respectively as compared to control levels. This decrease is rendered to be not significant on glibenclamide treatment showing percentage decrease of 3.30% and 7.75%, respectively. The estimated levels of serum globulin in diabetic rats showed significant decrease with a percentage of 24.17% compared to controls. However, non-significant fluctuations in globulin levels were achieved upon glibenclamide administration during the whole experimental period with percentage increase of 5.0% as compared to controls. A highly significant increase in serum urea concentration of diabetic animals has been observed. The highest increment reached was at the eighth week where urea recorded 97.33 mg/dl with a percentage increase of 154.99% as compared with control group. Glibenclamide did not manage to retain urea to normal level. Uric acid concentration was generally decreased in diabetic group and this decrease reached a significant value during the last two weeks of the study. Also, creatinine concentration was decreased significantly in diabetic animals recording a magnitude of 37.04% compared to control. However, glibenclamide administration managed to return uric acid and creatinine concentrations near to normal levels. Histopathological changes in kidney of diabetic rats were represented by condensed capillaries of glomeruli where the epithelia of these capillaries were hypertrophied and possessed pyknotic nuclei. Necrotic changes in proximal and distal convoluted tubules, loss of inner brush border lining proximal convoluted tubules, degeneration and infiltration of glomerular tufts by chronic inflammatory cells and red blood cells, swelling of tubular epithelium and enlargement of mesangial cells were also observed. On treatment with glibenclamide recovery was manifested in a general manner but limited parts of urineferous tubules designated earlier manifested diabetic changes of degeneration and cloudy swelling.

Key words: Diabetic rats, glibenclamide, Kidney structure, protein profile, non-protein nitrogen constituents.

INTRODUCTION Diabetes mellitus is a disorder in which the level of blood glucose is persistently raised above the normal range. It may result from either lack of pancreatic insulin secretion or resistance to insulin action. There
are many associated metabolic abnormalities with diabetes mellitus notably ketoacidosis, the progressive development of disease of capillaries of the kidney and retina, damage to the peripheral nerves and excessive arteriosclerosis (World Health Organization, WHO, 1980). On severe insulin lack, hyperketonaemia will developed together with alterations of lipid and protein turnover (Watkins et al., 1990). In addition, diabetes mellitus initiates loss of body weight (Runby et al., 1992 and Hotta et al., 1996).

The influence of diabetes on structure and function of the kidney together with protein profile is a matter of concern for many researchers. Porte and Halter (1981) recorded that hypoalbuminemia is a common problem in diabetic animals and is generally attributed to the presence of diabetic nephropathy. Hepatic levels of albumin and its mRNA were found to be decreased in diabetes mellitus (Jefferson et al., 1983; Peavy et al., 1985 and Wanke & Wong, 1991). Mansour et al., (2002) demonstrated that total protein and albumin were significantly decreased in alloxan diabetic rats. Elevated levels of urea were recorded in diabetes (Annamalia & Augusti, 1980 and Akimoto et al., 2000). However, uric acid and creatinine concentrations were shown to be decreased (Ganong, 1995).

Edress et al., (1979) studied the histological changes in the kidney of alloxan diabetic rats. The first changes were observed in the form of thickening of the glomerular basement membrane and deposition of hyaline substance around the walls of the glomerular blood capillaries and in the mesangial areas. Glomerular filtration rate is elevated and is correlated to the increased filtration surface (Hirose et al., 1980 and Christiansen, 1984). Several histological features correlating with renal function have been described in diabetic nephropathy. These include mesangial expansion, glomerular size, capillary filtration surface, obliteration of glomeruli and increased interstitial fibrosis (Steffes et al., 1989; Osterby et al., 1990 and Yanardag et al., 2002). In some studies, these features correlate with each other and with glomerular filtration rate and albuminuria (Lane et al., 1993).

Glibenclamide therapy in diabetic subjects was an attractive strategy for many investigators (Ashcroft and Ashcroft, 1992a; Kecskemeti et al., 2002 and Kulkarni et al., 2002). Although diabetes mellitus has become a widely spread disease in Gaza strip, only few reports addressed the problem. The current study has been intended to through more light on the relationship between diabetes and the changes in protein profile and kidney structure and
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function. Assessment of the therapeutic effect of glibenclamide all over the treatment period of eight weeks was also followed. Glibenclamide, a second-generation sulfonylurea, was the drug of choice as it is commonly used for the treatment of diabetes mellitus in Gaza Strip.

MATERIALS AND METHODS

Experimental Animals
Male albino rats were the chosen experimental animals for the present study. They were young adults weighing 90-110 gm. Animals were normal and healthy. They were housed in well-aerated cages under normal environmental conditions of temperature and humidity. Animals were fed on commercial balanced diet and tap water was offered ad libitum all over the experimental period.

Induction of diabetes and treatment
Animals were allocated into two major groups: A control group and an experimental group. The control group was orally administrated with saline solution. The experimental group was fasted for 24 hours and then rendered diabetic by a single intraperitoneal injection of a freshly prepared alloxan monohydrate solution at a dose of 150 mg/kg body weight (Halim et al., 1977). The chosen diabetic animals (Rats with blood glucose level >200 mg/dl) were subdivided into two sub-groups: The rats of the first sub-group remained without treatment and considered as diabetics. Animals of the second sub-group were orally administrated with glibenclamide at a dose of 36 mg/kg body weight, daily all over the experimental duration of eight weeks and considered as treated diabetics. Glibenclamide (commercially known as glucocare) was purchased from local pharmacies as tablets and then grinded using a mortar. The powder was dissolved in water and administrated to animals using a stomach tube with a smooth tip to protect the interior lining of the oral and buccal cavity from injury.

Morphological studies
Animals were individually weighed at weekly intervals in order to detect any changes in their body weights. A sensitive balance was used and weights were recorded to the nearest gram. Dead animals were recorded daily in order to calculate the percentage of mortality each week.

Physiological Studies
Animals from both control and experimental groups were decapitated weekly. Blood samples were collected in 10 ml plain tubes for serum preparation.
Clear serum samples were separated by centrifugation at 3000 r.p.m. for 20 min. Serum total protein was determined according to the biuret reaction as designated by Armstrong and Carr (1964). The kits were purchased from Biotech laboratories U.K. Serum albumin was determined using Randox reagent kits and following their instruction manual according to the method of Doumas et al., 1971. The concentrations of globulin were calculated by the following equation: Concentration of globulins (gm/dl) = Total protein – Albumin. Urea determination is based upon the cleavage of urea with urease (Berthelot’s reaction) according to Fawcett and Scott (1960). The kit was purchased from Boehringer Mannheim GmbH Diagnostica. Serum uric acid was determined using the SPINREACT reagent kits and following their instruction manual described by Fossatti et al., 1980. Serum creatinine was determined without protein precipitation according to Bartels et al., 1972 using the DiaSys reagent kits.

Histological Studies
Following decapitation, the kidney was dissected from the surrounding connective tissues and organs. It was then excised and immediately immersed in saline solution for the removal of blood. The preparation was then fixed in 10% buffered formalin (Lillie, 1954). Fixed tissue was dehydrated, cleared with xylene and completely impregnated with paraffin wax. The tissue was then sectioned by a rotary microtome at a thickness of 3µm, mounted and affixed to slides and later used for the routine haematoxylin and eosin stain (Harris, 1900).

Statistical Analysis
All the experimental data were statistically analyzed according to Hine and Wetherill (1975).

RESULTS
Morphological studies
Table 1 demonstrates the average weekly body weight of both control and experimental groups. It is obvious that control animals showed progressive increase in body weights with the lapse of time. The rate of increase in body weight amounted to 134.89% at the end of the experiment when calculated in terms of their original weights. Gradual increase in the body weight of diabetic rats took place till the end of the study where it reached a maximum value of only 47.20% of their original weight with a difference of 87.69% from controls. Also, rats treated with glibenclamide showed gradual increase in
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body weights till the end of the eight week. The increase amounted to 80.45% of their initial weights with a difference of 54.44% from control animals. No mortalities were recorded in both control and glibenclamide treated rats. However, in the sixth week of the experiment, the mortality rate increased in the diabetic rats and recorded 16.66%. Moreover, extra mortalities were recorded in the eighth week, with percentage of 20.0%.

Physiological studies

The mean values of serum total protein, albumin and globulin of both control and experimental animals are presented in table 1. Serum total protein level showed a highly significant decrease in diabetic rats throughout the study with a percentage value of 21.74% compared to controls. However, there was a non significant decrease in serum total protein of treated diabetic animals recording a percentage decrease of only 3.30% as compared to controls. Also, a highly significant decrease in serum albumin levels of diabetic rats was recorded compared to that of controls recording a percentage decrease of 20.19%. This decrease rendered to be not significant in animals treated with glibenclamide with a percentage decrease of 7.75% as compared to the control group. Serum globulin levels in diabetic rats exhibited significant decrease with a percentage of 24.17% compared to controls. However, globulin levels in treated diabetic animals showed non-significant fluctuations during the whole experimental period with percentage increase of 5.0% as compared to control group. Table 2 illustrates the average serum urea, uric acid and creatinine concentrations of both control and experimental groups. A highly significant increase in urea concentration of diabetic animals has been observed. The highest increment reached was at the eighth week where it recorded 97.33 mg/dl with a percentage increase of 154.99% as compared with control group. Nevertheless, the mean value of the treated diabetic animals for urea was also significantly increased throughout the study recording a percentage increase of 59.47% as compared to control group. Uric acid concentrations were generally decreased among diabetic animals and this decrease become significant and reached its maximum value of 23.70% during the last two weeks of the study. On glibenclamide treatment such decrease is rendered to be non-significant showing a percentage of 5.78% as compared to control. Serum creatinine levels of diabetic rats showed highly significant decrease commencing from the second week of the study with a percentage of 37.04% as compared to control group.
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The mean value of serum creatinine concentrations for the treated diabetic group showed non-significant decrease with a percentage of 5.56% as compared to control group.

**Histological studies**

Fig. 1 presented kidney section of control albino rat showing normal glomerulus surrounded by Bowman’s capsule, proximal and distal convoluted tubules. Histological examination of kidney from diabetic rats is shown in Fig. 2. Histopathological changes were represented by condensed glomerular capillaries where the epithelia of these capillaries were hypertrophoid and possess pyknotic nuclei. In addition, necrotic changes in proximal and distal convoluted tubules; cloudy swelling in the lining cells of proximal and distal convoluted tubules with pyknotic nuclei, loss of inner brush border of proximal convoluted tubules; completely eroded proximal convoluted tubules were also manifested. The glomerular tufts were obviously contracted, lobulated degenerated and infiltrated by chronic inflammatory cells and RBCs. The glomeruli were more or less shrunken. The urinary spaces became wide and the interstitial connective tissue was invaded by chronic inflammatory cells mainly lymphocytes. Finally, the nuclei of some deteriorated cells displayed obvious signs of karyorrhexis while a few of these nuclei were markedly karyolyzed. Treatment with glibenclamide rendered the kidney susceptible to recovery but at quite slow rate (Fig. 3). The prementioned lesions from diabetes induction were still designated in the earlier weeks of glibenclamide administration in the form of vacuolation within glomerular tuft; infiltration of glomeruli with inflammatory cells; degenerative changes and cloudy swelling within convoluted tubules; appearance of pyknotic nuclei in urineferous tubules and deposited extracellular hyaline material in between tubules. Although, glibenclamide managed to stimulate early regenerative features commencing from the fifth week of treatment yet some histological lesions still persisted as shrunken deeply stained glomeruli; altered urineferous tubules and obliteration, of some lumens. At the end of the investigation, recovery was manifested in a general manner except limited parts of urineferous tubules designated earlier manifested diabetic changes of degeneration and cloudy swelling. On the other hand, the glomeruli seemed to undergo recovery. Distribution of cells, visceral epithelial cells and mesangial cells within the glomeruli attained near to normal features.
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It could be deduced that diabetes causes pathological alterations in many physiological and histological aspects and glibenclamide was managed to alleviate most of them.

DISCUSSION

Morphological Studies

The observed decrease in body weight following experimental diabetes mellitus is in agreement with previous studies (Rungby et al., 1992; Hotta et al., 1996 and Badr-Eldin et al., 1998). This may be attributed to different side effects of inability to use carbohydrates including lypolysis, glycogenolysis and acidosis. It also may be attributed to disturbances in one or many metabolic pathways and due to deficiency of protein or disturbances in different enzymatic activities. However, diabetic rats treated with glibenclamide showed increase in body weight which may be explained by increased insulin secretion or increased food consumption (Fernstrom & Fernstrom, 1993 and Farouque & Meredith, 2003).

Physiological investigation

The decrease in serum total protein observed in diabetic rats is coincide with the findings of Peavy et al., (1985) and Wanke & Wong (1991). This decline may be due to the inhibited oxidative phosphorylation processes which lead to decrease of protein synthesis, increase in the catabolic processes and reduction of protein absorption. Two mechanisms may account for the alterations in protein synthesis in diabetic rats. First, the defect in hepatic protein synthesis resulting from insulin deficiency was most likely due to a decrease in the amount of mRNA bound to ribosomes, leading to a decrease in the hepatic polysome population (Tragl & Reaven, 1972 and Jefferson et al., 1983). Second is the reduction in the number of ribosomal protein synthesis (Wool et al., 1966), thus the capacity of the tissue for protein synthesis is decreased. Transport and uptake of amino acids in peripheral tissues are depressed, causing an elevated circulating level of amino acids particularly alanine which further enhance gluconeogenesis in the liver. Decline in ATP production and direct requirement for insulin-protein synthesis is decreased in all tissues (Ganong, 1995). The efficiency of glibenclamide to restore total protein concentrations is presumably due to its ability to increase insulin secretion (Annamalia & Augusti, 1980; Fernstrom & Fernstrom, 1993 and Farouque & Meredith, 2003). The proposed sites of action include hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein.
Fig. 1. Histological examination of kidney tissue of normal albino rats, showing: (A) the glomerulus (g), surrounded by Bowman’s capsule (▲); outer and inner visceral layers and a capsular space (♦). The proximal (P) and distal (d) convoluted tubules. [HX - E; X132]; (B) glomerular epithelium; mesangial cells & arterioles [HX - E; X330].

Fig. 2. Photomicrograph of kidney tissues of diabetic rats showing (A) necrotic lesions of both proximal and distal convoluted tubules.[HX - E; X132]; (B) contraction lobulation and degeneration of the glomerular tufts; wide urinary (capsular) space (♦) [HX - E; X132]; (C) shrunken and lobulated glomeruli infiltrated by chronic inflammatory cells and RBCs. [HX-E; X132]; (D) karyorrhexis of some deteriorative nuclei in proximal convoluted tubule (▼) [HX - E; X132].
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Fig. 3. Light micrograph of kidney tissue of rats treated with glibenclamide showing (A) vacuolations together with inflammatory cells and RBCs within the glomerular tuft [HX - E; X132]; (B) chronic inflammatory cells (†) invading the interstitial connective tissue. [HX - E; X66]; (C) remnants of necrotic cells (+) occupying the capsular space [HX – E; X 132]; (D) near to normal feature. Notice; the distribution of cells, visceral epithelial cells and masengeal cells within the glomeruli. (r) in other cells. [HX – E; X 132].

and decreased proteolysis by activation and synthesis of transaminases and other enzymes catalyzing amino acids transamination. The decrease in albumin level documented for diabetic rats may be due to liver massive necrosis, deterioration of liver function, hepatic resistance to insulin and glycogen impairment of oxidative phosphorylation (Ezzat et al., 1989; Guler et al., 1994 and Rao, 1995). Nevertheless, hypoalbuminemia is a common problem in diabetic animals and is generally attributed to the presence of diabetic nephropathy (Porte & Halter, 1981). Under the same experimental conditions serum globulin level revealed an appreciable decrease. Such results were substantiated by Tragl & Reaven (1972) and Wanke & Wong (1991).

Previous studies have shown that hepatic levels of albumin and its mRNA are decreased within 3 days following the onset of diabetes mellitus (Jefferson et al., 1983; Peavy et al., 1985 and Wanke & Wong, 1991). In contrast to
globulin, administration of glibenclamide to diabetic rats did not successfully restore albumin concentration to the normal level, suggesting the necessity for a longer duration of treatment. The highly significant increase in serum urea concentrations of diabetic rats may be due to depletion of serum protein, increase in the rate of circulating amino acids and deamination takes place that consequently leads to the formation of large amount of ammonia which is eventually converted to urea. The breakdown of amino acids during gluconeogenesis in the liver results in increased production of urea, fostering negative nitrogen balance (Ganong, 1995). Glibenclamide potentiated a substantial decrease in urea concentration, but this still far away from normal control levels. This result is in conformity with Annamalia & Augusti (1980) and Fernstrom & Fernstrom (1993) and may be primarily due to nephropathy. A general decrease in uric acid concentration in the diabetic rats was only vindicated during the latter weeks. Uric acid represents the ultimate catabolic of purines. Uric acid can be absorbed across the renal mucosa and excreted in urine as uric acid per se (Guggino et al., 1983). Nevertheless, glibenclamide intake enhanced the previous concentrations to meet with near to normal levels. Alloxan diabetic rats initiated significant decline in creatinine concentration that commenced from the second week onwards. Creatinuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus (Ganong, 1995). This decline was amended to near to normal levels following treatment with glibenclamide.

Histological studies
The observed histopathologic changes in the kidney of alloxan diabetic rats including condensed capillaries of glomeruli, necrotic changes, loss of inner brush border lining proximal convoluted tubules, degeneration glomerular tufts and infiltration by chronic inflammatory cells and red blood cells and enlargement of mesangial cells are in agreement with the findings of Edress et al., (1979); Mauer et al., (1983); Steffes et al., (1989); Spadella et al., (1996) and Yanardag et al., (2002). These changes may be due to an increase in the capillary permeability resulting in escape of plasma protein into the intercapillary spaces, and also in the mesangial areas. The presences of these abnormal exudates in the mesangium usually stimulate the process of proliferation of the mesangial cells leading to appearance of new cells. It appears that the mesangium expanding into an inherently large or perhaps secondarily enlarged glomerulus will less affect peripheral capillary surface
than the same mesangium volume in a smaller glomerulus (Ellis et al., 1986 and Osterby et al., 1988). Thus, the change in one component of the glomerulus must affect the volume available to other structures, e.g. glomerular capillaries. Mechanistically the mesangium impinges on the peripheral capillary surface and with sufficient pathological expansion, the enlarging mesangium will eventually comprise filtration surface and reduce glomerular filtration rate. Infiltration process begins shortly after an area of tissue has been damaged when capillaries and postcapillary venules in adjacent healthy tissue become dilated and blood flow within them slow. In general, it may be postulated that structural lesions of the glomerulus are the primary cause of loss of glomerular function in diabetic animals. Glibenclamide rendered the kidney susceptible to recovery but at quite slow rate. Recovery is in the form of appearance of regenerative cells in variable areas of the urineferous tubules. It is worth mentioning that the concurrent histological finding come as a supportive factor for the initially obtained biochemical studies. The maintained high urea levels might be a normal sequel to nephropathy. Nevertheless, Spadella et al., (1996) came to the conclusion that treatment through pancreas transplantation in alloxan- induced diabetic rats prevent the development of kidney lesions only after 6 months post transplantation. This statement substantiates the present results of the requirement of a much longer period of treatment for stimulation of obvious recovery.

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