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ANTIDIABETIC AND KIDNEY PROTECTIVE EFFECT OF ASPARAGUS RACEMOSUS IN ALLOXAN INDUCED DIABETES IN RATS

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ABSTRACT

The Asparagus racemosus was evaluated for its antidiabetic activities against alloxan induced hyperglycemia in rats. Thirty rats were used divided into 2 groups, normal control: Composed from 10 rats and diabetic group composed from 20 rats I.P.injection.of alloxan by dose 120mgm/kg b. w, This group subdivided into 2 subgroup: 1. Alloxan group: 10 rats as control negative. 2. Asparagus treated group: 10 rats administrated Asparagus racemosus powder by dose 500 mg/kg b. Wt. daily P.O. for 30days (Kumar et al., 2011 and Sara et al., 2013). Diabetes is recognized as a group of heterogeneous disorders with the common of hyperglycaemia and glucose intolerance, due to insulin deficiency, impaired of insulin action, or both (Harris and Zimmet, 1997). Diabetes mellitus (DM) is considered as impaired insulin secretion and (or) action. DM describes a group of metabolic diseases in which the person has high blood glucose level than normal, either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), increasingly thirsty (polydipsia) and hungry (polyphagia), (Teixeira et al., 2000). DM results in disturbances in carbohydrate metabolism (Barik et al., 2008) Daily administration of *Asparagus racemosus* to diabetic rats by dose (500 mg/kg b. wt.) for 30 days decreased serum glucose. *These findings indicate that anti hyperglycemic activity of Asparagus racemosus mediated by inhibition of carbohydrate digestion and absorption, together with*

enhancement of insulin secretion and action in the peripheral tissue (Hannan, et al., 2011).

KEYWORDS: Alloxan, Asparagus racemosus, kidney protection. Antidiabetes.

1-INTRODUCTION

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, Diabetes Mellitus (DM) is a state of chronic hyperglycemia is a common disease affecting over 124 million individuals worldwide, and if not treated, it is responsible for many complications affecting various organs in the body. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs. (Laakso 2001 and Quinn 2001)

Changes in human behavior and lifestyle over the last century have resulted increase in the incidence of diabetes (Laakso, 2001 and Zimmet et al., 2001).

Asparagus racemosus (Liliaceae) is a popular vegetable consumed in many parts of the world and grows naturally throughout India, Asia, Australia and Africa. It is commonly used for the treatment of diarrhea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac (Nadkarni, 1976).

Asparagus racemosus was used as a bitter, sweet, emollient, cooling, nervinetonic, constipating, galactogogue, diuretic, carminative, appetizer, stomachic, antispasmodic and tonic. It is also used in nervous disorders, dyspepsia, diarrhea, dysentery, tumors, inflammation, burning sensation, hyperdipisia, nephropathy, agalactia and general debility (Chetri et al.,2005 and Visavadiya et al., 2009).

This study was carried out to study the effect of Asparagus racemosus on the Alloxan induced diabetes in rats.

The steps of studyinclude

1- Determination the serum activities of

Fasting blood sugar (FBS), Urea (Ur) and Creatinine (Cr) *After (30) days of Asparagus administration.*

2- Detection the histopathological:- study the enhancement of Asparagus racemosus on the kidney toxicity in diabetic case.

2-MATERIALS AND METHODS

2.1 Experimental animals

A total number of 30 male albino rats, weighting 170-200 g were used in the experimental investigation of this study. Rats were obtained from the laboratory animal's research center, faculty of veterinary medicine, Benha University. Animals were housed in separate cages, fresh and clean water was supplied adlibtium. Rats were kept at a constant environmental and nutritional condition throughout the period of the experiment. Animals were left for 10 days for adaptation period before the beginning of the experiment.

2.2 Natural products

Asparagus racemosus powder was obtained from Shaanxi Pioneer Biotech Co. Ltd from China.

2.3 Drugs and dosage

2.3.1 Alloxan powder of European Egyptian Pharm Alexandria Egypt.

2.3.2 Kits for FBS obtained from Spinreact, Urea, Criatinine obtained from Diamond.

2.3.3 Experimental design

At the beginning of the experiment, rats were divided into 3 groups

Group 1: - normal control: Composed from 10 rats were fed normal diet.

Group 2: - diabetic group(alloxan group): Rats in this group were injected once with alloxan monohydrate in sterile normal saline at dose of 120mg/kg BW intraperitoneally (Oyedepo et al., 2013).

Induction of diabetes

The animals were fasted for 12 h. prior, and alloxan freshly prepared in saline was administrated intraperitoneally (i.p.) as a single dose of 120 mg/ kg.

Alloxan is capable to producing fetal hypoglycemia by massive pancreatic insulin release, so rats were treated with 20% of glucose solution (15-20 ml) intraperitoneally after 6 h.

The rats were kept for 24 h on 5% glucose solution bottles in cages to prevent hypoglycemia (Stanely et al., 1998).

Group – 3 (alloxan and A.R. group)

Rats were injected as group 2 by alloxan and treated with A.R. after alloxan injection by 48

hours Asparagus group: 10 rats taken Asparagus racemosus powder by dose 500 mg/kg daily. P.O. for (30) days. (Kumar et al., 2011and Sara et al., 2013).

2.3.4 Sampling

Blood samples were collected from all animals at 30 day from onset of treatment with Asparagus racemosus. The samples were allowed to coagulate and then centrifuged at 3000 rpm for 5 min; the serum separated was used for determination of FBS, Cr, and Urea activities.

The kidney part was fixed in 10%neutral buffered formalin for 48 hours. Thereafter, the fixed specimens were processed through the conventional paraffin embedding technique (dehydration in ascending grades of ethyl alcohol, clearing in different changes of xylene and embedding in different changes of paraffin was at (60°C). Paraffin block were prepared and 5 microns thick sections were stained by hematoxylin and eosin (H&E) according to the method described by (Culling, 1983).

2.3.5. Biochemical Analysis

Serum FBS,Cr, and Urea were determined according to the method of (Young. 2001).

2.3.6. Statical Analysis

Results data were analyzed using SPSS (Statistical package for social science; ver. 10.0) and the significance among the samples was compared at $P<0.05$.Results were represented as mean ± SD.

3-RESULTS

3.1. Effect of alloxan and *Asparagus racemosus* on serum fasting blood suger of rats (U/L):-

The data represented in table (1) showed that administration of alloxan nonsignificantly increase the serum sugar level.

Administration of Asparagus racemosus by dose of 500 mg/ kg P.O. decrease the serum sugar levels. When compared to alloxan group.

3.2. Effect of alloxan and Asparagus racemosus on serum creatinine and urea of rats.

The data represented in tables (1) showed that administration of alloxan nonsignificantly increase the serum creatinine levels and non-significantly increase the serum urea.

Administration of Asparagus racemosus by dose of 500 mg/ kg P.O. significantly decrease

the serum creatinine levels and significantly decrease the serum urea. When compared to alloxan group.

The creatinine and urea levels of Asparagus racemosus treated group is nearly to that of normal group.

3.3. Histopathological findings

Control group

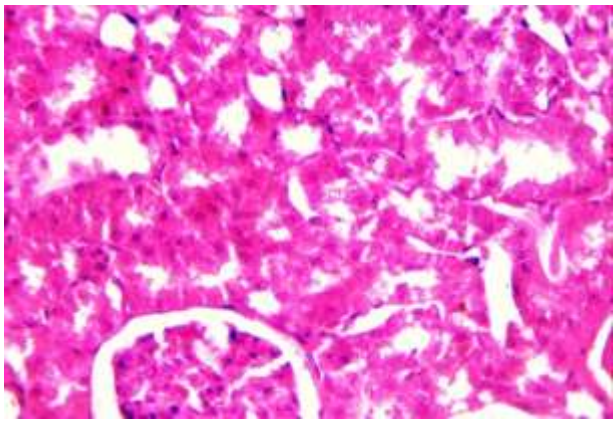

Fig. 1: Kidney of control rat showing normal histological structure H&E stain x20.

The Microscopical examination of kidnies of normal controlled rats howing normal histological structures (Fig 1).

Alloxan treated rats showed

The group of rats Admenstrated alloxan by dose 120 mg/kg showing the following pathological change

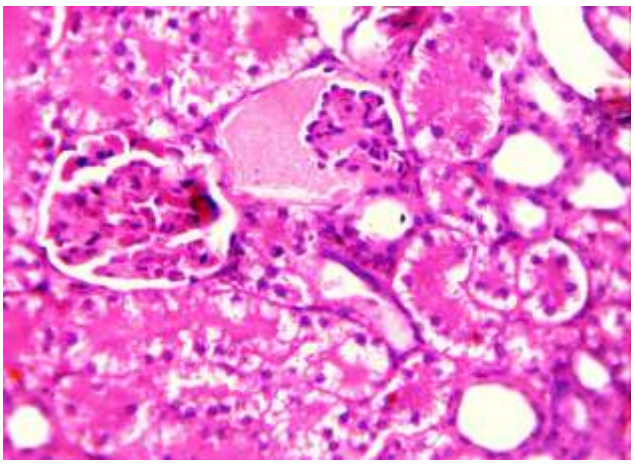

Fig. 2: Kidney of Alloxan admenstrated rat showing shrinkage of glomerlui H&E stain x200.

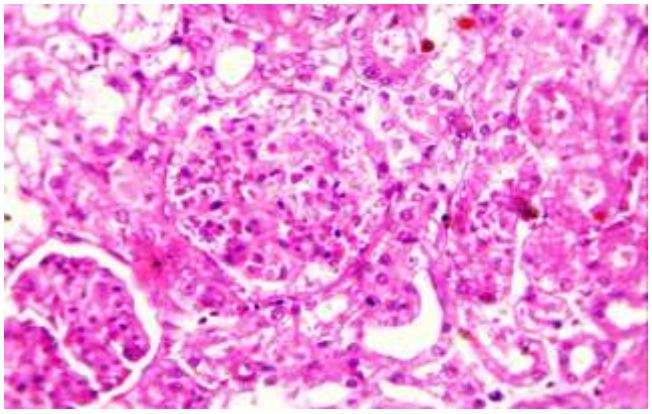

Fig. 3: Kidney of Alloxan admenstrated rat showing eosinophilic debris in Bowman space H&E stainx 200.

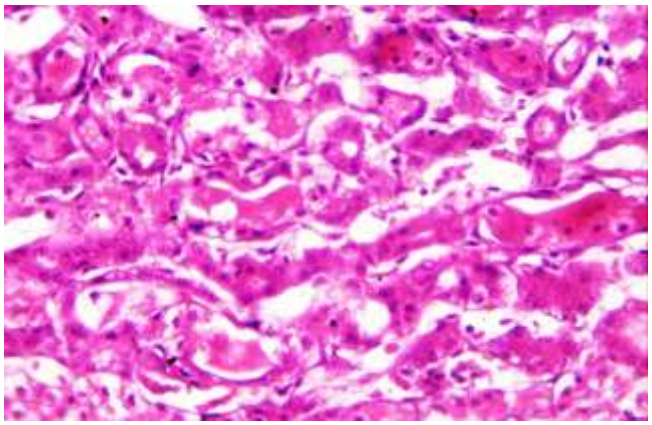

Fig. 4: Kidney of Alloxan admenstrated rat showing coagulation necrosis of renal tubules H&E stain x 200.

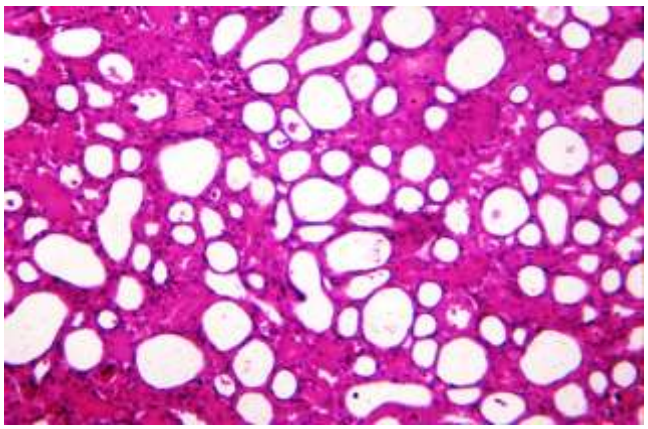

Fig. 5: Kidney of Alloxan admenstrated rat showing sever dilataion with systic formation H&E stain x200.

The kidnies of rats of this group were suffering from severe congestion and dilatation of the renal blood vessels and intertubular blood capillaries in addition to glomerulituft.

The most prominent microscopical findings of these kidnies were represented by shrinkage of the glomerlui tuft with presence of eosinophilic finely granular hemogenous substance in the Bowman space (Fig 2).

However some of the affected glomeruli showing disintegration of the glomerlui tuft with presence of eosinophilic debris in Bowman's space in addition to renal tubules showing complete discumation of the epithelial lining or showing vaculation of the epithelial lining the tubules (Fig 3).

However most of the renak tubules were suffeering from coagulative nicrosis with presence of eosinophilic cast in the lumen of renal tubules (Fig 4).

The renal tubules showing sever dilatation with systic formation (Fig 5).

Asparagus racemosus treated rats showed

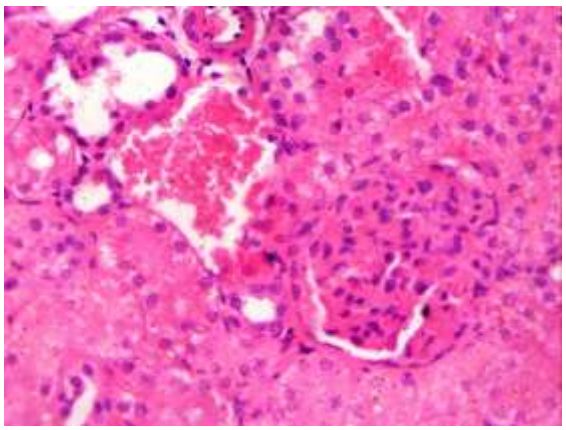

Fig. 6: Kidney of Asparagus administrated rat showing congestion of renal blood vessels. H&E stain x200.

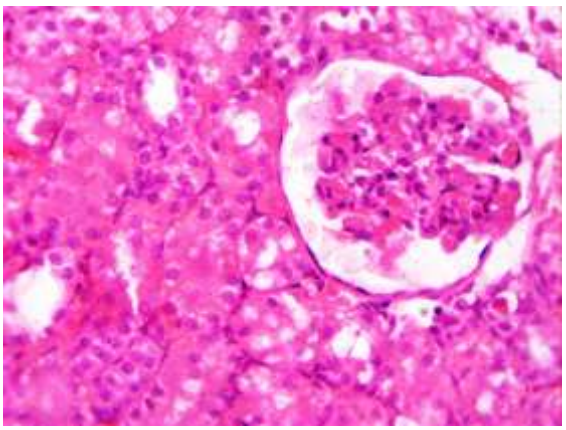

Fig. 7: Kidney of Asparagus administrated rat showing chrinkage of glomeular toftes. H&E stain.

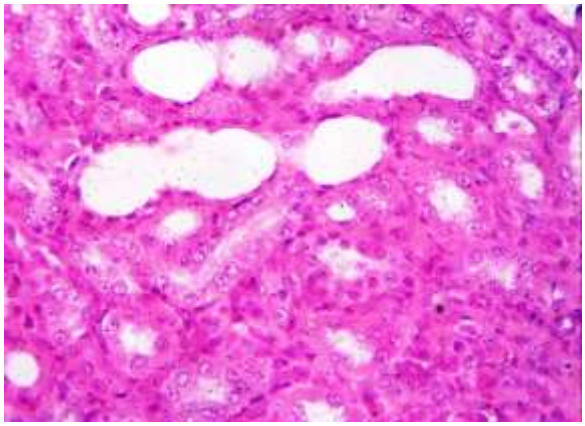

Fig. 8: Kidney of Asparagus administrated rat showing mild systic dilatation and mild degenerative changes. H&E stainx200.

Kidney of this group showing congestion of renal blood vessels and intertubular blood capillary (fig 6).

The glomerular showing mild shrinkage of their glomerular toftes with presents of fine eosinophilic homogenous substsnces in the Bowmans space.(fig 7).

The renal tubules were suffering from mild systic dilatation and mild degenerative changes (fig 8).

Table 1: The effect of Asparagus racemosus and silymarin on serum FBS, Cr, and Urea, in Alloxan induced diabetes in rats after (30) days:

	FBS	Cr	Urea
Control	*68.8±4.979*^{b}	*0.5600±0.051*^{b}	*38.8000±2.2676*^{b}
Alloxan	*222.8±24.40*^{a}	*2.0750±0.4148*^{a}	*146.62±28.2030*^{a}
Asparagus racemosus	*73.142 9±5.217*	*0.4857±0.077*	*44.1429±5.2751*^{b}

Data was expressed as mean±SE. SE = standard error.

The mean values with different super script letter within the same column were significantly differing at $P < 0.05$.

4-DISCUSSION

The obtained data demonstrated in table (1) revealed that the administration of Asparagus Racemosus to diabetic rats decrease the serum FBS, Cr, and urea activities when compared with alloxan treated group.

4.1. Effect of Asparagus racemosus and alloxan on serum Fasting blood suger (FBS)

The obtained data demonstrated in table (1) revealed that the administration of Asparagus racemosus to Diabetic rats decrease the serum fasting blood suger when compared with alloxan group.

The present study showed that oral administration of alloxan at a dose of 120mg/kg b.wt IP non significantly increase the serum sugar level.

Administration of alloxan causes severe damage to the insulin secreting β cells of pancreas which leads to hyperglycemia these results matched with that reported by Guno et al 2008.

That matched with Rahul et al 2012 they Said the treatment with Asparagus racemosus 100&250 mg/kg ameliorared plasma glucose level when compared to diabetic control rats.

As well as Raghavan et al., 2004 said 4 weeks treatment with Asparagus racemosus in diabetic rats significantly decreased in plasma glucose level which might be attributed to its anti-diabetic and insulin secretory activity.

4.2. Effect of Asparagus racemosus and Alloxan on Urea and Creatinine levels

The obtained data in table (1) demonstrated revealed that the administration of Asparagus racemosus to diabetic rats decreased the serum Urea and Creatinine levels, when compared with alloxan group.

Our results as that reported by Godwin and Alvin 2014 they said in diabetic control group, the blood urea level was significantly increased when compared to normal control group. In diabetic rats treated with Asparagus racemosus 400 mg / kg significantly decreased the blood urea level when compared to diabetic gr0up.as well as the blood creatinine level of diabetic conrtol group was increased significantly when compared to normal control group. But diabetic rats treated with Asparagus racemosus 200 mg/kg and 400 mg/kg showed significant decrease in bloodcreatinine level when compared to diabetic control group.

In diabetic rats treated with the Asparagus racemosus blood urea level was significantly decreased when compared to diabetic control group. Blood urea nitrogen is formed when protein breaks down, which is another marker of kidney function. When blood flows through the body, protein circulates to cells. Cells use the protein and excrete the waste products urea which is filtered out of the blood by kidneys Urea also contain nitrogen. In diabetic nephropathy urea and nitrogen stay in the blood. The Asparagus racemosus restored the elevated blood urea

nitrogen in diabetic rats Dabal, 2010.

But diabetic rats treated with Asparagus racemosus showed significant decrease in blood creatinine level when compared to diabetic control group.

Creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate (Ramesh et al 2007).

If serum creatinine levels increased due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume The Asparagus racemosus significantly reversed the elevated blood creatinine in diabetic rats (Patel et al., 2009).

Also Rahul et al., 2012 reported the diabetic rats exhibit marked increase in creatinine and urea levels as compared to normal rats and treated group with *Asparagus racemosus* 250 mg/kg showed significantly decreased plasma creatinine and urea levels as compared to the diabetic rats.

As well as Hannan et al., 2007 said 4 weeks treatment with Asparagus racemosus in diabetic rats significantly decreased in plasma creatinine and urea levels.

Oral administration of plant extract has revived the structure and function of kidney affected by diabetes. Regeneration of epithelium, expansion of glomeruli, disappearance of heamorrhages and cytoplasmic debris leads to decrease the levels of serum and creatinine were the major changes observed besides lowering blood glucose. This also reported by Kumar and Janardhana, 2011.

4.3. Effect of Asparagus racemosus and alloxan on kidney histopathology of rats

Godwin and Alvin, 2014 reported the histopathological observations of the rat kidneys revealed that the normal group rats showed normal glomeruli and kidney tubules with healthy epithelial cells. The kidney of diabetic control group rats shows thickening of vesicles, disrupted tubules, degeneration and necrosis of epithelial cells and intertubular haemorrhage. But the kidneys of diabetic rats treated with REAR 400 mg/kg showed regeneration of tubular epithelium and moderate intertubular haemorrhage.

Kiran et al., 2012 recorded diabetic rats treated with Asparagus racemosus significantly decreased the kidney weights when compared to diabetic control rats. The increase in

kidney weight was due to renal enlargement, which is one of the key features occuring during nephropathy, a hypertrophy and hyperfunction of the kidneys with typical increase in kidney size and glomerular filtration rate can be observed. This is due to the factors such as glomerular hypertrophy and nephromegaly (whole kidney enlargement), an early feature of both experimental and human diabetes occurs due to combination of tubular hypertrophy hyperplasia and interstitial expansion.

Godwin and Alvin, 2014 saw the histopathological observation of the rat kidneys revealed that the normal control group rats shows normal glomeruli and tubules with healthy epithelial cells. The kidneys of diabetic control rats shows thickening of vesicles, disrupted tubules, degeneration and necrosis of epithelial cells and inter tubular haemorrhage. But the kidneys of diabetic rats treated with different doses of *Asparagus racemosus* 50 mg/kg, 100 mg/kg and 200 mg/kg, showed regeneration of tubular epithelium depicting normal tubules with intact epithelium and presence of few RBCs in between the tubules. The report of histopathological studies of rat kidneys strongly supports the outcome of the study by restoring the kidney damage.

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