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RESEARCH ARTICLE

EFFECT OF *MORINGA OLEIFERA*, *HORDEUMVULGARE* AND THEIR MIXTURE ON MICROVASCULAR COMPLICATIONS OF DIABETES MELLITUS

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ABSTRACT

Rupture Diabetes mellitus (DM) is a heterogeneous disease. One of the chief injuries arising from hyperglycemia is injury to vasculature, which is classified as either small vascular injury (microvascular disease) or injury to the large blood vessels of the body (macrovascular disease). *Moringa oleifera* and *Hordeumleporinum* methanol extract mixture in Microvascular Complications (nephropathy disease).Models of STZ-induced diabetic nephropathy injected once into the tail vein of grouped Wistar rat with STZ 60mg/kg in sodium citrate buffer (1ml/kg). Biochemical assessment of renal injury by urine albumin excretion is considered to be one of the most sensitive markers of renal injury. Blood sample for estimation of creatinine, blood urea nitrogen, uric acid and total protein. Histopathological examination at the end period and Statistical analysis was performed as the mean± standard deviation (SD).

Aims and Objective: To evaluate the effect of *Moringa oleifera*, *Hordeumvulgare* and their mixture in Microvascular Complications of Streptozotocin (STZ) induced Diabetes Mellitus Wistar rat.

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INTRODUCTION

Diabetes is characterized by chronic hyperglycaemia caused by defects in insulin secretion, insulin action, or both, resulting in impaired function in carbohydrate, lipid, and protein metabolism (Vlad and Popa, 2012). Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy). Effective control of hyperglycemia in diabetic patients is critical for reducing the risk of micro- and macrovascular complications (Khandouzi *et al*., 2015). Natural sources play an important role in the management of diabetesmellitus, especially in developing countries, delaying the development of diabetic complications and correcting the metabolic abnormalities (Al-Logmani and Zari, 2011). *Moringa oleifera* and *Hordeumvulgare* are among the natural sources reported to have beneficial effects in the treatment of many diseases. Barley Grass (*Hordeum vulgare*) is the common source of the grain barley, but as a health food the powdered leaf is also very popular.

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Consumption of barley which contains many medicinally active phytocompounds is usually associated with improvement in health. Barley sprouts, which are the young leaves of barley harvested approximately 10 days after sowing the seeds, have recently received much attention as a functional food in numerous countries. The main constituents of Barley include important antioxidants such as vitamin E, phytic acid, selenium, tocotrienols, and various phenolic acids. After the consumption of Barley, these antioxidants are released at differential rate throughout the gastrointestinal tract over a long period of time. *Moringa oleifera* Lamarck (*Moringa*) is the cultivated species of the genus *Moringa* of the family *Moringaceae*. Several health benefits were reported as a result of supplementation with *Moringa leaves* (Mahajan *et al*., 2007; Hamza, 2010; Yassa and Tohamy, 2014). *Moringa* has also nutraceutical uses and is used in treatment of hypercholesterolemia and hyperglycemia, and also, as a nutritional supplementation, it can be prescribed as food appendage for coronary artery disease patients along with their regular medicines. (Rajanandh *et al*., 2012)

MATERIALS AND METHODS

*Moringa oleifera*leaves were collected from the campus of college and dried the leaves in 60c oven. *Hordeumvulgare*were cultivatedand the grass only harvested after 14th day. and dried

Table 1. Effect of treating diabetic nephropathy rats with methanolic extracts of *Moringa oleifera*, *Hordeumvulgare* and their mixture for 4 weeks on kidney functions and urine

Group	Creatinine mg/dl	Blood Urea Nitrogen mg/dl	Total Protein g/dl	Blood Glucose mg/dl	Urine albumin
Control	2.17±1.28	63.10±3.10	12.30±1.42	258±3.96	32.44±0.13
Moringa oleifera 50mg/kg	0.75±1.71	45.58±0.13*	8.14±3.96	100.8±5.20	21.32±0.12*
Hordeumvulgare 50mg/kg	0.95±1.89*	50.01±1.96	7.61±1.26**	94.48±3.36*	17.27±0.01**
Mixture (Moringa oleifera & Hordeumvulgare) 50mg/kg	0.87±2.32**	40.12±0.03**	7.84±2.05*	132±2.27	21.29±0.21
Mixture (Moringa oleifera & Hordeumvulgare) 1000mg/kg	0.428±2.32**	21.82±1.03***	5.62±2.05**	72.2±4.07**	15.31±0.31**

*Values are represented as mean ±SD, where n=6, ***P<0.001 as compare to normal control, **p<0.01 as compare to control. ANOVA analysis: within each row, means with different superscript.*

in oven and powdered for the extraction. Both dried powder sample were extracted by methanol extraction and evaporated with rotary evaporator

Preliminary Phytochemical Screening

Preliminary phytochemical screening was done for the presence of carbohydrates, proteins, saponins, alkaloids, flavonoids, tannins, tri-terpinoids and phenolic compounds according to the procedure described in "Textbook of Practical Pharmacognosy" by C.K. KOKATE

Microvascular Complication Diabetic nephropathy

Animals

Thirty Six Wister rats weighing 90-220gm were obtained from Dubai college animal house. All the animals were weighed and grouped into 5 groups(n=4) and kept under light-dark cycle and given drinking water *ad libitum*. All grouped animal were administration daily through oral route of methaonal extract of test sample until experiment period. Group 1 as control received normal saline; group 2 *Moringa oleifera* 50mg/kg, group 3 *Hordeumvulgare* 50mg/kg and group 4 &5 their mixture 1:1 *(Moringa oleifera Hordeumvulgare) 50&100mg/kg*

Diabeticnephropathy in rat

Models of STZ-induced diabeticnephropathy injected once into the tail vein with STZ 60mg/kg in sodium citrate buffer (1ml/kg). (Al-Malki, 2013) Following the STZ injection, rats should be given drinking water supplemented with sucrose (15 g/L) for 48 h, to limit early mortality as stores of insulin are released from damaged pancreatic islets., rats should be assessed for hyperglycaemia and those with fasting blood glucose of over 15 mmol/L (280 mg/dL), should be included in studies of diabetic nephropathy. To prevent subsequent development of ketonuria, diabetic rats should be given daily subcutaneous injections of long-acting insulin (2–4 U/rat) to maintain blood glucose levels in a desirable range (16–33 mmol/L, 300–600 mg/dL)

Blood Glucose Monitoring

Blood glucose levels were determined pretest and weekly. Hyperglycemia was evident by 2-3 weeks. Control glucose was in the 153 mg/dl±16 range and STZ-treated glucose levels were consistently in the 765±98 mg/dl range from Week 1-4.

Collection of blood samples and biochemical analysis from serum

At the end of the experiments on the 30thday, blood samples were collected 20 h from fasting using light ether anesthesia from retro orbital sinus puncture

Biochemical assessment of renal injury

Urine albumin excretion is considered to be one of the most sensitive markers of renal injury. Measurements of UAER normally require rodents to be maintained in metabolic cages for 24 h to collect urine. The albumin: creatinine ratio in urine can also be used to measure diabetic renal injury in rodents. Blood sample for estimation of creatinine, blood urea nitrogen, uric acid and total protein.

Histopathological Estimation

At the end of the experiment , all the macro & micro vascular groups animals were anesthetized by light ether decapitation of the animals, the kidney were removed and fixed in 10% neutral-buffered formaldehyde solution for histopathology studies.

Statistical Analysis

Statistical analysis was performed as the mean± standard deviation (SD). The results were analyzed for statistical significance by unpaired t-test followed by Dunnet ‟sposthoc test of significance. P value less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Effect of *Moringa oleifera*, *Hordeumvulgare* and their mixture on Serum level

The mean values of urea, creatinine, and uric acid in the serum of the positive control group (G1) were significantly ($P < 0.001$) increased as a result of induced diabetes shown in Table 3. Treating these diabetic rats with methanolic extracts of *Moringa oleifera*, *Hordeumvulgare* and their mixture *in* G2, G3, G4 and G5, respectively G3&G5 significantly ($P < 0.001$) decreased urea, creatinine, and uric acid levels compared with those of the positive control group (G1). The mixture G5 of their both methanolic extract of *Moringa oleifera* and *Hordeumvulgare* were more effective than that of alone G2&G3. Also, Table 1 shows that the mean values of urinary albumin of the control group were significantly ($P < 0.001$) increased compared to other groups. Treating the diabetic nephropathy rats in G2, G3, G4 and G5 with methanolic extract of *Moringa oleifera*, *Hordeumvulgare* and their mixture , respectively, significantly ($P < 0.001$) decreased urinary albumin and increased creatinine in urine when compared with those of the control (G1).

Histopathology studies of Kidney tissue

Microscopically, the histopathological examination of the kidney tissues of rats in the group control (Figure 1(a))., which

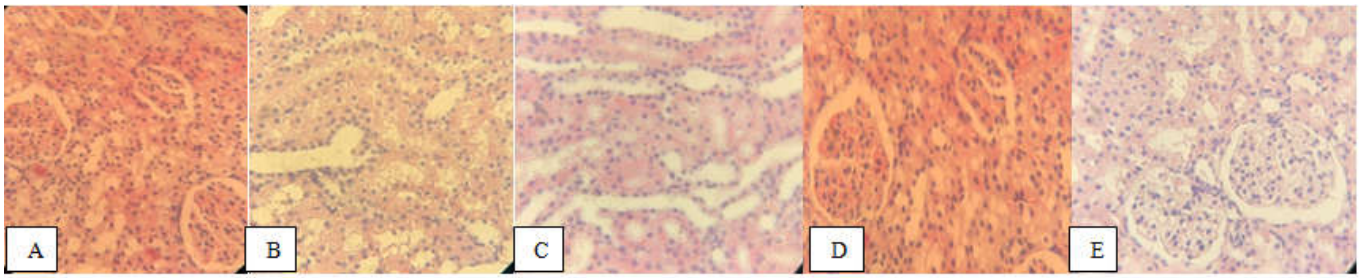

Figure 1: Shows the kidney tissue of rat from the control group 1 showing A collapsed glomerular tuft with marked tubular atrophy, interstitial inflammation, and interstitial hemorrhage, (c) & (d) kidney of diabetic rat treated with *Moringa oleifera*, *Hordeumvulgare mixture* methanol extract showing normal glomeruli and regenerated tubules with interstitial hemorrhage, and (e) kidney of diabetic rat treated with methanol *Moringa oleifera*, *Hordeumvulgare mixture* extract (G5) showing near normal renal cortical tissue.

Figure 1. Histopathology studies of *Moringa oleifera*, *Hordeumvulgare* and their mixture in diabetic nephropathy induced animal's kidney tissues

showed a collapsed glomerular tuft with marked tubular atrophy associated with interstitial inflammation and interstitial hemorrhage (Figure 1(b),(c),(d)&(e)). Meanwhile, the kidney sections of diabetic rats in G3 treated with the *Moringa oleifera*, *Hordeumvulgare* and their mixture of methanol extract for 4 weeks seemed to be restoring the normal appearance of glomeruli and regenerated tubules with interstitial hemorrhage and the kidney nearly restored the normal cortical tissue shown on (Figure 1(c) & (e)).

Conclusion

Diabetes mellitus is metabolic disorder leading to hyperglycemia, which later develops to micro- and macrovascular complications. The induction of experimental diabetes in the rats using chemicals which selectively destroy pancreatic β- cells is very convenient and simple to use as streptozotocin (STZ) that acts as diabetogenic agent mediated by reactive oxygen species. In the present study, induction of diabetes using streptozotocin (STZ) at a dose of 60mg/kg in rats of the control group showed significant increase in serum glucose level compared with the control group. The concurrent oral administration of *Moringa oleifera*, *Hordeumvulgare and their mixture* methanolic extract to the diabetic nephropathy rats of G2, G3 and G4, respectively, for 4 weeks significantly decreased glucose levels most probably due to their antioxidant chemical contents. STZ administration increased serum renal markers in rats, for example creatinine , urea and total protein level as a result of diabetic nephropathy which is considered a major complication of diabetes .34 The mixture of *Moringa oleifera*, *Hordeumvulgare* methanolic extract group 5 shows highly significant effect in blood serum level to decrease the creatinine , urea and total protein level compared to control group.

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