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Reno-Protective Effects of Egyptian Herbal Formula during Experimental Diabetes

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Abstract

Background: The long term effects of diabetes mellitus include progressive development of the specific complications of nephropathy that may lead to Diabetic nephropathy (DN).

The objective of this study was to investigate the development of Diabetes mellitus type 2 (T2D) in Streptozotocin-nicotinamide model followed by DN to estimate the Reno-protective effects of an Egyptian Herbal mixture formulation (HMF) consisting of (Panax ginseng, *Momordica charantia* and Gymnema sylvestre) on diet intake, bodyweight, water consumption, urine output, urine sugar, urine protein, fasting blood glucose, insulin, Glomerular filtration rate, glycosaminoglycans, renal oxidative stress marker malondialdehyde (MDA) in urine, blood and renal tissue of rats.

Method: White male albino rats weighing 150-200 gm about 4 month old were used for this study. 10 rats were fed a normal basal diet (Cr), 30 overnight fasted rats were Non insulin dependent diabetes mellitus (NIDDM)-induced by IMI of 60 mg/kg Streptozotocin (STZ) and thereafter 120 mg/kg nicotinamide was injected after 5 min. The rats found with permanent NIDDM were used for anti diabetic study and were equally divided into 2 subgroups each one include 10 rats. The first group with no supplement, the 2nd group received T2D+HMF. HMF was administered at 56 days (start time for treatments) for 8 weeks. Diet intake, bodyweight, water consumption, urine output, urine glucose, urine protein, fasting blood glucose, insulin level, Glomerular filtration rate, renal glycosaminoglycans were measured.

Furthermore, renal oxidative stress marker (MDA) and renal enzymes involved in the synthesis/degradation of glycosaminoglycans (L-glutamine fructose-6-phosglucosaminidas -phate aminotransferase, N-acetyl and b-glucuronidase) were analyzed.

Results: HMF showed a marked decrease in diabetes induced polydypsia, polyuria, urine sugar, hyperglycaemia, glomerular filtration rate, proteinuria and renal glycosaminoglycans. Increased activities of renal enzymes involved in the synthesis/degradation of glycosaminoglycans (L-glutamine fructose-6-phos-phate aminotransferase, N-acetyl glucosaminidase and b-glucuronidase) were significantly lowered by HMF supplementation during diabetes. Glycosaminoglycans and their components (aminosugar, uronic acid) revealed decreasing trends during diabetes and HMF effectively countered this decrease. The increase in renal oxidative stress markers (MDA) was significantly decreased by HMF supplementation during diabetes.

Conclusion: STZ induced-diabetes (DM) associated with hypoinsulinemia, renal function disturbances due to imbalance in synthesis/degradation of glycosaminoglycans and defective antioxidant stability that may have implications for the progress of DM and its related problems.

Treatment with HMF improved DM and its associated renal problems in different degrees through controlling the level of glycosaminoglycans and oxidative stress during diabetes thus prolonging late complications of diabetes.

Keywords: Diabetic nephropathy; Glycosaminoglycans; Oxidative stress

Abbreviations: AGE: Aqueous Ginseng Extract; BME: Bitter Melon Extract; DN: Diabetic Nephropathy; ESRD: End Stage Renal Disease; GAGs: Glycosaminoglycans; GFAT: Glutamine Fructose-6-Phosphate Amido Transferase; GFR: Glomerular Filtration Rate; GSE: Gymnema Sylvestre Extract; HMF: Herbal Mixture Formulation; MDA: Malondialdehyde; NAGase: N-Acetyl-β-D-Glucosaminidase; T2D: Diabetes Mellitus Type 2; UDP-GlcNAc: UDP-N-Acetylglucosamine

Introduction

Diabetic nephropathy (DN) remains the most common cause for end stage renal disease (ESRD) as the burden of diabetes increases worldwide. Nearly one-third of patients with diabetes develop nephropathy making early diagnosis critical in preventing long term kidney loss [1].

The kidney consists of four basic tissue types: vessels, glomeruli, tubules, and interstitium. Each of these types may be influenced by different pathophysiologic mechanisms associated with DN. Despite their variability, these mechanisms share common biochemical mechanisms including hyperglycemia and oxidative stress that progress to end-stage renal disease [2].

DN is characterized by Proteinuria that is considered to be a prognostic marker in renal disease. Proteinuria is the hallmark of diabetic nephropathy. Clinically evident proteinuria is considered after a phase of microalbuminuria where the albumin concentration is too low to be detected by dipstick method but can be measured by

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radioimmunoassay or immuno-turbidimetric method. When urinary albumin excretion exceeds >300 mg/day it is considered as overt proteinuria [3].

DN is characterized by hyperglycemia which is a primary cause of glomerular injury in patients with diabetic nephropathy as it responsible for the development and progression of diabetic nephropathy through metabolic derangements, including increased oxidative stress and accumulation of advanced glycation end products, as well as such hemodynamic factors as systemic hypertension and increased intraglomerular pressure [4].

Glycosaminoglycans (GAGs) are long unbranched mucopolysaccharides consisting of a repeating disaccharide unit. It has been proposed that hemodynamic alterations and structural changes in glomerular basement membrane glycosaminoglycans may play a role in the pathogenesis of DN [5]. Moreover, GAGs strongly influence thickness, integrity and permselectivity of the endothelial glycocalyx which its composition is strongly alterated in diabetes patients, who typically show early sign of renal damage [6]. Glucosamine is a precursor in the synthesis of GAGs. Glucosamine also is a component of the hexosamine pathway, which has been demonstrated to be a mechanism by which glucose leads to diabetic complications [7]. In the hexosamine pathway, glucose is metabolized to hexosamine via the transfer of an amide group from glutamine to fructose 6-phosphate to form glucosamine 6-phosphate. This subsequently is metabolized to hexosamines such as UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine, which are the building blocks for glycosaminoglycan synthesis. The rate-limiting step in the hexosamine pathway is the initial transfer of the amide group from glutamine, which is catalyzed by the enzyme glutamine: fructose-6-phosphate amidotransferase (GFAT; EC 2.6.1.16). GFAT activity is upregulated by high glucose and insulin levels [8]. N-Acetyl-β-D-glucosaminidase (NAGase) is an enzyme in lysosome which is contained abundantly in the renal tubular epithelia and is related to the degradation of mucopolysaccharides and glycoproteins.

It is recognized that the NAGase increases in case of diabetes. In the field of clinical and animal experiments, the measurement of the NAGase has attracted an interest in connection with diagnosis of various renal diseases and also as an indicator in examining nephrotoxicity [9]. Members of the glycosaminoglycan family vary in the type of hexosamine, hexose or hexuronic acid unit they contain. A uronic acid is a sugar acid with both a carbonyl and a carboxylic acid function while an amino sugar contains an amine group in place of a hydroxyl group [10].

Oxidative stress plays a crucial role in chronic complications of diabetes such as (DN).As a result of oxidative stress Peroxidation of lipid membrane occurs related to the pathogenesis of diabetes and its complications [11].

Lipid peroxide in the blood provides useful information for the prognosis of diabetes and its renal complications. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in diabetic patients [12].

Hence, DN is a multifactorial target of new therapies. Hypoglycemic agents that not only lower blood glucose but also stimulate the formation of GAGs. Also, Antioxidants are very important to prevent the effect of oxidative stress on the pathogenesis of DN [13].

Specific therapies to inhibit or reverse the progression of advanced stages of diabetic nephropathy have become an important issue in biomedical research. The ideal treatment/management for diabetes would allow the patient to remain not only symptom free but in good health with no or minimum abnormal metabolic state and to escape long term complications [14].

Ayurvedic formulations are used to treat a wide variety of diseases including diabetes mellitus as certain herbs were reported to lower blood glucose, although their efficiency is still debatable. Firstly, each herb contains a lot of components, few of which may be therapeutically effective. Secondly, different parts of an herb have different ingredient profiles [15]. Moreover, extraction methods may yield different active ingredients. Finally, herbal formulae containing multiple herbs may have synergistic effects [16].

Panax quinquefolius L (PQ. family Araliaceae) commonly known as American ginseng. The genus name of Panax ginseng is derived from the Greek terms 'pan', meaning all and 'axos' meaning cure. The species name was derived from the Chinese word 'jensheng', implying the herb whose roots resemble the human body [17].

Aqueous extracts of the root of ginseng (AGE) contain ginsenosides which are biologically active compounds. In addition to ginsenosides, some glycans and peptides isolated from ginseng root may also have hypoglycemic effect in mouse models of diabetes. In terms of stability, Ginsenosides can be administrated orally or through injection. When ginseng is taken orally; ginsenosides should be the major active compounds in the blood. Ginsenosides, the main pharmacologically active constituents of ginseng are derivatives of the triterpene dammarane structure [18].

Momordica charantia (MC, family Cucurbitaceae), commonly known as karela, bitter gourd, balsam pear, or bitter melon (BM) is a tropical and subtropical vine, widely grown as edible fruit among the most bitter of all vegetables [19].

Aqueous extracts of the fruit of *Momordica charantia*, Bitter melon extract (BME) include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids. The hypoglycemic constituents are a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids. These constituents are concentrated in the fruits which have also been shown to have the most pronounced hypoglycemic activity compared to other plant parts [20].

Gymnema sylvestre (GS, family Asclepiadaceous), is a tropical plant of the milkweed family with an ancient Sanskrit name meaning "destroyer of sugar". Aqueous ethanol extract of the leaves of Gymnema *sylvestre*, Gymnema *sylvestre* extract (GSE) contain gymnemic acid, tartaric acid, gurmarin, calcium oxalate, glucose, stigmasterol, betaine, and choline. The gymnemic acid components are believed to block the absorption of glucose in the small intestine. Both gurmarin (another constituent of the leaves) and gymnemic acid have been shown to block sweet taste in humans [21].

Material and Methods

Diet

Composition of the experimental diet (g/kg diet) was according to the formula of [22]. It included the normal diet for control rats (fat 5%, carbohydrates 65%, proteins, 20.3% fiber 5%, salt mixture and 3.7% vitamin mixture 1%). Diets were purchased from El-Gomhoria Company, Cairo, Egypt.

Experimental animals

30 white male rats (Sprague dawley strain) weighing 150-200 gm, about 4 month old were used for this study. Adult male rats were used, since male in generally are more susceptible to diabetes than females and young ones have a higher resistance to the diabetogenic effect of streptozotocin than adult.

Rats were purchased from the National Research Center, Cairo, Egypt. All animals were housed in stainless steel cages contain barriers for each rat for individual housing and the cage contain 5 rats and each rat had a tag number.

They kept under standard environmentally controlled, clean-air room with temperature 24 ± 5°C, illumination (12 h light/12 h dark cycles), a relative humidity of 60 ± 4%, and water and rodent chow were available ad libitum throughout the period of the investigation. They were housed for two weeks after their arrival in the laboratory for accommodation.

Our work was carried out in accordance with the guidelines of Beni Suef University for animal use and approved by Ethics and Animal Care Committee. These animals were used for induction of Diabetes mellitus.

Plant material preparation of the herbal formulation

Herbs were purchased from local Mohey El-Attar Company in El-Minia city. Identification and extractions of medicinal plants were completed in department of Pharmacognacy, faculty of pharmacy, El Minia University.

The preparation, composition dose of the herbal formula of each herbal extract in the HMF and the identification of their main chemical groups were realized as previously described as follow:

American ginseng (*Panax quinquefolius*) (300 mg/kg BW of rats /day interperitineally -administrated [23]. Bitter melon (*Momordica charantia*) (400 mg/kg BW of rats /day interperitineally -administrated according to [24] and Gymnema sylvestre 300 mg/kg BW of rats /day orally) [25].

Animals received daily intraperitoneal (IP) injections of (AGE, BME) and orally for GSE) for 28 consecutive days.

The extraction was done by water extract for *Panax quinquefolius* and *Momordica charantia* but in aqueous ethanol (50% 6I) for *Gymnema sylvestre*.

After isolation by several column chromatographic steps from the extracts and characterization by spectroscopic methods, the main compounds were identified as ginsenosides from Panax quinquefolius extraction. Charantin, Oleanolic acidglycoside, Citrulline and Vitamine c were isolated from *Momordica charantia* extraction.Triterpene saponine (Gymmmenic acid) Glabridin from Gymnema sylvestre extraction

Experimental induction of type 2 diabetes in rats

T2D was induced in overnight fasted rats by a single intraperitonial injection of 45-mg/kg streptozotocin, 15 min after the i.p. administration of 110-mg/kg-body weight of nicotinamide. STZ was dissolved in 0.1 M-citrate buffer (pH 4.5) and nicotinamide was dissolved in physiological saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The rats found with permanent NIDDM were used for the study [26].

Experimental procedure

In the experiment, a total of 30 male rats (20 STZ- nicotinamide induced diabetic surviving rats, 10 control rats) were used. The rats were divided into three groups of ten rats each: (i) control rats (vehicle treated); (ii) diabetic rats; (iii) diabetic rats administered HMF (i.p. for AGE, BME) accomapined with oral administration of GSE for 28 days.

At the end of 28 days, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation. Plasma was separated for the assay of glucose and insulin.

Our goal is to achieve Streptozotocin-induced diabetic model and its renal complications followed by treatment. This model provided us reliable method and resembles the clinical cases of diabetes and its treatments; also the period of treatment is safe and recommended in previous research.

Blood sampling

Rats were fasted overnight and the blood was collected in tubes containing heparin (20 U/ml of blood), either from the retro-orbital plexus prior to the grouping (under mild anaesthesia) or from the heart at the time of sacrifice, to measure the fasting blood glucose in plasma by the glucose oxidase method using a commercial kit.

Urine sampling

Urine was collected under a layer of toluene by keeping the rats in metabolic cages for a period of 24 h. Urine component levels were determined as follows: the content of reducing sugar present in the urine was measured by the 3, 5-dinitro salicylic acid method [27], protein by the sulfosalicylic acid method [28] and Glomerular filtration rate (GFR) was determined as described earlier.

Tissue sampling

Kidneys were dehydrated with acetone, defatted with petroleum ether using Soxhlet apparatus and powdered. For composition analysis, GAG samples from each group were hydrolyzed with 2 N trifluoro acetic acid for uronic acid at 100°C in sealed tubes in an oven for 8 h.

Amino sugar was estimated by the method of [29] and uronic acid by the carbazole method [30].

Renal lipid peroxidation was measured through malondealdehyde (MDA) levels according to [31].

Statistical analysis

Statistical analysis was carried out using Graph Pad Instat software (version 3, ISS-Rome, Italy). Groups of data were compared with ANOVA, followed by Tukey-Kramer (TK) multiple comparisons post-test. Values of $P<0.05$ were regarded as significant. Data were expressed as mean ± standard error (SEM).

Results and Discussion

Here we used STZ -Nicotinamide induced T2 diabetic animal model to examine the pathogenesis of diabetic nephropathy, since this model resembles some of the characteristic pathological changes of diabetic nephropathy.

The antidiabetic properties of *Panax quinifolium*, *Momordica charantia* and *Gymnema sylvestre* are very well documented in humans and experimental animals and our results further substantiated the antidiabetic properties of HMF in terms of controlling the diabetes

induced pathognomic features such as loss of body weight, increase in urine sugar, polyuria, and polydypsia conditions as shown in table 1. In addition, HMF significantly reduced fasting blood glucose in diabetic rats by as compared to diabetic control indicating its hypoglycaemic influence during diabetes (Table 2).

Effect of HMF on kidney weight and glomerular filtration rate and proteinuria

Renal hypertrophy was assessed by calculating the weight of both the kidneys per 100 g of bodyweight (Table 2). A significant increase in kidney weight was observed in diabetic rats as compared to control rat kidneys.

Administration of HMF significantly countered diabetes induced renal hypertrophy and the kidneys from treated rats (T2D+HMF).GFR and proteinuria elevated significantly in diabetic rats when compared to the control rats and Administration of HMF, showed a significant reduction in the GFR and proteinuria during diabetes treated group (T2D+HMF) compared to diabetic group (Table 2).

Effect of fenugreek on the activities of renal enzymes

Activity of renal enzymes involved in the synthesis/degradation of GAGs: (GFAT), (NAG) and b-glucuronidase) was examined in the kidney essentially as described previously.

The activity of GFAT, an enzyme involved in the synthesis of amino sugars, was significantly increased in diabetic group compared to the control group and HMF administration significantly controlled this increase in GFAT activity during diabetes. Activity of lysosomal enzymes NAG and b-glucuronidase were found to be significantly increased during diabetes as compared to its control (Table 3). Administration of HMF was significantly effective in preventing this increase during diabetes (T2D+HMF).

Effect of HMF on glycosaminoglycans

Composition of kidney GAGs was examined by estimating the amounts of amino sugars, uronic acid and sulfates (Table 3). The content of amino sugars decreased in diabetic rats compared to its control and HMF was effective in preventing this decrease during diabetes. The uronic acid content reflects the GAG content in the kidney and a decrease in the uronic acid content was observed in diabetic group compared to the control group and HMF administration effectively attenuated the decrease in uronic acid content during diabetes. Similarly, sulfation in the GAG decreased significantly in diabetic group compared to normal group. Further administration of HMF effectively prevented this decrease in sulfate content during diabetes (T2D+HMF).

Effect of HMF on renal oxidative stress marker MDA

The content of MDA increased in diabetic rats compared to its control and HMF was effective in preventing this decrease during diabetes (Table 4).

Group Paramete	Normal Diet	Diabetic	HMF
Diet intake (g/24 h)	14.10 ± 0.63	18.77± 0.76***a	16.23 ± 0.5***b
Gain in body weight (g)	107. 0 ± 4.71	-29.6 ± 5.32***a	7.9 ± 3.4***b
Water consumption (ml/24 h)	25.47 ± 1.67	98.2 ± 6.5***a	68.4 ± 3.3***b
Urine output (ml/24 h)	14.7 ± 0.62	81.4 ± 2.91***a	59.9 ± 1.99***b
Urine sugar (g/24 h)	0.007 ± 0.0008	6.96 ± 0.72***a	5.11 ± 0.50***b

a***Significantly different from control at P < 0.001
b***Significantly different from DM at P < 0.001

Table 1: Effect of HMF on diet intake, bodyweight, water consumption, urine output, and urine sugar in diabetic rats, compared to normal control (Values are expressed in mean ± SE, N= 10 for each group).

Group Paramete	Normal Diet	Diabetic	HMF
Fasting blood glucose (mg/dl)	99.4 ± 9.2	261.5 ± 35.1***a	214.0 ± 13.7***b
Glomerular filtration rate (ml/min)	0.77 ± 0.110	6.45 ± 0.628***a	2.98 ± 0.35***b
Kidney weight (g/100 g)	0.51 ± 0.017	1.54 ± 0.068***a	1.03 ± 0.162***b
Urinary protein, mg/d	10.23 ± 1.4	14.2 ± 2.3***a	11.1 ± 1.05***b

a***Significantly different from control at P < 0.001
b***Significantly different from DM at P < 0.001

Table 2: Effect of fenugreek on fasting blood glucose glomerular filtration rate and proteinuria in diabetic rats, compared to normal control (Values are expressed in mean ± SE, N= 10 for each group).

Group Paramete	Normal Diet	Diabetic	HMF
L-Glutamine fructose-6-phosphate aminotransferase (GFAT) (n moles of product formed/mg of protein/min)	0.93 ± 0.002	1.29 ± 0.008***a	1.00 ± 0.02***b
N-Acetyl B- glucosaminidase (NAG) (n moles of product formed/mg of protein/min)	41.7 ± 0.25	63.8 ± 0.31***a	49.1 ± 0.35***b
Amino sugar (mg/g dry kidney tissue)	462	350***a	386***b
Uronic acid (mg/g dry kidney tissue)	1250	854***a	1134***b

a***Significantly different from control at P < 0.001
b***Significantly different from DM at P < 0.001

Table 3: Effect of fenugreek on activities of some of the renal enzymes and GAGs in diabetic rats, compared to normal control (Values are expressed in mean ± SE, N= 10 for each group).

Group Paramete	Normal Diet	Diabetic	HMF
Renal M.D.A (n mol /g/hr)	3.95 ± 0.05	8.3 ± 0.07***a	6.53 ± 0.06***b

a***Significantly different from control at P < 0.001
b***Significantly different from DM at P < 0.001

Table 4: Effect of fenugreek on MDA in diabetic rats, compared to normal control (Values are expressed in mean ± SE, N= 10 for each group).

Discussion

In this study, we report the beneficial effect of HMF in controlling the progression of kidney damage by modulating the diabetes induced effects on hexosamine pathway, renal oxidative stress and glycoprotein deposition in STZ induced diabetic rats.

Effect of HMF on urine output

Enlargement of Kidney is an early feature in experimental and diabetes due to an increase in the capillary length and diameter and was correlated with the degree of glycaemic control [32]. We observed partial yet significant reduction in the kidney weight with HMF administration.

Hyper functional kidney with increase in GFR is reported in the early stages of diabetes [33]. Long term metabolic control is known to reduce kidney filtration in human diabetic subjects. In our study, GFR increased considerably in diabetic group.

HMF administration to diabetic rats showed significant reduction in kidney filtration. The consumption of BME might help in the protection of (GAGs), which promote proper functioning of the kidney by maintaining the glomerular filtration barrier, thereby delaying complications associated with diabetes. Their beneficial effect has been attributed to the high amount of dietary soluble fiber in the components of HMF [34]. In addition to stimulatory effect of AGE [35] or BME [36] or GSE [37] on nitric oxide synthesase due to their phenolic compound content.

STZ-induced diabetic rodents result in development of nephropathy similar to the early stage of human diabetic nephropathy [38]. 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 [39]. 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 [40]. The HMF administered to the diabetic rats successfully prevented the enlargement of the kidney also MC extract acted as an antioxidant thereby preventing the oxidative damage involved in the diabetic kidney [41].

Effect of HMF on blood glucose and insulin level

The observed beneficial effects of HMF could be attributed to Plasma glucose-lowering action of HMF that may be due to ginsenoside the major principles contained in AGE (Rh2) that has ability to increase insulin secretion by β cells as a result of the release of acetylcholine from nerve terminals that then stimulates muscarinic M3 receptors in pancreatic cells [42].

Moreover, Lectin, a component of BME has insulin-like activity. It lowers blood glucose levels by enhancing peripheral cellular uptake of glucose, increasing glucose utilization by the liver via improvement of glucose oxidation through activating glucose-6-phosphate dehydrogenase with decrease of gluconeogenesis via inhibition of two key enzymes, glucose-6-phosphatase and fructose-1,6-bisphosphatase [43]. Oleanolic acid glycosides a component of BME improved glucose tolerance in diabetics by preventing sugar from being absorbed into intestines [44]. Gymnemic acid, the major constintuent of GSE resemble glucose molecules, thus these acids fill the receptor locations on the taste buds thereby preventing its activation by sugar molecules present in the food, thereby curbing the sugar craving. Similarly, gymnemic acid molecules fill the receptor location in the absorptive external layers of the intestine thereby preventing the absorption of sugar molecules by the intestine, which results in a reduction in blood sugar levels [21]. Also, GSE appears to have the benefits on supporting blood glucose homeostasis of diabetic rats through increased serum insulin levels via repair or regeneration of the endocrine pancrease.

Effect of HMF on synthesis glycosaminoglycans

Hyper-functional kidney with increase in GFR, and renal hypertrophy due to increase in the capillary length and diameter are reported during diabetes [45].

A relatively large body of evidence supports the notion that glomerular capillary wall and mesangial alterations in diabetic nephropathy involve biochemical alterations of glycoproteins in these structures.

There is evidence of an increase in uronic acid content to diabetes mellitus. This situation is postulated to be a result of 1) increased GAG degradation or 2) decreased GAG synthesis in addition to alterations in the structure of proteoglycans of diabetic kidney. That is, a lower percentage of them form aggregates because GAG degrading enzyme activities are known to be elevated during diabetes [46].

The increase in amino sugars during diabetes could be due a significant increase in the UDP-sugar content which is building block of glycoproteins, GAGs and glycolipids or due the changes in the expression of GFAT, a rate-limiting enzyme of hexosamine biosynthetic pathway, which has been reported to be involved in GBM thickening. Also, excessive flux of glucose through the hexosamine biosynthetic pathway (HBP) implicated in the development of insulin resistance in peripheral tissues and complications of diabetic nephropathy [47].

GAG degrading enzyme, N-acetyl-b-glucosaminidase (NAG), is a lysosomal enzyme of the proximal canaliculi, a marker of tubular injury and is related to vascular complications [48]. Although the changes in the activities of NAG are not very consistent during previous studies the observed beneficial effect of HMF on the elevated enzyme activities is mainly attributed to the hypoglycaemic effect of HMF during diabetes.

HMF administration during diabetes effectively controlled the increase in GFAT activity due to inhibitory action of BME of on key enzymes involved in the synthesis or degradation, viz., GFAT, N-acetyl glucosaminidase (NAG and β Glucuronidase [49,50], thus playing important role in blocking the effect of hexosamine biosynthesis pathway induced glomerular injury, indicating its importance the management of diabetes

Effect of HMF on renal oxidative stress markers

Free radical reactions lead to lipid peroxidation that is mainly responsible for cell and tissue damages. A significant increase in TBA-reactive substances as an index of endogenous lipid peroxidation has

been noted in diabetic conditions. In addition, the measurement of TBA-reactive substances is frequently used to determine the oxidative stress level in diabetic patients [51].

As shown by present study, the levels of TBA-reactive substance in kidney of diabetic rats were significantly increased, whereas the administration of HMF significantly decreased these TBA-reactive substance levels compared to diabetic control rats. Therefore, the administration HMF was suggested to alleviate oxidative stress of diabetic pathological conditions through the inhibition of lipid peroxidation.

Protective effect of GSE against renal damage in experimental diabetic rats due to antioxidant properties of its constituents that include Flavonoids, Phenols, Tannis (Phenolic compounds) and Triterpenoids [52].

Also, the presence of glycosides, saponins, triterpines, steroids, vitamin C and A, phytochemicals such as momorchins, momordinol, momordicins, charantin, cucurbitacins, diosgenin, goyaglycosides, goyasaponins in BME that are characterized by their antioxidant activity [53].

On other hand, Ginsenoside the major constituent in AGE content that could induce the antioxidant enzymes which are important for maintaining cell viability by lowering the level of oxygen radical generated from intracellular metabolism.

AGE, also has the ability to intercalate into the cell membrane, changing its fluidity and inhibit lipid peroxidation by chelating transition metals and scavenging ROS.

The present results suggested that the HMF enhanced the antioxidant defence against reactive oxygen species produced under hyperglycemic conditions, hence protecting the kidney cells. Thus HMF is beneficial for the treatment of diabetic associated with renal function disturbance via the inhibition of oxidative stress and reverse the pathological changes occurring in the kidney as a result of hyperglycemia. [54].

Taken together, our findings suggest the beneficial role of HMF in controlling the progression of kidney damage and additional research to clarify the involvement of HMF or its bioactive components in the molecular pathways could offer potential insight into the cellular mechanisms responsible for diabetic related complications and developing new strategies for treatment.

Conclusion

It could be concluded that STZ- Nicotinamide induced T2D associated with low values of insulin and C- peptide, defective antioxidant stability and decreased GAGs levels which may have implications for the progress of microvascular complications as diabetes associated renal disturbances. Also treatment with (HMF) improved DM and its associated nephropathy.

Furthermore HMF has hypoglycemic, insulin sensitizing, antioxidant, vasodilator effects and consider as a way to surmount and indicate its use fullness as potential treatment in diabetes and its associated renal function disturbance. The results suggest that alterations in the activities of key metabolic enzymes of hexosamine pathway could be one of the biochemical rationale by which HMF attenuates the hyperglycemic and oxidative stress effects in diabetic rats.

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