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Original Research Article

Preclinical evaluation of kudzu root extract and maqui berry extract in streptozotocin induced nephropathy

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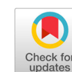

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ABSTRACT

Background: Nephropathy is a significant microvascular problem associated with diabetes. Present study is design to assess the role of Kudzu root extract and Maqui berry extract for their probable nephroprotective effects in streptozotocin induced diabetic rats.
Objective: Present study aims to screen the potential of Kudzu root extract and Maqui berry extract against diabetes induced nephropathy in experimental animals
Result: Diabetes nephropathy was developed in a group of male wistar rats (200-250 g) by a single dose of streptozotocin (55 mg/kg/i.p.). Nephropathy was assessed by biochemical parameters including blood glucose, total protein, albumin, urea, uric acid, creatinine, and total bilirubin in serum and urine. Oxidative stress markers such as lipid peroxidation, reduced glutathione and superoxide dismutase, were assessed along with the membrane bound ATPases. Kudzu root extract and Maqui berry extract administered to the nephropathy rats at a dose of 50 mg/kg/p. o and 100 mg/kg/p. o individually and in combination for 2 weeks. DN rats showed a significant elevation in creatinine, albumin, total protein, total bilirubin, uric acid, urea and oxidative stress markers such as lipid peroxidation and a significant reduction in superoxide dismutase and glutathione level. Treatment with Kudzu root extract at dose 50 mg/kg and 100 mg/kg as well as Maqui berry extract at dose 50 mg/kg and 100 mg/kg alone and their combination for 2 weeks notably altered the level of renal function biomarkers, oxidative stress markers and ATPases level towards near normal level.
Conclusion: The current results indicate that combined use of Kudzu root extract and Maqui berry extract demonstrates synergistic effects in alleviating renal injury by reducing hyperglycemia and oxidative stress markers.

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1. Introduction

Diabetes mellitus (DM) comprises a series of metabolic changes marked by hyperglycemia caused by impaired insulin secretion.[1] Chronic hyperglycemia, a metabolic disease known as DM, is a result of either decreased insulin secretion from pancreatic beta-cells or insulin resistance. Hyperglycemia in diabetic individuals leads to impaired mitochondrial function, advanced glycation end functions, and other variables that develop reactive oxygen species, which eventually stimulates DNA breakage, resulting in apoptotic destruction of cells.[2]

The gradual onset of renal failure in the context of persistent hyperglycemia is known as diabetic nephropathy (DN). Early microalbuminuria, renal hyperfiltration, hyper-perfusion, and enhanced permeability of capillaries to macromolecules and proteinuria with or without prolonged renal insufficiency that results in end-stage renal disease (ESRD) are indicative of the functional abnormalities associated with diabetic nephropathy.[3]

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Diabetic nephropathy is a prevalent microvascular consequence of diabetes. It is also the main trigger of end-stage kidney disease, which has been marked by a variety of renal dysfunctions, including mesangial enlargement, deterioration of renal basement membrane enlargement, oxidative damage, and inflammation-related dysregulation.[4] Diabetic nephropathy is marked by increasing decline in function of the kidneys, elevated levels of protein, interstitial fibrosis, and glomerulus dysfunction. Although the change in metabolic processes, renal hemodynamic, increased production of renin-angiotensin, and the inflammation are the known etiology of diabetic nephropathy.[5] The pathophysiology of DN is most likely caused by associations among metabolic and hemodynamic problems. Elevated generation of reactive oxygen species under hyperglycemic circumstances increases the synthesis of cytokines, growth regulators, and transcription elements involved in DN. Thus, oxidative stress can cause persistent inflammation, tubulointerstitial fibrosis, and kidney enlargement.[6] Elevated oxidative stress in an elevated glucose levels state activates several pathways, including the Rheb, AKT, and P38-MAPK pathways, which support kidney tissue fibrosis and inflammation caused by diabetes mellitus.[7]

Flavonoids have a wide range of beneficial properties and are a crucial ingredient in several kinds of nutraceutical, pharmacological, medical, and cosmetic substances. This is due to their powerful anti-oxidative, anti-inflammatory and free radical destruction abilities.[8] Pueraria lobata (Willd.), dried root Ohwi (Fabaceae), referred to as kudzu root is widely utilized in traditional Chinese medicine for the management of fever, acute diarrhoea, intestinal disorders, metabolic syndrome, and cardiovascular disorders. Common isoflavonoids from kudzu root, which involves puerarin, daidzin, and daidzein have been related with pharmacological actions such as antioxidant, cardiovascular protection, neuroprotection, anticancer, and anti-inflammation.[9] Maqui (Aristotelia chilensis) is a natural evergreen shrub found primarily in central and southern America, particularly Chile and Argentina. The species of plant belongs to the Elaeocarpaceae family. Its black - purple berry are commonly referred to world-wide for their exceptional antioxidant capabilities, found mostly in the fruit. Maqui contains high levels of antioxidants called flavonoid and biological characteristics Maqui is a highly nutritious fruit with numerous biologically active ingredients, including flavonoids, tannins, phenolic acids, stilbenes, and anthocyanins. Maqui is renowned for its ability to absorb oxygen radicals, inhibit the enzyme xanthine oxidase, and reduce intracellular levels of oxidative stress. The antioxidant activity is mostly owing to the polyphenolic component, notably anthocyanin-based compounds, which account for more than 80% of total polyphenols. Maqui berries inhibits reactive oxygen species, also known as ROS, synthesis, suppresses NO (nitric oxide) generation, and inhibits oxidative stress.[10] Considering a variety of beneficial effects of both the drugs, the study was planned to assess the activities of Kudzu root and Maqui berries in diabetic nephropathy.

2. Materials and Methods

2.1. Drugs and chemicals

Kudzu root extract and Maqui berry extract were obtained from Shree sai biotech, Indore, India. Streptozotocin (STZ) was purchased from Sigma Aldrich (USA). All chemical and necessary diagnostic kits were of standard class.

2.2. Experimental animals

Healthy adult male rats of Wistar strain (200-250g) were used in the experiment and each divide into eight groups containing 6 rats. The rats were procured from Laxmi Biotech Pune All rats placed separately in cages made of polypropylene with paddy husk used as bedding material. The animal were kept on standard laboratory condition as per the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (at temperature of 23 +2°C, relative humidity of 55% +10% and 12-12-h light & dark cycle). Animals had free access to water and standard laboratory feed (Nutrivet Lab, Pune, India) prior to the dietary alteration. The experimental protocol was reviewed and approved by Institutional Animal Ethics Committee (IAEC) of SSDJ College of Pharmacy, Neminagar, Chandwad (SSDJ/IAEC/22-23/01).

2.3. Induction of DN using STZ

Diabetic nephropathy was induced with a single injection of streptozotocin (55 mg/kg) dissolved in 0.2 ml of citrate buffer (0.1 M, pH 4.5). Diabetes was confirmed 72 hours after STZ administration, with blood glucose levels (BGL) measured using a digital glucometer (ACCU-CHEK, Roche Diabetes Care, Germany). The rats were then observed for four weeks to monitor the progression of nephropathy.

2.4. Experimental design

Eight groups of rats were formed, each group consisting of six rats. The group descriptions are as follows: Group I: control rat treated with vehicle alone, Group II: Rats administered with STZ (55 mg/kg/I.P.),[11] Group III: Rat treated with Kudzu root extract (50 mg/kg/p.o./day) dissolved in distilled water,[11] Group IV: Rat treated with Kudzu root extract (100 mg/kg/p.o./day) dissolved in distilled water,[11] Group V: Rat treated with Maqui berry extract (50 mg/kg/p.o./day) dissolved in distilled water,[12] Group VI: Rat treated with Maqui berry extract (100 mg/kg/p.o./day) dissolved in distilled water,[12] Group VII:

Rat treated with Kudzu root extract (25 mg/kg/p.o./day) plus Maqui berry extract (50 mg/kg/p.o./day) simultaneously in combination, Group VIII: Rat treated with Kudzu root extract (50mg/kg/p.o./day) plus Maqui berry extract (100 mg/kg/p.o./day) simultaneously in combination

All drug treatment was given for 2 weeks

2.5. Estimation of body weight, kidney weight, and kidney hypertrophy index

After the 2-week treatment, rats were sacrificed and kidney was isolated. The body and kidney weight of the rat were measured by electronic weighing balance and kidney weight was evaluated as per the given formula.[13]

$$Kidney\ hypertrophy\ index\ (\%) = \frac{Kidney\ weight}{Body\ Weight} \times 100$$

EQ : 1

2.6. Estimation of biochemical parameters in serum and urine

After 2nd week of treatment, rats were individually kept in metabolic cages for one day and urine sample was collected. Blood was collected from the retro-orbital nerve using micro-capillaries under ether anaesthesia, and the serum was separated through high-speed centrifugation. Urine and serum samples were kept at -20°C and used for various biochemical parameters. A glucometer was used to measure blood glucose levels. Total protein, Albumin, Urea, Uric acid, Creatinine, total bilirubin was analysed in serum and urine samples using commercial diagnostic kits. Creatinine clearance was calculated by the given formula.[14]

$$Creatinine\ clearance = urinary\ creatinine\ \frac{Urine\ volume}{Serum\ creatinine}$$

EQ : 2

2.7. Tissue homogenization

At the end of the treatment, the rats were euthanized using the decapitation method. The kidneys were then removed and rinsed in cold physiological saline. The kidney was used to estimate antioxidant enzymes. The kidney was finely chopped into small pieces in a chilled sucrose solution (0.25M). The renal tissues were homogenized in 10% w/v tris-hydrochloride buffer (10 mM, pH 7.4). The homogenate was centrifuged at 10000 rpm for 15 minutes at 0°C using a cooling centrifuge.[15] The supernatant was used to measure the levels of lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH). The evaluation of Na^+/K^+ ATPase was performed using a sedimentation-based approach.

2.8. Evaluation of oxidative stress markers

2.8.1. Assessment of lipid peroxidation (LPO)

The 2.0ml supernatant from kidney homogenate was added with 2.0ml of freshly produced 10%w/ TCA and placed in an ice bath for 15 minutes. Centrifuged the precipitate to separate it. 2 ml of the clear supernatant solution 2.0 ml of freshly produced TBA were added. A boiling water bath was used to heat the resultant solution for 10 minutes. After that, it was quickly refrigerated in ice for 5 minutes. The reaction between TBA and MDA produced a pink color, which was measured at 532 nm in comparison to the blank. A variable concentration of standard malondialdehyde (0-23 nM) was utilized. The results were given as nM of MDA/mg protein.[16]

2.8.2. Assessment of superoxide dismutase (SOD)

0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which 0.25ml of ice-cold ethanol and 0.15ml ice cold chloroform was added. Centrifuged the mixture at 2500 rpm for 15 minutes after thoroughly mixing it. After that, 0.5 ml of the supernatant was added to with 1.5 ml of carbonate buffer and 0.5 ml of EDTA solution. Epinephrine (0.4 ml) was added to start the reaction, and the optical density change/minute at 480 nm was evaluated in comparison to a blank. Protein units used to express the SOD concentration were U/mg. Change in optical density per minute when the enzyme is taken unit at 50% blockage of the transition from epinephrine to adrenochrome. The calibration curve was established using 10-125 SOD units.[17]

2.8.3. Assessment of reduced glutathione (GSH)

A 20% TCA solution was used to deproteinize the kidney homogenate (supernatant), which was subsequently centrifuged. Then 0.25 ml of the supernatant was mixed with 2 ml of the DTNB reagent. The final volume was adjusted to 3 ml using phosphate buffer. The resulting yellow color was measured at 412 nm. Various concentrations (10-50 μg) of standard glutathione were prepared. GSH mg/g protein was stated as the GSH concentration.

2.8.4. Assessment of Na+/K+ ATPase

In 0.2 ml homogenate, 1.0 ml of tris hydrochloride buffer and 0.2ml each of magnesium sulphate, sodium chloride, potassium chloride, EDTA, ATP were added. The mixture was incubated for 15 minutes at 36°C. The reaction was arrested by 1.0 ml of TCA (10%), and centrifuged. The enzymatic activity was represented as nM of IP liberated/gm protein/min, and the phosphorus level of the solution was determined.[18]

2.8.5. Statistical analysis

All variables were analysed by Graph Pad Prism (version 10.2.3) Following the application of ANOVA with Dunnett's post-hoc test, the data was presented as mean ± standard error of mean (SEM) (n= 6). A statistical significance level of $p < 0.05$ was used to determine differences among all groups tested.

3. Results

3.1. Effect of KRE and MBE and their combination on BGL

Seventy-two hours post- STZ injection, BGL was significantly (p<0.001) elevated in DN rats compared to control rats. This elevation persisted through the 4th week. DN rats treated with KRE (50mg/kg) showed more significant (p<0.001) reduction in BGL and MBE (50 &100mg/kg) alone showed a less significant (p<0.05) decrease in BGL, with the combination treatment resulting in a significant (p<0.01) reduction compared to untreated DN rats (Table 1).

Table 1: Kudzu root extract, Maqui berry extract and its combined effect on BGL

Groups	Blood glucose level (mg/dl)		
	72hr after STZ injection	**Final at week 4**	**Final after 6th week**
G - I	85 ± 4.824	88.67 ± 4.326	81.17 ± 4.070
G - II	257.5 ± 10.41	333.2 ± 9.024###	412.8 ± 2.227
G -III	296.7 ± 6.546	341.0 ±7.716***	141.3 ± 26.45***
G -IV	258.8 ± 10.72	482.3 ± 4.745***	354.5 ± 12.83*
G -V	257.3 ± 5.408	445.5 ± 6.158***	357.5 ± 4.151*
G -VI	243.0 ± 5.416	441.0 ± 16.69***	327.0 ± 3.856*
G -VII	267.3 ± 21.45	303.8 ± 22.69**	189.3 ± 26.62**
G -VIII	446.8 ± 23.46	444.2 ± 16.93*	367.8 ± 6.263**

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test #p<0.05, ##p<0.01, ###p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

3.2. Effect of Kudzu root extract, Maqui berry extract and their combination on urine volume, kidney weight and hypertrophy index

Urine volume, kidney weight and hypertrophy index at the end of 6th week. It was observed after 24 h urine volume (ml) of DN rats was significantly (p <0.001) increased whereas the kidney weight and hypertrophy index were significantly altered due to impairment in renal function when compared to control rats.

The treatment with Kudzu root extract (50 mg/kg p.o.), Maqui berry extract (100 mg/kg p.o.) and their combination to DN rats showed significant (p<0.001) decreased the urine volume and restored the elevated kidney weight and hypertrophy index to the normal level as compared with DN group (Table 2).

3.3. Effect of Kudzu root extract, Maqui berry extract and its combined effect on biochemical parameters in Urine

The total protein, albumin and urea level in urine were monitored at the end of 6th week. There were significant changes observed in the level of total protein, albumin and urea in DN rats. It was observed that DN rats showed significant (p<0.001) increased in the level of total protein, albumin and urea as compared with normal. Treatment with Kudzu root extract, Maqui berry extract and their combination (Kudzu root extract +Maqui berry) showed significant (p <0.001) declined in the level of renal biomarker when compared with DN rats. The uric acid, creatinine and total bilirubin level in urine were monitored at the end of 6th week. It was found that DN rats showed significant ($p < 0.001$) increased in level of uric acid, creatinine and total bilirubin in urine when compared to the control rats. Rats administered with Kudzu root extract, Maqui berry extract and combination of both showed significant changes in the level of uric acid, creatinine and total bilirubin whereas level of these biomarkers in all treatment group significantly ($p < 0.001$) reduced as compared to DN group after 2-week treatment (Table 3).

3.4. Effect of Kudzu root extract, Maqui berry extract and its combined effect on biochemical parameters in serum

The total protein, albumin and urea level in serum were monitored at the end of 6th week. There were significant changes observed in the level of total protein, albumin and urea in DN rats. It was observed that DN rats showed significant (p<0001) increased in the level of total protein, albumin and urea as compared with normal. Treatment with Kudzu root extract, Maqui berry extract and their combination (Kudzu root extract + Maqui berry) showed significant (p <0.001) declined in the level of renal biomarker when compared with DN rats. The uric acid, creatinine and total bilirubin level in serum were monitored at the end of 6th week. It was found that DN rats showed significant ($p < 0.001$) increased in level of uric acid, creatinine and total bilirubin in urine when compared to the control rats. Rats administered with Kudzu root extract, Maqui berry extract and combination of both showed significant changes in the level of uric acid, creatinine

Table 2: Kudzu root extract, Maqui berry extract and their combined effect on Kidney urine volume, kidney weight andHypertrophy index

Parameters	Groups Weeks	G-I	G-II	G-III	G-IV	G-V	G-VI	G-VII	G-VIII
Urine volume (ml)	0 day	16.8±0.92	20.5±0.77##	19.1±0.75*	16.3±0.56**	18.4±0.85**	17.7±0.90**	17.9±1.03**	19.1± 0.78**
	4th week	17.3±1.04	72.8± 0.91###	66.6± 1.11*	69.9± 0.78*	71.3± 0.67*	70.1± 0.64*	69.4± 1.42*	71.2± 0.68*
	6th week	16.8±0.92	83.3±1.02###	36.5±0.97***	53.3±1.58**	53.6±1.03**	47.7±0.57**	41.8±0.89***	36.6± 0.95***
Kidney weight (gm)		0.91±0.07	1.64±0.01###	1.29±0.08***	1.34±0.01***	1.33±0.01***	1.23±0.01***	1.19±0.09***	1.12± 0.01***
Hypertrophy index (%)		0.35±0.01	1.05±0.09###	0.43±0.09***	0.53±0.09***	0.55±0.01***	0.44±0.01***	0.41±0.09***	0.42± 0.01***

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test #p<0.05, ##p<0.01, ###p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

Table 3: Effect of Kudzu root extract, Maqui berry extract and its combined effect on biochemical parameters in Urine

Groups	Total protein (gm/dl)	Albumin (gm/dl)	Urea (mg/dl)	Uric acid mg/dl	Creatinine (mg/dl)	Total Bilirubin (mg/dl)
G-I	3.55±0.05	1.72±0.03	15.2±0.58	3.27±0.01	1.17±0.03	0.79±0.02
G-II	9.71±0.07###	5.66±0.02###	51.3±0.93###	8.15±0.01###	4.57±0.06###	4.17±0.02###
G-II	5.57±0.05***	3.59±0.04***	25.8±0.94***	4.41±0.02***	1.93±0.04***	2.24±0.05***
G-IV	6.95±0.08**	4.62±0.02**	36.6±0.94**	5.06±0.01**	3.04±0.03**	3.36±0.03**
G-V	7.09±0.06**	4.70±0.01**	40.2±0.78*	5.11±0.009**	3.09±0.03**	3.55±0.03**
G-VI	5.79±0.05***	3.55±0.06***	24.4±0.77***	4.51±0.01***	2.09±0.03***	1.94±0.02***
G-VII	4.57±0.01***	3.13±0.01***	22.2±0.59***	4.10±0.008***	1.53±0.01***	1.77±0.03***
G-VIII	4.82±0.01***	3.18±0.007***	24.7±0.75***	4.13±0.009***	1.55±0.01***	1.73±0.03***

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test #p<0.05, ##p<0.01, ###p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

and total bilirubin whereas level of these biomarkers in all treatment group significantly ($p < 0.001$) reduced as compared to DN group after 2-week treatment (Table 4).

3.5. *Effect of KRE and MBE alone and in combination on LPO, SOD and GSH level*

Lipid peroxide level was monitored in all groups. It was observed that the concentration of LPO in homogenate of Kidney of DN rats was elevated significantly($p<0.001$) when compared to control animals. DN rats administered with KRE & MBE showed significant($p<0.001$) reduction. concomitant of KRE and MBE does not show significant ($p<0.001$) reduction in LPO concentration after 2-week treatment when compared to DN rats.

There were significant changes observed in level of SOD in all mentioned groups. DN rats exhibited significant ($p<0.001$) declined in level of SOD due to alteration in oxidative stress markers when compared to the normal control groups. Treatment with KRE and MBE alone significantly($p<0.01$) ameliorated the activity of SOD, whereas combination therapy of both KRE and MBE displayed more significant($p<0.001$) increase in the level of SOD as compared with DN groups. Reduced glutathione level was monitored in all groups. There were significant changes observed in level of GSH in all mentioned groups. It was found that DN rats showed significant ($p<0.001$) decreased in GSH activity as compared to the normal rats. After the treatment with KRE (50mg/kg), MBE (100mg/kg) and combination of both (KRE+MBE) significantly($p<0.001$) increased the GSH level in sciatic nerve homogenate when compared with DN groups (Figure 1).

3.6. *Effect of KRE and MBE alone and in combination on Na+/K+ATPases levels*

There was significant($p<0.001$) reduction in the level of Na^+/K^+ ATPases in the Kidney homogenate when compared to the normal control group DN rats administered with KRE exhibited significant ($p<0.001$) increase in Na^+/K^+ ATPases level whereas rats treated KRE (100mg/kg) and MBE (50mg/kg) showed less significant ($p<0.01$) and combination of both does not show significant ($p<0.05$) elevation in Na^+/K^+ ATPases as compared to DN group (Figure 2).

Table 4: Effect of Kudzu root extract, Maqui berry extract and its combined effect on biochemical parameters in serum

Groups	Total protein (gm/dl)	Albumin (gm/dl)	Urea (mg/dl)	Uric acid mg/dl	Creatinine (mg/dl)	Total Bilirubin (mg/dl)
G-I	6.82±0.09	2.59±0.04	23.7±0.75	2.20±0.007	1.37±0.05	0.82±0.01
G-II	14.2±0.05$^{###}$	6.52±0.03$^{###}$	54.6±0.77$^{###}$	7.32±0.01$^{###}$	4.24±0.04$^{###}$	3.21±0.01$^{###}$
G-II	9.07±0.05***	3.89±0.04***	35.1±0.96***	4.42±0.01***	2.36±0.03***	1.75±0.03***
G-IV	11.7±0.05**	4.93±0.04**	40.6±0.62*	5.19±0.01**	3.33±0.02**	2.38±0.01**
G-V	12.8±0.03**	5.04±0.04**	41.8±0.66*	5.28±0.01**	3.39±0.02**	2.87±0.01*
G-VI	9.11±0.05***	3.30±0.03***	33.6±0.76**	4.25±0.01***	2.25±0.01***	1.70±0.02***
G-VII	7.33±0.01***	3.16±0.03***	30.6±0.41***	4.11±0.02***	2.22±0.03***	1.39±0.01***
G-VIII	7.62±0.02***	3.15±0.03***	32.4±0.66***	4.12±0.01***	2.24±0.01***	1.48±0.007***

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test $^{#}$p<0.05, $^{##}$p<0.01, $^{###}$p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

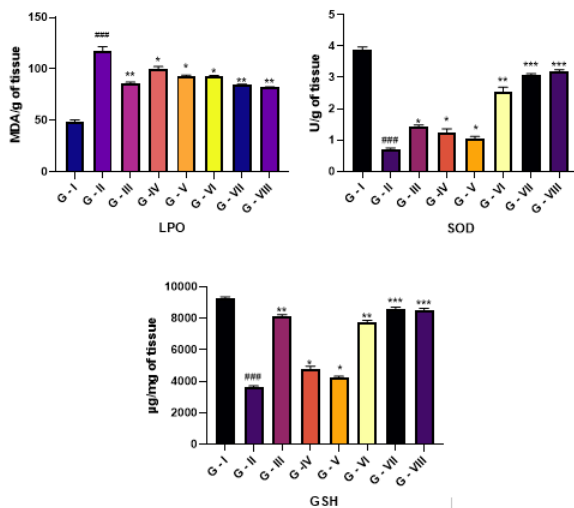

Figure 1: Kudzu root extract and Maqui berry extract and its combined effects on markers of oxidative stress in the renal tissue LPO, SOD and GSH

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test $^{#}$p<0.05, $^{##}$p<0.01, $^{###}$p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

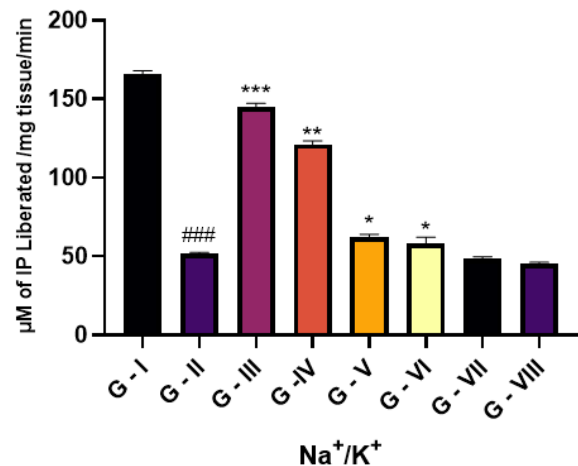

Figure 2: Effect of KRE and MBE alone and in combination on Na^{+}/K^{+} ATPases

Values are expressed as mean ± SEM (n=6). Analysis was performed by applying. ANOVA followed by Dunnett test $^{#}$p<0.05, $^{##}$p<0.01, $^{###}$p<0.001 compared to control and *p<0.05, **p<0.01, ***p<0.001 compared to DN group.

4. Discussion

The long-term hyperglycemia has been demonstrated to be the primary cause of all problems associated with diabetes mellitus, include diabetic nephropathy (DN). Research revealed that hyperglycemia is vital for the occurrence of oxidative stress.[19] The possible therapeutic benefits of antioxidants in diabetes and DN have been studied. Substances known as antioxidants decrease the generation of free radicals by reducing oxidative stress. Due to changes in numerous hemodynamic variables, hyperglycemia causes damage to the renal tissue.[20] In rats, diabetes induced by STZ is characterized by hyperglycemia. A potential cause for the elevated blood glucose levels in the current study could be the death of pancreatic beta-cells caused by STZ treatment to rats, which has been shown to result in insufficient release of insulin.[21]

In the current investigation, the experimental rats were given STZ intraperitoneally, which caused persistent diabetes because of the drug's cytotoxic effects on the pancreatic beta cells in the islets of Langerhans that produce insulin. Hyperglycemia from the degeneration of these beta cells caused oxidative damage in the tissue of the kidney.[22,23] In this investigation, high blood glucose level was noticed in STZ rats indicating severe destruction of beta cells. Studies have investigated the potential of kudzu root extract to reduce blood glucose levels, primarily due to its bioactive compounds, including isoflavones like puerarin.[24] Maqui berry extract, rich in anthocyanins and other polyphenolic compounds, has been explored for its

ability to manage blood glucose levels. The study suggested that the antioxidant properties of anthocyanins play a role in improving insulin sensitivity and glucose uptake.[25]

Studies on the antioxidant effects of kudzu root extract in rats have shown promising results, highlighting its potential to mitigate oxidative stress. Demonstrated that kudzu root extract significantly enhanced the antioxidant enzyme activities and reduced oxidative stress markers in diabetic rats, suggesting its protective role against oxidative damage.[26] Studies showed that administration of maqui berry extract to rats led to a significant increase in antioxidant enzyme activities and a decrease in lipid peroxidation, indicating its strong antioxidant capacity.[27] The body weight, polyphagia, and polydipsia of STZ-induced diabetic rats were significantly reduced. The loss of muscle mass, degradation of tissues proteins, and gluconeogenesis may be the causes of a decrease in weight following STZ injection.[28] Treatment with Kudzu root extract, Maqui berry extract along with their combination was reported to increase the body weight and altered feed and water intake of DN rats by reducing muscle tissue damage.

It is considered that renal hypertrophy is main pathological indicator of diabetes induced renal damage.[29] In present study it was noticed that total kidney weight and kidney index of DN rats was significantly increased when compared to control rats. Treatment with Kudzu root extract, Maqui berry extract and their combination significantly reduced the kidney tissue enlargement. The main causes of diabetic nephropathy include polyuria, proteinuria, and reduced renal function, which are shown by elevated urine creatinine levels. Renal damage persists in diabetic rats due to the kidneys' diminished capacity to sustain steady detoxifying and regulating processes, as seen by greater amounts of kidney markers in their blood and urine.[28] Administration of Kudzu root extract and Maqui berry extract to the DN rats displayed significant changes in biochemical parameters in urine and serum. In the groups treated with Kudzu root extract and Maqui berry extract, the elevated biomarker levels significantly decreased. However, in the group treated with a combination of the antioxidant's Kudzu root extract and Maqui berry extract these levels were reduced even more significantly than with individual treatments. DN rats exhibited reduced creatinine clearance due to damage to the tubular cells. Antioxidant therapy of Kudzu root extract and Maqui berry extract to the DN rats significantly increase the creatinine clearance. When STZ is administered, free radicals are produced, which can result in oxidative damage and diabetic nephropathy. This can affect the function of endogenous antioxidant indicators. The creation of free radicals as a result causes an imbalance between oxidants and antioxidants. The present investigation looked at indicators of oxidative stress, such as LPO, SOD, GSH. To inhibit the interaction between radicals and biological molecules, antioxidants should be in close proximity to the radical's source of creation, competing with free radicals for the biological substrate.[30] In the present study, high levels of reactive oxygen species (ROS) produced in diabetic rats interact with polyunsaturated fatty acids in cell membranes, oxidizing and increasing the level of lipid peroxidation. LPO is regarded as a critical OS indication in those with long-term diabetes. High glucose levels are caused by increased LPO, which damages beta cells.[31,32] It was found that after 2-week treatment of Kudzu root extract, Maqui berry extract and their simultaneous dose to the STZ rats, they demonstrated a notable reduction in LPO level as compared with the DN groups. Following a two-week treatment with Kudzu root extract, Maqui berry extract, and a combination of both in STZ rats, a significant decrease in lipid peroxidation (LPO) level was observed compared to the diabetic nephropathy (DN) groups.

SOD is the primary enzyme involved in the anti-oxidant defense mechanism. SOD plays a vital role in protecting the cells from ROS by converting superoxide radical anion ($O2^{\bullet-}$) to H_2O_2 via metal-catalyzed reaction.[33] In the current study, levels of superoxide dismutase (SOD) decreased significantly in rats with diabetic nephropathy (DN) due to oxidative stress induced by hyperglycemia. Treatment with Kudzu root extract at a dose of 50 mg/kg alone significantly increased SOD levels, also the Maqui berry extract at a dose of 100 mg/kg. However, the combination of these extracts showed a more significant increase in SOD levels compared to when either drug was used alone. In this study, diabetic nephropathy rats exhibited significantly reduced levels of glutathione compared to control rats. Treatment with Kudzu root extract at a dose of 50 mg/kg notably increased GSH activity, as did Maqui berry extract at 100 mg/kg. However, the combination of both antioxidants showed a more beneficial effect in increasing the depleted GSH level compared to using either drug alone. Due to structural changes in the cell membrane, the activities of the membrane-bound ATPases (Na^+/K^+ ATPase) were substantially reduced in STZ diabetic rats as compared to control rats.[34] It was observed that the administration of Kudzu root extract, Maqui berry extract, and their combination was found to enhance the activities of membrane-bound enzymes (Na^+/K^+ ATPase) by preserving ionic balance in the kidney. The combination of both antioxidants exhibited greater effect than individual drug.

5. Conclusion

The present research suggests that natural antioxidants like kudzu root extract and maqui berry extract may help control high blood sugar and reduce oxidative stress induced by diabetes mellitus. Treatment with kudzu root extract and maqui berry extract simultaneously showed a significant renoprotective effect by improving the function

of the renal antioxidant system and reversing morphological alterations. The combination of each antioxidant was found more effective than alone drug. The results of this study indicated that the combined action of kudzu root extract and maqui berry extract, due to their strong antioxidant properties, contributed to slowing the progression of diabetic nephropathy.

6. Source of Funding

None

7. Conflicts of Interest

There are no conflicts of interest by any of the author.

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