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Research Article

Virgin Coconut Oil Solubilised Curcumin Protects Nephropathy in Diabetic Rats

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ABSTRACT

Nephropathy is considered as one of the major secondary complications in diabetic patients. The goal of the current study was to perform preclinical screening of virgin coconut oil (VCO) solubilised curcumin in diabetes-induced nephropathy. Male albino rats of the Wistar strain were injected with a single dose of streptozotocin (STZ) (60 mg/kg/i.p). Diabetic nephropathy (DN) was developed after 4 weeks of STZ injection and the treatment was continued for further 4 weeks (i.e 8 weeks). DN rats were treated with VCO (8ml), VCO solubilised curcumin at a low dose (0.66mg/4ml/kg) and high dose (1.32mh/8ml/kg). DN was assessed by evaluating biochemical parameters such as blood glucose, total protein, albumin, urea, creatinine, and total bilirubin from serum and urine sample, whereas the activity of endogenous antioxidant and membrane- bound phosphatases were studied from kidney homogenate. VCO-solubilised curcumin significantly reduced blood and urine glucose level, increased body weight and reduced kidney weight and kidney hypertrophy. It also normalized urine volume, albumin, creatinine, total protein, total bilirubin and urea levels. Treatment also significantly improved antioxidants and membrane-bound phosphatase activities. In conclusion, compared to the individual treatment group, VCO solubilized curcumin significantly modifies the altered parameters toward normal. The potent antioxidant activity of these substances may be to blame for this defense.

Keywords: Virgin coconut oil; curcumin; diabetes; nephropathy; antioxidants

INTRODUCTION

Diabetes is a condition in which there is persistent hyperglycemia. According to the International Diabetes Federation 2019, it is anticipated that the number of diabetes patients worldwide will grow nearby 578 million by 2030.[1,2] It has been estimated that 20-40% of type I and type II diabetic individuals will suffer from diabetic nephropathy (DN) within 20-30 years.[3,4] DN is characterized by abnormally high levels of albumin excretion in the urine, diabetic glomerular lesions, and a decrease in the glomerular filtration rate.[5] Oxidative stress and vascular dysfunction are considered as key contributors to the development of DN. Most of the site-related kidney damage may be exacerbated by the levels of enzymatic antioxidants.[6,7] Antioxidants are the agents that interrupt chain reactions, which are the cell's major source of free radical production, and hence reduce the oxidation of free radicals by macromolecules. Literature showed that antioxidant supplements prevent nephropathy in diabetic rats.[8,9]

Curcumin is a yellow compound, a primary byproduct of turmeric and obtained from the *Curcuma longa* plant.[10] Curcumin possesses strong antioxidant and anti-inflammatory properties. It is a lipophilic polyphenolic molecule with a low molecular weight.[11] It has poor bioavailability when used alone. Virgin coconut oil contains high levels of polyphenols because of that it maintains normal antioxidant parameters in tissues. In the renal tissue, VCO increased SOD, CAT, and GPx activities, resulting in a consistent decrease in MDA levels.[12] Curcumin, in combination with VCO polyphenols, may have a higher bioavailability.[13] In nephropathy, several essential oil combinations have a synergistic impact. Long-term administration of combinations of antioxidant compounds may be a viable approach for reducing oxidative stress.

In this study authors proved that VCO solubilised curcumin exhibited a synergistic antioxidant effect against

ROS in diabetic nephropathy. The nephroprotective effects as well as the potential mechanism of VCO solubilised CUR on STZ-induced diabetic nephropathy were investigated.

MATERIALS AND METHODS

Drugs and Chemicals

Curcumin was obtained as a gift sample from ASOJ soft caps, Baroda, Gujarat and VCO were freshly prepared in the laboratory by hot press method. All other chemicals used in the study were purchased from standard suppliers.

Experimental animals

Male albino Wistar rats were used in the present study. The animals were housed in polypropylene cages in the animal house under the temperature 25±2°C and 12 hr light and 12 hr dark cycle. Water ad libitum and a standard pellet diet were provided to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the study was performed considering the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Protocol approval number is SSDJ/IAEC/2020-21/03

Dose fixation of curcumin in VCO

5 mg of curcumin was dissolved in 1 ml of VCO using a vortex mixer for 5 minutes. The liquid was then allowed to sit overnight before being centrifuged the following day at 10,000 RPM for 30 minutes. The residue was dried, the supernatant was collected, and the amount of curcumin was weighted. By subtracting the end weight from the initial weight, the soluble component of curcumin was estimated. One ml of VCO solubilizes 0.165 mg of curcumin, according to the observation. VCO was administered in two doses (4ml and 8ml/kg) based on previous study at our laboratory.[14] The soluble curcumin was determined at 0.66 mg and 1.32 mg, respectively.

Experimental Design

The rats were divided into 5 groups, each group contains 6 rats,

1. Group I: Control, received citrate buffer (as vehicle for STZ).
2. Group II: Diabetic Nephropathy, injected with a single dose of STZ (60mg/kg/i. p).
3. Group III: Animals were treated with VCO (8ml/kg by oral gavage for 4 weeks.
4. Group IV: Animals received a low dose of VCO solubilised CUR (0.66mg/4ml/kg) by oral gavage for 4 weeks.
5. Group V: This group of animals received a high dose of VCO solubilised CUR (1.32mg/8ml/kg) by oral gavage for 4 weeks.

Rats injected with a single dose of STZ were monitored for the development of diabetes. Diabetes was assessed by checking blood glucose level by digital glucometer, rats with blood glucose level more than 250mg/dl were considered as diabetic rats. Those rats that developed diabetes were observed for the development of nephropathy. Significant nephropathy was noticed in 4th week after the STZ injection, which was assessed by evaluating urine glucose and protein level. The treatment with CUR and VCO was continued for a further four weeks in the rats having DN.

Assessment of Diabetic Nephropathy

Assessment of General Parameters

The body weight, urine volume, water intake, feed intake and kidney weight were monitored in the beginning and at the end of treatment in each group. The hypertrophy index was calculated.

Biochemical Parameters

At the end of treatment, the animal was separately placed in metabolic cages for 24 hrs. for urine sample collection. The blood was collected from the retro-orbital plexus under mild anesthesia and serum was separated using centrifuge (2000 rpm for 15 min). The level of glucose, total protein, creatinine, albumin in serum and urine were estimated using a standard diagnostic kit using semi automated biochemical autoanalyzer (ROBONIK, India).

Antioxidant parameters

At the end of the study, rats were sacrificed by cervical dislocation, Kidney was removed and the homogenate was prepared. The tissue homogenate was used for the estimation of membrane-bound enzymes (Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase). The supernatant that remain after centrifugation was used for the estimation of antioxidants such as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) by using an antioxidant assay kit by Elabscience, USA.

Statistical analysis

Results were expressed as Mean ± S.E.M. One-way ANOVA followed by Dunnet 't' tests. Values are considered as significant $P<0.05$. Control compared with diabetic $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, Diabetic compared with treatments $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$. Analysis was performed using GraphPad prism-7 software.

Table 1: Effect of VCO Solubilised Curcumin on General Parameters

Groups	Body weight (g)	Kidney weight (g)	Water intake (ml)	Feed intake (g)	Urine Volume (ml)
I	259.33± 15.067	1.14± 0.026	50.53± 5.576	21.93± 0.77	6.6± 0.111
II	170.81±4.357***	1.76± 0.049***	109.83±3.419**	45.33±1.087***	80.66± 2.261***
III	181.94± 6.839	1.52± 0.081#	93.677± 4.506	38.09±1.652	53.6± 2.361#
IV	215.37± 4.311##	1.31± 0.073##	86.66± 3.137#	29.01±1.064#	41.33± 3.051##
V	231.29± 6.281##	1.24± 0.043##	78.09± 5.551##	27.16±1.741###	34.5± 1.648##

Values are expressed as Mean ± SEM. Values are considered as significant P<0.05. Control compared with diabetic *P<0.05, **P<0.01, ***P<0.001, Diabetic compared with treatments #P<0.05, ##P<0.01, ###P<0.001.

RESULTS

Effect of VCO solubilised curcumin on body weight, kidney weight, feed intake, water intake and urine volume

At the end of treatment, i.e., on 8th week body weight, feed intake, water intake, urine volume and kidney weight were recorded. As shown in Table 1, DN rats showed a significant reduction in body weight and a significant increase in kidney weight, water intake, feed intake and urine volume compared to control rats. Treatment with high low and high dose of virgin coconut oil solubilised curcumin showed significant increase in body weight and a significant reduction in the levels of kidney weight, water intake, feed intake and urine volume as compared to DN rats (Table 1). High dose of virgin coconut oil solubilised curcumin showed better protection as compared to alone VCO and low dose.

Effect of VCO Solubilised Curcumin on Urine Biochemical Parameters

At the end of treatment, i.e., on 8th week DN group showed a significant increase in the level of total protein and albumin whereas a significant decrease in the level of urine creatinine and urea were observed when compared with normal control rats. Treatment with VCO solubilised curcumin at low and high doses showed significant alteration in all the urine parameters towards normal (Table 2).

Effect of VCO Solubilised Curcumin on Serum Biochemical Parameters

Table 3 showed the altered serum biochemical parameters in DN rats. Rats with nephropathy showed a significant increase in blood glucose, creatinine, albumin and urea level whereas a significant reduction in the level of total protein was observed. Treatment with low and high dose of VCO solubilised curcumin dose dependently showed a significant reduction in blood glucose, creatinine, albumin and urea level whereas a significant increase in the level of total protein was observed. The higher dose was found to be more effective in attenuating the serum biochemical parameters compared to nephropathy rats (Table 3).

Effect of VCO Solubilised Curcumin on Endogenous Antioxidant Level

STZ-DN group showed a significantly decrease in the level of SOD, CAT and GSH in renal tissues. Treatment with VCO solubilised CUR showed a significantly increased in the level of SOD, CAT and GSH. The level of tissue nitrite was significantly increased in STZ-DN group and it was significantly decreased in treatment groups. The group V shows better increased in antioxidant level as compared to other treatment groups (Figure 1).

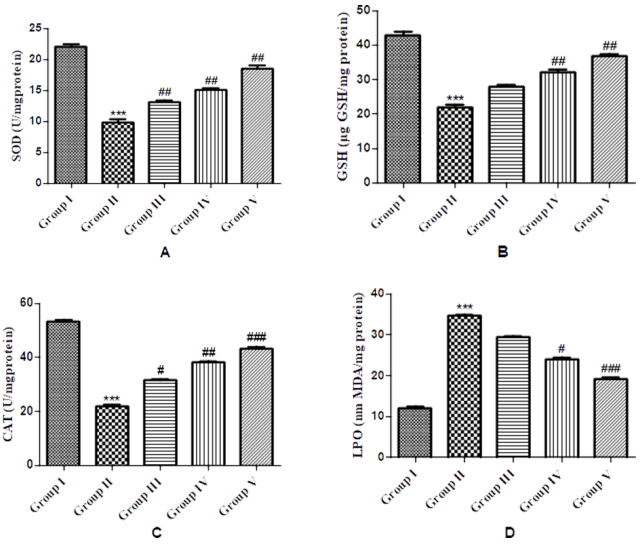

Fig. 1: Effect of Virgin Coconut Oil Solubilised Curcumin on Tissue Antioxidant Level Values are expressed as Mean ± SEM. Values are considered as significant P<0.05. Control compared with diabetic *P<0.05, **P<0.01, ***P<0.001, Diabetic compared with treatments #P<0.05, ##P<0.01, ###P<0.001.

Effect of VCO Solubilised Curcumin on Kidney ATPase Level

Figure 2 shows a significant increase in the activities of Na^+/K^+, Ca^{2+}, Mg^{2+} in STZ- DN group as compared to the control. The treatment with VCO solubilised CUR shows a significant decreased in the activities of ATPases as compared to VCO alone group and the STZ-DN group.

Table 2: Effect of VCO Solubilised Curcumin on Urine Biochemical Parameters

Groups	Total protein (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)	Urea (mg/dl)
I	2.54± 0.162	4.54±0.298	2.59±0.293	14.94±1.659
II	7.39± 0.347***	1.19± 0.944***	6.42±0.187***	10.25±2.194***
III	6.19±0.19	1.41±0.112	5.03±0.34#	11.06±0.743##
IV	5.07± 0.234#	2.64± 0.127##	4.35±0.134##	12.02±0.777##
V	3.55± 0.229##	3.25±0.151###	3.51±0.302###	12.82±0.271##

Values are expressed as Mean ± SEM. Values are considered as significant P<0.05. Control compared with diabetic *P<0.05, **P<0.01, ***P<0.001, Diabetic compared with treatments #P<0.05, ##P<0.01, ###P<0.001.

Table 3: Effect of Virgin Coconut Oil Solubilised Curcumin on Serum Biochemical Parameters

Groups	Glucose (mg/dl)	Total protein (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)	Urea (mg/dl)
I	127.75± 3.127	6.618± 0.345	1.316±0.094	1.846±0.177	14.3±1.07
II	461.92±25.461***	3.643±0.478***	3.576± 1.992***	4.501± 0.225***	35.826± 27.669***
III	345.42± 32.821	4.022±0.263#	2.901± 0.058	3.12± 2.441#	27.81±0.672#
IV	338.42± 22.721##	5.317±0.186##	1.845± 0.127##	2.548±0.333##	19.97±0.554##
V	296.18± 16.774###	5.498± 0.239###	1.482± 0.156##	2.388±0.222##	17.083±0.541###

Values are expressed as Mean ± SEM. Values are considered as significant P<0.05. Control compared with diabetic *P<0.05, **P<0.01, ***P<0.001, Diabetic compared with treatments #P<0.05, ##P<0.01, ###P<0.001.

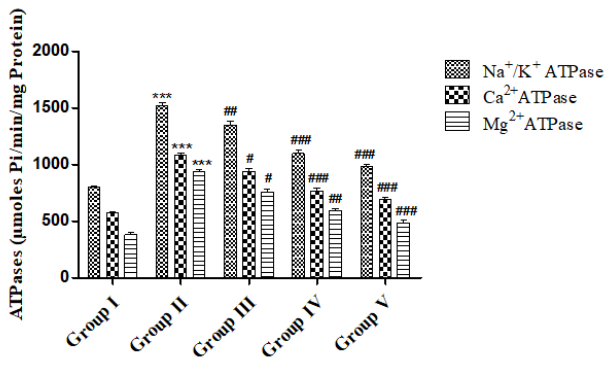

Fig. 2: Effect of VCO Solubilised Curcumin on Kidney ATPase Level Values are expressed as Mean ± SEM. Values are considered as significant P<0.05. Control compared with diabetic *P<0.05, **P<0.01, ***P<0.001, Diabetic compared with treatments #P<0.05, ##P<0.01, ###P<0.001.

DISCUSSION

Diabetes mellitus (DM) is a chronic endocrine disease characterized by a lack of insulin synthesis in the pancreas, which can be hereditary or acquired.[15] The present study was designed to investigate the protective effect of VCO solubilised curcumin in STZ-induced diabetic nephropathy. In the current study, characteristic DM symptoms, included body weight loss and an increase in renal tissue weight were observed. The treatment groups III, IV and V shows a significant increase in body weight whereas the water intake and feed intake of Group III, IV and V were found to decrease as compared to Group II. Polyuria is another symptom of DM caused by osmotic diuresis, in addition to persistent hyperglycaemia.[16] STZ-induced DN rats demonstrated an increased level of urine output. The treatment group rats showed reduced levels of urine output. Group II rats showed enhanced kidney weight as compared to treatment group rats. Treatment groups considerably reduced kidney weight. The above finding suggests that the treatment with VCO solubilised CUR may prevent kidney hypertrophy. Group V shows a more beneficial effect as compared to groups III and IV in body weight, feed intake, water intake, urine volume, kidney weight and hypertrophy index.

STZ-induced DN group shows significant alterations in urine and serum parameters. In serum, the level of creatinine, uric acid, and urea was increased as compared to the control group. This rise might be attributed to decreased excretory and regulatory renal function, which is required to keep these parameters in a continuous state of homeostasis. Also, the increase in serum creatinine level is a common characteristic of the development of DN.[17] In the urine the level of creatinine, uric acid, and urea was found to be decreased indicating the renal clearance is hampered. The treatment with VCO solubilised CUR positively affected these parameters, especially Group V containing a high dose.

In STZ-induced DN rats, serum total protein, and albumin concentration decrease significantly with an increase in urine as compared to the control group. As a result, albuminuria was linked to decreasing kidney function. 4 weeks of treatment with VCO solubilised CUR shows increases the level of total protein and albumin in serum with a decrease in urine. Also, the administration of STZ increases the level of glucose in serum and urine.[18] This might be

because the urine sample contains protein. As the protein builds up in the vessel, the metabolism of carbohydrates and lipids is disrupted. Glucose will not be digested and will be excreted in the urine.[19] The treatment group shows a significant decrease in the level of glucose. Group V shows a more beneficial effect.

Diabetes and its consequences, such as diabetic nephropathy, are exacerbated by oxidative stress. An imbalance between oxidants and antioxidants is referred to as oxidative stress.[19] The generation of reactive oxygen species increased as a result of oxidative stress (ROS). Hyperglycemia not only increases the generation of reactive oxygen species (ROS), but it also inhibits antioxidative processes by glycosylating antioxidative enzymes.[20] One method by which natural antioxidants prevent diabetic complications is by avoiding lipid peroxidation and maintaining a balance between the generation of ROS and antioxidant defenses.[21] Various reports showed that natural antioxidants and antioxidative enzymes show protective effect on oxidative stress in diabetic nephropathy.[6,22]

In the present study, the STZ-induced DN group shows a decrease in the level of antioxidant enzymes in renal tissue. The treatment with VCO solubilised CUR shows a significant increase in the level of SOD, CAT and GSH in tissue homogenate. Group V shows more beneficial effects as compared to other treatment groups. In diabetes, excessive nitric oxide (NO) production is linked to changes in renal structure and function. Increasing evidence suggests that levels of nitrite, an oxidized end product of NO, are rising, which might be due to the production of peroxynitrite by the interaction of NO with superoxide radicals.[23] In the present, study STZ induced DN rats show an increase in the level of tissue nitrite, whereas the level was found to be decreased in the treatment with VCO solubilised CUR.

The STZ-induced DN rat shows a significant increase in the level of membrane-bound enzymes like Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases. This rise might be a direct reaction to the absence of insulin, or it could be secondary compensatory alterations in the kidney in response to experimental diabetes' chronic glucose osmotic dieresis. Adaptive alterations in the altered electrochemical gradients across the renal tubular cells of STZ-diabetic rats might be represented by variations in renal tubular ATPases activity.[24] The treatment with VCO solubilised CUR shows a significant decrease in the level of tissue ATPases.

CONCLUSION

Streptozotocin-induced rats showed diabetic nephropathy by altering various biochemical as well as histological parameters. As opposed to separate treatment groups, virgin coconut oil solubilized curcumin prevents the transition toward normal. The potent antioxidant activity of these substances may be to blame for this defense. That is further depth study of virgin coconut oil and curcumin on a molecular level is required to get the nephroprotective effect of drugs.

Conflict of Interest

The authors declare no conflict of interests.

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