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Research article

Evaluation of nephroprotective and antioxidant potential of Cucumis pubescens in rats

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ABSTRACT

To find the effective way of treatment of diabetic nephropathy with herbal medicine with modern allopathic medicines. In the present research study we use streptozotocin model (60mg/kg) for inducing the diabetes and development and progression of Diabetic may leads to various diabetic complication; Nephropathy is one of the imperative complication leads to renal physiological alteration. Various nephropathy evaluation parameters were assessed in this research study by various methods. Cucumis pubescens an herb used for the treatment of the nephropathy along with the Antidiabetic drugs. The result obtained in the current research study showed that herbal medicine in experimentally induced diabetic nephropathy in rats significantly aid in nephropathic complications. At present therapeutic scenario there are a lot of allopathic medicines in the market for the treatment of diabetes mellitus, but due to severe complication with the drugs therapy move towards the Ayurvedic/Herbal treatment. At present some advance research is needed to explore the active phyto constituents for the treatment of various ailments.

Keywords: Diabetic, Cucumispubescens, Herbal medicine, Glucose.

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INTRODUCTION

Latest clinical trial and experimental studies reported that consumption of herbs, vegetables and product is accommodating in treatment and in the management of various metabolic disorder including diabetes in an uncomplicated and economically effective manner. From ancient time, peoples have used natural resource products like plants, herbs, animals, marine organisms, microorganisms as a medicine in the treatment of various disorders. According to relic report, utilization of plant by human being from ancient time approximate 60,000 years back.^(1,2) Utilization of natural resources in ancient time was very incredible act because lack of knowledge and identification of plants, sometimes peoples consumed toxic herbs that lead to various complications and even death. Synthesis of herbal medicine for the treatment of any aliment depends on skillfulness and experience of the practicing physician of the indigenous systems of medicine.

Traditional herbal medicine having medicinal plants, organic matter and minerals etc. Medicated preparation of traditional herbal also known as churans and vatti. (3-5) From prehistoric time herbal medicine are used globally from ancient time. There are a huge number of medicinal plant that having ultimate pharmacological profile.

Cucumis pubescens is an herbal based herb that shows remarkable effect in treatment of various disease and disorders. Cucumis pubescens Willd. is an seasonal, drained, dispersal, stem creeping, bony.

The plant is not cultivated it grow with other crops in between as weeds. (6-8) Mostly *Cucumis pubescens* grow with guar crop (*cyamopsis tetragonoloba*), sorghum field. Mostly *Cucumis pubescens* grow in deprived and arid grounds. Species of the plant mostly found in arid area of India. The plant having yellowish flower, hairy stem, outer covering of fruit having various color; green and brown striped in between yellow ripe fruit. The leaves of the plant are greenish in color. (8-10) The plant is rich source of various active constituents that shows ultimate pharmacological action. Leaves of the plant is rich source of a variety of carotenes, other active constituents are meloside A and L (A-6C-diglucosylapigenin, L-6C-diglucosylluteolin), vitamin C. Unripe fruit of the plant is bitter in taste and may cause eruptions of skin and strangury.

The unripe fruit is bitter, sour; may cause skin eruptions and strangury. The ripe fruit is sweet. The fruit having galactagogue, diaphoretic, laxative properties. (7,11-13) Fruit parts also used in various disorders. Biological hierarchy of the plant as in Table 1

Table 1: Biological classification order of the plant

Kingdom	Plantae		
Phylum	Tracheophyta		
Class	Magnoliopsida		
Order	Cucurbitales		
Family	Cucurbitaceae		
Genus	Cucumis		
Scientific name	CucumispubescensWilld		

MATERIALS AND METHODS

Drugs & Chemicals

Streptozotocin (STZ) (MP Biomedicals, India), ethanol, sulphanilamide, butanol, citric acid, petroleum ether, ethylene diaminetetraacetic (EDTA), Ellman's reagent, ferric chloride, ferrozine, glacial acetic acid, glucosidase, research laboratories Pvt. Ltd. New Delhi, india), Metformin (hydrochloride), NADPH, napthylethylene -diamine, p-nitrophenyl-α-D glucopyranoside pyridine (pNGP), potassium dihydrogen phosphate, potassium ferricyanide, sodium nitroprusside, sodium hydroxide, sodium lauryl sulphate, soluble starch and iodine (HI Media, Laboratories Pvt. Ltd, Mumbai, India) and all other chemicals used are analytical grade.

Collection and authentication of plant

The whole plant was collected from the local field of Hisar, Haryana during April, 2019 and a voucher specimen was deposited and authenticated by Dr S.S. Yadav, Assistant Professor, Department of Botony, Maharshi Dayanand University Rohtak, Haryana.

Preparation of extract

Fresh ripe fruit of *Cucumis pubescens* were collected and washed properly with tap water followed by distilled water, then shade dried at room temperature. Dry fruit was powdered using a mechanical grinder. The fruit powder was extracted in ethanol using Soxhlet assembly.

Experimental animals

Healthy Wistar albino rats (150-200g) of either sex were acquired from diseases free small animal house, Lala Lajpat Rai University of Veterinary and Animal science Hisar (Haryana). Experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) in its meeting was held on Oct, 2017 (CPCSEA Registration No.436/GO/ReBi/S/2001).

Development of diabetes mellitus (DM) Type II in rats

Administration of single dose Streptozotocine (60 mg/kg, i.p.) in night fasted animal. After STZ administration (6 hours) rats received 10% dextrose solution for next one day cycle. The blood glucose level was checked after 72 hours of Streptozotocin administration by using automatic glucose analyzer (Gluco Dr. Auto Glucometer). Blood sample was collected from pricking of tail by hypodermic sterile needle. The rats having (display) serum glucose level more than 250 mg/dl were selected for experimental design and animal named as diabetic rats.

Assessment of diabetic nephropathy

The Nephropathic complication develops, after 4 weeks the single administration (60mg/kg) of Streptozotocin, was evaluated in rats by measuring serum creatinine, uric acid and urea level by using commercially accessible kits.

Experimental groups of animal study Group for Diabetes Mellitus Type 2 (Streptozotocin induced Diabetes)

Group 1	Normal control rats
Group 2	STZ + NA (Diabetic control)
Group 3	STZ + NA + Metformin (50mg/kg) were administered
	to the diabetic rats
Group 4	Extract of Cucumis pubescens (200mg/kg, p.o)
Group 5	Extract of Cucumis pubescens (500mg/kg, p.o)

Sample collection

Blood sample

After end of fifty six days protocol design blood (2 ml) sample collected by using retro orbital technique. Blood sample was centrifuged (3000 rpm for 10 minutes) and serum was separated out from blood. Isolated serum was used to check the various biological parameters.

Organs collection and storage

After blood collection animals were sacrificed by cervical dislocation technique under mild anesthesia. Renal sample was washed in saline solution and stored in 10% formalin solution for future use.

Estimation of parameters

I. Glucose estimation

The blood glucose level was done by automatic glucose analyzer (Gluco Dr. Auto Glucometer) after 72 hours of Streptozotocin administration.

II. Other Nephropathy parameter

Serum creatinine level estimation, Blood urea nitrogen level, Urine protein estimation was estimated by using commercial available diagnostic kits.

Methods: Serum creatinine level = Alkaline picrate

Blood urea nitrogen level = Mod. Berthelot

III. Lipid Profile Estimation

Triglyceride (TG) level, High density lipoproteins (HDL) in the serum was estimated by using diagnostic kits.

IV. Renal Oxidative Stress

By using thio-barbituric acid reactive method (TBAR), reduced glutathione method (GSH).

Statistical analysis

All values were expressed as mean ± S.D. The result obtained from different group of animal were statistically analyze by using one way ANOVA, followed by Turkey's multiple comparison test. The 'p' value of less than 0.05 was considered statistically significant and the 'p' values were of two tailed.

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RESULTS AND DISCUSSION

In the present study animal having blood glucose level more than 250mg/dL, the animals are considered as diabetic rats' further investigation done on these animals. Treatment of the animal was started after 4 weeks of STZ administration with plant extracts at various doses (*Cucumis pubescence* 200 and 500 mg/kg). Animals were treated with the therapies continued for 4 weeks. The termination of the study was made after completion of 8 weeks and all the research parameter were evaluated at the end of the experimental procedure.

Blood glucose level

Blood glucose level was prominently high in diabetic rats as compared to normal rats. In the diabetic rats *Cucumis pubescens* blood glucose level was significantly reduced and showed prominent effect at a dose of 500 mg/kg as compared to at dose 200 mg/kg and diabetic control animal group. (Table 2)

Table 2: Effect of Cucumis pubescens on various parameters

Groups	Blood Glucose	Serum Creatinine	Blood Urea	Triglyceride	HDL
Normal control rats	102.7±3.98	0.75±0.02	10.51±0 .56	147.7±1.46	49.57± 0.88
STZ+NA (Diabetic control)	287.8±6.14 *	1.96±0.05* **	35.93±0 .38***	308.0±1.16	32.54± 0.90
STZ+NA+ Metformin (50mg/kg) were administered to the diabetic rats	118.7±4.32 ***	1.02±0.02* *	17.79±0 .34***	181.5±0.861	43.47± **** 0.50
Extract of Cucumis pubescens(200mg /kg, p.o)	270.09±4.5 9*	1.80±0.04* *	32.82±0 .88**	291.70 ±1.27*	34.72 ±1.85* *
Extract of Cucumis pubescens (500mg/kg, p.o)	242.07±3.5 1***	1.45±0.04* **	29.29±0 .87**	260.09 ±1.07**	35.75 ±1.84* *

Data is expressed as Mean ± SEM and analysed by using One Way ANOVA followed by Turkey's test. *** P<0.0001, **P<0.001 and *P<0.05 as compared to normal control. ****P<0.0001, **P<0.001 and *P<0.05 as compared to diabetic rats. NC- Normal control, DC- Diabetic control, TG-Triglyceride, HDL- High density lipoprotein

Serum creatinine and blood urea nitrogen level

In diabetic rats serum creatinine and blood urea nitrogen values are elevated as compared to normal group animals. Treatments with different doses of *Cucumis pubescens* showed prominent reduction in the levels of serum creatinine and blood urea nitrogen. Results showed that high dose of *Cucumis pubescens* was effective in the treatment of diabetic induced elevated level of serum creatinine and blood urea nitrogen (Table 2).

Lipid profile

Dysregulation of the lipids was found in diabetic animals. An augmented level of serum triglyceride level and decline in the level of HDL cholesterol. Extract of *Cucumis pubescens* prominently reduced the elevated level of triglyceride and increases the reduced level of HDL cholesterol. High dose of the extract showed effective regulation of the lipid profiles in diabetic rats. All the data were

showed in (Table 2).

Renal TBARS and GSH

In diabetic animals after completion of 8 weeks of STZ administration experimental protocol results showed that elevation in the level of renal TBARS and decrease in GSH level as compared to normal rats. These are the prominent oxidative stress index. Treatment of diabetic animals with *Cucumis pubescens* at low and high dose showed that decreases in the level of renal TBARS and increases the level of GSH were noted. At dose 500 mg/kg showed prominent effect in the management of oxidative stress. The results of the oxidative parameter were mentioned in Figures I and II

Figure 1: Value of Renal TBARS

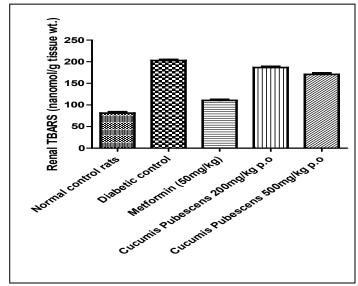
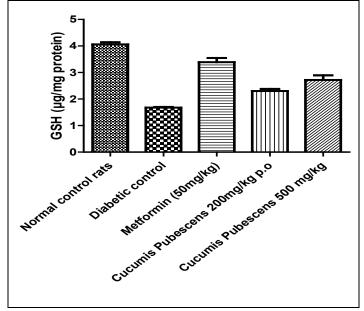


Figure 2: Value of Renal GSH



DISCUSSION

The present result of the research study shows the nephroprotective potential of *Cucumis pubescens* extract shows prominent effect in controlling diabetes. In single treatment therapy of allopathic medicine shows antidiabetic effect or in combination with plants

show synergistic effect in preventing the diabetic complication. *Cucumis pubescens* at 500 mg/kg oral dose reduced the elevated level of blood serum creatinine, blood urea level, serum protein in experimentally induced diabetic nephropathy in rats. *Cucumis pubescens* with high dose treatment therapy shows prominent and significant result in lowering the elevated level of TG, CH and increases the level of good cholesterol HDL levels (7,12). The plant extract also showed effective results in the management of oxidative stress in experimentally induced diabetic nephropathy in rats.

CONCLUSION

From the obtained results of the existing research study it may be concluded that advance investigation and further research is needed to explore to find additional beneficial effect and active constituents of the plant *Cucumis pubescens*. The above result shows that extract of plant shows tremendous nephroprotective activity. Ayurvedic formulation shows pharmacological desirable effect at therapeutic doses and does not produces any complicated reactions.

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CONFLICT OF INTEREST No

ETHICAL APPROVAL

CPCSEA Registration No.436/GO/ReBi/S/2001 dated on Oct, 2017.

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