

Original Article

Medical Science

Oral Administration of Aqueous Extract of *Trichosanthes cucumerina* may Prevent Diabetic Renal Abnormalities

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ABSTRACT [ENGLISH/ANGLAIS]

Diabetes mellitus has many possible complications including diabetic nephropathy, ketoacidosis, retinopathy, neuropathy, etc. This study was targeted at investigating the potentials of aqueous extract of *Trichosanthes cucumerina* (AET) in preventing renal abnormalities in diabetic rabbits. Twenty four adult rabbits randomly grouped into four (Groups A, B, C and D; n = 6*4) were used for this study. Group A (control) was neither made diabetic nor treated with AET. Group B was made diabetic (alloxan-induced). Group C and group D were also made diabetic (alloxan-induced), but were in addition orally administered 100 and 150 mg of AET/Kg Body Weight/Day respectively. All treatments were for eight weeks and the blood glucose level (BGL) was monitored throughout the research period, at one week interval. Twenty-four hours after the last treatment, 24-hour urine sample was collected from each rabbit, and the volumes (24hUV) were recorded. The rabbits body weights were re-determined, BGL re-measured, and they were sacrificed. Blood samples were collected. Urine urea level (UUL), urine creatinine level (UCL), urine glucose level (UGL), serum urea level (SUL), and serum creatinine level (SCL), urea clearance (UC), creatinine clearance (CC), and UC/CC were determined. The control and "test groups" were compared using Students' t-test. P < 0.05 was taken as significant. The results obtained show that diabetes significantly reduces body weight gain and kidney weight, UUL, UCL, UC, and CC increases 24hUV, UGL, SUL, and SCL. While oral administration of AET reduces these diabetic adverse effects.

Keywords: Diabetes, renal abnormalities, *Trichosanthes cucumerina*, renal clearance

RÉSUMÉ [FRANÇAIS/FRENCH]

Le diabète sucré a de nombreuses complications possibles, y compris néphropathie diabétique, une acidocétose, rétinopathie, neuropathie, etc. Cette étude visait à étudier les potentiels de l'extrait aqueux de *Trichosanthes cucumerina* (AET) dans la prévention des anomalies rénales chez les lapins diabétiques. Vingt-quatre lapins adultes hasard regroupés en quatre (groupes A, B, C et D; n = 6 * 4) ont été utilisées pour cette étude. Groupe A (contrôle) n'a été ni rendu diabétique, ni traité avec AET. Groupe B a été rendu diabétique (alloxane-induit). Groupe C et D du groupe ont également été rendus diabétiques (alloxane-induit), mais en plus administrée par voie orale 100 et 150 mg d'AET / kg de poids corporel / jour respectivement. Tous les traitements ont été pendant huit semaines et le niveau glucose sang (BGL) a été suivie pendant toute la période de recherche, à une semaine d'intervalle. Vingt-quatre heures après le dernier traitement, l'échantillon d'urine de 24 heures ont été recueillies auprès de chaque lapin, et les volumes (24hUV) ont été enregistrés. Le poids des lapins corps ont été ré-établi, BGL remesuré, et ils ont été sacrifiés. Des échantillons de sang ont été recueillis. Niveau de l'urée urinaire (UUL), le niveau de l'urine de la créatinine (UCL), le niveau de glucose dans l'urine (UGL), taux d'urée sérique (SUL), et le niveau de créatinine sérique (SCL), clairance de l'urée (UC), la clairance de la créatinine (CC), et l'UC / CC ont été déterminées. Le contrôle et les «groupes d'essai» ont été comparées à l'aide des étudiants t-test. P < 0,05 a été considérée comme significative. Les résultats obtenus montrent que le diabète réduit de manière significative le gain de poids et du poids des reins, UUL, UCL, UC, et augmente CC 24hUV, UGL, SUL, et SCL. Bien que l'administration orale de AET réduit ces effets indésirables diabétiques.

Mots-clés: Diabète, des anomalies rénales, *cucumerina Trichosanthes*, la clairance rénale

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Accepted/Accepté: March,
2011

Full Citation: ADEEYO
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James. Oral
Administration of
Aqueous Extract of
Trichosanthes cucumerina
may Prevent Diabetic
Renal Abnormalities.
World Journal of Young
Researchers 2011;1:4-9

INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder in which tissues are unable to utilize glucose due to unavailability of insulin or end-organ insulin receptor inactivity [1, 2] has many possible complications (e.g. diabetic nephropathy, retinopathy, neuropathy etc [3]) despite that many chemotherapeutic drugs [4], life style measures/modifications and insulin injections [5] have really proven to be very helpful. Decrease in body antioxidants as well as hyperglycemic excessive generation of free radicals induces oxidative stress which causes DM complications and organ damage [6].

This study was, therefore, targeted at investigating the possibility of using aqueous extract of *Trichosanthes cucumerina* (being a good source of antioxidants [7]) in preventing diabetic renal abnormalities by mopping up free radicals [8].

MATERIALS AND METHODS

Adult Rabbits (*Oryctolagus cuniculu*)

Twenty four adult rabbits, *Oryctolagus cuniculu*, [of both sexes, average Body Weight (BW) 1.734 ± 0.141 Kg] obtained from the animal house section of the Department of Physiology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, were used for this study. The animals were kept in the research section of the animal house of Department of Physiology, LAUTECH, Ogbomoso, Nigeria, and allowed to acclimatize over a period of two weeks.

Plant Materials

Trichosanthes cucumerina was obtained from Ogbomoso market, Oyo State, Nigeria, and authenticated at the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria.

Preparation of Aqueous Extract of *Trichosanthes cucumerina* (TC)

The preparation of aqueous extract of TC (AET) was done at the Central University research Laboratory, LAUTECH, Ogbomoso, Nigeria. The TC was dried under shade (ambient temperature was 30 – 40 oC. The dried TC was grinded into powder. One part of the grinded TC was boiled in sixteen parts of distilled water for 15 minutes, and then filtered before cooling, using muslin cloth. The filtrate was poured in an evaporating dish and placed on a water bath at 80 oC, and reduced pressure (70% atmospheric pressure) to remove the solvent. After

all the solvent had evaporated, the extract was scrapped and stored in a dry glass container at a temperature of 0 – 4 oC. Fresh solution of the extract was prepared in distilled water just before use to maintain its potency.

Induction of DM using Alloxan

DM was induced by single intraperitoneal administration of 200 mg of alloxan/Kg BW. Blood sugar level of each animal was then taken at 48th hour post alloxan administration. This was repeated on the 4th and 7th day, during which all the animals were confirmed diabetic (NIDDM positive).

Animal Treatment

The twenty four rabbits were randomly grouped into four (Group A, B, C and D, n = 6). Rabbits in group A served as the control and were neither made diabetic nor treated with AET. Group B rabbits served as diabetic control: they were made diabetic and not treated with AET. Rabbits in group C and D were also made diabetic, but were in addition orally administered 100 and 150 mg of AET/Kg BW/Day respectively. All treatments were for eight weeks, and the blood glucose level was monitored throughout the period of the research.

Animal Sacrifice and Collection of Samples

Twenty-four hours after the last treatment, each rabbit was transferred into a metabolic cage with accessories for collecting urine. Twenty-four hour urine sample was collected for each rabbit. Each rabbit was weighed, and blood glucose level was measured. The rabbits were then sacrificed by cervical dislocation. Blood samples (6 ml/rabbit) were collected by cardiac puncture, and poured in plain bottles. Serum was collected by centrifugation at 3000 rpm for 15 minutes ($g = 9.821$ m/s). Left and right kidneys were excised.

Collection of Data and Statistical Analysis

The weight gain (final weight – initial weight) and mean [(left + right)/2] kidney weights, and 24 hour urine volume were obtained and recorded. Urine and serum creatinine concentration were determined using alkaline picrate method described by Jaffe [9]. Urine and serum urea concentration were determined using diacetylmonoxime method described by Ceriotti et al. [10]. Renal clearance was then calculated using the formula "Clearance of Y = (Urine conc. of Y X 24 hr Urine volume)/Serum conc. of Y" as documented by Guyton and Hall [11].

The control and "Test groups" were compared using t-test. The significant level was set to $P < 0.05$.

RESULTS

The following results were obtained and are presented as mean \pm SEM and level of significance is taken at " $P < 0.05$ " (*), " $P < 0.001$ " (**), and/or " $P < 0.0001$ " (***).

Body Weight Gain (g), Kidney Weight (g) and Relative Kidney Weight (%)

Gain in Body Weight was found to be significantly ($p < 0.001$) lower in group B, while weight gain in groups C and D were not significantly ($p > 0.05$) different from that of the control. A similar trend was noted for kidney weight and the relative kidney weight which were significantly smaller in the diabetes group [Table 1].

Blood Glucose Level

BGL was significantly ($P < 0.0001$) high and relatively constant in the diabetes group (group B) throughout the eight weeks of the research in contrast to the significantly ($P < 0.0001$) high but constantly reducing BGL in the AET treated groups (group C and group D) with respect to the control [Figure 1].

24 Hour Urine Volume (24hUV), Urine Glucose Level (UGL), Urine Urea Level (UUL), Serum Urea Level (SUL), Urine Creatinine Level (UCL), Serum Creatinine Level (SCL)

24hUV of group B was significantly ($P < 0.05$) higher than that of the control, while there was no significant difference in 24hUV of both group C and group D with respect to the control [Table 2]. Glucosuria was found in all the treatment groups (except the control) in the magnitude that was significantly ($P < 0.0001$) highest in group B, higher in group C and not significantly higher in group D [Table 2]. UUL and UCL were significantly lower while SUL and SCL were found to be significantly ($P < 0.001$) higher in the diabetes group (group B) compared to the control.

Urea Clearance (UC), Creatinine Clearance (CC), Ratio of Urea Clearance to Creatinine Clearance (UC/CC)

UC and CC were significantly ($P < 0.05$) smaller in the diabetes group compared to the control, while UC and CC for groups C and D were not significantly different from those of the control [Table 3].

DISCUSSION

The significantly low weight gain in diabetes group (B) and the non-significant difference in weight gain in both group C and group D with respect to the control (A) [Table 1] show that diabetes significantly reduces body weight gain, while oral administration of AEF prevents this adverse effect of diabetes on body weight. This is in support of Chang and Halter [12] who documented that diabetes is characterized by metabolic disorders and deficient metabolic processes as well as improper tissue uptake of nutrients associated with diabetes [1, 2]. It can also be accounted for by the deficiency in the renal functions (e.g. renal re-absorption), which leads to excessive passage of urine (polyuria), and loss of nutrients in urine – aminouria, microalbuminuria, glycosuria, etc [13].

In a similar way, the significantly smaller kidney weight in the diabetes group (B) compared to the control (as well as with respect to the other treatment groups) [Table 1] could also be accounted for (although partly) by the metabolic disorders and deficient metabolic processes as well as improper tissue uptake of nutrients associated with diabetes [1, 2]. Since absolute and relative organ weights could often be used as an important empirical indicator of the normalcy/efficiency of most body organ systems [14, 15], therefore, the significantly smaller kidney weight, as well as the significantly smaller relative kidney weight in the diabetes group would reinforce the evidences that support the claims that diabetes could cause renal abnormalities. This could still be linked (also very vaguely linked) to the kidney-damaging effects of the hyperglycemia and the oxidative stress in diabetes mellitus [16]. This observation is in line with those of Falokun et al. [3] who established that kidney functions are compromised in uncontrolled diabetes mellitus. It may therefore be (at least somewhat) safe to say that it would be the nutritional and anti-oxidant contents of the administered aqueous extract of TC (AET) [8] that prevented the adverse effects of diabetes in the kidney weight and the body weight gain of group C and group D animals.

The significantly high and relatively constant blood glucose level (BGL) in the diabetes group throughout the eight weeks of the research in contrast to the significantly high but constantly reducing BGL in the AET treated groups (groups C and D) [Figure 1] further establishes the anti-hyperglycemic (invariably, the hypoglycemic) effects of AET. This suggests (although just in a moderately sufficient extent/degree) that AET may have

the ability to improve the functions of the B cells of pancreatic islet and/or improve glucose uptake by body tissues. However, the details and/or the molecular bases of how AET could improve the functions of the B cells of pancreatic islet and the uptake of glucose by body tissues could be explained neither by this present investigation nor by its observations.

The significantly higher 24hUV of group B compared to the control and the non-significant difference in 24hUV in both group C and group D with respect to the control [Table 2] show that diabetes significantly increases urine output, while oral administration of AET maintains the output by forcing 24hUV towards the normal average value. This is in favour of the documentations of Dobson [13] that diabetes causes an increase in urine output, and its highly suggestive that AET is able to regulate some renal functions/process (in diabetic condition) especially those that have to do with urine formation (which may include glomerular filtration rate, renal secretion and re-absorption of water, and/or renal secretion and re-absorption of salts). This could be linked to the antioxidant [7] and tissue protective abilities of AET [17], which (in this case) prevent renal damage that the diabetes could have caused.

Furthermore, the glucosuria found in all the treatment groups (except the control) is significantly highest in group B (diabetes), higher in group C (diabetes + 100 mg AET/Kg BW/Day) and not significantly higher in group D (diabetes + 150 mg AET/Kg BW/Day) [Table 2]. This indicated that AET reduces glucosuria that results from diabetes, and adds to the growing evidence that oral

administration of AET is able to protect/regulate body tissues and renal functions in DM. Similar explanations could fit the significantly low urine urea level (UUL) and urine creatinine level (UCL) found in the diabetes group (B) [Table 2]. The significantly low UUL and UCL would, therefore, have caused the significantly high serum urea level (SUL) and serum creatinine level (SCL) found in the diabetes group [Table 2], since serum level of these metabolic end products increases when their excretion in the urine is compromised (such as what is observed here). These are parallel to the observations of Januszewski et al. [18] who found that urinary albumin and PCL in diabetic rats were correlated with the increases in other markers of nephropathy.

Finally, the significantly smaller urea clearance (UC) and creatinine clearance (CC) in the diabetes group (group B) compared to the control (as well as with respect to the other treatment groups, groups C and D) [Table 3] adds to the evidences that diabetes affects renal functions [3], and further establishes that oral administration of AET at least significantly reduces the degree to which diabetes affects renal functions. These observations are more validated by the research outcomes of Caregaro et al. [19] who established that CC is relatively highly sensitive in detecting renal failure. The non-significant difference in the ratio of UC to CC (UC/CC) throughout the groups suggests that diabetes may affect all renal functions equally and that merely measuring some renal parameters (that point to renal function) may give a reasonable insight into how normal or how affected (i.e. the degree of normalcy) of the other renal parameters.

Table 1: This table shows Gain in Body Weight, Kidney Weight, and Relative Kidney Weight (RKW)

Group	Final Body Weight (Kg)	Initial Body Weight (Kg)	Gain in Body Weight (Kg)	Kidney Weight (g)	RKW (%)
A	1.95 ± 0.07	1.62 ± 0.06	0.33 ± 0.06	0.9897 ± 0.0441	0.0512 ± 0.0137
B	1.78 ± 0.08	1.67 ± 0.06	0.12 ± 0.08*	0.8029 ± 0.0465**	0.0452 ± 0.0097*
C	1.92 ± 0.07	1.65 ± 0.05	0.27 ± 0.08	0.9012 ± 0.0531	0.0471 ± 0.0104
D	1.93 ± 0.06	1.57 ± 0.06	0.37 ± 0.08	0.9072 ± 0.0548	0.0473 ± 0.0146

Group A: control; group B: diabetic; group C: diabetic and was administered 100 mg of AET/Kg BW/day; group D: diabetic and was administered 150 mg of AET/Kg BW/day.

Data are expressed in mean ± SEM. Significant difference compared with the control group is indicated by * $p < 0.05$ by independent-samples t-test.

In conclusion diabetes significantly reduces body weight gain and kidney weight, and affects renal functions

(causing increase in 24hUV, UGL, SUL, and SCL, and decrease in UUL and UCL, UC, and CC, which are the

easy-to-measure, and highly reliable, indicators of renal abnormality); while oral administration of AET prevents some and significantly reduces some of these diabetes adverse effects. This is attributable to the cyto-protective and anti-oxidant properties of AET. However, the exhaustive details and molecular bases of how AET

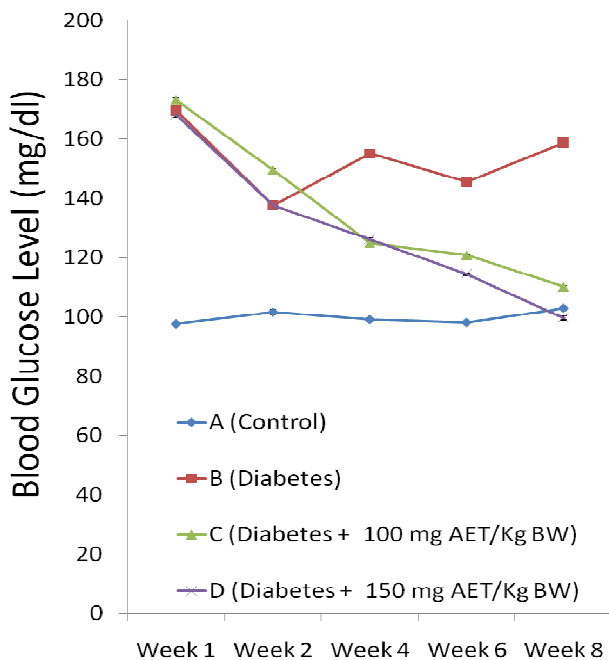
protects against diabetic nephropathy requires further research. And we would also recommend that future researcher should consider using some other (perhaps, more reliable) indicators of renal abnormalities which we could not consider in this study.

Table 2: This table shows 24 Hour Urine Volume (24hUV), Urine Glucose Level (before sacrifice UGL), Urine Urea Level (UUL), Serum Urea Level (SUL), Urine Creatinine Level (UCL), Serum Creatinine Level (SCL)

Group	24hUV	UGL	UUL	SUL	UCL	SCL
A	8.65 ± 0.29	0.00 ± 0.00	47.54 ± 0.66	18.42 ± 0.36	6.62 ± 0.21	1.85 ± 0.11
B	13.3 ± 0.34*	36.22 ± 0.64***	33.38 ± 0.53*	26.55 ± 0.33**	3.58 ± 0.18**	3.64 ± 0.17**
C	10.15 ± 0.22	26.67 ± 0.47***	38.07 ± 0.66	19.57 ± 0.43	5.55 ± 0.26	1.99 ± 0.58
D	9.18 ± 0.15	8.83 ± 0.59	41.6 ± 0.32	15.5 ± 0.32	5.82 ± 0.18	1.69 ± 0.17

Group A: control; group B: diabetic; group C: diabetic and was administered 100 mg of AET/Kg BW/day; group D: diabetic and was administered 150 mg of AET/Kg BW/day. Data are expressed in mean ± SEM. Significant difference compared with the control group is indicated by * $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$ by independent-samples t-test

Figure 1: This figure shows the variations in Blood Glucose Level (BGL) over the 8 weeks of the research.



Data are expressed in mean ± SEM. Significant difference compared with the control group is indicated by *** $p < 0.0001$ by independent-samples t-test

Table 3: This table shows Urea Clearance (UC), Creatinine Clearance (CC), Ratio of Urea Clearance to Creatinine Clearance (UC/CC)

Groups	UC	CC	UC/CC
A	21.36 ± 0.42	29.89 ± 0.45	0.72 ± 0.07
B	15.36 ± 0.32*	19.69 ± 0.50*	0.95 ± 0.12
C	23.16 ± 0.64	29.00 ± 0.58	0.81 ± 0.12
D	26.43 ± 0.50	47.76 ± 1.06	0.76 ± 0.10

Group A: control; group B: diabetic; group C: diabetic and was administered 100 mg of AET/Kg BW/day; group D: diabetic and was administered 150 mg of AET/Kg BW/day Data are expressed in mean ± SEM. Significant difference compared with the control group is indicated by * $p < 0.05$ by independent-samples t-test

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ACKNOWLEDGEMENT / SOURCE OF SUPPORT

Nil

CONFLICT OF INTEREST

No conflict of interest was declared by the authors

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