Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (4), 2024; 1158-1165

Nephroprotective effect of the combination of *Nisha* and *Amalaki* in Alloxan-induced hyperglycaemic rats

Research Article

Dhanya R[1*], Ansary PY[2]

1. Professor, Department of Dravyaguna Vigyana, Sumandeep Ayurved Medical College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat.
2. Professor, Department of Dravyaguna Vigyana, Govt. Ayurveda College, Thripunithura, Kerala. India.

Abstract

Background: Experimental studies have shown that *Nisha* (*Curcuma longa* L) rhizomes and *Amalaki* (*Phyllanthus emblica* L) fruits possess antidiabetic, antioxidant and nephroprotective activity. Their combination is effective in Type-2 Diabetes. The present study was an attempt to assess the nephroprotective activity of the combination of powder of *Nisha* rhizomes and *Amalaki* fruits in alloxan induced hyperglycaemic albino rats. Methods: The in-vivo study was conducted at the facility in Govt. Ayurveda College, Thiruvananthapuram. Wistar rats of both sexes weighing 150-200 gm were induced with hyperglycaemia using intraperitoneal injection of Alloxan monohydrate (120mg/kg). The test drug in the ratio 1:4 (*Nisha*:*Amalaki*) was given in three groups in the doses, 0.108g, 0.216g and 0.432g /200g body wt. They were compared with normal control (euglycaemic healthy animals), diabetic control (no treatment hyperglycaemic rats) and standard conventional antidiabetic group administered with Glibenclamide in the dose 0.18 mg/200g body wt. After 30 days, blood, urine and tissue samples were collected and analysed for Morphological, Biochemical parameters and Histopathological changes. Results and conclusion: Statistical evaluation was done using Kruskal-Wallis Test and Mann-Whitney Test. Test drug given in dose 0.108g corresponding to human dose 6 g showed the best results with respect to Morphological and biochemical parameters among all treated groups. Histopathological parameters- glomerular and tubular changes and presence of infiltrative cells- returned to normal in all the treated groups which shows that euglycemia is important for prevention of renal changes in diabetics. The study shows that the drug combination is effective in preventing Diabetes induced renal damages.

Keywords: Diabetic nephropathy, *Nishamalaki,* Glomerular sclerosis, Ayurveda, In vivo study.

Introduction

Chronic diseases are the leading cause of death and disability worldwide, accounting for almost 60% of all deaths and 43% of the global burden of disease. Four of the most prominent chronic diseases are cardiovascular diseases, cancer, chronic obstructive pulmonary disease, and type 2 diabetes (1). The International Diabetes Federation (IDF) have estimated that 451 million adults live with diabetes worldwide in 2017 with a projected increase to 693 million by 2045, if no effective prevention methods are adopted. India ranks number two in the countries with the largest numbers of adults with diabetes aged 20–79 years in 2021with 74.2 million diabetics which is projected to grow to 124.9 million till 2045(2). Chronic diseases, such as diabetes mellitus, are a significant burden on patients, their families, and care givers. It is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective insulin secretion. These metabolic disturbances result in acute and long-term diabetic complications, leading to premature death and disability. Diabetic nephropathy (DN) is one of the most frequent and severe complications of diabetes mellitus (DM) (3). Approximately forty percent of patients with DM develop DN, which is the most common cause of end-stage kidney failure and renal replacement therapy (4). Preventing the onset of these complications is crucial in managing DM.

Plants which are both antidiabetic and antioxidant will be a safe alternative or adjuvant in preventing DN. The combination of *Nisha* (*Curcuma longa* Linn.) and *Amalaki* (*Phyllanthus emblica* Linn.), is considered as best drug for the treatment of *Prameham* (Diabetes mellitus) in ancient Ayurvedic textbooks (5). *Nisha*, a well-known drug, has been used for centuries for its cosmetic, antidiabetic and antiallergic properties (6). *Amalaki*, another plant, known for its rejuvenating properties, is used in many diseases involving multiple systems (7). The hypoglycemic effect of the combination has been proven too (8). Both these drugs are cheap and available in plenty in almost all parts of India. So, this study aims to find out the nephroprotective activity of the drug combination in diabetes induced renal damage.

*** Corresponding Author:**

Dhanya R

Professor, Department of Dravyaguna Vigyana, Sumandeep Ayurved Medical College and Hospital, Sumandeep Vidyapeeth, Vadodara, Gujarat.

Email Id: dhanya_212@yahoo.co.in

Materials and Methods

Study drug

Table 1 : Collection, Processing and storage of Test Drugs

Drug Name	Site of collection	Time of collection:	Processing and storage
Curcuma longa Linn.	Haripad, Alappuzha dist. , Kerala	In the month of January when the leaves had just dried off	The rhizomes were cleaned, washed, and boiled till soft and shade dried. The dried rhizomes were then trampled upon to remove the outer skin and stored in air tight containers.
Phyllanthus emblica Linn.	Ayaparambu, Alappuzha Dist., Kerala	In the month of February.	They were washed, cut into pieces, stony endocarp was removed, and then fleshy mesocarp was taken. This fleshy mesocarp was shade dried and stored in air tight containers.

The drugs were authenticated at Drug Standardisation Unit, Government Ayurveda college, Thiruvanathapuram, Kerala. *Preparation of test drug:* Both the drugs were powdered separately into fine powder of 120 mesh size. The powder of *Nisha* and *Amalaki* were mixed in the ratio 1:4. The test drug in powder form was administered in various doses as suspensions with distilled water. *Dose of the test drug*: The dose for the test drug was calculated using the table constructed by Paget G. E. and Brans J.M. (1964) for evaluation of drug activities, by fixing the human dose of *churnam* (powder) as 12 gm (9). Half this probable dose and double this dose were also given to separate groups to find out the most effective dose in preventing nephropathy.

Selection and care of animals

The experiment was performed at animal house of Govt. Ayurveda College, Thiruvananthapuram. The study was approved by Institutional Animal Ethics Committee, Govt. Ayurveda College, Thiruvananthapuram (order No: 01/IAEC/AVC/2010. Healthy wistar strain albino rats of either sex weighing 150-200 gm were housed under standard laboratory condition in polyethylene cages giving 12hr dark and night cycle and fed by standard rat feed and water. The animals were acclimatized at laboratory hygienic condition for two weeks before starting the experiment. The bedding was of standard wood shaving material. It was changed every third day or as necessary. The animals were monitored twice daily. Weight was taken daily and activities were monitored. The animal handling was performed according to Good Laboratory Practice (GLP).

Chemicals

Alloxan monohydrate (S.D. Fine-Chem Limited, Mumbai), Glibenclamide (Daonil of Sanofi Aventis Pharma India.), Carboxymethylcellulose Sodium (Merck), Glucose Kit GOD-POD Method (Erba Mannheim), creatinine Kit -Jaffe's Method (Erba Mannheim), Urea (BUN) kit GLDH-Urease method (Erba Mannheim), Total protein Kit (Erba Mannheim),

Experimental design

Hyperglycaemia was induced in rats experimentally by administering Alloxan monohydrate.

Preparation of Alloxan monohydrate solution: 5 g of Alloxan monohydrate was dissolved in 50 ml normal saline so that 1 ml normal saline contains 100 mg alloxan monohydrate. It was given to the animals in a dose of 120 mg/kg body weight.

Procedure: 30 wistar albino adult rats of either sex (weighing 150-200 gm) were used in the study. The animals were observed for weight food intake, activities, and general health for two weeks. Diabetes was induced by using alloxan monohydrate after fixing its dose to 120mg/kg body weight. Freshly prepared alloxan monohydrate in sterile normal saline was injected intraperitoneally, after fasting the animals for 16 hrs. To prevent fatal hypoglycaemia, rats were treated with 20% glucose solution after three hrs followed by a supply of 5% glucose solution bottles in their cages for the next 24 hrs. After 72 hrs blood was collected by tail-clipping method. The animals who showed blood glucose levels>200 mg/dl were included in the study (11). They were randomly assigned to five groups. The first group contained five non-diabetic rats which were taken as normal control. Hyperglycaemic rats were grouped into five, each group containing five animals. They were caged separately and were marked for individual identification. The second group formed Diabetic control. The third group received double the effective dose of the drug combination (i.e.0.432/200 wt) and the fourth group received half the effective dose of the drug combination (0.108g/200g bdy wt). The fifth group was administered with the effective dose of the drug combination (0.216g/200 g bdy wt). The effective dose was calculated by taking 12 g as the effective adult human dose of choorna as per reference from Sharangdhara Samhita (9) and converting it to animal dose using Paget and Barnes table (multiplying with conversion factor 0.018for 200 g bdy wt rat -12x0.018= 0.216g for 200 g bdy wt)) the sixth group received conventional antidiabetic drug in the dose 0.18 mg/200g bdy wt (by taking the adult human dose as 10 mg and by multiplying it with Paget and Barnes table conversion factor). The test drug and conventional antidiabetic drug were administered for 30 days by oral route using an intra gastric tube.

Grouping of animals

Table 2: Grouping of animals

Group	Name of the group	No: of animals in each group	Drug given and dose of the drug
Group I	Normal control (NC)	5	No drug – vehicle
Group II	Diabetic control (DC)	5	No drug - vehicle
Group III	Treatment group I (TG-I)	5	Double the dose of the drug combination (i.e.0.432/200gbdy wt)
Group IV	Treatment group II(TG-II)	5	Half the dose of the drug combination (0.108g/200g bdy wt)
Group V	Treatment group III (TGIII)	5	One Dose of the drug combination (0.216g/200 g bdy wt)
Group VI	Treated with conventional antidiabetic drug (TCAD)	5	Glibenclamide in the dose 0.18 mg/200g bdy wt
	Total number of animals used	**30**	

The body weights of the animals were noted before and after the study. After 30 days, all the animals were put in metabolic cage and urine was collected. The urine of animals in each group was measured to know the volume of output and was analyzed for urine protein and urine creatinine. After that the animals were fasted for 12hrs. anaesthetized and they were sacrificed. Blood samples from jugular arteries were collected and kidneys were removed. Blood glucose level and biochemical parameters for kidney damage such as Blood urea nitrogen (BUN) and Serum Creatinine were estimated. Kidneys were subjected to histopathological changes with regarding to glomerular changes, tubular changes and presence of infiltrative cells.

Blood glucose is estimated by GOD/POD method. In this test, the intensity of the pink coloured complex formed as a result of oxidation of glucose is used to estimate the glucose level. Standard solution is prepared by mixing 1ml of working reagent and 10µl of standard solution. Test solution is prepared by mixing 1ml of working reagent and 10µl of test sample. Blank solution is prepared by mixing 1 ml working reagent and 10 µl of distilled water. These solutions were mixed well and the optical density after 10 minute incubation was read. The results were calculated using the formula, Glucose Concentration in mg/dl=

Serum creatinine and urine creatinine were estimated using Jaffe's method. Standard solution is prepared by mixing 1000µl of working reagent and 100µl of standard solution. Test solution is prepared by mixing 1000µl of working reagent and 100µl of test sample. These solutions are mixed well and initial absorbance (A_1) 20 seconds after mixing and final absorbance (A_2) 80 seconds after mixing are recorded. The results were calculated using the formula, Creatinine in mg/dl= where $\Delta A = A_2 - A_1$ and concentration of standard is 2 mg/dl

BUN was estimated using GLDH-Urease method. Standard solution is prepared by mixing 1000µl of working reagent and 20µl of standard solution. Test solution is prepared by mixing 1000µl of working reagent and 20µl of test sample. These solutions are mixed well and initial absorbance (A_1) 20 seconds after mixing and final absorbance (A_2) 80 seconds after mixing are recorded. The results were calculated using the formula, Urea in mg/dl= where $\Delta A = A_2 - A_1$ and concentration of standard is 50 mg/dl. To convert from mg/dl of Urea to mg/dl of BUN multiply the results by 0.467.

Protein was estimated using Biuret Method. In this test, the intensity of the violet-coloured complex is used to estimate the protein level. Standard solution is prepared by mixing 1ml of working reagent and 10µl of standard solution. Test solution is prepared by mixing 1ml of working reagent and 10µl of test sample. 1 ml working reagent was taken as blank solution. These solutions were mixed well and the optical density after 5 minute incubation was read against the reagent blank. The results were calculated using the formula, Protein Conc. in g/dl=

Histopathological examination: Kidneys were removed and kept in 10% formalin solution for histopathology studies. For histopathological studies two blocks of tissues were taken from different sites of the kidney. Paraffin sections were prepared and stained with haematoxylin and eosin in methodology. Arbitrary scores were used to assess renal changes Arbitrary scores used are Severe (+ + +), Moderate (++), Mild (+), Absent (0).

Assessment of hyperglycemia, kidney damage and efficacy of the drug were evaluated with the help of urine output, morphological, biochemical and histopathological parameters.

Statistical Analysis: The changes seen to assessment parameters in treatment groups were compared with the normal control, diabetic control and conventional antidiabetic drug treated groups. The difference between each peers group was calculated using "Mann-Whitney Test", and the difference between all studied groups was assessed by "Kruskal-Wallis Test".

Results

The results can be summarised as follows. DC showed significant reduction in general health and activities and body weight. A significant increase in volume of urine, and biochemical parameters and pathological changes in kidney occurred in them when compared to NC. Compared to DC, all the test groups treated with any medicine showed significant reduction with respect to all measured parameters. The animals treated with half the effective dose (TG-II) showed greatest response to the treatment with respect to general health and activities, polyuria, weight gain and

biochemical parameters. They also showed statistically significant difference in BUN, Serum Creatininie and Urine Protein when compared to TCAD. Compared to TCAD, TG-III showed better means with respect to weight gain and in all biochemical parameters. TG-I and TCAD showed equal response. There were no marked pathological changes in TG-I, TG-II, TG-III and TCAD.

General health and other conditions during the study

The health of animals in groups TG-I, TG-II, TG-III and TCAD was compared with that of DC and NC. DC showed severe weight loss and reduced physical activities, reddening of paws and reddish secretion in the medial canthus. The urine collected from them was frothy and turbid. The treatment groups after diabetes induction, showed weight loss and decreased physical activities. Upon starting treatment, the animals regained health slowly. TG-II group regained health and weight completely. The urine collected from this group was clear whereas other groups showed slight turbidity of urine.

Urine Volume: The mean value of urine volume in NC was nine ml and that in DC was 18 ml. The mean value of urine volume in TG-II (animals given half the dose drug) and TCAD (animals given standard anti-diabetic drug) had returned to 10ml i. e. almost equal to the mean value of NC. All the treatment groups had recovered from polyuria with respect to DC.

Urine Creatinine: has increased significantly in DC (3.10) when compared to NC (0.48). All the treatment groups showed a significant decrease in Urine Creatinine with respect to DC. The mean Urine Creatinine value of treatment group –II (0.56) and Treatment group III (0.62) was almost similar to the mean value of normal control (0.48) as they share similar superscripts.

Urine Protein: In DC (37.50) Urine Protein has increased significantly with respect to NC (9.40). All treatment groups showed a significant reduction in Urine Protein. Treatment group II (animals given half the dose) showed the greatest response to the drug with respect to Urine Protein.

Effectiveness of trial on various urine parameters

Table: 3 Effectiveness of trial on various urine parameters

Group	Volume of urine		Group	Urine creatinine		Group	Urine protein	
	Mean (in ml)	S.D		Mean (in	S.D		Mean(in g/	S.D
NC[a]	9.00	0.00	NC[a]	0.48	0.07	NC[c]	9.40	1.06
DC[d]	18.00	2.00	DC[c]	3.10	0.10	DC[d]	37.50	1.49
TG-I[b]	13.00	1.73	TG-I[b]	0.68	0.05	TG-I[a]	28.60	1.91
TG-II[a,c]	10.00	1.00	TG-II[a,b]	0.56	0.06	TG-II[e]	18.10	1.15
TG-III[b]	13.33	1.53	TG-III[a,b]	0.62	0.09	TG-III[b]	24.40	2.69
TCADa,b,c	10.00	2.65	TCAD[b]	0.68	0.06	TCAD[a,b]	27.20	2.67
p	0.022*			0.027*			0.008*	

Effectiveness of trial on various Morphological parameters

Body weight: There is no statistically significant change in gain of body weight or percentage of gain in body weight among the groups. Animals in the Diabetic control had 17.83% weight loss. Treatment group II i.e. animals given half the effective dose showed 8% weight gain.

Kidney volume: There is no significant change in volume of kidney among the groups.

Kidney weight: There is no significant change in weight of kidney among the groups.

Table: 4 Effectiveness of trial on various morphological parameters

Group	Gain of body weight		Group	Percentage gain of body weight		Group	Kidney volume		Group	Kidney weight	
	Mean (in	S.D.		Mean	S.D.		Mean (in	S.D		Mean	S.D
NC[a]	5.00	5.00	NC[a]	3.23	3.34	NC[a]	1.17	0.25	NC[a]	1.42	0.41
DC[a]	-30.00	18.03	DC[a]	-17.83	12.22	DC[a]	1.70	0.36	DC[a]	1.66	0.21
TG-I[a]	-21.67	20.82	TG-I[a]	-14.48	13.83	TG-I[a]	1.07	0.38	TG-I[a]	1.38	0.20
TG-II[a]	15.00	15.00	TG-II[a]	8.57	8.57	TG-II[a]	1.43	0.06	TG-II[a]	1.31	0.01
TG-III[a]	-11.67	45.37	TG-III[a]	-9.43	26.93	TG-III[a]	1.13	0.40	TG-III[a]	1.20	0.39
TCAD[a]	-13.33	27.54	TCAD[a]	-8.89	18.36	TCAD[a]	1.33	0.35	TCAD[a]	1.33	0.30
p	0.182			0.208			0.336			0.534	

Effectiveness of trial on various Biochemical investigations:

Blood sugar: Blood sugar level has increased significantly in DC with respect to All the treatment groups and TCAD showed significant decrease in blood sugar with respect to DC i.e. 249.67, 204.67, 201 and 246.33 respectively. Treatment group-II and III (animals

given half the dose and probable dose of drug) showed best response compared to TG-I and TCAD.

BUN: BUN has increased considerably in DC (143.57) when compared to NC (12.67). All the treatment groups showed significant decrease in BUN with respect to DC. Moreover treatment group –II (animals given half the effective dose of drug) showed the best response in decreasing BUN as seen from the table below.

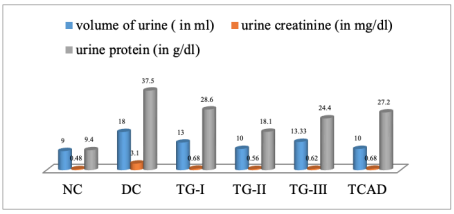
Serum Creatinine: The mean S.Creatinine value was 0.5mg/dL and I.47mg/dL in NC and DC respectively. S.creatinine has increased significantly in DC group. TG-I (0.87) and TG-III (0.77) showed similar response when compared to TCAD whereas TG-II significantly decreased Serum Creatinine.

Table: 5 Effectiveness of trial on various Biochemical investigations

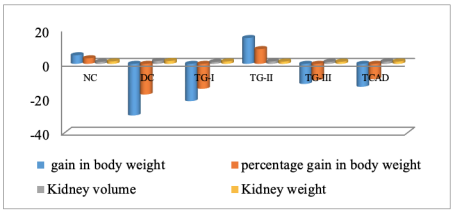
Group	Blood Sugar		Group	BUN		Group	Serum Creatinine	
	Mean(in mg/dL)	S.D		Mean (in mg/dL)	S.D		Mean (in mg/dL)	S.D
NC^{e}	84.67	10.69	NC^{d}	12.67	4.20	NC^{c}	0.50	0.00
DC^{a}	413.67	57.14	DC^{e}	143.57	3.65	DC^{d}	1.47	0.06
TG-I^{b}	249.67	8.50	TG-I^{a}	68.70	8.40	TG-I^{a}	0.87	0.06
TG-II^{b,c}	204.67	44.07	TG-II^{b}	41.83	3.45	TG-II^{b}	0.67	0.06
TG-III^{c,d}	201.00	36.51	TG-III^{b,c}	49.67	7.73	TG-III^{a,b}	0.77	0.06
TCAD^{b,d}	246.33	103.06	TCAD^{a,c}	82.53	44.08	TCAD^{a}	0.87	0.06
p	0.027*			0.008*			0.007*	

Graphs representing the effectiveness of trial on different parameters

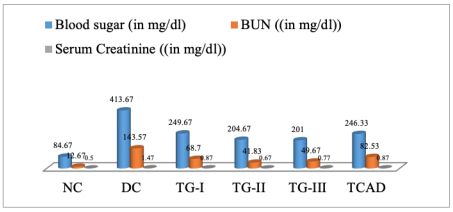
Graph: 1 Effectiveness of trial on Urine parameters



Graph: 2 Effectiveness of trial on morphological parameters



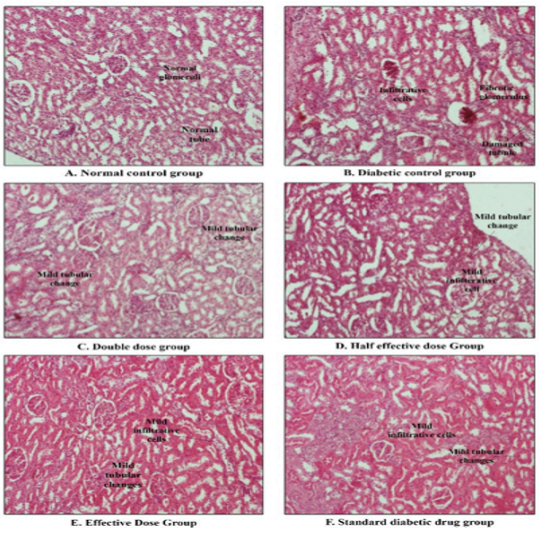
Graph: 3 Effectiveness of trial on various Biochemical investigations



Effectiveness of trial on Histopathological changes

Glomerular changes, Tubular changes, Presence of infiltrative cells: There is highly significant sclerotic changes in glomeruli, significant pathological changes in renal tubules and presence of infiltrative cells in DC. All the treatment groups and TCAD returned to normalcy with regard to all Histopathological changes. (Figure 1).

Figure 1: Photographs of the cross-section of kidneys of animals in each group



Discussion

The study showed that continuous intake of the test drug in half the effective dose (6 g) is effective in correcting diabetes-related renal damage. The study also confirmed that any mode of treatment reducing hyperglycemia offers protection against renal damage in Diabetes. The biochemical parameters like blood sugar, BUN, S. creatinine, U. Creatinine, and Urine Protein can be managed by using the powder of the combination of *C.longa* and *P.emblica* in the ratio 1:4.

Histopathological investigations revealed significant pathological lesions in diabetic control compared to normal control. There was glomerular necrosis, tubular changes, and infiltration of inflammatory cells in Diabetic control kidneys which shows that uncontrolled Diabetes can lead to structural changes in major structures of the kidney like glomeruli, tubules, etc. All treatment groups and TCAD returned to normalcy with respect to glomerular changes, tubular changes, and infiltration of inflammatory cells.

Chronic hyperglycemia during diabetes causes gyration of body proteins, leading to secondary complications affecting eyes, kidneys, nerves, and arteries. Oxidative stress may play a role in the development and progression of complications, making the prevention of diabetic nephropathy an important

issue (10). Herbal drugs, such as *Nisha* and *Amalaki*, offer hope in managing these conditions due to their safety profile and properties like antioxidant, antidiabetic, nephroprotective, etc. Studies have shown that *Curcuma longa* possesses antioxidant, nephro-protective, and anti-diabetic properties (12-13). Amalaki is a well-known antidiabetic, anti-inflammatory, antioxidant, and nephroprotective drug (14-15). The combination has also been shown to lower Insulin resistance in Diabetics (16)

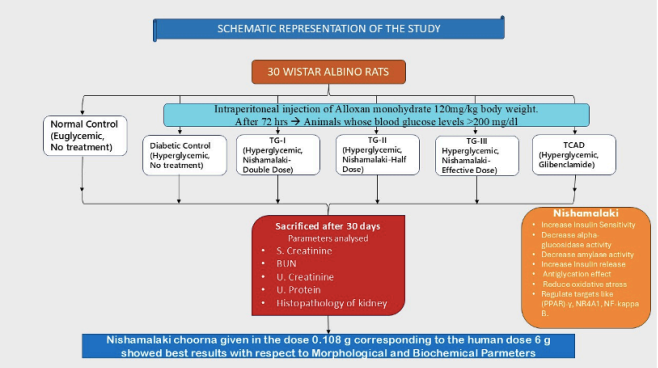
Selection of formulation and proportion of test drug: In *Prameharoga*, the use of *Nisha choorna*(powder) or *kalka*(paste) mixed with *Amalaki swarasa*(juice) is prescribed by *Brhattrayis* and other classics. Despite this indication, the *choorna* of *Amalaki* was taken for this study due to the unavailability of fresh fruits in all seasons and for the convenience of administration. In some of the earlier antidiabetic studies also powder formulation was used and found effective. The proportion was fixed to one part of *Nisha*: four parts of *Amalaki* because the standard ratio of adding *Choorna* or *Kalka* to *Swarasa* is 1:4 as per *Sarngadhara Samhita* (17).

Grouping: A Normal control and diseased control group was kept to assess the change in parameters in an uncontrolled state of Diabetes compared to that of a normal animal. The study drug was given in three doses- effective dose, half the effective dose and double the effective dose as an optimal dose for the prevention of nephropathy in diabetics was not available.

Experimental model to study Diabetic nephropathy: Hyperglycemia was induced in rats experimentally by administering Alloxan monohydrate. The alloxan-treated animals exhibit severe hyperglycemia, glycosuria, hyperlipidemia, polyphagia, polydipsia, and other symptoms of uncontrolled diabetes and also develop various complications such as nephropathy, cardiomyopathy, well-marked retinopathy, and others (18). Diabetic rats are kept for a period of 21 days or more, to develop diabetic nephropathy by researchers. Studies have shown that diabetic nephropathy develops in diabetic rats as early as 21 days. So, it seems that a period of one month is ample time for development of diabetic nephropathy in diabetic rats. The haemodynamic changes induced by increased blood sugar causes proteinuria, glomerulosclerosis, tubulointerstitial fibrosis in patient's kidneys.

Probable mode of action: Research shows that oxidative stress and glycated proteins, originating from long-standing hyperglycemia, may play a role in the development of diabetes. So, hypoglycemic agents coupled with antioxidants may help in managing Diabetes and its complications. A network pharmacology based study involving 201 phytochemicals and 262 targets of Nisha and Amalaki showed that curcumin and its derivatives, bis-(4-hydroxycinnamoyl)methane, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one, calebin A, and quercetin regulated various DM targets like AKT1, GSK3B, (PPAR)-γ, NR4A1, NF-kappa B, JUN, Mcl-1, Bcl-2,and IL-2. In addition, Nisha and Amalaki may regulate T2DM by modulating glucose and lipid metabolism, β-cell survival and proliferation, regulation of insulin resistance, inflammation, apoptosis, and cell cycle through PI3K-Akt, TNF, FoxO, Jak-STAT, MAPK, and NF-kappa B signaling pathway(19). *C. longa* has antidiabetic, Protein glycation inhibitory potential and LDL oxidation inhibitory potential(20). The chemical constituents present in *C. longa*, Curcumin, demethoxycurcumin, bis-demethoxy-curcumin, and ar-turmerone, suppressed the increase blood glucose level and contributed to the effect via peroxisome proliferator-activated receptor (PPAR)-gamma activation(21). These chemicals also have protective action from Advanced Glycation End Products-induced (AGEs- induced) Mesangial cell apoptosis and oxidative stress(22). It also has antiglycation activity, beta cell protective activity(22-23). *P.emblica* also has beta cell protective action and stimulates the secretion of insulin (24-25). It inhibited the activities of α-amylase, α-glucosidase, and dipeptidyl peptidase-4 and displayed antioxidant potential(26).Because of its high phenolic content, it has Antiglycation and antioxidant properties. Thus, the combination of *Phyllanthus emblica* and *Curcuma longa* may provide a nephroprotective effect through various mechanisms, such as increasing insulin sensitivity, decreasing α glucosidase activity, decreasing amylase activity, beta cell protection, increasing insulin release, antiglycation effect, reducing oxidative stress and regulating targets like (PPAR)-γ, NR4A1, NF-kappa B.

Figure 2: Schematic representation of the study



It is further recommended that Advanced biomolecular studies can be done to have a better understanding of the mechanism of action of the drug combination with detailed analysis of the pharmacokinetics. Curative effect of the drug in Diabetic Nephropathy can also be sought for with an experimental model and afterwards by a clinical trial.

Conclusion

Chronic diabetes if unchecked leads to complications. Oxidative stress and upregulation of inflammation mediators and associated pathways have been found to be the causative factors for Diabetic Nephropathy. Plants and compounds in them can reduce the AGE's, act as antioxidant, modulate immune factors of diabetes and reduce systemic and organ-specific inflammation. The combination of *Nisha* and *Amalaki* is

a famous Ayurvedic preparation having antidiabetic activity. The present study shows that the combination has nephroprotective effect in Diabetes mellitus. The drug combination in half the effective dose (6 g) is found to be most effective in giving protection against renal damage due to Diabetes mellitus.

Declaration of competing interest: the authors report no conflict of interest.

Acknowledgment: Scientists and staff of Drug Standardisation Unit, Thiruvananthapuram, Staff of Animal House, Govt. Ayurveda College, Thiruvananthapuram.

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