

Original Research Article

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Studies of Anti-Diabetic Effect of *Morinda citrifolia* Fruit Juice on Alloxan Induced Diabetic Rat

Nitin D. Jadhav, Debi P. Mishra*, Abhinna K. Behera,
Sudhir R. Rajurkar and Bhagirath V. Ballurkar

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and
Animal Sciences, Parbhani, India

*Corresponding author

ABSTRACT

Diabetes is a metabolic disorder due to insufficiency of insulin (absolute/relative) and generally classified as IDDM and NIDDM. Present study was conducted to observe the anti-diabetic effect of *Morinda citrifolia* fruit juice. 48 wistar rats of either sex comprising of four groups were taken for the study. Three groups of 36 rats was induced diabetes with single i/p administration of 5% w/v of alloxan monohydrate in normal saline. One group was kept as healthy control (Group-I) and one as diabetic control (Group-II). For comparison of the effect of *Morinda citrifolia* fruit juice @ 2 mg/kg Bwt. (Group-IV) with normal standard drug i.e. metformin @ 100 mg/kg Bwt. (Group-III). All the treatments were given through oral route. Parameter studied were serum Glucose, Triglyceride, Total cholesterol, HDL, LDL and Haemoglobin at 1st, 14th, 28th days after commencement of treatment. After induction of diabetes serum glucose and triglyceride concentration increased and total cholesterol, HDL, LDL concentration decreased at statistically significant level where as there was no statistical change in haemoglobin level. The group treated with *Morinda citrifolia* fruit juice showed better result but the best result was obtained with the standard anti-diabetic drug.

Keywords

Anti-Diabetic,
Morinda citrifolia,
Fruit Juice,
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Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world's population. It is characterized by dysregulation in the carbohydrate, protein and fat metabolisms caused by the complete or relative insufficiency of insulin secretion and/or insulin action (O' Brien and Granner, 1996). Diabetes mellitus is currently a major life style disease for now a days and has public health importance, because its incidence and prevalence are elevated and increasing, reaching epidemic proportions. Diabetes can be diagnosed by presence of

four classical signs that include polyurea, polyphagia, polydipsia and, foremost, hyperglycemia. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the produced insulin (Gardner and Shoback, 2011).

There are three main types of diabetes mellitus-

(1) Type 1 DM - Results from the body's failure to produce enough insulin, previously

referred to as insulin dependent diabetes mellitus (IDDM). (2) Type 2 DM - Begins with insulin resistance, a condition in which cells fail to respond to insulin properly, previously referred to as non-insulin dependent diabetes mellitus (NIDDM). (3) Gestational diabetes - Begins when pregnant women without a previous history of diabetes develop a high blood glucose level.

Untreated diabetes can cause many complications. Acute complications include diabetic keto-acidosis and nonketotic hyperosmolar coma (Kitabchi *et al.*, 2009). Serious long term complications include heart disease, stroke, kidney failure, foot ulcers, and damage to the eyes. Diabetes doubles the risk of cardiovascular disease (Sarwar *et al.*, 2010) and 75% of deaths in diabetics are due to coronary artery disease (O’Gara *et al.*, 2013). Damage to nerves known as diabetic neuropathy, lead to numbness and altered pain sensation. There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5 fold greater rate of decline in cognitive function (Cukierman *et al.*, 2005). Diabetes mellitus is currently a major public health concern, because its incidence and prevalence are elevated and increasing, reaching epidemic proportions. As at 2013, 382 million people have diabetes worldwide. This is equal to 8.3% of the adult population (Yuankai and Hu, 2014).

Morinda citrifolia Linn (Rubiaceae) also known as Indian mulberry (Noni) is a small evergreen tree. The leaves are 8-10 inches long, oval shaped; dark green and shiny, with deep veins (Rivera *et al.*, 2011). Fruit juice of *M. citrifolia* is a well known health drink and has various pharmacological properties including anti-diabetic, antioxidant and anti-inflammatory (Harada *et al.*, 2010) antitumour activity (Hirazumi and Furusawa, 1999).

Materials and Methods

Materials

Experimental animals

The present study was conducted in 48 Wistar rats of either sex, age 4-6 weeks. The wistar rats of in-house; Animal house facility of College of Veterinary and Animal Sciences, Parbhani were used. Animals were selected after physical and behavioral examination. The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol.

The Wistar rats were kept under constant observation for at least five days before commencement of the experiment. All necessary management procedures were adopted to keep the animals free from stress.

Drugs and chemicals

Alloxan

The alloxan was used for induction of diabetes in Wistar rats.

Chemical name : Alloxan
Empirical formula : $C_4H_2N_2O_4 \cdot H_2O$
Batch no : 033/0326/P1
Vehicle : Normal Saline

Metformin

Metformin was used as standard oral hypoglycemic drug as is most commonly used oral hypoglycemic agent.

Compound name: Glycomet
Chemical name: Metformin
Hydrochloride
Batch No : 28006694
Vehicle : Deionised water

Plant material

***Morinda citrifolia* L.**

Botanical name : *Morinda citrifolia*
Family : *Rubiaceae* (coffee family)
Common name: Indian Mulberry,
Great morinda
English : Noni
Hindi : Bartundi
Sanskrit : Ayushka
Marathi : Nagakunda
Gujarati : Surangi

***Morinda citrifolia* L. (Noni) fruits juice**

Morinda citrifolia L. fruit juice prepared by the College of Food Science and Technology, MKV, Parbhani was used in the present study.

Methods

Experimental design

Table 1 shows experimental design, distribution of different groups and treatment given. Each group contained 12 animals of either sex.

Induction of diabetes

Out of 48 healthy wistar rats, 36 rats from group II to IV were fasted overnight; diabetes was induced by Intraperitoneal administration of alloxan monohydrate (S. D. Fine Chem. Ltd., Mumbai) in physiological saline as 5 percent W/V at the dose rate of 100 mg/kg body weight. After one week, diabetic status was confirmed in the alloxan administered rats by estimating plasma glucose level >200 mg/dl.

Collection of sample

Blood samples were collected in aliquots on the 0 day, 14th day & on the day of animal

sacrifice (28th day), from retro-orbital plexus with the help of capillary tube for estimations of blood biochemical parameters.

Parameter studied

The serum was separated from blood and following parameters were estimated by using serum biochemistry semi-auto analyzer (Model- Chem.7).

Blood glucose (GLU) : (GOD/POD method)

Triglyceride (TG) : (GOD/POD method)

Total cholesterol (TC) : (CHOD/POD method)

High density lipoprotein (HDL) : (CHOD/POD by HDL precipitating agent)

Low density lipoprotein (LDL) : Total cholesterol minus HDL

Blood haemoglobin level was measured by: (Sahli's Acid haematin method)

Statistical analysis

The data obtained from various parameters from all groups was analyzed by as per the method suggested by Panse and sukhatme (1967) using Factorial Randomized Block Design (FRBD).

Results and Discussion

All the results obtained were presented in tabulated format for appropriate analysis. After statistical analysis, data of different groups were presented taking critical difference (CD) at both 5% and 1% confidence level. After intra-group and inter-group comparison designations like significant (S), non-significant (NS) and highly significant (HS) were given.

Superscripts a,b,c.... shows significant difference within the column (between different groups on specific day) at p=0.05.

Superscripts p,q,r.... shows the significant difference within the row (between different days in a specific group) at p=0.05.

Table.1 Experimental design

Group N= 12	Treatment	Dose	Route
I	Healthy control	Normal saline as vehicle	-
II	Alloxan	100 mg/kg body weight	Intraperitoneal
III	Alloxan	100 mg/kg body weight	Intraperitoneal
	Metformin	100 mg/kg body weight	Oral in deionised water
IV	Alloxan	100 mg/kg body weight	Intraperitoneal
	<i>Morinda citrifolia</i> L. fruit juice	2 ml/kg body weight	Oral

All the animals were maintained for the experimental period of 28 days

Table.2 Blood glucose level (Mean ± S.E, mg/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day (Mean±S.E)	14 th day (Mean±S.E)	28 th day (Mean±S.E)		
I	Healthy control	90.43 ^{dp} ± 2.84	89.29 ^{dp} ± 2.73	89.18 ^{dp} ± 2.03	NS	At 5% 8.65 At 1% 11.48
II	Diabetic control	422.30 ^{bpq} ± 6.77	428.79 ^{ap} ± 10.05	412.66 ^{aq} ± 7.25	S	
III	Metformin @ 100 mg/kg BW	443.42 ^{ap} ± 9.98	307.90 ^{bq} ± 5.79	129.69 ^{cr} ± 2.77	HS	
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	396.72 ^{cp} ± 7.83	287.47 ^{cq} ± 8.07	155.88 ^{br} ± 5.80	HS	
Stat		HS	HS	HS		
CD		At 5%- 10.0 At 1%-13.24				

Table.3 Serum Triglyceride concentration (Mean ± SE, mg/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day	14 th day	28 th day		
I	Healthy control	93.81 ^{cp} ± 3.25	93.96 ^{dp} ± 2.45	90.35 ^{cp} ± 3.01	NS	At 5% 6.86
II	Diabetic control	213.80 ^{ap} ± 7.24	217.56 ^{ap} ± 5.77	215.27 ^{ap} ± 5.95	NS	
III	Metformin @ 100 mg/kg BW	196.15 ^{bp} ± 4.60	170.94 ^{bq} ± 4.37	124.91 ^{br} ± 2.34	HS	At 1% 9.09
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	197.03 ^{bp} ± 6.74	162.38 ^{cq} ± 6.02	129.66 ^{br} ± 5.12	HS	
Stat		S	HS	HS		
CD		At 5%-7.92 At 1%-10.50				

Table.4 Serum total cholesterol concentration (Mean ± S.E, mg/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day	14 th day	28 th day		
I	Healthy control	85.84 ^{ap} ± 3.37	85.85 ^{ap} ± 3.57	85.50 ^{ap} ± 3.32	NS	At 5% 3.91
II	Diabetic control	62.13 ^{bp} ± 2.16	61.92 ^{cp} ± 2.56	59.11 ^{bp} ± 2.08	NS	
III	Metformin @ 100 mg/kg BW	56.20 ^{cr} ± 2.09	70.71 ^{bq} ± 2.29	83.97 ^{ap} ± 3.18	HS	At 1% 5.18
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	59.01 ^{bcr} ± 3.36	74.77 ^{bq} ± 3.21	87.97 ^{ap} ± 3.37	HS	
Stat		HS	HS	S		
CD		At 5%-4.52 At 1%-5.98				

Table.5 Serum HDL concentration (Mean ± S.E, mg/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day	14 th day	28 th day		
I	Healthy control	29.28 ^{ap} ± 1.02	29.84 ^{ap} ± 0.52	29.09 ^{bp} ± 0.62	NS	At 5% 2.07
II	Diabetic control	19.81 ^{bp} ± 0.84	19.84 ^{cp} ± 0.49	18.97 ^{cp} ± 0.95	NS	
III	Metformin @ 100 mg/kg BW	18.86 ^{cp} ± 1.12	24.40 ^{bq} ± 0.98	28.94 ^{br} ± 1.13	HS	At 1% 3.58
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	22.20 ^{bp} ± 3.72	30.97 ^{aq} ± 3.89	41.04 ^{ar} ± 4.19	HS	
Stat		HS	HS	HS		
CD		At 5%-3.13 At 1%-4.14				

Table.6 Serum LDL concentration (Mean ± S.E, mg/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day	14 th day	28 th day		
I	Healthy control	56.56 ^{ap} ± 3.50	56.01 ^{ap} ± 3.45	56.41 ^{ap} ± 3.74	NS	At 5% 3.25
II	Diabetic control	42.31 ^{bp} ± 2.19	42.08 ^{cp} ± 2.48	40.13 ^{cp} ± 2.18	NS	
III	Metformin @ 100 mg/kg BW	37.33 ^{cr} ± 2.13	46.30 ^{bq} ± 3.00	55.03 ^{ap} ± 3.50	HS	At 1% 4.30
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	36.81 ^{cq} ± 2.03	43.81 ^{bp} ± 2.02	46.94 ^{bp} ± 1.72	S	
Stat		HS	HS	HS		
CD		At 5%-3.74 At 1%-4.97				

Table.7 Hb values (Mean ± S.E, g/dl) on different days in experimental rats of different groups

Gr.	Treatment	Days			Stat	CD
		1 st day	14 th day	28 th day		
I	Healthy control	14.31 ^{ab} ± 0.51	14.00 ^{ab} ± 0.40	13.66 ^b ± 0.44	NS	At 5% 0.75
II	Diabetic control	14.48 ^{ab} ± 0.69	14.40 ^{ab} ± 0.69	14.53 ^a ± 0.66	NS	
III	Metformin @ 100 mg/kg BW	15.00 ^a ± 0.56	14.77 ^a ± 0.58	14.68 ^a ± 0.58	NS	At 1% 0.99
IV	<i>Morinda citrifolia</i> L. fruit juice @ 2ml/kg BW	13.96 ^b ± 0.32	13.89 ^b ± 0.33	13.71 ^b ± 0.33	NS	
Stat		S	S	S		
CD		At 5%-0.87		At 1%-1.15		

After induction of diabetes, serum glucose (Table 2) and triglyceride (Table 3) concentration increases significantly above normal physiological limits. The observations obtained may be due to abnormal carbohydrate and lipid metabolism due to diabetes. However after 28 days of treatment protocol statistically positive results were obtained in all the treatment groups. Our findings were in agreement with the results obtained by Nayak *et al.*, (2011) and Lee *et al.*, (2012).

After induction of diabetes total cholesterol (Table 4), serum HDL (Table 5) and serum LDL (Table 6) concentration decreases significantly below normal physiological limits. Many scientific workers have reported an increase in serum total cholesterol and serum LDL level due to diabetes in humans. However the observations we obtained in wistar rats due to diabetes may be due species variation and in agreement with the results obtained by Ebara *et al.*, (1994) and Pinheiro *et al.*, (2011).

There was no significant change noticed with respect to haemoglobin (Table 7) in all the

treated groups. After treatment protocol in group-III, IV gradual improvement in health condition was noted.

In conclusion single intra-peritoneal administration of alloxan monohydrate after overnight fasting can successfully induce diabetes in wistar rats. Due to induction of diabetes, serum glucose and triglyceride level increase and total cholesterol, serum HDL, serum LDL level decreases. There was no significant change noted in respected to haemoglobin level in any of the treated groups. The group which was treated by *Morinda citrifolia* juice gave better result. But the best result was obtained by the treatment standard anti-diabetic drug i.e. metformin.

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