Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(6):550-553

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Determination of LD_{50} of Naringenin for its effects on diabetic nephropathy in rats-A pilot study

Mathi Selvam and Murugaiyah Kaliyaperumal*

Department of Biochemistry, Enathi Rajappa College, Bharathidansan University, Tamil Nadu, India

---

ABSTRACT

Diabetic nephropathy is a combined effect of various genetic and environmental factors. Prolonged duration of diabetes, poor glycaemic control and hypertension are major risk factors for diabetic nephropathy. Naringenin, is a biphenolic compound belonging the naturally occurring flavanone, it have been investigated including anticarcinogenic, antimutagenic, anti-inflammatory and antioxidant. However, to our knowledge no reports have recorded the precise biological action of Naringenin against on diabetic nephropathy in rats. Therefore, the present study was designed with an aim to determine the LD_{50} of naringenin for its effects on diabetic nephropathy in rats by the method of Miller and Tainter. The LD_{50} value of Naringenin was calculated by both arithmetically and graphically according to the method of Ghosh. Study shows LD_{50} value of Naringenin, the 0 % mortality was found to be 100 mg/ kg bwt and also the 100 % mortality was found in the dose of 600mg/kg bwt. After calculating the LD_{50} values for the Naringenin, we have then fixed $1/10^{th}$ of the LD_{50} values of Naringenin as pharmacological doses. We have concluded that Naringenin administered at a dose of 50mg/kg bwt were effective than the rest of the doses particularly when given orally to experimental animals.

Keywords: LD_{50}, Naringenin, acute toxicity studies, diabetic nephropathy

---

INTRODUCTION

Diabetic nephropathy (DN) affects approximately one-third of all diabetic patients. Most devastating complication of diabetes is nephropathy, which causes 14% of all deaths in diabetes patients and accounts for 40% of end-stage renal cases [1, 2]. DN is the common cause of chronic kidney failure and end stage of renal disease [3]. DN, a frequent and major micro vascular complication of diabetes mellitus, in many countries of the world [4]. Several factors, such as hyperglycemia, hyperlipidemia, oxidative stress and inflammatory cytokines, contribute to the progression of renal damage in DN [5, 6]. Diabetic nephropathy results from the combined effects of various genetic and environmental factors. Prolonged duration of diabetes, poor glycaemic control and hypertension are major risk factors for both diabetic nephropathy and cardiovascular disease [7-9].

Flavonoids are widely recognized as naturally occurring antioxidants that can inhibit lipid oxidation in biological membrane. They usually contain one or more aromatic hydroxyl groups, which is responsible for their antioxidant activity [10]. Naringenin (figure 1), is a biphenolic compound belonging the naturally occurring flavanone, widely distributed in tomatoes, cherries, grapefruits and predominantly present in citrus fruits [11]. It has been shown to possess potential health benefits as an anticarcinogenic [12], antimutagenic [13], anti-inflammatory [14] and antioxidant [15]. However, to our knowledge no reports have recorded the precise biological action of naringenin

against on diabetic nephropathy in rats. Therefore, the present study was designed with an aim to determine the LD_{50} of naringenin for its effects on diabetic nephropathy in rats.

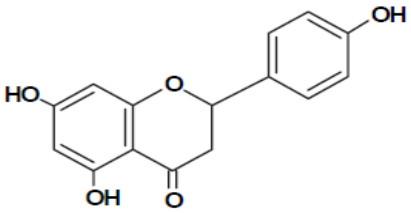


Figure 1. Structure of Naringenin

EXPERIMENTAL SECTION

Animals

Male albino Wistar rats ((130-140g) were used for the study. The animals were acclimated to laboratory housing conditions under 12hr light and dark cycles for 2 weeks prior to the commencement of the treatment under standard laboratory conditions (Room temperature 25±2°C and relative humidity 50–60 %). They were housed in polypropylene cages with stainless steel grill top, bedded with rice husk and offered commercial standard pellet diet and tap water *ad libitum*. The animals used in the present study were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Chemicals

(±) Naringenin (95%) were procured from Sigma Chemical Co. (St. Louis, Missouri, USA), stored at 2–4°C and protected from direct sunlight. All other chemicals used were purchased from standard local commercial suppliers and were of analytical grade.

Acute toxicity studies

Male Wistar Albino rats were divided into number of experimental groups (lower doses and higher doses groups), 10 animals for each group for acute toxicity studies. All animals were allowed to fast by withdrawing the food and water for 18 hr. Naringenin were freshly suspended in carboxy methylcellulose at the following concentrations 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300 and 1400 mg/kg b.wt were orally administered to rats to find out the LD_{50} values of them. The animals were provided with food and water immediately after the Naringenin administration.

RESULTS

The LD_{50} value of Naringenin was calculated by both arithmetically and graphically according to the method of Ghosh [16]. We have excluded the lower doses from the dose that cause zero percent mortality (100 mg/kg bwt) and higher doses from the dose that cause 100% mortality (600 mg/kg bwt) for calculation. The methods of determination of LD_{50} dose of Naringenin are given in tables 2. The graphical representation of LD_{50} of the Naringenin is given in figure 2.

Arithmetic method

In the interpretation of toxicity data, LD_{50} value was determined by the Karber's method described by Ghosh [16]. The interval mean of the number dead in each group of animals was multiplied with the differences between doses for the same interval and the product was noted. Results from doses larger than the least dose lethal to all animals in a group and doses smaller than the maximal tolerated dose were excluded. The sum of the product was divided by the number of animals in a group and the resultant quotient was subtracted from the least lethal dose to calculate LD_{50}.

The LD_{50} of Naringenin was calculated by the following formula.

$$LD50 = \text{Maximum dose (100\% dead)} - \frac{\text{Product (a x b)}}{\text{No. of animals in each group}}$$

***Graphical method* [17]**

For the interpretation of the toxicity data, the observed percentage animals that had died at each dose level is then transformed to probit by referring to the appropriate table (table 1) [16] and the values thus obtained are plotted against log dose. The results were made fitted with straight line after regression analysis of the probits (figure 2).

Table 1. Transformation of Percentages to probits

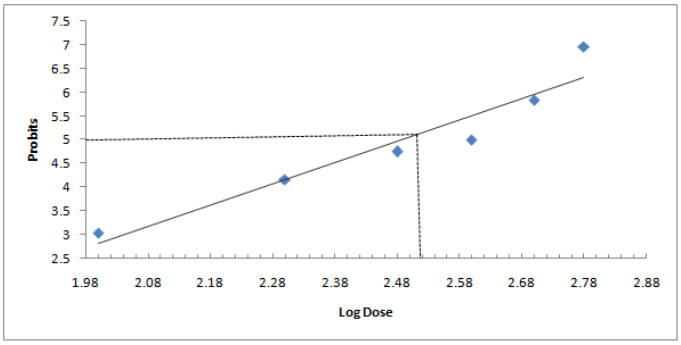
%	0	1	2	3	4	5	6	7	8	9
0	--	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	6.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	6.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 2. Method of determination of LD_{50} of Naringenin

Groups	Dose* mg/kg	Number of dead animals	Arithmetic method			Graphical method			
			Dose Difference (a)	Mean mortality (b)	Product (a x b)	Log dose	Dead (%)	Corrected (%)#	Probits
1	100	0/10	-	-	-	2.0	0	2.5	3.04
2	200	2/10	100	1.0	100	2.30	20	20	4.16
3	300	4/10	100	3.0	300	2.48	40	40	4.75
4	400	6/10	100	5.0	500	2.60	60	60	5.25
5	500	9/10	100	7.5	750	2.70	90	90	6.28
6	600	10/10	100	9.5	950	2.78	100	97.5	6.96
Total (a x b) = 2600									

**The data below 100 mg/kg body weight and above 600 mg/kg body weight were omitted for calculation; #Corrected formula for 0% dead = 100X 0.25/n; for 100% dead = 100 X (n-0.25)/n, where n is the number of animals in each group; LD_{50} of Naringenin = 600 - (2600/10) =340 mg/kg bwt*

Figure 2. Graphical representation of LD_{50} of Naringenin (LD_{50} = antilog 2.52 = 331.13 mg/kg.bwt)



DISCUSSION

DN is characterized by glomerular and tubules hypertrophy, thickening of the basement membranes, accumulating of extracellular matrix components, glomerulosclerosis as well as tubulo-interstitial fibrosis in mesangium and interstitium [18, 19]. Toxicity test examine toxic effects when a chemical is absorbed into the body. The most common test of acute (short-term) toxicity is the LD_{50} test. Many different substances are tested in this way, including all drugs [20]. The aim of the present study was to determine the LD_{50} of Naringenin given orally in rats for evaluating the protective effects on DN. Our acute toxicity study shows LD_{50} value of Naringenin, the 0% mortality was found to be 100 mg/ kg bwt and also the 100% mortality was found in the dose of 600mg/kg bwt. The animals were observed continuously for the first four hours followed by every 2h up to 24h for any change in behavior and manifestation of symptoms. After calculating the LD_{50} values for the Naringenin, we have then fixed

$1/10^{th}$ of the LD_{50} values of Naringenin as pharmacological doses. We found that Naringenin administered at a dose of 50mg/kg bwt were effective than the rest of the doses.

CONCLUSION

After calculating the LD_{50} values for Naringenin, dose dependent studies were carried out to find out effective pharmacological dose of the plant products for further experimental studies. From this study we have concluded that the pharmacological dose of Naringenin is 50 mg/kg bwt. We have therefore chosen 50mg/kg bwt of Naringenin for our experimental studies.

Acknowledgement
We wish to express our thanks to Mr. M.Marimuthu Chairman, and Mr. P.Ganesan Secretary, Enathi Rajappa College, pattukkottai for providing us the necessary facilities in the department to carry out our work successfully.

REFERENCES

[1] CRW Edwards, Baird JD, Frier BM, Shepherd J, Toft AD. *New York: Churchill Livingstone*, **1995.**
[2] AN Lasaridis, Sarafidis PA. *Am J Hypertens*, **2003**; 16, 689–97.
[3] FN Ziyadeh, Sharma K. *Journal of the American Society of Nephrology*, **2003**, 14, 1355–1357.
[4] M Molitch, DeFronzo R, Franz M, Keane W, Mogensen C, Parving H, Steffes M. *Diabetes Care*, **2004**, 27, S79.
[5] P Fioretto, Steffes M, Brown D, Mauer S. *Am J Kidney Dis*, **1992**, 549.
[6] IM Shah, Mackay SP, McKay GA. *Curr Med Chem*, **2009**, 16, 997–1016.
[7] Diabetes Control and Complications Trial Research Group. *N Eng J Med* **1993,** 329 (14), 977-986.
[8] R Gaede, Lund-Anderson H, Parving HH. Pedersen O. *N EngJ Med*, **2008**, 358 (6), 58O-S91.
[9] RR Hoi Man, Paui SK, Bethei MA *et al. W Ens / J Mecí*, **2008**, 35905, 1577-1589.
[10] FAA Van Acker, Schouten O, Haenen GR, Van Der Vijgh WJF, Bast A. *FEBS Lett*, **2000**, 473, 145–148.
[11] S Kawaii, Tomono Y, Katase E, Ogawa K, Yano M. *J Agric Food Chem*, **1999**, 47, 3565–3571.
[12] S Kanno, Tomizawa A, Hiura T, Osanai Y, Shouji A, Ujibe M,Ohtake T, Kimura K, Ishikawa M. *Biol Pharm Bull*, **2005**, 28, 527–530.
[13] WL Bear, Teel RW. *Anticancer Res*, **2000**, 20, 3609–3614.
[14] S Hirai, Kim YI, Goto T, Kang MS, Yoshimura M, Obata A, Yu R, Kawada T. *Life Sci*, **2007,** 81, 1272–1279.
[15] PG Pietta. *J Nat Prod*, **2000**, 63, 1035–1042.
[16] MN Ghosh. In Satistical Analysis, Fundamentals of Experimental Pharmacology, 2nd ed, Calcutta: Scientific Book Agency, **1984**, 189.
[17] LC Miller, Tainter ML. *Proc Soc Exp Bio Med*, **1944,** 57, 261.
[18] P Fioretto, Steffes M, Brown D, Mauer S. *Am J Kidney Dis*, **1992**, Off. J. Natl. Kidney Found, 20, 549.
[19] IM Shah, Mackay SP, McKay GA. *Curr Med Chem*, **2009**, 16, 997–1016.
[20] R Turner R, Acute toxicity: The determination of LD50. In *Screening Methods in Pharmacology*, Academic Press, New York, **1965**, 300.