

Phytochemical and Hypoglycemic Screening of Seeds and Peel of *Nephelium longan* Fruits

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Abstract

Phytotherapy is considered to be lowest lethal or has no side effects in contrast to current allopathic medicines. The intention of the present investigation was to assess the therapeutic efficacy of *Nephelium longan* extract in an animal model of diabetes. So in this research we have performed phytochemical screening of methanolic extracts of peel (MNLP)

and seeds (MNLS) of *N. longan* along with their hypoglycemic study in 18 h fasted normal and alloxan induced diabetic rats. Plant extracts at a dose of 150 mg/kg and 300 mg/kg body weight were administered orally in fasting glucose loaded rat with regard to normal control during 2 h study period and in alloxan induced (110 mg/kg body weight i.p.) diabetic rat in comparison with reference drug Metformin hydrochloride (150 mg/kg) during 7 days test period. Preliminary phytochemical screening showed that MNLP and MNLS exhibited positive response to alkaloids, tannins and flavonoids. Findings confirmed that the continuous post-treatment for 7 days with both extracts showed significant ($P < 0.05$) hypoglycemic activity in OGTT and normoglycemic rats and insignificant antidiabetic effects in alloxan induced rat models in dose dependent manner. Further, isolation and establishment of exact mechanism of action of precise compounds from MNLP and MNLS have to be continued in the recent future.

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1. Introduction

From the ancient time man has used different parts of plants in healing and prevention of numerous ailments. Traditionally all medicinal preparations were derived from plants, whether in the plain form of plant parts or in the more composite form of crude extracts, combinations, etc. (Ayyanar and Ignacimuthu, 2009). Plant derived medicines are extensively used because they are comparatively safer than the synthetic alternatives, they are simply available and cheaper (Iwu et al., 1999). Medicinal plants are vital sources for novel chemical compounds with potent pharmacological activities (Farnsworth, 1989). They act as natural reservoirs for

antidiabetic, anti-inflammatory, analgesic, antidiarrheal, antipyretic agents. In the developed countries 25 percent of the medical drugs are based on plants and their derivatives and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries (Ayyanar and Ignacimuthu, 2009). Diabetes mellitus is a chronic metabolic disorder distinguished by hyperglycemia, glycosuria, hyperlipidaemia, negative nitrogen balance, and occasionally ketonemia (Tripathi, 2010). The extensive pathological alteration escorts to obstacles similar to retinopathy, microangiopathy, and nephropathy (Akhtar

and Ali, 1980). Globally the prevalence of diabetes mellitus is increasing. Although research for diabetes has resulted in newer insulin preparations and medications, improved systems for insulin delivery and self-monitoring of blood glucose, and more aggressive treatment of diabetes complications and comorbid conditions (Daly, 2003), majority of the oral hypoglycemic agents, presently in use, produce severe side effects like hypoglycemic coma and hepatorenal disturbances. Hence, there is a burning need to explore safer and more efficient hypoglycemic mediators. Still today management of diabetes with no side effects is a great challenge to the medical community.

There is continuous hunt for alternative drugs. Hence, it is prudent to search for options in herbal medicine for diabetes as well. Conventional antidiabetic plants might offer new oral hypoglycemic agents, which can counteract the high expenditure and poor accessibility of the present medicines for many rural peoples in developing countries.

Nephelium longan (Family, Sapindaceae; Bengali name, Kathlichu) is a tree of 30 or 40 ft in stature and 45 ft in width, with rough-barked trunk to 2 and 1/2 ft thick and long, spreading, slightly drooping and heavily foliated branches. Longan is a subtropical fruit, closely allied to the glamorous lychee also known as dragon's eye or eyeball is largely grown in China and South East Asia, including Thailand, Vietnam and the Philippines (Menzel et al., 1995). The color of fruit peel is brown or light-brown with white translucent flesh. The seed is round and black with a spherical white spot at the base. The flesh is sweet and juicy; therefore, it can be consumed in both fresh and processed products, such as canned longan in syrup or as dried fruit.

The flesh extracts have been reported to use in stomachic, insomnia, neurasthenic neurosis and also act as febrifuge, vermifuge and antidot (Morton, 1987). The extract of longan arillus exhibited anxiolytic-like, sedative and analgesic effects (Okuyama et al., 1999). Peels and seeds extract showed CNS depressant and antinociceptive properties (Ripa et al., 2012). The plant extracts also found to be anti-mutagenic (Minakata, et al., 1985) anticarcinogenic (Sherine, et al., 2010) antibacterial, cytotoxic and antioxidant effects (Ripa et al., 2010). Ellagitannins, corilagin, acetonyl-geraniin were reported in seeds of longan (Cheng et al., 1995; Hsu et al., 1994;

Rangkadilok et al., 2005). Longan arillus was shown to contain adenosine (Okuyama et al., 1999) and gallic acid (Rangkadilok et al., 2005).

Keeping all these in view and since conventional medicine is an imperative source of potentially useful novel compounds for the development of chemotherapeutic agents; we have investigated the methanolic extracts of peels and seeds of the fruits of *N. longan* for their hypoglycemic effect.

2. Material and Methods

2.1. Collection and Identification of Plant

In this investigation, the fresh fruits of *N. longan* were collected from, Dhaka, Bangladesh in August, 2012. The fresh leaves and fruits of longan were identified by Dr. Mahbubur Rahaman, Associate Professor of Department of Botany, Rajshahi University, Rajshahi and National Herbarium of Bangladesh, whose voucher specimen no. is 36664 and is maintained in our laboratory for future reference. From the collected fruits peel and seeds were separated and dried for one week and pulverized into a coarse powder with a suitable grinder. The powder was stored in an airtight container, and was kept in a cool, dark and dry place for analysis.

2.2. Preparation of Extracts

Approximately 400 g of powdered materials of both the peel and seed were taken in two different clean, flat bottomed glass containers and were deepened in 800 ml of 95% methanol. The containers with their contents were sealed and kept for a period of 7 days associated with occasional shaking and stirring. The two mixtures then underwent a coarse filtration by a piece of clean, white cotton plug and were filtered through Whatman filter paper (Bibby 200, Sterilin Ltd., UK). The filtrates (methanolic extract) obtained were evaporated using rotary evaporator. The methanolic portion of the peel delivered a reddish brown gummy precipitate which was designated as MNLP; whereas, the seed portion yield brown mass which was named as MNLS. The extracts were transferred to two different closed containers for further use and fortification.

2.3. Chemicals and drugs

Alloxan (Fluka, Germany), Tween-80, normal saline solution (Beximco Infusion Ltd., Bangladesh), Metformin (Square Pharmaceuticals Ltd., Bangladesh), were procured and used in the experiment. All

chemicals in this investigation were of analytical reagent grade.

2.4. Phytochemical analysis

The MNLP and MNLS were subjected to qualitative chemical screening for the identification of bioactive constituents (tannins, alkaloids, flavonoids etc.) using standard procedures (Trease and Evans, 2001).

2.5. Animals

Young Long-Evans rats of either sex weighing about 80-120gm were used to conduct the research. The rats were procured from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRDB). They were kept in standard environmental condition (at $24.0 \pm 0^\circ\text{C}$ temperature & 55-65% relative humidity and 12 hours light/dark cycle) for two weeks for acclimation and fed ICDDRDB formulated rodent food and tap water ad libitum. All animals were fasted over night before tests while providing tap water ad libitum.

2.6. Ethical Approval

The guidelines followed for animal experiment were accepted by the institutional animal ethical committee (Zimmermann, 1983).

2.7. Oral toxicity studies

An acute oral toxicity study was followed according to "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of methanol extract. Long Evans rats (N=6, 150-200 g) overnight fasted for 18 were used for the study. Different doses of plant extracts up to 1600 mg/kg, p.o. was administered and animals were observed for the first 3 hours of administration and mortality recorded within 48 hours.

2.8. Induction of diabetes in rats

Diabetes was created by a single dose subcutaneous injection of freshly prepared alloxan monohydrate dissolved in normal saline to overnight fasted rodents. Blood glucose level (BGL) was measured by using one-touch glucometer and diabetes was confirmed after 72 hr of alloxanisation. Rats which showed hyperglycemia (BGL > 10 mmol/l) were chosen for research.

2.9. Experimental Design

2.9.1. Oral Glucose Tolerance Test (OGTT)

In the beginning, hypoglycemic activity of plant extracts was checked in overnight fasted normal rats, which were uniformly alienated

into six groups of each. Normal control group received only vehicle (1 normal saline p.o.) and standard group received 1 ml of reference drug Metformin in the vehicle (150 mg/kg, p.o.), while group from third to sixth were administered with 1 ml of MNLP and MNLS at 150 and 300 mg/kg, p.o. doses respectively. Subsequent 30 min post extract administration all the rodents were fed with glucose (2 g/kg). Blood samples were taken from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, GmbH, Germany).

2.9.2. Effect of methanolic extracts in Normoglycemic rats

The rats were divided into six groups of 6 animals (n=6) each. Group I served as control and received saline water. Group II served as standard control, received Metformin in the vehicle (100mg/kg, p.o.). Group III, IV, V, VI received 150 and 300 mg/kg MNLS and MNLP orally respectively. Blood glucose levels were determined at 0, 1, and 2 hr following treatment from tail vein.

2.9.3. Study on Alloxan-induced diabetic rats

The experimental rodents were randomly divided into six groups consisting of 6 rats in each group. The groups were denoted as group-I, group-II, group-III, group- IV, group-V and group-VI. Each group of rats received a specific treatment. Test samples at a dose of 150 mg/kg and 300 mg/kg body weight of rats were used to evaluate the hypoglycemic activity. Standard Metmorfoin was used at a dose of 100mg/kg body weight. Before administering the drugs, each rat was weighed properly and the doses were adjusted accordingly. In the evaluation of the hypoglycemic effect, the blood glucose level of the experimental animals were measured at 0 hour by tail tipping method (Durschlag et al., 1996) using a glucometer (Bioland G-423S). Then the control, standard, MNLS and MNLP extracts and their different fractions were administered orally to the experimental animals with the help of feeding needle. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 1, 3 and 7 respectively.

3. Statistical Analysis

All the values in the test are expressed as mean \pm standard error of the mean (SEM). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc

Dunnett's tests with the Statistical Package for Social Sciences (SPSS) program (SPSS 16.0, USA). Dissimilarity between the means of the various groups were measured significant at $P < 0.05$.

4. Result

4.1. Phytochemical analysis

The extracts gave positive tests for tannins,

alkaloids, and flavonoids (Table 1).

4.2. Acute toxicity

In the acute toxicity test, the plant extracts were found to be safe up to doses of 1.6 g/kg. Behavior of the animals was strictly observed for the first 3 in the next 48 h. The extracts did not affect any behavioral change or mortality on rats during 48 h inspection.

Table 2: Oral glucose tolerance test in rat for *N.longan* fruit

Group	Initial (mmol/L)	1 h (mmol/L)	2 h (mmol/L)	3 h (mmol/L)
G-I (Control)	8.32 ± 0.11949	6.67 ± 0.07149	4.92 ± 0.12494	3.83 ± 0.14063
G-II (Standard)	$9.47 \pm 0.18559^*$	7.65 ± 0.36492	4.22 ± 0.29486	3.02 ± 0.19221
G-III (MNLS-150)	$9.87 \pm 0.35653^*$	$8.58 \pm 0.33903^*$	$7.53 \pm 0.39129^*$	$6.47 \pm 0.33632^*$
G-IV (MNLS-300)	$10.18 \pm 0.19322^*$	$8.33 \pm 0.16667^*$	$6.43 \pm 0.26667^*$	$5.2 \pm 0.26667^*$
G-V (MNLP-150)	$10.73 \pm 0.34416^*$	$9.33 \pm 0.39215^*$	$8.05 \pm 0.39306^*$	$6.98 \pm 0.26384^*$
G-VI (MNLP-300)	$10.33 \pm 0.28944^*$	7.47 ± 0.19944	5.63 ± 0.24037	3.58 ± 0.37454

4.3. Oral glucose tolerance test

In oral glucose tolerance test, we have observed that both extracts was active and comparable to that of the glucose treated control group in both doses (Table-2). A good anti-hyperglycemic effect of

MNLP was observed at 300mg/kg dose in the first hour after glucose loading in rats under OGTT. This effect was still present 180 min after the oral administration of glucose. MNLS was also shown to be hypoglycemic at higher dose.

Table 1: Phytochemical constituents of different extracts of *D.longan* fruit seeds

Extracts	Flavonoid	Tannin	Alkaloid	Saponin	Carbohydrate
MNLS	+	+	+	-	-
MNLP	+	+	+	-	-

Here, (+): Present; (-): Absent

4.4. Normoglycemic study

In case of normoglycemic test we have observed that administration of single dose of both MNLS and MNLP in normal rats showed reduction in blood glucose levels at different time intervals compared to 0 h of the same

group. As shown in Table-3, oral administration of MNLS (300 mg/kg) and MNLP(300 mg/kg) caused a significant ($P < 0.05$) reduction in glucose levels respectively at 2 h and 3h as compared to normal control group.

Table 3: Effect of on Blood Glucose in Normoglycemic rat

Group	Design of Treatment	Dose mg/kg	Blood glucose levels (mmol/l)		
			0hr	1hr	2hr
I	N.saline		7.216 ± 0.16415	7.116 ± 0.15794	7.133 ± 0.15635
II	Standard	150	$9.65 \pm 0.46601^*$	6.7 ± 0.52915	$3.77 \pm 0.32318^*$
III	MNLS-150	150	8.166 ± 0.25647	7.18 ± 0.26635	6.03 ± 0.31588
IV	MNLS -300	300	7.883 ± 0.27131	$5.72 \pm 0.38333^*$	$4.08 \pm 0.39616^*$
V	MNLP-150	150	7.866 ± 0.25906	6.7 ± 0.30441	$5.65 \pm 0.33441^*$
VI	MNLP-300	300	7.883 ± 0.27131	5.87 ± 0.28008	$4.47 \pm 0.24037^*$

All values are expressed as mean \pm STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. * $P < 0.05$ significant compared to control.

Table 4: Blood glucose level of alloxan induced diabetic rat after treatment with MNLS & MNLP

Group	Design of Treatment	Dose mg/kg	Blood glucose levels (mmol/l)		
			1 st day	3 rd day	7 th day
I	N.saline		7.0667 ± 0.07149	6.9500 ± 0.04282	7.0833 ± 0.03073
II	Control (diabetic)		15.0000 ± 0.36515 *	17.5833 ± 0.20069 *	19.7500 ± 0.17078 *
II	Standard	150	12.466 ± 0.67511 *	5.533 ± 0.27039	3.58 ± 0.35816
III	MNLS-150	150	12.466 ± 0.67511 *	5.533 ± 0.27039	3.58 ± 0.35816 *
IV	MNLS -300	300	17.67 ± 0.42164 *	11.95 ± 0.69940 *	8.08 ± 0.49018
V	MNLP-150	150	15.83 ± 1.19490 *	10.33 ± 0.76012 *	6.88 ± 0.27131
VI	MNLP-300	300	16. ± 0.68313 *	11.50 ± 0.76376 *	7.17 ± 0.16667

All values are expressed as mean ± STD (n=6); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05 significant compared to control

4.5. Effect on blood glucose level of hyperglycemic rats

Table 4 illustrates the effect of graded doses of methanolic extracts of seeds and peel of *N. Longan* on blood glucose level of hyperglycemic rats. There was significant decrease in the blood glucose levels at 1st, 3rd day (P<0.05) with 150 and 300mg/kg doses of MNLS and MNLP extracts were reported.

5. Discussion

From the dawn of human civilization herbal medicines have been used for the management of diabetes and persist to be presently established as a choice of therapy. Noticeably huge numbers of anti-diabetic herbs are identified through folklore, however pharmacological assessment by scientific methods is obligatory to establish the antidiabetic activity. The study of such medicines might propose a natural key to unlock a diabetologist's pharmacy for the future (Venkateshwarlu et al., 2009). The economic disaster, elevated expenditure of industrialized medicines, disorganized community access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the issues contributing to the vital role of medicinal plants in health care (Johann et al., 2007). Although huge number of antidiabetic medicines is now available in pharmaceutical market, still remedies from medicinal plant are used with success to treat this disease. Plant drugs and herbal formulations are repeatedly considered to be less toxic and free from side effects than synthetic ones. According to WHO, hypoglycemic agents of plant source used in traditional medicine are essential. The attributed anti-hyperglycemic property of these plants is due to their capability to refurbish the role of pancreatic tissues by causing a boost in insulin output or diminish in the intestinal

absorption of glucose. Therefore, healing with herbal drugs to protect β-cells and smoothing out oscillation in glucose levels. In common there is very tiny biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently implicated as having antidiabetic effects. The research for alternate remedies (from the plant kingdom) for diabetes mellitus will continue all over the world as the disease poses many challenges not only to the physician but also to the researcher. The current research is a preliminary estimation of hypoglycemic and antihyperglycemic activity of methanolic extracts of peel (MNLP) and seeds (MNLS) of *N. longan* at 150 and 300 mg/kg in rodents. The phytochemical screening revealed that the plant extracts showed positive result to alkaloids, tannins and flavonoids. It has been well-established that most medicinal plants contain phenolic compounds and bioflavonoids that have outstanding antioxidant and antidiabetic properties. We also knew that diabetes is of two categories out of which one is genetically based and other as a result of dietary indiscretion (Dave and Katyare, 2002). Alloxan leads to a fall in insulin release there by a drastic diminution in plasma insulin concentration leading to stable hyperglycemic states (Yasodha et al., 2008). It induces diabetes by dose dependent destruction of β-cells of islets of langrhans (Szkudelski, 2001; Murthy et al., 2004). So, in the present study alloxan was chosen to create diabetic condition in rat and significant hyperglycemia was achieved within 48 hours after alloxan (110g/kg b.w. i.p) injection. The results (Table 2) proved that after a single administration of glucose 2gm/kg in rat, there was a significant reduction (p < 0.05) of fasting blood glucose level during the 3 h study period for both

extracts at 150mg/kg and 300 mg/kg doses. In alloxanised rats, administration of MNLS and MNLP at the above mentioned doses up to 7 days was able to correct this abnormality about to normal range. Additionally, MNLS and MNLP illustrated similar significant lowering of blood glucose level in OGTT and normoglycemic rats. These observations suggest that the experimental extract might acquire insulin like effect on peripheral tissues either by promoting glucose consumption metabolism or inhibiting hepatic gluconeogenesis since alloxan treatment causes permanent destruction of β cells (Pari and Venkateswaran, 2002).

In acute toxicity animals of any group did not show. As no mortality or toxic effect was observed during 48 hours acute toxicity experiment, LD₅₀ was not calculated.

Any toxic effect numerous authors stated that phenolic acids, flavonoids, steroids/terpenoids, are known to be bioactive antidiabetic principles (Oliver-Bever, 1986; Rhemann and Zaman, 1989). Since the phytochemical analysis of the experimented crude extracts has shown the presence of potent phytochemicals like alkaloids, flavonoids and tannin. Therefore the crude extracts might be new sources of expansion of new plant based therapy for management of diabetes. But we still don't know which chemical components are exactly accountable with the aforesaid effects so to find out the lead compounds responsible for aforesaid activities from the above plant are in progress.

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