

Anti-inflammatory and Anti-diarrheal Effects of Methanolic Extracts of Seeds and Peel of *Nephelium longan* Fruits in Rats

FARHANA ALAM RIPA, and AFROZA HABIB

For author affiliations, see end of text.

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ABSTRACT

Herbal medicines are playing a vital role in the remedy of numerous diseases. Preliminary phytochemical screening of the methanolic extracts of seeds (MNLS) and peel (MNLP) of *Nephelium longan* revealed the presence of alkaloids, tannins and flavonoids. For antidiarrheal screening, we have followed Castor-oil-induced method whereas to check anti-inflammatory property, we used carrageenan-induced rat paw edema in Long-Evan rats. Two doses (250 and 500 mg/Kg) of the extracts and one dose of indomethacin as reference was used. Both extracts significantly ($p < 0.05$) reduced the formation of oedema induced by carrageenan in a dose-depending manner. MNLP and MNLS also exhibited anti-diarrheal action in dose-depending manner and all the results were found to be significant ($p < 0.05$) in comparison to loperamide. The outcomes indicate the potent anti-inflammatory and anti-diarrhoeal effects of *N. longan* extracts on living models which are comparable with those of standard drugs such as indomethacin and loperamide respectively and support their conventional uses as medicine.

Keywords: *Nephelium longan*, Anti-inflammatory, Anti-diarrhoeal activity

Regardless of the great progresses observed in the modern medicine in latest decades, plants still make an important role in health care. Medicinal plants have become the hub of intense study in terms of conservation and as to whether their traditional uses are sustained by actual pharmacological effects or they simply use them based on folklore [1-3]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [4]. Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. *Nephelium longan* (Family, Sapindaceae; Bengali name, Kathlichu) is a tree of 30 or 40 ft in stature. Longan is a subtropical fruit, closely allied to lychee also famous as dragon's eye or eyeball and is largely grown in China, South East Asia, Thailand,

Vietnam and the Philippines [5]. The flesh of the fruit 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 [6]. The extract of longan arillus exhibited anxiolytic-like, sedative and analgesic effects [7]. Peels and seeds extract showed CNS depressant and antinociceptive properties [8]. The plant extracts also found to be antimutagenic [9], anticarcinogenic [10], antibacterial, cytotoxic and antioxidant [11]. Ellagitannins, corilagin and acetonyl-geraniin were reported in seeds of longan [12-14]. Longan arillus was shown to contain adenosine [7] and gallic acid [14]. The current study has been designed to check the anti-inflammatory and anti-diarrheal activity of methanolic extracts of peels and seeds of *N. longan* fruits.

MATERIALS AND METHODS

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. 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.

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 yielded a brown mass which was named as MNLS. The extracts were transferred to two different closed containers for further use and fortification.

Chemicals and drugs

Carrageena (Sigma chemicals, USA), Tween-80, Castor oil (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), loperamide and indomethacin (Square Pharmaceuticals Ltd., Bangladesh) were procured and used in the experiment. All chemicals in this investigation were of analytical reagent grade.

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 [15].

Animals

Young Long-Evans rats of either sex weighing about 80-120 gm 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 (ICDDRB). 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

ICDDRB formulated rodent food and tap water *ad libitum*. All animals were fasted overnight before tests while providing tap water *ad libitum*.

Ethical Approval

The guidelines followed for animal experiment were accepted by the institutional animal ethical committee [16].

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 Evan 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.

Carrageenin- induced paw edema model

Thirty six rats weighing 150-200 g were accommodated in colony cages in an animal house, at an ambient temperature of $25 \pm 2^\circ\text{C}$, with 12 h light/dark cycle. They were allowed standard laboratory feed and water *ad libitum*. Rats were alienated into six groups (n = 6); Group I served as control received 0.9% normal saline in 3% Tween 80 suspension while Group II was treated with indomethacin (10 mg/kg) and; Group III- VI orally received MNLS and MNLP extracts at 250 and 500 mg/kg body weight. Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contained Tween 80 in the right paw of rats. The paw volume was precised at 0, 0.5, 1, 2 and 3 h after carrageenan injection using a plethysmometer. Boost in the linear diameter of the right hind paws were taken as a sign of paw oedema. Oedema was evaluated in terms of the difference in the zero time linear diameter of the inject hind paw and its linear diameter at time *t* (i.e.30, 60, 120, 180 min) following carrageenan administration.

Castor oil-induced diarrhea test

Awouter et al. [17] method was followed for screening of anti-diarrheal effect of experimented extracts. After measuring weights, thirty (30) rats were fasted for 18 h with free access to water and randomly separated into ten groups containing five rodents each. Each plant crude extract was administered orally at 250, and 500 mg/kg body weight. Group-I (control) received only normal saline (5 mL/kg bodyweight), while the 2ND group received the standard drug, Loperamide (5 mg/kg body weight). Group-III to Group-VI received MNLS and MNLP extracts at the above mentioned doses. One hour later, all the animals received 1 mL/rat of castor oil orally by gavage. The animals were kept in separate metabolic cages to scrutinize fecal matter consistency and frequency of defecation for 4 h. Feces

Table 1. Anti-inflammatory effect of MNLS/MNLP on carrageenin-induced paw edema in rat

Treatment	Dose (mg/kg b.w)	Before Inflammation (mm)	After treatment in inflamed mice (mm)				
			0 min	30 min	60 min	120 min	180 min
Control	-	3.87 ± 0.01506	5.01 ± 0.01770	5.44 ± 0.01759	5.25 ± 0.02290	5.22 ± 0.02535	5.16 ± 0.02509
Standard	10	3.59 ± 0.01493 *	4.61 ± 0.01983 *	4.49 ± 0.01626 *	4.33 ± 0.05289 *	4.21 ± 0.03250 *	4.11 ± 0.04561 *
MNLS-250	250	3.88 ± 0.01138	5.06 ± 0.02982	4.89 ± 0.03252 *	4.75 ± 0.02578 *	4.64 ± 0.02701 *	4.58 ± 0.02701 *
MNLS-500	500	3.97 ± 0.01384 *	5.03 ± 0.01838	4.75 ± 0.01095 *	4.6 ± 0.01125 *	4.40 ± 0.01764 *	4.32 ± 0.01563 *
MNLP-250	250	3.89 ± 0.01424	5.09 ± 0.03363	4.91 ± 0.03073 *	4.79 ± 0.03194 *	4.69 ± 0.03347 *	4.64 ± 0.03194 *
MNLP-500	500	4.01 ± 0.02066 *	5.08 ± 0.03510	4.82 ± 0.04890 *	4.66 ± 0.04836 *	4.494 ± 0.05735 *	4.42 ± 0.05839 *

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

Table 2. Effect of MNLS & MNLP extract on castor oil-induced diarrhoea in rat

Group	Dose	Total no of diarrheal faeces in 4 h	Percentage
G-I (Control)	Distilled water	30.6 ± 2.52190	-
G-II (Standard)	2 mg/kg	10.6 ± 1.20830 *	65.36
G-III (MNLS-250)	250	15.4 ± 0.60000 *	49.67
G-IV (MNLS-500)	500	12 ± 0.70711 *	60.78
G-V (MNLP-250)	250	15.2 ± 0.66332 *	50.33
G-VI (MNLP-500)	500	11 ± 0.44721 *	60.05

All values are expressed as mean ± STD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. **p* < 0.05 significant compared to control

were collected with an absorbent sheet of paper placed below the transparent cages. The total number diarrheal feces expelled were compared with the control group. The total score of diarrheal faeces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhea. The percent (%) inhibition of defecation was calculated using the subsequent formula.

$$\% \text{ Inhibition of defecation} = [(A - B) / A] \times 100$$

A = Mean number of defecation produced by castor oil

B = Mean number of defecation produced by drug or extract

Statistical analysis

All the values in the test are expressed as mean ± 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.

RESULTS AND DISCUSSION

Phytochemical screening of dried crude extracts of seeds and peel of *N. longan* gave positive tests for

tannins, alkaloids, and flavonoids. 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.

Carrageenin-induced paw edema test was performed to evaluate the anti-inflammatory activity of the methanolic extracts of peel and seeds of *N. longan*. There was a dose-dependent, significant reduction in carrageenin-induced rat paw edema at 250 and 500 mg/kg of extracts and at 10 mg/kg indomethacin over a period of 240 min as shown in Table 1. Carrageenin provoked edema is generally used as an investigational animal model for acute inflammation and is supposed to be biphasic, among which the first phase is initiated by the release of histamine and kinins and then prostaglandin in the later phase [18]. So, the effect of the methanolic extracts against inflammations produced by these individual mediators was studied. Flavonoids and other phenolics compounds of plant derivatives have been reported as antioxidants and as scavengers of free radicals [19,20] which can also exert anti-inflammatory effects [21]. The extract successfully suppressed the inflammation formed by histamine, bradykinin, prostaglandins and serotonin.

On the other hand in castor oil-induced diarrhoeal experiment in rodents, MNLS and MNLP extracts at the

doses of 250 and 500 mg/kg, diminished the total number of feces as well as the total number of diarrhoeic feces in a dose-dependent manner (Table 2). These results were shown to be statistically significant ($p < 0.01$). The results were almost similar to the effect of extensively-used antidiarrheal drug, loperamide when tested at 2 mg/kg. Castor oil diminishes the fluid absorption, enhances secretions in small intestine and colon, and influences smooth muscle contractility in the intestine. It produces diarrhea by ricinoleic acid [22] elevated prostaglandin biosynthesis. Prostaglandin participates in the patho-physiological functions in gastrointestinal tract [23]. The tested seed extracts reduced the castor oil induced diarrhea may be through the inhibition of prostaglandin biosynthesis.

Finally we may say that all experimented extracts of seeds and peel of *N. longan* fruits have potent anti-inflammatory and antidiarrheal activities. However, further research is needed in order to find out the precise mechanisms and responsible chemical constituents for the above-mentioned pharmacological activities. In the near future, we will conduct experiments with purified fractions of the above extracts for further pharmacological and toxicological characterization.

REFERENCES

- Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. Hydroxyl radical and hypochlorous acid scavenging activity of small centaury (*Centaurium erythraea*) infusion. A comparative study with green tea (*Camellia sinensis*). *Phytomedicine* 2003; 10:517-22.
- Rakh MS, Chaudhari SR. Evaluation of CNS depressant activity of *Momordica dioica* Roxb wild fruit pulp. *Int J Pharm Pharmacol* 2010; 2:124-6.
- Fransworth NR. Ethnopharmacology and future drug development: the North American experience. *J Ethnopharmacol* 1993; 38:45-152.
- Houghton PJ. The role of plants in traditional medicine and current therapy. *J Altern Complement Med* 1995; 1:131-43.
- Menzel C, Watson B and Simpson D. Longan, In: *The Australia New Crops Newsletter*; School of Land and Food Sciences (Dr. Rob Fletcher), The University of Queensland Gatton College: Brisbane (1995) 4:14-5.
- Morton J. Longan In: *Fruits of warm climates*, Florida Flair books, Miami, FL, USA 1987; 259-62.
- Okuyama E, Ebihara H, Takeuchi H, Yamazaki MA. The anxiolytic-like principle of the Arillus of *Euphoria longana*. *Plant Med* 1999; 65:115-19.
- Ripa FA, Haque M, Bulbul IJ, Al-Sharmin A, Begum Y, Habib A. Screening of central nervous system (CNS) depressant and antinociceptive activities of methanolic extracts of the peel and seed of *Nephelium longan* fruits. *Afr J Pharm Pharmacol* 2012; 611:848-54.
- Minakata H, Komura H, Tamura SY, Ohfuné Y, Nakanishi K, Kada T. Antimutagenic unusual amino acids from plants. *Cell Molec life sci* 1985; 41:1622-3.
- Sherine G, Siddharth VB, Erich AL, Irfan SA, Atiya A, Brian TC, Kenneth LW. Cytotoxicity screening of Bangladeshi medicinal plant extracts on pancreatic cancer cells. *BMC Comple Altern Med* 2010; 10:52.
- Ripa FA, Haque M, Bulbul IJ. *In vitro* Antibacterial, Cytotoxic and Antioxidant Activities of Plant *Nephelium longan*. *Pak. J Biol Sci* 2010; 13:22-7.
- Cheng JT, Lin TC, Hsu FL. Antihypertensive effect of corilagin in the rat. *Can J Phys Pharmacol* 1995; 73:1425-9.
- Hsu FL, Lu FH, Cheng JT. Influence of acetylgeraniin, a hydrolyzable tannin from *Euphoria longana*, on orthostatic hypotension in a rat model. *Planta Med* 1994; 60: 297-300.
- Rangkadilok N, Worasuttayangkurn L, Bennett RN, Satayavivad J. Identification and quantification of polyphenolic compounds in Longan (*Euphoria longana* Lam.) fruit. *J Agric Food Chem* 2005; 53:1387-92.
- Trease G, Evans M. Pharmacopoeial and related drugs of biological origin. In: *A Textbook of Pharmacognosy*. 15th ed. London:WB Saunders, 2001; pp. 262-70.
- Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983; 16:109.
- Awouter F, Neimegeers CJE, Lenaert FM, Janssen PAJ. Delay of castor oil diarrhoea in rats; A new way to evaluate inhibitors of prostaglandin's biosynthesis. *J Pharm Pharmacol* 1978; 30:41-5.
- Castro J, Sasame H, Sussman H, Buttette P. Diverse effect of SKF52 and antioxidants on CCl4 induced changes in liver microbial P-450 content and ethyl-morphine metabolism. *Life Sci* 1968; 7:129-36.
- Lien EL, Ren S, Bui HH, Wang R. Quantitative structure activity relationship analysis of phenolic antioxidants. *Free Radic Biol Med* 1999; 26: 285-94.
- Van Acker FAA, Schouten O, Haenen GRMM, Van der Vijgh WJF, Bast A. Flavonoids can replace -tocopherol as an antioxidant. *FEBS Lett* 2000; 473:145-8.
- Geronikaki AA, Gavallas AM. Antioxidant and anti-inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. *Comb Chem High Throughput Screen* 2006; 9: 425-42.
- Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and castor oil-induced diarrhoea. *J Pharmacol Exp Ther* 1994; 268:291-5.
- Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of N^G-nitro-L-arginine methyl ester. *Br J Pharmacol* 1994; 113:1127-30.

CURRENT AUTHOR ADDRESSES

Farhana Alam Ripa, Department of Pharmacy, BRAC University, Mohakhali, Dhaka-1212, Bangladesh. E-mail: ripa.seu@gmail.com (Corresponding Author)

Afroza Habib, Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh.