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*Full Length Research Paper*

# Screening of central nervous system (CNS) depressant and antinociceptive activities of methanolic extracts of the peel and seed of *Nephelium longan* fruits

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**In recent years, research on medicinal plants has engrossed a lot of interest worldwide. Large body of proof has been accumulated to reveal promising potential of medicinal plants used in different traditional, complementary and alternative systems. Keeping this in mind, the present study was carried out to scrutinize the antinociceptive and central nervous system (CNS) depressant activity of the methanol extracts of seed (MNLS) and peel (MNLP) of *Nephelium longan* in young Long-Evans rats. Both types of tests were conducted after oral administration at doses of 250 and 500 mg/kg body weight of rats. CNS activity was evaluated by watching the fall of rats in locomotor and exploratory activities. We also used open field and hole cross tests. The analgesic activity was examined using acetic acid-induced writhing and formalin tests. The results of the statistical analysis showed that the experimental extracts had significant (P < 0.01 and 0.05,) dose dependent CNS depressant and antinociceptive activities. In the acetic acid-induced writhing model, MNLP at dose of 500 mg/kg exerted a maximum of 54.43% inhibition of writhing response, whereas MNLS gave 52.531% inhibition which are comparable to 58.86% inhibition of writhing by standard drug, indomethacin (10 mg/kg). In case of CNS depressant activity, both extracts displayed dose dependent suppression of motor activity and exploratory behavior in the tested models. The results of the study indicate that the plant possesses strong analgesic and central nervous system depressant activity.**

**Key words:** *Nephelium longan*, antinociceptive, indomethacin, CNS depressant activity.

## INTRODUCTION

The empirical use of plants as medicine can be traced back to over five millennia to ancient documents of early civilizations, such as in China, Egypt, India and the Near East, but is certainly as old as mankind (Hamburger and Hostettmann, 1991). These medicines, initially took the form of traditional forms of formulations, such as tinctures, teas, poultices, powders and other herbal formulations (Balick and Cox Plants, 1997). Although, indigenous knowledge systems faces the threat of rapid disappearance under the influence of Western culture, the World Health Organization (WHO) estimates that even today, 80% of the world's populations exclusively rely on traditional medicine; especially in developing countries where resources to build up a primary health care system are still lacking (Farnsworth et al., 1985). In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. Isolated active constituents are used for applied research. The first step towards this goal is the screening of plants used in popular medicine. For the last few decades, phytochemistry (study of plants) has been making rapid progress and herbal

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Products are becoming popular. Pain is formally defined as an unpleasant sensory and emotional incident coupled with real or likely tissue injure. Pain acts as a word of warning sign against disorder of the body and has a practical function. However, it is the most important indicator that brings the patient to physician. Analgesics mitigate pain as a symptom, without affecting its reason (Akter et al., 2008). Currently, available analgesic drugs, such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are being searched at different laboratories throughout the world.

*Nephelium longan* (Family, Sapindaceae; Bengali name, Kathlichu) is a tree of 30 or 40 ft in height and 45 ft in width, with rough-barked trunk to 2 and 1/2 ft thick and long, spreading, slightly drooping and heavily foliaged branches. Longan is a subtropical fruit, which is extensively grown in China and South East Asia, including Thailand, Vietnam and the Philippines (Menzel et al., 1995).

Botanical synonyms for this species include *Dimocarpus longan* Lour., *Euphoria longan* Steud., *Euphoria longana* Lam. and *Nephelium longana* Cambess. Closely allied to the glamorous lychee, in the family Sapindaceae, the longan or lungan, also known as dragon's eye or eyeball and as *Mamoncillo chino* in Cuba, has been referred to as the "little brother of the lychee". In Thailand, the matured fruit can be harvested from July to September. The fruit peel is brown or light-brown with white translucent flesh. The seed is round and black with a circular 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. In Chinese medicine the flesh of the longan is used as a stomachic, febrifuge, vermifuge and also as an antidote for poison (Mortin, 1987). There is also limited information on the analysis of the bioactive compounds in longan fruit. A decoction of the dried flesh is also used as a tonic and for the treatment of insomnia and neurasthenic neurosis (Mortin, 1987). The seeds have previously been shown to contain the hydrolyzable tannins (ellagitannins) corilagin and acetonyl-geraniin (Cheng et al., 1995; Hsu et al., 1994). A methanol extract of longan arillus exhibited anxiolytic-like effects; it was shown to contain adenosine, which may also account for the reported sedative and analgesic effects (Okuyama et al., 1999). It has also been reported to contain gallic acid (Rangkadilok et al., 2005). The plant has also got anti-mutagenic (Minakata et al., 1985), anticarcinogenic (Sherine et al., 2010) antibacterial, cytotoxic and antioxidant effects (Ripa et al., 2010). Keeping this in view and since traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents; we have investigated the peels and seeds of the fruits of *N. longan* for their CNS depressant and antinociceptive activities in suitable animal models.

## MATERIALS AND METHODS

### Plant

In this present research, the fruits of *N. longan* were collected from Narayanganj, Dhaka, Bangladesh in August, 2011 and were identified by Dr. M. A. Razzaque Shah Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh, whose voucher specimen is maintained in our laboratory for future reference. From the collected fruits, peels 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 the extract

Around 250 g of powdered materials of both peel and seed were taken in two different clean, flat bottomed glass containers and were immersed in 500 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 material 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.

### Animals

Young Long-Evans rats of either sex weighing about 80 to 100 g, aged 8 to 14 weeks were used for the research. The rats were purchased 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 ± 0°C temperature and 55 to 65% relative humidity and 12 h light/12 h dark cycle) for one week for acclimation after their purchase and were fed ICDDRB formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

### Chemicals and drugs

Acetic acid (Merck, Germany), Tween-80 (BDH Chemicals, UK), formalin (CDH, India), normal saline solution (Beximco Infusion Ltd., Bangladesh), indomethacin and diazepam (Square Pharmaceuticals Ltd., Bangladesh) were procured and used in the experiment. All chemicals in this investigation were of analytical reagent grade.

### Acute toxicity test

For acute toxicity, we followed the method of Hilaly et al. (2004) with slight modifications in experimental rats. Rats that fasted for 16 h were randomly divided into groups of six rats per group for both extracts. Graded doses of the investigated extracts MNLS and

MNLP (200, 400, 800 and1600 mg/kg) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and were noticed over a period of 48 h for signs of acute toxicity. The number of deaths within this duration was recorded.

### CNS depressant activity

#### *Hole cross test*

The method was followed as described by Takagi et al. (1971). A cage with a fixed steel partition in the middle position having a size of 30 × 20 × 14 cm was taken. A hole of 3 cm diameter was made at a height of 7.5 cm in the midpoint of the cage. Thirty rats were divided into six groups with five rats in each group. Group I rats were given only vehicle (1% Tween-80 in water) 10 ml/kg (p.o.), animals of Group II received diazepam (as a standard drug) at 1 mg/kg body weight (p.o.), while rats of Group III and Group IV were treated with 250 and 500 mg/kg body weight (p.o.) of the MNLP; rodents of Groups V and VI received MNLS at a dose of 250 and 500 mg/kg body weight (p.o.), respectively. The number of passages of a rat through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of test drugs.

#### *Open field test*

We used open field test to monitor behavioral responses in rat that were placed in a novel and bright arena. Rodents tend to stay away from brightly illuminated areas. The experiment also assesses a range of anxiety-induced, locomotor activity and exploratory behaviors. The animals were treated as the earlier stated method. The test was carried out according to the technique described by Gupta et al. (1971). The floor of an open field of half square meter was divided into a series of squares, each colored alternatively black and white. The apparatus had a 40 cm wall. The number of squares visited by the animals was calculated for 3 min, at 0, 30, 60, 90 and 120 min subsequent to oral administration of the experimental crude extracts.

### Analgesic activity

#### *Acetic acid induced writhing method*

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test. Acetic acid-induced writhing test in rats (Sharma et al., 2010) for analgesic effect of the tested samples was conducted. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. Test samples (250 and 500 mg/kg body weight), vehicle (1% Tween-80 in water) and indomethacin (10 mg/kg) were administered orally 30 min prior to intra-peritoneal administration of 0.7% acetic. Then, the rodents were observed for specific contraction of body referred to as 'writhing' for the next 20 min (Sharma et al., 2010). Full writhing was not always completed by the animal, because sometimes the animals start to give writhing, but they do not finish it. This incomplete writhing was taken as half-writhing. Accordingly, two half-writhing were considered as one full writhing. The number of writhes in each treated group was compared to that of a control group while indomethacin (10 mg/kg) was used as a reference drug (positive control). The percent inhibition (% analgesic activity) was calculated by:

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

Where A = Average number of writhing of control per group; B = Average number of writhing of test per group.

#### *Formalin test*

Since different analgesics act differently in the early and late phases, the formalin test can be used to clarify the possible mechanism of an antinociceptive effect of a proposed analgesic (Tjolsen et al., 1992; Woode et al., 2011). So, here, antinociceptive activity of the extracts was determined by means of the formalin test as described by Sharma et al. (2010). Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MNLS and MNLP (250 and 500 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.). The rats were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the tenure between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was calculated with a stop watch.

### Statistical analysis

All the values in the test are presented as mean ± standard error of the mean (SEM). The data were statistically analyzed using analysis of variance (ANOVA) and post-hoc Dunnett's tests with the Statistical Package for Social Sciences (SPSS) program (SPSS 16.0, USA). Differences between the means of the various groups were considered significant at $P < 0.01$ and 0.05.

## RESULTS

### Acute toxicity

In the acute toxicity test, the plant extracts were found to be non-toxic up to doses of 1.6 g/kg. Behavior of the animals was strictly observed for the first 3 h, then at an intermission of every 4 h during the next 48 h. The extracts did not cause any behavioral change or mortality on rats during 48 h inspection.

### Effects of methanolic extract of fruits of *N. longan* on CNS depressant activity

Hole cross test and open field test was conducted to find out the depressive action of the tested extracts on CNS in rats (Gupta et al., 1971). Both MNLS and MNLP significantly diminished the locomotor activity.

#### *Hole cross test*

In the animals treated with MNLP and MNLS, extracts were given orally in two different doses (250 and 500 mg/kg) which significantly diminished the locomotion activity in a dose-dependent manner and the effect was comparable with that of the standard drug diazepam. The

**Table 1.** CNS depressant activity of methanolic extract of peel and seed of *N. longan* fruit in hole cross test in rats.

| Group | Treatment | Dose, route | Number of movements | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 min | 30 min | 60 min | 90 min | 120 min |
| Group-I (Control) | 1% Tween-80 in water | 10ml/kg, p.o | 12.8 ± 1.16 | 13.0 ± 1.41 | 13.6 ± 0.93 | 14.2 ± 0.86 | 14.0 ± 0.55 |
| Group-II (Standard) | Diazepam | 1mg/kg, p.o | 11.2 ± 0.58 | .0 ± 0.71* | 4.0 ± 0.84* | 2.4 ± 0.81* | 1.8 ± 0.37* |
| Group-III (Extract) | MNLP | 250 mg/kg, p.o | 16.2 ± 1.02** | 14.2 ± 0.73 | 12.6 ± 0.68 | 10.2 ± 0.73* | 8.6 ± 0.51* |
| Group-IV (Extract) | MNLP | 500 mg/kg, p.o | 14.2 ± 0.58 | 9.8 ± 0.73 | 6.4 ± 0.51* | 4.2 ± 0.37* | 2.2 ± 0.37* |
| Group-V (Extract) | MNLS | 250 mg/kg, p.o | 16.2 ± 1.02** | 12.0 ± 0.32 | 10.0 ± 1.0** | 7.2 ± 1.2* | 5.2 ± 1.07* |
| Group-VI (Extract) | MNLS | 500 mg/kg, p.o | 14.2 ± 0.58 | 8.4 ± 0.60* | 5.2 ± 0.37* | 3.6 ± 0.51* | 1.6 ± 0.40* |

All values are expressed as mean ± SEM (n = 5); one-way analysis of variance (ANOVA) followed by Dunnet's test. *P < 0.01 and **P < 0.05 significance compared to control.

locomotor activity lowering effect was manifested at the 2nd observation (30 min) period and was sustained up to the 5th observation period (120 min) for both extracts in both doses. The extracts produced reduction in spontaneous motor activity, and this effect may be attributed to CNS depression, as depression of locomotor activity is common to most neuroleptics. The CNS was depressed till the completion of observation period, and the results were statistically significant (Table 1).

### *Open field test*

In this test, the extracts exhibited spontaneous decline in locomotion in the tested rodents from the second to last study period at both dose levels (250 and 500 mg/kg body weight) given orally. With time, the effect of the extracts was increased and a noticeable result was found at 120 min for both administrated test samples. Test animals showed significant decrease in the number of movement in the dosages of 250 and 500 mg/kg (19.0 ± 1.45 and 15 ± 1.05, respectively for MNLP and 19.0 ± 1.0 and 14.8 ± 1.16 in the order for MNLS as compared to 118.0 ± 1.58 in the control group and 9.6 ± 0.51 in the standard group) at 120 min of administration of the extracts. So, the extracts showed dose dependent CNS depressant activities which were statistically significant (Table 2).

## Analgesic activity

### *Acetic acid-induced writhing test*

Table 3 shows the analgesic effect of methanolic extracts of the peel of the fruits of *N. longan* on rats in acetic acid-induced writhing method. At both doses, the MNLP and MNLS extracts exhibited significant reduction (P < 0.01) of writhing induced by the acetic acid after oral administration in a dose dependant manner. The percent inhibitions were recorded after oral administration at two different doses of experimental extracts and standard drug, indomethacin (10 mg/kg body weight). In the case of indomethacin, it was 58.87%, whereas for MNLP at 250 and 500 mg/kg body weight dose, it was found to be 37.341 and 54.43%, respectively; meanwhile, for MNLS at 250 mg/kg body weight, it was recorded at 36.075% and for 500 mg/kg dose at 52.531%. From the aforementioned results, it can be noticed that both extracts at a higher doses showed higher potency which are comparable to the standard drug. Both extracts at the dose of 500 mg/kg body weight were found more potent.

### *Formalin test*

The methanolic extracts of the peel and seeds of the fruits of *N. longan* (250 and 500 mg/kg, p.o.) significantly suppressed the licking activity in either phases of the formalin-induced pain in rats (Table 4) in a dose dependant manner. The reference antinociceptive drug, indomethacin (10 mg/kg) also significantly inhibited the licking activity against both phases of formalin-induced

**Table 2.** CNS depressant activity of methanolic extract of the peel and seed of *N .longan* fruit in open field test in rats.

| Group | Treatment | Dose, route | Number of movements | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 min | 30 min | 60 min | 90 min | 120 min |
| Group-I (Control) | 1% Tween-80 in water | 10 ml/kg, p.o | 118.4±1.21 | 118.0 ± 1.30 | 115.4 ± 0.51 | 117.4 ± 1.17 | 118.0 ± 0.71 |
| Group-II (Standard) | Diazepam | 1 mg/kg, p.o | 117.2 ± 1.16 | 64.6 ± 1.44* | 40.8 ± 0.58* | 18.8 ± 0.86* | 9.6 ± 0.51* |
| Group-III (Extract) | MNLP | 250 mg/kg, p.o | 124.8 ± 2.48 | 96.2 ± 5.18* | 68.6 ± 5.64* | 38.0 ± 2.92* | 19.0 ± 1.0* |
| Group-IV (Extract) | MNLP | 500 mg/kg, p.o | 136.0 ± 2.92* | 98.0 ± 2.57* | 48.8 ± 3.73* | 23.0 ± 0.84* | 14.8 ± 1.16* |
| Group-V (Extract) | MNLS | 250 mg/kg, p.o | 124.8 ± 2.48 | 99.6 ± 6.11* | 68.8 ± 5.16* | 37.6 ± 3.01* | 19.0 ± 1.45* |
| Group-VI (Extract) | MNLS | 500 mg/kg, p.o | 133.8 ± 2.15* | 98.2 ± 1.77* | 49.2 ± 3.91* | 23.2 ± 0.80* | 15 ± 1.05* |

All values are expressed as mean ± SEM (n = 5); one-way analysis of variance (ANOVA) followed by Dunnet's test. * P < 0.01 significance compared to control.

**Table 3.** Analgesic activity of methanolic extract of peel and seed of *N. longan* fruit by acetic acid induced writhing method in rats.

| Group | Treatment | Dose, route | No. of writhing | Percent inhibition |
|---|---|---|---|---|
| Group-I (Control) | 1% Tween-80 in water | 0.1 ml/10 g body weight | 26.3 ± 0.56 | - |
| Group-II (Standard) | Indomethacin | 10 mg/kg | 10.83 ± 1.22* | 58.86 |
| Group-III (Extract) | MNLP | 250 mg/kg, p.o | 16.50 ± 1.02* | 37.341 |
| Group-IV (Extract) | MNLP | 500 mg/kg, p.o | 12.0 ± 0.73* | 54.43 |
| Group-V (Extract) | MNLS | 250 mg/kg, p.o | 16.83 ± 0.60* | 36.075 |
| Group-VI (Extract) | MNLS | 500 mg/kg, p.o | 12.5 ± 0.67* | 52.531 |

All values are expressed as mean ± SEM (n = 6); one-way analysis of variance (ANOVA) followed by Dunnet's test. *P < 0.01 significance compared to control.

nociception. But, the extracts of the seeds of *N. longan* fruits at the dose of 500 mg/kg body weight showed more licking activity against late phase of formalin-induced pain than that of the standard drug, indomethacin.

## DISCUSSION

Numerous synthetic CNS depressant and analgesic drugs are available in the market for the management, control and/or treatment of patients. However, most of these synthetic drugs are not only inaccessible and unaffordable, but many of them possess toxic effects. It is therefore necessary to look inward for the development of inexpensive, effectual and secure CNS depressant and analgesic agents from plants and other natural resources. The results of this study indicate that MNLP and MNLS possess CNS depressant and analgesic activity (250 and 500 mg/kg p.o.). These results exhibit that the extracts significantly diminished the locomotor activity as shown by the data of the open field and hole cross tests. The locomotor activity is a test to appraise the level of excitability of the CNS (Mansur et al., 1980), and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system (Ozturk et al., 1996). The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.

**Table 4.** Effect of the methanolic extract of the peel and seed of *N. longan* fruit on hindpaw licking in the formalin test in rats.

| Group | Treatment | Dose, route | Early phase (s) | Late phase (s) |
|---|---|---|---|---|
| Group-I (Control) | Distilled water | 10 ml/kg | 35.67 ± 1.38 | 46.0 ± 1.03 |
| Group-II (Standard) | Indomethacin | 10 mg/kg | 16.83 ± 0.91* | 21.83 ± 0.70* |
| Group-III (Extract) | MNLP | 250 mg/kg | 21.0 ± 1.06* | 25.33 ± 1.45* |
| Group-IV (Extract) | MNLP | 500 mg/kg | 18.67 ± 2.25* | 22.33 ± 1.14* |
| Group-V (Extract) | MNLS | 250 mg/kg | 25.33 ± 0.67* | 23.83 ± 0.70* |
| Group-VI (Extract) | MNLS | 500 mg/kg | 19.17 ± 0.74* | 20.33 ± 0.67* |

All values are expressed as mean ± SEM (n = 6); one-way analysis of variance (ANOVA) followed by Dunnet's test. *P < 0.01 significance compared to control

Different anxiolytic, muscle relaxant and sedative-hypnotic drugs, elucidate their action through $GABA_A$, therefore, it is possible that extracts of *N. longan* fruits may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts (Kolawole et al., 2007). Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders (Bhattacharya and Satyan, 1997). Earlier investigation on phytoconstituents and plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system, which led to the assumption that they can act as benzodiazepine-like molecules (Verma et al., 2010). So, it is probable that phytoconstituents are responsible for its CNS depressant activity.

In the current study, we evaluated the MNLP and MNLS extracts for their analgesic property by acetic acid induced writhing and formalin techniques. The mechanisms for testing analgesic were selected such that both centrally and peripherally mediated effects were investigated. Acetic acid-induced writhing model signifies pain sensation by triggering localized inflammatory response. Such pain initiates the release of free arachidonic acid from tissue phospholipids (Ahmed et al., 2001). The acetic acid induced writhing response is a well known procedure to evaluate peripherally acting analgesics. The response is thought to be reconciled by peritoneal mast cells (Ronaldo et al., 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain et al., 2006). So, the abdominal constrictions produced after administration of acetic acid is linked to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the plant extracts may contain analgesic principles acting on the prostaglandin pathways leading to reduction of pain sensation in rat. We have also investigated the analgesic effect of the experimental plant crude extracts by formalin test to evaluate both peripheral and central activity. In the early phase of formalin test, the pain began due to the direct stimulation of the sensory nerve fibers by formalin, while in the late phase, pain induced due to different inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins (Dharmasiri et al., 2003). This test is considered to be a more convincing analgesic model which is better correlated with clinical pain (Tjolsen et al., 1992; Ghannadi et al., 2005). In this study, the extract caused a dose-dependent decrease in licking time (Table 4) by rats injected with formalin signifying the analgesic effect of the extracts. In an earlier study (Reanmongkol and Itharat, 2007), ethanol aqueous extracts of this plant have been reported not to affect the formalin induced licking activity.

Lack of mortality and signs of toxicity up to 5 times the maximum effective dose of MNLP and MNLS extracts demonstrate that the fruit has broad safety boundary. In future, we will do more research on *N. longan* fruits to find out more pharmacological activities in the plant.

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