International Journal of Phytopharmacology

Research Article

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

ACUTE ORAL TOXICITY STUDY IN RATS WITH METHANOLIC LEAF EXTRACT OF *LAURUS NOBILIS L.* ON HISTOPATHOLOGICAL CHANGES

Chandrasekaran Arcot Ravindran* and S. Ravichandran

P.S.V. College of Pharmaceutical Science and Research, Orappam Village & Post, Bargur Taluk, Krishnagiri District - 635108, Tamil Nadu.

ABSTRACT

To determine the effect of methanolic leaf extract of Laurus nobilis L on histopathological changes in acute oral toxicity in Wister albino rats. As described by Akunna et al the acute oral toxicity study of methanolic and chloroform leaf extract of *Laurus nobilis L* was conducted using the Organization for Economic Cooperation and Development (OECD) (2000) Guidance Document on Humane End points that should reduce the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure. Briefly, animals were divided into 2 groups, 5 animals were weighed and individually identified in each group. Group A treated with methanolic extract and group B were treated with chloroform extract. Group A: The first animal was given the test dose 5 mg per kg body weight of methanolic leaf extract of *Laurus nobilis L* (MELN). The second animal was given the test dose 50 mg per kg body weight of chloroform leaf extract of *Laurus nobilis L* (MELN). The third and fourth animals were concurrently dosed and the fourth and fifth animals sequentially dosed 300 and 2000 mg per kg body weight and fifth animal were kept as control. Similarly Group B were treated with chloroform extract in the similar fashion of dosing. The results were evaluated as follows (S = Survival, X = death). The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a total period of 14 days. All observations were systematically recorded with individual records maintained for each animal. There were no deaths of mice dosed at 2000 mg/kg body weight of the plants extract both within the short and long outcome of the limit dose test of Up and Down method. Effect of methanolic and chloroform leaf extract of *Laurus nobilis L* (MELN and CELN) on histopathological changes in acute oral toxicity in rat's transverse section of Brain, Kidney and Liver showed normal histology upon administration of 2000 mg/kg of Leaf Extract. It was found to be normal epithelium and intact interstitium in rat's transverse section of Brain, Kidney and Liver. The LD50 was calculated to be greater than 3000 mg/kg body weight orally.

Key words: Acute Toxicity Studies, *Laurus Nobilis L* (MELN), Histopathological studies.

Corresponding Author: **Chandrasekaran Arcot Ravindran** Email: arcsekaran123@gmail.com

INTRODUCTION

Plants have always been a major component of traditional system of healing in developing countries, which have also been an integral part of their history and culture. Medicinal plants offer alternative remedies with tremendous opportunities. Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and that these can be used to prevent and cure several human diseases. Even today, majority of the world population depends on herbal healthcare practice (WHO, 1998). Natural products are the keystone of health care delivery especially in resource poor settings. Currently statistics estimates that about

Access this article online

DOI: http://onlineijp.com/

DOI: http://dx.doi.org/10.21276/ijp.2020.11.2.1

Quick Response code

Received:05.02.20 **Revised**:22.02.20 **Accepted**:15.03.20

eighty percent of the world's population relies on traditional medicine for health care products (Farnsworth NR *et al*., 1985; Appidi JR *et al*., 2008). There are many family of plants contribute their natural sources to cure many diseases, the one among the family is Lauraceae .The Lauraceae comprise 32 genera and about 2000-2500 species. *Laurus nobilis L*., (bay) a member of the family named Apollo's Laurel in mythology, is a plant native to the Southern Mediterranean region including Hatay Turkey. This *Laurus nobilis L*., (bay) plant are taken out during the spring season and either potted or plunged in nursery. The rich peaty soil with plenty of water and congenial moist atmosphere near the sea coast are favorable conditions for fast and luxriant growth1. The leaves of L. nobilis are plucked and dried under shade for use as a flavouring material in a variety of culinary preparations, especially in French cuisine. The leaves contain an essential oil of aromatic, spicy odour and flavour which can be isolated by steam distillation. The oil is a valuable adjunct in the flavouring of all kinds of food products, particularly meats, sausages, canned soups, baked goods, confectionery, etc (Guenther E, 1953). The leaves of L. nobilis L. are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence (Kivcak B and T. Mert, 2002).

Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to perform toxicity tests in appropriate animal models, and acute toxicity studies are just one of a succession of toxicity tests that are used (Sally Robinson, 2007). This study was performed to assess the short-term toxicity of MELN in Wister albino rats when administered by gavage as a single oral dose. Current work provides information on the potential health hazards of the test article with respect to oral exposure. Data from this study may serve as a basis for classification and/or labeling of the test article.

MATERIAL AND METHODS

Plant material

The leaf of *Laurus nobilis L* were collected from Bujang Valley, Archeological site, Merbok, Kedah, Malaysia. Taxonomic identification was made from USM, Malaysia (Specimen herbarium no: 11250). A voucher specimen is preserved in our laboratory for further reference at school of Pharmaceutical sciences, University Sains Malaysia.

Preparation of plant extracts

The powdered plant materials were successively extracted with methanol by hot continuous percolation method in a Soxhlet apparatus for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Phytochemical screening

On preliminary screening of leaves of *Laurus nobilis L* the extracts showed the positive Shinoda test for flavonoids, Molisch test for glycosides, a positive Liebermann–Burchard reaction for steroid (Liebermann C. Zur *et al*., 1885) and a positive Noller test for triterpenoids (Noller CR *et al*., 1942), which were confirmed by thin layer chromatography with the solvent system benzene–ethylacetate (1:1) over silica gel G (Stahl E, 1969).

Animal Grouping and Experimental Protocol

Forty male adult wistar rats weighing 200-250 g were used for the study. The rats were randomly divided into four groups of twelve rats each. Group A served as the control and the rats were neither treated nor rendered cryptorchid. Group B rats were treated alone with BLE. Rats in group C were only rendered bilaterally cryptorchid while rats in group D were rendered bilaterally cryptorchid and also treated orally with 60 mg/kg body weight of BLE daily for fifty six days. Procedure to induce cryptorchidism was performed as described by Saalu LC *et al*., 2007.

Animals and Animal Husbandry

Description, Identification and Housing

Young adult, *Wister* albino rats were approximately 8-13 weeks of age at experimental initiation. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel rages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals.

Environment

The animal room temperature and relative humidity ranges were 68-74°F and 30-53%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment Light timers were set to maintain a 12-hour light/I2-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

Food

Certified Rodent, Chow (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various

environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet.

Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted annually and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study.

Acclimation

Upon receipt the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing.

Dosing

On day-1, the animals chosen for the limit test were weighed and fasted overnight on day 0, the test article was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe.

Clinical Observations

Test animals were observed for clinical abnormalities two times on study day 0 (postdose) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

Body Weights

Individual body weights were obtained the limit test for the animals prior to fasting (day -1), prior to dosing on day 0 and on days 7 and 14. Body weight means and standard deviations were calculated using statistical tools.

Analysis of data

Data from the limit test were analyzed and an LD50 value estimated as follows: < 50% Mortality: LD50 was estimated as greater than the administered dose.= 50% Mortality: LD50 was estimated as equal to the administered dose. > 50% Mortality: LD50 was estimated as less than the administered dose.

Animal Sacrifice and sample collection

The rats were weighed and then anaesthetized just before they were sacrificed. The Kineys, Brain and liver of the rats were removed and were fixed in 10% formol-saline for histological examination.

Assessment of acute toxicity studies (OECD, 2002)

The test substance was administered orally to a group of experimental animals at one of the defined doses. The substance will be tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level

Animals were divided into 2 groups, 5 animals were weighed and individually identified in each group. Group A treated with methanolic leaf extract and group B were treated with chloroform extract. Group A: The first animal was given the test dose 5 mg per kg body weight of methanolic leaf extract of *Laurus nobilis L* (MELN). The second animal was given the test dose 50 mg per kg body weight of chloroform leaf extract of *Laurus nobilis L* (MELN). The third and fourth animals were concurrently dosed and the fourth and fifth animals sequentially dosed 300 and 2000 mg per kg body weight and fifth animal were kept as control. Similarly Group B was treated with chloroform extract in the similar fashion of dosing.

The test substance was administered in a single dose by gavage using a stomach tube. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The time interval between treatment groups will be determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Body

weight changes showed that animals in control group increase in weight significantly (Table 3).

Histological evaluation (Shivananda Nayak *et al*., 2006)

Histological analysis as described by Saalu LC *et al*., 2007, the organs were cut in slabs of about 0.5 cm thick and fixed in Bouin's fluid for a day after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 57°C. Serial sections of 5 µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven-dried. Light microscopy was used for the evaluations.

Statistical analysis

Results of the biochemical estimations are reported as means S.E.M. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA), Student's t-test was used for determining significance (Woolson RF, 1987).

RESULT AND DISCUSSION

Acute Oral Toxicity

The single-dose oral toxicity of two different types of extracts of Bay leafs (*Laurus nobilis L*) was evaluated in Wister albino rats. A acute oral toxicity studies was performed in which one group of five male and five female rats received a single oral administration of the test article at a dose of 2000 mg/kg body weight Following dosing, were observed daily and weighed weekly. A gross histopathological examination was performed on all the test animals at the time of scheduled euthanasia (day 14). The Figure 1 shows Effect of methanolic leaf extract of *Laurus nobilis L* (MELN) on histopathological changes in acute oral toxicity in rats Brain, Kidney and Liver. Figure 1(a), 1(b) and 1(c) represents the transverse section of Brain, Kidney and Liver showing normal histology upon administration of 2000mg/kg of Leaf Extract and Figure 2 shows the Effect of Chloroform extract of Laurus nobilis (MELN) on histopathological changes in acute oral toxicity in rats Brain, Kidney and Liver. Figure no. 2(a), 2(b) and 2(c) represents the transverse section of Brain, Kidney and Liver showing normal histology upon administration of 2000mg/kg of Leaf Extract.

Table 1. Effect of Leaf Extracts on Behavioral Changes in Acute oral toxicity in rats

S. No.	Symptoms	Leaf Extract I (2000mg/kg)	Leaf Extract II (2000mg/kg)
1	Death	--	--
Central Nervous System			
2	Convulsions	--	--
3	Tremor	--	--
4	Straub tail	--	--
5	Sedation	--	--
6	Excitation	--	--
7	Jumping	--	--
8	Abnormal gait	--	--
9	Motor in-coordination	--	--
10	Altered muscle tone	--	--
11	Akinesia	--	--
12	Catalepsy	--	+
13	Loss of balance	--	--
14	Fore-paw treading	--	--
15	Writhing	--	--
16	Stereotypy	--	--
17	Altered fear	--	--
18	Altered respiration	--	--
19	Aggression	--	--
20	Analgesia	--	--
21	Body Temperature	--	--
Autonomous Nervous System			
22	Head movements	--	--
23	Scratching	--	--
24	Altered reactivity to touch	--	--
25	Loss of righting reflex	--	--

26	Loss of corneal reflex	--	--
27	Defecation/Diarrhea	--	--
28	Salivation	--	--
29	Lacrimation	--	--
30	Myosis/ Mydriasis	--	--
31	Loss of traction	--	--

Table 2. The results of Acute Toxicity Test for both the extracts (Up and Down Procedure) in Rats

Test serial number	Animal Identity	Dose of MoE mg/kg	Short term result (48hrs)	Long term results (14days)
1	REP	2000	s	s
2	LEP	2000	s	s
3	TC	2000	s	s
4	RLT	2000	s	s
5	LLT	2000	s	s
6	I	2000	s	s

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RLT = Right leg tagged; LLT=Left Leg tagged, I = Intact rat

Table 3: The gross anatomical parameters

Anatmical Parameters	Control B	BLE-alone
Initial Body weight	200.03±1.5	200±5.3
Final Body weight	205.12±1.2	204.3±2.1
Bodyweight difference	05.09	04.3

Figure 1. Effect of methanolic leaf extract of *Laurus nobilis.L* (MELN) on histopathological changes in acute oral toxicity in rats Brain, Kidney and Liver. Figure no. 1(a), 1(b) and 1(c) represents the transverse section of Brain, Kidney and Liver showing normal histology upon administration of 2000mg/kg of Leaf Extract

FIGURE 1: Effect of Methanolic Extract II on Brain, Kidney and Liver

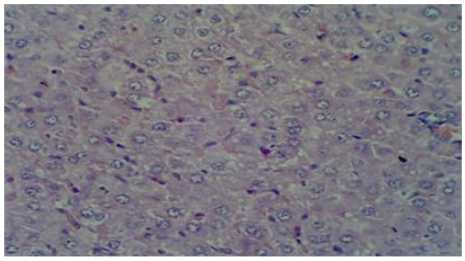

Figure 1a:Effect of methanolic extract of *Laurus nobilis* on histopathological changes in Brain

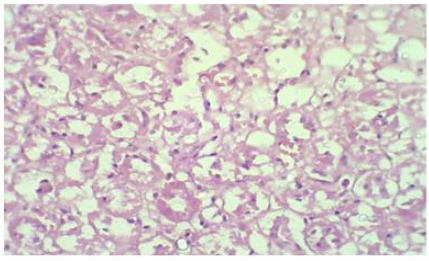

Figure 1b: Effect methanolic extract of *Laurus nobilis* on histopathological changes in Kidney

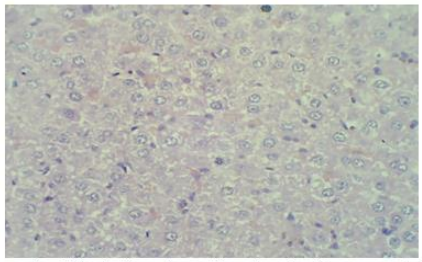

Figure 1c: Effect of methanolic extract of *Laurus nobilis* on histopathological changes in Liver

Figure 2. Effect of Chloroform leaf extract of *Laurus nobilis.L* (MELN) on histopathological changes in acute oral toxicity in rats Brain, Kidney and Liver. Figure no. 2(a), 2(b) and 2(c) represents the transverse section of Brain, Kidney and Liver showing normal histology upon administration of 2000mg/kg of Leaf Extract

Figure 2: Effect of Chloroform Extract II on Brain, Kidney and Liver

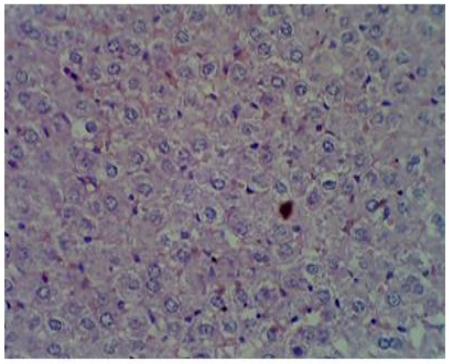

Figure 2a: Effect of chloroform extract of *Laurus nobilis* on histopathological changes in Brain

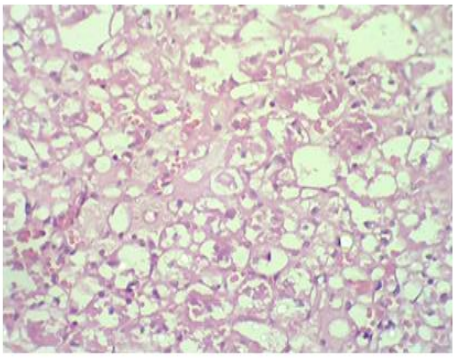

Figure 2b: Effect chloroform extract of *Laurus nobilis* on histopathological changes in Kidney

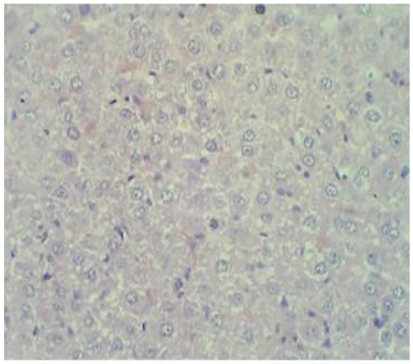

Figure 2c: Effect of chloroform extract of *Laurus nobilis* on histopathological changes in Liver

Effect of leaf extracts on behavioral changes in acute oral toxicity in rats was shown in Table 1, Table 2 shows the results of Acute Toxicity Test for both the extracts (Up and Down Procedure) in Rats (Jalalpure SS *et al*., 2003; Ahrom Ham *et al*., 2011). No mortality occurred during the acute toxicity studies. A slight body weight loss was noted for one female rat during the study day 7-14 body weight interval. However, since the animals were young adults and still growing, body weight gain was noted for all other animals during the test period (Table 3). Under the conditions of this test, the acute oral LD50 of was estimated to be greater than 3000 mg/kg in the rat. A slight body weight loss was noted for one female rat during the study day 7-14 body weight interval (Jouda Mediouni-Ben Jemâa *et al*., 2011; Ali-Shtayeh M.S *et al*., 2000).

CONCLUSION

No mortality occurred during the acute toxicity studies. A slight body weight loss was noted for one female rat during the study day 7-14 body weight interval. Results reported in this work showed that methanolic leaf extract of *Laurus nobilis.L* (MELN) on Brain, Kidney and Liver showing normal histology upon administration of 2000mg/kg of Leaf Extract. Effect of chloroform leaf extract of *Laurus nobilis.L* (CELN) on histopathological changes in acute oral toxicity in rats Brain, Kidney showed normal histology upon administration of 2000 mg/kg of Leaf Extract.

REFERENCES

Ahrom Ham, Jongheon Shin, Ki-bong Oh, Sung-Jin Lee, Kung-Woo Nam, Uk Koo, Kyeong Ho Kim, Woongchon Mar. Neuroprotective Effect of the n-Hexane Extracts of Laurus nobilis L. in Models of Parkinson's Disease. *Biomol Ther*. 19(1), 2011, 118-125.

Ali-Shtayeh MS, Yaniv Z, Mahajna J. Ethnobotanical survey in the Palestinian area: A classification of the healing potential of medicinal plants. *J. Ethnopharmacol*. 73, 2000, 221-232.

Appidi JR, Grierson DS, Afolayan AS. An ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape Province, South Africa. *Pakistan Journal of Biological Sciences*. 11(15), 2008, 1961-1963.

Challenging the regulatory requirement for acute toxicity studies in the development of new medicines, A workshop report, by Kathryn Chapman, NC3Rs; Sally Robinson, Astra Zeneca, 2007.

Farnsworth NR, Akerele O, Soejarto DD, Bingel AS, Guo Z. Medicinal plants in therapy. *WHO Bulletin*. 63, 1985, 965-981.

Guenther E. The Essential Oils; In: Oil of Bay. Van Nostrand Company Inc, New York; 1953: 378-396.

Jalalpure SS, Patil MB, Prakash NS, Hemalatha K, Manvi FV. Hepatoprotective activity of fruits of Piper longum L. *Indian J Pharm Sci*. 65, 2003, 363-366.

Jouda Mediouni-Ben Jemâa, Nisrine Tersim, Mohamed Larbi Khouja. Composition and Repellent Efficacy of Essential Oil from Laurus nobilis against Adults of the Cigarette Beetle Lasioderma serricorne (Coleoptera: Anobiidae). *Tunisian Journal of Plant Protection*. 6(1), 2011, 29-42.

Kivcak B and T Mert. Preliminary evaluation of cytotoxic properties of Laurus nobilis leaf extracts. *Fitoterapia*. 73, 2002, 242-243

Liebermann C Zur, Kenntniss Der Cochenille und des Cochenillecarmins. Berichte der deutschen chemischen Gesellschaft. 18(1), 1885, 1021–1024.

Noller CR, RA Smith, GR Harris, JM Walker. Saponins and sapogenins some color reactions of triterpenoid sapogenins. *J. Am. Chem. Soc*. 64, 1942, 3047-3047.

OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the OECD guidelines for the testing of chemicals organisation for economical co-operation and development. Paris. June 2000.

Saalu LC, Adesanya AO, Oyewopo AO, Raji Y. An evaluation of the deleterious effect of unilateral cryptorchidism on the contralateral normally descended testis. *Sci. Res. Essays*. 2(3), 2007, 074-8.

Shivananda Nayak, Poorna Nalabothu, Steve Sandiford, Vidyasagar Bhogadi, Andrew Adogwa. Evaluation of wound healing activity of Allamanda cathartica. L. and Laurus nobilis. L. extracts on rats. *BMC Complementary and Alternative Medicine*. 6, 2006, 12-17.

Stahl E. Thin-layer chromatography -a laboratory handbook, second ed. 1969; Berlin: Springer.

Woolson RF. Statistical methods for the Analysis of Biomedical Data Wiley series of probability and mathematical statistics, 1987; 315-32

World Health Organization: Quality control methods for medicinal plant materials. Published by WHO, Geneva, 1998.