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Data Report

Safety studies of LAURESH® a standardized *Laurus nobilis* leaf extract

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ABSTRACT — The erroneous assumption that herbal products is generally safe for consumption, is a major factor leading to the increased of herb-induced liver injury (HILI). Even though *Laurus nobilis* or laurel is a commonly used spice, the safety aspect for its consumption is under-studied. To bridge this gap of knowledge, the mutagenicity, acute toxicity, and subacute toxicity of LAURESH®, which is a standardized laurel leaf extract were evaluated. Mutagenicity study using two *S. typhimurium* strains, TA100 and TA98 indicated that LAURESH® does not cause base substitution and frameshift mutation, thus suggesting that LAURESH® is non-mutagenic. While acute oral toxicity on mice established the LD_{50} at no less than 2,000 mg/kg of body weight, and a 28-day subacute toxicity test on rat revealed the NOAEL to be 1,000 mg/kg/day. Furthermore, blood chemistry, urinalysis, necropsy, and histopathological data from subacute toxicity study on rats does not show adverse event that could be attributed to LAURESH®, thus indicating that LAURESH® is unlikely to cause HILI. Taken together, the findings from this study and previous clinical study on LAURESH®, in combination with the historic use of laurel and previous toxicity studies conducted on laurel leaves extract, strongly suggest that LAURESH® is safe for human consumption.

Key words: Bay leaf, Deacetyl laurenobiolide, *Laurus nobilis*, LAURESH®, Laurel, Safety

INTRODUCTION

Laurel, also known as bay leaf, is an aromatic evergreen shrub from the Lauraceae family. It is scientifically known as Laurus nobilis and is native to the Mediterranean coast (Parthasarathy *et al.*, 2008; Ross, 2001; Siriken *et al.*, 2018). Commonly known as an ingredient or spices in Mediterranean and Southeast Asian cuisines, laurel is also well known for their medicinal properties (Bianchi, 2015). Due to their economic values, laurel is cultivated in many regions which includes southern Europe, western Asia, northern Africa and America (Parthasarathy *et al.*, 2008; Ross, 2001). On the term of chemical contents, laurel is known to contain volatile compounds, sesquiterpenoids, flavonoids, proanthocyanidins, and simple phenolic compounds (Alejo-Armijo *et al.*, 2017; Caputo *et al.*, 2017; Fidan *et al.*, 2019), which may directly and indirectly contribute to its biological activities. Laurel is traditionally used for rheumatism, cough, cardiac diseases, sprains, viral infections, diarrhea, gastrointestinal problems, and antiseptic (Alejo-Armijo *et al.*, 2017; Caputo *et al.*, 2017; Fidan *et al.*, 2019; Fang *et al.*, 2005). Whereas, scientific investigations of laurel have led to the discovery of its many pharmacological usages which includes, antioxidant (Dias *et al.*, 2014; Ereifej *et al.*, 2016), anticonvulsant (Sayyah *et al.*, 2002), analgesic (Sayyah *et al.*, 2003), anti-inflammatory (Sayyah *et al.*, 2003), antimutagenic (Samejima *et al.*, 1998), immunostimulant (Bilen and Bulut, 2010), antiviral (Bilen and Bulut, 2010), antibacterial (Fidan *et al.*, 2019; Fukuyama *et al.*, 2011; Ino *et al.*, 2013; Nabila *et al.*, 2022; Yang and Ino, 2013), antifungal (Patrakar

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et al., 2012), and etc. Apart from its medicinal usage, laurel is also commonly used in the fragrance industry (Alejo-Armijo *et al.*, 2017) and its preservative properties imparted by antioxidant and antibacterial activities have also led to the wide usage of laurel as an additive for food preservation (El *et al.*, 2014).

Traditionally use as an antiseptic and in addition to its well-documented antimicrobial activity (Alejo-Armijo *et al.*, 2017; Fidan *et al.*, 2019; Fukuyama *et al.*, 2011; Ino *et al.*, 2013; Nabila *et al.*, 2022; Yang and Ino, 2013), laurel has become an attractive resource for oral care. Developed as an ingredient for oral care, LAURESH® is a standardized laurel leaf extract that contain no less than 1% of deacetyl laurenobiolide, which is an anti-bacterial sesquiterpenoid found in *L. nobilis* (Tada and Takeda, 1971). *In vitro* studies have shown that LAURESH® and its standardizing compound, deacetyl laurenobiolide possess anti-bacteria activity against periodontal disease-related bacteria such as *Porphyromonas gingivalis*, *Actinomyces viscosus*, *Actinobacillus actinomycetemcomitans*, and *Prevotella intermedia* (Fukuyama *et al.*, 2011; Yang and Ino, 2013). Additionally, anti-bacteria activity against carries-related bacteria such as *Streptococcus mutans*, *S. salivarius*, *S. sangius*, and *S. mitis* were also confirmed from *in vitro* studies of deacetyl laurenobiolide (Ino *et al.*, 2013). LAURESH® has also been demonstrated to suppress halitosis via inhibition of the growth of anaerobic bacteria (Yang and Ino, 2013). Additionally, clinical trial revealed that LAURESH® also improves saliva buffering within the normal range. By maintaining the salivary pH above 5.5, LAURESH® can prevent tooth decalcification, which is one of the factors that can lead to caries. Apart from preventing caries, the same clinical trial also revealed the LAURESH® improves the gingival index, which is an index for gauging the health of gingivae (Kuniyoshi *et al.*, 2021). The evidences from studies conducted on LAURESH® thus highlights its potential as an oral care ingredient.

Due to the recent rise in herb-induced liver injury (HILI) cases, which come in connection with the increased popularity of self-herbal treatment, there are growing concerns on the safety of herbal usages even among commonly used herbs (Amadi and Orisakwe, 2018; Teschke *et al.*, 2021). Although laurel leaves are generally recognized as safe (GRAS)-certified by the Food and Drug Administration (FDA) of the United States of America, and a clinical trial conducted using LAURESH® showed no adverse event (Kuniyoshi *et al.*, 2021), further confirmation of the safety of LAURESH® remains a necessity. Considering that LAURESH® is an attractive ingredient from herbal origin with proven potential for oral care, it is imperative to prove its safety for consumers and to further open the door for its future development. Hence, to bridge the knowledge gap on LAURESH® safety, we sought to reveal the mutagenicity, acute toxicity, and subacute toxicity of LAURESH®.

MATERIALS AND METHODS

Preparation of LAURESH®

Cultivated laurel leaves of European origin were used in the production of LAURESH®. Briefly, one hundred gram of laurel leaves were mixed with 1.4 L of 85% (v/v) ethanol, heated to reflux for two hours and filtered. The extracted liquid was vacuum concentrated at 50°C and freeze-dried to yield LAURESH®.

Experimental animals

Single dose acute oral toxicity study

Twenty ICR mice (*Mus musculus*; 10 male & 10 female), aged 5-weeks were purchased from Japan SLC. Inc, (Shizuoka, Japan) for single dose acute oral toxicity study. Mice were grouped in five and housed in polycarbonate cages, and kept at 23°C ± 2 under an artificial 12 hr light/dark cycle. Mice were given diet (Nosan Corporation, Kanagawa, Japan) and water *ad libitum*. Mice were quarantined and acclimatized for one week prior to experiment.

28-day repeated dose subacute oral toxicity study

Thirty-six (18 male & 18 female) Sprague-Dawley rats (*Rattus norvegicus domestica*; Crl:CD [SPF]), aged 5-weeks were purchased from The Jackson Laboratory Japan, Inc. (Formerly Charles River Laboratories Japan, Inc.) for 28-day repeated dose subacute oral toxicity study. Rats were house individually in wire-mesh cages (W19.7 x D26.3 x H18.0 cm) (TOYO-LABO, Tokyo, Japan) and changed weekly. Rats were kept at between 20-26°C and relative humidity between 35-70% under an artificial 12 hr light/dark cycle (light on at 07:00 and light of at 19:00). The air in the animal room were changed 12 times per hour. Rats were given diet (Oriental Yeast, Tokyo, Japan) and water *ad libitum* except during fasting. Rats were quarantined and acclimatized for one week prior to experiment.

Standardizing compound analysis

The standardizing compound of LAURESH®, deacetyl laurenobiolide, was analyzed using HPLC. In brief, 25 mg of LAURESH® was dissolved in 5 mL 50% EtOH (v/v) under sonication. Dissolved LAURESH® was then filtered using 0.45 μm filter. Standard deacetyl laurenobi-

olide was prepared by dissolving 10 mg of deacetyl laurenobiolide (Tokiwa Phytochemical, Chiba, Japan) in small amount (about two drops) of EtOAc. Dissolved solution was top up to 10 mL with MeOH. The column used for the HPLC analysis was CAPCELL PACK UG120 4.6 mm I.D. × 250 mm 5 μm (Shiseido, Tokyo, Japan). Water was used as eluent A and acetonitrile was used as eluent B. HPLC was carried out under the following gradient elution sequence: 35% B for 30 min, 35%-100% B for 20 min, and 100% B for 10 min. The flow rate is at 1 mL/min, UV detection at 200 nm, column oven temperature at 40°C, and the injection volume is 10 μL.

Mutagenicity

The mutagenicity of LAURESH® was conducted by BoZo Research Center Inc. (Tokyo, Japan) in accordance to Ames test protocol (Ames *et al.*, 1975). LAURESH® was evaluated using the following bacteria strains, *Salmonella typhimurium* TA100, and TA98. *S. typhimurium* TA100 was used to indicate the base substitution mutation, and *S. typhimurium* TA98 to indicate the frameshift mutation. LAURESH® was tested using concentration at 19.5, 78.1, 313, 1,250, 2,500, 5,000 μg/plate on abovementioned tester strains. DMSO which was used to dissolve LAURESH® was used as negative control, while 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2; Wako, Osaka, Japan) and benzo[α]pyrene (B[α]P; AccuStandard Inc, Connecticut, USA) were used as positive control. Bacteria was preincubated to be 1×10^9/mL, and LAURESH® and positive controls were added to the media with 0.1 mol/L phosphate buffer (pH 7.4) or S9 Mix, which was used to examine metabolic activation. Then, they were incubated at 37°C for 48 hr, and the number of revertant colonies was counted.

Single dose acute oral toxicity study

The single dose acute oral toxicity study was approved and conducted by Japan Food Research Laboratories (Tokyo, Japan) in accordance to the OECD guidelines for the Testing of Chemicals 420 (OECD, 2002). Five male and five female mice were randomly allocated to each of the LAURESH® and control group. Test solution was prepared by adding water to LAURESH® and homogenized (KINEMATICA, Switzerland). The solution was then adjusted to 100 mg/mL. Prior to administration of test solution, mice were fasted for four hours followed by measurement of each mouse body weight. Mice in test group were given a single dose of test solution that is equivalent to 2,000 mg/kg per body weight by oral gavage, while mice in control group were given water at 20 mL/kg. The mice were observed once a day for 14 days and the body weight of each mouse was recorded on the 7th and 14th day. After 14 days all mice were euthanized. Endpoint evaluation included clinical sign of toxicity, changes in body weight, and mortality. All mice were used for evaluation purposes. Comparison between LAURESH® and control group were performed at a significance level of 5% using t-test.

28-day repeated dose subacute oral toxicity study

The 28-day repeated dose subacute oral toxicity study was conducted by Biosafety Research Center Inc. (Shizuoka, Japan) according to the Criteria for Reliability of Application Materials (Ordinance for Enforcement of the Law Concerning Ensuring Quality, Efficacy and Safety of Pharmaceuticals and Medical Devices, Article 43) and Partial Revision of Guidelines for Repeated Dose Toxicity Studies (April 5, 1999 Pharmaceutical Examination No. 655) of Pharmaceutical Safety Bureau, Ministry of Health, Labour and Welfare, Japan. Additionally, this study was reviewed and approved prior to initiation by the Institutional Animal Care and Use Committee of Biosafety Research Center Inc.

In brief, six male and six female rats were allocated to each of the LAURESH® and control group based on their body weight on day 1, and randomly assigned using a computer system package for safety studies (LATOX-F/V5). The body weights of the rats used in this study ranged from 222 to 241 g for males and from 135 to 159 g for females, respectively. Control group was given water while test groups were given either 600 or 1,000 mg/kg/day of LAURESH® by oral gavage. The general condition of each rat was observed twice a day (before and after dosing). Rats were weighed prior to dosing on day 1 (before grouping), 4, 8, 11, 15, 18, 22, 25, and 28. In addition, the body weight gain between day 1 and 28 was calculated. After the end of the study period (day 29), rats were sacrificed for necropsy. All rats were used for evaluation purposes.

Blood sampling

All the animals scheduled to undergo necropsy were subjected to fasting from the evening on the day prior to blood sampling and the subsequent necropsy. Blood samples were collected from the abdominal aorta of animals under isoflurane anesthesia. Blood and plasma samples were used for hematology. Blood samples were collected into blood-collecting tubes (INSEPACK II-D, Tokuyama Sekisui) containing an anticoagulant (EDTA-2K). Plasma samples were prepared from blood samples collected into blood-collecting tubes (Venoject II, Terumo) contain-

ing an anticoagulant (3.2% sodium citrate solution) by centrifugation at 1,700 × g for 13 min at room temperature. For the blood chemistry, serum samples were used. These samples were prepared from blood samples collected into blood-collecting tubes (Venoject II) containing a Gel and Clot activator by centrifugation at 1700 × g for seven minutes at room temperature.

Hematology and blood chemistry

Blood and plasma samples were used for hematology. The hematocrit, hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio, reticulocyte count, platelet count, white blood cell count, differential leukocyte ratios, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, and large unstained cell count blood parameters containing EDTA-2K were analyzed using a Hematology System (ADVIA120, Bayer), and prothrombin time and activated partial thromboplastin time were determined using plasma samples containing 3.2% sodium citrate solution using a coagulation analyzer (STA Compact, Roche). The differential leukocyte ratios were determined using flow-cytometric measurement with peroxidase staining and dual angle laser flow-cytometry. Blood smear specimens were also prepared using May-Grünwald-Giemsa staining and stored.

For the blood chemistry, serum samples were used. The total protein, glucose, triglyceride, total cholesterol, blood urea nitrogen, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase,alkaline phosphatase, γ-glutamyl transpeptidase, calcium, inorganic phosphorus, sodium, potassium, chloride, albumin ratio, α1-globulin ratio, α2-globulin ratio, β-globulin ratio, γ-globulin ratio, albumin ratio/total globulin ratio, albumin concentration, α1-globulin concentration, α2-globulin concentration, β-globulin concentration, and γ-globulin concentration serum parameters were determined using an automatic analyzer (Hitachi 7170, Hitachi), an electrolyte analyzer (EA07, A&T) or an electrophoresis analyzer (Epalyzer 2 plus, Helena Laboratories).

Urinalysis

Animals were placed in individual urine-sampling cages with food and water, and fresh urine (within three hours after urination) and pooled urine (24 hr) samples were collected. The pH, occult blood, ketone bodies, glucose, protein, bilirubin, urobilinogen were examined using fresh urine samples with the Ames test strip (N-Multistix SG-L, Siemens Healthcare Diagnostics), and the reagent strips were interpreted using an automatic strip reader (CLINITEK Advantus, Siemens Healthcare Diagnostics). Urinary volume, color, osmotic pressure, sediment, sodium concentration, potassium concentration, chloride concentration, total sodium excretion, total potassium concentration, and total chloride concentration were examined using the 24-hr urine samples.

The volume and color of the urine samples were examined, and the samples were then centrifuged at approximately 400 × g for five minutes to separate the supernatant and residue (sediments). The supernatant was used to measure the urinary electrolytes (sodium, potassium and chloride) and osmotic pressure. The urinary electrolytes were determined using the above-mentioned electrolyte analyzer (EA07), and the total excretion value of each electrolyte was calculated. The osmotic pressure was determined using an osmotic pressure analyzer (Osmotic Pressure AUTO&STAT™ OM-6030, Arkray Factory). While, erythrocytes, leukocytes, squamous cells, transitional epithelial cells and renal tubular epithelial cells from the residue was stained using the new Sternheimer method to prepare urine sediment microscope slides and the following parameters were examined microscopically.

Necropsy and histopathological examination

The pathological examinations consisted of organ weight measurement, a full macroscopic examination (necropsy), and a histopathological examination. The animals for scheduled necropsy were necropsied after blood sampling and euthanasia by exsanguination under isoflurane anesthesia.

Organ weights were obtained for the brain (excluding olfactory bulb), heart, lung (including bronchi), liver, kidneys, spleen, testes, salivary glands (sublingual glands and mandibular glands), adrenal glands, ovaries, pituitary gland, thymus, and uterus. The organ weight to body weight ratio (relative organ weight) was calculated from the body weight measured on the day of necropsy and the organ weight (absolute organ weight/final body weight × 100).

Lungs (including bronchi), heart, aorta, kidneys, liver, thymus, spleen, pancreas, lymph nodes (mesenteric and cervical), adrenal glands, salivary glands (sublingual glands and mandibular glands), pituitary gland, brain (cerebrum, cerebellum, and brainstem; excluding olfactory bulb), spinal cord (cervical, thoracic, and lumbar), tongue, stomach, duodenum, jejunum, ileum (including Peyer's patch), cecum, epididymides, seminal vesicles (including coagulating glands), prostate (including urethra), ovaries, oviducts, uterus, vagina, urinary bladder, skeletal muscle (fermoral region), sciatic nerve, skin,

mammary gland, trachea, esophagus, thyroids glands, parathyroids glands, sternum, femur (left), bone marrow (sternum and femur), nasal cavity (nasoturnibate), Zymbal's glands, and macroscopically abnormal organs/tissues were fixed with 10% neutral buffered formalin solution. Testes were pre-fixed in formalin-acetic acid solution and the eyes (including optic nerve) and Harderian glands were pre-fixed in Davidson's solution followed by 10% neutral buffered formalin solution.

Specimens for histopathological examination were prepared from all animals. Fixed lungs (including bronchi), heart, kidneys, liver, thymus, spleen, pancreas, adrenal glands, brain (cerebrum, cerebellum, and brainstem; excluding olfactory bulb), stomach, duodenum, testes, ovaries, nasal cavity (nasoturbinate), and macroscopically abnormal organs tissues samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin and were examined microscopically. All histopathological findings, including the types and severity, were directly recorded using a pathology system (PATHOTOX).

Statistical analysis

The body weight, body weight gain, food consumption, hematological data, blood chemical data, urinalysis data (volume, osmotic pressure, electrolytes) and absolute/relative organ weights were initially analyzed using the Bartlett's equal variance test. Dunnett's multiple comparison test was used in the case of equal variance (non-significant), and Steel's test was used in the case of unequal variance (significant), to test the significant difference between the control group and each treatment group. The significance level was 5% for Bartlett's test of equal variance, and 5% and 1% for two-sided tests. No statistical tests were used to compare clinical signs, or gross and histopathological findings.

RESULTS

Standardizing compound analysis

The presence and content of deacetyl laurenobiolide in LAURESH® was confirmed by comparing the retention time and area under the curve (AUC) of commercial deacetyl laurenobiolide, respectively, using HPLC analysis. HPLC chromatogram of LAURESH® show the presence of a peak at 46.3 min (Fig. 1a), which is similar to the retention time of commercial deacetyl laurenobiolide (Fig. 1b). Further, analysis of AUC revealed that LAURESH® contains no less than 1.0% of deacetyl laurenobiolide.

Mutagenicity

Ames test was used to assess the mutagenicity of LAURESH® to *Salmonella typhimurium* TA100 and TA98. *S. typhimurium* TA100 was used to indicate the base substitution mutation, and *S. typhimurium* TA98 was used to indicate the frameshift mutation. Test samples were 19.5, 78.1, 313, 1,250, 2,500, 5,000 μg/plate of LAURESH®. DMSO was used as negative control, and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) and benzo[α]pyrene (B[α]P) were used as positive control. The increases in the number of reverse mutant colonies were less than 2-fold of the number in negative control, and no dose-dependent increase was observed regardless of the metabolic activation (Table 1). Thus, the result of Ames test revealed that LAURESH® was non-mutagenic.

Single dose acute oral toxicity study

The single dose acute oral toxicity study showed no abnormality and death during the test period. Likewise, there was no difference in body weight between the LAURESH® group and control group for both male and female mice during the 14 days period (Table 2). Furthermore, necropsy conducted at the end of the test period also showed no abnormality to both male and female mice.

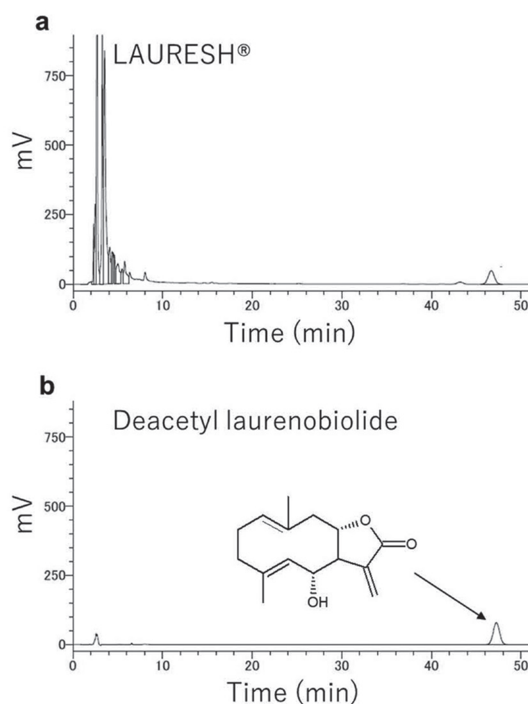

Fig. 1. HPLC chromatogram of LAURESH® (**a**) and deacetyl laurenobiolide (**b**).

Table 1. Comparison of Ames test results of LAURESH® and negative control.

S9 Mix	Sample	Conc. (μg/plate)	The average number of revertant colonies: Base substitution mutation TA100 (*n* = 2)	Frameshift mutation TA98 (*n* = 2)
-	Negative control		106	17
	LAURESH®	19.5	103	14
		78.1	111	14
		313	94	17
		1,250	112	16
		2,500	114	20
		5,000	118	15
+	Negative control		115	40
	LAURESH®	19.5	124	29
		78.1	122	32
		313	116	24
		1,250	102	32
		2,500	115	24
		5,000	108	18

Table 2. Comparison of acute toxicity test results on mice before and after administration of 2,000 mg/kg of LAURESH®.

Sex	Group	Body weight (g): Before administration	After administration: Day 7	Day 14
Male	LAURESH® (*n* = 5)	33.2 ± 1.2	38.9 ± 2.0	41.4 ± 2.4
	Control (*n* = 5)	33.2 ± 1.4	38.3 ± 2.5	41.3 ± 2.9
Female	LAURESH® (*n* = 5)	26.2 ± 0.9	29.2 ± 1.4	31.9 ± 1.2
	Control (*n* = 5)	26.5 ± 1.0	29.3 ± 1.0	31.1 ± 1.0

All values are shown as mean ± standard deviation.

Hence, based on the obtained results, the LD_{50} of LAURESH® under the condition set in this study was no less than 2,000 mg/kg.

28-day repeated oral dose toxicity study

No animal mortality occurred during the administration period, and there were no observable changes in general condition that could be attributed to the effects of LAURESH®. One male rat in the 600 mg/kg/day LAURESH® group experienced neck and back injuries from Day 22-29. However, this observation also occurred in untreated animals, so it was not attributed to the effects of LAURESH®. Furthermore, no significant difference to the body weight (Table 3) and daily food consumption (data not shown) between the control group and LAURESH® group were observed.

Hematological test showed that female rats in the 1,000 mg/kg/day of LAURESH® group had statistically higher basophil ratio than control (Table 4). Additionally, the prothrombin time was significantly lower in male rats in both 600 and 1,000 mg/kg/day of LAURESH® group than control group (Table 4). While, activated partial thromboplastin time was only significant to male rats in 1,000 mg/kg/day of LAURESH® group than control group (Table 4). Regarding the blood chemistry analyses (Table 5) and urinalysis (Table 6), there were no significant difference between LAURESH® group and control group.

Pathological examination indicated female rat in 1,000 mg/kg/day of LAURESH® group showed significantly higher relative liver weight than control group (Table 7). On the other hand, necropsy examinations findings include brown spots in the lungs, cysts and depressions in the kidneys, and crust on the skin (data not shown). Since all lesions were isolated, localized, and occurred only in a few incidences, these incidences were considered to be spontaneous lesion frequently observed in rats. Hence, the necropsy examinations indicated that

Table 3. Mean body weight of rats on a 28-day repeated oral dose subacute toxicity study.

Day of treatment	Male			Female		
	Control (n = 6)	LAURESH® (mg/kg/day)		Control (n = 6)	LAURESH® (mg/kg/day)	
		600 (n = 6)	1,000 (n = 6)		600 (n = 6)	1,000 (n = 6)
1	234 ± 6	234 ± 5	234 ± 6	148 ± 5	148 ± 8	149 ± 6
4	257 ± 9	261 ± 8	257 ± 6	157 ± 9	154 ± 6	159 ± 5
8	287 ± 14	297 ± 11	292 ± 11	169 ± 10	165 ± 7	171 ± 5
11	313 ± 17	327 ± 15	321 ± 13	180 ± 11	176 ± 6	181 ± 7
15	341 ± 21	357 ± 19	349 ± 15	190 ± 14	185 ± 8	192 ± 8
18	361 ± 24	377 ± 19	371 ± 17	198 ± 16	187 ± 9	200 ± 8
22	380 ± 29	400 ± 20	392 ± 22	209 ± 19	198 ± 8	211 ± 9
25	389 ± 32	410 ± 19	401 ± 26	209 ± 16	206 ± 10	215 ± 9
28	403 ± 32	425 ± 17	416 ± 30	218 ± 18	207 ± 10	224 ± 10
Gain	169 ± 30	191 ± 14	182 ± 25	70 ± 15	59 ± 11	76 ± 9

All values are shown as mean ± standard deviation.

Table 4. Hematology of rats on a 28-day repeated oral dose subacute toxicity study.

Item	Male			Female		
	Control (n = 6)	LAURESH® (mg/kg/day)		Control (n = 6)	LAURESH® (mg/kg/day)	
		600 (n = 6)	1,000 (n = 6)		600 (n = 6)	1,000 (n = 6)
Hematocrit (%)	46.7 ± 1.4	46.7 ± 1.3	47.4 ± 1.5	45.5 ± 1.3	46.1 ± 2.0	45.0 ± 1.4
Hemoglobin (g/dL)	16.2 ± 0.6	16.1 ± 0.5	16.5 ± 0.7	16.2 ± 0.3	16.3 ± 0.5	15.9 ± 0.4
Red blood cell count (x10^6/mm^3)	8.02 ± 0.34	8.02 ± 0.31	8.19 ± 0.18	7.95 ± 0.48	8.04 ± 0.35	7.86 ± 0.36
Mean corpuscular volume (µm^3)	58.2 ± 0.8	58.1 ± 1.8	57.9 ± 0.9	57.4 ± 2.5	57.4 ± 1.4	57.4 ± 1.1
Mean corpuscular hemoglobin (pg)	20.2 ± 0.3	20.1 ± 0.8	20.1 ± 0.5	20.5 ± 1.0	20.3 ± 0.5	20.2 ± 0.6
Mean corpuscular hemoglobin concentration (%)	34.7 ± 0.5	34.7 ± 0.4	34.7 ± 0.4	35.7 ± 0.6	35.4 ± 0.6	35.3 ± 0.7
Reticulocyte (%)	2.3 ± 0.4	2.3 ± 0.6	2.3 ± 0.4	2.1 ± 0.7	1.9 ± 0.4	21 ± 0.6
Reticulocyte (x10^9/L)	179.6 ± 32.3	180.6 ± 43.9	185.1 ± 29.8	165.6 ± 49.5	155 ± 27.1	159.7 ± 42.0
Platelet count (x10^3/mm^3)	1219 ± 113	1155 ± 126	1201 ± 126	1226 ± 253	1122 ± 80	1204 ± 136
White blood cell count (x10^3/mm^3)	8.04 ± 0.94	9.06 ± 3.06	9.01 ± 2.58	5.75 ± 1.56	8.02 ± 2.46	6.40 ± 2.93
Differential leukocyte ratio - Neutrophil (%)	12.5 ± 3.1	13.2 ± 5.5	13.1 ± 3.5	15.7 ± 7.8	16.5 ± 5.8	14.9 ± 5.5
Differential leukocyte ratio - Lymphocyte (%)	82.4 ± 2.9	81.8 ± 4.9	81.4 ± 4.0	79.5 ± 7.5	78.6 ± 5.7	79.7 ± 5.6
Differential leukocyte ratio - Monocyte (%)	2.9 ± 0.6	2.4 ± 0.5	3.2 ± 0.5	2.1 ± 0.6	2.4 ± 0.4	2.8 ± 0.5
Differential leukocyte ratio - Eosinophil (%)	0.9 ± 0.4	1.1 ± 0.6	0.8 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.2 ± 0.3
Differential leukocyte ratio - Basophil (%)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1*
Differential leukocyte ratio - Large unstained cells (%)	1.1 ± 0.3	1.3 ± 0.6	1.2 ± 0.2	1.0 ± 0.4	1.0 ± 0.2	1.2 ± 0.5
Neutrophil (x10^3/mm^3)	0.98 ± 0.16N	1.19 ± 0.58	1.12 ± 0.24	0.91 ± 0.48	1.33 ± 0.64	0.88 ± 0.24
Lymphocyte (x10^3/mm^3)	6.65 ± 0.98	7.43 ± 2.70	7.40 ± 2.41	4.56 ± 1.23	6.29 ± 1.97	5.15 ± 2.58
Monocyte (x10^3/mm^3)	0.24 ± 0.06	0.21 ± 0.05	0.29 ± 0.06	0.12 ± 0.03	0.20 ± 0.08	0.19 ± 0.11
Eosinophil (x10^3/mm^3)	0.07 ± 0.04	0.09 ± 0.03	0.07 ± 0.03	0.09 ± 0.04	0.10 ± 0.02	0.08 ± 0.05
Basophil (x10^3/mm^3)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Large unstained cells (x10^3/mm^3)	0.09 ± 0.03N	0.13 ± 0.09	0.10 ± 0.02	0.06 ± 0.03	0.08 ± 0.04	0.08 ± 0.07
Prothrombin time (sec.)	13.0 ± 1.3	10.5 ± 0.7**	11.1 ± 1.0*	8.8 ± 0.3	9.0 ± 0.1	8.7 ± 0.3
Activated partial thromboplastin time (sec.)	24.1 ± 2.1	21.6 ± 1.9	21.3 ± 1.3*	17.5 ± 1.0	17.5 ± 1.2	18.1 ± 0.7

All values are shown as mean ± standard deviation. Significant difference from control; *$P \leq 0.05$, **$P \leq 0.01$ (Dunnett's multiple comparison test). N: Non parametric analysis.

no negative attribute that could be derived from LAURESH®. On the other hand, histopathological examination detected sporadic occurrences of microgranulomas in the liver (Fig. 2a-d), as well as tubular regeneration in the kidneys (Fig. 2b-h) of both male and female rats. However, the liver's microgranuloma and tubular regeneration in the kidney does not only occurs in rats given 1,000 mg/kg/day of LAURESH® (Fig. 2b & 2d) but also

Table 5. Blood chemistry of rats on a 28-day repeated oral dose subacute toxicity study.

Item	Male			Female		
	Control ($n = 6$)	LAURESH® (mg/kg/day)		Control ($n = 6$)	LAURESH® (mg/kg/day)	
		600 ($n = 6$)	1,000 ($n = 6$)		600 ($n = 6$)	1,000 ($n = 6$)
Total protein (g/dL)	5.86 ± 0.18	5.86 ± 0.11	5.78 ± 0.17	5.99 ± 0.20	6.10 ± 0.21	6.17 ± 0.21
Albumin (g/dL)	3.10 ± 0.15	3.04 ± 0.10	3.06 ± 0.08	3.50 ± 0.20	3.41 ± 0.24	3.54 ± 0.27
Globulin (g/dL)	2.76 ± 0.14	2.82 ± 0.09	2.72 ± 0.10	2.49 ± 0.22	2.70 ± 0.12	2.64 ± 0.20
Albumin ratio/Total globulin ratio	1.13 ± 0.09	1.08 ± 0.06	1.13 ± 0.03	1.42 ± 0.19	1.27 ± 0.13	1.35 ± 0.18
Glucose (mg/dL)	182 ± 25	169 ± 19	178 ± 28	128 ± 15	116 ± 9	129 ± 19
Triglyceride (mg/dL)	87 ± 40N	62 ± 9	75 ± 28	19 ± 4	16 ± 9	23 ± 12
Total cholesterol (mg/dL)	61 ± 10	57 ± 9	57 ± 10	65 ± 7	65 ± 20	72 ± 15
Blood urea nitrogen (md/dL)	13.6 ± 1.9	12.4 ± 2.4	13.4 ± 1.4	18.0 ± 1.9	16.9 ± 2.2	17.4 ± 5.2
Creatinine (md/dL)	0.26 ± 0.03	0.29 ± 0.04	0.28 ± 0.04	0.34 ± 0.03	0.30 ± 0.03	0.32 ± 0.03
Total bilirubin (md/dL)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Aspartate aminotransferase (U/L)	71 ± 7	76 ± 9	75 ± 9	71 ± 7N	75 ± 15	64 ± 4
Alanine aminotransferase (U/L)	28 ± 4	25 ± 5	26 ± 6	22 ± 3	24 ± 4	22 ± 4
Alkaline phosphatase (U/L)	543 ± 71	576 ± 102	553 ± 97	430 ± 113	355 ± 66	373 ± 44
Calcium (mg/dL)	9.78 ± 0.26	9.90 ± 0.17	9.81 ± 0.27	9.53 ± 0.27	9.67 ± 0.38	9.74 ± 0.37
Inorganic phosphorus (md/dL)	7.51 ± 0.57N	7.53 ± 0.46	8.24 ± 1.33	6.74 ± 0.72	7.41 ± 0.58	7.06 ± 0.50
Sodium (mmol/L)	141.7 ± 1.0	142.5 ± 0.7	142.2 ± 1.0	142.0 ± 0.7	142.7 ± 1.0	142.0 ± 1.0
Potassium (mmol/L)	4.71 ± 0.11N	4.54 ± 0.17	4.76 ± 0.35	4.53 ± 0.18	4.52 ± 0.36	4.47 ± 0.24
Chloride (mmol/L)	104.6 ± 1.0	104.6 ± 1.2	104.1 ± 1.0	107.4 ± 1.9	107.2 ± 1.6	107.0 ± 1.1

All values are shown as mean ± standard deviation. N: Non parametric analysis.

Table 6. Urinalysis of rats on a 28-day repeated oral dose subacute toxicity study.

Item	Male			Female		
	Control ($n = 6$)	LAURESH®		Control ($n = 6$)	LAURESH®	
		600 mg/kg/day ($n = 6$)	1,000 mg/kg/day ($n = 6$)		600 mg/kg/day ($n = 6$)	1,000 mg/kg/day ($n = 6$)
Volume (mL)	19.4 ± 4.0	16.4 ± 10.0	18.0 ± 7.7	11.7 ± 5.3	10.7 ± 4.2	9.9 ± 4.5
Specific gravity	1.040 ± 0.009	1.053 ± 0.018	1.045 ± 0.014	1.050 ± 0.015	1.047 ± 0.012	1.056 ± 0.019
Sodium (mmol/L)	107.1 ± 34.4	132.7 ± 49.4	116.1 ± 43.1	120.4 ± 31.3	124.9 ± 43.0	141.7 ± 55.1
Potassium (mmol/L)	187.8 ± 46.5	252.3 ± 86.4	211.4 ± 61.9	234.0 ± 75.6	211.0 ± 59.9	257.4 ± 101.4
Chloride (mmol/L)	138.1 ± 37.5	187.5 ± 60.9	155.4 ± 57.3	183.8 ± 55.6	168.5 ± 51.7	204.2 ± 79.9
Sodium (mmol/day)	1.97 ± 0.38	1.82 ± 0.20	1.83 ± 0.17	1.27 ± 0.22	1.20 ± 0.23	1.27 ± 0.35
Potassium (mmol/day)	3.49 ± 0.38	3.47 ± 0.42	3.44 ± 0.58	2.42 ± 0.21	2.07 ± 0.46	2.29 ± 0.59
Chloride (mmol/day)	2.56 ± 0.38	2.61 ± 0.37	2.45 ± 0.29	1.91 ± 0.19	1.64 ± 0.40	1.83 ± 0.50

All values are shown as mean ± standard deviation.

in control group (Fig. 2a & 2c). The sporadic occurrences in both groups indicates that the abovementioned histopathological findings were unrelated to LAURESH®. Additionally, steatosis in the liver (Fig. 3b) was detected in a single female rat given 1,000 mg/kg/day of LAURESH®. No other occurrences of steatosis were observed in the livers of the remaining rats. As a reference, Fig. 3a illustrates a liver without steatosis. Considering it was a single occurrence, this finding was also deemed unrelated to LAURESH®. Inflammatory changes in the lungs of female rats (Fig. 3c-d) and kidneys of male rats (Fig. 2e-f) were also observed. The inflammatory changes were deemed inconsequential as it occurs sporadically in rats from control (Fig. 3c & 3e) and LAURESH® group (Fig. 3d & 3f). Similarly, sporadic mineralization detected in kidneys of male rats from control group (Fig. 3g) and LAURESH® group (Fig. 3h), were also deemed unrelated to LAURESH® since it occurs in both groups and only found in a few rats. A microscopic examination of gastric mucosa was also performed, in which no abnormal findings were observed. Taken together, histopathological examination also indicated that administration of LAURESH® does not considered to be toxic.

Table 7. Absolute and relative organ weights of rats on a 28-day repeated oral dose subacute toxicity study.

Item	Male			Female		
	Control	LAURESH® (mg/kg/day)		Control	LAURESH® (mg/kg/day)	
	(n = 6)	600 (n = 6)	1,000 (n = 6)	(n = 6)	600 (n = 6)	1,000 (n = 6)
Body weight (g)	376 ± 32	396 ± 15	388 ± 28	201 ± 16	194 ± 9	208 ± 9
Brain (g)	2.18 ± 0.09	2.16 ± 0.09	2.11 ± 0.10	1.91 ± 0.05	1.91 ± 0.07	1.92 ± 0.04
Brain (%)	0.582 ± 0.547	0.547 ± 0.028	0.546 ± 0.034	0.955 ± 0.056	0.985 ± 0.059	0.926 ± 0.052
Heart (g)	1.28 ± 0.06	1.28 ± 0.08	1.27 ± 0.07	0.74 ± 0.05	0.78 ± 0.06	0.78 ± 0.07
Heart (%)	0.341 ± 0.020	0.324 ± 0.022	0.328 ± 0.009	0.371 ± 0.018	0.403 ± 0.040	0.374 ± 0.019
Lungs (g)	1.36 ± 0.13	1.41 ± 0.10	1.39 ± 0.06	0.97 ± 0.06	0.96 ± 0.06	0.97 ± 0.06
Lungs (%)	0.362 ± 0.015	0.356 ± 0.027	0.359 ± 0.020	0.484 ± 0.014	0.494 ± 0.036	0.465 ± 0.021
Liver (g)	11.51 ± 1.61	11.88 ± 0.81	12.07 ± 1.13	5.71 ± 0.64	5.67 ± 0.35	6.38 ± 0.59
Liver (%)	3.053 ± 0.199	3.002 ± 0.130	3.107 ± 0.147	2.845 ± 0.096	2.920 ± 0.111	3.067 ± 0.197*
Kidneys (g)	2.81 ± 0.11	2.85 ± 0.11	2.79 ± 0.27	1.59 ± 0.24	1.59 ± 0.09	1.68 ± 0.19
Kidney (%)	0.750 ± 0.050	0.720 ± 0.021	0.718 ± 0.040	0.790 ± 0.072	0.821 ± 0.048	0.807 ± 0.066
Spleen (g)	0.62 ± 0.11N	0.70 ± 0.03	0.63 ± 0.06	0.42 ± 0.06N	0.40 ± 0.02	0.44 ± 0.10
Spleen (%)	0.164 ± 0.028	0.177 ± 0.012	0.164 ± 0.021	0.207 ± 0.016N	0.205 ± 0.012	0.212 ± 0.040
Adrenal glands (mg)	53 ± 3	59 ± 12	51 ± 9	52 ± 4	58 ± 9	61 ± 9
Adrenal glands (%)	0.014 ± 0.001N	0.015 ± 0.003	0.013 ± 0.003	0.026 ± 0.002	0.030 ± 0.004	0.029 ± 0.003
Testes (g)	3.18 ± 0.52N	3.37 ± 0.14	3.21 ± 0.10	NA	NA	NA
Testes (%)	0.856 ± 0.188N	0.854 ± 0.047	0.829 ± 0.068	NA	NA	NA
Ovaries (mg)	NA	NA	NA	75 ± 22	84 ± 12	86 ± 29
Ovaries (%)	NA	NA	NA	0.037 ± 0.009	0.043 ± 0.006	0.041 ± 0.012
Pituitary (mg)	11 ± 3	12 ± 2	11 ± 1	12 ± 3	12 ± 2	13 ± 2
Pituitary (%)	0.003 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Thymus (mg)	568 ± 120	556 ± 101	492 ± 135	449 ± 78	463 ± 85	453 ± 40
Thymus (%)	0.150 ± 0.024	0.141 ± 0.026	0.126 ± 0.031	0.223 ± 0.029	0.239 ± 0.046	0.218 ± 0.016
Uterus (mg)	NA	NA	NA	451 ± 116	414 ± 80	493 ± 147
Uterus (%)	NA	NA	NA	0.226 ± 0.063	0.214 ± 0.047	0.238 ± 0.077
Salivary glands (g)	0.64 ± 0.04	0.69 ± 0.08	0.63 ± 0.04	0.41 ± 0.02N	0.40 ± 0.02	0.43 ± 0.07
Salivary glands (%)	0.172 ± 0.012	0.174 ± 0.020	0.163 ± 0.014	0.204 ± 0.016	0.206 ± 0.011	0.206 ± 0.024

All values are shown as mean ± standard deviation. Significant difference from control; $*P \leq 0.05$ (Dunnett's multiple comparison test). N: Non parametric analysis. NA: Not applicable.

DISCUSSIONS

A common misconception about herbal usage is that, due to their natural origin, they are often considered to be safe and without the accompanying negative side-effects. In contrast to this belief, herbal products are not necessarily safe, and the safety aspect can also be influenced by the dosage. Furthermore, herbal products used in the form of supplements and other form of herbal preparations are often known to cause HILI (Stournaras and Tziomalos, 2015). The prevalence of HILI in on the rise as complementary medicines is gaining popularity, which is made worse due to the reason that herbal products are more than often used under self-medication and/or without a medical supervision. More than often the misconception that herbal products are generally safe as well as the general lack of safety studies are the main factor that led to rise in HILI prevalence (Schoepfer *et al.*, 2007; Stickel *et al.*, 2005). Laurel which is more generally used as a spice or flavoring in culinary than as dietary supplements (Bianchi, 2015), is not exempted from the misconception and its safety aspect is understudied. The quantity of laurel used in cooking and in dietary supplements can differ greatly, which means that its safety for culinary use cannot be assumed to be equivalent to its safety for use in supplements (Kara *et al.*, 2021). *In lieu* with the current gap in the knowledge of laurel safety, we seek to disclose the data on the mutagenicity, as well as acute and subacute toxicity of LAURESH®, which is a standardized extract of laurel leaves.

Comparison between the HPLC chromatogram of LAURESH® and deacetyl laurenobiolide indicated the presence of deacetyl laurenobiolide in LAURESH®, which is one of the bioactive components of LAURESH®

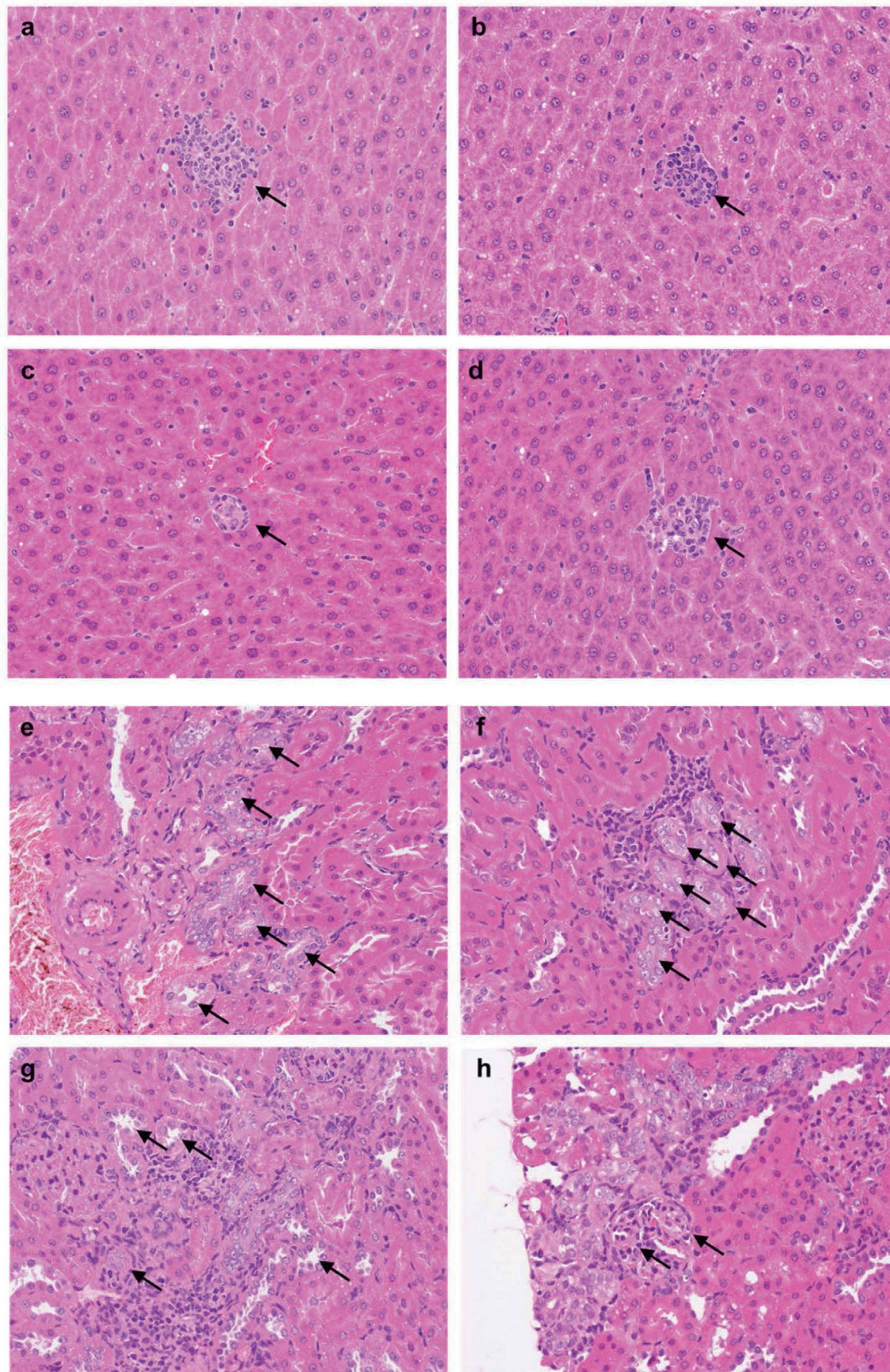

Fig. 2. Histopathological examination of livers and kidneys of both male and female rats (40x magnification). Control male rat (**a & e**), male rat given 1,000 mg/kg/day of LAURESH® (**b & f**), Control female rat (**c & g**), female rat given 1,000 mg/kg/day of LAURESH® (**d & g**). Small granuloma in the liver exemplified by black arrows (**a-d**). Tubular regeneration in kidney exemplified by black arrows (**e-g**). Pictures taken at 40x magnification.

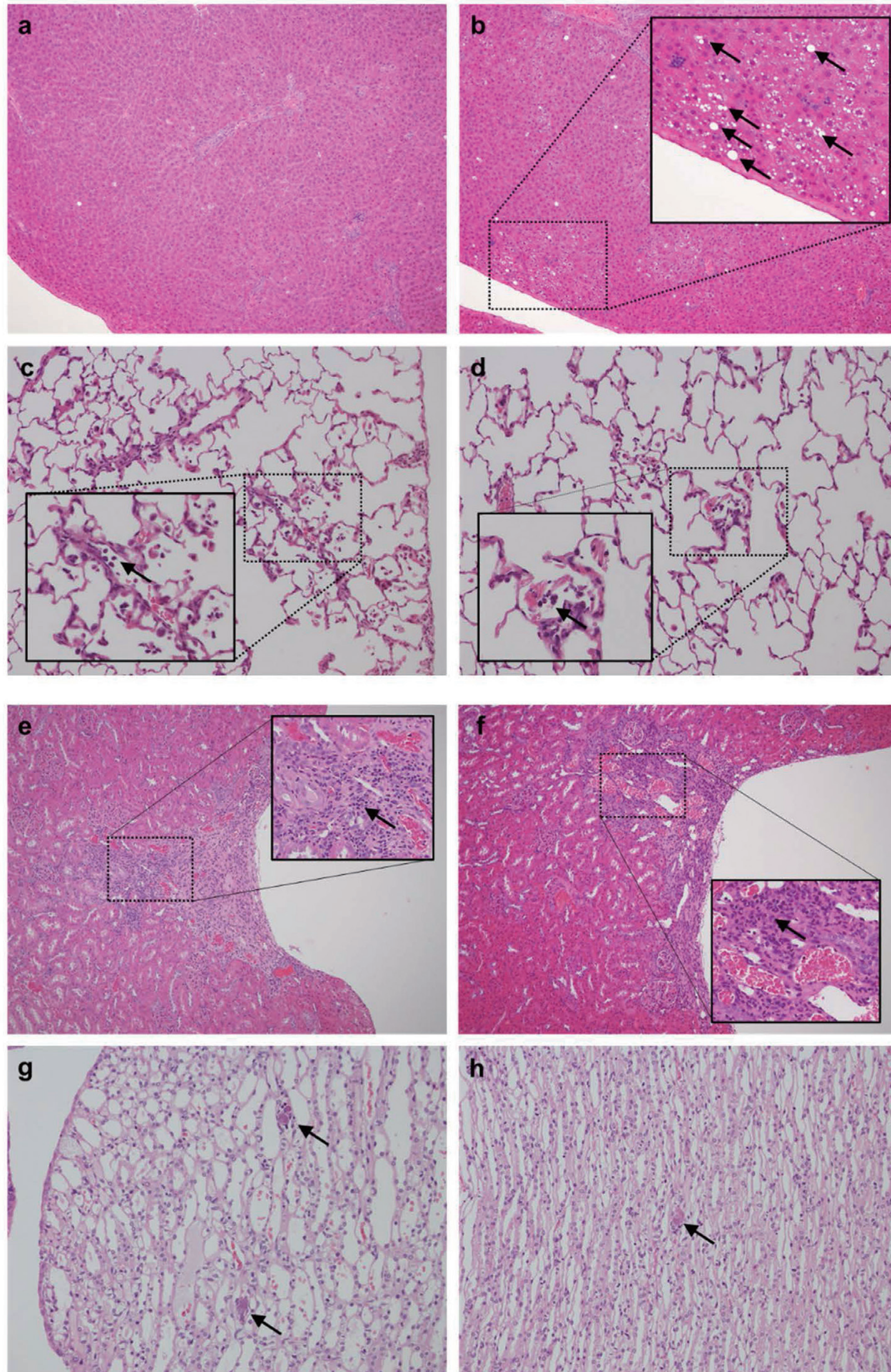

Fig. 3. Histopathological examination of livers (10x magnification) and lungs (20x magnification) of female rats (**a**-**d**). Control female rat (**a & c**), female rat given 1,000 mg/kg/day of LAURESH® (**b & d**). Fat droplets in the liver exemplified by black arrows (**b**) Immune cells infiltration in lungs exemplified by black arrows (**c**-**d**). Histopathological examination of kidneys of male rats (**e**-**h**). Immune cells infiltration in kidneys exemplified by black arrows (10x magnification) (**e**-**f**). Control male rat (**e & g**), male rat given 1,000 mg/kg/day of LAURESH® (**f & h**). Mineral deposits in the kidneys exemplified by black arrows (20x magnification) (**g**-**h**).

(Kuniyoshi *et al.*, 2021). HPLC analyses revealed that the LAURESH® used in the safety study described in this manuscript contained no less than 1.0% of deacetyl laurenobiolide, which is consistent with the amount of deacetyl laurenobiolide in standardized LAURESH®. The mutagenicity of LAURESH® was assessed using two *S. typhimurium* strain, TA100 and TA98. Results from Ames test indicated that LAURESH® does not cause base substitution and frameshift mutation. Based on the results, LAURESH® is suggested to be non-mutagenic. Not only that LAURESH® is non-mutagenic, a study conducted on laurel revealed that 3-kaempferyl p-coumarate isolated from laurel leaves showed anti-mutagenic activity against a known dietary carcinogen, 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Samejima *et al.*, 1998). However, in another study on anti-mutagenic activity, aqueous laurel extract was not effective against another carcinogen, *N*-methyl-*N*-nitrourea (Tatsuzaki *et al.*, 2014). On the other hand, the ability of LAURESH® in eliciting acute toxicity was evaluated using single dose acute oral toxicity study conducted on five male and five female mice allocated to each of the LAURESH® and control group. No adverse event was observed during the 14 days test period. Hence the LD_{50} of LAURESH® is no less than 2,000 mg/kg under the condition set in this study. In view that the test dosage of 2,000 mg/kg for this single dose acute oral toxicity study and a safety factor set at 100-fold, the equivalent dosage for a human with 60 kg body weight is at 1,200 mg. In another acute toxicity study conducted on female Wistar rats, the aqueous extract of *L. nobilis* leaves yielded similar LD_{50} at 2,000 mg/kg (Senou *et al.*, 2021). In the same study, no significant change to the serum creatinine, serum ALT transaminases, and hemoglobin was observed, as such the study concluded that *L. nobilis* leaves extract was not toxic to the liver and kidneys. On an additional note, the LD_{50} of aqueous extract of the powdered *L. nobilis* seeds introduced intraperitoneally to albino mice was determined to be at 13.66 g/kg (Afifi *et al.*, 1997). Collectively, the mutagenicity and acute toxicity test of LAURESH® resonate well with other studies conducted on *L. nobilis* leaves extract, thus further reinforcing the non-mutagenic and non-acute toxicity safety aspect of LAURESH®.

A 28-day repeated oral dose toxicity study was conducted on male and female at with 0, 600, and 1,000 mg/kg/day of LAURESH®. In the test, no mortality and abnormality in the general condition, body weight, food consumption, blood chemistry, urinary, necropsy, and histopathological examinations that could be attributed to LAURESH® in both male and female rats. Hematological examination revealed that female rat in 1,000 mg/kg/day of LAURESH® group showed high basophil ratio (Table 4). However, the change in actual number was minor, therefore it was considered to be non-toxic. In the same examination, prothrombin time was significantly lower in male rats in both 600 and 1,000 mg/kg/day of LAURESH® group than control group (Table 4). While, activated partial thromboplastin time was only significant to male rats in 1,000 mg/kg/day of LAURESH® group than control group (Table 4). These changes were deemed inconsequential due to the reason that the changes were in a lessening manner. On the other hand, pathological examination of female rat in 1,000 mg/kg/day of LAURESH® group showed significantly higher relative liver weight than control group (Table 7). However, there are no observed corresponding abnormalities observed from the liver necropsy. A single occurrence of steatosis in the liver was found in the liver of a female rat in 1,000 mg/kg/day of LAURESH® group (Fig. 2b). This observation is also considered inconsequential as it was not observed in other rats. Additionally, blood chemical examination also showed no changes to related liver functions. Taken together, the higher relative liver weight of female rats in 1,000 mg/kg/day of LAURESH® group were deemed to be inconsequential. As described, the 28-day repeated oral dose toxicity study revealed that LAURESH® is non-toxic to rats even at 1,000 mg/kg/day. Hence, the no-observed-adverse-effect-level (NOAEL) for LAURESH® is at 1,000 mg/kg/day. Considering that the NOAEL for LAURESH® is at 1,000 mg/kg/day and by applying the safety factor of 100-fold to NOAEL, the equivalent intake for a human with 60 kg body weight is at 600 mg.

Liver injury related to consumption of herbal products is referred to as HILI. The clinical symptoms of HILI are similar to those of drug-induced liver injury, and thus diagnose with the same methods (Amadi and Orisakwe, 2018). Thus, increase in liver parameters such as serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin concentration can be also used as an indication for HILI (Navarro and Senior, 2006). No changes were observed to the serum ALP, AST, ALT, and total bilirubin in rats administered with 600 and 1,000 mg/kg/day of LAURESH® during the test period (Table 5). Additionally, previous clinical study of LAURESH® conducted on 44 healthy Japanese adults showed that intake of 108 mg/day of LAURESH® did not change the serum ALP, AST, ALT, and total bilirubin level (Kuniyoshi *et al.*, 2021). Coupled together, these studies on LAURESH® strongly suggest that LAURESH® is unlikely to elicit HILI. As an additional note, clinical study by Kuniyoshi *et al.*, 2021,

also revealed that it is safe to take 108 mg/ day of LAURESH® for 4 weeks. The clinical study of LAURESH® revealed that eosinophilia, eosinophil count, and potassium were significantly higher, as well as a lower glucose in LAURESH® group. However, these fluctuations were within the normal range, thus it was considered as inconsequential from ingestion of LAURESH®.

In summary, Ames test revealed that LAURESH® is non-mutagenic, while *in vivo* studies established its LD_{50} at no less than 2,000 mg/kg and NOAEL to be 1,000 mg/kg/day. Taken together, this study suggested that LAURESH® is non-toxic. Proper scientific studies on the toxicity and safe dosage of herbal products are crucial to reduce the potential health risk for human consumption. The findings from this study and previous clinical study on LAURESH® (Kuniyoshi *et al.*, 2021), in combination with the historic use laurel and previous toxicity studies conducted on laurel leaves extract further reinforce that LAURESH® poses no health risk for human consumption.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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