Supplementation of *Laurus nobilis* Attenuate Ethanol-induced Psychomotor Alterations in Rats

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Abstract – *Laurus nobilis* (*L. nobilis*) is traditionally used as an herbal medicine to treat various diseases. Ethanol (EtOH) consumption entails physiological, mental and psychomotor alterations. The aim of the present study was to assess the effects of *L. nobilis* in attenuating the EtOH-induced psychomotor alterations. *L. nobilis* was administered to SD rats, 30 minutes before EtOH administration (4 g/kg), at doses of 25, 50 and 100 mg/kg. Evaluations of psychomotor activity in the open-field, accelerating rota-rod, wire, and swimming ability were done at 1, 2, 4 and 8 hours after EtOH administration. In addition, blood ethanol and acetaldehyde levels were also measured. Pre-treatment of *L. nobilis* significantly improved EtOH-induced psychomotor alterations and decreased blood ethanol and acetaldehyde levels. These findings suggest that *L. nobilis* might be an effective substance to attenuate the harmful effects of EtOH, particularly psychomotor alterations, and can potentially be considered as a functional food.
Keywords – *Laurus nobilis*, Ethanol, Psychomotor alterations, Behavioral alterations, Acetaldehyde

Introduction

The consumption of ethanol (EtOH)-containing beverages has traditionally been accepted as part of individual and social practices across all cultures worldwide.[1] EtOH is primarily taken because it induces a sense of well-being (euphoric effects) and relaxation (anxiolytic effects).[1,2] It is considered to be one of the most used and abused substances worldwide.[3] The high prevalence of EtOH consumption is thought to be influenced by media, advertisements, friends or the family.[4]

Despite its euphoric effects, consumption of EtOH causes hazardous effects that may seriously affect a person's health and welfare arising to social and economic problems. EtOH use entails unfavorable consequences such as physical, mental and psychomotor alterations.[5,6] Of particular interest is its ability to induce psychomotor alterations which are immediately manifested 1 to 4 hours after EtOH ingestion.[7,8] These behavioral alterations predispose one to serious or even fatal injuries. Thus,

there is a rising need for an immediate intervention. In addition, due to the social practices, norms, customs and traditions that are associated with alcohol consumption, it is difficult for a person to say "no" when offered alcoholic drinks during social or family gatherings. Hence, a substance that can reduce or counter EtOH-induce psychomotor alterations is needed. However, at present, there is no effective treatment to counteract these EtOH-induced psychomotor alterations. Herbal medicines are favored for its preventive and/or therapeutic efficacies with fewer side effects.[9,10] Experimental application of herbal medicines has also been practiced to reduce hangover symptoms and cure alcohol dependence.[11]

Laurus Nobilis (*L. nobilis*), commonly known as bay leaf, is an evergreen tree belonging to the Lauraceae family and is widely cultivated in many countries with moderate and subtropical climate.[12] *L. nobilis* is traditionally used as an herbal medicine because of its antibacterial, anti-fungal, and antioxidant effects.[13-16] Studies showed that *L. nobilis* (especially sesquiterpene lactones) inhibits NO production (anti-inflammatory), and enhances liver gluthatione S-transferase (GST) activity.[17-19] Furthermore, Matsuda *et al*. demonstrated that costunolide, a sesquiterpene lactone isolated from the extract of *L. nobilis*,

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inhibited gastric emptying which contributed to the inhibition of blood-ethanol elevation in rats.[17] Therefore, we hypothesized that *L. nobilis* might also have beneficial effects on EtOH-induced psychomotor alterations. This was based on the findings that behavioral alterations are directly related to the rise in ethanol and acetaldehyde levels in the blood.[8,20-22]

Recently, hangover pills or over-the-counter (OTC) drugs were used to cure or prevent EtOH-induced psychomotor alterations however, very little scientific evidences support the effectiveness of these complementary treatments.[23] Thus, the present study aims to assess the effect of *L. nobilis* in attenuating EtOH-induced effects, particularly on its associated psychomotor alterations. Various behavioral tests were employed (open-field test, accelerating rota-rod test, hanging wire test, and cold swimming test) to evaluate the effects of *L. nobilis* on EtOH-induced psychomotor alterations. Moreover, blood ethanol and acetaldehyde concentrations were also measured.

Experimental

Subjects – Sprague Dawley male rats, 8 weeks old, were obtained from Charles River, Japan via Hanlim Laboratory Animals Co. (Hwasung, Korea). They were housed in groups in a temperature-controlled (22 ± 2 ℃) and humidity-controlled ($55 \pm 5\%$) animal room on a 12/12 h light/dark (07:00-19:00 h light) schedule. Food and water were provided ad libitum, except the night before and during behavioral and hematological testing. Rats were allowed to acclimatize to the laboratory setting, for at least 7 days, before the commencement of any experiments. Six to eight animals were used in each experimental group. Animal treatment and maintenance were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23 revised 1985) and the Animal Care and Use Guidelines of Sahmyook University, Korea.

Test materials – Ethanol was purchased from Duksan Pure Chemicals (Korea). It was diluted with distilled water to a concentration of 25% (w/v) then administered orally. *Laurus nobilis* (*L. nobilis*) was kindly supplied by CHEILDEDANG Co. (Seoul, Korea). Leaves of *L. nobilis* were grounded to obtain a concentrated water extract, and then spray dried. The dried powder extract of *L. nobilis* was dissolved in distilled water to make 25, 50 and 100 mg/mL immediately before oral administration.

Experimental designs – Animals were divided into 5 different groups (control, EtOH, *L. nobilis* 25, 50 and 100

mg/kg) of 6-8 rats. Rats were pre-treated with *L. nobilis* (25, 50 and 100 mg/kg/mL, p.o.) or vehicle (distilled water) 30 minutes before 4 g/kg EtOH (25% w/v) oral administration. Pre-experimental data showed that the dose of 4 g/kg of EtOH made the most relevant level of alterations in behavioral functions (data not shown). Control group was given with the vehicle only. All the tests were done at four different time points (1, 2, 4 and 8 hours) after EtOH administration.

Behavioral test

Open field test – Animals were placed in an open-field arena, consisting of a square Plexiglass container (42×42 cm) with a field bordered of 42 cm high sidewalls. Open-field test was used to evaluate locomotor activity. Prior to the evaluation, animals were habituated to the box for 2 minutes to remove the bias of novelty. The observed parameters were the distance travelled and the duration of movement which were recorded for 5 minutes.[24,25] Ethovision (Noldus, Netherlands) system was used to record animal movement and behavior.

Accelerating rota-rod test – Motor control, balance and coordination were tested using accelerating rota-rod. [26,27] Rats were trained 4 times to run on the rota-rod at an accelerating speed from 10 to 30 rpm, two times a day for two days before the actual experiments. This test was performed as described by Ramezani *et al.* with slight modification.[25] The last training results were recorded and served as the baseline data. Rats were reloaded on the rod when they fell from it. Latency time and falling frequency were recorded for 5 minutes. If the rats remained on the rod until the time they were removed, latency time would be 300 seconds (s) with 0 falling frequency.

Hanging wire test – The hanging wire test was used to assess limb strength and balance.[28] All the rats were trained 2 times before the actual experiments. Baseline data were measured on the test day before *L. nobilis* or vehicle administration. Rats were lifted by the tail, allowed to grasp a horizontally fixed wire (148 cm length × 55 cm height × 0.5 cm diameter) with their forepaws and released. Rats were reloaded on the wire when they fell from it. Latency time and falling frequency were recorded for 2 minutes. If the rats remained on the wire until the time they were removed, latency time would be 120 s with 0 falling frequency.

Cold swimming test – Swimming ability was tested in an open tank filled with water (8 ± 2 ℃) for 10 minutes. Experimental procedures were performed in a stable environment with distraction kept in minimum. After experiments, rats were removed from the water, dried with towels and placed under a warmer until they got dry

then returned back to their respective cages. Water temperature was monitored before every experiment. After close observation of rats' drowning behavior, swimming latency time was recorded.

Hematological (blood) test

Blood ethanol and acetaldehyde level were assessed with headspace coupled with gas chromatography/mass spectrometry (HS-GC/MS).

Reagents – Standard materials of acetaldehyde and ethanol were obtained from Sigma (St. Louis, MO). Methanol and acetonitrile were purchased from Merck (Darmstadt, Germany); citric acid, sodium citrate, d-glucose, sodium chloride, and sodium fluoride were obtained from Sigma (St. Louis, MO), all general reagent grades.

Headspace (HS) procedure and gas chromato-graphy/mass spectrophotometry (GC/MS) conditions – For the HS procedure, whole blood samples were obtained from male SD rats (8 weeks) by heart puncture and stored at –4 °C. 1,000 µL of samples, vehicle or standard materials were placed in each sterile headspace vial. 0.5 g NaCl and 0.1 g NaF were added to each vial. A 500 µL dextrose solution was also placed in each vial. The vials were sealed with rubber caps, mixed, and stabilized to permit the HS to reach equilibrium. The HS trap conditions were as follows: needle temperature, 100 °C; transfer-line temperature, 110 °C; oven temperature, 70 °C; shaker, on; high pressure injection, off; thermostating time, 10 minutes; pressurization time, 1 minute; withdrawal time, 0.2 minute; injection pressure, 25 psi; column pressure, 25 psi; injection time, 0.03 minute; GC cycle time, 13.5 minutes; injection volume, 1 µL. The carrier gas was Helium (He).

Gas chromatography/mass spectrophotometry (GC/MS) analysis was carried out with a GC/MS (model Clarus 600T, PerkinElmer) equipped with a DB-5MS capillary

column (60 m × 0.25 mm × 250 µm). The GC conditions were as follows: injector temperature, 110 °C; injection volume, 1 µL. Helium (He), Hydrogen (H_2) and air were used as the carrier gas at flow rate of 10 mL/min. The injection mode was split with a flow rate of 40 mL/min after 0.5 minutes.

Statistical analysis – All data were expressed as the mean ± S.E.M. (standard error of mean) and analyzed using one-tailed unpaired t-test. All test values of $p < 0.05$ were considered statistically significant. All statistical analyses were conducted using GraphPad Prism Version 5.02 software (California, USA).

Results

Effect of *L. nobilis* on Open-field test – Exploratory activity was determined based on total distance moved (cm) and total movement duration (sec). Fig. 1. shows that EtOH-treated group significantly decreased exploratory activity (1 h to 8 h, $p < 0.001$) compared to control group. Pre-treatment of *L. nobilis* significantly increased exploratory activities on distance moved [25 mg/kg (4 h and 8 h, p < 0.05), 50 mg/kg (2 h, p < 0.05; 4 h, p < 0.01; 8 h, p < 0.001), 100 mg/kg (1 h and 2 h, p < 0.05; 4 h, p < 0.01; 8 h, p < 0.001); Fig. 1A] and movement duration [25 mg/kg (1 h, 2 h and 4 h, p < 0.05; 8 h, p < 0.01), 50 mg/kg (1 h and 2 h, p < 0.05; 4 h, p < 0.01; 8 h, p < 0.001), 100 mg/kg (1 h and 2 h, p < 0.01; 4 h and 8 h, p < 0.001); Fig. 1B] in a dose-dependent manner, as compared to the EtOH group.

Effect of *L. nobilis* on accelerating rota-rod test – The control group of rats that received vehicle showed similar latency time and falling frequency in rota-rod test at all-time points (baseline to 8 hours; Fig. 2). Rats administered with EtOH (EtOH group) showed a
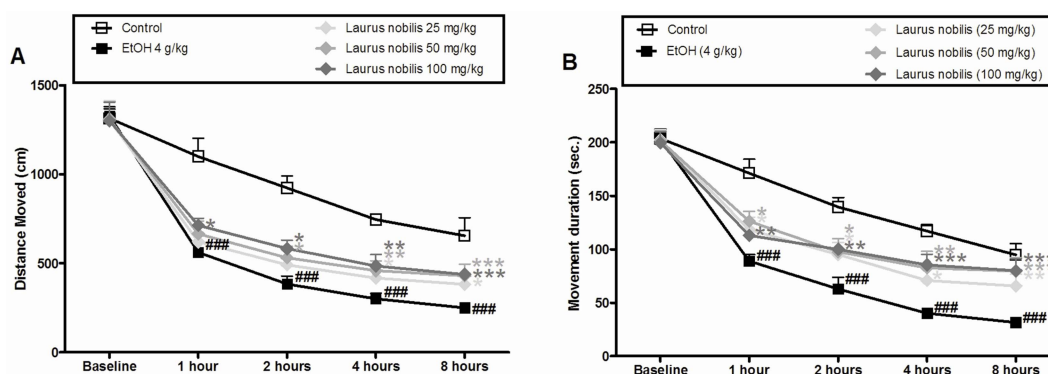

Fig. 1. The effect of *Laurus nobilis* (*L. nobilis*) on Open-field test. *L. nobilis* was orally pre-treated 30 minutes before EtOH administration on open-field test. Each point represents Mean ± SEM for $n = 8$. ### $p < 0.001$ vs. control group and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. ethanol-treated group by unpaired t test.
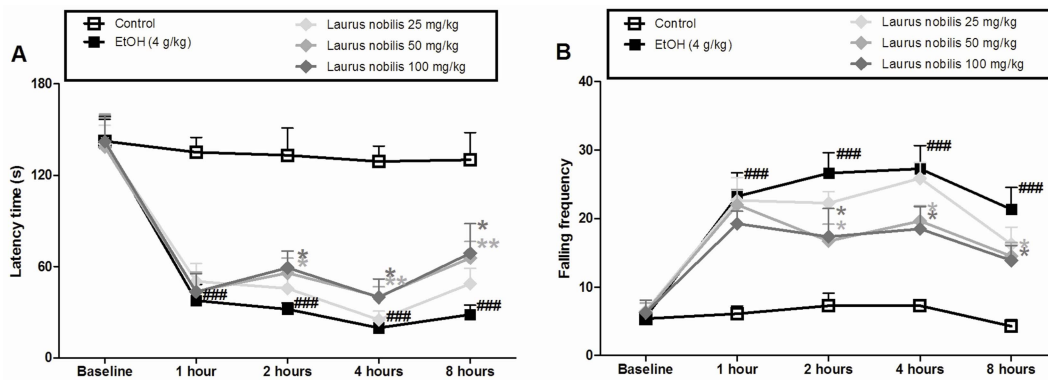
Fig. 2. The effect of *Laurus nobilis* (*L. nobilis*) on Accelerating Rota-rod test. *L. nobilis* was orally pre-treated 30 minutes before EtOH administration on accelerating rota-rod test. Each point represents Mean ± SEM for $n = 8$. ### $p < 0.001$ vs. control group and *$p < 0.05$ and **$p < 0.01$ vs. ethanol-treated group by unpaired t test.
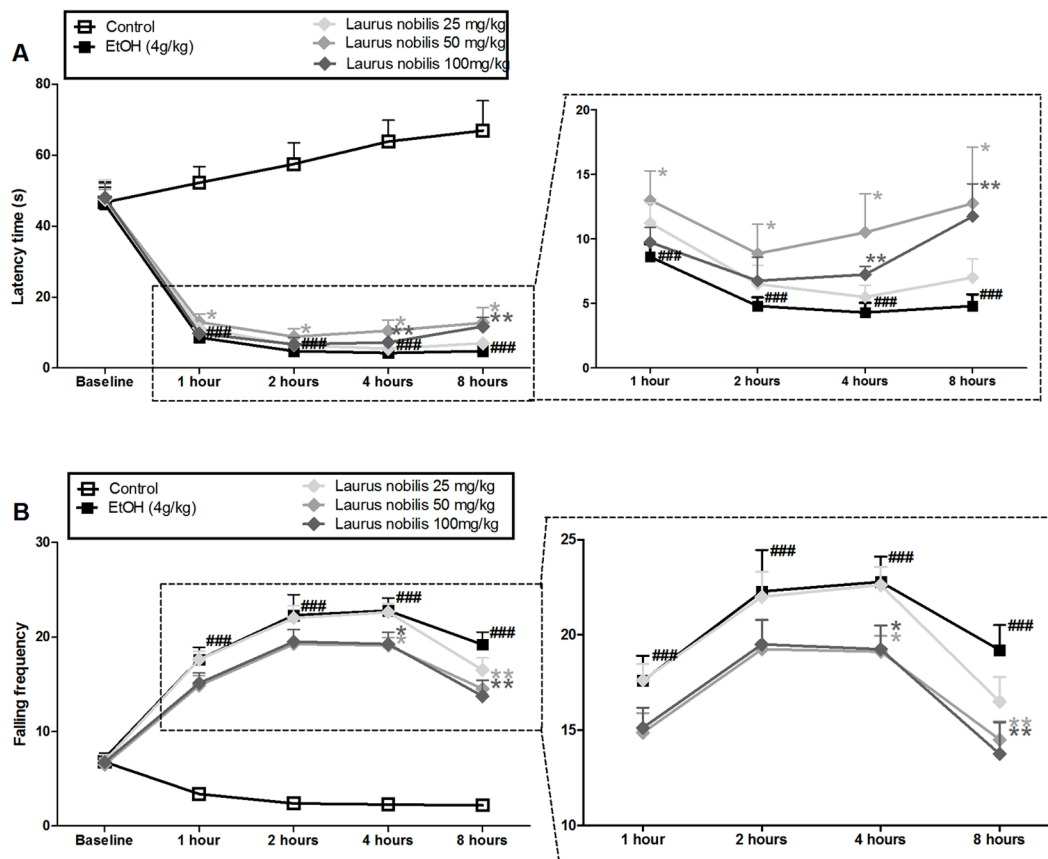

Fig. 3. The effect of *Laurus nobilis* (*L. nobilis*) on Hanging Wire test. *L. nobilis* was orally pre-treated 30 minutes before EtOH administration on hanging wire test. Each point represents Mean ± SEM for $n = 8$. ### $p < 0.001$ vs. control group and *$p < 0.05$ and **$p < 0.01$ vs. ethanol-treated group by unpaired t test.

significant decrease (1 h to 8 h, $p < 0.001$; Fig. 2A) in latency time and a significant increase in falling frequency (1 h to 8 h, $p < 0.001$; Fig. 2B) compared to control group. Impaired rota-rod performance was altered in *L. nobilis*-treated group that shows a significant increase in latency time [50 mg/kg (2 h, $p < 0.05$; 4 h and 8 h, $p <$

0.01), 100 mg/kg (2 h, 4 h, and 8 h, $p < 0.05$; Fig. 2A] and a significant decrease in falling frequency [50 mg/kg (2 h, 4 h, and 8 h, $p < 0.05$), 100 mg/kg (2 h, 4 h, and 8 h, $p < 0.05$; Fig. 2B] compared to EtOH group.

Effect of *L. nobilis* on hanging wire test – Muscle strength was evaluated on hanging wire. Fig. 3. shows

Table 1. The effect of *Laurus nobilis* (*L. nobilis*) on Blood ethanol concentration

Group/Time	EtOH	*Laurus nobilis*		
Dose (mg/kg)	4000	25	50	100
1 hour	2.91 ± 0.28	2.58 ± 0.17	$2.33 \pm 0.07*$	$1.75 \pm 0.25**$
2 hours	2.79 ± 0.28	2.42 ± 0.19	$1.91 \pm 0.09**$	$1.74 \pm 0.23**$
4 hours	2.56 ± 0.18	2.22 ± 0.14	$1.51 \pm 0.32**$	$1.52 \pm 0.23**$
8 hours	2.28 ± 0.27	2.10 ± 0.16	$1.05 \pm 0.22**$	$1.20 \pm 0.19**$

L. nobilis was orally pre-treated 30 minutes before EtOH administration on blood ethanol analysis. Each point represents Mean ± SEM for $n = 7$. *$p < 0.05$ and ** $p < 0.01$ vs. ethanol-treated group by unpaired *t* test.

Table 2. The effect of *Laurus nobilis* (*L. nobilis*) on Blood acetaldehyde concentration

Group/Time	EtOH	*Laurus nobilis*		
Dose (mg/kg)	4000	25	50	100
1 hour	0.55 ± 0.21	$0.50 \pm 0.02*$	$0.48 \pm 0.03*$	$0.50 \pm 0.01*$
2 hours	0.49 ± 0.03	0.49 ± 0.03	0.43 ± 0.02	0.44 ± 0.01
4 hours	0.42 ± 0.03	0.39 ± 0.03	0.36 ± 0.07	0.37 ± 0.02
8 hours	0.33 ± 0.01	$0.27 \pm 0.03*$	$0.25 \pm 0.03**$	$0.27 \pm 0.02**$

L. nobilis was orally pre-treated 30 minutes before EtOH administration on blood acetaldehyde analysis. Each point represents Mean ± SEM for $n=7$. *$p < 0.05$ and ** $p < 0.01$ vs. ethanol-treated group by unpaired *t* test.
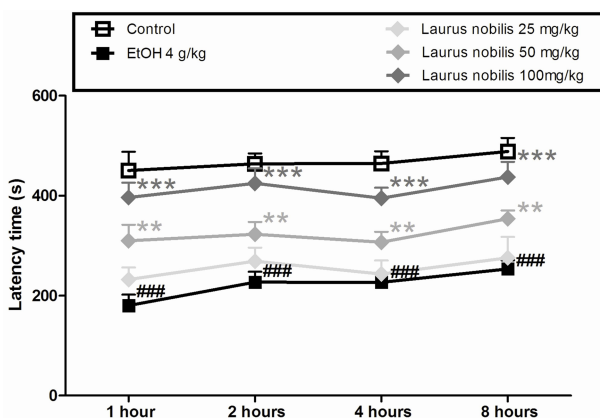

Fig. 4. The effect of *Laurus nobilis* (*L. nobilis*) on Cold Swimming test. *L. nobilis* was orally pre-treated 30 minutes before EtOH administration on cold swimming test. Each point represents Mean ± SEM for $n = 8$. ### $p < 0.001$ vs. control group and **$p < 0.01$ and ***$p < 0.001$ vs. ethanol-treated group by unpaired *t* test.

that EtOH group significantly decreased in latency time and increased in falling frequency (1 h to 8 h, $p < 0.001$) compared to control group. Pre-treatment of *L. nobilis* significantly increased in latency time [50 mg/kg (1 h to 8 h, $p < 0.05$), 100 mg/kg (4 h and 8 h, $p < 0.01$); Fig. 3] and significantly decreased in number of falls [50 mg/kg (4 h, $p < 0.05$; 8 h, $p < 0.01$), 100 mg/kg (4 h, $p < 0.05$; 8 h, $p < 0.001$); Fig. 3] compared to EtOH group.

Effect of *L. nobilis* on cold swimming test – EtOH consumption increases the risk of hypothermia because of its action as a vasodilator.[26,29,30] Fig. 4. shows that EtOH

administration decreased swimming time of rats at all-time points (1 h to 8 h, $p < 0.001$) compared to control group. However, with pre-treatment of *L. nobilis*, swimming latency time significantly increased in a dose-dependent manner compared to EtOH group as an indicative of rat's endurance and tolerance [50 mg/kg (1 h to 8 h, $p < 0.01$), 100 mg/kg (1 h to 8 h, $p < 0.001$); Fig. 4].

Effect of *L. nobilis* on blood ethanol and acetaldehyde concentrations – Table 1 and Table 2 show the effects of *L. nobilis* on blood ethanol and acetaldehyde levels after EtOH administration. *L. nobilis* significantly inhibited elevation of blood ethanol [50 mg/kg (1 h, $p < 0.05$; 2 h to 8 h, $p < 0.01$), 100 mg/kg (1 h to 8 h, $p < 0.01$); Table 1]. As ethanol level decreased, elevation of blood acetaldehyde level was also inhibited by *L. nobilis* pre-treatment [25 mg/kg (1 h and 8 h, $p < 0.05$;), 50 mg/kg (1 h, $p < 0.05$; 8 h, $p < 0.01$), 100 mg/kg (1 h, $p < 0.05$; 8 h, $p < 0.01$); Table 2]. These results show that animals treated with *L. nobilis* before the oral administration of EtOH presented a lower blood ethanol and acetaldehyde level than the ones treated with EtOH only.

Discussion

Consumption of EtOH produces psychomotor alterations which predispose one to serious or even fatal injuries.[6] Despite these unfavorable/ harmful effects, EtOH consumption still seems to be inevitable because it has become an essential part of human practices, culture and

socialization.[1,31] Thus, a substance which can counter these detrimental side-effects would be greatly beneficial. Psychomotor alterations induced by EtOH are primarily because of the accumulation of EtOH in the blood.[5,8] Upon ingestion, EtOH is easily absorbed in the mucosal lining of the gastrointestinal tract and diffuses into the systemic circulation. In addition, during the process of EtOH metabolism, acetaldehyde as a toxic metabolite is produced, which can further contribute to these psychomotor alterations.[32] The dose of EtOH used in the present study, was sufficient to induce psychomotor alterations which are generally manifested. Indeed, as compared to the control group, the EtOH group demonstrated significant alterations in exploratory activity (open-field test), motor coordination (accelerating rota-rod test), muscle strength (hanging wire test), and swimming ability (cold-swimming test).

Laurus nobilis (*L. nobilis*), one of the most widely used culinary spices in most Western countries, has gained an attention on account of its numerous beneficial effects and is known to potently inhibit ethanol absorption in ethanol-loaded rats. In the present study, *L. nobilis* counteracted EtOH-induced alterations in exploratory activity (Fig. 1), motor coordination (Fig. 2), muscle strength (Fig. 3), and swimming ability (Fig. 4). Decrease in blood ethanol (Table 1) and acetaldehyde (Table 2) levels were also observed in the *L. nobilis*-treated groups. Based on these findings, we can infer that *L. nobilis* relieves EtOH-induced psychomotor alterations in relation to the decrease in blood ethanol and acetaldehyde levels.

Supporting the present findings is a study by Matsuda *et al.*,[17] reporting that *L. nobilis* can inhibit blood ethanol elevation. They reported that costunolide, a sesquiterpene lactone isolated from *L. nobilis*, showed potent inhibition of ethanol absorption. Ethanol blood levels remained low due to *L. nobilis*' (costunolide's) inhibition of gastric emptying and increase in gastric fluid secretion, diluting ethanol's concentration.[17,18] Thus, it is likely that the observed beneficial effects of *L. nobilis* in EtOH-induced psychomotor alterations are mediated by costunolide. Costunolide might have inhibited ethanol absorption, resulting to a reduced blood ethanol levels and consequently the amelioration of EtOH-induced psychomotor alterations.

Another possibility is that, the beneficial effects of *L. nobilis* against ethanol-induced psychomotor alterations might be due to its antioxidant properties, which were reported by previous studies.[13,14] Reactive oxidative species (ROS) are produced during EtOH metabolization which interferes with the body's normal defense mechanism, causes oxidative stress and can lead to cell injury or death. Moreover EtOH consumption is said to influence free radical formation, wherein this free radicals are thought to cause behavioral alterations.[33,34] Previous studies have reported that damages induced by EtOH can be prevented with antioxidant therapy, on or before alcohol consumption.[35] Thus, antioxidants are vital in preventing damages induced by EtOH, and adequate antioxidant supplementation is necessary to maintain or enhance physical performance.[36] Taken together, *L. nobilis* may have ameliorated EtOH-induced psychomotor alterations through its antioxidant effects.

In conclusion, the present study showed that pre-treatment of the extract of *Laurus nobilis* attenuated EtOH-induced psychomotor alterations in rats. Moreover, blood ethanol and acetaldehyde level elevations were inhibited in the *L. nobilis* -treated groups. For this reason, this study demonstrated that *L. nobilis* could attenuate EtOH-induced behavioral alterations and can potentially be considered as a functional supplemental food for alleviating EtOH-induced behavioral disturbances.

Acknowledgements

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