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Research Article

INHIBITORY EFFECT OF HESPERIDIN ON VOLUNTARY ETHANOL DRINKING AND PREFERENCE IN SWISS ALBINO MICE

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ABSTRACT

Objectives: This investigation is expected to evaluate the potential of hesperidin in inhibiting ethanol (EtOH)-induced behavioral sensitization and its effect on voluntary EtOH consumption and preference in Swiss albino mice.

Methods: Behavioral sensitization was induced using EtOH (2 g/kg), and the voluntary EtOH drinking preference test was employed to assess the effects of hesperidin. Mice were treated with hesperidin at doses of 4, 8, and 16 mg/kg (oral), and changes in EtOH intake and preference were recorded.

Results: Hesperidin significantly reduced EtOH consumption in rats at doses of 8 mg/kg and 16 mg/kg ($p<0.001$), with effects observed from days 31 to 34. Acute dosing at 4 mg/kg also decreased EtOH intake ($p<0.001$). Ondansetron showed similar efficacy ($p<0.001$). No significant group differences were found during the restricted access phase. Furthermore, no significant changes in water intake were observed during the restriction (days 19–28) or treatment phases (days 29–34; $p>0.05$), with hesperidin (4–16 mg/kg) and ondansetron (4 mg/kg) showing no effect (two-way repeated measures analysis of variance).

Conclusion: Hesperidin effectively attenuates EtOH -induced behavioral sensitization as well as reduces the rewarding effects of EtOH, suggesting its potential as a natural therapeutic agent for managing alcohol-related illnesses.

Keywords: Behavioral sensitization, Ethanol drinking preference, Hesperidin, Ethanol addiction.

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INTRODUCTION

Alcoholism, or alcohol use disorder (AUD), is a chronic condition marked by compulsive drinking, loss of control over alcohol intake, and withdrawal-related emotional distress [1]. The condition poses a substantial public health challenge, contributing significantly to the global disease burden. As stated by the World Health Organization (2018), alcohol misuse accounts for approximately 5.9% of all global deaths, surpassing the mortality burden caused by illicit drug use. Long-term alcohol consumption is implicated in a spectrum of neuropsychiatric, hepatic, gastrointestinal, cardiovascular, and oncological complications [2]. In addition, it aggravates socioeconomic concerns, including accidents, domestic violence, reduced workplace productivity, and increased healthcare costs [3].

Alcohol addiction is underpinned by a complex neurobiological framework, involving alterations in multiple neurotransmitter systems. Repeated exposure to ethanol (EtOH) induces behavioral sensitization, a phenomenon that reflects enhanced locomotor and motivational responses to subsequent drug exposures. This process is mediated by interactions between various neurotransmitters and neuromodulators, notably dopamine, glutamate, GABA, serotonin, nitric oxide, and several neuropeptides such as corticotropin-releasing hormone and gonadotropin-releasing hormone [4,5]. Dysregulation of peroxisome proliferator-activated receptors (PPARs) and nicotinic acetylcholine receptors has also been implicated in the reinforcement and relapse associated with EtOH consumption [6].

Despite growing insights into the pathophysiology of alcohol dependence, the development of effective pharmacotherapies remains limited. The current treatment strategies, including disulfiram, naltrexone, and acamprosate, exhibit variable efficacy and are often associated with poor compliance and high relapse rates [7]. One of the major barriers to advancing therapeutic options is the limited commercial interest and perceived low market profitability of anti-addiction drugs [8]. This underscores the need to explore novel, safe, and affordable natural compounds that can modulate the neurochemical pathways involved in alcohol addiction.

Hesperidin, a bioflavonoid glycoside abundant in *Citrus* spp. such as *Citrus aurantium*, *Citrus sinensis*, and *Agathosma serratifolia*, has attracted significant pharmacological interest. It exhibits a broad spectrum of biological activities including neuroprotective, anxiolytic, antidepressant, antiepileptic, anti-Alzheimer, hepatoprotective, cardioprotective, anti-inflammatory, and anticancer effects [9,10]. The neurobehavioral effects of hesperidin have been linked to its modulation of monoaminergic pathways, oxidative stress, and neuroinflammation [11].

Given its multimodal mechanisms of action, hesperidin may represent a promising candidate for attenuating the reinforcing properties of EtOH. In particular, its anxiolytic and neuroprotective effects may counteract the negative affective states and neural adaptations that drive compulsive alcohol use. Therefore, the current investigations aim in the direction of investigate the inhibitory outcome of hesperidin on voluntary EtOH consumption and preference using Swiss albino mice as an experimental model.

MATERIALS AND METHODS

Materials

Dried peels of *C. sinensis* were purchased from a local Ayurvedic pharmacy store. EtOH (99% v/v) was purchased from Merck Ltd., India.

Ondansetron injection (Emeset; Cipla Ltd.) was purchased from a local pharmacy store. Saccharin was purchased from Sciore Laboratories Ltd., Pune, India.

Drug solutions and administration
Hesperidin was isolated from dried peels of *C. sinensis* and ondansetron injection was utilized in the investigation. All chemicals and drugs were dissolved in 0.9% saline. EtOH was diluted to prepare solutions of 3%, 6%, and 10% v/v, each supplemented with 0.2% w/v saccharin to enhance palatability. Fresh drug solutions were prepared daily before administration.

Animals
Male Swiss Albino mice (Mus musculus, n=6–10), aged 3–4 weeks and weighing 25–35±2 g, were utilized. They were housed under standard conditions at Sciore Pvt. Ltd., with a 7-day acclimation period. Experiments were approved by the IAEC (Approval No. SC/IAEC/2122/027) and conducted per CPCSEA guidelines. Separate groups were used for each experimental set [12]. Only healthy animals that completed a 7-day acclimatization period under standard laboratory conditions were enrolled.

Methods
EtOH drinking preference test
The EtOH preference study employed the well-established two-bottle choice paradigm with slight modifications. Mice (16–20 g) were used. Animals were group-housed for 1 week before the experiment and then randomly assigned into groups (n=8). To prevent food-related bottle preference, food pellets were distributed near both bottles [13-16]. During the EtOH preference test, mice were required to exhibit consistent fluid intake and tolerance to increasing EtOH concentrations during the habituation phase (days 1–18) to be included in subsequent phases. Animals were randomly assigned to groups (n=8) following habituation.

Habituation phase (day 1–18)
During this phase, mice were gradually habituated to increasing concentrations of EtOH. One bottle contained tap water with 0.2% w/v saccharin, while the further contained EtOH (3%, 6%, 10% v/v) in 0.2% w/v saccharin solution. Each concentration was presented for 6 consecutive days. Bottles were interchanged daily to eliminate positional bias, and fluid intake was measured every 24 h. Body weights were recorded every 4 days. Leakage and evaporation losses were accounted for using identical bottles placed in empty cages [14]. Mice showing signs of illness, injury, or abnormal behavior during the acclimatization or habituation phases were excluded. In addition, animals that failed to consume adequate volumes of fluid or did not tolerate EtOH solutions were excluded from the treatment phase.

Restriction phase (day 19–28)
Following habituation, EtOH access was restricted to 12 h during the dark cycle (19:00–07:00 h). During this period, rats received 10% EtOH with 0.2% saccharin or 0.2% saccharin alone. Water was removed 1 h before EtOH access, and saline (10 mL/kg, i.p.) was given 1 h prior [15].

Treatment phase (day 29–34)
During the treatment phase, animals received saline (10 mL/kg) or hesperidin (4, 8, or 16 mg/kg, i.p.) for 6 days. EtOH preference was evaluated by offering 10% EtOH+0.2% saccharin versus 0.2% saccharin alone for 12 h during the dark cycle. Intake was measured, and EtOH consumption (g/kg) was calculated. Final data were collected on day 34, followed by sacrifice and gross necropsy [13,16].

Data examination
Data are represented as mean±standard error of the mean. Statistical examination was done utilizing two-way analysis of variance (ANOVA) monitored by Tukey's *post hoc* test. $p<0.05$ was deliberated significant [16].

RESULTS

Effect of hesperidin on voluntary EtOH intake
During the restricted access phase (Day 19–28), a gradual increase in EtOH consumption was observed. However, two-way ANOVA analysis publicized no statistically significant modifications in EtOH intake among the groups during this period (Fig. 1). In contrast, during the treatment phase, hesperidin at a dosage of 16 mg/kg (orally) produced a marked reduction in EtOH consumption from day 30 to 34, which was highly significant ($p<0.001$) (Fig. 2). In addition, hesperidin at 8 mg/kg (orally) significantly lowered voluntary EtOH intake between day 31 and 34 ($p<0.001$). Ondansetron treatment also led in the direction of a significant diminution in EtOH preference from day 32 to 34 ($p<0.001$). Furthermore, administration of hesperidin at 4 mg/kg (orally), 30 min before the EtOH access test, significantly reduced voluntary EtOH intake compared to the EtOH control group, particularly from day 32 to 34 ($p<0.001$).

Effect of hesperidin on water intake
No significant changes in water intake were observed during the restriction phase (days 19–28, Fig. 3). Hesperidin (4, 8, 16 mg/kg, oral) and ondansetron (4 mg/kg, oral) also had no effect on voluntary

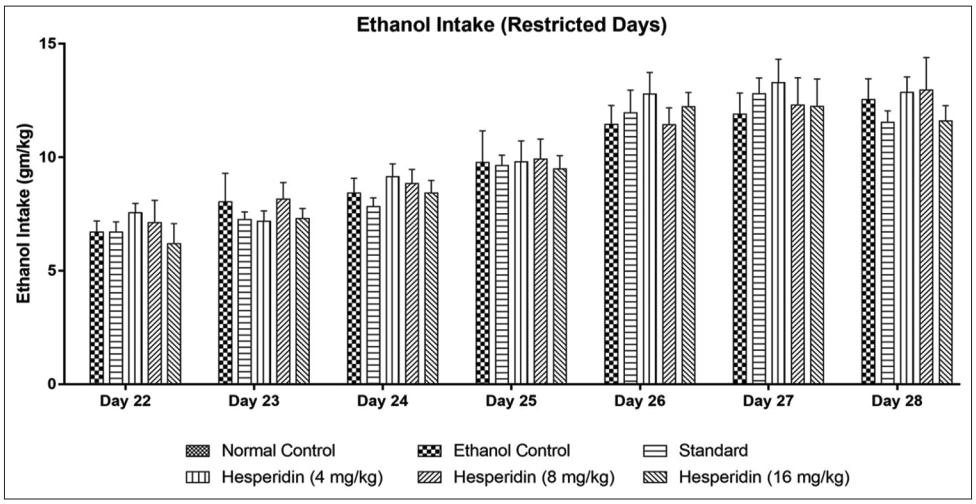

Fig. 1: Voluntary ethanol (EtOH) intake (grams per kilogram body weight per day, g/kg/day) in Swiss albino mice (n=8 per group) during the restricted access phase (Days 19–28). A gradual increase in EtOH consumption was observed across all groups, but two-way analysis of variance revealed no statistically significant differences between groups during this period. Data are presented as mean±standard error of the mean

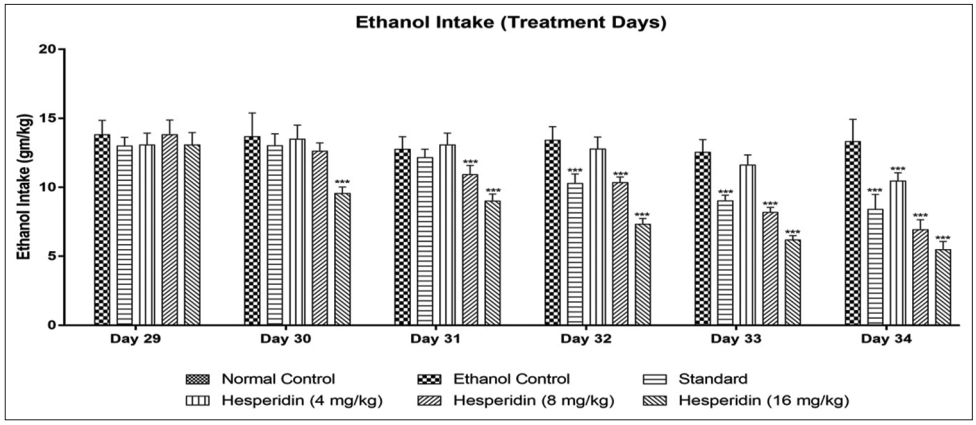

Fig. 2: Effect of hesperidin administered orally at 16 mg/kg on voluntary ethanol (EtOH) intake (grams per kilogram body weight per day, g/kg/day) in Swiss albino mice during the treatment phase (days 29–34). Hesperidin at 16 mg/kg caused a significant reduction in EtOH consumption from day 30 to day 34 compared to the vehicle (EtOH control) group. Data are shown as mean±standard error of the mean. ***p<0.001 versus vehicle-treated control (two-way analysis of variance with Tukey's *post hoc* test)

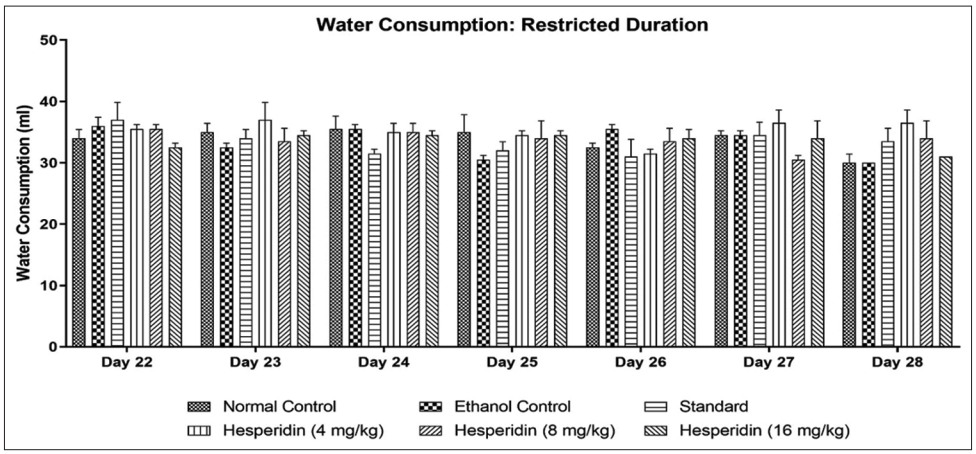

Fig. 3: Voluntary water intake (milliliters per day, mL/day) in Swiss albino mice during the restricted access phase (days 19–28). No significant changes in water intake were observed between groups throughout this period. Data are presented as mean±standard error of the mean

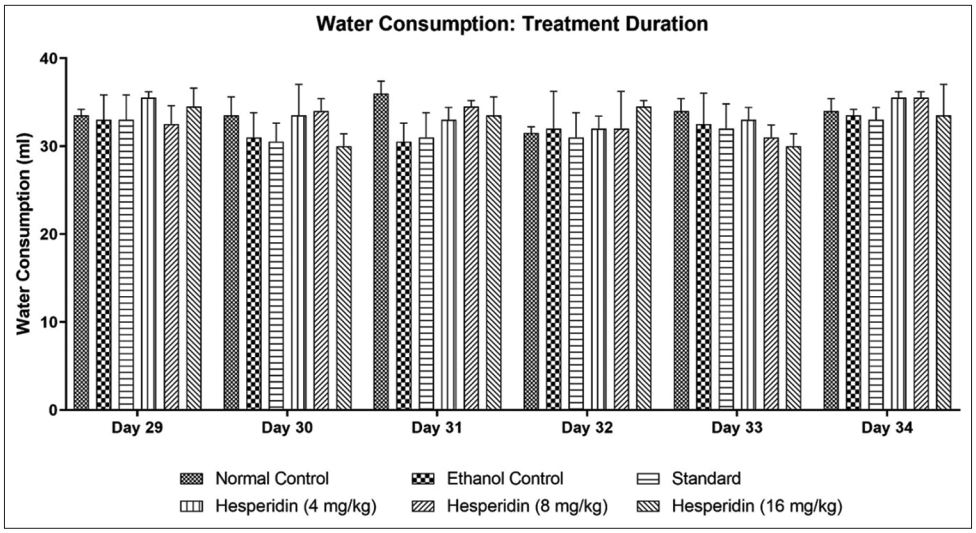

Fig. 4: Effect of hesperidin (4, 8, and 16 mg/kg, orally administered, orally.) and ondansetron (4 mg/kg, orally) on voluntary water intake (milliliters per day, mL/day) in Swiss albino mice during the treatment phase (Days 29–34). No significant differences in water intake were observed between treatment groups, as analyzed by two-way repeated measures analysis of variance. Data are presented as mean±standard error of the mean

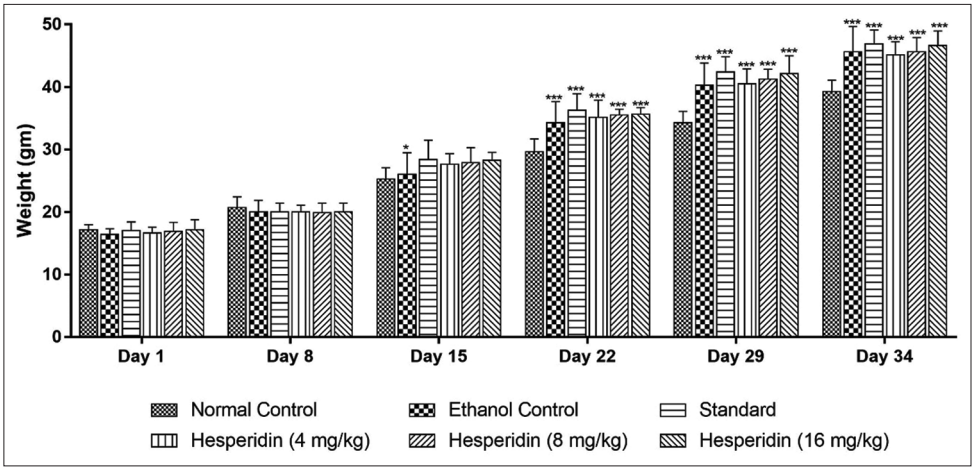

Fig. 5: Changes in body weight (grams) of Swiss albino mice across the entire experimental period. A significant increase in body weight was observed in all groups over time (p<0.001, two-way analysis of variance). Data are presented as mean±standard error of the mean

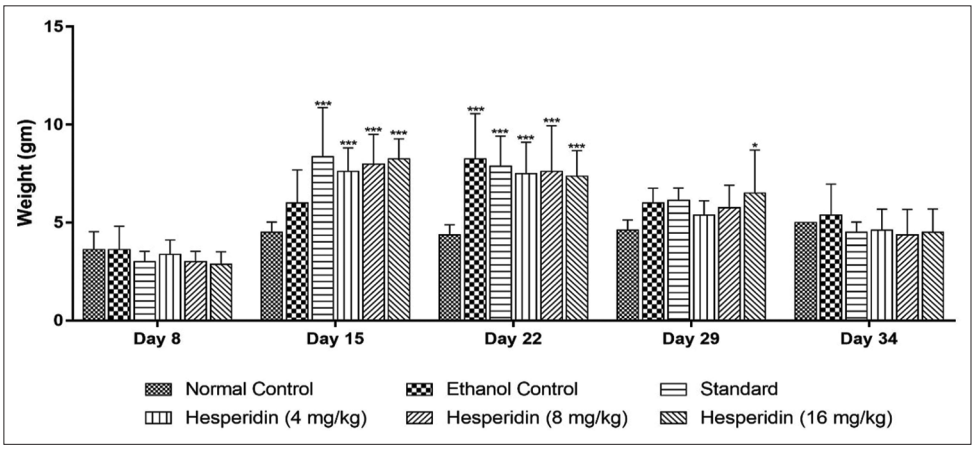

Fig. 6: Changes in body weight (grams) of Swiss albino mice during the treatment phase. A significant increase in body weight was observed in all groups over time (p<0.001, two-way analysis of variance). Data are presented as mean±standard error of the mean

water intake during treatment (Days 29–34), as per two-way repeated measures ANOVA (Fig. 4).

Body weight

Throughout the experiment, a significant increase in body weight was observed across all groups of mice (p<0.001), indicating notable weight gain and changes over time (Figs. 5 and 6).

DISCUSSION

This study examined the impact of hesperidin on voluntary EtOH intake using a restricted access two-bottle choice model in Swiss albino mice. During the restriction phase (day 19–28), all groups developed a consistent EtOH preference, with no significant intergroup differences, confirming baseline homogeneity (Fig. 1). Hesperidin treatment (Day 29–34) significantly and dose-dependently reduced EtOH consumption, with the 16 mg/kg dose showing highly significant effects from day 30 onward (p<0.001), followed by the 8 mg/kg and 4 mg/kg doses showing significant reductions during later days (Fig. 2). These effects were comparable to ondansetron, a 5-HT_3 antagonist used as a positive control [17]. Notably, hesperidin did not affect water intake or body weight (Figs. 3-6), suggesting its action was specific to EtOH preference rather than general fluid or food intake.

The mechanistic basis of hesperidin's anti-addictive effect may involve the modulation of multiple neurotransmitter systems linked to alcohol addiction. EtOH preference and relapse involve dysregulation of serotonergic, dopaminergic, glutamatergic, and PPAR-γ pathways [18,19]. Hesperidin has been shown to modulate 5-HT_2B and 5-HT_3 receptors, aligning with the serotonergic mechanism of ondansetron [20,21]. Furthermore, hesperidin activates PPAR-γ, a nuclear receptor implicated in reducing drug-seeking behavior [22,23], as seen in models of neurotoxicity and cardiac dysfunction [24-26]. It also inhibits glutamate release and protects against hippocampal excitotoxicity, potentially contributing to reduced EtOH reinforcement [27].

In addition, hesperidin's anxiolytic, antidepressant, and neuroprotective properties including 5-HT_1A receptor modulation and antioxidant effects may further support its efficacy in mitigating the emotional and neurochemical drivers of alcohol-seeking behavior [28-30]. A behavioral study in zebrafish larvae has also shown hesperidin's ability to reverse alcohol-induced anxiety [31,32]. Overall, the findings indicate that hesperidin dose-dependently reduces voluntary EtOH intake without adverse effects, possibly through serotonergic, glutamatergic, and PPAR-γ pathways. This highlights its potential as a natural treatment for AUDs [33-35].

CONCLUSION

The outcomes of the current investigations provide pharmacological confirmation supporting the inhibitory effects of hesperidin on voluntary EtOH consumption in mice, as demonstrated by the voluntary EtOH drinking test. The attenuation of EtOH preference by hesperidin may involve modulation of the serotonergic, glutamatergic, or PPAR-γ systems. Notably, both hesperidin and ondansetron significantly reduced EtOH intake without affecting water consumption, signifying

a specific effect on EtOH-related motivation rather than general fluid intake. These findings highlight hesperidin as a promising candidate for mitigating the motivational properties of EtOH. However, supplementary investigations are warranted to elucidate the precise mechanisms and receptor pathways involved in its anti-addictive effects. Future studies should explore the receptor-specific actions of hesperidin, especially its role in serotonergic, glutamatergic, and PPAR-γ pathways. In conclusion, hesperidin shows promise as a therapeutic option for managing EtOH addiction, pending further research.

AUTHOR'S CONTRIBUTION

DR. Kailasam Koumaravelou signed the experimental framework, and data analysis, and finalized the manuscript. Mr. Krushna Kashinathrao Zambare conducted experimental work and managed the data collection.

CONFLICT OF INTEREST

All authors have nothing to declare.

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Nil.

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