
Toxicological, Biochemical and Histopathological Evaluation of the Ethanolic extract of Seagrass-*Enhalus acoroides* in Albino wistar rats

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Highlights

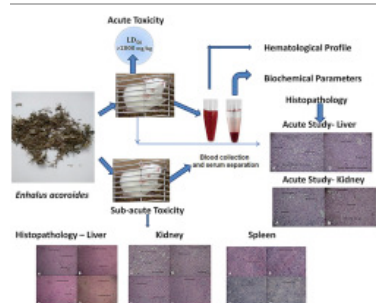
- Acute toxicity i.e. LD₅₀ *Enhalus acoroides*, was more than 2000 mg/kg in wistar albino rats.
- 28 days repeated dose sub-acute toxicity showed NOAEL (No observed adverse effect level) at a dose of 400 mg/kg in wistar albino rats.
- No significant differences in hepatic marker enzymes, indicates the hepato-safety of the *Enhalus acoroides*.
- Haematological, Biochemical and Histopathological parameters did not show significant differences in *Enhalus acoroides* treated wistar rats.
- Based on this scientific appraisal, it can be concluded that *Enhalus acoroides* have a high margin of safety and can be further used for pre-clinical trials.

Abstract

The purpose of this research was to examine the acute toxicity (14 days) and sub-acute toxicity (28 days) studies of ethanolic extract of *Enhalus acoroides* (EEEE) on male wistar albino rats. A single dose of 2000 mg/kg of the extract was administered to the male rats for acute study and for sub-acute study, dose levels of 100, 200 and 400 mg/kg/b.wt. of the extract was administered daily by oral gavage to the rats respectively. During the treatment periods, body weight, water and food consumption, clinical signs, mortality, biochemistry, hematology, organ weights, gross findings, and histopathology were examined. Imperative organs are subjected to histopathology. Upto 2 g/kg b.w. of EEEA did not show any mortality in the acute toxicity studies. Our results demonstrated that sub-acute treatment of the crude extract of *Enhalus acoroides* revealed there were no significant changes in the haematological and serum biochemical parameters seen in the treated groups compared to the control group. Histological studies revealed no abnormalities in liver, kidney and spleen tissue at the high dose level of EEEA. In conclusion, both the acute

and sub-acute toxicity studies proved no clinical toxic symptoms and histopathological lesions, which show Ethanolic extract of *Enhalus acoroides* are safety at normal therapeutic doses.

Graphical abstract



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Introduction

Medicinal plants are regularly used as therapeutic sources because of the simple and easy accessibility and also low in cost (Silva et al., 2014). As the natural herbal products are safe and free from side effects, the people consume it for medicinal purposes. Enormous different compounds are present in the medicinal plants, but some compounds with great complications are also seen (Nasri and Shirzad, 2013). Though herbs can be employed in treating most of the diseases, repeatedly they are consumed without knowing the scientific validation and substantiation of their pharmacological effects (Carneiro et al., 2014).

The current allopathic practices gradually turned to the site for good scientific and accomplished observational efforts of researchers in oxidative stress. During the last three decades, drugs based on antioxidants and finding the mechanism of action for the deterrence and management of complicated diseases like Alzheimer's disease and carcinoma have appeared (Aqil et al., 2006). However, the origin of its development remains embedded in traditional medications (Mchesney et al., 2007).

Herbal products are devoid of toxic side effects which are derived from nature, are often coupled with allopathic drugs that are often consumed in established drugs (Pushpa Latha et al., 2010). However, the level of toxicity should be detected for accurate and acknowledged herbal drugs, like the established traditional drugs that are appropriately validated; the toxicity is not assessed often in the conventional herbal drugs (Smart et al., 2011). In general, people give importance to the remedial property of the medicinal drugs and abandon their toxic effects to different organs.

Due to effective pharmacological activities, less toxicity and cost effective and rich in antioxidant properties, this increases the recent scientific development of medicinal herbs, (Auddy et al., 2003). The research in herbal toxicity has increased anxiety among the globe to figure and implement the different guidelines to monitor and prevents toxicity due to herbal drugs. Currently, the research increases on searching novel compounds from seagrasses which are rich in antioxidants like polyphenols, terpenoids, flavonoid, tannins and saponins, since the mechanism of action begins at the target cellular level.

One such seagrass is *Enhalus acoroides* (Linn.f.) Royle, belongs to the family Hydrocharitaceae, has dark green long linear leaves with 1.0–1.5 cm width and 50–170 cm length. The calorific values of *E.acoroides* were comparable to sweet potato, peas, bengal gram, and potato. Literature also showed *E.acoroides* contains higher antioxidant activity, higher activity of scavenging free radicals and higher reducing power property and also effectively used for the treatment of various oxidative stress related problems. From the studies, *E.acoroides* present in the region of South China shows the properties like anti-feedant, anti-bacterial and anti-larval activities in the biochemical analysis and identified 11 pure compounds which include 5 sterols and 4 flavonoids (Ragupathi Raja Kannan et al., 2010). Recent studies statement reveals that antioxidant compounds are abundant in seagrass (Gillan et al., 1984).

In our past report we acknowledged the existence of phytochemicals from the EEEA which contains carbohydrates, terpenoids, steroids, alkaloids, flavonoid, and tannins (Amudha et al., 2017). Certain bio-active components were also isolated from

E.acoroides including 1-nonadecene (17.15%), n-tetracosanol-1 (11.48%), 1-octadecene (10.06%), 2-pentadecanone (7.87%), behenyl alcohol (7.33%) (Amudha et al., 2018).

From the literature, it was proven the great potential and several applications of *E.acoroides* as a medicinal herb. However, data about toxicity of the crude extract from the whole seagrass of *E.acoroides* are still limited. The pharmacological and toxicological properties of the seagrass still need to be found better expose of the risks of their use, and ensuring the safety for the people. So for the safety evaluation of ethanolic extract of *E.acoroides*, acute and sub-acute toxicity studies were worked out to examine the toxicity after single dose of EEEA and 28-days repetitive oral dose of EEEA in wistar rats.

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Section snippets

Plant material

The whole seagrass of *Enhalus acoroides* was collected from Devipattinam, Ramanadhapuram District, Tamilnadu during the month of June 2016. The seagrass was authenticated in ICAR by Dr. N. Kaliaperumal M.Sc., Ph.D., Scientist-in-charge, CMFRI.

Obtention of extract

The collected *E.acoroides* leaves washed with distilled water carefully to get rid of the traces of impurities. The leaves were shade dried, mechanically grinded and powdered. The grinded powder was initially soaked into 1:2 ratio ethanol for three days with

Results

The panel experts of OECD defines the acute toxicity study as “the adverse effect occurring within a short time of (oral) administration of a single dose of a substance or multiple doses given within 24h”. And sub-acute toxicity as “the advance effects occurring as result of the repeated daily (oral) dosing of a chemical to experimental animal for part (not exceeding 10%) of the life span” (Lee and Dixon, 1978).

Acute toxicity tests provide preliminary information on the toxic nature of a

Conclusion

The present investigation showed that the ethanolic extract of the whole parts of *Enhalus acoroides* did not present significant toxicity when administered in a single highest dose, being considered safe by the OECD. However, when various doses were given for 28 days, were found some changes in biochemical, haematological, and histological parameters, but not statistically significant. In conclusion, the current investigation demonstrates that at doses consumed in the traditional medicine, the

Declaration of interest

The authors declare that there is no conflict of interest.

Submission declaration

The present work has not been published previously in any form and not under consideration for publication elsewhere.

Authors contributions

- The author P.Amudha executed the acute and sub-acute toxicity experiment, euthanasia of the animals and writing of the manuscript.
- The author Dr.V.Vanitha designed the protocol, assistance in animal euthanasia and supervised the research work.

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