Effect of Feeding Aqueous Extracts Of *Sargassum Crassifolium* and *Ulva Fasciata* on Biochemical and Hematological Parameters of Laboratory Mice

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Abstract
The consumption of seaweeds has increased over the recent past. However, certain effects of seaweeds are yet to be investigated. The objective of the present study was to evaluate the safety and the effects of feeding aqueous extracts of *Sargassum crassifolium* and *Ulva fasciata*, on some selected haematological parameters including differential blood count, packed cell volume (PCV), concentrations of total serum protein, serum aspartate transaminase (AST) and serum alanine transaminase (ALT) in laboratory mice. Two groups of six mice were orally administered with aqueous extracts of the two seaweed species, separately. A dose of 454.5mg/kg body weight was given once a day for 9 days and observed up to 12 days. A control group (n=5) was also maintained without treatment. Both extracts did not show any toxic effects in an acute toxicity test. There was a significant increase ($p<0.05$) in the neutrophil count in mice 12 days after commencing treatment with aqueous extracts of *Ulva fasciata* (n=5, 31.55% ± 4.1%) compared with the control (n=5, 19.0% ± 3.4%). However, the consequent decrease in the monocytes and lymphocytes with the Ulval extract was not significant ($p>0.05$) 12 days post-treatment. There was no significant ($p>0.05$) change in the total serum protein concentration, at the end of the study period. Serum concentrations of AST and ALT did not show significant changes ($p>0.05$) after feeding the extracts of *Ulva fasciata* and *Sargassum crassifolium*. In conclusion, aqueous extracts of *Sargassum crassifolium* and *Ulva fasciata* did not exhibit any toxicity in laboratory mice, *in vivo*, at the dose tested.

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Introduction
Seaweeds or marine macro algae are relatively simple photosynthetic plants, constituting commercially important, renewable resources that have applications in medicine, food, animal feed and as bio fuels (Bocanegra *et al.*, 2009). They produce different chemically active metabolites (biogenic compounds) such as halogenated compounds, alcohols, aldehydes and terpenoids that have antibacterial, anti-macro fouling and antifungal properties (Kolanjinathan *et al.*, 2014). In this regard, seaweeds have a wide range of therapeutic possibilities, both internally and externally. Seaweeds are used for the treatment of various diseases such as allergy, cancer, ulcers, arthritis, and hypotension. They have also been used as an herbal medicine to suppress inflammation due to their trace mineral content.

Sargassum spp. and *Ulva* spp. have been found to possess a variety of biological effects with a potential for its use in medicine. However, toxicity data is essential for preclinical trials of any medicine.

The objective of the present study was to evaluate the safety and the effects of feeding aqueous extracts of *Sargassum crassifolium* and *Ulva fasciata*, on some selected haematological parameters including differential blood count, packed cell volume (PCV), total serum protein concentration, serum aspartate transaminase (AST/SGOT) concentration and serum alanine transaminase (ALT/SGPT) concentration, in laboratory mice. The study was for a short term toxicity evaluation with parameters that can indicate effects on liver, blood and immune modulation of seaweeds.

Materials and Methods
Sample collection
Samples of *Sargassum crassifolium* and *Ulva fasciata* were collected from Koggala algae bed, in the Southern Province of Sri Lanka. The samples were washed thrice with sea water followed by washing with tap water.

Preparation of extracts
Collected samples were dried in the shade and were then dried in a drying oven at 40^{0}C for an hour. Next, the samples were pulverized separately into a fine powder using a household grinder. Two grams (2 g) of each sample was then soaked in 40 ml of distilled water and sonicated at 25 ^{0}C for one hour (a lapse of 10

minutes was allowed after every 15 minutes of sonication). The resulting solution was centrifuged for 10 minutes at 1500rpm at 40^{0}C. The supernatant was kept at 4 ^{0}C until used.

Experimental animals

White, male and female laboratory mice weighing 18-30 g were used in this study. Animals were maintained in polypropylene cages bedded with soft rice husk (6 mice/cage) with 12 h light and 12 h dark cycle. Mice were fed with a commercially broiler starter ration. They were acclimatized for 3 days, prior to the treatment. Animal maintenance was carried out in an animal house at the Faculty of Medicine, University of Peradeniya.

Seventeen mice were randomly assigned to three groups, 6 each for each seaweed treatment and 5 for the control. Each mouse was numbered according to the guidelines of the Animal House. Prior to the feeding trial, two blood smears were prepared from haphazardly selected mice by using tail tip blood. All mice had free to access water and food. Group 1 (control group) was maintained without any seaweed treatment. *Sargassum crassifolium* and *Ulva fasciata* extracts were orally administrated for group 2 and group 3 mice, respectively, once a day for nine days with the extracts at a dose level of 454.5 mg/kg body weight. Animals were observed and were weighed on 3rd, 7th and 12th days after the treatment. Blood smears were prepared from tail tip blood.

Estimation of hematological parameters: Differential count of white blood cells was carried out and PCV was evaluated by using a hematocrit centrifuge.

Biochemical assays: Serum ALT and AST were assayed using 10s-UV/VIS spectrophotometer (Thermoscientific™, Germany) and commercial test kits (Human Gesellschaftfür Biochemica und Diagnostica MBH, Germany) were used for these analyses. Serum total protein was determined by using RHC-200 ATC refractometer.

Statistical analysis: Microsoft Excel 2010 and SPSS version 20.0 (Generalized Linear Models) software programs were employed in the analysis of biochemical and haematological data.

Results and Discussion

The extracts of *Sargassum crassifolium* and *Ulva fasciata* did not seem to cause any gross lesions and the organ sizes remained normal at the 12th day after the treatment. At the end of the study period, there was no significant change ($p>0.05$) in the PCV and total serum protein (TSP) concentration after feeding aqueous extracts of *Ulva fasciata* (PCV, 30.5 ± 6.3 and TSP, 5.4 ± 1.1) and *Sargassum crassifolium* (PCV, 37.7 ± 4.7 and TSP, 5.4 ± 0.2) compared with the control (PCV, 33.0 ± 5.0 and TSP, 5.2 ± 0.8).

Alam and Qasim (1994) reported that *Sargassum bovianum, Caulerpa faridii* and *Gracilaria corticata* species did not cause any marked changes in blood parameters, only up to 5% concentration in animal feed. At both 10% and 20% levels, a significant decrease in blood hemoglobin, raised erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) levels were noted compared to control diet.

According to reports, a number of rats have died either due to excessive water loss through diarrhea and vomiting or a certain nervous system breakdown, during the feeding trial of *Sargassum bovianum, Caulerpa faridii, Gracilaria corticata* species (Alam and Qasim, 1994). However, feeding trials of laboratory mice with *Ulva fasciata* and *Ulva lactuca* have shown positive results (Alam and Qasim, 1994).

Moreover, in the present study, the serum concentrations of AST and ALT did not show any significant changes ($p>0.05$) after feeding with the extracts (n=6) of *Ulva fasciata* (AST, 207.9 ±140.6 and ALT, 38.0 ±19.3) and *Sargassum crassifolium* (AST, 142.8 ± 52.4 and ALT, 25.4 ± 9.5), compared with the control (AST, 130.1± 120.1 and ALT, 34.9 ± 19.9).This finding indicated that there seem to be no adverse effects of feeding aqueous extracts of *Ulva fasciata* and *Sargassum crassifolium* on the liver of mice.

The study also revealed a significant increase ($p<0.05$) in the neutrophil count in mice after 12 days treatment with aqueous extracts of *Ulva fasciata* (n=5, 31.5% ± 4.1%) compared with the control (n=5, 19.0% ± 3.4%). However, the consequent decrease in the monocytes and lymphocytes with the *Ulva* extract was not significant, 12 days after commencing treatment.

Conclusions

Aqueous extracts of *Sargassum crassifolium* and *Ulva fasciata* did not exhibit any toxicity in laboratory mice, *in vivo*, at the dose tested. However, further studies with different doses of feeding need to be conducted in order to arrive at firm conclusions.

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