

# Vitamin E and Trolox™ reduce toxicity of Aloe barbadensis Miller juice in Artemia franciscana nauplii but individually are toxic at high concentrations

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## Citation

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## Abstract

This study reports on the acute toxic effects of Aloe barbadensis Miller (Aloe vera) juice as well as high doses of the antioxidants vitamin C, vitamin E and Trolox™ (a water soluble vitamin E analogue) to the salt water crustacean Artemia franciscana. Aloe vera juice exposure resulted in acute toxicity, being capable of inducing mortality at dilutions as low as 4 % juice and having an LC50 at 24 h of  $4.6\% \pm 0.3$ . 6% Aloe vera juice dilutions were capable of causing 100% mortality within 4 h of exposure to A. franciscana. All of the antioxidants tested were also toxic to A. franciscana when tested in high doses. Toxicity of the antioxidants at 24 h was in the following order of toxicity: vitamin C (LC50 203.1  $\mu\text{g/ml} \pm 11.3$ ) > Trolox™ (LC50 = 283.3  $\mu\text{g/ml} \pm 25.8$ ) > vitamin E (only low toxicity was observed at 24 h with the tested concentrations). However, in lower doses vitamin E and Trolox™ were non-toxic and could block the toxicity induced by Aloe vera juice. Vitamin E was more effective than Trolox™ at blocking Aloe vera juice induced toxicity. Treatment of A. franciscana with antioxidants prior to exposure to juice was significantly more effective than the simultaneous treatment of antioxidant and the toxin. These data suggest that the lethality induced by Aloe vera juice is due to oxidative stress which can be blocked by antioxidant addition.

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## INTRODUCTION

Aloe barbadensis Miller (Aloe vera) has a long history of use for medicinal and dietary purposes and as a major component of many cosmetic preparations. Amongst its therapeutic uses, Aloe vera been shown to have anti-inflammatory activity [1,2] immunostimulatory activity [3], antibacterial activity [4], antiviral activity [5], accelerated wound healing [6] and a reduction of radiation induced skin reactions [7]. However, there have also been reports of toxic effects of Aloe vera leaf components. Various low molecular weight components of Aloe vera gel have been reported to be cytotoxic to fibroblasts [8] and both normal human skin cells and tumour cells in vitro [9]. Recently, we have found toxicity of Aloe vera leaf gel components towards Artemia franciscana nauplii [10] and a range of bacteria and fungi [11]. The mechanism of toxicity was not established in these studies but the induction of oxidative stress was considered likely.

Many active constituents have been isolated from A.

barbadensis leaves and their biological activities studied. The anthraquinones, anthrones and chromones in particular have received much recent attention and have been shown to be effective at counteracting various diseases [2,12]. Yen et al. [13] have investigated the antioxidant activity of various anthraquinones. In particular, aloe emodin was found to inhibit lipid peroxidation. Tian and Hua [14] have demonstrated that aloe emodin and aloin may act as either a pro-oxidant or an antioxidant, their action being dependent on their concentration. Therefore these compounds may act in either a protective or toxic manner at different concentrations. It has been suggested that the mode of toxicity for certain Aloe vera gel components is oxidative stress induction [14]. Conversely, at lower concentrations, these same components act as antioxidants and protect from oxidative stress [15].

The use of antioxidants such as vitamin E and vitamin C to reduce the effects of oxidative stress has received much recent attention. However, these studies have proved confusing, with some studies showing therapeutic effects [16,17], whilst other studies indicate that these antioxidants may be toxic [18]. It has been shown in a variety of human

and animal models that the effects of vitamin E and vitamin C are dose dependent, with low doses functioning as antioxidants and blocking toxicity, while high doses induce toxicity through oxidative stress [ 19 ].

The *Artemia nauplii* (brine shrimp larvae) lethality bioassay [ 20 ] has been used to examine the toxicity of a wide variety of plant compounds. It is an efficient, inexpensive and relatively rapid way to detect toxic compounds, requiring only low amounts of sample. This test correlates well with cytotoxic activity of some human tumours and, therefore has the potential to detect new antitumour agents [ 21 ]. The current report aims to examine the mechanism of Aloe vera induced toxicity in brine shrimp and determine if it is due to oxidative stress. This is achieved by determining whether the toxicity of Aloe vera juice can be blocked with the addition of antioxidants. In so doing, this report also examines whether specific vitamin antioxidants can induce toxicity in the *Artemia nauplii* bioassay, as has been found in human and other animal models [ 18,19 ].

## **MATERIALS AND METHODS**

### **CHEMICAL REAGENTS**

Aloe vera juice was obtained from Aloe Wellness Pty Ltd, Australia and was stored at 4 °C until use. Aloe vera juice was serially diluted in deionised water for use in the *A. franciscana* bioassay. Vitamin E (α-tocopherol; Sigma, purity > 96%) was dissolved in 60% methanol to give a 10 mg/ml stock. This stock was serially diluted in deionised water for use in the bioassay. Trolox™ (Sigma, purity >97%) was prepared as a 1.5 mg/ml stock in 60% methanol and was diluted in deionised water for use in the bioassay. Vitamin C (L-Ascorbic acid, AR grade, Chem-Supply) was dissolved and diluted in deionised water. Aloe emodin (Sigma, purity >95%) was prepared by dissolving in distilled water to give a concentration of 500 µg/ml and serially diluting in deionised water. All reagents were prepared fresh before use.

### **REFERENCE TOXINS FOR BIOLOGICAL SCREENING**

Potassium dichromate (AR grade, Chem-Supply) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in artificial seawater for use in the *A. franciscana nauplii* bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) was obtained from Sigma-Aldrich with a mixture of cis (76.6%) and trans (23.0%) isomers and prepared as a 4 mg/ml stock in distilled water. The stock was serially diluted in artificial

seawater for use in the bioassay.

### **BIOLOGICAL SCREENING**

Toxicity was tested using a modified form of the *Artemia nauplii* lethality assay developed by Meyer et al. [ 20 ] for the screening of active plant constituents. *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 litre synthetic seawater under artificial light at 25°C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. 400 µl of seawater containing approximately 25 (mean 25.2, n = 286, SD 8.6) nauplii were added to wells of a 48 well plate and immediately used for bioassay. *A. barbadensis* juice was diluted serially in deionised water. 400 µl of the diluted juice and the reference toxins were transferred to the wells and incubated at 25 ± 1°C under artificial light (500 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate. All concentrations of treatments also were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 72 h all nauplii were sacrificed and counted to determine the total number per well. The LC<sub>50</sub> with 95% confidence limits for each treatment was calculated using probit analysis [ 22 ].

### **ANTIOXIDANT CO-TREATMENT**

To determine the ability of antioxidants to block the toxic effect of Aloe vera juice, vitamin C was freshly prepared in deionised water as a 400 µg/ml solution. Vitamin E and Trolox™ were each dissolved in 60 % methanol and diluted in deionised water to a concentration of 400 µg/ml. Aloe vera juice was diluted in deionised water to give a 24 % solution. The 24 % juice was added to an equal volume of the relevant antioxidant (400 µg/ml) and mixed well. 400 µl of the juice/antioxidant mixtures was added to wells of a 48 well plate containing 400 µl of seawater containing *A. franciscana nauplii*, resulting in test concentrations of 6%

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juice and 100 µg/ml antioxidant in the bioassay. The mortality was monitored at regular intervals. All tests were performed at least three times in triplicate.

### ANTIOXIDANT PRE-TREATMENT

Aloe vera juice was prepared as a 24 % solution and the antioxidants were prepared as 400 µgml<sup>-1</sup> solutions as described for the co-treatment experiments. 200 µl of the test antioxidants were added to wells of a 48 well plate containing 400 µl of seawater containing *A. franciscana* nauplii and incubated at 25°C for 4 h. Following the 4 h antioxidant pre-treatment, 200 µl of 24 % juice was added to the wells resulting in a final concentration of 6 % juice and 100 µg/ml in the bioassay. The mortality was monitored at regular intervals. All tests were performed at least three times in triplicate.

### STATISTICS

The Paired T-Test was used to calculate statistical significance between control and treated groups with a P value < 0.05 considered to be statistically significant.

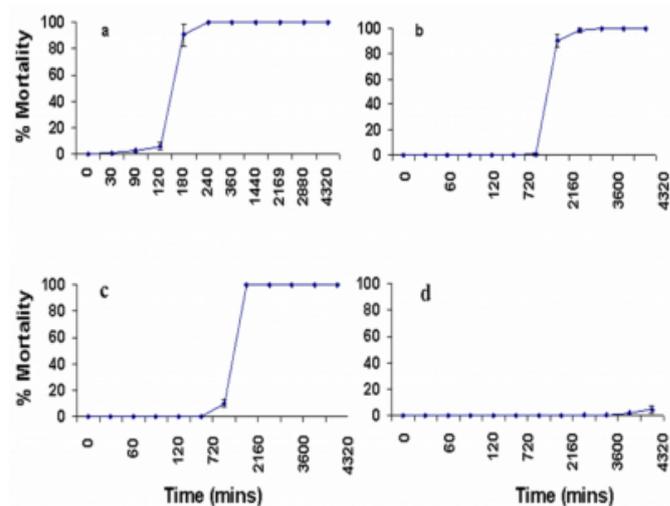
## RESULTS

### ALOE VERA JUICE TOXICITY

A 50 % dilution of Aloe vera juice was found to induce 100 % mortality within 4 h in the *A. franciscana* bioassay. Neither of the reference toxins, Mevinphos (2000 µg/ml) nor potassium dichromate (800 µg/ml) produced notable mortality compared to the negative controls in 4 h. A time course study was performed to determine the rate at which 50 % Aloe vera juice could induce toxicity in the bioassay. As is seen in Figure 1a, the onset of Aloe vera juice toxicity (as defined by mortality) was seen at approximately 120 min, and approximately 240 min was required for 100 % mortality. In contrast, both Mevinphos (Figure 1b) and potassium dichromate (Figure 1c) took much longer to exert their effect. The onset of Mevinphos toxicity was approximately 12 h and more than 36 h was required for 100% mortality. Similarly, potassium dichromate toxicity was not evident until 12 h and approximately 24 h was required for 100 % mortality. Spontaneous mortality in all seawater controls was < 1% at 24 h (Figure 1d). Due to the rapid toxicity of aloe vera juice, we have reported LC<sub>50</sub> values for 4 h (Table 1). To enable comparison to the LC<sub>50</sub> values of the reference toxins the LC<sub>50</sub> at 24, 48 and 72 h are also reported.

**Figure 1**

Figure 1: Acute toxicity to . of (a) Aloe vera juice (50 % dilution in deionised water), (b) Mevinphos (2000 µg/ml) (c) potassium dichromate (800 µg/ml), (d) artificial seawater control. All bioassays were performed in at least triplicate.



**Figure 2**

Table 1: Brine shrimp larvicidal activity of Aloe vera juice, reference toxins Mevinphos and potassium dichromate and the antioxidants vitamin C, vitamin E and Trolox™.

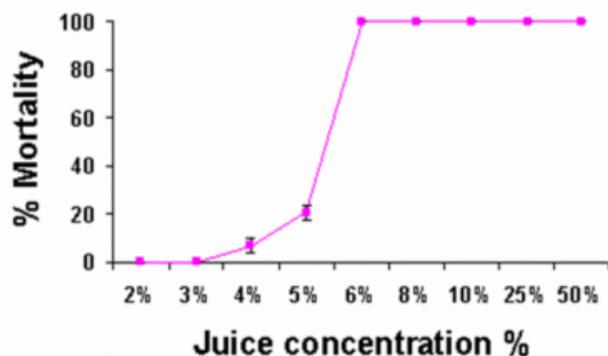
Treatment	LC <sub>50</sub> value in µg/ml* (95 % confidence interval) at time (h)			
	4	24	48	72
Aloe vera juice	5.4 ± 0.3 %	4.6 ± 0.3 %	4.4 ± 0.4 %	4.3 ± 0.2 %
Mevinphos	-	1336 ± 70	501 ± 33	109 ± 12
Potassium Dichromate	-	73 ± 4	12 ± 4	3.7 ± 0.3
Vitamin C	608.3 ± 39.7	203.1 ± 11.3	196.9 ± 17.9	182.0 ± 10.1
Vitamin E	-	-	-	96.0 ± 6.3
Trolox™	453.2 ± 25.2	283.3 ± 25.8	279.8 ± 18.2	275.0 ± 15.3

\*Values are expressed as mean (µg/ml unless otherwise stated) ± S.D.

Figure 2 shows the effect of Aloe vera juice dose on mortality in the *A. franciscana* bioassay. *A. franciscana* nauplii were exposed to dilutions of Aloe vera juice for 4 h and the % mortality determined. The induction of toxicity was evident when the *A. franciscana* were exposed to approximately 4 % Aloe vera juice. 6 % juice was the lowest dose capable of inducing 100% mortality within the 4 h period. The LC<sub>50</sub> at 4 h for Aloe vera juice was 5.4 % ± 0.3 (Table 1).

**Figure 3**

Figure 2: Dependence of Aloe vera juice concentration on lethality. Aloe vera juice was diluted freshly prior to use in deionised water. 400  $\mu$ l of juice dilution was added to 400  $\mu$ l of saline containing *A. nauplii*. All bioassays were performed in at least triplicate.

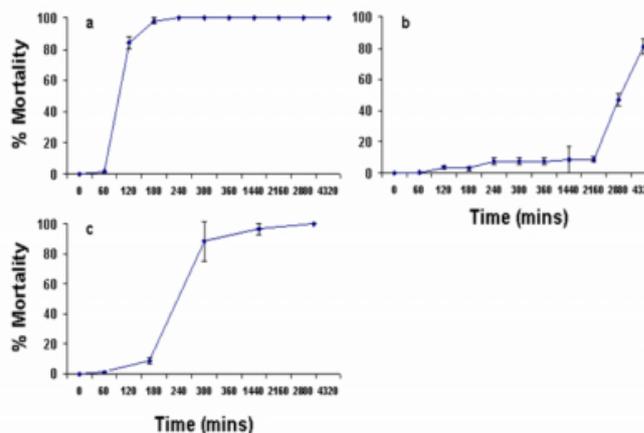


### TOXICITY OF ANTIOXIDANTS

To determine whether vitamin C and E and the water soluble vitamin E analogue Trolox™ were toxic in the *A. franciscana* nauplii bioassay, high doses of these antioxidants were tested in the bioassay (vitamin C, 1250  $\mu$ g/ml; vitamin E, 320  $\mu$ g/ml; Trolox™, 500  $\mu$ g/ml). Vitamin C induced toxicity inside 90 min with 100 % mortality seen in 180 min (Figure 3a). Both vitamin E (Figure 3b) and Trolox™ (Figure 3c) were slower in inducing toxicity. For both vitamin E and Trolox™, the onset of toxicity was seen within 240 min, however only low mortality was seen at this time. The induction of 100 % mortality took much longer to achieve. Trolox™ took 48 h to induce 100 % mortality (96.4 %  $\pm$  3.6). Vitamin E was even slower at inducing mortality, with approximately 80 % mortality at the 72 h test period.

**Figure 4**

Figure 3: Acute toxicity to *A. franciscana* nauplii of (a) 1250  $\mu$ g/ml vitamin C, (b) 320  $\mu$ g/ml Vitamin E, (c) 500  $\mu$ g/ml Trolox™. 400  $\mu$ l of antioxidant dilutions were added to 400  $\mu$ l of saline containing nauplii. All bioassays were performed in at least triplicate.



The effect of antioxidant concentration on mortality was measured at various times. Table 1 shows that the LC<sub>50</sub> of the vitamin C (203.1  $\mu$ g/ml  $\pm$  11.3) was substantially lower than the LC<sub>50</sub> of the reference toxin Mevinphos (1336  $\mu$ g/ml  $\pm$  70) at 24 h, demonstrating its relative toxicity. However, potassium dichromate had an LC<sub>50</sub> of 73  $\mu$ g/ml  $\pm$  4.0 at 24 h, nearly three fold more toxic than the vitamin C. Interestingly, whilst the toxicity of vitamin C was observed very rapidly, it reached its maximum lethality within 24 h. Only small decreases in LC<sub>50</sub> were seen over the next 2 days. In contrast, while both Mevinphos and potassium dichromate took longer to exert their effects, the mortality due to these toxins continued to increase over time. Whether these contrasting effects are due to a difference in the mechanism of toxicity of these compounds, or whether the extracts active compounds are labile and lose effect over time is not evident from these studies. Future studies need to focus on the mechanism by which the extract compounds exert toxicity.

Trolox™ was capable of inducing toxicity rapidly with a 4 h LC<sub>50</sub> of 453.2  $\mu$ g/ml  $\pm$  25.2. Trolox™ reached its maximum toxicity within 24 h and only small decreases in LC<sub>50</sub> were seen over the next 2 days. Vitamin E treatment resulted in low *A. franciscana* mortalities at all doses tested within the first 48 h, accordingly only the 72 h LC<sub>50</sub> for vitamin E treatment is reported here (Table 1). Vitamin E had a 72 h LC<sub>50</sub> in the *Artemia* bioassay of 96  $\mu$ g/ml  $\pm$  6.3.

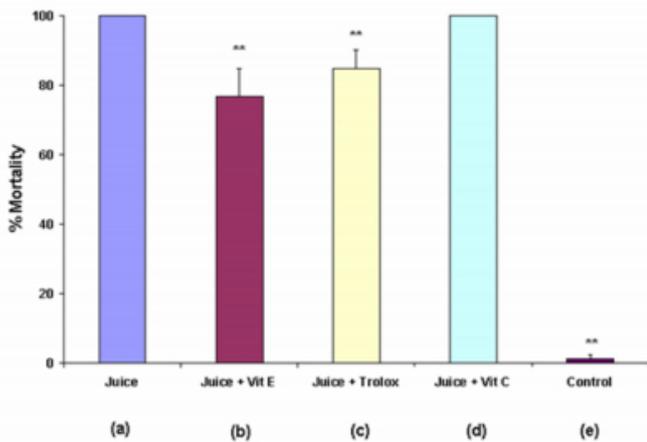
### EFFECTS OF ANTIOXIDANT CO-TREATMENT ON ALOE VERA JUICE TOXICITY

If the toxic effects of Aloe vera juice to *A. franciscana* are at least in part due to the induction of oxidative stress, then treatment of the *A. franciscana* nauplii with sub lethal doses of antioxidants would be expected to decrease the toxicity of the juice. The effects of simultaneous exposure of Aloe vera juice and various antioxidants are shown in Figure 4.

Vitamin C did not decrease the toxicity of Aloe vera juice in the *A. franciscana* bioassay. In effect, vitamin C appeared to enhance the toxicity of the juice. Studies within this laboratory have shown that vitamin C speeds up the toxic effect of the juice (unpublished data). In contrast, vitamin E was capable of decreasing Aloe vera gel toxicity by 23.3% ( $\pm 8.1\%$ ). The water soluble vitamin E analogue, Trolox™, also decreased Aloe vera juice toxicity, although to a lesser degree ( $15.3\% \pm 5.4$ ).

#### Figure 5

Figure 4: The effects of simultaneous treatment of antioxidants with Aloe vera juice on mortality in the *A. franciscana* nauplii bioassay. (a) 24 % Aloe vera juice was added to an equal volume of artificial seawater and 400  $\mu\text{l}$  of the mixture was tested in the bioassay. 400  $\mu\text{g/ml}$  solutions of (b) vitamin E, (c) Trolox™ and (d) vitamin C were added to equal volumes of 24 % Aloe vera juice and 400  $\mu\text{l}$  of each mixture was tested in the *A. franciscana* bioassay. (e) 400  $\mu\text{l}$  of artificial seawater was used as a control. All bioassays were performed in at least triplicate. \*\* indicates a statistically significant difference to treatment with juice alone.



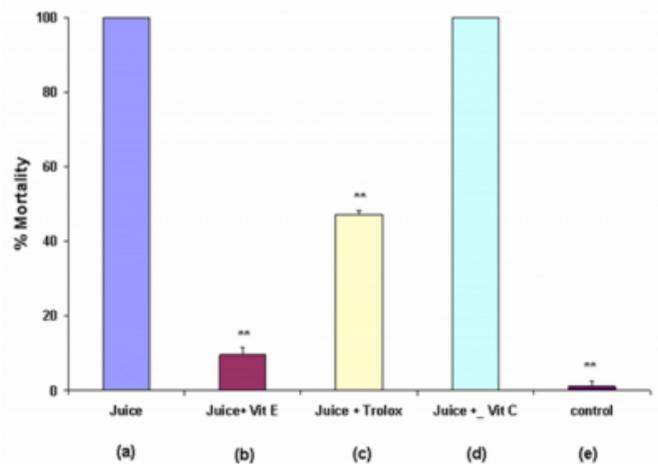
### EFFECTS OF ANTIOXIDANT PRE-TREATMENT ON ALOE VERA JUICE TOXICITY

Pre-treating *A. franciscana* nauplii with the vitamin antioxidants proved more effective at blocking the toxicity of Aloe vera juice than simultaneous treatment. Figure 5 shows the decrease in Aloe vera juice induced toxicity when

*A. franciscana* nauplii were pre-treated with the antioxidants for 4 h prior to addition of Aloe vera juice. As with the antioxidant co-treatment, vitamin C was ineffective at blocking Aloe vera juice toxicity. Indeed, vitamin C accelerated the onset of toxicity of Aloe vera juice (unpublished data) to *A. franciscana* nauplii. Vitamin E proved extremely effective at blocking Aloe vera juice toxicity at the doses tested. Pre-treatment of *A. franciscana* nauplii with vitamin E resulted in a  $90.4\% \pm 2.1$  decrease in Aloe vera juice toxicity. Trolox™ was also effective at blocking toxicity, reducing *A. franciscana* mortality by  $52.8\% \pm 0.9$ .

#### Figure 6

Figure 5: The effects of antioxidant pre-treatment on Aloe vera induced toxicity in the *A. franciscana* bioassay. 200  $\mu\text{l}$  of (b) vitamin E, (c) Trolox™ and (d) vitamin C were added to 400  $\mu\text{l}$  of artificial seawater containing *A. franciscana* nauplii and incubated at 25  $^{\circ}\text{C}$  for 4 h. 200  $\mu\text{l}$  of seawater was added to the (a) positive juice control and the (e) negative control. Following the 4 h pre-treatment, 200  $\mu\text{l}$  of 24 % Aloe vera juice was added to the juice control and to each test and the mortality was monitored over time. 200  $\mu\text{l}$  artificial seawater was added to the negative control instead of Aloe vera juice. All bioassays were performed in at least triplicate. \*\* indicates a statistically significant difference to treatment with juice alone.



### DISCUSSION

The current study demonstrated the ability of Aloe vera juice to induce mortality in *A. franciscana*. Aloe vera juice exposure resulted in acute toxicity, being capable of inducing mortality at dilutions as low as 4 % juice, with an  $\text{LC}_{50}$  at 24 h of  $4.6\% \pm 0.3$ . At 6 % dilutions, Aloe vera juice was capable of causing 100 % mortality within 4 h of exposure to *A. franciscana*.

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Many studies have reported on the antioxidant and pro-oxidant potential of *A. barbadensis* extracts [ 15,23 ]. The current study has demonstrated the ability of Aloe vera juice to alter redox state. Aloe emodin in particular has high inhibitory free radical scavenging activity [ 13 ] and can inhibit linoleic acid peroxidation [ 2 ]. However, other studies have also reported on the toxic effects of Aloe vera components [ 8,9 ]. Recent studies within this laboratory have confirmed toxic effects of Aloe vera leaf gel components against *A. franciscana* and a broad spectrum of microbial agents (unpublished data).

These seemingly conflicting reports on the protective/toxic effects of Aloe vera components may be due to the different concentrations used in these various studies. Tian and Hua [ 14 ] have reported on the concentration dependence effects of two common Aloe vera components. Aloin has a pro-oxidant effect at low concentrations and had an antioxidant effect at higher concentrations. In contrast, aloe emodin was shown to function as a pro-oxidant only at high concentrations. Thus, the acute toxicity induced by Aloe vera juice in the current study may be due to a relatively high level of aloe emodin and/or aloin present in the juice. Interactions between the various components within the crude juice may also play a role in converting otherwise antioxidant molecules into pro-oxidants in the juice.

Similarly, other well studied antioxidants have also been shown to have opposing effects at different concentrations. Previous studies have shown the therapeutic effect of many vitamin antioxidants [ 16,24 ], whilst other work indicated that these antioxidants may be toxic [ 18 ]. As for the Aloe vera active phenolics, the antioxidant/pro-oxidant effects of these vitamins also seem to be dependent on their concentrations. Tafazoli et al. [ 16 ] have shown that a variety of vitamin E analogues, including  $\alpha$ -tocopherol and Trolox™, behave as antioxidants at low concentrations and convert to pro-oxidants as the concentration increases. Trolox™ has also been shown to have direct therapeutic effects in *Lumbriculus variegatus* being capable of blocking copper toxicity at low concentrations whilst itself being toxic at high doses [ 24 ].

The current study demonstrates the toxicity of high doses vitamin C and vitamin E (and its analogue Trolox™) in *A. franciscana* nauplii. Exposure to high doses of vitamin C was particularly effective at inducing *A. franciscana* nauplii mortality. This may be due to causes other than the conversion of vitamin C from a free radical scavenger to an electron donor. Studies within this laboratory have shown *A.*

*franciscana* nauplii to be sensitive to pH changes (unpublished data). The addition of vitamin C in the doses used in these experiments resulted in a pH decrease of up to 2 pH units. Thus, it was likely that the mortality induced by vitamin C in these experiments was due to the pH decrease associated with its addition in this system.

Whilst vitamin E exposure did not induce the level of mortality seen for vitamin C, vitamin E was itself toxic to *A. franciscana* nauplii at high concentrations (320  $\mu\text{g/ml}$ ). This lethality was slow in its onset, taking 72 h to become evident (compared to mortality for 1250  $\mu\text{g/ml}$  vitamin C becoming evident within 2 h). Concentrations of vitamin E below 100  $\mu\text{g/ml}$  were not lethal to *A. franciscana* nauplii, even at these extended times. The toxic effect seen for vitamin E was likely due to a conversion of vitamin E from a free radical scavenger to an electron donor. Vitamin E is the major lipid soluble antioxidant of biomembranes. Its antioxidant activity is dependent upon its ability to donate hydrogen from a hydroxyl group on its chromone ring to free radicals [ 25 ], thus reducing membrane lipid peroxidation. However, under some conditions (eg. at high concentrations) vitamin E may convert to pro-oxidant activities and itself induce oxidative stress [ 16 ].

Trolox™ was also toxic to *A. franciscana* nauplii in the higher doses tested. Trolox™ is a phenolic antioxidant originally designed as a food preservative due to its free radical trapping capability [ 26 ]. Its structure is similar to that of  $\alpha$ -tocopherol but lacks the hydrophobic tail, making it the more hydrophilic than other vitamin E analogues. Like vitamin E, the ability of Trolox™ to function as either an antioxidant or a pro-oxidant is concentration dependent, with higher concentrations favouring a pro-oxidant function [ 26 ]. In their 2005 study, Tafazoli et al. [ 16 ] revealed Trolox™ to be a substantially more effective pro-oxidant than vitamin E. The results reported in the current paper support Trolox™ inducing oxidative stress more effectively than vitamin E. Trolox™ was capable of inducing mortality in *A. franciscana* nauplii in a much shorter time (4 h) compared to vitamin E (72 h) at the same concentration.

Although both vitamin E and Trolox™ were toxic to *A. franciscana* nauplii at high concentrations, this study also demonstrated the ability of vitamin E and Trolox™ to reduce or protect *A. franciscana* nauplii against Aloe vera induced oxidative stress at lower concentrations. Both vitamin E and Trolox™ were able to block Aloe vera juice induced toxicity when they were added to the *A. franciscana*

nauplii simultaneously with the juice. This ability to block the juice induced oxidative stress indicated the ability of vitamin E (or Trolox™) to scavenge free radicals in/induced by the juice. Vitamin E provided a more effective protectant than Trolox™ at the dose tested (100 µg/ml), being capable of blocking 23.3% ( $\pm$  8.1%) mortality. The water soluble vitamin E analogue, Trolox™ (100 µg/ml), also decreased Aloe vera juice toxicity, although to a lesser degree (15.3%  $\pm$  5.4). Whether this difference in levels of protection of the vitamin E analogues was due to differences in their mechanisms of action was not established in these tests. It was possible that structural differences may make Trolox™ more susceptible to conversion from an antioxidant to a pro-oxidant and thus less effective at blocking lethality.

Vitamin E and Trolox™ proved more effective at blocking oxidative stress induced mortality when the *A. franciscana* nauplii were exposed to the antioxidant for 4 h prior to addition of the Aloe vera juice. Vitamin E pre-treatment resulted in a 90.4%  $\pm$  2.1 decrease in mortality (compared to a 23.3%  $\pm$  8.1 decrease for simultaneous treatment). Similarly, Trolox™ pre-treatment blocked 52.8 %  $\pm$  0.9 mortality, a significant decrease in mortality from the 15.3 % ( $\pm$  5.4) decrease seen for simultaneous treatment. Presumably the antioxidants are more effective given time to enter the cells. This is not surprising, given the role of vitamin E analogues in blocking cell membrane lipid peroxidation. The more hydrophobic nature of vitamin E compared to Trolox™ may mean that it may more effectively interact with membrane lipids, accounting for its greater effectiveness at blocking Aloe vera juice induced oxidative stress.

In conclusion, the current study demonstrated the toxicity of Aloe vera juice towards *A. franciscana* nauplii. The identity of the toxic components of the juice are not reported here but previous studies in this laboratory (unpublished data) indicated that a number of toxic compounds, including aloe emodin, may be responsible for this toxicity. This work provide further support that these toxic components exert an effect through the induction of oxidative stress, which could be blocked/counteracted by antioxidants.

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