Frontiers in Life Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tfls20

Investigation of the effect of K. alvarezii on antioxidant enzymes, cell viability and DNA damage in male rats

N. Nagarani [a] & A. K. Kumaraguru [a]

[a] Department of Marine & Coastal Studies , School, of Energy, Environment and Natural Resources, Madural Kamaraj University , Madurai , TN , 625021 , India

To cite this article: N. Nagarani & A. K. Kumaraguru (2012) Investigation of the effect of K. alvarezii on antioxidant enzymes, cell viability and DNA damage in male rats, Frontiers in Life Science, 6:3-4, 97-105, DOI: 10.1080/21553769.2013.811123

To link to this article: http://dx.doi.org/10.1080/21553769.2013.811123

Frontiers in Life Science, 2012
Vol. 6, Nos. 3–4, 97–105, http://dx.doi.org/10.1080/21553769.2013.811123

Taylor & Francis
Taylor & Francis Group

Investigation of the effect of *K. alvarezii* on antioxidant enzymes, cell viability and DNA damage in male rats

N. Nagarani* and A.K. Kumaraguru

Department of Marine & Coastal Studies, School, of Energy, Environment and Natural Resources, Madural Kamaraj University, Madurai, TN 625021, India

(*Received 21 April 2012; final version received 30 May 2013*)

In recent years, a significant number of novel metabolites with potent pharmacological properties (antioxidant, antitumor, anti-HIV) have been discovered from marine organisms. *Kappaphycus alvarezii* (Doty) is a marine alga belonging to order: Gigartinales and family: Solieriaceae. It has been invasive in the Gulf of Mannar since 2000, and has a high content of the steroid phenol. This is the first report on its antioxidant properties using an animal model. This study aimed to explore the antioxidant and antigenotoxic/protective role of *K. alvarezii* growing in southeast coast of India in the male rat. The findings indicate that an extract of *K. alvarezii* is not nephrotoxic at the dose levels. The algal antioxidant activity shows similar correlation (89%; $R^2 = 0.8963$) at varying temperature to the known antioxidant. *K. alvarezii* extracts exhibit significant protective effects against DNA damage induced by H_2O_2, which might be related to antioxidant activity. In conclusion, our results suggest that long-term daily administration of *K. alvarezii* extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and cell damage. Our results support the use of *K. alvarezii* in the food and pharmaceutical industries.

Keywords: antigenotoxicity; antioxidant; comet assay; DPPH; *Kappaphycus alvarezii*; Gulf of Mannar

Introduction

Kappaphycus alvarezii (Doty 1973) (Rhodophyta, Gigartinales), synonymously known as *K. cottonii*, is a red alga. This alga is an introduced species and a noxious aquatic weed in Hawaii. However, recent molecular studies have revealed the most abundant species in Hawaii reported to cause an invasion on the coral reefs is actually *Eucheuma denticulatum* and not *K. alvarezii* (Zucarello et al. 2006). It is one of the most important sources of carrageenan used in a variety of commercial applications, e.g. gelling, thickening, and stabilizing agents, especially in food products such as frozen desserts, chocolate milk, instant products, yogurt, jellies and in sauce preparation. Aside from these functions, it is used in pharmaceutical formulations, cosmetics, and industrial applications such as mining (Hayashi et al. 2007). *K. alvarezii* has been introduced into many different tropical places, since it is the main source of carrageenan in the world, coastal populations can benefit from its cultivation. Most notably, it is increasingly being cultivated on the southeastern coasts of India.

Kappaphycus spp. have been reported to display antitumor activity (Huamao & Song 2005), heavy metal chelation (Suresh Kumar et al. 2007, 2008a), nitrate reductase activity (Granbom et al. 2004), *in vivo* antioxidant activities (Nagarani & Kumaraguru 2012), and *in vitro* antioxidant activities (Chew et al. 2008; Suresh Kumar et al. 2008b). Phytochemical studies on species demonstrated the presence of high protein, steroids, carotenoids, vitamins and minerals, etc. (Rajasulochana et al. 2009; Nagarani and Kumaraguru 2012).

There have been insufficient phytochemical and animal studies conducted on *K. alvarezii*. The present study aimed to gather further knowledge regarding the effects of *K. alvarezii* extract on vertebrates, in order to establish possibilities for its pharmacological use. Furthermore, the present study evaluated the protective effects of the alga against cytotoxicity using trypan blue exclusion. Single-cell gel electrophoresis (comet) assay was used to evaluate the genotoxicity and antigenotoxicity of *K. alvarezii*. Thus, the aim of the present study was to elucidate the potential beneficial role of marine algae as a food using the male rat as a model animal.

Materials and methods

Sample preparation

The marine alga *Kappaphycus alvarezii* was collected during August 2010, from the Mandapam coast (latitude 9°17′ N, longitude 79°22′ E), Gulf of Mannar. The sample was identified at the Center for Marine and Fisheries Research

*Corresponding author. Email: nagaranikannan@yahoo.co.in

Institution (CMFRI), Mandapam and the voucher specimen (DMCSKA01) was maintained in the Department Museum.

Algal extract preparation

The thalli of *K. alvarezii* were cut into pieces, sun dried and powdered in a grinder to 40-mesh size powder. The algae were extracted with water, vacuum dried and stored at room temperature (stable up to 100°C; Nagarani & Kumaraguru 2012) or 20°C until use. The extract was filtered through Whatman No. 4 filter paper to obtain particle free extract. The residue was re-extracted twice and filtered (to elute/collect the excess suspended molecules). The extracts were pooled, concentrated and dried under a vacuum and the dried extract was used for exploring its potential activity.

Phytochemical screening

Phytochemical screening of various extracts was carried out according to the standard methods as described by Trease and Evans (1996) and Harborne (1973) for alkaloids, tannins, flavonoids, steroids, saponins and cardiac glycoside.

Inhibition of DPPH radical

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Koleva et al. 2002). One milligram per milliliter concentrations of purified extract were added to an equal volume of methanolic solution of DPPH (100 mM). After 15 minutes at room temperature, the absorbance was measured at 517 nm. The experiment was repeated three times. Buthylated hydroxytolune (BHT) and vitamin C were used as standard controls. The stability of the *K. alvarezii* extract was determined at temperatures of 25, 50, 75, and 100°C. Briefly the extract was incubated at different temperatures for 30 min, and then subjected to the assay (Rehman et al. 2003).

Preclinical studies

Adult male albino rats of Wistar strain (240 ± 20 g) were obtained from Animal Behavior Department, School of Biological Sciences, Madurai Kamaraj University, Madurai, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2°C) and 12 h light/1 2h dark condition. They were fed standard pelleted rat diet (Amrut rat/mice feed M/s Pranav Agro Industries, Sangli-Maharastra, India) and were given free access to water. Rats received humane care in compliance with the guidelines of the University and Institutional Legislation, regulated by the International Research and Review Board (IRB), Ethical Clearance (EC), Biosafety and Animal Welfare Committee.

The experimental animals were randomly divided into three groups as follows: Group A received an appropriate volume of water by intraperitoneal injection on alternate days. This served as the control. Group B received an appropriate volume of the extract by intraperitoneal injection on alternate days at a curative dose of 100 mg kg^{-1} body weight. Group C was given an intraperitoneal injection with 200 mg kg^{-1} body weight of aqueous extract of *K. alvarezii* on alternate days. The experiment was conducted for 60 days. Five rats in each group were sacrificed 24 h after the last dose. The livers were removed, washed in phosphate buffer and stored at −80°C for enzyme analysis. The chosen dosage is based on extrapolation from estimated exposure levels for humans as described by Llewellyn et al. (1998).

Biochemical oxidative stress analysis

Livers and blood were washed with saline, and homogenized by Tris-HCl buffer. Supernatant fractions of liver homogenate were used for measuring activities of the reduced glutathione (GSH) (Kuo et al. 1983), glutathione peroxidase (GPx) (Lawrence & Burk 1976), superoxide dismutase (SOD) (Marklund & Marklund 1974), catalase (Aebi 1974) and lipid peroxidation (Ohkawa et al. 1979).

Cell viability assay

Dye exclusion is a simple and rapid technique measuring cell viability but it is subject to the problem that viability is being determined indirectly from cell membrane integrity. Thus, it is possible that a cell's viability may have been compromised (as measured by capacity to grow or function) even though its membrane integrity is (at least transiently) maintained. Conversely, cell membrane integrity may be abnormal yet the cell may be able to repair itself and become fully viable. Viability of the muscle cell was counted using trypan blue exclusion method (Anderson et al. 1994) ($n = 5$/exposure).

$$\text{Viable cells}(\%) = \frac{\text{total number of viable cells per ml of aliquot}}{\text{total number of cells per ml of a aliquot}} \times 100$$

Preparation of oxidatively damaged red blood cells

Samples of venous (2 ml per animal) blood were obtained from the male rat using heparin as the anticoagulant. After centrifugation to remove plasma and leukocytes, the red blood cells (RBCs) were washed once with phosphate buffered saline (PBS: 5 mmol l^{-1} phosphate, 0.15 mol l^{-1} NaCl, and 5 mmol l^{-1} dextrose, pH 7.4) and resuspended to a final hematocrit of 20% in PBS with 2 mmol l^{-1} sodium azide to inhibit catalase. After incubation at 30°C for 10 minutes, measured quantities of H_2O_2 were added to the

RBC suspension to obtain final concentration in the range of 100 and 200 μmol l^{-1}, and the cells were incubated for an additional 15 minutes at 4°C to avoid repair of the induced oxidative DNA damage (Collins et al. 2004). The oxidative reaction was quenched by washing the cells with PBS containing 5 mmol l^{-1} dithiothreitol (DTE), followed by 5-minute incubation in the same buffer. Control RBCs underwent the same regimen with one exception: no H_2O_2 was added to these cells. H_2O_2 was added to the reaction vessel to initiate oxidant damage; this served as a negative control. The same toxicants were incubated with 100 and 200 ppm of algal extract separately, i.e. 0.1 and 0.2 mg ml^{-1} RBC.

Single-cell gel electrophoresis (SCGE) assay (comet assay)

The alkaline comet assay was performed according to the experimental design of Singh et al. (1998) and improved by Tice (1995). Briefly, microscope slides were dipped briefly into 1.5% hot (60°C) normal melting agarose prepared in phosphate-buffered saline (PBS). The slides were dried overnight at room temperature and then stored at 4°C until used. Subsequently, freshly collected, heparinized peripheral blood (20 μl) was suspended in 120 μl of 0.5% low melting point agarose in PBS at 37°C and pipetted onto a microscope slide pre-coated with a layer of normal melting point agarose. This mixture was allowed to set at 4°C for 10 min and the slides then immersed in a freshly prepared cold (4°C) lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris and 1% lauroyl sarcosine, and the pH adjusted to pH 10.0–10.5 with NaOH; 1% Triton X-100 and 10% dimethyl sulfoxide were added immediately before use) at 4°C for 1 h. After lysis, the slides were placed in an electrophoretic tank containing 300 mM NaOH and 1 mM EDTA, pH > 13.0 for 30 min, and electrophoresis was then done at 25V, 350 mA for 40 min. Soon after neutralization (3 × 5 min in 0.4 MTris, pH 7.5 at 4°C), the slides were stained with ethidium-bromide (20 μg ml^{-1}), fixed in 100% methanol for 5 min.

To evaluate the extent of DNA damage, images of 1000 randomly selected cells were analyzed from each sample using a Zeiss Cannon fluorescence microscope (filter 510–560 nm, barrier filter 590 nm) connected with a digital camera. Imaging was performed by using a computerized image analysis system (Comet Assay version 1.1) which acquires images, computes the integrated intensity profile for each cell, estimates the comet cell components (head and tail) and evaluates a range of derived parameters (Jaloszynski et al. 1997). All of the slides were prepared and analyzed in duplicate.

These include tail length, % DNA in migrated in comet tail and tail moment (an index of DNA damage which considers both tail length and the fraction of DNA in the comet tail). To check for toxicity or an effect on DNA, all the extracts were tested alone (without test chemical).

Scoring

There was a close relation between the subjective visual score and the measurements of the percentage of DNA in the tail by image analysis. In 90% of cells, the percentage of DNA in the tail for different visual grades of damage fell in the following non-overlapping ranges: grade 0 (no damage), < 5%; grade 1 (low damage), 5–25%; grade 2 (medium damage), 25–45%; grade 3 (high damage), 45–70%; grade 4 (very high damage), > 70%.

Randomly selected lymphocytes were graded visually for each slide. A total damage score as arbitrary unit (AU) for the slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells of grade 4) (Collins 2004).

Statistical analysis and correlations

The mean values and the standard deviations were calculated from the data obtained from three independent experiments. Statistical differences at $p < 0.05$ were considered to be significant. The coefficient of determination (r^2) was calculated using SPSS and Microsoft Excel.

Results

Phytochemical screening

Phytochemical screening study of *K. alvarezii* extract revealed that the extract had significant quantities of alkaloids, flavonoids, steroids, and tannins, and an absence of terpenoids. Total phenolic content was 28.47 mg GAE (standard, gallic acid equivalent)/100 g dw; protein was 1.623 ± 0.04 mg g^{-1} fresh weight; carotene was 6.24 mg/100 g.

The bioactive fractions against DPPH free radicals were dried. The extract was characterized by thin layer chromatography using silica gel coated TLC plates (Merck) with mobile phase, ethyl acetate: chloroform: hexane 2:9:3. Chromatograms were evaluated under UV light at 600–660 nm to detect the presence of steroids. The presence of steroid if any was further confirmed by spraying the plates with hot vanillin in sulfuric acid.

Residues of 30 mg were dissolved in acetone for proton NMR studies. The residue of *K. alvarezii* has a bunch of signals in the aliphatic region of the ^{1}H NMR spectrum (Figures 1(a) and 1(b)). As there were no responsible signals in the aromatic or olefinic region, the presence of flavonoids in the extract was safely ruled out. Absence of signal in the regions between 4 and 6 indicated the absence of glycosides or unsaturated terpenoids. Obviously the signals in the region 0.5–2.0 ppm suggest the presence of steroidal identity in the extracts. The signal around 3.8 ppm could be due to the hydrogen geminal to oxygen probably hydroxyl

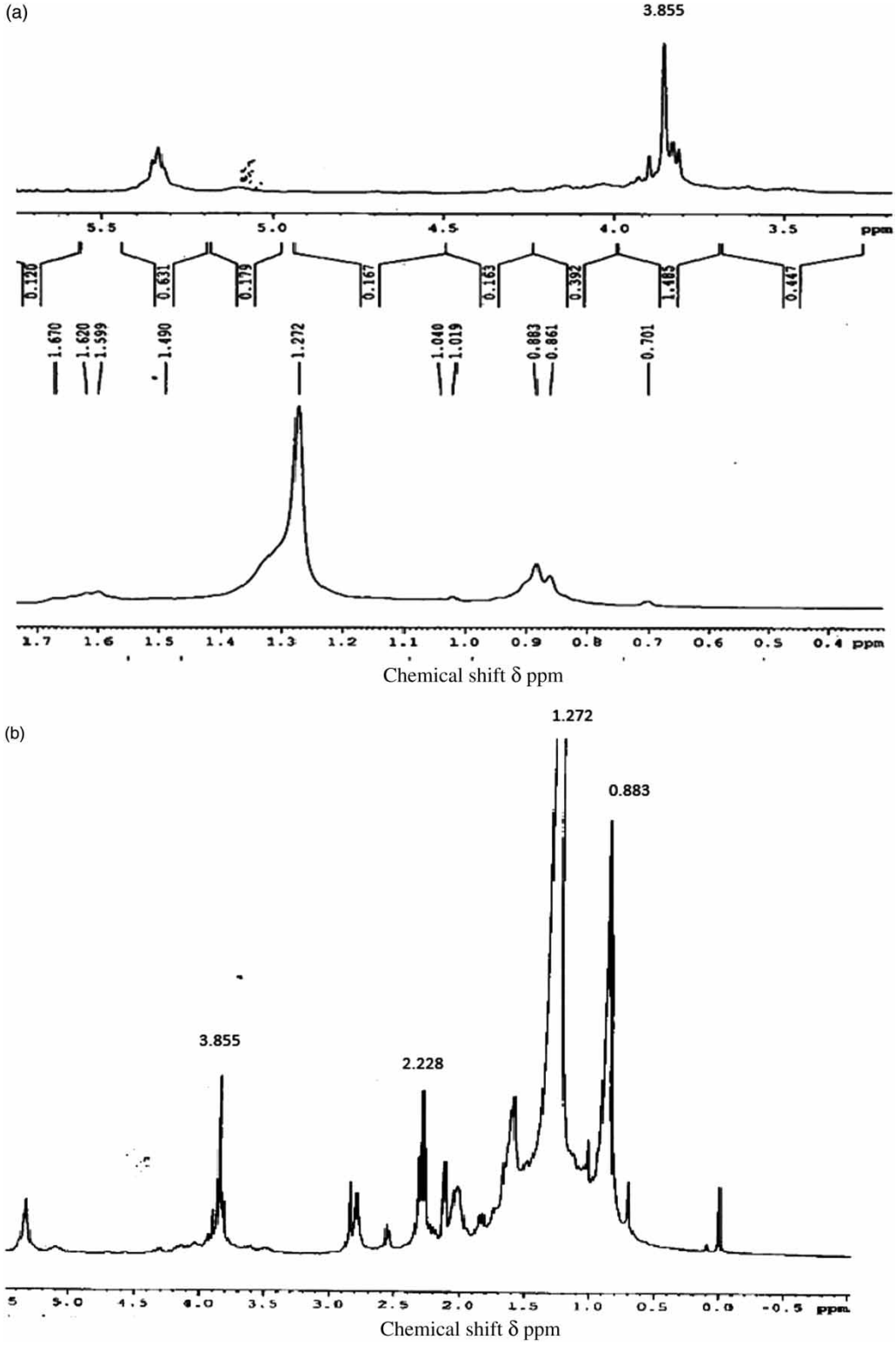

Figure 1. Proton NMR of *K. alvarezii* methanolic extract.

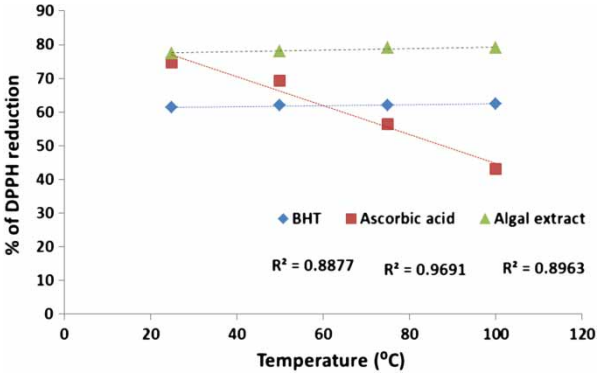

Figure 2. Effect of alga against DPPH free radicals at different temperatures.

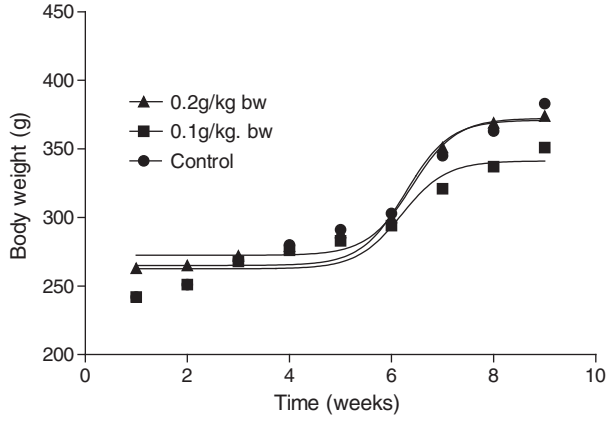

Figure 3. Body weight gain of rats in exposed to algal extracts. Values are means of five animals and error bars are not shown. No statistical difference between treatment and control groups.

group present in the steroidal nucleus. From the spectral data it was concluded that the compounds exhibiting antioxidant activity are probably oxygenated steroids. The oxygenated sterols of *K. alvarezii* exhibited 69% of inhibition against the DPPH radical.

The DPPH scavenging activity of *Kappaphycus alvarezii* extract is presented in Figure 2. The minimum concentration (1 mg ml^{-1}) of the extract that exhibited maximum (< 80%) free radical scavenging activity was chosen for the animal experimental study. The algal antioxidant activity shows similar correlation (89%; $R^2 = 0.8963$) at varying temperature to the known antioxidant (BHT), which confirms the stability of the extract.

Body weight

The aqueous extract of *K. alvarezii* was found to be practically non-toxic (no lethal effect and observed abnormalities) and its LD_{50} could not be calculated since the treatment did not cause any death response. LD_{50} values were previously calculated for five doses ranging from 50, 100, 150, 200 and 250 mg kg^{-1} body weight. The animals treated with the extract did not manifest any overt signs of toxicity and there was no significant difference in the weight of the treated animals compared to the control.

The body weight gain curves of rats are shown in Figure 3, where growth curves of alga-treated rats are compared with those of control groups. There were no statistically significant ($p < 0.05$) differences in the body weight loss of rats in treatment and corresponding control groups. The mean body weight of rats was 242 ± 4.2 g and 383 ± 3.6 g of initial and final control rats. There was no significant difference in food and water intake of rats in both control and treated groups.

Effect of algae on serum and liver antioxidant status of experimental groups versus control group

Oxidative stress parameters, such as thiobarbituric acid reactive substances (TBARS) in rat declined following algal treatment in comparison to control groups. Result of alga on lipid peroxidation (LPO) in rat indicated a significant decrease in the hepatic LPO with a concomitant increase in glutathione (GSH) and catalase (CAT) activities (Table 1). Given its role in the antioxidant defense system, catalase activity was expected to increase. Activity was only detected in liver tissue, and no significant change in activity occurred in serum. When alterations in lipid peroxidation were examined, a significant decrease in serum LPO (42.8 and 84.4%, in group B and C to that of group A; $p < 0.01$) and SOD (4–1.2% in serum ($p < 0.0001$) and liver ($p < 0.0001$) respectively in group B (Table 1), followed by an increase in the activities of CAT (61.3%) and total GSH content (89.89%, $p < 0.0001$) as a result of algal administration, suggesting its antioxidative property (Table 1).

Catalase activity in rat serum did not change significantly in response to algal treatment from that of the control rats. The increase in CAT whole blood was not remarkable after treatment for 60 days ($p < 0.0001$); however, after treatment for 60 days, the rat showed a marked increase in CAT activity in the liver ($p < 0.5$). There was significantly more ($p < 0.0001$) GPx activity in serum than in control (Table 1).

Cell viability

Relationship between percentages of tail DNA measured using Comet Score version 1.1 software and visual scores of oxidative DNA damage representing each class (0–4) were measured and visually scored by the same operator. The mean comet tail with respect to the percentage of DNA damage was represented in Figure 5. Consequently the comet class 0 represents > 5% damage, Class 1 (20%), Class 2 (35%), Class 3 (57%) and Class 4 with 85% mean values (where $Y = 19.3x - 17.9$; $R^2 = 0.9064$), respectively (Figures 5 and 6).

Table 1. Oxidative stress analysis of *K. alvarezii* treated male rat ($n = 5$) (mean ± SE).

	LPO (mM cm^{-1} of MDA)	SOD (units/mg of protein)	CAT (units/mg of protein)	GSH (nmole)	GPx (units/mg of protein)
Serum					
Control	6.28 ± 0.1*	336.3 ± 5.9	7.19 ± 0.19	1361.6 ± 3.84	48.0 ± 1.5
Dose I	4.44 ± 0.47	304.3 ± 7.1	7.10 ± 0.15	1053.3 ± 38.2*	144.0 ± 3.2
Dose II	1.18 ± 0.11*	244.6 ± 2.6	7.24 ± 0.20*	1012.3 ± 12.6	159.0 ± 2.7
Liver					
Control	1.30 ± 0.18*	265.6 ± 6.8	4.79 ± 0.27	1247.6 ± 8.96*	131.2 ± 1.6
Dose I	1.06 ± 0.06*	255.0 ± 4.5	6.05 ± 0.10	976.6 ± 17.4	91.3 ± 1.8
Dose II	0.98 ± 0.13	166.0 ± 6.8	8.17 ± 0.12	675.0 ± 5.77	40.01 ± 0.9

Dose I = 100 mg kg^{-1} of body weight; dose II = 200 mg kg^{-1} of body weight.
*$p < 0.05$, whereas other $p < 0.0001$ within groups.

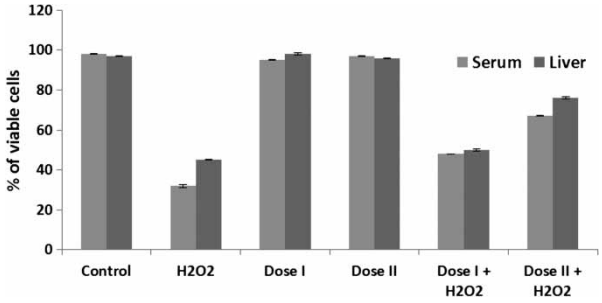

Figure 4. Cell viability assay.

The cytotoxicity studies on the viability of the cells are depicted in Figure 4. The data clearly indicate that hydrogen peroxide is cytotoxic by providing free radicals to the *in vitro* environment. We observed only 35% of viable cells with less tail formation, i.e. DNA fragmentation ($p = 0.075$) (Figure 7). No significant cell death was noticed in either algal dose. The algal extract also increased the viability of the cells treated with hydrogen peroxide. In support to the viable of the blood cells the frequency of DNA damage was higher on hydrogen peroxide exposure (< 80%) and algal extract exposed cells dose not showed any significant changes in induction of single strand breakage, only class 0 and 1 were mostly observed during scoring. The sudden drop in DNA damage induction was observed in both algal concentrations ($p < 0.05$).

Discussion

The additive and synergistic effects of phytochemicals in seaweeds have been proposed as being responsible for their potent antioxidant and anti-cancer activities. Among the compounds detected in the *Kappaphycus alvarezii* extracts are carrageenan, steroids (Nagarani & Kumaraguru 2012), tannins, phenolic acids and flavonoids, which have been described as antioxidant agents when assessed separately (Rajasulochana et al. 2009).

The NMR data of *K. alvarezii* showed the presence of a steroid group unlike that reported by Vallinayagam et al. (2009) on proton-NMR signals corresponding to polyunsaturated esters (7.263 ppm, 5.371 ppm and 1.254 ppm in *Sargassum wightii*; 7.239 ppm, 5.322 ppm and 1.227 ppm in *Gracilaria edulis*), and poly-saturated alcohol (7.23 ppm 1.256 and 1.226 ppm, 0.827 ppm in *Padina gymnospora*). These signals were absent in the *K. alvarezii* NMR data, confirming the presence of oxygenated steroidal identity and the absence of all other groups as reported by Vallinayagam et al. (2009). Sterols are important structural components of cell and organelle membranes of higher organisms. They regulate membrane fluidity and permeability as well as membrane-associated metabolic processes. Oxysterols and oxyphytosterols, as present in the human body, may be derived from absorption of oxidized sterols present in food, as well as from endogenous origin (Sanders et al. 2000). Oxysterols have been ascribed a number of important roles in connection with antiperoxidation, cholesterol

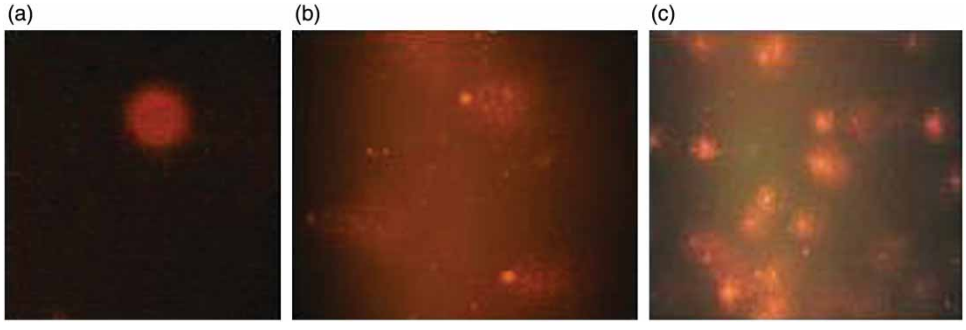

Figure 5. Photos of comets observed during SCGE showing: (a) control DNA; (b) class IV comets; (c) cell burst.

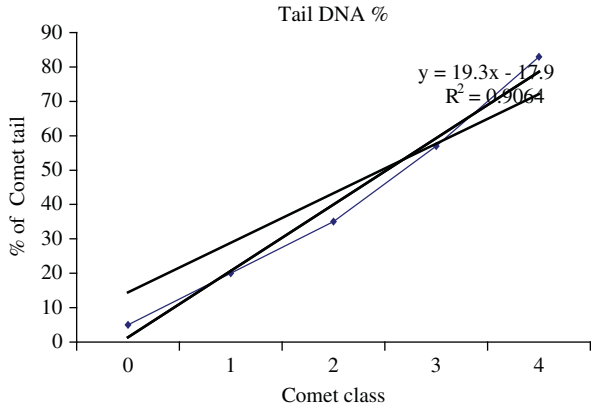

Figure 6. Comet class with respect to the percentage of comet tail during visual scoring.

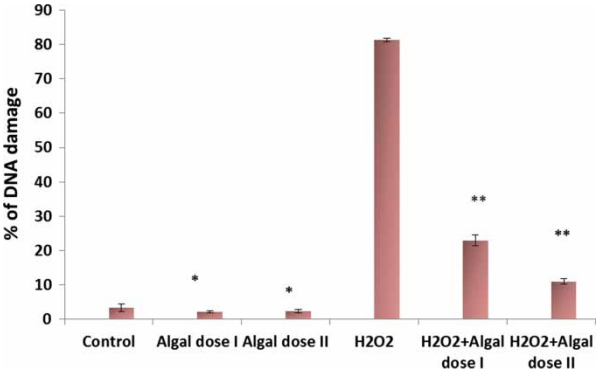

Figure 7. Comet assay scores for rat blood cells treated with *Kappaphycus alvarezii* extract and/or 100 μmol of hydrogen peroxide. For each treatment 1000 nuclei were scored ($n = 1000$ cells/treatment).*Significant difference related to the control groups for damage frequency at $p < 0.05$; **$p < 0.001$ (one-way ANOVA followed by Tukey test).

turnover, atherosclerosis, apoptosis, necrosis, carcinogenesis, inflammation, immunosuppression and development of gallstones (Panda et al. 2009).

The measurement of radical scavenging activity of any antioxidant is commonly determined by the DPPH method since it is a quick, reliable and reproducible method to assess the *in vitro* antioxidant activity of pure compounds as well as plant extracts *in vivo*. The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule. Algal extracts showed highly significant scavenging activities by reducing the stable radical $DPPH^{\cdot}$ to yellow-colored diphenyl picrylhydrazine. This could be explained by their scavenging ability to donate a hydrogen atom from their hydroxyl groups. A linear correlation between DPPH scavenging activity and temperature variation showed a statistically significant correlation ($R^2 = 0.8963$) with natural (vitamin C; $R^2 = 0.9691$) and synthetic antioxidant (BHT; 0.8877). These data are in accordance with earlier research (Park et al. 2009; Mukherjee et al. 2011; Salar & Seasotiya 2011), suggesting that a high temperature does not influence antioxidant activity in *K. alvarezii*.

Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids (Valko et al. 2006).The reduction of MDA in *K. alvarezii* groups at the end of the study might represent antioxidative augmentation and prove that this alga has excellent antioxidant activity, as previously measured (Nagarani & Kumaraguru 2012). The results of the study suggested that *K. alvarezii* consumption may decrease lipid peroxidation induced by free radicals.

Determinations of the *in vivo* antioxidant activities of alga in rat liver and serum are presented in Table 1. Both SOD and CAT play an important role in defense mechanisms against the harmful effects of reactive oxygen species (ROS) and free radicals in biological systems. GSH content was another important parameter that revealed oxidative damage in both liver and serum, and TBARS are cytotoxic products that are a hallmark of lipid peroxidation. The fact that *K. alvarezii* reduced TBARS, SOD, and GSH, and increased CAT and GPx levels to their control levels indicated that it may prevent the peroxidation of lipids by its antioxidant properties.

SOD was found to be decreased in the treated animal's serum and liver. SOD is considered to be a stress protein which is synthesized in response to oxidative stress (McCord 1990). The low level of SOD in the animal might be due to the absence of oxidative stress caused by the extract. An increase in the level of SOD activity leads to various diseases. Significantly high SOD was observed in breast cancer (Kokaglu et al. 1989) and in Hodgkin's disease (Abdel-Aziz & El-Naggar 1997). SOD plays an important role in the elimination of ROS and protects cells against the deleterious effects of super oxide anions derived from the peroxidative process in liver and kidney tissues (Fridovich 1995), and the observed decrease in SOD activity suggests that the *K. alvarezii* has an efficient protective mechanism and does not induce any toxic mechanism.

The increase in catalase activity is crucial in counteracting the accumulation of H_2O_2 and thus suppressing the formation of the very potent and reactive OH radical. The significant decrease in superoxide dismutase activity of these rats was intriguing and is probably due to the ability of antioxidant compounds in *K. alvarezii* to scavenge free radicals and therefore decrease generation of ROS and lower oxidative stress (Hussin et al. 2007; Nagarani & Kumaraguru 2012). The reduced level of catalase and glutathione peroxide might be due to the excess production of anions in response to the extract of *K. alvarezii* as previously reported in *Terminalia chebula* (Sarkar et al. 2012). Glutathione (GSH) concentration was also reduced in the animals treated with the extract. It is suggested that the utilization of glutathione by glutathione peroxidase (GPx) catalyses the oxidation of GSH to GSSG (Gibanananda & Hussain 2002). This oxidation reaction occurs at the expense of H_2O_2.

Cell viability measurements assess healthy cells in a sample. Cells that take up a dye are pronounced nonviable, whereas those that do not are viable. A high viability is regarded by many as a prerequisite for the comet assay. However, trypan blue does not measure viability, but simply indicates whether cell membranes are intact. Cells with damaged membranes are trypan blue positive, but recover and survive (Collins 2004; Jha 2008). Dead or dying cells can undergo rapid DNA fragmentation, which should be expected to increase DNA migration in the comet assay. It is therefore mandatory to perform concurrent cell viability test. According to Hartmann et al. (2003) it is important to measure cytotoxicity when screening for genotoxicity with the comet assay, as the occurrence of cells with completely fragmented chromatin could lead to false-positive test results.

ROS can damage DNA and lead to mutation and chromosomal damage. *K. alvarezii* extracts exhibit significant protective effects against DNA damage induced by H_2O_2, which might be related to antioxidant activity. Similar reports were previously reported in *Sargassum wightii* (Josephine et al. 2008), *Ulva rigida and Fucus vesiculosus* (Cleide et al. 2007; Celikler et al. 2008).

Extensive efforts are being made to investigate therapeutic substances capable of reducing the genotoxicity of various natural and man-made mutagens in human life (Turkez et al. 2005; Turkez & Geyikoglu 2010). Concomitant treatment with antioxidants provided protection against oxidative damage by mutagens in experimental animals (Abubakar et al. 2003; Esparza et al. 2003; Geyikoglu et al. 2005; Turkez & Geyikoglu 2010). Qi et al. (2006) showed the strong antioxidant activity (*in vitro*) of high sulfate content in polysaccharide extracted from *Ulva pertusa*. Fabiani et al. (2008) reported that specific compounds (phenol), when used both as purified compounds and in complex crude extracts in olive oil, prevented H_2O_2-induced DNA damage.

Their interactions and possible synergistic effects may facilitate the protective effects observed. The present study showed that the use of crude extracts can be more advantageous than the use of isolated compounds. The interaction between phytochemicals in the extracts showed efficacy in reducing mutagenicity and improved the protective effects.

Conclusion

It is suggested that correct processing could be used to enhance the amount of bioactive compounds and antioxidant capacity of *K. alvarezii* extracts, which exhibit significant protective effects against DNA damage induced by H_2O_2, perhaps related to antioxidant activity. In conclusion, our results suggest that long-term daily administration of *K. alvarezii* extract offers enhanced antioxidant potential and protection against tissue lipid peroxidation and cell damage. However, further clinical studies are necessary to assess the benefits and safety of sulfated polysaccharide extracts before use in human beings, and approval by Food and Drug Administration (FDA) is needed before they are marketed.

Acknowledgements

N. Nagarani thanks MKU for financial support to complete this work. The authors are grateful to Dr Sripathi Kandula and Dr Selvam, School of Biological Sciences, Madurai Kamaraj University for their guidance in animal handling.

References

Abdel-Aziz AF, El-Naggar MM. 1997. Superoxide dismutase activities in serum and white blood cells of patients with some malignancies. Cancer Lett. 113:614.

Abubakar MG, Taylor A, Ferns GAA. 2003. Aluminium administration is associated with enhanced hepatic oxidant stress that may be offset by dietary vitamin E in the rat. Int J Exp Pathol. 84:49–54.

Aebi H. 1974. Catalase estimation. In: Bergmeyer HU, editor. Methods of enzymatic analysis. New York: Verlag Chemic, Weinheim; p. 673–684.

Anderson DT, Yu W, Phillips BJ, Schmezer P. 1994. The effect of various antioxidant and other modifying agents on oxygen-radical generated DNA damage in human lymphocytes in the comet assay. Mutat Res. 307:261–271.

Celikler S, Yildiz G, Vatan O, Bilaloglu R. 2008. *In vitro* Antigenotoxicity of *Ulva rigida* C. Agardh (Chlorophyceae) extract against induction of chromosome aberration, sister chromatid exchange and micronuclei by mutagenic agent MMC1. Biomed Environ Sci. 21:492–498.

Chew YL, Lim YY, Omar M, Khoo KS. 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol. 41:1067–1072.

Cleide L-S, Gusmão CLS, Takahashi CS. 2007. Genotoxic and antigenotoxic effects of *Fucus vesiculosus* extract on cultured human lymphocytes using the chromosome aberration and Comet assays. Genet Mol Biol. 30(1):105–111.

Collins AR. 2004. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol. 26:249–261.

Doty MS. 1973. Farming the red seaweed *Eucheuma* for carrageens. Micronesica. 9:59–73.

Esparza L, Gomez M, Romeu M, Mulero M, Sanchez DJ, Mallol J, Domingo JL. 2003. Aluminum-induced pro-oxidant effects in rats: protective role of exogenous melatonin. J Pineal Res. 35:32–39.

Fabiani R, Rosignoli P, Bartolomeo AD, Fuccelli R, Servili M, Montedoro GF, Morrozzi G. 2008. Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. J Nutr. 138:1411–1416.

Fridovich I. 1997. Superoxide anion radical, superoxide dismutases and related matters. J Biol Chem. 272:18515–18517.

Geyikoglu F, Turkez H, Keles MS. 2005. The role of fruit juices in the prevention of aluminum sulphate toxicity in human blood in vitro. Fres Environ Bull. 14:878–883.

Gibanananda R, Hussain SA. 2002. Oxidants. Ind J Exp Biol. 40:1213–1232.

Granbom M, Chow F, Lopes PF, de Oliveira MC, Colepicolo P, de Paula EJ, Pedersén M. 2004. Characterisation of nitratereductase in the marine macroalga *Kappaphycus alvarezii* (Rhodophyta). Aquat Bot. 78(4):295–305.

Harborne JB. 1973. Phytochemical methods. London: Chapman and Hall Ltd.; p. 49–188.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S. 2003. Recommendations for conducting the *in vivo* alkaline Comet assay. 4th International Comet Assay Workshop. Mutagenesis. 18:45–51.

Hayashi E, Paula JD, Chow F. 2007. Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical water of Sao Paulostate. Brazil J Appl Phycol. 19:393–399.

Huamao Y, Song J. 2005. Preparation, structural characterization and *in vitro* antitumor activity of *kappa*-carrageenan oligosaccharide fraction from *Kappaphycus striatum*. J Appl Phycol. 17:7–13. doi:10.1007/s10811-005-5513-8.

Hussin M, Abdul-Hamid A, Mohamad S, Saari N, Ismail M, Bejo MH. 2007. Protective effect of *Centella asiatica* extract and powder on oxidative stress in rats. Food Chem. 100: 535–541.

Jaloszynski P, Kujawsk M, Czub-Swierczek M, Markowska J, Szyfter K. 1997. Bleomycin-induced DNA damage and it's removal in lymphocytes of breast cancer patients studied by comet assay. Mutat Res. 385:223–233.

Jha AN. 2008. Review: ecotoxicological applications and significance of the comet assay. Mutagenesis. 23(3):207–221, 2008. doi:10.1093/mutage/gen014.

Josephine A, Nithya K, Amudha G, Veena CK, Preetha SP, Varalakshmi P. 2008. Role of sulphated polysaccharides from *Sargassum Wightii* in Cyclosporine A-induced oxidative liver injury in rats. BMC Pharmacol. 8:4.

Kokaglu E, Aktuglu G, Belce A. 1989. Leukocyte superoxide dismutase levels on acute and chronic leukemias. Leukemias Res. 13:457.

Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 13:8–17.

Kuo CH, Maita K, Sleight SD, Hook JB. 1983. Lipid peroxidation: a possible mechanism of cephaloridine-induced nephrotoxicity. Toxicol Appl Pharmacol. 67:78–88.

Lawrence R, Burk RF. 1976. Glutathioneperoxidase activity in selenium deficient rat liver. Biochem Biophy Res Comm. 71:952–958.

Llewellyn GC, McCay JA, Brown RD, Musgrove DL, Butterworth LF, Munson AE, White KL. 1998. Immunological evaluation of the mycotoxin patulin in female B6C3F1 mice. Food Chem Toxicol. 36:1107–1115.

Marklund S, Marklund G. 1974. Involvement of superoxide anion radical in the auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem. 47: 469–474.

McCord JM. 1990. Is superoxide dismutase a stress protein? In: Burbon R, Rice-Evans C, Blake D, Window C, editor. Stress protiens in inflammation. London: Richalien; p. 125.

Mukherjee S, Pawar N, Kulkarni O, Nagarkar B, Thopte S, Bhujbal A, Pawar P. 2011. Evaluation of free-radical quenching properties of standard Ayurvedic formulation Vayasthapana Rasayana. BMC Complement Altern Med. 11:38–30.

Nagarani N, Kumaraguru AK. 2012. Chemical characterization, temperature stability and enzymatic studies on edible marine algae *Kappaphycus alvarezii* (Doty). J Aquat Food Prod Technol. 21:480–492.

Ohkawa H, Ohishi N, Yagi K. 1979. Assays of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95:351–358.

Panda S, Jafri M, Kar A, Meheta BK. 2009. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*. Fitoterapia. 8: 123–126.

Park JH, Park YK, Park E. 2009. Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. Plant Foods Hum Nutr. 64:244–249.

Qi H, Zhang Q, Zhao T, Hu R, Zhang K, Li Z. 2006. In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). Bioorg Med Chem Lett. 16(9):2441–2445.

Rajasulochana P, Dhamotharan R, Krishnamoorthy P. 2009. Primary phytochemical analysis of *Kappaphycus* sp. J Am Sci. 5(2):91–96.

Rehman Z, Salariya AM, Habib F. 2003. Antioxidant activity of ginger extract in sunflower oil. J Sci Food Agric. 83:624–629.

Salar RK, Leena Seasotiya L. 2011. Free radical scavenging activity, phenolic contents and phytochemical evaluation of different extracts of stem bark of *Butea monosperma* (Lam.) Kuntze. Front Life Sci. 5(3–4):107–116.

Sanders DJ, Minter HJ, Howes D, Hepburn PA. 2000. The safety evalutation of phytosterol esters part 6. The comparat ive absorption and tissue distribution of phytosterols in the rat. Food Chem Toxicol. 38:485–491.

Sarkar R, Hazra B, Mandal N. 2012. Reducing power and iron chelating property of *Terminalia chebula* (Retz.) alleviates iron induced liver toxicity in mice. BMC Complement Altern Med. 12:144.

Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of levels of DNA damage in individual cells. Exp Cell Res. 175:184–191.

Suresh Kumar K, Ganesan K, Subba Rao PV. 2007. Heavy metal chelation by non-living biomass of three color forms of *Kappaphycus alvarezii* (Doty) Doty. J Appl Phycol. 20(1):63–66.

Suresh Kumar K, Ganesan K, Subba Rao PV. 2008a. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed. Food Chem. 107:289–295.

Suresh Kumar K, Ganesan K, Subba Rao PV. 2008b. Heavy metal chelation by non-living biomass of three color forms of *Kappaphycus alvarezii* (Doty) Doty. J Appl Phycol. 20:63–66. doi:10.1007/s10811-007-9181-8

Tice RR. 1995. The single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. In: Phillips DH, Venitt S, editors. Environ mutagenesis. Oxford: Bios Scientific Publishers; p. 315–339.

Trease GE, Evans WC. 1996. Pharmacognosy. 13th ed. London (UK): Bailliere Tindall; p. 282–396.

Turkez H, Geyikoglu F. 2010. The anti-genotoxic effect of taurine on aluminum sulphate induced DNA damage in human peripheral lymphocytes. IUFS J Biol. 69:25–32.

Turkez H, Geyikoglu F, Keles¸ MS. 2005. Biochemical response to colloidal bismuth subcitrate: dose-time effect. Biol Trace Elem Res. 105:151–158.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. 2006. Free radical metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 160:1–40.

Vallinayagam K, Arumugam R, Ragupathi Raja Kannan R, Thirumaran G, Anantharaman P. 2009. Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. Global J Pharmacol. 3(1):50–52.

Zuccarello GC, Critchley AT, Smith J, Sieber V, Lhonneur GB, West JA. 2006. Systematics and genetic variation in commercial *Kappaphycus* and *Eucheuma* (Solieriaceae, Rhodophyta). J Appl Phycol. 18:643–651.