Journal of Pharmacy & Pharmacognosy Research
E-ISSN: 0719-4250
editor@jppres.com
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Chile

Menéndez, Roberto; García, Teidy; Garateix, Anoland; Morales, Ruth A.; Regalado, Erik L.; Laguna, Abilio; Valdés, Olga; Fernández, Miguel D.
Neuroprotective and antioxidant effects of Thalassia testudinum extract BM -21 , against acrylamide -induced neurotoxicity in mice
Journal of Pharmacy & Pharmacognosy Research, vol. 2, núm. 3, mayo-junio, 2014, pp. 53-62
Asociación de Académicos de Ciencias Farmacéuticas de Antofagasta
Antofagasta, Chile

© 2014 Journal of Pharmacy & Pharmacognosy Research, 2 (3), 53-62
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Neuroprotective and antioxidant effects of *Thalassia testudinum* extract BM-21, against acrylamide-induced neurotoxicity in mice

[*Efectos neuroprotectores y antioxidantes del BM-21 extracto obtenido de* Thalassia testudinum*, sobre la neurotoxicidad inducida por acrilamida en ratones*]

Roberto Menéndez[a#*], Teidy García[a#], Anoland Garateix[a], Ruth A. Morales[a], Erik L. Regalado[b], Abilio Laguna[b], Olga Valdés[b], Miguel D. Fernández[a]

[a]Department of Pharmacology; [b]Department of Analytical Chemistry, Center of Marine Bioproducts (CEBIMAR). Calle Loma entre 35 y 37, Alturas del Vedado, Plaza de la Revolución, La Habana, CP. 10400, Cuba. [#]These authors equally contributed to this work.
*E-mails: roberto.menendez@infomed.sld.cu, rmenendezsoto@gmail.com

Abstract

Context: Acrylamide (ACR) neurotoxicity is associated with the enhancement of lipid peroxidation and the reduction of the antioxidative capacity distal axon and nerve terminal regions. The aqueous ethanolic extract of the marine plant *Thalassia testudinum*, named BM-21, have shown antioxidant, anti-inflammatory and analgesic properties.

Aims: To determine the neuroprotective and the antioxidant effects of BM-21, standardized to thalassiolin B content (5.8 ± 0.9%), on acrylamide (ACR)-induced distal axonopathy in male OF-1 mice.

Methods: Animals were administered with ACR (70 mg/kg, s.c., 4 weeks), and BM-21 was co-administered p.o at the doses of 4, 40 and 400 mg/kg. The effect of BM-21 on neurobehavioral indexes (rota-rod test), compound muscle action potential (CMAP) of the sciatic nerve and oxidative stress parameters were investigated.

Results: BM-21 significantly prevented the neurobehavioral sings of neurotoxicity and the alteration of CMAP amplitude and velocity. The lowest dose (4 mg/kg) failed to ameliorate these parameters whereas the highest dose (400 mg/kg) was the most active. BM-21 (400 mg/kg) significantly restored total hydroperoxides (THP) and glutathione (GSH) in the sciatic nerve as well as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. Additionally, the extract also modified THP, GSH and the activity of SOD in cerebellum and brain towards the standard values.

Conclusions: BM-21 given at doses that prevented ACR-induced neurotoxicity also produced antioxidant effect in the sciatic nerve, cerebellum and brain. Thus, the neuroprotective activity of BM-21 in this model seems to be mediated at least partly by its antioxidative properties.

Keywords: Acrylamide-induced axonopathy; oxidative stress; BM-21; *Thalassia testudinum*.

Resumen

Contexto: La neurotoxicidad inducida por acrilamida (ACR) se asocia al incremento de la peroxidación lipídica (POL) y a la reducción de la capacidad antioxidante de zonas distales de axón y terminales nerviosas. El extracto hidroetanólico de la planta marina *Thalassia testididum* (BM-21) posee efectos antioxidantes, anti-inflamatorios y analgésicos.

Objetivos: Determinar los efectos neuroprotectores y antioxidantes del BM-21 estandarizado acorde al contenido de thalassiolina B (5,8 ± 0,9%), sobre la axonopatía distal inducida por ACR en ratones OF-1.

Métodos: Los animales fueron administrados con ACR (70 mg/kg, s.c, 4 semanas) y con BM-21 (4, 40 y 400 mg/kg, p.o) y se investigaron los efectos sobre tiempo de permanencia en la varilla rotatoria, potencial de acción muscular complejo (PAMC) del nervio ciático y sobre parámetros del estrés oxidativo.

Resultados: El BM-21 previno significativamente la aparición de los signos de neurotoxicidad conductual y las alteraciones de la amplitud y la velocidad del PAMC. La dosis inferior administrada (4 mg/kg) fue inefectiva, mientras que la máxima ensayada (400 mg/kg) mostró la mayor eficacia. A esta dosis fueron restauradas significativamente las concentraciones de hidroperóxidos totales y de glutatión reducido y la actividad de la superóxido dismutasa en el nervio ciático, cerebelo y cerebro y la de glutatión peroxidasa en nervio ciático

Conclusiones: El BM-21 administrado a dosis en que previno la neurotoxicidad inducida por ACR también indujo efectos antioxidantes en nervio ciático, cerebelo y cerebro, por lo que sus efectos neuroprotectores parecen estar mediados al menos parcialmente, por sus propiedades antioxidantes.

Palabras Clave: Axonopatía inducida por acrilamida; estrés oxidativo; BM-21; *Thalassia testudinum*.

ARTICLE INFO

Received | Recibido: May 7, 2014.
Received in revised form | Recibido en forma corregida: June 18, 2014.
Accepted | Aceptado: June 21, 2014.
Available Online | Publicado en Línea: June 22, 2014
Declaración de Intereses | Declaration of interests: The authors declare no conflict of interest.
Financiación | Funding: This work was supported in part by the IFS Research Grant (project F/4237-1) and by the project CITMA 047/2012 (Cuba).

INTRODUCTION

Acrylamide (ACR) is neurotoxic in humans and experimental animals and produces skeletal muscle weakness and ataxia that is related to selective damage to distal axon and nerve terminal regions (Miller and Spencer, 1985). ACR has extensive used in many fields in industrial manufacturing and has been found in relatively high concentrations a variety of cooked and heat processed foods frequently consumed by humans. Therefore, the increasing and continuous exposure of humans to ACR may lead to nerve damage; hence strategies to protect against ACR-mediated neurotoxicity are relevant.

The mechanisms involved in ACR neurotoxicity are scarcely understood; however, recent studies have shown that are associated with the enhancement of lipid peroxidation (LPO) and the reduction of the antioxidative capacity in nerve tissue mostly caused by a primary depletion of reduced glutathione (GSH) (Yousef and El-Demerdash, 2006; Zhu et al., 2008). New studies have revealed the inability of ACR to activate the nuclear factor erythroid 2-related factor 2-antioxidant-responsive element (Nrf2-ARE) pathway in the nerve terminal that makes them especially sensitive to oxidative damage (Zhang et al., 2011). Accordingly, it seems likely that consumption of natural antioxidants may exert beneficial effects upon ACR-induced neurotoxicity. In this regard, experimental studies have shown that *Panax ginseng* extract (Mannaa et al., 2006), *Carica papaya* aqueous extract (Sadek, 2012), *Bacopa monieri* (Shinomol et al., 2013) and melatonin derivatives (Ahmeda et al., 2010) protect against ACR neurotoxicity due to antioxidative action.

Marine species have proven to be rich sources of antioxidants such as polyphenols and hence, they have attracted attention in the search for natural antioxidants. A previous study reveals that polyphenols are the major class of compounds present in the aqueous-methanolic extract of *Thalassia testudinum* leaves, named BM-21. A skin protective activity-guide fractionation of BM-21 resulted in the isolation of thalassiolin B (chrysoeriol 7-b-D-glucopyranosyl-2"-sulphate), the most abundant active component of the extract (Regalado et al., 2009). In a second phytochemical analysis (Regalado et al., 2012) other phenolic compounds were identified as chrysoeriol-7-O- β – D -glucopyranosyl-2″-sulfate (thalassiolin B), apigenin 7-O-β-D-glucopyranosyl-2″-sulfate (thalassiolin C), chrysoeriol 7-O-β-D-glucopyranoside, apigenin 7-O-β-D-glucopyranoside, dihydroxy-3',4'-dimethoxyflavone 7-O-β-D-glucopyranoside, luteolin-3'-sulphate, chrysoeriol and apigenin. As expected, BM-21 was found to exhibit strong antioxidant activity *in vitro* in four different free radical scavenging assays ($HO^{\bullet}$, $RO_2^{\bullet}$, $O_2^{-\bullet}$ and $DPPH^{\bullet}$) and inhibited lipid peroxidation (Regalado et al., 2012). The extract also displayed hepatoprotective effect *in vitro* against ter-butilhidroperoxide (*ter*-BOOH), LPS, ethanol and CCl_4 in primary cultured rat hepatocytes mainly due to antioxidant action (Rodeiro et al., 2008). In addition, the extract standardized to thalassiolin B (5.8 ± 0.9%), has been reported to have antinociceptive effects *in vivo* mediated by the inhibition of acid-sensing ionic channels (ASICs) (Garateix et al., 2011). Results have shown that thalassiolin B was entirely responsible for skin protective effect and mostly accounted for the antioxidant (Regalado et al., 2009) and the antinociceptive activity and the inhibition of ASIC channels (Garateix et al., 2011). In view of these reports, the aqueous-ethanolic extract of fresh *T. testidinum* was standardized to thalassiolin B content for further study its potential cytoprotective effect in animal models.

In the present work, it was evaluated the protective action of BM-21 against ACR-induced neurophysiological and oxidative stress disturbances in mice. Primary was investigated whether or not the oral administration of BM21 (4, 40 and 400 mg/kg) prevented ACR-induced peripheral neurotoxicity in mice (70 mg/kg s.c) by measuring abnormalities in motor coordination (rota-rod test) and electrophysiological examination of sciatic nerve compound muscle action potential (CMAP). It was further studied the *in vivo* antioxidant status in the sciatic nerve, cerebellum and brain following ACR and BM-21 administration.

MATERIALS AND METHODS

Plant material and preparation

Fresh *Thalassia testudinum* Banks & Soland ex König leaves were collected in the month of March 2010 from "La Concha" beach (22° 05' 45" N, 82° 27' 15" W). The plant was authenticated by Dr. Areces J.A. (Institute of Oceanology, La Havana, Cuba) with a standard sample preserved in the Cuban National Aquarium (No. IdO39). The extract was prepared as before described (Garateix et al., 2011). Briefly, whole dry and ground *T. testudinum* leaves were continuously extracted (three times) with EtOH-H_2O (50:50, v:v) during 24 h at room temperature. The combined extracts were filtered and concentrated under reduced pressure and temperature 30 to 40°C to yield BM-21. Thalassiolin b was quantified through the standard method previously reported (Garateix et al., 2011). The percentage of thalassiolin b was within the range previously reported (5.7 ± 0.9%).

Materials

All chemicals were from Aplychem (Darmstadtz, Germany). Reagent kits for the determination of superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) were from Randox (Crumlin, U.K.). All the other chemicals and reagents were of the highest purity available. Spectrophotometer UV/visible-120-2 was from Shimadzu Corporation (Tokyo, Japan). The stimulator model SEN-1101 and the oscilloscope model VC-11 were obtained from Nihon Khoden (Tokyo, Japan).

Animals

Male OF-1 mice (20-25 g) were supplied by the Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB, Santiago de Las Vegas, Habana, Cuba). Prior to experiment animals were adapted for seven days to laboratory conditions (controlled temperature 25 ± 2°C, relative humidity 60 ± 10% and 12 h light/dark cycles). Tap water and standard diet for rodents supplied by CENPALAB were freely provided. All procedures were conducted according to the European Commission guide-lines for the use and cared of laboratory animals and approved by the Committee for Animal Care in Research of the Center. The minimum number of animals and duration of observation required to obtain consistent data were employed.

ACR-induced neurotoxicity

The dose of ACR, the route and duration of exposure corresponded to the progressive worsening of neurobehavioral indices previously described in OF-1 mice (Acevedo et al., 2000). After acclimation, mice having at the beginning of the experiment 20-25 g, were assigned at random to one of the following groups (15 animals per experimental groups): (1) treated with saline (s.c) and distilled water (p.o); (2) treated with ACR dissolved in normal saline (70 mg/kg s.c, 5 times/week) and distilled water (p.o); and groups 3-5, treated with freshly prepared BM-21 once-a-day (4, 40 and 400 mg/kg, respectively) plus ACR (70 mg/kg, s.c, 5 times/week). The doses of BM-21 were fixed based on earlier studies on the anti-nociceptive effects of this extract (Garateix et al., 2011). Animals had free access to food and water throughout the experimental period. BM-21 was dissolved in distilled water to appropriated concentration and administered by gastric gavage. Distilled water, BM-21 and ACR were administered at 0.01 mL/g body weight. The administration of BM-21 was initiated three days prior to ACR and then, BM-21 and ACR were administered simultaneously for four weeks. Body weights were determined weekly whereas deaths were daily monitored by simple visual inspection. Motor coordination was determined at the beginning of the experiment (baseline) and at the end of week 1, 2, 3 and 4 of treatment by using a modified device of Dunham and Miya (1957). Animals were placed in a bar of 3 cm diameter that rotated at 8 rpm and test was terminated either when the mice fell from the rod or at 2 min. The test was repeated three times at 60 min intervals for each mouse and the averaged times were used for statistical comparisons. A blind observer performed this behavioral test. Electro-physiological parameters of CMPA were examined at the beginning and 4 weeks after the beginning of the experiment. CMPA was recorded according a modified method (Acevedo et al., 2000) originally described by Hopkins and Gillart (1971). The signal was sent to an oscilloscope and

digitized using an AD converter Digidata 1200. Data analyses were performed by means of Analnew version 7.0 (Soto and Vega, 1987) and stored by the microprocessor system. Two parameters were used to evaluate CMAP that were calculated as described previously (Zhu et al. 2008); peak amplitude (CMAPA, in millivolts, mV) and duration (CMAPD in milliseconds, ms).

Study of oxidative stress parameters in ACR-induced neurotoxicity

In a second experiment mice weighted 20-25 g were administered with ACR and BM-21 with the same procedure than the first experiment, except that BM-21 was administered solely at 400 mg/kg. This dose was selected based on our previous experiment that showed maximum protection. The number of animals per experimental groups was chose according to the survival rate observed in the first experiment. Animals were distributed at random in control; ACR; BM-21 plus ACR. To check efficacy of BM-21, the rota-rod performance was done at the beginning and four weeks after ACR administration. At the end of the experimental period, the animals were euthanized under slight anesthesia and sciatic nerves were rapidly dissected and rinsed in cold 0.9% NaCl. To obtain enough material for sciatic nerve three animals in each experimental group were randomly pooled. The brain and cerebellum of eight animals of each group were selected at random. Homogenates were prepared by homogenizing tissue samples in cold PBS in ice, in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 4 000×g for 20 min at 4°C, and the resultant supernatant was used.

Estimation of GSH concentration

The procedure described by Ellman (1959) was followed. Concentration of GSH was determined using a molar absorption coefficient of 14 150 M/cm.

Estimation of total hydroperoxides (THP)

THP were determined by the FOX method (Nourooz-Zadeh et al., 1996). Hydroperoxide content was determined using a molar absorption coefficient of 4.3×10^4 M/cm.

Estimation of SOD and GSH-Px activity

The activity of these enzymes in homogenates of the sciatic nerve was determined colorimetrically according to the methods described by the commercial assay kits.

Statistical analysis

Data are presented as mean ± SEM along with the number of animals. Statistical analysis was performed using Graph-pad prism 5.0 software. Global comparison was performed using One Way ANOVA and when significant results were obtained, pair wise testing was done by the help of Bonferroni's Multiple Comparison Test. Neurological deficits were presented as median ± 95% confidence interval and analyzed using non-parametric Kruskal–Wallis test followed by post-hoc Dunn's multiple comparison tests. Statistical analysis mortality and body weight were performed with the statistics software SPSS (Version 10) for Windows. The results of mortality were examined for significance using the Fisher's Exact Test. $P<0.05$ was considered statistically significant.

RESULTS

Table 1 shows the effects of different treatments on survival rates. The mortality in the ACR-treated animals at the end of the treatment reached 46.4%. In the group treated with 4 mg/kg, mortality was as for ACR-treated, whereas, in the group treated with 40 mg/kg, the death rate decreased, although there are still remain no significant difference between this group and the control. In the group administered Table 2 presents the results of mean body weight at the beginning of the study (basal) and 4 weeks after treatment. No difference in the initial body weight was observed in any experimental group whereas a severe reduction in body weight gain was observed in the ACR-treated animals relative to the control group. Also, a reduction in body weight relative to controls was observed in the group of 4 and 40 mg/kg. However, BM-21 (400 mg/kg) attenuates the reduction of body weight gain since on the week 4, and mice treated with

400 mg/kg showed no significant differences when compared with controls.

The results of the rota-rod test (Fig. 1) showed that mice treated with ACR displayed severe neurotoxicity. Two weeks after ACR exposure no significant reduction of endurance time in rota-rod was observed in ACR-treated group in comparison to control. However, as treatment continues (weeks 3 and 4) time endurance gradually decreased in ACR treated corresponding to the progressive worsening of motor coordination. At week 4, the dose of 4 mg/kg and 40 mg/kg behaved similarly than ACR treated group, even when a slight amelioration was observed at 40 mg/kg. In contrast, the extract at 400 mg/kg demonstrated a significant beneficial effect on motor coordination all over the experimental period.

Fig. 2 shows the results of electrophysiological indices in the sciatic nerve after 4 weeks of ACR exposure. Alteration in the group treated with ACR was observed that was characterized by a marked and significant decrease in amplitude and increase of duration. CMAP of mice administered with BM-21 4 mg/kg was almost similarly modified by ACR. The BM-21 (40 mg/kg) treated group showed mild to moderate degree of recuperation of amplitude and duration whereas BM-21 at 400 mg/kg restored both parameters towards the normal level.

Studies in homogenates of the sciatic nerve, brain and cerebellum (Figs. 3 and 4) showed a significant enhancement of oxidative stress after ACR administration as revealed by the significant increment of THP, the decrease of GSH and the decrease of SOD activity. Following treatment with BM-21 at 400 mg/kg THP, GSH and the activity of the antioxidant enzymes were restored to nearly normal.

Table 1. Effects of BM-21 on animal mortality per week in mice treated subcutaneously with ACR.

Treatment	Weeks				Mortality (%)
	1	2	3	4	
	Death				
	Y/N	Y/N	Y/N	Y/N	
Control	0/15	0/15	0/15	0/15	0
ACR 70 mg/kg	0/15	0/15	5/10*	7/8**	46.6
BM-21 4 mg/kg	0/15	0/15	3/12	7/8**	46.6
BM-21 40 mg/kg	0/15	0/15	4/11	5/10*	33.3
BM-21 400 mg/kg	0/15	0/15	0/15^{+}	1/14^{+}	6.6

*P<0.05, **p<0.001 comparison *vs.* control; $^{+}$p<0.05 comparison *vs.* ACR-treated group. Fisher's Exact Test.

Table 2. Effects of BM-21 on body weight gain in mice treated subcutaneously with ACR.

Treatment	Weight (g)		Body weight increase (%)
	Baseline	Week 4	
Control	23.23 ± 0.36	35.22 ± 0.72	51.6 ± 7.8 (N = 15)
ACR 70 mg/kg	23.80 ± 0.44	27.91 ± 1.29***	16.0 ± 14.0 (N = 8)
BM-21 4 mg/kg	23.06 ± 0.26	27.24 ± 0.79***	19.5 ± 12.4 (N = 8)
BM-21 40 mg/kg	22.94 ± 0.29	29.66 ± 0.73***	31.0 ± 12.0 (N = 10)
BM-21 400 mg/kg	23.99 ± 0.42	34.14 ± 0.53^{+++}	45.1± 9.5 (N = 14)

The start number of animal *per* group was 15. Data are mean ± SEM. (N) number of animals. ***P<0.001, comparison *vs.* control group; $^{+++}$p<0.001, comparison *vs.* ACR-treated group (one Way ANOVA followed by Tukey's Multiple Comparison Test).

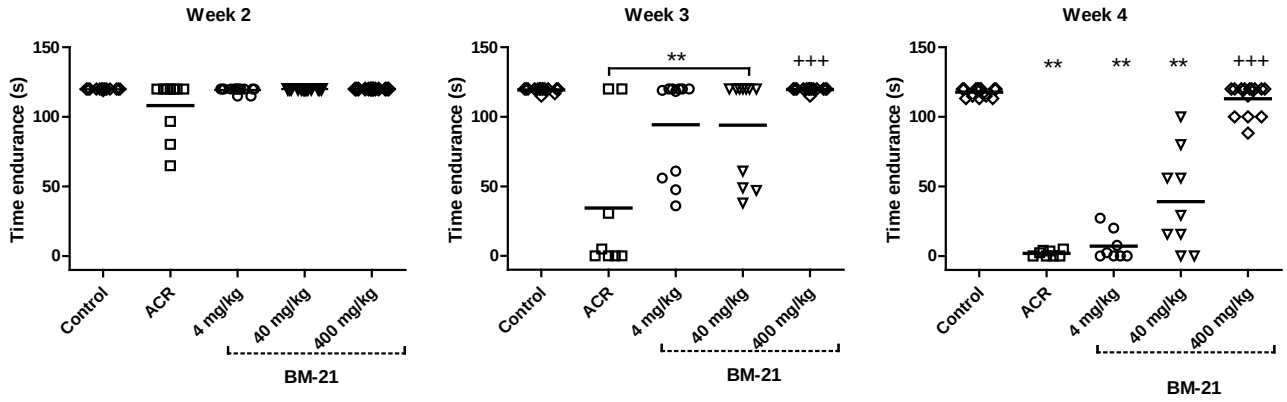

Figure 1. Effects of BM-21 on time endurance in rota rod test. Geometric symbols represent values for individual mice. Horizontal lines represent median values for time endurance. **P<0.01, ***p<0.001 comparison vs. control group; $^{+++}$p< 0.001 comparison vs. ACR group (both tested using non-parametric Kruskal–Wallis test followed by post-hoc Dunn's Multiple Comparison Test).

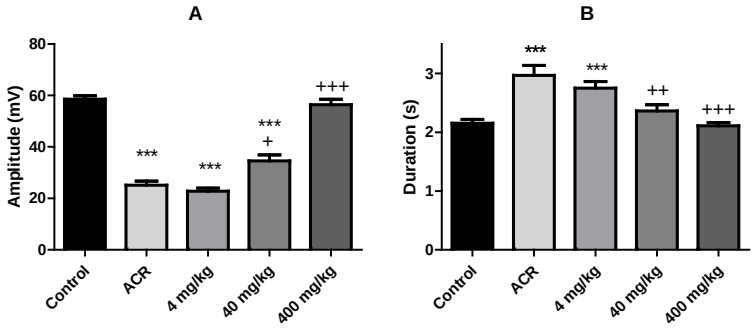

Figure 2. Effects of BM-21 on compound muscle action potential amplitude (ACMAP, Fig. 2A) and duration (DCMAP, Fig. 2B) after 4 weeks of treatment. Each column represents the mean values ± SEM (N = 15, controls; N = 8, ACR treated; N = 8, N = 10 and N = 14, ACR + BM-21 at 4, 40 and 400 mg/kg respectively). ***P<0.001, comparison *vs.* control; $^{++}$p<0.01; $^{+++}$p<0.001, comparison *vs.* ACR treated group (tested using one-way ANOVA followed by Tukey's Multiple Comparison Test).

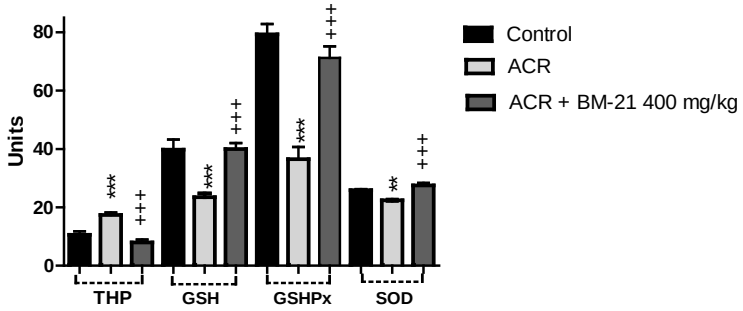

Figure 3. Concentration of THP (µmol/mg protein), GSH (µmol/mg protein) and the activity of GSH-Px (µmol NADPH/min/mg protein) and SOD (U/mg de protein) in homogenates of the sciatic nerve, cerebellum and brain of control, ACR-intoxicated and ACR-intoxicated mice treated with BM-21 (400 mg/kg). Each column represents the mean values ± SEM (N = 7). **P<0.01, ***p<0.001 control group *vs.* ACR-treated group; $^{+++}$p<0.001 ACR-treated *vs.* BM-21 treated group (tested using one-way ANOVA followed by Tukey's Multiple Comparison Test).

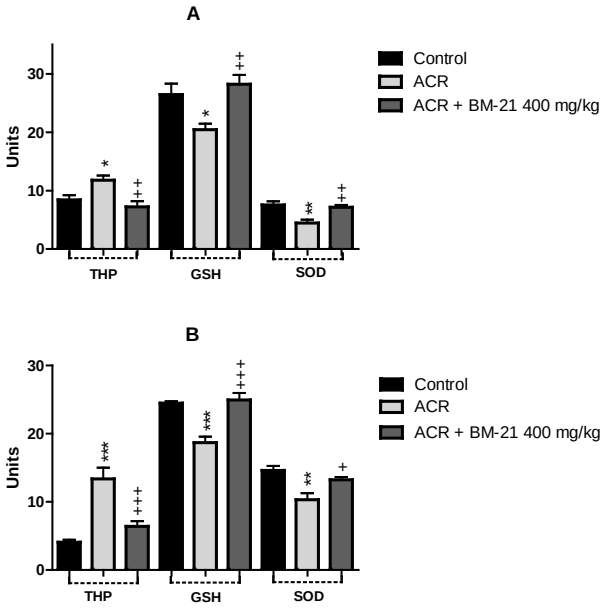

Figure 4. Concentration of THP (µmol/mg protein), GSH (µmol/mg protein) and the activity of SOD (U/mg de protein) in homogenates of cerebellum **(A)** and brain **(B)** of control, ACR-intoxicated and ACR-intoxicated mice treated with BM-21 (400 mg/kg). Each column represents the mean values ± SEM (N = 7).*P<0.05, **p<0.01, ***p<0.001 control group *vs.* ACR-treated group; $^{+++}$p<0.05, $^{+++}$p<0.01, $^{+++}$p< 0.001 ACR-treated *vs.* BM-21 treated group (tested using one-way ANOVA followed by Tukey's Multiple Comparison Test).

DISCUSSION

Considerable experimental data from rodent studies have shown than ACR exposure produces pronounced neurotoxicity (Matsuoka et al., 1996; Ko et al., 1999; Mannaa et al., 2006; Zhu et al., 2008) and that the oxidative stress in nervous tissue is linked to such neurotoxicity (Yousef and El-Demerdash, 2006; Zhu et al., 2008). All these have converted ACR-neurotoxicity into a suitable experimental model to evaluate the potential intervention of new neuroprotective agents with antioxidant effects. Hence, the focus of the present work was to evaluate the effect of BM-21 against ACR-induced neurophysiological disorders and the possible mechanisms underlined.

As expected from previous work (Acevedo et al., 2000) these results showed that ACR exposure elicited typical behavioral deficits which began two weeks after administration and kept on increasing as treatment continued for up to 4 weeks. In such a condition of the maximum exposition, mice developed severe limb paralysis, ataxia and skeletal muscle weakness that were evident by the marked decline of motor coordination observed in rota-rod test. These findings also suggest that behavioral deficit appear to correlate well with the electro-physiological observations because ACR markedly decrease amplitude and velocity CMAP at the end of the administration period that is in agreement with previous findings (Acevedo et al., 2000; Zhu et al., 2008).

The molecular mechanism of ACR-induced neurotoxicity remains poorly understood; however, oxidative stress may account for the ACR-induced neuropathy. Several findings have shown that conjugation with GSH is the principal mechanism for the detoxification of ACR and glycidamide (GLY), its major oxidized metabolite. As GSH plays an important role in the anti-oxidant defense and the regulation of cellular events (Wu et al., 2004), depletion of neural GSH content might be one of the primary events in ACR-induced neuropathy. In addition, a recent investigation have suggested the inability if nerve tissue to respond to oxidative stress through ARE pathway, making nerve fibers especially sensitive to ACR intoxication (Zhang et al., 2011). Therefore, the continuous administration with ACR can lead to an overall increase in intracellular ROS and oxidative damage in nerve.

Previous works have convincingly demonstrated that the enhancement of LPO and the reduction of the antioxidative capacity in nerve tissue correlate well with the alterations of electrophysiology indices of the sciatic nerve and behavioral disturbances of rats administered with ACR (Zhu et al., 2008). In line with these data, the present results showed decreased concentrations of GSH, increased levels of LPO in the sciatic nerve (expressed as the increased of THP) and decreased of SOD and GSH-Px activity in ACR intoxicated animals. The decrease in antioxidant enzyme activity might be a consequence of GSH depletion due to continuous ACR exposure because free radicals when present in high concentrations are capable of interacting with these enzymes and inactivating them (Pigelot et al., 1990; Vessey and Lee, 1993). Consequently, the impairment of the activity of these enzymes may additionally contribute to an overall oxidative

stress and oxidative cell injury of the sciatic nerve.

BM-21 administration to mice receiving ACR resulted in a significant improvement in the behavioral and electrophysiological alterations of CMAP. Also, the extract attenuated the LPO induced by ACR in the sciatic nerve. In previous works have been demonstrated the antioxidant properties of BM-21 on a wide range of radical species and experimental models (Rodeiro et al., 2008; Regalado et al., 2009; Regalado et al., 2012). Thus, protective effects of BM-21 may be caused by its antioxidant properties by means of which susceptible sciatic nerve fibers produced less exposed to oxidative stress leading to reduced cell damage and improvement of nerve functions.

The rota-rod paradigm is a reliable test to study motor function and balance that requires an unaltered central function and motor coordination (Van Meer and Raber, 2005). The current results showed that the motor function became abnormal in ACR intoxicated mice within three weeks after intoxication that is consistent with previous reports (Nakagawa-Yagi et al. 2001). This support the notion that ACR intoxication is associated with selective nerve terminal damage not only at the peripheral level but also in CNS (Miller and Spencer, 1985) that contributes to neurological dysfunction. Under these conditions, mice intoxicated with ACR showed increased oxidative stress in brain and cerebellum. These results are in conformity with previous results (Zhu et al., 2008; Ahmeda et al., 2010; Lakshmi et al., 2012; Shinomol et al., 2013). However, the level of oxidative stress and the abnormal motor coordination were brought to near normal by the treatment with BM-21. Thus, it seems likely that BM-21 also enhanced resistance to free radical attack generated by ACR administration in CNS.

The results so far discussed have been related to central and peripheral neurotoxicity as the most sensitive effect induced by ACR intoxication. However, findings of several laboratories revealed typical symptoms of systemic toxicity by ACR exposure. It is well documented that once absorbed, ACR is passed readily through cell membranes and widely distributed among tissues and then transformed into GLY (Barber et al. 2001; LoPachin and DeCaprio, 2005). As conjugation with GSH is the main mechanism for the detoxification of ACR and GLY (Tong et al. 2004) the broad tissue distribution of ACR may cause GSH depletion at various tissues triggering them to an imbalanced antioxidant status. Hence, an overall increase of oxidative mechanisms by ACR may lead to LPO, cell injury and multisystem toxicity. Consistent with this, ACR induces in addition to neurotoxicity, oxidative stress in various organs and tissues (Ali et al., 1983; Srivastava et al., 1983; Khan et al., 1999; Mannaa et al., 2006; Sadek, 2012; Sahai, 2012). Treatment with BM-21 to intoxicated mice attenuated body weight lost and a dramatically reduced of mortality. Even though the mechanism of action of such effects is beyond the objective of the present work, on the basis of the actual results, it is probable to speculate that treatment with BM-21 may adequately protect intoxicated mice through its antioxidant effects as occurred with *Panax ginseng* extract (Mannaa et al. 2006), the leaf powder of *Bacopa monnieri* (Shinomol et al. 2013), and papaya fruit extract (Sadek, 2012).

As mentioned before, the presence of flavonoids has been previously established in BM-21. In addition to thalassiolin A, several others flavones identified as components of BM-2 are known to exhibit a broad spectrum of pharmacological properties including antioxidant and cytoprotective actions (Raj Narayana et al. 2001). Hence, the presence of these active biological principles in the extract may be accounting for the biological effect of BM-21 that could involve, at least partially, antioxidant and/or free radicals scavenging activities. However, due to the wide range of biochemical and pharmacological properties of polyphenols on the nervous system (Spencer, 2008) further studies are still required to prove the entire action of BM-21.

CONCLUSIONS

This study is the first to demonstrate the neuroprotective and the antioxidant of BM-21, the aqueous-ethanolic extract from the sea grass *T. testudinum* on ACR-induced axonopathy. A key finding of the present study is that the behavioral deficits and the electrophysiological

alteration in the sciatic nerve elicited by ACR were significantly reduced by oral administration of BM-21. The extract gave at an adequate dose to prevent ACR-induce neurotoxicity also produces antioxidant effects in the sciatic nerve, brain and cerebellum. Thus, the antioxidant effect of BM-21 may ameliorate the free radical associated deleterious effects induced by the continue exposure to ACR in these tissues. These data suggest that neuroprotective activity of BM-21 in this model seems to be mediated at least partly by its antioxidative properties.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors are grateful to Ms. Addys Palmero for excellent technical assistance for the study.

This work was supported in part by the IFS Research Grant (Project F/4237-1) and by the project CITMA 047/2012 (Cuba).

REFERENCES

Acevedo ME, Pérez-Saad H, León N, García JD (2000) Efecto protector de una mezcla de gangliósidos en la polineuritis por acrilamida en ratones. Rev Neurol 30: 1096-1100.

Ahmeda HH, Elmegeed GA, El-Sayed E-SM, Abd-Elhalim MM, Shousha WG, Shafic RW (2010) Potent neuroprotective role of novel melatonin derivatives for management of central neuropathy induced by acrylamide in rats. Eur J Med Chem 45: 5452-5459.

Ali SF, Hong JS, Wilson WE, Uphouse LL, Bondy SC (1983) Effect of acrylamide on neurotransmitter metabolism and neuropeptide levels in several brain regions and upon circulating hormones. Arch Toxicol 52: 35-43.

Barber D S, Hunt J R, Ehrich MF, Lehning EJ, LoPachin RM (2001) Metabolism, toxicokinetics and hemoglobin adducts formation in rats following subacute and subchronic acrylamide dosing. Neurotoxicology 22: 341-353.

Dunham NM, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 46: 208-209.

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70-77.

Garateix A, Salceda E, Menéndez R, Regalado EL, López O, García T, Morales RA, Laguna A, Thomas OP, Soto E (2011) Antinociception produced by *Thalassia testudinum* extract BM-21 is mediated by the inhibition of acid-sensing ionic channels by the phenolic compound thalassiolin B. Mol Pain 7: 10.

Hopkins AP, Gilliat AW (1971) Motor and sensory nerve conduction velocity in the baboon: normal values and changes during acrylamide neuropathy. J Neurol Neurosurg Psychiat 34: 415-426.

Khan MA, Davis CA, Foley GL, Friedman MA, Hansen LG (1999) Changes in thyroid gland after acute acrylamide exposure. Toxicol Sci 47: 151–157.

Ko M-H, Chen W-P, Lin-Shiau S-Y, Hsieh ST (1999) Age-dependent acrylamide neurotoxicity in mice: morphology, physiology, and function. Exp Neurol 158: 37-46.

Lakshmi D, Gopinath K, Jayanthy G, Anjum S, Prakash D, Sudhandiran G (2012) Ameliorating effect of fish oil on acrylamide induced oxidative stress and neuronal apoptosis in cerebral cortex. Neurochem Res 37: 1859-1867.

LoPachin RM, DeCaprio AP (2005) Protein adduct formation as a molecular mechanism of neurotoxicity. Toxicol Sci 86: 214-225.

Mannaa F, Mosaad AA-W, Hanaa H, Ahmed H, Park MH (2006) Protective role of *Panax ginseng* extract standardized with ginsenoside Rg3 against acrylamide-induced neurotoxicity in rats. J Appl Toxicol 26: 198-206.

Matsuoka M, Matsumura H, Igisu H (1996) Creatine kinase activities in brain and blood: possible neurotoxic indicator of acrylamide neutotoxicity. Occup Environ Med 53: 468-471.

Miller MS, Spencer PS (1985) The mechanisms of acrylamide axonopathy. Annu Rev Pharmacol Toxicol 25: 643-666.

Nakagawa-Yagi Y, Dong-Kug C, Nobuo O, Shin-ichi S, Motohide S, Takashi M, Takashi I, Yoshiyuki S (2001) Discovery of a novel compound: insight into mechanisms for acrylamide-induced axonopathy and colchicine-induced apoptotic neuronal cell death. Brain Res 909: 8-19.

Nourooz-Zadeh J, Tajaddini-Sarmadi J, Ling KLE, Wolff SP (1996) Low-density lipoprotein is the major carrier of lipid hydroperoxides in plasma. Biochem J 313: 781-786.

Pigelot E, Corbisier P, Houbion A (1990) Glutathione peroxidase, superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Res Rev: 1-7.

Raj Narayana K, Sripal Reddy M, Chaluvadi MR, Krishna DR (2001) Bioflavonoids classification, pharmacological, biochemical effects and therapeutical potential. Indian J Pharmacol 33: 2-16.

Regalado EL, Menendez R, Valdes O, Morales RA, Laguna A, Thomas OP, Hernandez Y, Nogueiras C, Kijjoa A (2012) Phytochemical analysis and antioxidant capacity of BM-21, a bioactive extract rich in polyphenolic metabolites from the sea Grass *Thalassia testudinum*. Nat Prod Commun 7: 47-50.

Regalado EL, Rodriguez M, Menendez R, Concepcion AA, Nogueiras C, Laguna A, Rodriguez AA, Williams DE, Lorenzo-Luaces P, Valdes O, Hernandez Y (2009) Repair of UVB-damaged skin by the antioxidant sulphated flavone glycoside thalassiolin B isolated from the marine plant *Thalassia testudinum* Banks ex Konig. Mar Biotechnol (NY) 11: 74-80.

Rodeiro I, Donato MT, Martinez I, Hernandez I, Garrido G, Gonzalez-Lavaut JA, Menendez R, Laguna A, Castell JV, Gomez-Lechon MJ (2008) Potential hepatoprotective effects of new Cuban natural products in rat hepatocytes culture. Toxicol In Vitro 22: 1242-1249.

Sadek KM (2012) Antioxidant and immunostimulant effect of *Carica papaya* Linn. aqueous extract in acrylamide intoxicated rats. Acta Inf Med 20: 180-185.

Sahai V (2012) Histological changes in liver of albino mice due to chronic administration of acrylamide. Indian J Fund Appl Life Sci 2: 51-54.

Shinomol GK, Raghunath N, Bharath MM, Muralidhara (2013) Prophylaxis with *Bacopa monnieri* attenuates acrylamide induced neurotoxicity and oxidative damage via elevated antioxidant function. Cent Nerv Syst Agents Med Chem 13: 3-12.

Soto E, Vega R (1987) A Turbo Pascal program for on line spike data acquisition and analysis. J Neurosci Methods 19: 61-68.

Spencer PES (2008) Flavonoids: modulators of brain function? Br J Nutr 99 E-Suppl 1: ES60-ES77.

Srivastava SP, Das M, Seth PK (1983) Enhancement of lipid peroxidation in rat liver on acute exposure to acrylamide and styrene a consequence of glutathione depletion. Chem Biol Interact 45: 373-380.

Tong GC, Cornwell WK, Means GE (2004) Reactions of acrylamide with glutathione and serum albumin. Toxicol Lett 147: 127-131.

Van Meer P, Raber J (2005) Mouse behavioural analysis in systems biology. Biochem J 389: 593-610.

Vessey DA, Lee KH (1993) Inactivation of enzymes of the glutathione antioxidant system by treatment of cultured human keratinocytes with peroxides. J Invest Dermatol 100: 829-833.

Wu G, Fang YZ, Yang S, Lupton J R, Turne ND (2004) Glutathione metabolism and its implications for health. J Nutr 134: 489-492.

Yousef MI, El-Demerdash FM (2006) Acrylamide-induced oxidative stress and biochemical perturbations in rats. Toxicology 219: 133-141.

Zhang L, Gavin T, Barber D S, LoPachin RM (2011) Role of the Nrf2-ARE pathway in the acrylamide neurotoxicity. Toxicol Lett 205: 1-7.

Zhu J-Y, Zeng T, Zhu Y-B, Yu S-F, Wang Q-S, Zhang L-P, Guo X, Xie K-Q (2008) Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. Neurochem Res 33: 2310-2317.