Short Communication

Antioxidant content and activity of the Indian fresh-water pearl mussel in the prevention of arthritis in an experimental animal model

Mousumi Chakraborty1†, Sourav Bhattacharya1†, Raghwendra Mishra2, Debarati Mukherjee2 and Roshnara Mishra1*

1Department of Physiology, University of Calcutta, 92, Acharya Prafulla Chandra Road, Kolkata 700009, India
2Department of Biochemistry, N.R.S. Medical College and Hospital, Kolkata 700014, India

(Submitted 1 August 2011 – Final revision received 3 October 2011 – Accepted 10 November 2011 – First published online 6 January 2012)

Abstract

The flesh of the Indian fresh-water mussel, Lamellidens marginalis (LM; Lamarck, 1819), is the byproduct of pearl culture and a cheap protein source. The present study investigated the antioxidant content of this ethnomedicinally cited species to outline its importance in food security and disease prevention. LM was found to be rich in polyphenol antioxidants with good correlation with its reducing capacity. LM also showed a significant free-radical-scavenging activity, H2O2-scavenging activity and Fe-chelating activity. To study the effect of this dietary antioxidant against oxidative stress, we took inflammatory arthritis as a model. LM-treated arthritis rats showed a higher antioxidant defence system with elevated superoxide dismutase, total thiol, glutathione S transferase, glutathione peroxidase, total antioxidant status and catalase concentration of haemolysate. Oxidative stress markers like serum thiobarbituric acid-reacting substances, methyl glyoxal, NO and total oxidant status levels were decreased in LM-treated arthritis rats. Hence, the dietary antioxidants of LM were found to be effective in the prevention of oxidative stress in inflammatory arthritis. In conclusion, LM, the cash-crop byproduct, provides a rare opportunity for income and nutrition, not only by providing cheap and available energy, protein and dietary factors, but also by providing antioxidants effective against chronic inflammatory disease.

Key words: Lamellidens marginalis: Antioxidants: Arthritis: Nutrition

In developing countries, where commercial farming is essential for income, food security is being compromised as a consequence of deviation from subsistence production(1). The low-income-group population has therefore come to rely more on cheap energy from foods devoid of antioxidants and other dietary factors(2). Developing countries have thus become the cohabitation of diseases like malnutrition and obesity(3), cancer and chronic diseases. Hence, to address this problem, we should look for foods which can help generate income as well as provide proper nutrition and food security.

In the present paper, we have studied the antioxidant content and activity of a commercially important fresh-water pearl mussel, the Lamellidens marginalis (LM; Lamarck, 1819). This species can be considered as an important food source to solve the contradiction between commercial farming and food security, as the energy- and protein-rich flesh of the mussel is a byproduct of pearl culture. Substantiated by the antioxidant content, rarely found in animal protein, the flesh can be a complete package of energy and dietary factors besides being cheap, ethnic and easy to culture.

LM is available in the densely populated food-insecure inlands of India, Bangladesh, Burma, Sri Lanka and Nepal. The edible foot portion has been reported to have ethnomedicinal usage (4). Mussels are unique in their content of phenol proteins (5) and have anti-inflammatory effects in experimental models and clinical studies(6). Hence, we studied the antioxidant content of mussel and explored the effect in in vitro and in an adjuvant-induced arthritis model. The antioxidant defence system of marine mussels was reported in the perspective of pollution and heavy-metal toxicity(7).

Abbreviations: ~SH, total thiol; ABTS, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; FCA, Freund’s complete adjuvant; GPx, glutathione peroxidase; GST, glutathione S transferase; LM, Lamellidens marginalis; SOD, superoxide dismutase; TAS, total antioxidant status; TOS, total oxidant status.

* Corresponding author: R. Mishra, fax +91 33 2351 9755/2241 3288, email roshnaramishra@gmail.com
† The authors made an equal contribution to this study.
However, the dietary significance of the antioxidant has so far not been reported. This information is a rare example of integration of commerce and nutrition, both for energy and disease prevention, as a perfect piece to fit in the jigsaw of integrated nutritional approach.

Materials and methods

Chemicals

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium (ABTS) salt, EDTA, reduced glutathione, metaphosphoric acid, Folin–Ciocalteau reagent, 1-chloro-2,4-dinitrobenzene, potassium ferricyanide, ferrozin, pyrogalol, tert-butyl hydroperoxide, ferric chloride, Freund’s complete adjuvant (FCA) and indomethacin, Trolox, O-dianisidine dihydrochloride, xylene orange and 1, 2-diamino benzene were purchased from Sigma.

Animals

Wistar strain male albino rats, about 9–12 weeks old (120 ± 10 g), were used for the in vitro experiments. The animals were collected and housed in a controlled environment (room temperature: 23 ± 2°C, relative humidity: 60 ± 5%, 12 h day–12 h night cycle) and fed ad libitum with a balanced diet and water. All animal experiments were approved by the Departmental Animal Ethical Committee and were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on animals, Government of India.

Collection of sample and preparation of extract

Live adult fresh-water mussels were collected from the local market of Kolkata, India, and the species was identified as LM (voucher specimen no.: M26322/5) from the Mollusca Section of Zoological Survey of India, New Alipore, Kolkata, India. Aqueous extract of LM was prepared and expressed as per µg dry weight for in vitro experiments, and as per mg wet weight for in vivo experiments.

Determination of total phenol, reducing power, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium radical-scavenging activity, total antioxidant capacity, hydrogen peroxide-scavenging and metal-chelating activity

The total phenol content of the extract was determined by Folin–Ciocalteau’s reagent, according to the method of Taga et al. The phenol content was evaluated from gallic acid standard curve (5–200 µg) and the value was expressed in terms of gallic acid equivalents.

Reducing power of the extract was determined according to Oyaizu and expressed in terms of ascorbic acid equivalent.

The ABTS+ radical-scavenging activity of LM was assessed according to Re et al. and total antioxidant capacity was determined by comparing the ABTS+ radical-scavenging activity with trolox (0–2 µM) standard.

H2O2-scavenging activity of the extract was determined according to Nahavi et al. and expressed as percentage of H2O2 scavenged.

The Fe2+-chelating activity of the extract was determined according to Ebrahimi zadeh et al. The percentage inhibition of Fe2+-ferrozine complex formation by the extract was calculated according to the formula:

\[
\text{Inhibition} (%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100,
\]

where Abscontrol and Abssample are the absorbance of control and absorbance of sample, respectively. The metal-chelating activity of LM at the dose 100 mg/ml was expressed in terms of ascorbic acid equivalents.

Induction of oxidative stress associated with inflammatory arthritis and evaluation of oxidative stress defence system and pro-oxidant markers

Arthritis was induced in rats and the experiment was carried out as described by Chakraborty et al. Experimental animals were divided on the next day after adjuvant injection into the following five groups, 1–V, for respectively, the saline-injected normal control, FCA-injected arthritic control, arthritic animals supplemented with LM 1 (500 mg/kg per d, per oral), LM 2 (1 g/kg per d, per oral) and indomethacin (1 mg/kg per d, per oral). Treatment was given from 1 to 13 d. On the 15th day, blood was collected, and serum (as per mg of protein) and haemolysate (as per mg of Hb) markers of oxidative stress were assessed.

Serum superoxide dismutase (SOD) activity was estimated by measuring the percentage inhibition of the pyrogalol auto-oxidation by SOD according to the standard method. Here, one unit of SOD was defined as the enzyme activity that inhibits the auto-oxidation of pyrogalol by 50%. Catalase activity of haemolysate was estimated using the method of Beers & Sizer. Serum sulphhydril group content was assayed as described by Elman. Serum glutathione peroxidase (GPx) activity was estimated using the method of Paglia & Valentine. Serum glutathione transferase (GST) activity was estimated using the method of Habig & Jakoby. Serum total antioxidant status (TAS) value was estimated using the method of Re et al. Serum lipid peroxidation level by Buege & Aust and serum NO synthease activity by the method of Granger et al. Total oxidant status (TOS) was assessed using the method of Erel. Oxidative stress index, the ratio of TOS and TAS, was measured by Harma et al. and expressed as arbitrary units. To perform the calculation, the result unit of TAS was changed to µM Trolox equivalents, and oxidative stress index value was calculated as follows: oxidative stress index = (TOS, µM-H2O2 equivalent) × 100/(TAS, µM-Trolox equivalents).

Statistical methods

All the results were expressed as means with their standard errors, n = 6. The level of significance was determined by...
one-way ANOVA followed by Tukey’s post hoc test. A value of
$P<0.05$ was considered as significant. Pearson’s correlation
coefficient ($r$) was evaluated between total phenol content
and reducing power of LM. All statistical analyses were
performed using Origin 7 and MS-Office Excel 2007 software
packages.

Results

Antioxidant content of Lamellidens marginalis extract

The total phenol content of LM was found to be 82.81
(± 0.75) μg gallic acid equivalent per mg of LM. Reducing
power of per mg LM was comparable with 16.56 (± 1.06) μg of ascorbic acid. Correlation between phenol content
and reducing power of LM ($r$ 0.98) was found to be significant
($P=0.003$).

LM showed ABTS$^+$-scavenging activity and $H_2O_2$-scaven-
ging activity dose dependently. The half maximal inhibitory
concentration value of LM for ABTS$^+$-scavenging activity was
7.81 mg/ml (1.299 mM-Trolox equivalents) and for $H_2O_2$-
scavenging activity was 0.343 mg/ml.

LM showed 19-74% inhibition in $Fe^{3+}$–ferrozin complex
formation at the dose of 100 mg/ml concentration which was
found to be 7.086 μg EDTA equivalents.

In vivo antioxidant activity of Lamellidens marginalis
extract in Freund’s complete adjuvant-induced arthritis
model after oral supplementation

LM treatment significantly restored antioxidant defence sys-
tems, SOD, total thiol (~SH), GST level in serum, and catalase
concentration of haemolysate were changed significantly in
the arthritis group of animals, as shown in Table 1. Serum
SOD, ~SH, GST, GPx, TAS level and catalase concentration
of haemolysate were found to be significantly decreased
($P<0.05$) in adjuvant-injected arthritic rats when compared
with normal rats after the 15th day of FCA injection. LM1,
LM2 and indomethacin-treated rats showed significant increases ($P<0.05$) in serum SOD, ~SH, GST, GPx level and
catalase concentration on the 15th day after FCA injection
when compared with arthritic rats.

The pro-oxidant markers, namely, serum thiobarbituric acid-reacting substance level, nitric oxide and TOS level,
were found to be significantly increased ($P<0.05$ in adjuvant-
treated arthritic rats when compared with normal rats after
the 15th day of FCA injection. LM1, LM2 and indomethacin-
treated rats showed significant decreases ($P<0.05$ in serum
thiobarbituric acid-reacting substances, TOS and nitric oxide
level on the 15th day after FCA injection when compared
with arthritic rats (Table 1).

Discussion

An epidemiological association of decreased incidence of
age-related diseases in humans with diets rich in polyphenols
and antioxidants (24) has made antioxidant-rich plant-based
foods more valued than antioxidant-scarce animal protein (25),
which is a paradox in protein–energy malnutrition. Phenolic
antioxidants of the Indian fresh-water pearl-producing mussel, LM, can address this problem and help in food security,
as it is a more nutritious (26) alternative to meat, is cheaper,
and is available as a byproduct of the cash-crop, pearl. Prabhakar & Roy (22) showed that the foot portion of the mollus-
cus is eaten for its ethnomedicinal benefits in North-Bihar,
India. Polyphenol proteins have been reported to be secreted
from the specialised phenol glands of the mussel foot (23).
Polyphenols were reported as the main antioxidants of mussels in
pollution defense (27). Polyphenol antioxidants are found in
plants and none so far has been reported in animals/meat
consumed as food. These observations have led us to explore
the antioxidant activity of the foot-pad of LM and to study its
correlation with the phenol content of the foot-pad.

Thus, the experiments were designed to determine the
total phenol content, its correlation with the free-radical-
scavenging and antioxidant activity of LM extract in the

| Table 1. Effect of Lamellidens marginalis (LM) and standard on serum antioxidant marker levels in Freund’s complete adjuvant-induced arthritis animal model
<p>|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Arthritis</th>
<th>Standard</th>
<th>LM1 (500 mg/kg per d, PO)</th>
<th>LM2 (1 g/kg per d, PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Arthritis</td>
<td>Standard</td>
<td>LM1 (500 mg/kg per d, PO)</td>
<td>LM2 (1 g/kg per d, PO)</td>
<td></td>
</tr>
<tr>
<td>Parameter</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>TBARS (nmol MDA/mg protein)</td>
<td>0.09</td>
<td>0.01</td>
<td>0.17*</td>
<td>0.01</td>
<td>0.10†</td>
</tr>
<tr>
<td>~SH (μmol/mg protein)</td>
<td>1.24</td>
<td>0.02</td>
<td>3.99*</td>
<td>0.07</td>
<td>1.07†</td>
</tr>
<tr>
<td>GST (μmol/mg protein)</td>
<td>1.39</td>
<td>0.02</td>
<td>0.43*</td>
<td>0.03</td>
<td>1.35†</td>
</tr>
<tr>
<td>GPx (mU/mg protein)</td>
<td>22.49</td>
<td>0.39</td>
<td>10.75*</td>
<td>0.32</td>
<td>23.98†</td>
</tr>
<tr>
<td>SOD (U/min per mg protein)</td>
<td>0.19</td>
<td>0.01</td>
<td>0.11*</td>
<td>0.01</td>
<td>0.18†</td>
</tr>
<tr>
<td>Catalase (μmol/mg Hb)</td>
<td>7.87</td>
<td>0.54</td>
<td>4.88*</td>
<td>0.26</td>
<td>7.39†</td>
</tr>
<tr>
<td>NO (μM)</td>
<td>2.41</td>
<td>0.32</td>
<td>4.48*</td>
<td>0.19</td>
<td>2.73†</td>
</tr>
<tr>
<td>TAS (mm-Trolox equivalents)</td>
<td>2.54</td>
<td>0.05</td>
<td>2.17*</td>
<td>0.02</td>
<td>2.38†</td>
</tr>
<tr>
<td>ROS (μM H_2O_2 equivalents)</td>
<td>5.04</td>
<td>0.59</td>
<td>5.77*</td>
<td>0.83</td>
<td>6.59†</td>
</tr>
<tr>
<td>OSI (arbitrary units)</td>
<td>0.20</td>
<td>0.03</td>
<td>0.725*</td>
<td>0.04</td>
<td>0.28†</td>
</tr>
</tbody>
</table>

PO, per oral; TBARS, thiobarbituric acid-reacting substances; MDA, malonaldehyde; ~SH, total thiol; GST, glutathione transferase; GPx, glutathione peroxidase; SOD, super-
oxidase dismutase; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

* Mean values were significantly different from control ($P<0.05$, one-way ANOVA).
† Mean values were significantly different from arthritis ($P<0.05$, one-way ANOVA).
in vitro system, followed by its antioxidant efficacy in the adjuvant-induced arthritis model in rats.

Table 1 shows that LM can be considered as a food rich in antioxidants with a significant correlation between phenol content and reducing powers ($r = 0.98$; Fig. 1), indicating that the antioxidant property of LM is highly dependent on its phenol content. To study the association of this dietary antioxidant with the prevention of in vitro oxidative stress, we selected the FCA-induced inflammatory arthritis model that mimics human rheumatoid arthritis along with the feature of severe reactive oxygen species generation \(^{(28)}\). The oxidative burst takes place within the activated macrophage to combat foreign antigens in arthritis. NO is a good indicator of this oxidative burst within activated macrophages. SOD, catalase and peroxides in the presence of reduced glutathione and convert it to oxidised form. Thus, alteration of this total system indicates alteration of the body’s oxidative and antioxidant systems, markedly altered during this process. GPx regenerates the reduced glutathione from its oxidative form to sustain the antioxidant status of the body. Significantly decreased GPx level in the arthritis animal indicated the over-usage of enzyme to regenerate reduced glutathione to resist against reactive oxygen species. GST reduces organic peroxides in the presence of reduced glutathione and converts it to oxidised form. Thus, alteration of this total system indicates alteration of the body’s oxidative and antioxidant status as found in the present experiment with FCA-induced animals, which was restored due to LM treatment, thereby indicating LM to have antioxidant potential.

In summary, it could be concluded that the edible portion of LM, the pearl culture byproduct, contains antioxidants, particularly the unique phenolic proteins that are effective in the prevention of arthritis, a chronic inflammatory disease. Hence, this makes LM a suitable candidate in the design of an integrated nutritional approach.

Acknowledgements

The present work was partly funded by University of Calcutta, and Council of Scientific and Industrial Research (File No.: 09/028(031)/2010-EMRI dated 29-03-2011). M. C. and S. B. were involved in data collection, data analysis, data interpretation, literature search and manuscript preparation. R. M. was involved in data collection analysis and manuscript preparation. D. M. was involved in data collection. R. M. was also involved in the study design, data interpretation, literature search and manuscript preparation. None of the authors had any conflict of interest in connection with the present study.

References


