Original Article

Inhibitory effect of *Capparis zeylanica* Linn. on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia

Amrendra Kumar Chaudhary1*, Ruchi Solanki2, Vandana Singh3, Umesh Kumar Singh1

1Khavel Subharti College of Pharmacy, S.V. Subharti University, Meerut-250005, UP, India; 2School of Pharmaceutical Sciences, Shobhit University, Meerut-250110, UP, India; 3Translam Institute of Pharmaceutical Education and Research, Meerut, -250001, India

ABSTRACT

*Capparis zeylanica* Linn, a ‘Rasayana’ drug is used for its memory enhancing effects in the traditional Ayurvedic system of medicine. The aim of this study was to evaluate acetylcholinesterase (AChE) inhibitory and memory enhancing activities of *Capparis zeylanica* Linn. The *in-vitro* and *ex-vivo* models of AChE inhibitory activity were used along with Morris water maze test to study the effect on memory in rats. The anticholinesterase effect of methanolic and aqueous extracts of *Capparis zeylanica* was measured by spectrophotometric Ellman method at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml and brain monoamine oxidase (MAO-A and MAO-B) activity was assessed by Naoi’s method. The results of *in-vivo* and *ex-vivo* AChE assay revealed that methanolic and aqueous extracts of *Capparis zeylanica* inhibit AChE activity, whereas these extracts did not alter MAO activity at any concentration tested as compared to moclobemide and L-deprenyl. The results indicate that *Capparis zeylanica* improves scopolamine-induced memory deficits through inhibition of AChE activity, and not by direct MAO inhibition.

**Keywords** *Capparis zeylanica* Linn, Rasayana drug, anticholinesterase, MAO activity, amnesia, scopolamine

INTRODUCTION

*Capparis zeylanica* Linn, belonging to family Capparidaceae, is commonly known as Indian caper; found throughout India and has been used as a ‘Rasayana’ drug in the traditional Ayurvedic system of medicine. Rasayana plants are said to prevent ageing, re-establish youth, strengthen life, generate power, impart intelligence, aid improper digestion, clear complexion, enhance brain power, vital energy, eye sight, memory and prevent diseases, all of which imply that they establish youth, strengthen life, generate power, impart intelligence, aid improper digestion, clear complexion, enhance brain power, vital energy, eye sight, memory and prevent diseases, all of which imply that they increase the resistance of the body against any onslaught (Govindrajan et al., 2005; Puri et al., 2003). The plant *Capparis zeylanica* is reported to have alkaloids, triterpenes, flavanoids, steroidal substances and saponins. Phytochemical screening of the leaf extracts showed the presence of alkaloids, flavonoids, saponin glycosides, terpenoids, tannins, proteins and carbohydrates (Sharaf, 1997; Satyanarayan et al., 2008). The roots of *Capparis zeylanica* contain alkaloid, phytosterol, acids and mucilage. Fatty acids like ricinolenic acid, malvalic acid, stericulic acid, linoleic acid were also obtained (Mahmood et al., 1991; Haque et al., 2004). In Northern India, the leaves of *Capparis zeylanica* are widely used as counter-irritant, febrifuge and as a cataplasm in swellings, boils and piles (Chopra, 1969; Kirtikar et al., 1987). In Ayurveda, the *Capparis zeylanica* is used traditionally as cooling, cholagogue, bitter, stomachic, sedative, anti-hyrdrotic and also used in cholera, neuralgia, hemiplegia and rheumatism. Recent *in-vivo* and *in-vitro* studies have indicated antioxidant (Agarwal et al., 2009), anti-microbial (Chopade et al., 2008), analgesic (Upaganlawar et al., 2008), antipyreic (Ghule et al., 2007), anti-inflammatory (Chaudhary et al., 2004), anti-spasmodic (Mishra et al., 2007), immunomodulatory (Ghule et al., 2006) activities of *Capparis zeylanica*.

In a previous work we have evaluated the effect of methanolic and aqueous extracts of *Capparis zeylanica* Linn. leaves on spatial learning and memory in rats using Morris water maze task. Results of the previous studies indicated that scopolamine-induced amnesia was reversed by *Capparis zeylanica*, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in rat brain (Solanki et al., 2012). However till now, no scientific data is available in support of acetylcholinesterase (AChE) inhibitory and memory enhancing activities of *Capparis zeylanica*.

Therefore, with the aim to investigate the possible mechanism of its memory enhancing activity, in the present study methanolic and aqueous extracts of *Capparis zeylanica* Linn. leaves was further evaluated using *in-vitro* and *ex-vivo* models of AChE inhibitory activity and brain monoamine oxidase (MAO-A and MAO-B) activity along with Morris water maze test to study the effect on memory.

MATERIALS AND METHODS

Drugs and Chemicals

Scopolamine, galantamine, DTNB (dithiobisnitrobenzoic acid), electric eel acetylcholinesterase, acetylthiocholine iodide, serotonin, benzylamine, L-deprenyl and moclobemide were obtained from Sigma-Aldrich, U.S.A. Piracetam was purchased

©2012 by Association of Humanitas Medicine

**Address for Correspondence:** Amrendra Kumar Chaudhary

E-mail: amrendragpharma@gmail.com

**Received** March 2, 2012; **Accepted** May 23, 2012; **Published** May 31, 2012
doi: http://dx.doi.org/10.5667/tang.2012.0008
©2012 by Association of Humanitas Medicine

TANG / www.e-tang.org

2012 / Volume 2 / Issue 2 / e19
from UCB India Pvt. Ltd, India. Petroleum ether, methanol, chloroform, ammonia, sulphuric acid, felling solution B, sulphur powder precipitated, glacial acetic acid, butanol, fuming nitric acid, mercury and hydrochloric acid were procured from Rankem, New Delhi, India.

**Animals**

Adult (7 months of age) Wistar rats (160 - 240 g) and Swiss albino mice (20 - 40 g) of either sex were obtained from Indian Veterinary Research Institute, Bareilly, U.P, India. The animals were housed in polypropylene cage under standard conditions (25 ± 2°C, 12 h light and dark cycle) with free access to standard pellet feed (Ashirwad Industries, Mohali, Punjab) and water *ad libitum*. All the experimental procedures and protocols involving animals were reviewed by the Institutional Animal Ethical Committee (Registration number: 1279/ac/09/CPCEA) and were in accordance with CPCEA guidelines.

**Collection and authentication of plant material**

The leaves of *Capparis zeylanica* were obtained from a commercial supplier and authenticated by Prof. H. B Singh, Scientist F and Head, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen has been deposited at Raw Materials Herbarium & Museum (NISCAIR/RHMD/consult/2009-10/1305/108 dated November 03, 2009).

**Preparation of extracts**

The powdered leaves (150 g) were placed in Soxhlet apparatus and extracted successively using petroleum ether (40 - 60°C) and methanol for approximate 3 h. The solvent was completely removed under reduced pressure till the semi solid mass was obtained. After extraction with petroleum ether and methanol, remaining residue of leaves was air-dried. The dried marc was cold macerated at room temperature for 7 days with constant stirring and then filtered through a filter paper to obtain water extract. The extracts were stored in the refrigerator and a weighed amount was suspended in distilled water with 1% Tween 80 prior to administration (Ghule et al., 2006).

**Preliminary phytochemical testing**

Preliminary phytochemical screening of the methanolic and aqueous extract of the leaves of *Capparis zeylanica* was done to test the presence of the active chemical constituents such as alkaloids, glycosides, flavonoids, tannins, amino acid, carbohydrates, phenolic compounds, fats and fixed oils (Khandelwal, 2004).

**Acute toxicity study**

The *Capparis zeylanica* extract was administered orally in the dose of 50, 100, 150, 200, 400 and 800 mg/kg to different groups of mice (n = 6). After administration of extracts, mice were allowed food and water *ad libitum*. All the animals were observed for possible mortality cases and behavioral changes for 24 h (Lorke, 1983).

**In-vitro acetylcholinesterase assay**

The methanolic and aqueous extracts of leaves of *Capparis zeylanica* were tested for their in-vitro acetylcholinesterase inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Ellman et al., 1961). Electric eel acetylcholinesterase was used, and acetyl thiocyanate iodide (ATCI) was used as a substrate of the reaction. 5, 5-dithiobis (2-nitrobenzoic acid (DTNB) was used for the measurement of AChE activity. Briefly, 150 μl of 0.1 M sodium phosphate buffer (pH 8.0), 10 μl test compound solution (in ethanol), and 20 μl of enzyme solution (0.09 units/ml) were mixed and incubated for 15 min at 25°C. 10 μl of DTNB (10 mM) was then added and reaction was initiated by the addition of substrate (10 μl of ATCI, 14 mM solution). The hydrolysis of the ATCI was measured by the formation of the colored product 5-thio-2-nitrobenzoate anion formed by the reaction of DTNB and thiocyanate, which is released by the hydrolysis of enzyme. The formation of the colored product was measured at 410 nm after 5 min. Galantamine, a standard AChE inhibitor was used as positive control, which was dissolved in ethanol. Percent acetylcholinesterase inhibition was calculated (Ahmed et al., 2009).

\[
\text{Percentage inhibition} = \left( \frac{E - S}{E} \right) \times 100
\]

Where, *E* is the activity of enzyme without test sample and *S* is the activity of enzyme with test sample. All the experiments were done in triplicate (Orhan et al., 2009).

**Ex-vitro estimation of acetylcholinesterase activity**

Animals were pre-treated with methanolic and aqueous extracts of *Capparis zeylanica* (50, 100 and 150 mg/kg) orally for 7 days and on the day of experiment the same doses were administered 1 h before the experiment. To assess the anti-AChE activity of methanolic (MCZ) and aqueous (ACZ) extract of the leaves of *Capparis zeylanica*, all groups of rats were decapitated for enzymatic estimation, after the probe trial. The animals were sacrificed by means of quick decapitation without anaesthetia and the brain was isolated immediately. The dissection for discrete regions of brain (frontal cortex and hippocampus) was carried out. AChE inhibitory activity of MCZ and ACZ was measured as described in in-vitro assay method (Ellman et al., 1961) in frontal cortex and hippocampus. The discrete brain regions were homogenized in ice cold 0.1 M phosphate buffer (pH 8.0) using homogenizer. The homogenates were centrifuged at 1000 x g for 10 min at 4°C, and supernatant was used as a source of enzyme in AChE assay. The total acetyl cholinesterase activity in the aliquot of the homogenate was estimated. The aliquot was mixed with phosphate buffer (pH 8.0). To this, the substrate acetyl thiocyanate iodide and dithiobisnitrobenzoic acid (DTNB) reagent were added. Acetyltiothiocyanate iodide was hydrolyzed to thiocyanate and acetate by AChE. Thiocyanate reacted with DTNB reagent to produce a yellow colour. The rate of formation of thiocyanate from acetyltiothiocyanate iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The rate of colour (yellow) development is the measure of the AChE activity. Change in absorbance per minute of the sample was read at 410 nm. The enzyme activity is expressed as the ‘*n*’ moles of substrate hydrolyzed/minute/mg of protein (Ahmed et al., 2009). The protein contents were determined in the brain samples using Lowry (1951) method.

\[
R = \frac{\delta OD}{E \times mg of protein}
\]

Where R is the rate of enzyme activity in ‘*n*’ mole of acetyltiothiocyanate iodide hydrolyzed per minute per mg of protein. \(\delta OD\) is the change in absorbance per minute and *E* is the extinction coefficient, which is 13600 M\(^{-1}\) cm\(^{-1}\).

**Measurement of MAO-A and MAO-B activity**

**Monoamine oxidase (MAO) preparation from mouse brain**

The crude MAO was prepared using Naoi’s method. The male mice (20 - 25 g) were decapitated, and the brains were homogenized in an ice cold 20 ml of 0.25 M sucrose solution containing a 10 mM potassium phosphate buffer (pH 7.4) and 25 g) were decapitated, and the brains were homogenized in an ice cold 20 ml of 0.25 M sucrose solution containing a 10 mM potassium phosphate buffer (pH 7.4) and
centrifuged at 1,200 g for 5 min at 4°C. The supernatant was collected and further centrifuged at 16,000 g for 20 min at 4°C. The crude mitochondrial pellet was washed using a 10 mM sodium phosphate buffer (pH 7.4) and suspended in the same buffer.

**In-vitro assay for MAO activity**
The monoamine oxidase type A and B, with serotonin and benzyl amine as a substrate, were assayed spectrophotometrically (Naoi et al., 1987; Ro et al., 2001). The activity of monoamine oxidase-A was determined by quantifying the oxidative product of 5-hydroxytryptamine (serotonin), which has an absorbance peak at 280 nm. Similarly, the activity of monoamine oxidase-B was determined by quantifying the oxidative product of benzyl amines, which has an absorbance peak at 242 nm (Nag et al., 2001). MAO activity of monoamine oxidase (serotonin), which has a quantifying oxidative product of benzyl amine as a substrate, were assayed in vitro assay for MAO activity.

**RESULTS**

**Yield of plant extract**
The yield of petroleum ether, methanol and aqueous extracts of *Capparis zeylanica* was found to be 3.07, 9.03 and 12.17%, respectively.

**Phytochemical testing of Capparis zeylanica**
Preliminary phytochemical screening indicated presence of alkaloid, flavonoids, saponins, terpenoids, steroids and tannins were present in methanolic extract of *Capparis zeylanica*. Whereas aqueous extract showed the presence of alkaloid, flavonoids, saponins, terpenoids, steroids and carbohydrates.

**Acute toxicity**
The mice treated orally with 50, 100 and 150 mg/kg of methanolic and aqueous extracts of *Capparis zeylanica* was found to be normal. The acute toxicity study revealed that the extracts have good margin of safety and did not show lethal effects on the animals up to the doses of 800 mg/kg.

**Effect on brain MAO activity**
The methanolic and aqueous extracts of leaves of *Capparis zeylanica* (MCZ and ACZ respectively) were tested for their in-vitro MAO inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Table1). The extracts did not inhibit

---

**Table 1. In-vitro acetylcholinesterase and MAO inhibitory activity of extracts**

<table>
<thead>
<tr>
<th>Conc. (mg/ml)</th>
<th>Parameter</th>
<th>Treatment</th>
<th>IC_{50} (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>MCZ</strong></td>
<td><strong>ACZ</strong></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>MAO-A</td>
<td>1.58 ± 0.480</td>
<td>1.67 ± 0.511</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>1.48 ± 0.607</td>
<td>1.48 ± 0.607</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>13.27 ± 1.958</td>
<td>10.55 ± 0.922</td>
</tr>
<tr>
<td>0.3</td>
<td>MAO-A</td>
<td>2.68 ± 0.096</td>
<td>2.22 ± 0.912</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>1.91 ± 0.817</td>
<td>1.91 ± 0.817</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>21.03 ± 0.806</td>
<td>17.46 ± 0.929</td>
</tr>
<tr>
<td>1.0</td>
<td>MAO-A</td>
<td>3.25 ± 0.553</td>
<td>3.42 ± 0.585</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>2.75 ± 1.020</td>
<td>2.75 ± 1.020</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>31.42 ± 1.979</td>
<td>27.17 ± 1.861</td>
</tr>
<tr>
<td>3.0</td>
<td>MAO-A</td>
<td>3.01 ± 0.911</td>
<td>3.01 ± 0.911</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>3.24 ± 1.211</td>
<td>3.24 ± 1.211</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>38.35 ± 1.194</td>
<td>33.86 ± 0.990</td>
</tr>
<tr>
<td>10.0</td>
<td>MAO-A</td>
<td>4.25 ± 0.210</td>
<td>4.25 ± 0.210</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>7.28 ± 2.254</td>
<td>7.28 ± 2.254</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>50.80 ± 0.912</td>
<td>46.78 ± 3.074</td>
</tr>
<tr>
<td>30.0</td>
<td>MAO-A</td>
<td>4.85 ± 0.921</td>
<td>4.85 ± 0.921</td>
</tr>
<tr>
<td></td>
<td>MAO-B</td>
<td>7.55 ± 0.843</td>
<td>7.55 ± 0.843</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>55.22 ± 0.492</td>
<td>52.96 ± 2.188</td>
</tr>
<tr>
<td></td>
<td><strong>MAO-A</strong></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>MAO-B</strong></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><strong>AChE</strong></td>
<td>12.32</td>
<td>19.49</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM

Not calculated due to its less activity. The data were analyzed with Graph Pad Prism 3.0 computer software statistical analysis using Two-way ANOVA followed by Bonferroni post tests. Median inhibitory concentrations (IC_{50} values) are represented with 95% confidence intervals (CI). A value of p < 0.05 was considered to be statistically significant.
Effect on in-vitro acetylcholinesterase activity

The methanolic and aqueous extracts of leaves of Capparis zeylanica were tested for their in-vitro acetylcholinesterase inhibitory effect at 0.1, 0.3, 1.0, 3.0, 10 and 30 mg/ml concentrations (Table 1). The extracts showed a mild inhibition as compared to galantamine. The most active one was noted to be methanolic extract having IC$_{50}$ value at 12.32 mg/ml, aqueous extract displayed a similar activity with IC$_{50}$ 19.49 mg/ml.

Ex-vivo acetylcholinesterase assay in frontal cortex and hippocampus

After testing the spatial reference memory by Morris water maze test, ex-vivo assay was performed for assessing the AChE activity. The analysis showed that brain AChE activity was different between groups [F$_5$, 40 = 232.72, p < 0.0001] in different brain regions [F$_1$, 80 = 4.41, p < 0.05] (Table 2 and 3). Further, post-hoc analysis revealed that both the extracts of Capparis zeylanica (50, 100 and 200 mg/kg) dose-dependently decreased AChE activity in prefrontal cortex and hippocampus compared to control.

DISCUSSION

Cholinergic neurons in the central cholinergic system possess an important function in the process of learning and memory which is influenced by drugs effective in this system (Everitt et al., 1997). Therefore, the measurement of acetylcholinesterase activity has been extensively used as an experimental model to screen pure molecules or extracts with possible efficiency against dementia.

Many anti-cholinergic drugs, such as scopolamine induces a transient disruption of memory in humans and experimental animals by blocking postsynaptic muscarinic receptors (Drachman et al., 1974). This effect can be antagonized by cholinomimetics, such as physostigmine (Megazzini et al., 1965) which increase brain ACh contents. To find out whether Capparis zeylanica has any cholinergic activity, the MCZ and ACZ were evaluated for anti-AChE activity in in-vitro and ex-vivo models. In the present study, MCZ and ACZ pre-treatment for 7 days (50, 100 and 150 mg/kg) inhibited AChE activity in the frontal cortex and hippocampus regions. Anti-cholinesterase activity of both the extracts of Capparis zeylanica may facilitate cholinergic neurotransmission which is important for learning and memory functions.

Therefore, cholinergic hypofunction represents as one of the major problems resulting in cognitive impairment. The results of ex-vivo AChE assay revealed that Capparis zeylanica extracts possess AChE inhibitory activity in hippocampus and frontal cortex, which suggests their ability to cross blood brain barrier and by inhibiting AChE enzyme providing longer time for acetylcholine to stimulate postsynaptic muscarinic receptors. Enhancing cholinergic transmission is likely to offer beneficial effects which may improve patient's conditions. Taking these facts together anti-AChE activity of Capparis zeylanica may be an important mechanism associated with improvement of learning and memory functions in rats.

Studies have suggested subtle functions of the different amineergic systems in spatial memory formation. Under certain circumstances, stimulation of the noradrenergic system enhances MWM acquisition. The selective monoamine oxidase B (MAO-B) inhibitor L-deprenyl thus alleviated scopolamine-induced acquisition and probe trial performance deficits (Yavich et al., 1993). The results from the present study show that Capparis zeylanica extracts did not alter MAO activity at any concentration tested as compared to moclobemide and L-deprenyl. Thus, it can be concluded that the nootropic activity of Capparis zeylanica is not a result of direct MAO inhibition but may be, at least in part, mediated by AChE inhibition. However, these contentions need to be further explored before coming to any conclusion on precise mechanism of action.

Preliminary phytochemical screening showed that flavonoids and terpenoids were the main chemical constituents of Capparis zeylanica extracts. Flavonoids and terpenoids contribute synergistically to the neuroprotection mainly via antioxidant activity (Mu et al., 2007). Probably, these compounds are considered to play major role in memory improvement effect of this plant. The present study suggests that Capparis zeylanica may possibly play an important role in

<table>
<thead>
<tr>
<th>Location</th>
<th>Control</th>
<th>Scopolamine</th>
<th>MCZ-50</th>
<th>MCZ-100</th>
<th>MCZ-150</th>
<th>P-500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>35.99 ± 0.792</td>
<td>40.92 ± 2.214$^a$</td>
<td>28.45 ± 0.176$^{cd}$</td>
<td>23.43 ± 0.879$^{cd}$</td>
<td>17.95 ± 0.578$^{b,cd}$</td>
<td>13.14 ± 0.439$^{b,cd}$</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>34.32 ± 0.391</td>
<td>39.03 ± 0.784$^b$</td>
<td>26.65 ± 0.142$^{cd}$</td>
<td>21.83 ± 0.957$^{cd}$</td>
<td>16.75 ± 0.862$^{b,cd}$</td>
<td>12.88 ± 0.391$^{b,cd}$</td>
</tr>
</tbody>
</table>

After probe trial in Morris water maze test, AChE assay was carried out in frontal cortex and hippocampus. Piracetam was used as the standard drug. The results are expressed as mean ± standard error mean (SEM). The data were analyzed with Graph Pad Prism statistical analysis using Two-way ANOVA followed by Bonferroni post tests. A value < 0.05 was considered statistically significant. $^a$ p < 0.001 compared with control, $^b$ p < 0.01 compared with control, $^c$ p < 0.001 compared with scopolamine, $^d$ p < 0.01 compared with ACZ-50, $^e$ p < 0.01 compared with ACZ-100, $^f$ p < 0.001 compared with ACZ-150 and $^g$ p < 0.001 compared with ACZ-150.
preventing cholinergic dysfunctions. To our knowledge this is the first report providing evidence for the AChE inhibitory and memory enhancing effect of the *Capparis zeylanica* in scopolamine-induced amnesia in rats.

**ACKNOWLEDGEMENTS**

Authors would like to thank Prof. (Dr.) Ranjit Singh of School of Pharmaceutical Sciences, Shobhit University for their technical assistance.

**CONFLICT OF INTEREST**

The authors have no conflicting financial interests.

**REFERENCES**


Mishra SN, Tomar PC, Lakra N. Medicinal and food values of *Capparis* a harsh terrian plant. Indian J Traditional Knowledge. 2007;6:230-238.


Naoi M, Nagatsu T. Quinoline and quininaldines as naturally occurring inhibitors specific for type A monoamine oxidase. Life Sci. 1987;40:1075-1082.


Woo HI, Seong SH, Seon AL, Xiang HH, Kyong SL, Myung KL, Bang YH, Jai SR. Monoamine oxidase inhibitory 2012 / Volume 2 / Issue 2 / e19