EVIDENCE-BASED USE OF SEA BUCKTHORN FRESH JUICE FOR PATIENTS WITH TRAUMATIC BRAIN INJURY. A PILOT STUDY

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Abstract

Sea buckthorn (Hippophaë rhamnoides L.) berries are widely used for their nutritional value and therapeutic effects. The aim of our paper was the evolution of phenolic content (by means of spectrophotometric methods), antioxidant capacity (scavenger activity towards DPPH, ABTS·+, free radicals and ferric reducing power) and evidence regarding sea buckthorn fresh juice association in the management of hospitalized patients with traumatic brain injury. According to our results, sea buckthorn fresh juice is a source of phenolic compounds (0.28 g total phenolic content expressed as tannic acid equivalents/100 mL fresh juice and 0.09 g flavones expressed as hyperoside equivalents/100 mL fresh juice), with antioxidant activity. Our clinical results have shown beneficial effects (a decrease in lactate and sodium blood levels and an increase in Glasgow coma score) of sea buckthorn fresh juice (given together with specific medication) in the management of patients with traumatic brain injury.

Rezumat

Fructele de cătină (Hippophaë rhamnoides L.) sunt întrebuințate atât pentru aportul nutrițional cât și datorită efectelor terapeutice variate. Scopul lucrării a constat în evaluarea conținutului în compuși fenolici (prin metode spectrofotometrice), a activității antioxidante (chelatarea radicalilor liberi DPPH, ABTS·+), capacitatea de reducere a ferului) și a beneficiilor terapeutice ale sucului de cătină la pacienți spitalizați cu neurotraumă. Rezultatele obținute au arătat că sucul de cătină este o sursă de polifenoli (0,28 g polifenoli totali exprimați în echivalenți de acid tanic/100 mL suc și 0,09 g flavone exprimate în echivalenți de hiperozidă/100 mL suc), cu efect antioxidant. Rezultatele clinice au arătat o îmbunătățire a stării de sănătate (scăderea concentrațiilor serice de sodiu și lactat, creșterea scorului Glasgow) a pacienților cu neurotraumă, la care s-a administrat pe lângă medicația specifică și sucul de cătină.

Keywords: sea buckthorn berry juice, polyphenols, antioxidant capacity, traumatic brain injury

Introduction

Traumatic brain injury (TBI) represents one of the most serious public health problems worldwide, accounting for 10% of the world’s deaths [24]. Moreover, neurotrauma is suspected to contribute to neurodegenerative diseases, chronic traumatic encephalopathy, epileptic seizures or post traumatic diabetes [1, 5, 11]. Recent researches have shown that almost 51% of patients with TBI suffer of electrolyte imbalances and the mortality rates are higher for patients with hyponatremia [2, 16, 19]. Hyponatremia leads to an increase in extracellular osmolarity (beyond 280 mOsmol/kg) and further activation of osmoreceptors in the hypothalamus. Activation of osmoreceptors favours the release of antidiuretic hormone and arginine, which in turn increase the reabsorption of water in the distal tubule and collecting duct [11]. Moreover, TBI patients have increased blood and cephalorachidian lactate levels, which are correlated to brain injuries severity [27]. It is well known that physiological increased lactate levels trigger reperfusion of ischemic brain tissues after TBI [29]. However, severe TBI leads to neuronal death, which in turn accounts for high lactate levels, which become an unfavourable prognostic factor [15, 20, 22]. According to recent studies natural compounds (mainly polyphenols) positively influence several events involved in TBI (neuroinflammation, apoptosis, oxidative stress etc.) [13, 21, 28].

Sea buckthorn (Hippophaë rhamnoides L.) berries are a source of bioactive compounds with a wide range of therapeutic effects (hypercholesterolemic, hypoglycaemic, antioxidant, anti-inflammatory, etc.) [4, 12, 14, 23, 31]. Taking into consideration the scientific data, the aim of our research was the evaluation of phenolic content, antioxidant capacity and evidence regarding sea buckthorn fresh juice association in the management of patients with TBI.
Materials and Methods

Materials

Sea buckthorn juice (SSBJ) was obtained from fresh ripe fruits, collected in September 2018, from Prahova County, Romania. Juice extraction was performed at room temperature with an electric juice extractor (Philips HR18701/70, Netherlands).

All chemicals were purchased from Sigma-Aldrich and used without further purification.

Spectrophotometric determination of total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method, as previously described [6, 10]. Results were expressed as g tannic acid/100 mL SBJ, based on a calibration curve (2 - 9 µg/mL, \( R^2 = 0.9999, n = 8 \)).

Spectrophotometric determination of flavones content

Flavones were determined based on their reaction with aluminium chloride as previously described [10]. Results were expressed as g hyperoside/100 mL SBJ, based on a calibration curve (7 - 34 µg/mL, \( R^2 = 0.9999, n = 12 \)).

Determination of antioxidant capacity

The antioxidant capacity was evaluated by means of scavenger activity towards 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS'+) free radicals and ferric reducing power, as previously described [6, 10]. The concentration range was 0.6 - 12 µL SBJJ (for DPPH and ferric reducing power assays) and 0.6 - 6 µL SBJJ (for ABTS'+ assay). The antioxidant activity was expressed as EC\(_{50}\) (µL), which represents the SBJJ concentration that inhibits with 50% the activity of DPPH/ABTS' + free radicals or the SBJJ scavenging of a 0.5 absorbance (for ferric reducing power).

The antioxidant activity was also expressed as ascorbic acid equivalents (mM ascorbic acid/100 mL SBJJ), based on vitamin C calibration curves: 0.005 - 0.04 mg/mL, \( R^2 = 0.9975, n = 5 \) (DPPH method); 0.01 - 0.07 mg/mL, \( R^2 = 0.9973, n = 6 \) (ABTS' + method) and 0.008 - 0.04 mg/mL, \( R^2 = 0.9969, n = 5 \) (ferric reducing power).

Experimental protocol for SBJJ administration to TBI patients

Neuronal resuscitation protocol (“Floreasca” Emergency Hospital, Bucharest, Romania protocol) was used for SBJJ administration to TBI patients, hospitalized in the Anaesthesia and Intensive care unit (NCH-STI). Patients were divided into two groups. The control group consisted of five patients that received only common medication. The treatment group consisted of 26 TBI patients that received SBJJ besides common medication. Patients were given (by nasogastric intubation) 10 mL SBJJ /kg bw/day in the morning (8.30 - 10.30 a.m.). The patients did not receive food two hours before and after administration of SBJJ. Patients were monitored for 20 days, regarding their blood sodium/lactate levels (which were determined in the hospital’s laboratory) and Glasgow coma score. The clinical protocol was approved by the Local Medical Ethics Committee of “Floreasca” Emergency Hospital. An informed consent form was signed prior to administration of SBJJ by patients’ family members.

Statistical analysis

Spectrophotometric and antioxidant assays were carried out in triplicate. Results are presented as mean ± standard deviation (SD) and were determined in Microsoft Office (Excel, 2010). The statistical analysis for clinical data was performed using the open source software R. The data set was longitudinal and its main purpose was to characterize the changes observed over time (20 days) in three continuous response variables: blood sodium levels, blood lactate levels and Glasgow coma score. It was also important to highlight the main differences between control and treatment groups. As the conditions of normality and homoscedasticity were partially fulfilled, a robust method for two-way repeated measures ANOVA based on the trimmed means has been used [17]. We have also applied post hoc comparisons based on a single effect which uses a bootstrap approach [8, 9]. Moreover, in the statistical analysis we followed the evolution of the Glasgow median score in relation to the average values of the two parameters (blood sodium lactate levels over 20 days), using Pearson coefficient, because the assumption of bivariate normality of residuals was fulfilled. A value of \( p < 0.05 \) was considered the threshold for a statistically significant difference.

Results and Discussion

As shown in Table I, SBJJ is a source of phenolic compounds. Our results regarding the total phenolic content are similar to Tian Ye et al. that found 20.5 - 25.5 mg gallic acid/100 mL fresh juice (equivalent to 0.2049 - 0.2499 mg tannic acid/100 mL fresh juice) [26], but much lower compared to Mendelova A et al. that found 2 - 2.92 g gallic acid/1 L of fresh juice (equivalent to 1.99 - 2.91 g tannic acid/100 mL fresh juice) [18]. Our flavones content is moderate, but a comparison with other authors’ results was difficult, since most studies report the flavones content only for freeze-dried berries [12].

Our results regarding the antioxidant activity, showed that SBJJ has the ability to scavenge DPPH, ABTS' + free radicals and ferric reducing power properties. The scavenger activity towards DPPH varied between 29.11% (for 0.6 µL) and 85.07% (for 12 µL). The results are higher, compared to Tian Ye et al. that only found 29 - 31% inhibition [26]. Regarding the ABTS' + assay, SBJJ scavenged the free radical in a dose dependent manner; the highest inhibition (91.36%) was achieved for 6 µL fresh juice. The higher antioxidant activity towards ABTS' + free radical, compared to DPPH, might be the consequence of a different
mechanism of action. Concerning ferric reducing power, the highest absorbance (0.5518) was found for the highest concentration (12 µL fresh juice). As shown in Table I, the highest antioxidant activity was observed using ABTS⁺ assay, followed by DPPH and ferric reducing power methods. Important antioxidant activity for SBBJ was also reported by other authors [12, 14, 18, 23]. We assume that flavones [7], phenolcarboxylic acids [12], proanthocyanidins [31], carotenoids (mainly epoxide forms which are soluble in water) [14, 25] and mineral elements [4] are responsible for SBBJ overall antioxidant capacity.

Table I

| SPECTROPHOTOMETRIC ASSAYS | Total phenolic content (g tannic acid/100 mL) | 0.2895 ± 0.0162 |
|----------------------------|-----------------------------------------------|-----------------|
| Flavones                   | Flavones (g hyperoside/100 mL)                | 0.0934 ± 0.0104 |
| ANTIOXIDANT ASSAYS         | Method                                        | DPPH            |
|                            | EC₅₀ (µL)                                     | 4.70 ± 0.2687   |
|                            | Ferric reducing power                         | 11.66 ± 0.1272  |
|                            | Ascorbic acid equivalents                     | EC₅₀ (µL)       |
|                            | Ascorbic acid equivalents (mM ascorbic acid/100 mL) | 4.2732 ± 3.3491 |

SBBJ – sea buckthorn fresh juice, EC₅₀ – SBBJ concentration (µL) that inhibits with 50% the activity of DPPH, ABTS⁺ free radicals or the SBBJ concentration that provides 0.5 absorbance (for ferric reducing power). Results are expressed as mean ± SD (n = 3).

Regarding our clinical results, for sodium blood levels response variable, one can note (Figure 1A) significant differences between groups (p = 0.0049) and significant differences relative to time (p = 0.002), but an insignificant interaction between time and group factors. Significant post hoc differences between groups were observed on days 1, 3, 4, 7, 8 (p < 0.05). For lactate blood levels response variable (Figure 1B), we observed significant differences between groups (p = 0.00012) and significant differences relative to time factor (p = 0.002) and also a significant interaction between time and group factors (p = 0.009). Significant post hoc differences between groups were observed on days 1, 2, 3, 4, 5, 11, 13, 18, 19, 20 (p < 0.05). For the Glasgow coma score response variable (Figure 1C) we obtained extremely significant differences between groups (p < 0.0001) and significant differences regarding time factor (p = 0.0011) and also a significant interaction between time and group factors (p = 0.0066). Significant post hoc differences between groups were observed during the whole period of SBBJ administration (p < 0.003).

The average evolution of each response variable relative to time factor is highlighted for each analysed group (control/treatment) in Figure 2. As for our results (Figures 3 and 4), one can observe that the magnitude of the Pearson coefficient indicates an extremely high negative correlation (between Glasgow coma score and sodium/lactate blood levels) in the tested group (lactate blood levels = -0.961, CI 95% = (-0.98, -0.90) where p < 0.0001 and sodium blood levels = -0.96, CI 95% = (-0.98, -0.90) where p < 0.0001) and a medium negative value in the control group (lactate blood levels = -0.62, CI 95% = (-0.83, -0.25) where p = 0.003 and sodium blood levels = -0.68, CI 95% = (-0.86, -0.35) where p = 0.008), interpretations given by guidelines of Cohen (Figure 3). The linear and quadratic regression functions that best estimate the dependency between the Glasgow coma score and lactate/sodium blood levels variables are presented in Figure 4. For the control group the adjusted r-squared values are relatively low: lactate blood levels explain...
35.9% of Glasgow coma score variability ($p = 0.003$), while sodium blood levels explain 44% ($p = 0.0008$). For the treatment group the adjusted r-squared values are extremely high: lactate blood levels explain 92.5% of Glasgow coma score ($p < 0.0001$) and sodium blood levels explain 91.8% ($p < 0.0001$). Residual normality, independence and homoscedasticity were fulfilled. The quadratic model was chosen based on the Akaike information criterion (AIC).

![Interaction Plot of a Na serial incidence](image1)

**Figure 2.** Interaction of all variables (for control and treatment groups) relative to time factor

![Interaction Plot of a lactat serial incidence](image2)

![Interaction Plot of a Glasgow score incidence](image3)

![Figure 3.](image4)

Correlation between variables

![Figure 4.](image5)

V Variation of Glasgow coma score for analysed groups

We assume that polyphenols are responsible for SBBJ beneficial effects (decreased sodium and lactate blood levels and increased Glasgow coma score) in patients with TBI. TBI involves hypoxia, cell death, mitochondrial
dysfunction, oxidative stress, neuroinflammation and metabolic dysfunction [5]. According to recent research, flavones from sea buckthorn fresh juice (quercetin glycosides) [7] reduce the expression of Bax and cleaved caspase-3 proteins (involved in apoptosis), decrease the level of pro-inflammatory cytokines and increase the activity of antioxidant enzymes [30]. Kaempferol [7] was found to improve tricarboxylic acid cycle flux, neurons viability and mitochondrial integrity [13]. Among phenoliccarboxylic acids found in sea buckthorn juice [12], chlorogenic acid has shown strong anti-inflammatory effects in TBI, through down-regulation of TLR4 (toll-like receptor)/Nf-κb signalling pathways [3].

Conclusions

Sea buckthorn juice is a source of bioactive compounds, with antioxidant activity and beneficial effects (a decrease in lactate and sodium blood levels and an increase in Glasgow coma score) for hospitalized patients with traumatic brain injury.

Conflict of interest

The authors declare no conflict of interest.

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