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**PROTOCOL FOR NEURAL RESUSCITATION
AFTER SERIOUS ASSAULT
A NEW THERAPEUTIC APPROACH TO SEVERE TRAUMATIC
BRAIN INJURY
ABSTRACT OF DOCTORAL THESIS**

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TABLE OF CONTENTS

List of published scientific papers.....	5
List of abbreviations.....	6
Introduction.....	7
1. Traumatic brain injury. Pathophysiology and therapeutic attitude.....	10
1.1. Brain injuries.....	11
1.1.1. Focal injuries.....	12
1.1.2. Secondary injuries.....	13
1.1.2.1. Excitotoxicity.....	13
1.1.2.2. Mitochondrial dysfunction.....	14
1.1.2.3. ROS release and lipid peroxidation.....	15
1.1.2.4. Neuroinflammation.....	16
1.1.2.5. Axonal degeneration.....	16
1.1.2.6 Glial scarring and myelin-associated axonal growth inhibitors.....	16
1.1.2.7 Cell death by apoptosis of neurons and oligodendrocytes.....	17
1.1.2.8. Affection of autophagy and lysosomal pathways.....	17
1.2. Therapies in posttraumatic brain injury.....	18
1.2.1. Protection of neurons and glia against toxicity.....	18
1.2.2. Calcium channel blockers and calcium channel blocker enzymes.....	19
1.2.3. Treatment of chemical stress for neurons and glia.....	19
1.2.4. Anti-inflammatory and anti-apoptotic agents.....	20
1.2.5. Erythropoietin.....	20
2. Natural compounds. Therapeutic intervention in TBI.....	21
3. Discussion of clinical cases that have benefited from the Floreasca Treatment Protocol.....	32
Clinical case 1.....	33
Clinical case 2.....	35
Clinical case 3.....	38
Clinical case 4.....	43
Clinical case 5.....	45
Clinical case 6.....	47

Clinical case 7.....	50
Clinical case 8.....	56
Clinical case 9.....	59
Clinical case 10.....	66
4. Influence of fresh sea buckthorn juice administration on biochemical parameters in patients with traumatic brain injury.....	77
4.1. Comparative phytochemical analysis of sea buckthorn fruit and juice.....	77
4.1.1. Flavone dosage.....	77
4.1.2. Total polyphenols dosage.....	79
4.1.3. Carotenoids dosage.....	81
4.2. Determination of antioxidant action in the acellular system.....	83
5. Influence of dietary supplementation on biochemical parameters in patients with traumatic brain injury.....	102
6. Considerations on potential mechanisms of action induced by phytocomplexes administered to patients with neurotruma.....	124
6.1. Potential mechanisms induced by the phytocomplex in sea buckthorn juice.....	124
6.2. Potential mechanisms induced by the phytocomplex in Ursomax.....	133
General conclusions.....	137
Originality degree.....	139
Research perspectives.....	140
Bibliography.....	141
Annexes.....	163

INTRODUCTION

The accident is a product of chance. Its unpredictable nature makes it a factor that intervenes at any time, for example at an intersection, while respecting the traffic rules, another vehicle runs a red light from the opposite direction. The accident is serious. The driver is trapped, requiring rapid extrication and intervention of Smurd. We are dealing with a polytrauma. The thorax, abdomen, upper and/or lower limbs and, last but not least, the head are probably affected. The countdown to rescue and recovery begins. The entire procedure is carried out according to a protocol accepted by the medical world. As we know after the assessment of the traumatic pathology, we start the treatment aiming not only to save the patient's life but also his neuromotor recovery. The life that by a favourable circumstance has been saved, must give him the existential motivation to feel again the emotions and pleasures of life, at least at the same level compared to life before the accident.

Herbal therapies have emerged due to the urgent need to improve the life quality of patients, especially patients who, until the accident, were deeply involved in professional, family and social life. Any occurring delay comes at a cost that the patient and the managing team have to bear. Herbal therapy is an active intervening factor in polyvalent therapy by reducing oxidative stress and inflammatory factors involved in the cytokine cascade - herbal therapy reduces tumour necrosis factor, nitric oxide, reduces blood-brain barrier permeability, decreases brain water content (anti-oedema).

Based on these premises, the main objective of the thesis was to highlight the role of dietary supplements in improving the symptoms induced by traumatic brain injury and in the recovery of these patients.

The specific thesis objectives were:

- ✚ Systematization of literature data on traumatic brain injury;
- ✚ Systematisation of literature data on natural compounds that have proven their efficacy in laboratory tests in terms of beneficial effects induced as pre- or post-treatment in experimental animals;
- ✚ Systematization of literature data on potential neuronal protective mechanisms induced by various natural compounds;
- ✚ summary presentation of 10 clinical cases that benefited from the Floreasca Treatment Protocol;

- ✚ presentation of my personal point of view in each clinical case, patients being strictly cared for under my coordination;
- ✚ phytochemical research aimed at quantitative phytochemical screening of sea buckthorn fruit and juice;
- ✚ determination of the antioxidant action of sea buckthorn juice, an important parameter in reducing oxidative stress;
- ✚ Evaluation of the influence induced on some biochemical parameters as well as the Glasgow score when administering sea buckthorn juice to neurotrauma patients;
- ✚ Evaluation of the influence induced on some biochemical parameters as well as the Glasgow score when Ursomax supplement and sea buckthorn juice are administered to neurotrauma patients;
- ✚ predicting potential mechanisms of action;
- ✚ linking all the results obtained and disseminating them in journals in the international scientific stream.

THE CURRENT STATE OF KNOWLEDGE

1. TRAUMATIC BRAIN INJURY

PATHOPHYSIOLOGY AND THERAPEUTIC ATTITUDE

Traumatic brain injury (TBI) is a major cause of death. In 2005, more than 3117000 patients were reported with posttraumatic neurological, psychosocial and sequelae complications [1]. Clinical manifestations include comatose state, seizures, headache, aphasia, amnesia, behavioural abnormalities. The incidence of traumatic brain injury is in the civilian population. Penetrating foreign body trauma can pass through the calvarium and traverse the dura and brain parenchyma. With regard to closed head trauma, tearing produces focal damage, cerebral haemorrhage, cerebral oedema and ischaemia. The severity of neurological damage correlates with the size, speed, trajectory and strength of the foreign body penetrating the brain. Brain tissue superinfection can be a relatively common complication. Head trauma interpreted in the context of complex polytrauma may be associated with respiratory failure, pneumonia (by aspiration), loss of brain tissue if there is a continuity solution [2]. "Roughly speaking" brain injuries can be primary, produced by mechanical forces during trauma, and secondary, tissue and cellular injuries secondary to primary trauma.

Brain tissue damage in the primary phase is not reversible, the goal of treatment initiated is

to stabilize the trauma and prevent damage in the secondary phase [3]. Therapeutic intervention causes loss of neurons and glial tissue around the epicenter where there is already inflammatory response, excitotoxicity, oxidative stress and apoptosis [4].

2. NATURAL COMPOUNDS. THERAPEUTIC INTERVENTION IN TBI

In this chapter, the information will be targeted on natural compounds isolated from different types of plant raw materials, highlighting the benefits induced in the pre-treatment, treatment or post-treatment of different forms of TBI. The research for the following should be mentioned: allicin [5], apocynin [6], baicaloside [7], caffeine [8], curcumin [9], crocin [10], ellagic acid [11], epigallocatechin-3-gallate [12], luteolin [13], naringenin [14], quercetin [15] etc.

PERSONAL RESEARCH

RESEARCH HYPOTHESIS

In emergency hospitals, polytrauma is a common occurrence. The involvement of craniocerebral pathology adds to the severity of the complex picture of polytrauma: abdomen, thorax, upper/lower limbs. The tendency in a patient who comes in extremely serious general condition is to return him to normal functionality, monitoring cardiovascular, respiratory parameters, the patient being under analgesia. Creating this therapeutic "comfort" often comes at an exaggerated price, as the analysis of a neurological diagnosis can be done on a conscious and cooperative patient. Severe cases or severe polytrauma come in a comatose state, often profound, which may require analgesia, making it impossible to determine a true clinical neurological status. Of course we have use the benefits of imaging, which can report the real damage to the brain as well as the cranial cavity. Only when we stop the analgetic sedation can we get useful data for a Glasgow score.

Our experience in Floreasca Hospital, in the Neurosurgery Department, has taught us that early neuroprotective treatment is essential.

The patients we received represent road traffic accidents, railroad accidents, physical assaults, etc. Patient survival is the top priority of the ICU, but the surviving patient needs a nervous system capable of efficiently processing and issuing commands compatible with the patient's environment. The result of activity on the Neurosurgery ITS is quantified in the number of patients who are discharged conscious and cooperative. Neurological sequelae are devastating and turn a previously normal human life into a boundless nightmare.

We used a therapeutic protocol which, when applied to patients, proved surprisingly effective in patients who had already been admitted to the Intensive Care Unit and who had progressed

favourably in the areas of respiratory, circulatory and digestive pathology, but neurologically remained with a GCS at a level difficult to accept for a young person and for whom life still had much to offer. We had patients aged 19/20/35/50/60 years, practically the whole age range, in whom the application of the protocol led to an unexpectedly good evolution. It is the **sea buckthorn juice** obtained by mechanical pressing, which must necessarily be administered fresh, orally or nasogastric tube (when the patient is in a coma), and which after use in countless cases never had incompatibilities or side effects, produced by the substance itself. Gastroesophageal reflux may exist, but I have annihilated it by administering prokinetics or positioning the patient in the ICU bed. **Ursomax, the second product of the protocol**, is also administered orally or via nasogastric tube and we also had no incompatibilities or side effects. The patients given this protocol, as demonstrated by the patients' daily progress, showed a trend of improvement in their Glasgow score, with improvement in their ability to understand and carry out orders, their ability to understand what was going on around them and, moreover, to integrate. Polytrauma is a misfortune that occurs at some point in life, but the perpetuation of the misfortune with a tendency to become permanent, turns life into a nightmare. It is the obligation of doctors to understand this situation and to help patients to overcome this drama in their lives, returning exactly where they left off - a normal environment, where the patient had a family, friends, etc. Our results at Floreasca give us the courage to consider that life of each person is the most important thing ever, for him and for society, and we should do everything to generate the driving force that helps him overcome this dramatic struggle he has gone through.

3. DISCUSSION OF CLINICAL CASES WHICH BENEFITED FROM FLOREASCA TREATMENT PROTOCOL

In this chapter we have presented in detail only 10 clinical cases of patients with different types of neurotrauma, who were additionally submitted to the Floreasca Protocol. All the intervention with the two types of supplements, sea buckthorn juice and Ursomax had the EC approval of the SCUB.

CLINICAL CASE 1

Personal observation of the evaluated case. When the patient with numerous haemorrhagic foci, both in the subarachnoid and intra-parenchymatous area, is predisposed by a possible anticoagulant treatment to their confluence and to the organisation of a haematoma with a compressive effect inside the cranial cavity, the alternative of administering sea buckthorn juice, intervenes in the most effective way, by several mechanisms: it stops the advance of coagulation

in traumatic foci where inflammatory and procoagulant factors are released at the same time; it stops oxidative stress and limits lipo-peroxidative oxidation; vitamin C and polyphenols, in their natural state, in sea buckthorn bring an additional antioxidative efficiency.

CLINICAL CASE 2

Personal observation of the evaluated case. CE, young patient with a family, victim of a serious accident, is admitted on 28.08.2016 with the diagnosis: “Severe craniocerebral polytrauma. Haemorrhagic brain injury ...” with long-term alteration of consciousness. Being a polytrauma, the concomitant multidisciplinary approach in STI-NCH. During the first 3 weeks the patient received from the Floreasca Protocol - Ursomax, 2 capsules/3 times/day, adding later the sea buckthorn juice (10ml/kg - weight). The evolution of the patient had a slower onset, being observed, at the September consultations, myopic pupils, isochore, reactive to light stimuli, trunk reflexes present (positive cough), positive Babinski, hyperreflexia bilateral extremities, discrete mobilization of both lower limbs. Being a young patient and a cervical trauma with odontoid fracture type 2 subclass B, the mobilization of the patient was performed within the limited parameters by the physiotherapist observing an improvement of the general condition so that at the neurological examination of 2.11.2016 we have a conscious, true patient, with difficult cooperation and with a brain MRI examination with haemorrhagic contusions and diffuse axonal lesions (post acute TBI). The subsequent evolution of the patient was excellent.

CLINICAL CASE 3

Personal observation of the evaluated case. Patient aged 22, admitted on 28.12.2016 with the diagnosis: “Road traffic accident. Severe craniocerebral polytrauma. Acute subdural haematoma of left hemisphere Diffuse cerebral oedema Haemorrhagic contusion of brain stem. Apart from neurosurgical diagnosis there was also renal capsule rupture, right pneumothorax, pelvis fracture, lumbar spine fracture, segment 6 liver contusion, epileptic disease”. The vital prognosis was reserved since admission. Depakine 500mg/2 times/day was required for the whole period in the STI. Progress of this patient was slow, persistent efforts were made to mobilize him at least 3 times/day and to administer Floreasca Protocol, sea buckthorn juice (10ml/kg weight) + Ursomax. I must admit that this case gave us the satisfaction and privilege of giving a chance of survival to this young man.

CLINICAL CASE 4

Personal observation of the evaluated case. Patient aged 19, admitted on 27.05.2017 at Floreasca Hospital following a road accident with a complex injury balance: severe TBI, diffuse

axonal injuries, chest trauma with multiple rib fractures and bilateral drained hemopneumothorax, left hemidiaphragm rupture, upper splenic and left renal pole contusion, pelvis fracture. The diagnosis in its complexity tells us about the kinetics that produced the trauma. It is clear that the patient being in a deep coma required ventilatory support and intervention of the appropriate specialist teams. Brain CT describes the presence of peri-mesencephalic cisterns with small areas of right frontal haemorrhagic contusion. The patient was admitted to Glasgow 3. Receives the Floreasca Protocol with family consent, sea buckthorn juice (500ml, divided into 3 doses: 200/200/100ml, hours: 8-11-13 and Ursomax (2 cp/3 times/day). The patient is cooperative, becomes interested in what is going on around him, asks questions and waits for answers, cooperates with the physiotherapy staff, executes commands and mobilizes upper and lower limbs.

CLINICAL CASE 5

Personal observation of the evaluated case. Patient aged 22, victim of a road traffic accident, with altered general condition, GCS=4p, with diagnosis: Polytrauma, Severe traumatic brain injury, Diffuse haemorrhagic brain injury, type Marshall II. The evolution of this case demonstrates the excellent collaboration between the classical protocols and the Floreasca protocol. Being a young patient, with remarkable functional reserves, the improvement of the general condition occurred at a fast pace, allowing us to recommend the referral of the patient to the Neuromuscular Recovery Clinic.

CLINICAL CASE 6

Personal observation on the case evaluated. GMV, 21-year-old patient, victim of a road accident, admitted on 28.08.18 with the diagnosis: Severe traumatic brain injury. Diffuse haemorrhagic lesion type Marshall II. On 30.09.18, with the family's consent, the Floreasca Protocol is recommended: fresh sea buckthorn juice (10ml/kg weight, administered in 3 tranches: 8-12-14) and Ursomax (2capsules /3 times/ day). Favourable evolution soon followed, repeated neurological examinations describing an evolution of the patient who on 27.10 was already conscious, cooperative, afebrile, spontaneously breathing, BP=145/96mmHg, AV=107bpm.

CLINICAL CASE 7

Personal observation on the case evaluated. PMR, a 19-year-old patient with no pre-existing pathology, is admitted to Floreasca Hospital with the diagnosis: Road traffic accident Severe craniocerebral polytrauma Glasgow coma 4p Diffuse subarachnoid haemorrhage Diffuse cerebral oedema Cervical spinal trauma with C4-C5 subluxation grade 1. As the patient's general condition is very serious, it is decided with the family's consent to apply the Floreasca protocol

(fresh buckthorn juice (10ml/kg of buckthorn in 3 times, 8-12-14 hours) and Ursomax (2 cp /3 times/day)), together with the complex treatment in intensive care. The evolution after discharge was favourable.

CLINICAL CASE 8

Personal observation on the case evaluated. Patient aged 39 years, admitted on 01.08.2020 at Floreasca clinical hospital following an assault while on duty, the patient being a taxi driver. He is brought in altered state of consciousness, GCS=11p, anisocoria, right eye>left eye, left brachial deficit. Cranial CT describes right TP epidural hematoma underlying a cranial fracture focus. On 07.08, taking into account the evolution of the patient and the subsegmental risks we propose to the family the Floreasca protocol: fresh sea buckthorn juice (10 ml/kg body) administered 3 times a day as follows: 250ml at 8 am, 250ml at 12 am and 200ml at 2.30 pm and Ursomax (2 cp/3 times/day) on nasogastric tube. On 18.09.20, we have a conscious, cooperative, slightly drowsy patient, still brady psychic, no frowning, pupils equal, preserved swallowing for solids and liquids, right hemiparesis, 2/5 upper limb, 3/5 pelvic limb. ROT present. On 9/22 patient is conscious, cooperative, no motor deficits, breathing spontaneously, efficient, no peripheral oedema. This seemingly hopeless complex case with neurosurgical and pulmonary complications has managed to make a salutary recovery with a team who monitored him and provided specialist support. It should be noted that our patient had a liver B virus infection with impaired anti-infective defense performance. It is obvious that the Floreasca protocol helped him a lot. After discharge from the hospital, the patient had a normal evolution with a remarkable integration into society and family.

CLINICAL CASE 9

Personal observation on the case evaluated. The 39-year-old patient is admitted on 12.12.19 to Floreasca Clinical Hospital with the diagnosis: Polytrauma due to railway accident. Severe craniocerebral trauma. Skull base fracture. On admission, the patient presents extremely serious general condition, analgetic sedated, residual curarized, myopic pupils, anisocoria, right eye>left ey, slow reactive, oro-tracheally intubated, mechanically ventilated under pressure. I recommended with family consent, Floreasca protocol: fresh sea buckthorn juice (10ml/kg body, divided into 3 doses at 8-12-14.30) and Ursomax (2 capsules /3 times/ day). As can be seen, the Floreasca protocol accelerated the neuromotor recovery mechanisms and in the few days of treatment the nervous system showed adaptive abilities in dynamics. In the period of about 1 month and a half, while under treatment in STI-NCH, the effective GCS score doubled. This period is approx. equal to the period of admission to POLI I and POLI II wards, from where he was

transferred with GCS=6p, to STI-NCH ward. This extremely difficult case proves the essential contribution of phytotherapy in complementarity with the principles of classical allopathic medicine. Practically, in a month and a half, the patient managed to survive the dysfunctions caused by the serious accident, thanks to the professionalism and dedication of the medical teams, the important functions of the body stabilizing. Our mission was to push the value of this GCS=6p score to a value where the patient had the power to process the information and give the right solution.

CLINICAL CASE 10

Personal observation on the case evaluated. MF, patient 20 years old, is admitted on 30.04.21, in Floreasca Emergency Clinical Hospital, for Polytrauma with severe TBI at the level of basal nuclei, there is presence of haemorrhagic lesion of 14/8mm, important diffuse cerebral oedema with disappearance of cortical grooves and cisternal, infra and supratentorial spaces. The patient being in extremely serious condition, with the family's consent, the Floreasca Protocol is applied, consisting of: fresh sea buckthorn juice (10ml/kg weight/day, administered in 3 doses: 250ml at 8.00 am; 250ml at noon and 200ml at 2.30 pm, as well as Ursomax (2 capsules /3 times/day). The evolution of the general condition was classified as "psycho-cognitive status and marked psychomotor agitation", which caused a lack of communication between mother and daughter. After the consultation at home, we recommended further treatment based on the Floreasca Protocol. I would like to mention that the Floreasca Protocol has been stopped since the second half of July 2021, without my consent. The resumption of the protocol at home meant a remarkable neurocognitive leap, which consisted, first of all, in the cessation of agitation, as well as a significant increase in the capacity of reception, synthesis and ideational association.

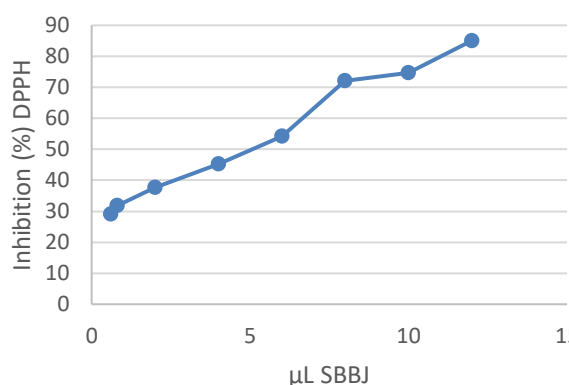
4. INFLUENCE OF FRESH SEA BUCKTHORN JUICE

ADMINISTRATION ON BIOCHEMICAL PARAMETERS IN PATIENTS WITH TRAUMATIC BRAIN INJURY

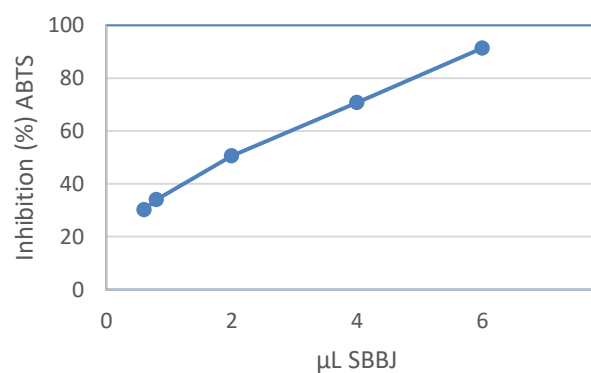
This chapter presents phytochemical research on sea buckthorn juice, a very important product in the supplementary therapy of neurotrauma patients. The content of flavones, total polyphenols and carotenoids was quantitatively determined. The antioxidant action induced by the phyto-complex in sea buckthorn juice was also evaluated. To achieve this scientific goal, the scavenger capacity on free radicals (DPPH and ABTS) and iron reducing capacity (Fe^{+3}) were determined. These methods have also been used by other authors in the evaluation of antioxidant activity for different types of plant products, plant extracts [16].

Antioxidant activity determined for sea buckthorn juice

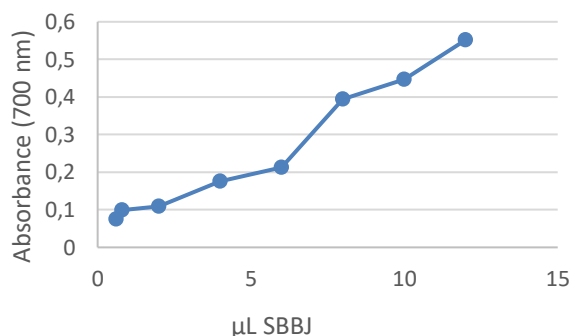
Method	DPPH	ABTS* +	Iron reduction
EC ₅₀ (μL)	4.70 ± 0.2687	2.1250 ± 0.1060	11.66 ± 0.1272
Ascorbic acid equivalents (mM ascorbic acid/100 mL fresh juice)	4.2732 ± 3.3491	19.5275 ± 7.9394	1.0748 ± 0.0853



A



B



C

FIGURE 1.
Evaluation of antioxidant action
Caption: A - DPPH, B - ABTS* +,
C - iron reduction

The results showed the scavenger capacity of sea buckthorn juice on DPPH and ABTS radicals, as well as its reducing properties on iron.

As shown in Figure 1A, the DPPH free radical scavenging activity ranges from 29.11% (for 0.6 ml) to 85.07% (for 12 ml). The DPPH free radical scavenging capacity was higher compared to the values published by Tian Ye et al. who found an inhibitory value of only 29-35% [17]. Regarding ABTS radical, fresh sea buckthorn juice produced scavenging of this radical in a dose-dependent manner, the highest inhibition of 91.36% was obtained for 6 ml (Fig. 1B). The antioxidant activity is higher against ABTS radical compared to DPPH, possibly a consequence of

a different mechanism of action. It is well known that the DPPH method is mainly based on electron transfer and is usually applied to hydrophilic antioxidants (flavones, phenol-carboxylic acids, proanthocyanidins, etc.), whereas the ABTS assay has a mixed mechanism, both electron and proton transfer. Lipophilic compounds (carotenoids) are also responsible for the antioxidant effect generated [18]. Regarding iron reduction (Fig. 1C), the highest absorbance of 0.5518 was found at the highest concentration of fresh juice - 12 ml. According to the data contained in Table IV.5, the highest antioxidant activity was highlighted by the ABTS method, followed by DPPH and iron reduction.

The neuronal resuscitation protocol (Floreasca protocol) aimed at administering fresh sea buckthorn juice (obtained by cold pressing) in order to prevent the production of secondary lesions in patients with neurotrauma admitted to the Floreasca Emergency Clinical Hospital, Anesthesia Intensive Care Clinic (NCH-STI).

It should be noted that a batch of 26 patients received fresh sea buckthorn juice in addition to the medication specific to the condition. Administration was via nasogastric tube every morning between 8.30-10.30, 10ml/kg weight / day. It is important that the patient does not receive food within 2 hours before and after administration of the sea buckthorn juice. In exceptional cases, if the patient is prone to gastric stasis, gastric rinses should be made on the nasogastric tube with about one litre of aniseed and fennel tea, after which Prodigest is administered, 4 tablets each (the suspension obtained after dispersing them in water, administered through the nasogastric tube). In the absence of medicinal teas, physiological saline can be used. After this, the buckthorn juice is administered. Patients were monitored for 20 days, parameters assessed were serum sodium and lactate (hospital medical laboratory) and Glasgow coma score, compared to a control group of 5 patients who were given only the condition-specific medication.

Sodium lactate was measured from blood serum by EAB determination at least once a day (usually in the morning).

Serum Na was assessed by serum ionogram (also taken in the morning). Sodium ion is determinant in assessing plasma osmolarity involved in the evolution of cerebral oedema of the brain subjected to severe trauma.

The data analysis shows a normalization of the serum sodium parameter, leading to a reduction of cerebral oedema with improvement of respiratory and cardiac functional parameters and, conclusively, the improvement of the Glasgow score. Normalization of the serum lactate parameter is observed. The return of this parameter to normal values is in line with the clinical

evolution of patients undergoing specific treatment under the Floreasca Protocol. The gradual evolution of the Glasgow score demonstrated the effectiveness of the protocol applied to patients with neurotrauma. I should mention that all patients were admitted with a low Glasgow score (comatose state) with a favourable evolution. Statistical analysis was performed using open source software R. The dataset is longitudinal, characterized by repeated measurements, performed on the same individual over 20 days. The main purpose of this longitudinal study is to characterize the changes observed in time (20 days) for 3 continuous response variables: Serum Na, Serum lactate, Glasgow score. It is also important to highlight the differences between the two groups: control and tested. For the serum Na response variable we obtained significant differences between groups (p value=0.0049) and significant differences for the time factor (p value=0.002) but a non-significant interaction between the time and group factors. Significant post hoc differences between groups were observed on days 1,3,4,7,8. For the serum lactate response variable we obtained significant differences between groups (p value=0.00012) and significant differences relative to the time factor (p value=0.002) and also a significant interaction between the two time factors and the group (p value=0.009). Significant post hoc differences between groups were observed on days 1,2,3,4,5,11,13,18,19,20. For the Glasgow score response variable we obtained highly significant differences between groups (p value<0.0001) and significant differences relative to the time factor (p value=0.0011) and also a significant interaction between the two factors time and group (p value=0.0066). Post hoc significant differences between groups were observed in all 20 days of the study. In the second part of the statistical analysis we followed the evolution of the median Glasgow score in relation to the mean values of the two parameters Na and Lactate over the 20 days. We used Pearson's parametric test because the assumption of normality and bivariate residuals is met. It can be seen that the Pearson-type statistical effect size indicates an extremely high negative correlation in the tested group (-0.962 Lactate and -0.96 Na) and a negative mean value in the control group (-0.62 Lactate and -0.68 Na), interpretations given by Cohen. The p-values for Pearson coefficients are highly significant (p-value<0.001).

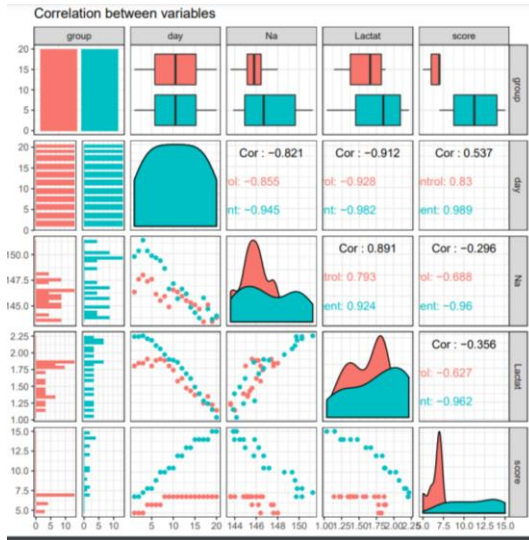


FIGURE 2.

Correlation of all evaluated parameters (Na, Lactate, Glasgow score/time/groups)

5. INFLUENCE OF DIETARY SUPPLEMENTS ON BIOCHEMICAL PARAMETERS IN PATIENTS WITH TRAUMATIC BRAIN INJURY

In another personal research study, we looked at the beneficial effects of giving neurotrauma patients another type of dietary supplement. Our project started from building a consistent relationship of continuity between pre-injury lifestyle with post-injury lifestyle through the combined effort of a team with long experience in polytrauma (ICU, neurosurgery, general surgery, thoracic surgery, ENT, ophthalmology, orthopaedics, internal medicine, cardiology, haematology, physiotherapy, psychology and 24/7 monitoring to avoid disorders and treat them when occurring). Achieving the pre-accident - post-accident arc of normality involves restoring the normal physiology of the body and in particular of the central nervous system for the reconstruction of family life and socio-professional life in a more plastic form, the individual regains his identity in society. In order to achieve all these goals, the neural resuscitation protocol (protocol of "Floreasca Emergency Hospital", Bucharest, Romania) was also used to administer the Ursomax supplement to patients with neurotrauma, admitted in the same Anaesthesia and Intensive Care Unit (NCH-ITS) at Floreasca Emergency Hospital. As the partial results were not therapeutically remarkable, it was decided to combine it with sea buckthorn juice. Patients followed the treatment for 21 days. The suspension (15-20 ml) resulting from dispersion of the contents of 2 tablets in distilled water and 10 ml/kg weight /day of sea buckthorn juice were administered by nasogastric tube. The administration was done 3 times a day and the patients did not receive food 2 hours before and after the supplements.

Throughout the monitoring period, sodium, potassium, haemoglobin, leucocytes, lymphocytes, urea, creatinine, C-reactive protein and Glasgow score were determined on blood samples collected from patients. The clinical results obtained were satisfactory, even when the GCS was 3, which implies the presence of multiple organ dysfunction.

Preliminary statistical analysis found that:

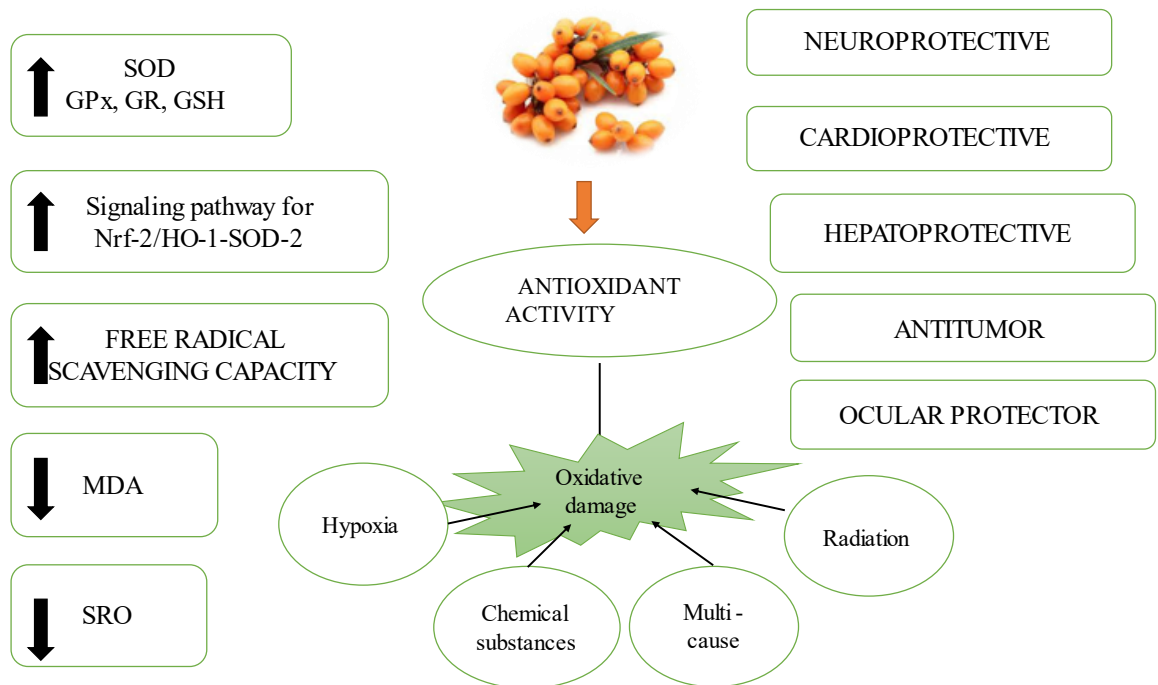
- ✓ Negative correlations between serum sodium and C-reactive protein, correlation coefficient = -0.46, p-value for statistical significance was 0.0382;
- ✓ Negative correlations between serum potassium and creatinine, correlation coefficient = -0.62, p-value = 0.0026;
- ✓ Positive, statistically significant correlations between serum potassium and C-reactive protein, correlation coefficient = 0.48, p-value = 0.0264;
- ✓ positive, statistically significant correlations between blood urea and serum creatinine, correlation coefficient = 0.55, p-value = 0.0098.

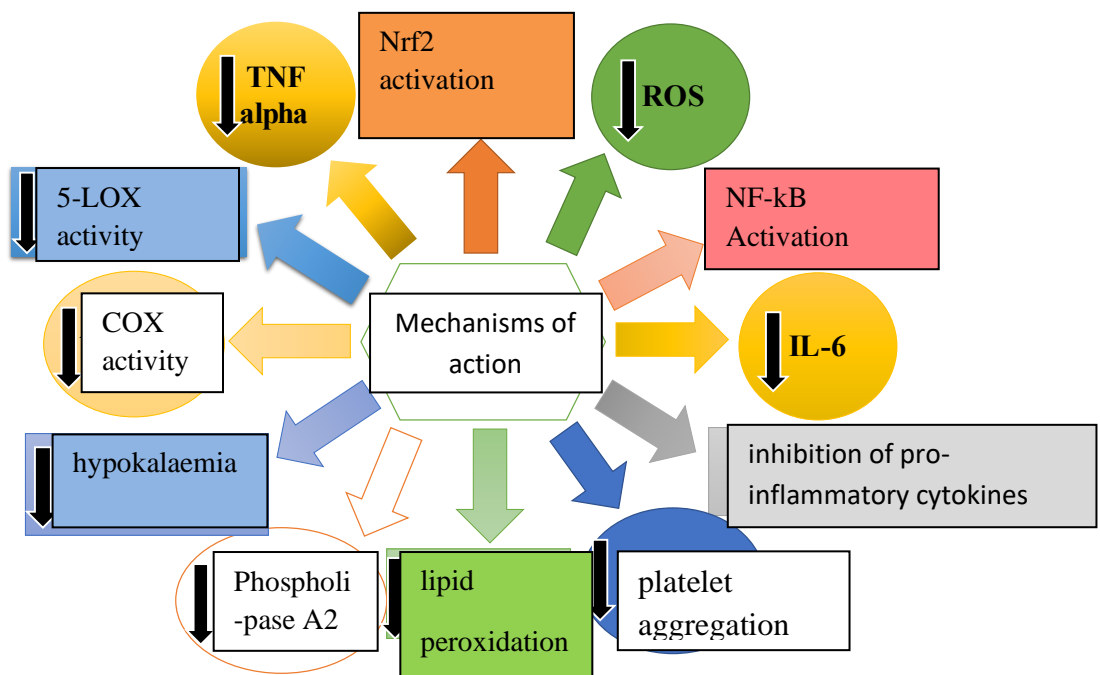
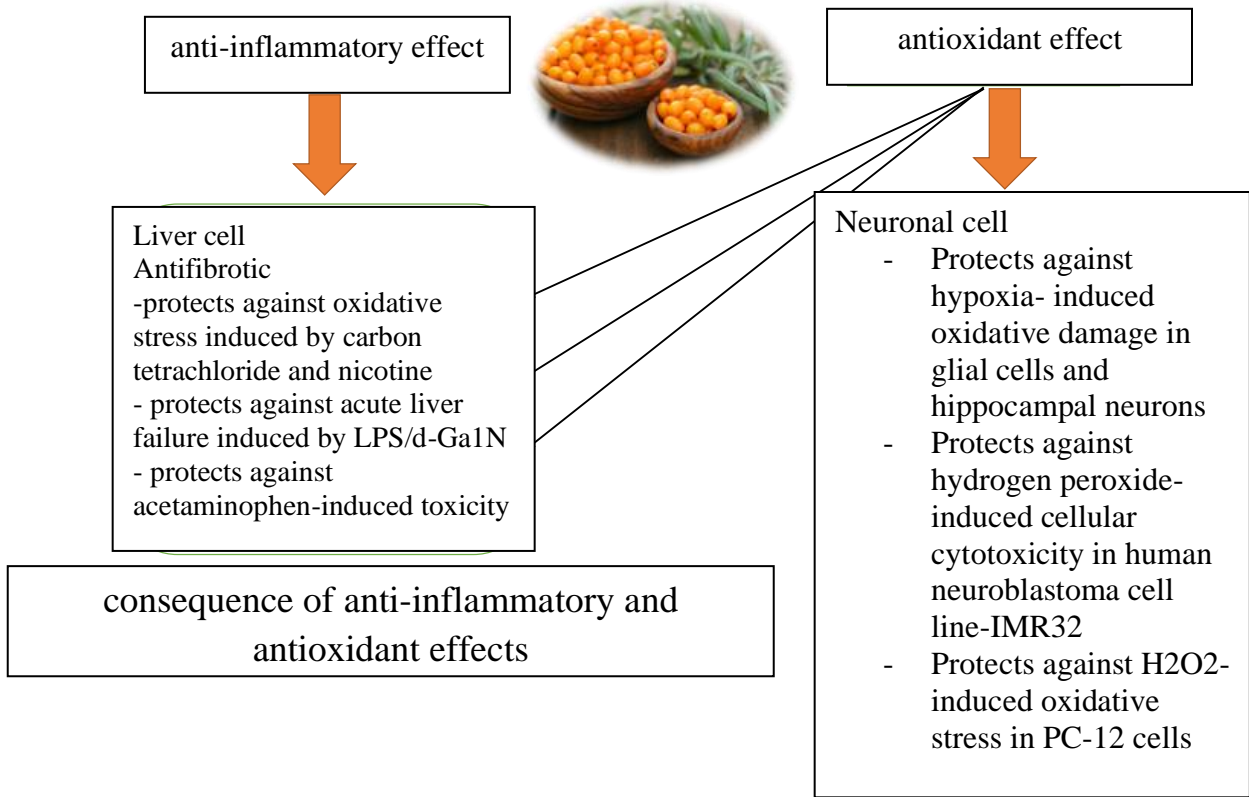
The correlation of sodium/potassium and C-reactive protein serum value was also highlighted by Yilmaz et al., the authors of the study found that there is a direct relationship between high sodium intake, inflammatory process and C-reactive protein. Therefore, the negative correlation between serum sodium and C-reactive protein and the positive correlation between serum potassium and C-reactive protein should be noted, which is probably due to the compensatory mechanism of urinary excretion in patients with high salt intake [213]. The positive relationship between serum potassium and C-reactive protein could be due to impaired renal function in some patients. On day 13 of treatment, a relationship between urea levels and SCG was identified, correlation coefficient = 0.43, p-value = 0.0527. On day 19 of treatment, there was a negative correlation between serum creatinine and C-reactive protein, correlation coefficient = -0.50, p-value = 0.0223, and between C-reactive protein and SCG, correlation coefficient = -0.54, p-value = 0.0399.

In conclusion, the combination of Ursomax supplement and sea buckthorn juice developed beneficial effects in patients with neurotrauma, with the influence of all parameters assessed being consistent with the Glasgow score.

6. CONSIDERATIONS ON POTENTIAL MECHANISMS OF ACTION INDUCED BY PHYTOCOMPLEXES ADMINISTERED TO NEUROTRAUMA PATIENTS

Patients given this protocol, as demonstrated by the daily developments, tended to improve their Glasgow score, with an improved ability to understand and carry out the orders given, with the ability to understand what was happening around them and, moreover, to integrate. Polytrauma is a misfortune that occurs at some point in life, but the perpetuation of the misfortune with a tendency to become permanent, turns life into a nightmare. It is the obligation of doctors to understand this situation and help patients to overcome this drama in their lives, returning exactly where they left off - a normal environment, where the patient had a family, friends, etc. Our results at Floreasca give us the courage to consider that the life of each person is the most important thing ever, for him and for society, and we should do everything to generate the driving force that helps him overcome this dramatic struggle he has gone through. If we were to summarise the whole range of biologically active compounds and the beneficial effects of taking sea buckthorn juice and Ursomax they would look like this:





GENERAL CONCLUSIONS. DEGREE OF ORIGINALITY . RESEARCH PERSPECTIVES

GENERAL CONCLUSIONS

The following conclusions can be drawn from the studies carried out:

- We have summarised the most important aspects of brain trauma and the therapeutic approaches to this multiple pathology;
- We systematized literature data on active principles administered in laboratory experiments to experimental animals that have undergone head trauma;
- we have followed the beneficial effects induced by the administration of sea buckthorn juice and Ursomax supplement in patients with neurotrauma admitted to the Neurosurgery Department of Floreasca Emergency Hospital;
- we selected 10 clinical cases of patients admitted with craniocerebral trauma to the Neurosurgery Department of Floreasca Emergency Hospital, who were administered the Floreasca Protocol (fresh sea buckthorn juice 10ml/kg body, divided into 3 doses at 8-12-14.30 and Ursomax - 2cp /3 times/day);
for each clinical case presented, I have also inserted my personal point of view, regarding the evolution of the patients to whom these supplements were administered; I mention that all the cases selected were under personal observation;
- Phytochemical research carried out on sea buckthorn juice/fresh sea buckthorn fruit justifies its association with neurotrauma patients, thus it has a content of 0.0934 ± 0.0104 /100 ml flavones, expressed as hyperoside, 0.2895 ± 0.0162 /100 ml total polyphenols, expressed as tannic acid, 3.0940 ± 0.23 mg β -carotene /100 g fresh fruit and 0.1595 ± 0.014 mg lycopene/100 g fresh fruit;
- The determination of the antioxidant action by the three methods - DPPH, ABTS, iron reduction, justifies the antioxidant effect, thus the DPPH free radical scavenging activity varies between 29.11% (for 0.6 ml) and 85.07% (for 12 ml); as for the ABTS radical, fresh sea buckthorn juice produced scavenging of this radical in a dose-dependent manner, the highest inhibition of 91.36% was obtained for 6 ml; antioxidant activity is higher for ABTS radical compared to DPPH, possibly as a consequence of a different mechanism of action; it is well known that the DPPH method is mainly based on electron transfer and is usually applied to hydrophilic antioxidants (flavones, phenolcarboxylic acids, proanthocyanidins,

etc.), while the ABTS assay has a mixed mechanism, both electron and proton transfer; lipophilic compounds (carotenoids) are also responsible for the antioxidant effect generated; as far as iron reduction is concerned, the highest absorbance of 0.5518 was found at the highest concentration of fresh juice - 12 ml;

- we followed the effect induced by the administration of sea buckthorn juice on some biological parameters and Glasgow score: for the serum Na response variable we obtained significant differences between groups (p value=0.0049) and significant differences relative to the time factor (p value=0.002) but an insignificant interaction between the time and group factors; for the serum lactate response variable we obtained significant differences between groups (p value=0.00012) and significant differences relative to the time factor (p value=0.002) and also a significant interaction between the time and group factors (p value=0.009); for the Glasgow score response variable we obtained highly significant differences between groups (p value<0.0001) and significant differences relative to the time factor (p value=0.0011) and also a significant interaction between the two factors time and group (p value=0.0066);
- We investigated the effects of Ursomax and sea buckthorn juice supplementation on biochemical parameters and Glasgow score in neurotrauma patients: negative correlations between serum sodium and C-reactive protein, correlation coefficient = -0.46, p-value for statistical significance was 0.0382; negative correlations between serum potassium and creatinine, correlation coefficient = -0.62, p-value = 0.0026; positive, statistically significant correlations between serum potassium and C-reactive protein, correlation coefficient = 0.48, p-value = 0.026; positive, statistically significant correlations between blood urea and serum creatinine, correlation coefficient = 0.55, p-value = 0.0098;
- we have identified potential mechanisms of action that may account for the beneficial effects induced in neurotrauma patients

DEGREE OF ORIGINALITY

The intensive care unit in any hospital is the place where it all works, the last frontier between TO BE and NOT TO BE. Success in intensive care is measured by the number of lives saved. Where therapeutic protocols in internal medicine, cardiology, general surgery, neurosurgery fail, intensive care begins. Having metabolic, neurological, haemodynamic, respiratory etc. monitoring capabilities, it actively intervenes in the regulation of disturbances in all these pathophysiological compartments to bring organic dysfunctions back to normal. Those who have been practising

intensive therapy for many years are less familiar with the therapeutic intervention of medicinal plants, because the medico-legal responsibility is monitored by international laws that establish, first of all, the essential Hippocratic condition - *Primum Non Nocere Deinde Salutare*. Most of the drugs used in intensive therapy have strict indications with known half-life, which allows their controlled use. For certain herbs to be used in this very special field, their therapeutic properties need to be evident and recognised in the international literature in order to fit in with the professional medical act that actually saves the patient in a critical situation.

The use of ketosis in intensive care therapy started from an ambivalent therapeutic experience carried out for many years, which allowed us to approach the case of intensive care therapy in a responsible and professional way, without harming any factor that comes into the equation of our treatment. The written agreement, as well as the detailed explanations, have always convinced advocates of the soundness of the therapeutic rationale, and indeed things have worked as predicted. Neurosurgical trauma was the ground on which we used therapeutic protocols that led not only to saving patients but also to achieving neurological performance, allowing them to return to a way of life close to everyday reality. As in the case of the sea buckthorn, Ursomax represents the call that plants from the Carpathian area reconfigure, in critical situations, the return of parameters to the level of normality through the activity of the active principles of *Allium ursinum*, *Taraxacum officinale*, *Urtica dioica* at the level of cellular and mitochondrial membranes.

Sea buckthorn, as far as I know, has never been used in intensive care units in our country. Its use in an advanced discipline was motivated by the exceptional qualities of the therapeutic principles existing in this plant. I confess that I was pleasantly surprised to find that patients under treatment with fresh sea buckthorn juice and Ursomax had an unexpectedly good evolution, which convinced me that the therapeutic arsenal can be improved for the benefit of patients.

RESEARCH PERSPECTIVES

I believe that the Floreasca protocol is just a promising start for a future major therapeutic approach in ICU.

Combining other types of supplements to reduce the recovery time of neurotrauma patients is the new goal.

Selective bibliography:

1. Marik PE, Varon J, Trask T, Management of head trauma. *Chest*, 2002; 122:699-711
2. Bramlett HM, Dietrich WD, Pathophysiology of cerebral ischemia and brain trauma: similarities and differences *J Cereb Blood Flow Metab*, 2004;24(2):133 - 50.
3. Johnston MV, Excitotoxicity in perinatal brain injury. *Brain pathology*, 2006. <https://doi.org/10.1111/j.1750-3639.2005.tb00526.x>
4. Northington FJ, Graham EM, Martin LJ, Apoptosis in perinatal hypoxic-ischemic brain injury: how important is it and should it be inhibited? *Brain Research Reviews*, 2005;50(2):244-257
5. Jones NC, Constantin D, Gibson CL, Prior MJ, Morris PG, Marsden CA, Murphy S, A detrimental role for nitric oxide synthase-2 in the pathology resulting from acute cerebral injury. *J Neuropathol. Exp. Neurol*, 2004;63:708-720
6. Ansari MA, Roberts KN, Scheff SW, A time course of NADPH-oxidase up-regulation and endothelial nitric oxide synthase activation in the hippocampus following neurotrauma. *Free Radic. Biol. Med.* 2014;77: 21-29
7. Kaltschmidt B, Widera D, Kaltschmidt C, Signaling via NF-kappaB in the nervous system. *Biochim. Biophys. Acta*, 2005;1745:287-299
59. Al Moutaery K, Al Deeb S, Ahmad Khan H, Tariq M, Caffeine impairs short-term neurological outcome after concussive head injury in rats. *Neurosurgery*, 2003;53:704-711
8. Sharma S, Ying Z, Gomez-Pinilla F, A pyrazole curcumin derivative restores membrane homeostasis disrupted after brain trauma. *Exp. Neurol*, 2010;226:191-199
- Kopan R, Ilagan MX, The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*, 2009;137:216-233
10. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K, A new model of diffuse brain injury in rats. Part I: Pathophysiology and biomechanics. *J Neurosurg*, 1994;80:291-300
11. Itoh T, Imano M, Nishida S, Tsubaki M, Mizuguchi N, Hashimoto S, Ito A, Satou T, (-)-Epigallocatechin-3-gallate increases the number of neural stem cells around the damaged area after rat traumatic brain injury. *J. Neural. Transm*, 2012;119:877-890
12. Sawmiller D, Li S, Shahaduzzaman M, Smith AJ, Obregon D, Giunta B, Borlongan CV, Sanberg PR, Tan J, Luteolin reduces Alzheimer's disease pathologies induced by traumatic brain injury. *Int. J. Mol. Sci*, 2014;15:895-904

13. Cui QJ, Wang LY, Wei ZX, Qu WS, Continuous naringin treatment benefits the recovery of traumatic brain injury in rats through reducing oxidative and inflammatory alterations. *Neurochem. Res.* 2014;39:1254-1262
14. Yao C, Zhang J, Liu G, Chen F, Lin Y, Neuroprotection by (-)-epigallocatechin-3-gallate in a rat model of stroke is mediated through inhibition of endoplasmic reticulum stress. *Mol. Med. Rep.* 2014;9:69-76
15. Jurca T, Pallag AM, Marian E, Mureşan ME, Stan RL, Vicaş LG, The histo-anatomical and the polyphenolic profile of antioxidant complex active ingredients from three *Viola* species *Farmacia*, 2019; 67(4):634-640
16. Tian Y, Pukanen A, Alakomi HL, Uusitupa A, Saarela M, Yang B, Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. *Food research international*, 2018; 106: 291-303
17. Dudonné S, Vitrac X, Coutierré P, Woillez M, Mérillon JM, Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays *Journal of Agricultural and Food Chemistry*, 2009; 57(5): 1764-1778
18. Yilmaz R, Akoglu H, Altun B, Yildirim T, Arici M, Erdem Y, Dietary salt intake is related to inflammation and albuminuria in primary hypertensive patients *Eur J Clin Nutr*, 2012; 66: 1214-1218

List of published scientific papers

Articles published in ISI-listed journals

Godeanu SC, Marin D, Stănciulescu L, Gane D, Văleanu A, Ghica M, Lupuliasa D, Gîrd CE, Efficacy evaluation of the association of some vegetal extracts in patients suffering from traumatic brain injury. Pilot study (II), *Pharmacy*, 2021, Vol. 69(6):1139-1144; <https://doi.org/10.31925/farmacia.2021.6.18>

Godeanu CS, Costea T, Ghica M, Lupuliasa D, Gîrd CE, Evidence-based use of sea buckthorn fresh juice for patients with traumatic brain injury. A pilot study, *Farmacia*, 2020, 68(3): 541-546, <https://doi.org/10.31925/farmacia.2020.3.21>

Papers presented at national and international conferences with publication in abstract form

Godeanu CS, Stănciulescu EL, Popescu M, Grintescu IM, A novel approach to treatment of haemorrhagic cerebral vascular accidents. *Euroanaesthesia*, Geneva, 3-6.05.2017, Abstract published in the *European Journal of Anaesthesiology*, Volume 34, Supplement 55 (The abstract has been published in the e-Supplement of the *European Journal of Anaesthesiology* (Volume 34, Supplement 55, June 2017) under the Accepted Abstract Number reference)

Godeanu CS, Marin DE, Druță V, Năstatse P, Stănciulescu EL, The role of phytotherapy in the complex management of trauma. National interdisciplinary conference with international visibility - Comprehensive approaches to pain and vertebral-medullary pathology in the context of the International Day of Myelic Injuries, Techirghiol, 2017. Abstract published in *Balneo Recherche Journal*, Balneara Publishing, 8(3):164